Phytochemistry, Vol. 27, No. 3, pp. 757–759, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

THE CYCLIZATION OF 8′-HYDROXY ABSCISIC ACID TO PHASEIC ACID *IN VIVO*

B. V. MILBORROW, N. J. CARRINGTON and G. T. VAUGHAN

School of Biochemistry, University of New South Wales, P.O. Box 1, Kensington, NSW, 2033 Australia

(*Received* 29 *July* 1987)

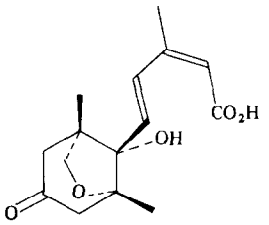
Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; abscisic acid; phaseic acid; cyclization; biosynthesis.

Abstract—The cyclization of 8′-hydroxyabscisic acid to phaseic acid was investigated by feeding *RS*-[3′,5,7-$^2D_6$]abscisic acid to tomato (*Lycopersicon esculentum*) shoots, the 1′-*S*-enantiomer was metabolized to dihydrophaseic acid-4′-*O*-β-D-glucopyranoside (DPAGS) which was isolated as the acetylated methyl ester and subjected to 500 MHz ^{1}H NMR. The ^{2}H atom from C-3′ of abscisic acid (ABA) was confined to the axial 3′-*pro*-*S* position of DPAGS (numbered as in ABA) and so the carbanion presumed to be formed at C-3′ during cyclization is protonated from the oxymethylene bridge, β-face. Enolization of the 3′-hydrogen atoms of phaseic acid in D_2O at pH 10.55 exchanged the axial hydrogen atom (α-face) preferentially. Thus the carbanion present at this position during cyclization is protonated from the opposite side. This suggests that the reaction that gives rise to phaseic acid is catalysed enzymically.

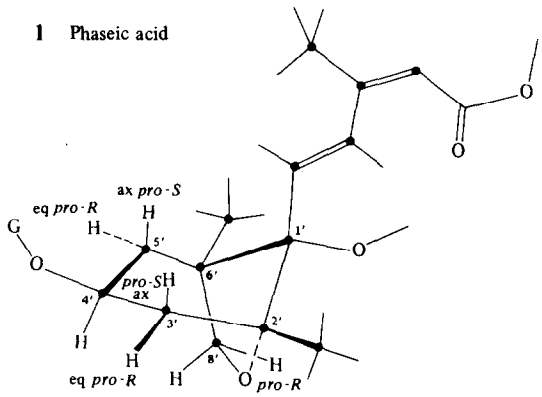
INTRODUCTION

The original isolate of 8′-hydroxyabscisic acid [all compounds are numbered as in abscisic acid (ABA)] rearranged to give phaseic acid (PA) (**1**) after methylation and solution in $CDCl_3$ [2] and since then several attempts to re-isolate it have yielded PA. Recently, a GC-MS analysis of cowpea (*Vigna unguiculata*) fruits [3] identified 8′-hydroxyabscisic acid and an 8′-(3-hydroxy-3-methylglutaryl) derivative has been characterized from the fruits of *Robinia pseudacacia* [4], it gave PA on alkaline hydrolysis. The rearrangement to PA *in vitro* has been established but how the cyclization occurs *in vivo* has not been examined hitherto.

The mechanism of formation of PA was investigated by analysing the origin of the hydrogen atoms at C-3′ of dihydrophaseic acid (DPA) (**2**). The 4′-glucoside of this compound is formed abundantly, its H atoms are not prone to exchange and the presence of the 4′-H atom enables the absolute configuration of the C-3′ H atoms responsible for the NMR signals to be established unambiguously.



1 Phaseic acid



2 Dihydrophaseic acid G = β-D-glucopyranosyl residue

RESULTS

The ^{1}H signals of the C-3′ and C-5′ hydrogen atoms of PA (Fig. 1) were identified by decoupling experiments in D_2O. The axial C-3′ shows clear ω-coupling ($J = 2.5$ Hz) to the 8′-*pro*-*R* hydrogen atom although this is not as clear on the C-3′ as on the C-8′ because the former shows a further, very slight coupling to the 9′-Me. The splitting ($J = 4$ Hz) on the upfield C-3′ and C-5′ signals of PA is ω-coupling between the two equatorial partners across C-4′. The downfield positions of both C-3′ signals with respect to their 5′-partners is attributed to the oxygen atom of the methylene bridge at C-2′. The hydrogen atoms adjacent to the 4′-ketone of PA are more easily exchanged than are those of ABA [5]. At pH 10.55 the axial C-3′ hydrogen atoms had almost completely exchanged and the axial C-5′ had been partly exchanged within 30 min and all four were replaced within five hr.

The considerable differences in the relative positions of the ^{1}H signals between PA and AcMeDPAGS (Table 1) are attributed to interactions between the carbonyl oxygen atoms of the tetraacetyl glucosyl residue and the C-3′ and C-5′ hydrogen atoms of the latter compound. The 18 Hz coupling between the two C-3′- and an identical coupling between the two C-5′-hydrogen atoms of PA establishes that both centres are in a very similar environment. In AcMeDPAGS there is 14 Hz coupling

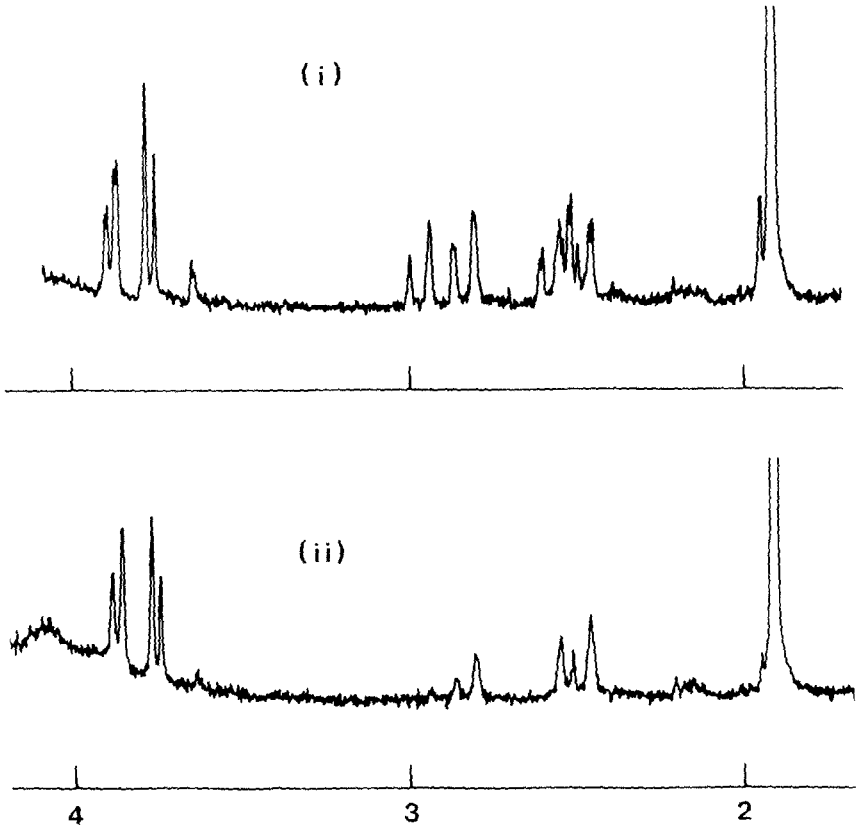


Fig. 1. ^{1}H 300 MHz NMR of PA in 0.05 M Na-Pi in D_2O, pH 6.9 (i) and after 30 min exchange at pH 10.55 (ii). More than half of the axial 5′-*pro-S* H atoms (δ 2.83) have been replaced by D while almost all of the axial, 3′-*pro-S* (δ 2.47) and 5′-*pro-R* (δ 2.54) ^{1}H has been lost. The weak coupling of the 5′-*pro-S* with the 8′-*pro-S* atom can be seen in (i) but exchange of the 5′-axial protium causes the 8′ signals (δ 3.9) to sharpen (ii).

Table 1. Relative positions of the signals of the 3′- and 5′-H atoms of PA (D_2O, 300 MHz) and AcMeDPAGS ($CDCl_3$, 500 MHz) (N.B. The Cahn, Ingold, Prelog rules reverse the nomenclature at C-3′ when the 4′-ketone of PA is reduced to a hydroxyl to make DPA)

	PA		AcMeDPAGS		
	δ	J(Hz)		δ	J(Hz)
3′-*pro-S* eq.	2.58 *dd*	18.3, 4	*3′-*pro-R* eq.	2.118 *dd*	14.3, 6.7
*3′-*pro-R* ax.	2.97 *d*	18.3	†3′-*pro-S* ax.	1.620 *dd*	14.3, 11
†5′-*pro-R* eq.	2.49 *dd*	18.8, 4	5′-*pro-R* eq.	1.946 *dd*	14, 6.7
5′-*pro-S* ax.	2.82 *d*	18.8, 2	5′-*pro-S* ax.	1.728 *dd*	14, 11, 2.1

* Lowest field.
† Highest field.

between axial and equatorial H atoms at C-3′ and C-5′ and 6.7 Hz coupling between the C-4′H and the equatorial C-3′ (δ 2.118) and C-5′H atoms (δ 1.946). The larger 11 Hz diaxial coupling between the C-4′H and the C-3′-*pro-S* (δ 1.620) and C-5′-*pro-S* (δ 1.728) confirms their assignments which were also determined by selective decoupling experiments.

The sample of AcMeDPAGS derived from [3′,5′,7′-D_6]ABA shows the signal of the equatorial, C-3′-*pro-R* hydrogen atom (δ 2.118) to be as strong (103%) as in unlabelled material (Fig. 2) while the signals of the axial C-3′ and the C-5′ hydrogen atoms are equally weak (36% of an undeuteriated H atom's signals). This establishes that it is the equatorial, 3′-*pro-R*- of AcMeDPAGS that is derived from the medium during the cyclization reaction that forms PA. The Cahn, Ingold, Prelog rules make the equatorial 3′-*pro-R* of DPA the 3′-*pro-S* of PA.

DISCUSSION

The cyclization of 8′-hydroxy ABA to form PA can be expected to proceed in two stages, in the second of which a carbanion at C-3′ would add an H^+ from the medium. Models indicate that the oxymethylene bridge and the C-

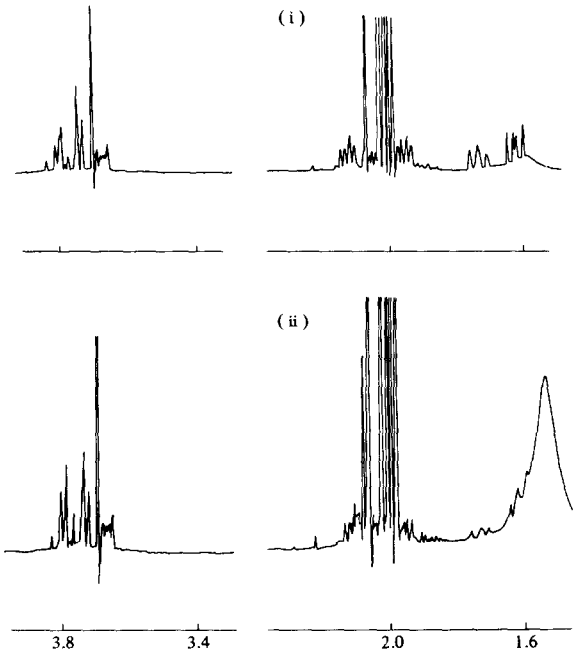


Fig. 2. ^{1}H 500 MHz NMR of AcMeDPA glucoside (*i*) and the spectrum of material derived from [3′,5′,7′-D_6]ABA in $CDCl_3$ (ii). Coupling constants and decoupling experiments established that the signal at δ 2.128 was that of the 3′-*pro-R* H atom. Smaller signals at δ 1.620 (5′-*pro-R*, eq), δ 1.728 (5′-*pro-S*, ax) and δ 1.946 (3′-*pro-S*, ax) arise from endogenous, unlabelled compound.

7′ Me would cause similar steric interaction at C-3′ so the addition of the H^+ would be expected to occur from either side if the reaction were catalysed physically. A carbanion would also be formed at C-3′ during the base-catalysed enolization of PA but when PA was treated with base in D_2O it was the axial, C-3′-*pro-R* hydrogen atom that exchanged with the medium while the equatorial C-3′-*pro-S* was retained. Both were eventually replaced. Thus there appears to be an influence favouring protonation at C-3′ from the α-face of the ring [C-3′ *re* in ABA or upper as drawn in (1)]. In contrast to this, the protonation of the carbanion during cyclization occurred solely from the β-face (originally the *si* face of C-3′ in ABA). This suggests that the cyclization is catalysed enzymically *in vivo*.

EXPERIMENTAL

Shoots of tomato (*Lycopersicon esculentum* c.v. Grosse Lisse) plants (150 mm, 200 g), that had been grown in a humid glasshouse and kept well watered to minimize the production of metabolites of ABA, were fed an aqueous solution of ABA adjusted to pH 7.0 with KOH (10 ml, 5.0 mg *RS*-ABA or 5 mg *RS*-[3′,5′,7′-D_6]ABA). After the solutions had been taken up the plants were kept in a moist atmosphere, in the light, for five days and were then extracted as described previously [6].

Crystalline dihydrophaseic acid 4′-*O*-β-D-glucopyranoside (DPAGS) was isolated by HPLC: Techsil-10 C_{18} column EtOH–H_2O–HAc, 40:459:1; DPAGS R_f 14 min at 2 ml/min. The DPAGS was methylated with diazomethane and rechromatographed on the same column in EtOH–H_2O (3:19), v/v; DPAGS Me R_f 15.6 min, 14–20 min collected. It was then acetylated and rechromatographed on a Techsil-10 silica column in isopropanol–hexane, 1:9; 4 ml/min, R_f 11.6 min (10–14 min collected). *RS*-[3′,5′,7′-D_6]ABA (5 mg) was prepared by exchange in M NaOD in D_2O, 1.0 ml, 25°, 6 hr [7].

The 500 MHz ^{1}H NMR spectra of unlabelled and D labelled samples of acetylated AcMeDPAGS (200 μg) were obtained in a Bruker AM-500 NMR spectrometer in $CDCl_3$ (0.5 ml) locked on the D of $CDCl_3$ and calibrated on the residual ^{1}H signal of $CHCl_3$. ^{1}H 300 MHz spectra of a sample of PA (100 μg), also isolated from tomato plants, was obtained in a Bruker 300 in D_2O calibrated on an external TMS standard. The PA was neutralized with NH_3, dried thoroughly and then dissolved in Na-Pi buffer, 0.05 molar, in D_2O, 6.0, as measured by a conventional pH meter. Exchange of the C-3′- and C-5′ atoms with the medium was followed after adjusting the pH to 10.55 with NaOD.

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