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The conformation of abscisic acid by n.m.r. and a revision of the proposed mechanism for cyclization during its biosynthesis

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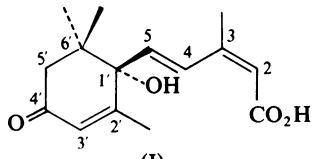
The n.m.r. spectrum of abscisic acid (ABA) formed from [1,2-$^{13}C_2$]acetate by the fungus *Cercospora rosicola* shows ^{13}C–^{13}C coupling between C-6′ (41.7 p.p.m.; 36 Hz) and the downfield 6′-methyl group (6′-Me) (24.3 p.p.m., 36 Hz). This 6′-Me, therefore, is derived from C-3′ of mevalonate [Bennett, Norman & Maier (1981) *Phytochemistry* **20**, 2343–2344]. An i.n.e.p.t. (insensitive nuclei enhanced by polarization transfer) pulse sequence demonstrated that the downfield ^{13}C signal is produced by the 6′-Me that gives rise to the upfield ^1H 6′-Me signal (23.1 d). The absolute configuration of this, the equatorial 6′-Me group, was determined as 6′-*pro*-R by decoupling and n.O.e. (nuclear-Overhauser-enhancement) experiments at 300 MHz using ABA, ABA in which the axial 6′-*pro*-*S* 5′-hydrogen atom had been exchanged with ^2H in NaO^2H and the 1′,4′-*cis*- and 1′,4′-*trans*-diols formed from these samples. The configuration at C-1′ and at C-6′ are now compatible with a chair-folded intermediate during cyclization, as proposed for β- and ε-rings of carotenoids. ABA in solution exists, as in the crystalline form, with the ring in a pseudo-chair conformation. The side chain is axial and the C-3 Me and the C-5 hydrogen atoms are predominantly *cis*(*Z*).

The first investigations of ABA biosynthesis sought to find a clear, qualitative difference between its stereochemistry and that of carotenoids. If such a difference existed, then the formation of ABA (I) by cleavage of a carotenoid could be discounted.

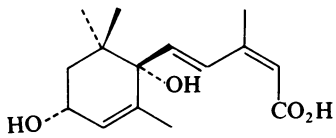
One of the features investigated was the direction of cyclization, and it was found that, like the other features examined, the absolute configuration of the *geminal* 6′-Me groups of ABA (Milborrow, 1975) was the same as that determined for the analogous Me groups of β-carotene (Bu'Lock *et al.*, 1970), i.e. the Me group that projects towards the observer structure in (I) was deduced to have been derived from C-2 of mevalonate in both compounds. The stereochemistry of cyclization of a bacterial β- and an algal ε-ring of C_{40} carotenoids has recently been conclusively shown by Britton *et al.* (1977, 1979) to be the reverse of that originally assigned (Bu'Lock *et al.*, 1970) to the β-ring from β-carotene made by the fungus *Phycomyces blakesleeanus*. Consequently either one assignment is incorrect or the β-cyclase of the fungus operates to give the opposite stereochemistry of the *geminal* Me groups. There are two ways in which this latter possibility could occur: one, the cyclizations could take place with complete, mirror-image symmetry, or, two, the reaction could proceed via a boat-folded intermediate rather than via the more planar, chair-folded intermediate proposed for the β- and ε-rings of C_{40} carotenoids (Britton *et al.*, 1977, 1979) and for the β-, ε- and γ-rings of C_{50} carotenoids (Swift & Milborrow, 1981). The stereochemistry at C-1′ of a precursor of ABA has been determined by Neil *et al.* (1982) to be the same as at the analogous C-6 position in the ε-ring of C_{40} carotenoids, so the cyclization in ABA cannot be a mirror image of that occurring in the C_{40} carotenoids. The stereochemistry that was proposed for the 6′-Me groups of ABA (Milborrow, 1975), taken together with the absolute configuration at C-1′ of the precursor 1′-deoxy-ABA (Neil *et al*, 1982), requires a boat-folded intermediate during cyclization.

(+)-*S*-ABA has recently been shown by Assante *et al.* (1977) to be formed by the fungus *Cercospora rosicola*, and Bennett *et al.* (1981) have analysed the ^{13}C n.m.r. spectrum of ABA formed by the fungus from [1,2-$^{13}C_2$]acetate. The 6′-Me signal at 23.1 p.p.m. lacked ^{13}C–^{13}C satellites, whereas the other 6′-Me signal, at 24.3 p.p.m. (coupling con-

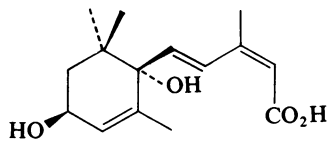
Abbreviations used: ABA, abscisic acid; n.O.e., nuclear Overhauser enhancement; i.n.e.p.t., insensitive nuclei enhanced by polarization transfer; Me, methyl; h.p.l.c., high-pressure liquid chromatography.



(I)
(+)-*S*-Abscisic acid



(II)
(+)-1′,4′-*cis*-Diol of abscisic acid



(III)
(+)-1′,4′-*trans*-Diol of abscisic acid

stant, $J = 36$ Hz), possessed them. This established their derivation from C-2 and C-3 Me of mevalonate respectively. It has now been possible to relate the ^{13}C C-6′-Me signals to their counterparts in the ^{1}H spectrum and thereby determine the absolute configuration of the C-6′-Me group derived from C-2 of mevalonate. This has necessitated the revision of the stereochemistry of the cyclization mechanism proposed for ABA (Milborrow, 1975). The revised assignments at C-6′ are compatible with chair-folding of the intermediate during cyclization.

Experimental

Compounds

The (±)-1′,4′-*cis*- (II) and (±)-1′,4′-*trans*-diols (III) were formed from (±)-ABA by $NaBH_4$ in ice-cold ethanol/water (2:1, v/v); for [5′-$^{2}H_1$]ABA the ethanol was 1:1 (v/v) with 0.2M-sodium phosphate buffer, pH 6.5. The diols were separated on silica-gel t.l.c. plates developed in toluene/ethyl acetate/acetic acid (25:15:2, by vol.); 1′,4′-*cis*-diol had R_F 0.3; 1′,4′-*trans*-diol had R_F 0.45. They were methylated with ice-cold ethereal diazomethane and acetylated with pyridine/acetic anhydride (2:1, v/v). Methyl esters of the 4′-*O*-acetyl diols were isolated as colourless crystals after h.p.l.c. in hexane/isopropyl alcohol (197:3, v/v) on a Waters' 7.8mm (internal diameter) × 300mm μPorasil column.

N.m.r

^{1}H n.m.r. spectra were recorded on a Bruker CXP-300 spectrometer operating at 300MHz in [^{2}H]chloroform in 5mm tubes with tetramethylsilane as an internal reference. Spectra were recorded with 16000 data points, 3000Hz spectral width, 90° pulse of typically 6μs, and a 3s recycle time. For n.O.e. difference spectra a 3s recycle time was used after a 4s irradiation. The decoupling frequency was placed in resonance for 16 scans and then off resonance for 16 scans and repeated. The spectra were then subtracted. The n.O.e. measurements were carried out in N_2-degassed [^{2}H]chloroform.

The methyl esters of the 4′-*O*-acetyl 1′,4′-diols of ABA isomerized at C-4′ on being left, with consequent confusion of the spectra, and so they were repurified by h.p.l.c. before each experiment.

Deuterium exchange

The 5′-protons of the methyl ester of ABA (Me-ABA) exchanged equally in [^{2}H]methanol (1ml) to which 100μl of 10M-$NaO^{2}H$ or ^{2}HCl were added. The 2′-Me and 3′-protons did not exchange until 200μl were added. Selective exchange of the downfield, axial (5′-*pro-S*) proton occurred over 4–6h when free ABA was dissolved in $^{2}H_2O$ and the 'pH' was raised to 11 by the addition of 10M-$NaO^{2}H$ (as measured with a normal pH-meter glass electrode).

Results and discussion

Relationship of the ^{13}C to the ^{1}H C-6′-Me signals

The 60MHz ^{1}H n.m.r. spectrum of ABA was first described by Ohkuma *et al.* (1965), and it was crucial for the elucidation of the structure (Tables 1*a* and 1*b*). High-field spectrometers enable the fine structure of the spectrum to be seen, but it has not been reported until now. Although the ^{13}C and ^{1}H spectra were obtained on racemic ABA and derivatives, all assignments are given for the natural (+)-*S*-enantiomer (I).

The determination of the absolute configuration of the C-6′-Me group of ABA derived from C-2 of mevalonate involves two steps now that its ^{13}C signal has been recognized (Bennett *et al.*, 1981). The first step is to relate this ^{13}C signal to the appropriate ^{1}H signal. The second step is to determine the relative configuration of the hydrogen atoms and methyl groups of the ring and relate them to the chiral centre at C-1′ whose absolute configuration has been determined (Ryback, 1972; Isoe *et al.*, 1972; Mori, 1973; Koreeda *et al.*, 1973).

Table 1. *^1H n.m.r. data for (a) Me- and (b) free ABA*

(*a*) ^1H n.m.r. data for Me-ABA ([^2H]chloroform; tetramethylsilane as internal standard)

	Chemical shift (δ) (p.p.m.)	Coupling constant (J) (Hz)
C-2 m*	5.753	0.44
		0.26
		0.23
C-3 Me d	2.009	0.44
C-4 dd	7.871	5.36
		0.26
C-5 dd	6.152	5.36
		0.23
C-2′ Me d	1.923	0.47
C-3′ m	5.942	1.46
		0.37
C-5′ (eq.) dd	2.288	5.71
		0.37
C-5′ (ax.) d	2.478	5.71
		0.27
C-6′ Me (eq.) s	1.014	
C-6′ Me (ax.)	1.110	0.27
Me ester s	3.706	

(*b*) ^1H n.m.r. spectra of free ABA in ^2H_2O; reference is tetramethylsilane in carbon tetrachloride in a sealed capillary

	Chemical shift (δ) (p.p.m.)	Coupling constant (J) (Hz)
C-2	5.809	
C-3 Me	1.929	
C-4	7.160	16.11
C-5	5.973	16.11
C-2′ Me	1.887	
C-3′	6.032	
5′ (ax.)	2.613	17.09
5′ (eq.)	2.199	17.09
C6′ Me (ax.)	1.012	
(eq.)	1.012	

* Abbreviations: ax., axial; eq., equatorial; d, m etc. have their usual n.m.r. meanings.

Conformation of the ring of ABA

Two opposite, self-consistent interpretations of the stereochemistry of the C-5′ and C-6′ ring substituents of ABA are possible, depending on the flexing of the ring; i.e., whether the side chain is axial or equatorial. The X-ray structure of (±)-ABA in crystalline form has been determined and the side chain was shown to be axial (Schmalle *et al.*, 1977; Ueda & Tanaka, 1977). It was considered necessary to provide unambiguous evidence that ABA takes up the same conformation (Fig. 1) in [^2H]chloroform solution as in the crystal. This was accomplished by analysing the n.m.r. spectra of the 1′,4′-*cis*- (II) and 1′,4′-*trans*-diols (III) of Me-ABA (Tables 2 and 3) and relating their structure to ABA. The absolute configuration at C-4′ of the diols has been established by formation of the 1′,4′-*cis*-diol by catalytic hydrogenation of the 1′,4′-epidioxide (Cornforth *et al.*, 1967). This allows the configuration of the substituents at C-5′ and C-6′ of the diols to be related by n.m.r. to the known configuration of the hydrogen atom at C-4′. However, this requires the determination of the relationship between the C-5′ ^1H signals of ABA and the diols formed from it. In dilute ^2HCl and NaO^2H in [^2H]methanol the C-5′ protons of Me-ABA exchange at equal rates with the medium, but the free acid in ^2H_2O, at pH 11, exchanges its axial 5′-proton more rapidly than the equatorial 5′-proton. Singly ^2H-labelled [5′-^2H]ABA was prepared and reduced in buffered $NaBH_4$ (pH 6.5) to give the isomeric diols, each with a considerable excess of one ^2H at C-5′. The downfield 5′-proton signal in the n.m.r. spectrum of ABA (2.613 d, ^2H_2O) was virtually absent (Fig. 2) and the downfield 5′-proton signal of the 1′,4′-*cis*-diol was also absent. In contrast the upfield 5′-proton signal of the 1′,4′-*trans*-diol was missing (1.701 d, [^2H]-chloroform).

Crystals of racemic ABA melt at 191°C and those of (+)-ABA at 160°C; consequently it was considered possible that the ring could take up a different conformation in solution in comparison with that in the crystal, so the n.m.r. spectra were analysed in detail. Harada (1973) noted that the pseudo boat conformation of ABA is excluded by the coupling ($J = 0.27$ Hz) between the downfield (axial) C-5′ (2.477 δ) proton and the downfield C-6′ (axial) methyl group (1.110 d) (analogous couplings were also detected in both diols; Tables 2 and 3). In either pseudo-chair conformation these groups are planar with the C-5–C-6 bond, whereas in a pseudo boat structure there is no planarity and such coupling could not occur (Fig. 3).

In ABA and both diols, coupling would be expected between the 6-*pro-R* Me (axial) and the 5′-*pro-R* (axial) proton if the dienoic acid side chain were equatorial or between the 6′-*pro-S* Me (axial) and the 5′-*pro-S* (axial) proton if the side chain were axial.

Long-range coupling would be expected between the equatorial 5′-proton and the C-3′ proton of all three compounds, as these substituents would be planar in either conformation.

The discrimination between the two orientations was accomplished by n.O.e. and by decoupling experiments.

N.O.e.

There was a clear n.O.e. in the spectrum of Me-ABA between the C-5′ protons and the upfield 6′-Me group such that irradiation of the Me signal gave exactly equal enhancement of the C-5′ protons. On the other hand the downfield 6′-Me showed an n.O.e. on the upfield 5′-proton only (Table 4). There was also an n.O.e. between the C-

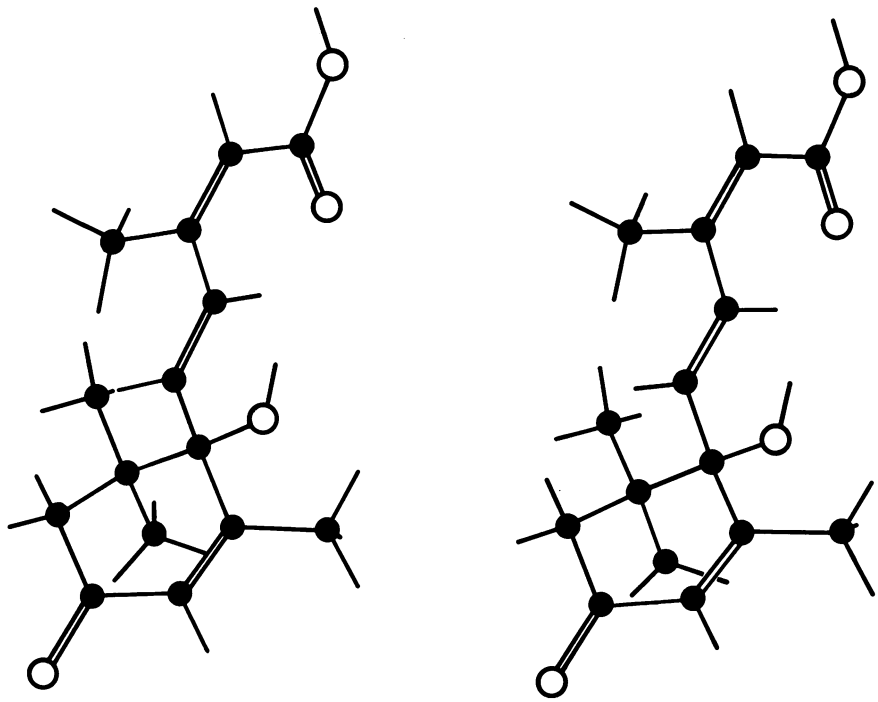


Fig. 1. *Stereoscopic view of abscisic acid showing the ring in a pseudo-chair conformation with the side chain axial*

Table 2. *^1H n.m.r. spectral data for 4′-O-acetyl-1′,4′-cis-diol of Me-ABA {[^2H]chloroform, tetramethylsilane (TMS) as internal standard}*

Abbreviations are as for Table 1.

	Chemical shift (δ) relative to TMS (p.p.m.)	Coupling constant (J) (Hz)
C-2 m		1.27
	5.708	0.68
		0.79
C-3 Me d	2.004	1.27
C-4 dd	7.764	16.12
		0.79
C-5 dd	6.034	16.12
		0.68
C-2′ Me	1.700	1.71
		0.98
C-3′ m	5.596	3.39
		0.98
		0.03
C-4′ m	5.307	6.20
		5.75
		3.39
		1.71
C-5′ (ax.)	1.859	14.24
		6.20
		<0.2
C-5′ (eq.)	1.744	14.24
		5.75
C-6′ Me (ax.)	1.042	<0.2
C-6′ Me (eq.)	0.954	
Me ester	3.707	
4′-*O*-Ac Me	2.054	

5 proton and the axial C-5′ proton, and a clear but weaker n.O.e. between both the C-4 proton and the C-5′ axial proton. These relationships can be accommodated by the conformation of the ring of ABA in a pseudo-chair form only where the side chain is axial; thus the *pro-R*- C-6′ Me is equatorial and the 5′-*pro-S* (downfield) proton is axial (Fig. 1). This is the same orientation as that of the racemate in the crystal form. Further support for this assignment of the conformation of the ring can be deduced from the more rapid exchange of the axial C-5′ proton in alkaline ^2H_2O as found by Williams *et al.* (1963).

The signals of the C-5′ protons of 1′-deoxy-ABA (Neil *et al.*, 1982) and ABA and also the C-6′ Me groups of these two compounds have closely similar chemical shift and coupling constants (Table 5). This suggests that the 1′-tertiary hydroxy group of ABA has little influence and that these chemical shifts are primarily determined by other features of the molecules.

Decoupling experiments

A second line of evidence for the conformation shown in Fig. 1 is provided by the ^1H spectra of the 1′,4′-*cis*- (II) and 1′,4′-*trans*-diols (III). The signal of the 4′-*R* hydrogen atom of (II) is coupled equally with the signals of the C-5′ hydrogen atoms. The bond of the 4′-hydrogen atom, therefore, must bisect the angle between the bonds of the C-5′-

Table 3. *^1H n.m.r. spectral data for 4'-O-acetyl-1',4'-trans-diol of Me-ABA ([^2H]chloroform, tetramethylsilane (TMS) as internal standard)*

Abbreviations are as for Table 1.

	Chemical shift (δ) relative to TMS (p.p.m.)	Coupling constant (J) (Hz)
C-2	5.713	1.28
		0.81
		0.68
C-3 Me	2.023	1.28
C-4	7.756	16.10
		0.81
C-5	6.149	16.10
		0.68
C-2' Me	1.685	1.95
		1.58
C-3'	5.530	2.67
		1.58
		1.43
C-4	5.374	9.46
		6.61
		1.95
C-5' (ax.)	1.701	13.23
		9.46
		0.6
C-5' (eq.)	1.867	13.23
		6.61
		1.43
6'-Me (ax.)	1.083	0.6
6'-Me (eq.)	0.924	
Me ester	3.709	
4'-*O*-Ac Me	2.069	

hydrogen atoms of (II) and so the ring must exist as a pseudo-chair. On the other hand, the upfield (i.e. axial) 5'-*pro-S* proton of the 1',4'-*trans*-diol shows stronger ($J = 9.46$ Hz) coupling to the C-4' proton than does the downfield (i.e. equatorial, 5'-*pro-R*) proton ($J = 6.61$ Hz), as would be expected from their diaxial positions in the 1',4'-*trans* isomer. The downfield 5'-proton (equatorial) also shows coupling to that at C-3', confirming the equatorial position of the former. These relationships can be accommodated only when the ring is in a pseudo-chair conformation. Both diols show coupling between the axial 5'-proton and the axial 6'-Me, but no coupling between the equatorial 5'-proton and the equatorial 6'-Me. Thus ABA and its isomeric 1',4'-diols all have the conformation in solution depicted in Fig. 1.

Recent work on the uptake carrier for ABA in bean roots indicates that the α-face (nomenclature of Rose *et al.*, 1980) of the ring of (+)-ABA (from which the side chain projects) is adjacent to the carrier molecule (B. V. Milborrow & P. H. Rubery, unpublished work). This is surprising because the α-face and axial side chain make a concave surface. The equal growth-inhibitory activity of the (+) and (−) enantiomers of ABA has been attributed to the opposite faces of the (+) and (−) molecules lying on the active site, but there is no evidence at present to indicate which face binds to the site of growth-inhibitory activity. The conformation of ABA that has been determined in solution may not necessarily be the conformation when bound to either the carrier or the growth-inhibitory sites.

Configuration of the side chain

Harada (1973) cites n.m.r. data for the side chain of 2-*trans*-ABA that suggest that it exists unequally in two conformations: the C-5 proton and the C-3 Me showed a 12.7% n.O.e., whereas that between the C-4 proton and the C-3 Me was 6.8%. In ABA itself an n.O.e. of 5.0% between the C-5 proton and the C-3 Me was detected, whereas that between the C-3 Me and the C-4 proton was 1.2%; consequently, at least in free solution, the side chain is predominantly C-3 Me; C-3; C-4; C-5 *E*.

Neither the uptake carrier nor the site of inhibitory activity respond to the 2-*trans* isomer or ABA Me ester, so a carboxy group is required to interact with a site quite close to the ring. As with the conformation of the ring we have no evidence to suggest that the conformation of the side chain of ABA in solution is identical with the conformation when attached to the active site.

Cyclization of ABA

Bennett *et al.* (1981) reported that the ^{13}C n.m.r. spectrum of (+)-ABA biosynthesized by the fungus *Cercospora rosicola* from [1,2-^{13}C]acetic acid showed ^{13}C–^{13}C coupling between the downfield 6'-Me signal (24 p.p.m.) and that of C-6' (41.7 p.p.m.). This identifies the former as being derived from the C-3 Me of mevalonate and the other 6'-Me signal, at 21 p.p.m., as that derived from C-2 of mevalonate.

The relationship between the ^1H and the ^{13}C signals of the 6'-methyl groups has now been established by a modified i.n.e.p.t. procedure (Morris & Freeman, 1979) in which the proton signal of the C-6' Me at 1.109 δ of a sample of unenriched Me-ABA was selectively inverted before the i.n.e.p.t. pulse (J. Redman & M. Batley, unpublished work). The ^{13}C signal of the carbon atom whose protons had been irradiated was then inverted (Fig. 4). The experiment was carried out by Dr. J. Redmond and Dr. M. Batley of Macquarie University, North Ryde, N.S.W., Australia, whose help is gratefully acknowledged.

Thus irradiation of the downfield 6'-Me ^1H signal (1.109 d) caused the inversion of the *upfield* (23.1 p.p.m.) ^{13}C signal of the 6' *geminal* pair. The downfield ^{13}C signal of ABA formed from [1,2-^{13}C]acetate showed ^{13}C–^{13}C coupling, hence the

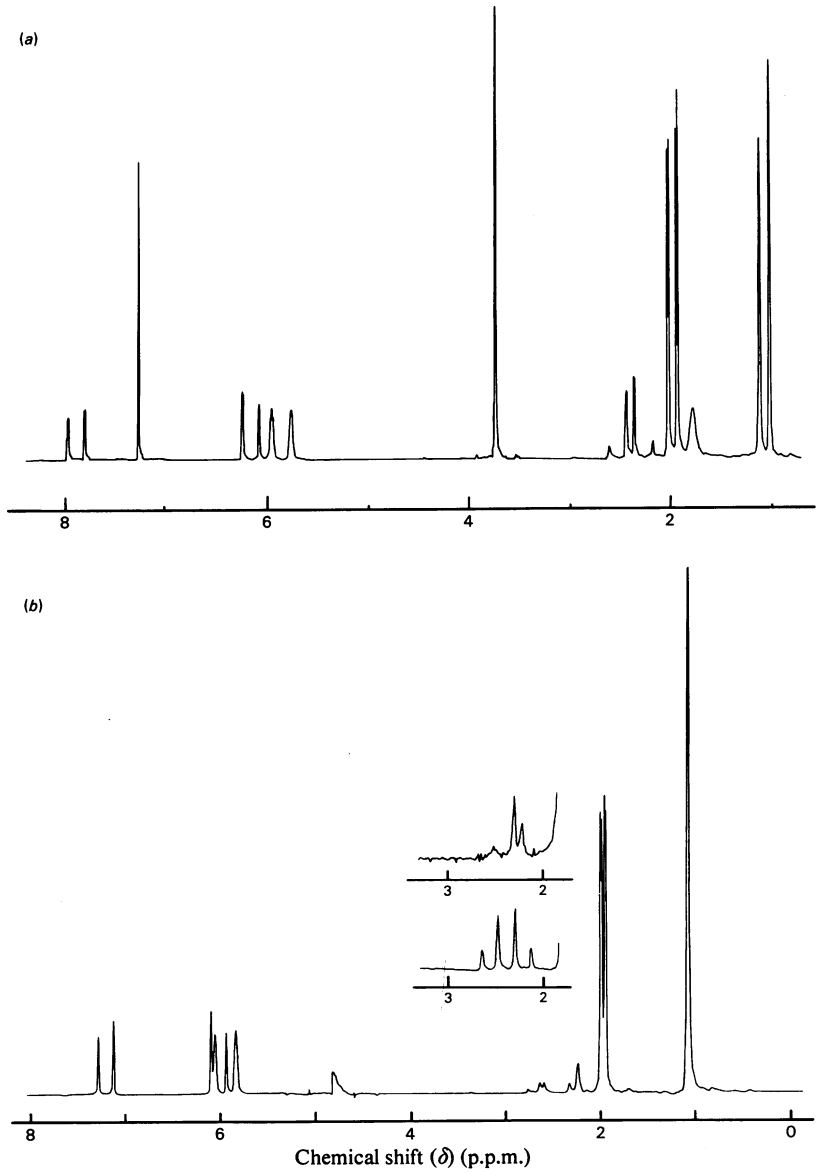


Fig. 2. *^1H n.m.r. spectra for (a) ABA methyl ester and (b) free ABA*

(*a*) ^1H n.m.r. spectrum (100MHz) of Me-ABA in [^2H]chloroform. (*b*) ^1H n.m.r. spectrum of ABA (100MHz) in ^2H_2O + NaO^2H, pH11 (as measured by a conventional glass electrode) after 8h. The axial 5′-proton has been largely replaced (65%) by ^2H, the equatorial 5′-proton much less (27%). The C-3′ and the C-2′ Me protons are stable at this pH. The inset shows the 5′ region of a similar sample of exchanged ABA in [$^2H_5$]pyridine.

6′-Me derived from C-2 of mevalonate (downfield ^1H, axial) is the 6′-*pro-S* Me of ABA. This reverses the previous assignment and makes the stereochemistry at C-1′ and C-6′ compatible with a chair-folded intermediate during cyclization (Fig. 5). All attempts so far to acetylate the 1′-hydroxy group of ABA have failed, and yet it is glucosylated *in vivo* (Loveys & Milborrow, 1981). Its equatorial position leaves it well free of any other substituent, so the failure to acetylate is unlikely to be attributable to steric effects.

This is the same arrangement and stereochemistry as that determined for the β- and ε-rings of the C_{40} carotenoids (Britton *et al.*, 1977, 1979).

The stereochemistry at C-6′ of ABA (Milborrow, 1972), based on the loss of ^3H from the methyl group of ABA when hydroxylated to give phaseic acid, suggested that the *pro-S*-C-6′ Me of ABA was derived from C-2 of mevalonate. This was superceded by the opposite arrangement when Kuhn–

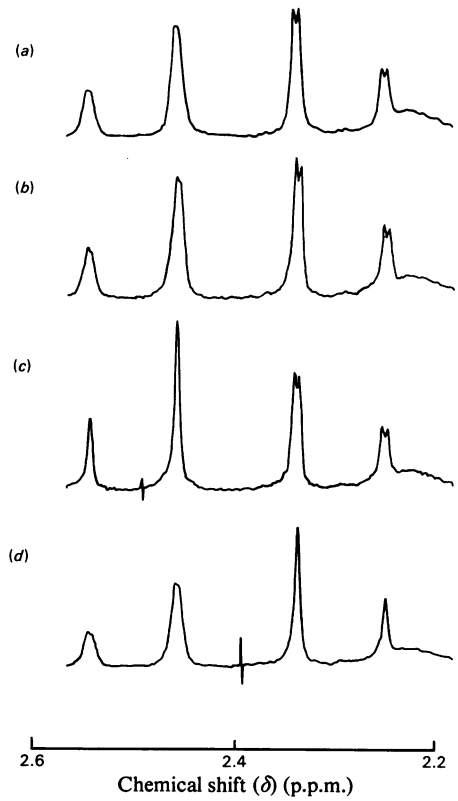


Fig. 3. *Spectra of C-5′ protons*
(*a*) The ^1H signals of the 5′-protons of ABA in [^2H]-chloroform. (*b*) The upfield 6′-Me irradiated; no effect on the C-5′ signals. (*c*) Downfield 6′-Me irradiated; the axial, 5′-*pro-S* proton's signal is decoupled. (*d*) C-3′ proton irradiated; the equatorial, 5′-*pro-R* proton's signal is decoupled.

Table 4. *N.O.e. effects in Me-ABA* ([^2H]*chloroform*)
Abbreviations are as for Table 1.

Irradiation	Chemical shift (δ) (p.p.m.)	Observed n.O.e. (%)	
C-6′ Me (eq.)	1.01	C-5′ H (eq.)	1.5
		C-5′ H (ax.)	1.5
		C-5 H	1.9
		C-4 H	0.7
C-6′ Me (ax.)	1.11	C-5′ H (eq.)	1.4
		C-3′ H	0.5
C-2′ Me	1.92	C-3′ H	4.7
		C-4 H	1.5
C-3 Me	2.01	C-2 H	6.8
		C-5 H	5.0
		C-4 H	1.2
C-5′ H (eq.)	1.28	C-6′ Me (eq.)	3.7
		C-6′ Me (ax.)	3.7
C-5′ H (ax.)	2.48	C-6′ Me	6.0
		C-5 H	11.7
C-3′ H	5.94	C-2′ Me	7.3
C-2 H	5.75	C-3 Me	8.8
C-5 H	6.15	C-5′ H (ax.)	5.4
		C-3 Me	9.3
C-4 H	7.87		

Table 5. *Comparison of n.m.r. data of ABA and 1′-deoxy-ABA*
Data for the latter are taken from Neil *et al.* (1982). δ is the chemical shift in p.p.m. *J* is the coupling constant

	1′-deoxy-ABA		ABA		Difference
	δ	*J* (Hz)	δ	*J* (Hz)	δ
C-5′	2.14	16	2.25	17.4	0.11
	2.38	16	2.53	16.4	0.15
C-6′ Me	0.97		1.02		0.05
	1.07		1.11		0.04

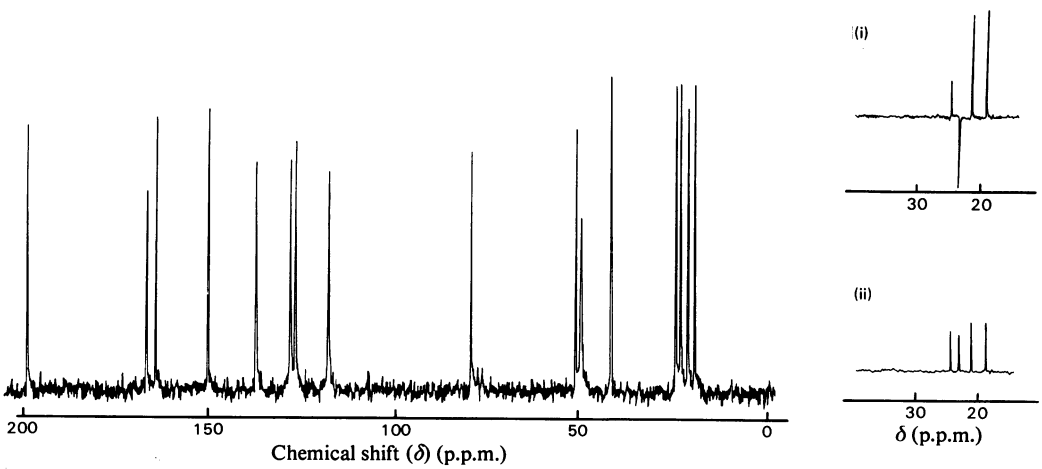
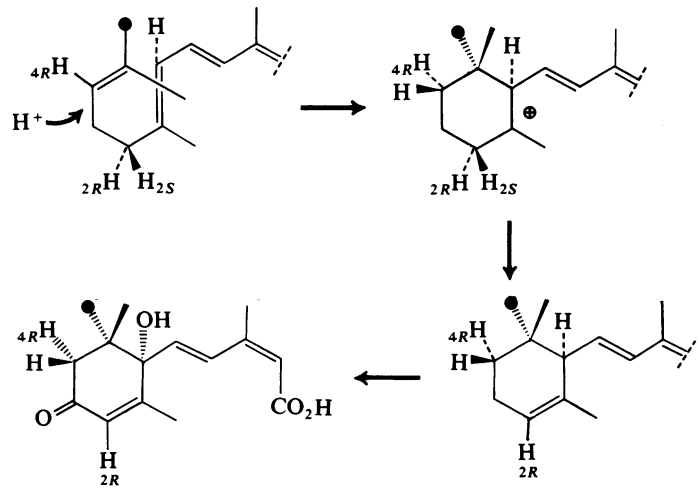


Fig. 4. ^{13}C *spectrum of Me-ABA* (*100 MHz*) *in* [^2H]*chloroform* (^1H *decoupled*)
The inset shows (i) the methyl group region of the 200 MHz spectrum and (ii) the same region with the i.n.e.p.t. sequence in operation. The protons of the downfield C-6′-Me group (1.1098d) were irradiated and this causes the inversion of the ^{13}C signal of this group. N.B. It is the upfield 6′-Me signal of the *geminal* pair (24.3d) that is inverted. C-6′-Me (equatorial, *pro-R*):23.1d; C-6′:41.78; C-3-Me:21.4d; C-2′-Me:19.1d.



(+)-*S*-ABA

Fig. 5. *Representation of the cyclization reaction that occurs during the biosynthesis of ABA*
The stereochemistry at C-1′, C-3′ and C-6′ has been determined, whereas the configuration of the H^+ added to C-5′ is based on that reported for C_{40} carotenoids at the analogous position. The addition of H^+ from the medium to C-5′, from what will become the α-face of the ring, and the loss to the medium of the 2-*pro-S* hydrogen atom of mevalonate from C-3′ of ABA (Milborrow, 1975), suggests that the presumptive β-face of the precursor interacts with the cyclase. If this is so, then what will become C-1′ and the side chain must attach to the enzyme before the C-5′, C-6′ part of the ring comes into position.

Roth oxidation of phaseic acid, derived from [2-^{14}C]mevalonate, gave labelled acetate and so indicated that the 6′-*pro-R* Me was derived from C-2 of mevalonate. Strong mineral acid caused considerable rearrangement of dihydrophaseic acid (Milborrow & Vaughan, 1982) and so it is possible that a reaction of this kind occurred during the oxidation of phaseic acid by the Kuhn–Roth procedure and was responsible for the formation of the small amounts of ^{14}C-labelled acetate detected.

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