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CONJUGATES OF THE 1′,4′-DIOLS OF ABSCISIC ACID WITH GLUCOSE

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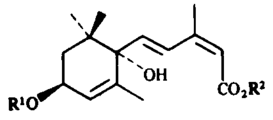
IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Lycopersicon esculentum*: Solanaceae; tomato shoots; 1′,4′-*trans*-diol of ABA; 1′,4′-*cis*-diol of ABA; 1′*S*,4′*S*-4′-dihydroabscisic acid-4′-*β*-D-glucoside; *β*-D-glucosyl 1′*S*,4′*R*-4′-dihydroabscisate; *β*-D-glucosyl 1′*S*,4′*S*-4′-dihydroabscisate, metabolism; conjugation.

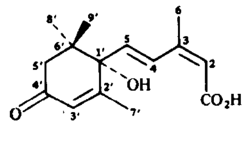
Abstract—The 4′-glucoside and glucose ester of the 1′,4′-*trans*-diol of ABA (1′*S*,4′*S*-4′-dihydroabscisic acid 4′-*β*-D-glucopyranoside and *β*-D-glucopyranosyl 1′*S*,4′*S*-4′-dihydroabscisate) were isolated and characterized as metabolites of the (±)-1′,4′-*trans*-diol of ABA supplied to tomato shoots. In addition to the conjugates of the natural, (+)-enantiomer of the *trans*-diol the corresponding conjugates were formed with the (−)-enantiomer (1′*R*,4′*R*-4′-dihydroabscisic acid 4′-*β*-D-glucopyranoside and 4′-*β*-D-glucopyranosyl 1′*R*,4′*R*-4′-dihydroabscisate). The two pairs of diastereomers were separated by reversed-phase HPLC. The 1′,4′-*cis*-diol glucose ester (4′-*β*-D-glucopyranosyl 1′*S*,4′*R*-4′-dihydroabscisate) was isolated and characterized as a metabolite of both (+)- and (−)-1′,4′-*cis*-diol of ABA. However, the diastereomeric forms of the *cis*-diol glucose ester were not separated by chromatography. The *cis*-diol glucose ester was unstable in acidic solutions and isomerized to the *trans*-diol glucose ester.

INTRODUCTION

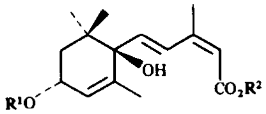
The 1′,4′-*trans*-diol of ABA **1** and the 1′,4′*cis*-diol of ABA **2** are metabolites of exogenous ABA supplied to a variety of plants [1, 2]. Both diols occur naturally [2, 3, 4] and *trans*-diol **1** was released by basic hydrolysis of extracts of avocado fruit, suggesting the presence of a base-labile conjugate of the *trans*-diol in that tissue. Studies of the metabolism of exogenous 1′,4′-diols revealed that unidentified conjugates of the diols were present [2]. The isolation and characterization of these metabolites is reported here.



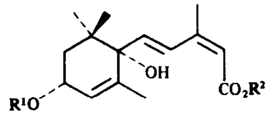
	R¹	R²
1	H	H
16	Ac	Me
4	Glc	H
8	Glc	Me
10	Glc(Ac)_4	H
12	Glc(Ac)_4	Me
6	H	Glc
14	Ac	Glc(Ac)_4



3 (+)-*S*-abscisic acid



	R¹	R²
5	Glc	H
9	Glc	Me
11	Glc(Ac)_4	Me
13	Glc(Ac)_4	Me
7	H	Glc
15	Ac	Glc(Ac)_4



	R¹	R²
2	H	H
17	Glc	H
17	H	Glc
18	Ac	Glc(Ac)_4

RESULTS AND DISCUSSION

Pea and tomato shoots supplied with exogenous 1′,4′-diols of ABA produced ABA, the glucose ester of ABA (ABAGE) and the 4′-glucoside of dihydrophaseic acid (DPAGS), probably via the oxidation of the diols to ABA rather than by a direct route [2]. In addition, four uncharacterized products of the 1′,4′-*trans*-diol of ABA **1** and several products of the 1′,4′-*cis*-diol of ABA **2** were found. The amounts of these conjugates were increased by feeding large amounts of the diols to tomato shoots. An increase in the proportion of ABAGE to phaseic acid was also observed by feeding ABA at levels *ca* 100 times the endogenous concentrations [5].

The 4′*-glucoside and the glucose ester of the* 1,4′*-trans-diol*

Thin layer chromatography of the uncharacterized radiolabelled metabolites, their derivatives, and products of basic hydrolysis was used to obtain structural information on these compounds. Compounds **4** and **5**, their methyl esters **8** and **9**, *O*-acetyl, methyl esters (**12**, **13**) and *O*-acetyl derivatives (**10**, **11**) had similar mobilities in two solvent systems. Neither **4** nor **5** were hydrolysed by base. Compounds **6** and **7** did not react with diazomethane but after acetylation the products (**14**, **15**) showed a marked increase in R_f. After basic hydrolysis **6** and **7** yielded a radiolabelled product that cochromatographed with the 1′,4′-*trans*-diol of ABA **1**. The derivatives of **6** and **7** had similar mobilities in two solvent systems.

The UV spectra, measured by a diode-array detector during HPLC, also showed the similarity within each pair of metabolites. The normalized UV spectra between 200 and 350 nm were identical for compounds **4** and **5** (λ_{max} 265 nm), their methyl esters **8**, **9** (λ_{max} 267 nm) and their *O*-acetylated methyl esters (λ_{max}267 nm). Similarly **6** and **7** (λ_{max} 273 nm) and the *O*-acetyl derivatives **14**, **15** λ_{max} 275 nm) had identical UV spectra. The chromatographic characteristics of **4** and **5** were similar to those of glucosides such as DPAGS [6]. Compounds **4** and **5** react with diazomethane to form methyl esters; they are resistant to hydrolysis by base and the UV spectra are similar to those of DPAGS (λ_{max}265 nm). Compounds **6** and **7** had similar properties to ABAGE, being susceptible to basic hydrolysis and not reacting with diazomethane. The UV spectra had similar absorbance maxima to ABAGE (λ_{max} 273 nm) and *O*-acetyl-ABAGE (λ_{max} 275 nm) except the spectra of **6** and **7** lack the shoulder at 240 nm, present in the spectrum of ABAGE, and attributed the α,β-unsaturated ketone chromophore.

Mass spectrometry

The mass spectra of methylated and acetylated 4′-glucosides of the 1′,4′-*trans*-diol (**13** and **14**) are almost identical, with only slight differences in the abundance of some fragment ions, consequently, the discussion of the features of the mass spectrum of **13** applies to those of **14**. The methane chemical ionization spectrum of **13** has ions corresponding to M + 1 (*m/z* 611), M + 29 (*m/z* 639) and M + 41 (*m/z* 651) which are frequently observed when methane is the ionizing gas and so the M_r is 610. The fragment ions at lower mass are also seen in the mass spectrum of glucose tetraacetate or the *trans*-diol methyl ester.

The fragmentation of D-glucose pentaacetate has been described [7] and involves the loss of four molecules of acetic acid (60 mass units) and one of ketene (42 mass units). The acetylated sugar residue shows similar fragmentation with ions at *m/z* 331, 289, 271, 169 and 109. The aglycone shows fragment ions similar to those of the *O*-acetyl 1′,4′-*trans*-diol of ABA methyl ester **16** [8]. The peak at *m/z* 263 corresponds to 4′-desoxy ABA which is often the highest mass ion observed for Me-*trans*-diol or its *O*-acetyl derivatives. The other ions (at *m/z* 245, 231, 137 and 125) and their relative intensity similar to those obtained with MeAc-*trans*-diol **16** and the pentadienoic acid side chain methyl ester, *m/z* 125, is evidence that the C-1 carboxyl is methylated. A sample of **13** derived from **4**, which was biosynthesized from the *trans*-diol with deuterium in the 4′-position, had a M_r of 611 with the ions form the aglycone increased by one mass unit (except *m/z* 125) but the ions from the sugar residue were unchanged.

A M_r of 610 and the mass spectrum is consistent with the metabolite's being the 1′,4′-*trans*-diol of ABA conjugated by the 4′-hydroxyl group to a hexose residue. Conjugation through the tertiary C-1 hydroxyl group is unlikely because this would leave the secondary 4′-hydroxyl group free to be acetylated and a molecular weight of 652 would be expected. Under the conditions used to acetylate the glucose residue, the 4′-hydroxyl group of the *trans*-diol is readily acetylatable but the tertiary 1′-OH was not acetylated (even in the presence of catalysts, 4-pyrrolodinopyridine or 4-dimethylaminopyridine, with isopropenyl acetate).

The mass spectra of the acetylated glucose esters of the *cis*- and *trans*-diols of ABA (**14**, **15**) have many common features. They are dominated by the loss of acetic acid ($[MeCOOH + H]^+ = m/z$ 61) from the acetylated 4′-hydroxyl group of the diol residue to give the apparent tetraacetyl glucose ester of 4′-desoxy ABA ($M_r = 578$). This is indicated by the occurrence of ions at *m/z* 579 $[M + H]^+$ and *m/z* 580 for the material derived from [4′-^{2}H]-*trans*-diol. The lower mass fragment ions are those of glucose (*m/z* 331, 271, 169, 109) and the *trans*-diol (*m/z* 231 or 232).

NMR spectroscopy

^{1}H NMR spectra of the *O*-acetyl methyl derivatives of **4** and **5** (**12** and **13**) were obtained on a 300 MHz instrument. Unlike spectra of glucosides run at 100 MHz [8, 9], the signals of the glucosyl protons were well resolved allowing the configuration of the sugar residue to be determined. Two-dimensional homonuclear shift correlated spectroscopy (COSY) was used to assign the position of the protons in the glucose molecule by determining which signals were coupled. The coupling constants for the pyranose ring protons ($J_{1,2} = 8$ Hz, $J_{2,3} = 10$ Hz, $J_{3,4} = 9$ Hz, $J_{4,5} = 10$ Hz) are the same as those measured for glucose pentaacetate. Furthermore, the coupling constants are of the same order as that measured by Coxon [10] for diaxial, vicinal protons of carbohydrates ($J = 8.6$–11 Hz). This gives the configuration of the sugar residue as β-glucose (C-1-*S*). The integral of the spectrum indicated that there was only one glucose residue in the molecule and this was confirmed by the presence of only one anomeric proton signal. The chemical shifts of the glucosyl protons of **12** and **13** have similar shifts to those of pentaacetyl glucose. The major exception is the anomeric proton which is upfield in **12** and **13** by *ca* 1 δ and reflects the difference in conjugation of C-1 to acetate or the 4′-hydroxyl group of the *trans*-diol of ABA. The chemical shifts of the glucosyl protons of **12** are similar to those of **13** except that the C-5 glycosyl proton of **13** is shifted upfield by 0.2δ, attributed interaction with the diol residue.

The proton signals of **12** and **13** show a close correspondence to the signals of *O*-acetylmethyl 1′,4′-*trans*-diol of ABA **16** [10]. The methyl signals of **12** at δ0.91, δ1.03, 1.57, 2.02 and 3.73 are in similar positions to the same groups of 4′-*O*-acetyl-*trans*-diol methyl ester **16** (C-9′ Me- δ0.92, C-8′ Me- δ1.08, C-7′ Me- δ1.69, C-6 Me- δ2.02, C-10 Me- δ3.71). The signals of the sidechain protons of **12** correspond to those of acetyl *trans*-diol methyl ester (C-2 δ5.71, C-4 δ7.76 and C-5 δ6.15). The ring proton signals correspond except that the 4′-proton of **12** (4.3–4.4) is downfield 1δ from its position in the spectrum of 4′-*O*-acetyl *trans*-diol (δ5.37) but close to the position of the 4'-proton of DPAGS (δ4.1–4.3). This provides evidence that the 4'-hydroxyl group of **12** is conjugated to glucose and is further evidence that it is not acetylated.

The 300 MHz ^{1}H NMR spectra of the *trans*-diol glucose esters **14**, **15** and ABAGE were obtained in [$^2H_5$] pyridine. The spectrum of ABAGE was similar to the 100 MHz spectrum of ABAGE [$^2H_5$]pyridine reported by Loveys and Milborrow [9]. In [$^2H_5$]pyridine the glucosyl protons were poorly resolved in comparison with those of *O*-acetyl *trans*-diol glucosides in $CHCl_3$. All four glucose esters had signals for the C-2 to C-6 glucosyl protons between δ3.8 and δ4.4. However, the signal for the anomeric, C-1 proton: a well-resolved doublet at δ6.2 with a coupling constant of 8 Hz showed that the glucosyl residue was attached via a β-configuration.

The main difference between the spectra of the diol glucose esters and ABAGE is the presence of a broad signal, at 4.44δ to 4.48δ in the spectra of the diols, which is attributable to the C-4' proton. The signals for the C-5' protons also show coupling to the C-4' proton. Overall, as expected, the NMR spectra of **14** and **15** are very similar but apart from the C-4' and C-5' proton signals the remainder of the spectrum is close to that of ABAGE.

Reduction of ABAGE to the glucose esters of 1,4'-cis-*and* 1,4'-trans-*diol of ABA*

A sample of ABAGE, formed from *RS*-ABA by tomato shoots, was reduced with sodium borohydride to prepare glucose esters of the *cis*- and *trans*-diols. The reduction was performed in phosphate buffer (0.1 M, pH 6.0) to avoid basic hydrolysis of the glucose ester. Reversed-phase HPLC, with the same conditions as those used to separate the metabolites of *trans*-diol, was used to separate the reduction productions of ABAGE (Fig. 1). Two peaks with identical retention times to **7** and **16** (12 and 13 min, respectively) were seen as well as unreduced ABAGE (19 min) and another peak at 38 min (the glucose ester of the *cis*-diol, **17**). The compounds eluting at 12 and 13 min had the same UV, mass and NMR spectra as **7** and **6**.

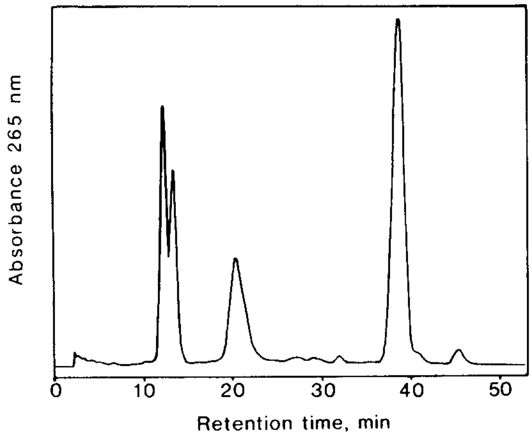


Fig. 1. HPLC separation of the reduction products of ABA glucose ester. The products of ABA glucose ester reduced with $NaBH_4$ were chromatographed on an 8 × 250 mm Techsil 10 C_{18} HPLC column. The mobile phase was EtOH–0.2% aq. HOAc (3:17) for 22 min followed by a linear gradient into EtOH–0.2% aq. HOAc (1:3) over 15 min a 4 ml/min. Peaks at 12 and 13 min are the (−)- and (+)-*trans*-diol glucose ester respectively. ABA glucose ester (19 min), *cis*-diol glucose ester (38 min).

Hydrolysis of the glucose conjugates of the trans-*diol*

Under basic conditions (EtOH–2 M KOH, 2:1, 2 hr at 40°), which are sufficient to hydrolyse the glucose esters **6** and **7**, compounds **4** and **5** released a small amount (5–12%) of a product with the same UV spectrum and HPLC retention time as the 1',4'-*trans*-diol of ABA **1**. However, all of the conjugates **4**–**7** were susceptible to acid hydrolysis and were completely hydrolysed by 1 M HCl at 100° for 6 hr. The sugar released by all conjugates was identified as glucose by comparison of the GC retention time and mass spectrum of the TMS-*O*-methyloxime and that of a glucose standard.

The stereochemistry of the trans-*diol residue*

The *trans*-diol released from **4**–**7** was methylated, oxidized to Me-ABA, reduced to the diols and the Me-*cis*-diol resolved into its enantiomers. However, using this procedure, the results were ambiguous with radioactivity in both enantiomers of the *cis*-diol derived from the conjugates, probably caused by the racemization of the diols which occurs in acidic solutions [8]. Similarly, feeding (+)-*trans*-diol and (−)-*trans*-diol to different batches of tomato shoots and separating the metabolites by HPLC did not give clear cut results. The comparison of metabolites with similar mobilities by separate HPLC runs led to uncertainties about the results. To overcome these problems relatively large amounts of (−)-[2-^{14}C]- and (+)-[G-^{3}H]-*trans*-diol were mixed and supplied to one batch of tomato shoots. The labelled enantiomers were diluted with a large amount of cold racemic material so that the amount of each enantiomer was practically identical. In addition, the diols were prepared by the reduction of the radiolabelled abscisic acids with sodium borodeuteride so that the diols were labelled with deuterium in the 4'-position.

The products of the mixture of (+)-[^{3}H] and (−)-[^{14}C]-*trans*-diol fed to tomato shoots and analysed after seven days show, as expected a large proportion of *trans*-diol conjugates (Fig. 2). The chromatogram of ^{3}H-labelled compounds indicated that compounds **4** (13 min) and **6** (19 min) were derived from (+)-[^{3}H]*trans*-diol. Other compounds containing tritium were *trans*-diol, ABA, ABAGE and small amounts of DPAGS. The products of (−)-[^{14}C]*trans*-diol were compounds **5** and **7**, ABA, ABAGE and *trans*-diol. The *trans*-diol glucose esters and 4'-glucosides were purified by HPLC as the methyl, acetyl derivatives. The purified compounds **5** and **7** contained 14-carbon label; **4** and **6** contained tritium. However, ^{3}H-labelled compounds contained a small amount (*ca* 3%) of 14-carbon and a similar, small proportion of tritium was found in the principally ^{14}C-labelled compounds. This small enrichment of the originally opposite enantiomer in these metabolites was probably produced by racemization of the diols during their preparation, which involved two (acidic) oxidation steps. Therefore, natural (+)-*trans*-diol is metabolized to the 4'-glucoside and the glucose ester. In accordance with the guidelines for the naming of conjugates of ABA [9] the glucoside **4** and glucose ester **6** are: (+)-1'*S*,4'*S*-4'-dihydroabscisic

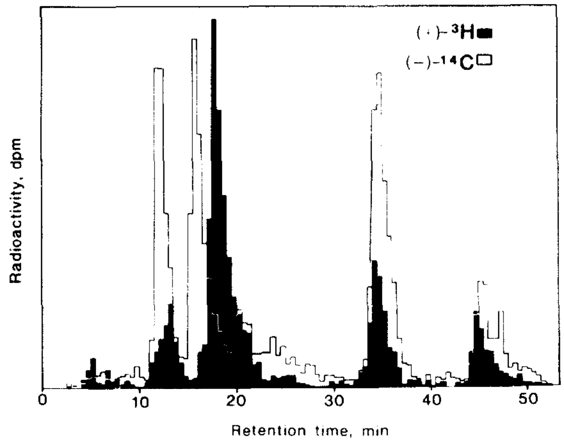


Fig. 2. The metabolites of (+)-[G-^{3}H]- and (−)-[2-^{14}C]-*trans*-diol of ABA. The products of (+)-[G-^{3}H]- and (−)-[2-^{14}C]*trans*-diol of ABA, fed to tomato shoots, were separated by the HPLC conditions described in Fig. 1. Fractions (2 ml) were collected and radioactivity determined by liquid scintillation counting. Full scale for ^{3}H is 3000 dpm and for ^{14}C is 500 dpm.

acid-4-β-D-glucopyranoside and β-D-glucopyranosyl 1′*S*,4′*S*-4′-dihydroabscisate respectively.

When racemic *trans*-diol is used in feeding experiments the epimers of the natural metabolites are formed and are separable by reversed-phase HPLC. This type of separation has not been observed for the 1′-glucoside or the glucose ester of ABA, although both epimers are formed when racemic ABA is fed [11]. The separation of the glucose esters of the (+)- and (−)-*trans*-diol provides an alternative method for producing (+)- and (−)-ABA. Plants fed large quantities of racemic ABA form ABA glucose ester which can be carefully reduced with sodium borohydride, and then the (+)- and (−)-*trans*-diol glucose esters can be separated by normal phase HPLC, hydrolysed in base and oxidized to give (+)- and (−)-ABA.

The glucose ester of 1′,4′-cis-diol of ABA

A metabolite of the *cis*-diol of ABA had the same UV spectrum and retention time as the *cis*-diol glucose ester **17** produced by the reduction of ABA glucose ester. This metabolite, after acetylation, produced from both (+)-[G-^{3}H]- and (−)-[2-^{14}C] *cis*-diol, co-chromatographed with acetylated *cis*-diol glucose ester on an 8 (i.d.) × 250 mm Techsil 5 C_{18} HPLC column eluted with methanol/0.2% aqueous acetic acid (3:1, v/v) at 2.0 ml/min ($R_t = 8.8$ min; $\lambda_{max} = 275$ nm).

The mass spectrum of *O*-acetyl *cis*-diol glucose ester **18** is similar to that of the *O*-acetyl *trans*-diol glucose esters **6**, **7** in that the spectrum is dominated by the ion at *m/z* 61 (acetic acid from the 4′-*O*-acetyl group). No molecular ion was evident but ions attributable to 4′-desoxy ABA glucose ester (*m/z* 579) and fragment ions of the *cis*-diol and tetraacetyl glucose residues are present. A sample of [4′-^{2}H]-*cis*-diol retained the 4′-deuterium atom and in the mass spectrum of the *O*-acetylated and derivative ions at *m/z* 580 and 232 replaced the ions at *m/z* 579 and 231.

The ^{1}H NMR spectrum of the *cis*-diol glucose ester **17** in [$^2H_5$]pyridine shows the presence of the C-4′ proton signal (δ4.43–4.54) but the remainder of the signals, with the exception of the C-5′ proton [(δ2.14 (eq.), 1.86 (ax.)], are less like those of ABAGE than the spectra of the *trans*-diol glucose esters.

The metabolite was hydrolysed in base to products having retention times and UV spectra similar to glucose and *cis*-diol on reverse-phase HPLC-diode array detection. The TMS derivative of the *O*-methyloxime of the sugar residue released by acidic hydrolysis had the retention time on capillary gas chromatography and a similar mass spectrum to that of a standard of the TMS-methyloxime of glucose.

The *cis*-diol glucose ester **17** in weakly acidic solutions isomerized to the (+)- and (−)-*trans*-diol glucose ester **5** diastereomers which were separated by reversed-phase HPLC to give a chromatogram similar to Fig. 1. This isomerization was observed for the synthesized compound as well as the metabolite isolated from tomato shoots and is further confirmation that diastereomers of the *cis*-diol glucose ester were formed but were not separable by HPLC. This is surprising in view of the facile resolution of the *cis*-diol by HPLC on chiral stationary phase columns and their inability to resolve the *trans*-diol [12]. The glucose ester of the (+)-*cis*-diol **17** is more correctly, (+)β-D-glucopyranosyl 1′*S*,4′*R*-4′-dihydroabscisate.

The *cis*- and *trans*-diols have been identified previously [3, 2] as naturally occuring compounds and have been shown to be formed from ABA fed to tomato and pea shoots [1]. These results have been established that glucosides and glucose esters of the diols are also formed from endogenous materials and so reduction of ABA and conjugation of the diols with glucose may represent a minor, alternative pathway of inactivation of ABA. Earlier work [1] showed that relatively small amounts of diols were formed in tomato compared with pea shoots so the importance of this pathway may vary widely between different species.

EXPERIMENTAL

RS-[2-^{14}C] ABA [25.6 mCi/mmol] and *RS*-[G-^{3}H] ABA [33.2 Ci/mmol] were from the Radiochemical Centre, Amersham. The reduction of ABA, the resolution of (±)-Me-*cis*-diol of ABA into its enantiomers, and the production of (+)- and (−)-ABA were as previously described [8].

Plant material and feeding. Seven-week old seedlings (200–300 mm high) tomato (*Lycopersicon esculentum* cv. Grosse Lisse) were cut at ground level and placed in 1–2 ml solutions of radiolabelled compounds.

The metabolism of 1′,4′-[2-^{14}C]-trans-*diol of ABA.* 2 μCi (±)-1′,4′-[2-^{14}C]*trans*-diol of ABA [70 μCi/mmol] was supplied to 17 tomato shoots (56 g). The metabolites were extracted four days after feeding and subjected to a preliminary clean-up procedure as described elsewhere [12].

Separation of the metabolites by HPLC. The metabolite fraction was chromatographed on an 8 × 250 mm Techsil 10 C_{18} HPLC column (HPLC Technology). The mobile phase was EtOH–0.2% aq. HOAc (3:17) for 22 min followed by a linear gradient into EtOH–0.2% aq. HOAc (1:3) over 15 min at 4 ml/min. The effluent was monitored by a diode-array detector as described in detail elsewhere [8]. A subsample (1/40) was chromatographed separately to determine the retention times of radiolabelled products. Effluent was collected in 2 ml fractions to which 0.5% 2,5-diphenyloxazole (PPO) in Triton X-100–toluene (2:1) was added and radioactivity was detected by liquid scintillation counting. The labelled products had the following retention times: dihydrophaseic acid 4′-glucoside (DPAGS)-6 min, **7**-12 min, **6**-13 min, **5**-16.3 min, **4**-19 min, ABA glucose ester (ABAGE)-20.3 min, **1**-33.7 min, ABA-37.8 min.

TLC of the metabolites of the 1′,4′-*diols of ABA and their derivatives*

Radiolabelled fractions of the metabolites were chromatographed underivatized after methylation, acetylation (pyridine–Ac_2O (1:1) 40°, 30 min) and basic hydrolysis (ammonia [s.g. 0.880] 40°, 30 min). Samples were applied in 10 mm strips to the origin of 100 × 100 mm silica gel 60 F_{254} HPTLC plates (Merk). Two solvent systems were used to develop the plates: solvent system A—toluene–EtOAc–HOAc (25:15:2), solvent system B—$CHCl_3$–MeOH–H_2O (75:22:3). Radiolabelled compounds were detected by autoradiography. R_f values:

Solvent system A—**7** (0), **6** (0), **5** (0), **4** (0), **8** (0.2), **9** (0.3), **15** (0.66), **14** (0.65), **11** (0.50), **10** (0.51), **13** (0.59), **12** (0.6). After basic hydrolysis -7, **6** (0.41), **5**, **4** (0), **1** (0.41).

Solvent system B—**7** (0.39), **6** (0.40), **5** (0.26), **4** (0.28), **9** (0.56), **8** (0.56), **15** (1), **14** (1), **11** (0.75), **10** (0.77), **13** (0.91), **12** (0.97). After base hydrolysis: **7** (0.63), **6** (0.63), **5** (0.26), **4** (0.28), **1** (0.63).

Derivatization and isolation of the conjugates of the 1′,4′-*trans-diol of ABA*. **4** and **5** were methylated with CH_2N_2 in Et_2O (0°) and purified by HPLC on an 8 × 250 mm Techsil 10 C_{18} column with mobile phase of EtOH–0.2% aq. HOAc (1:3) at 4 ml/min. R_ts: **7** (6.5 min), **6** (7.1 min), **5** (8.4 min), **4** (9.3 min), **9** (17 min), **8** (20 min). **8** and **9** were acetylated as above and chromatographed on the same column in EtOH–0.2% aq. HOAc (9:11) at 4 ml/min. R_ts **13** (8.5 min), **12** (9.5 min). **13** and **12** were further purified by normal-phase HPLC on an 8 × 2509 mm Techsil 10 silica column with *iso*PrOH–hexane (1:24) at 4 ml/min—**13** (13.5 min), **12** (15.7 min). **7** and **6** were acetylated and chromatographed on an 8 × 250 mm Techsil 5 C_{18} HPLC column with MeOH–0.2% aq. HOAc (3:1) at 2 ml/min–**15** (8.7 min), **14** (8.7 min).

Metabolism of the 1′,4′-[2-^{14}C]cis-(*diol of ABA*. 2 μCi (±)-1′,4′-[2-^{14}C]cis-diol of ABA [70 μCi/mmol] was fed to 15 tomato shoots (47 g). After 4 days the metabolites were extracted and separated by HPLC using the same procedures as for the metabolites of the *trans*-diol of ABA. The major metabolite **17** had a R_t of 38 min. **17** was acetylated and the product **18** chromatographed on an 8 × 250 mm Techsil 5 C_{18} had a R_t of 8 min.

Metabolism of (−)-[^{14}C]-*and* (+)-[^{3}H]cis-*and* trans-*diol of ABA*. 0.3 μCi (−)-[2-^{14}C] ABA [25.6 mCi/mmol], (+)-[G-^{3}H] ABA [44 mCi/mmol] and 40 mg (±)-ABA were mixed and reduced with $NaBD_4$ at 0°C for 30 min in MeOH–H_2O (2:1). The diols were separated by TLC on silica gel 60 F_{254} (Merk) developed in toluene–EtOAc–HOAc (25:15:2) R_f values: *cis*-diol **2** (0.32), *trans*-diol **1** (0.58), ABA (0.65). **1** and **2** were further purified by reverse phase HPLC on an 8 × 250 mm Techsil 10 C_{18} column with MeOH–0.2% aq. HOAc (2:3) at 4 ml/min. 0.07 μCi (−)-1′,4′-[2-^{14}C]*trans*-diol of ABA [4 μCi/mmol] and 0.17 μCi (+)-1′,4′[G-^{3}H]*trans*-diol of ABA [9.52 μCi/mmol] were supplied to 11 tomato shoots (20 g). 0.05 μCi (−)-1′,4′[2-^{14}C]*cis*-diol of ABA [4 μCi.mmol] and 0.15 μCi (+)-1′,4′-[G-^{2}H]*cis*-diol of ABA [9.52 μCi/mmol] were supplied to 12 tomato shoots (21 g). The metabolites were extracted and analysed as described above.

Hydrolysis of the conjugates and identification of the sugar residue as the TMS-O-*methyl oxime*. Samples were hydrolysed in 50 μl M HCl (100°, 6 h) under N_2. The acid was evaporated under N_2 and the *O*-methyloxime was formed by the method of ref. [13] and silylated as described in ref. [14]. The TMS-*O*-methyloxime was analysed by GCMS. GC conditions: 0.33 mm × 25 m BP-1 capillary column held at 100° for 1 min and programmed to rise at 6°/min to 300°. TMS-*O*-methyloxime of glucose had a R_t of 19.8 min.

Synthesis of the 1′,4′-cis-*and* 1′,4′-trans-*diol glucose esters*. ABS glucose ester was isolated from tomato shoots supplied with *RS*-ABA [12] and reduced with $NaBH_4$ in 100 μl MeOH–KPi buffer, pH 6.0 (2:1) at 0° for 50 min. The pH of the mixture was readjusted by the addition of a saturated solution of NaH_2PO_4 after each addition of NaH_2PO_4 (3 mg at 0, 5, 10, 20 and 30 min). The products were separated by HPLC on an 8 × 250 mm Techsil 10 C_{18} column with a mobile phase of EtOH–0.2% aq. HOAc (3:17) for 22 min and a linear gradient into EtOH–0.2% aq. HOAc (1:3) over 15 min. The R_ts of the products were: **7** (12 min), **6** (13 min), ABAGE (19 min), **17** (38 min).

NMR spectroscopy. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker CXP-300 spectrometer operating in the pulsed Fourier transform mode with quadrature detection. Chemical shifts were measured digitally, using TMS as an internal standard.

Methylated acetylated 4′-*glucoside of* (+)-1′,4′-trans-*diol of ABA* (**12**). ^{1}H NMR (300 MHz, $CDCl_3$) δ0.91 (3H, *s*, 9′-H), δ1.03 (3H, *s* 8′-H) δ1.56 (3H, *s*, 7′-H), δ1.7–1.9 (2H, *m*, 5′-H), δ2.02 (3H, *s*, 6-H), 1.99, 2.00, 2.02, 2.09 (3H, *s*, G-OAcMe), δ3.73 (3H, *s*, 1-h), δ3.91 (1H, *ddd*, $J_{4,5} = 10$ Hz, $J_{5,6B} = 2$ Hz, $J_{5,6A} = 5$ Hz, G5-H), δ4.14 (1H, *ddd*, $J_{5,6A} = 2$ Hz, $J_{6A,6B} = 12$ Hz, δ4.25 (1H, *dd*, $J_{5,6A} = 5$ Hz, $J_{6A,6B} = 12$ Hz, G6-H_A), δ4.3–4.4 (1H, *m*, 4′-H), δ4.76 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ4.97 (1H, *dd*, $J_{1,2} = 8$ Hz, $J_{2,3} = 10$ Hz, G2-H), δ5.07 (1H, *t*, $J = 10$ Hz, G4-H), δ5.29 (1H, *t*, $J = 9$ Hz, G3-H), δ5.64 (1H, *d*, 3′-H), δ5.73 (1H, *s*, 2-H), δ6.13 (1H, *d*, $J_{4,5} = 16$ Hz, 5-H), δ7.73 (1H, *d*, $J_{4,5} = 16$ Hz, 4-H).

Methylated acetylated 4′-*glucoside of* (−)-1′,4′-trans-*diol of ABA* (**13**). ^{1}H NMR (300 MHz, $CDCl_3$) δ0.92 (3H, *s*, 9′-H), δ1.03 (3H, *s*, 8′-H), δ1.57 (3H, *s*, 7′-H), δ1.7–1.9 (2H, *m*, 5′-H), δ2.01 (3H, *s*, 6-H) δ2.0, 2.01 (2), 2.06 (3H, *s*, G-OAcMe), δ3.71 (3H, *s*, 1-H), δ3.71 (1H, *ddd*, $J_{4,5} = 10$ Hz, $J_{5,6B} = 2$ Hz, $J_{5,6A} = 5$ Hz, G5-H), δ4.12 (1H, *dd*, $J_{5,6B} = 2$ Hz, $J_{6A,6B} = 12$ Hz, G6-H_B), δ4.3–4.4 (1H, *m*, 4′-H), δ4.75 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ4.98 (1H, *dd*, $J_{1,2} = 8$ Hz, $J_{2,3} = 10$ Hz, G2-H), δ5.05 (1H, *dd*, $J_{3,4} = 9$ Hz, $J_{4,5} = 10$ Hz, GA-H), δ5.32 (1H, *t*, $J = 9$ Hz, G3-H), δ5.62 (1H, *d*, 3′-H), δ5.72 (1H, *s*, 2-H), δ6.13 (1H, *dd*, $J_{4,5} = 16$ Hz, 5-H) δ7.75 (1H, *dd*, $J_{4,5} = 16$ Hz, 4-H).

(−)-1′,4′-trans-*Diol of ABA glucose ester* (**7**). ^{1}H NMR (300 MHz, [$^2H_5$]pyridine) δ0.93 (eH, *s*, 9′-H), δ1.13 (3H, *s*, 8′-H), 1.61 (3H, *s*, 7′-H), δ1.75 (3H, *s*, 6-H) 1.88–2.04 (2H, *m*, 5′-H), δ3.81–4.35 (5H, *m*, G2-G6H), δ4.44–4.58 (1H, *m*, 4′-H), δ5.58 (1H, *s*, 2-H), δ5.96 (1H, *s*, 3′-H), δ6.16 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ6.38 (1H, *d*, $J_{4,5} = 16$ Hz, 5-H), δ8.31 (1H, *d*, $J_{4,5} = 16$ Hz, 4-H).

(+)-1′,4′-trans-*Diol of ABA glucose ester* (**6**). ^{1}H NMR (300 MHz, [$^2H_3$]pyridine) δ0.93 (3H, *s*, 9′-H), δ1.13 (3H, *s*, 8′-H0, δ1.61 (3H, *s*, 7′-H), δ1.75 (3H, *s*, 6-H), δ1.88–2.03 (2H, *m*, 5′-H), δ3.81–4.33 (5H, *m*, G2-G6-H), δ4.46–4.54 (1H, *m*, 4′-H), δ5.59 (1H, *s*, 2-H), δ5.96 (1H, *s*, H-3′), δ6.18 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ6.38 (1H, *d*, $J_{4,5} = 16$ Hz, 5-H), δ8.29 (1H, *d*, $J_{4,5} = 16$, 1 Hz, 5-H), δ8.29 (1H, *d*, $J_{4,5} = 16$, 4 H).

1′,4′-cis-*Diol of ABA glucose ester* (**17**) ^{1}H NMR (300 MHz, [$^2H_5$]pyridine) δ0.90 (3H, *s*, 9′-H), δ1.16 (3H, *s*, 8′-H), δ1.67 (3H, *s*, 7′-H), δ1.79 (3H, *s*, 6-H), δ1.86 [2H, *m*, 5′-H (ax.)], δ2.14 [2H, *m*, 5′-H(eq.)], δ3.84–4.33 (5H, *m*, G2-G6-H), δ4.43–4.54 (1H, *m*, 4′-H), δ5.64 (1H, *s*, 2-H), δ6.02 (1H, *s*, 3′-H), δ6.21 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ6.27 (1H, *d*, $J_{4,5} = 16$ Hz, 5-H) δ8.39 (1H, *d*, $J_{4,5} = 16$ Hz, 4-H).

Abscisic acid glucose ester. ^{1}H NMR (300 MHz, [$^2H_5$]pyridine) δ0.94 (3H, *s*, 9′-H), δ1.09 (3H, *s*, 8′-H), δ1.65 (3H, *s*, 7′-H), δ1.80 (3H, *s*, 7′-H), δ1.18 [2H, *m*, 5′-H(eq.)], δ2.54 [2H, *m*, 5′-H (ax.)], δ3.81–4.31 (5H, *m*, G2-G6-H), δ5.67 (1H, *s*, 2-H), δ6.00 (1H, *s*, 3′-H), δ6.18 (1H, *d*, $J_{1,2} = 8$ Hz, G1-H), δ6.40 (1H, *d*, $J_{4,5} = 16$ Hz, 5-H), δ8.30 (1H, *d*, $J_{4,5} = 16$ Hz, 4-H).

Mass spectrometry Analyses were carried out with a Finnigan 3200 Quadrupole mass spectrometer interfaced to a Finnigan Incos 2300 Data System. Methane was used as reagent gas (ion source pressure 0.08–0.9 Torr).

Methylated acetylated 4′-glucoside of (+)-1′,4′-trans-*diol of ABA* (**12**). CIMS (CH_4, probe) 100 eV, *m/z* (rel. int.) 651 $[M+41]^+$ (0.2), 639 $[M+29]^+$ (1), 611 $[M+H]^+$ (0.2), 593 $[M+H-H_2O]^+$ (1), 331 (31), 289 (2), 271 (4), 263 (100), 245 (1), 231 (74), 169 (18), 137 (4), 125 (24), 109 (8), 61 (6).

Methylated acetylated 4′-glucoside of (−)-1′,4′-trans-*diol of ABA* (**13**). CIMS (CH_4, probe) 100 eVm *m/z* (rel. int.) 651 $[M+41]^+$ (0.2), 639 $[M+29]^+$ (1), 611 $[M+H]^+$ (0.2), 593 $[M+H\text{-}H_2O]^+$ (1), 331 (31), 289 (2), 271 (5), 263 (100), 245 (4), 231 (64), 169 (20), 137 (4), 125 (23), 109 (8), 61 (14).

Methylated acetylated 4′-glucoside of (+)-1′,4′-$[4'\text{-}^2H]$ trans-*diol of ABA* CIMS (CH_4, probe) 100 eV, *m/z* (rel. int.) 652 $[M+41]^+$ (0.5), 640 $[M+29]^+$ (2), 612 $[M+H]^+$ (0.5), 594 $[M+H-H_2O]^+$ (3), 331 (24), 271 (4), 264 (100), 246 (2), 232 (60), 169 (11), 138 (4), 125 (13), 109 (4), 61 (7).

O-Acetyl glucose ester of (+)-1′,4′-trans-*diol of ABA* (**14**). CIMS (CH_4, probe) 100 eV, *m/z* (rel.int.) 579 $[M+H-HOAc]^+$ (0.1), 331 (26), 271 (7), 231 (11), 169 (25), 127 (3), 109 (11), 61 (100).

O-Acetyl glucose ester of (−)-1′,4′-trans-*diol of ABA* (**13**). CIMS (CH_4, probe) 100 eV, *m/z* (rel.int.) 579 ($[M+H-HOAc]^+$ (0.6), 331 (98), 271 (18), 231 (24), 169 (47), 133 (4), 109 (16), 61 (100).

O-Acetyl glucose ester of (+)-1′,4′-$[4'\text{-}^2H]$-trans-*diol of ABA*. CIMS (CH_4, probe) 100 eV, *m/z* (rel. int.) 580 $[M+H-HOAc]^+$ (0.2), 331 (97), 271 (16), 232 (32), 169 (55), 109 (16), 87 (21), 61 (100).

O-Acetyl glucose ester of 1′,4′-cis-*diol of ABA*. CIMS (CH_4, probe) 100 eV, *m/z* (rel. int.) 579 $[M+H-HOAc]^+$ (0.1), 331 (29), 271 (4), 231 (9), 169 (18), 109 (10), 61 (100).

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