

Preparation and analysis of some acetosugar esters of abscisic acid and derivatives¹

J. Balsevich, G. Bishop, S.L. Jacques, L.R. Hogge, D.J.H. Olson, and N. Laganière

Abstract: Racemic abscisic acid (ABA), the *cis* and *trans* 1', 4'-diols (ABA diols) derived from ABA by reduction of the 4' ketone, and the corresponding 4'-O-acetates were converted into various acetosugar esters by reaction of their cesium salts with the 1-chloroacetosugars derived from glucose, galactose, lactose, and maltose. Analytical separations of the acetosugar esters using high-performance liquid chromatography (LC) on reverse-phase columns were developed. Continuous flow secondary ion mass spectra (CFSIMS) of the various acetosugar esters were obtained and an LC/CFSIMS protocol employing multiple reaction monitoring was used to detect ABA acetoglucose ester in an acetylated extract obtained from plant cells that had been treated with ABA.

Key words: abscisic acid, acetosugar esters, synthesis, chromatography, mass spectrometry.

Résumé : La réaction des sels de césium de l'acide abscissique (AAB) racémique, des diols-1', 4' *cis* et *trans* (diols de l'AAB) obtenus par réduction de la 4'-cétone ainsi que des 4'-O-acétates correspondants avec les 1-chloroacéto sucres dérivés de glucose, galactose, lactose et maltose, a permis de les transformer en esters d'acétosucres. On a développé une méthode analytique qui permet de séparer les esters d'acétosucres par chromatographie liquide (CL) à haute performance sur des colonnes en phase inversées. Pour chacun des esters d'acétosucres, on a obtenu les spectres de masse des ions secondaires en écoulement continu (SMISÉC) et un protocole de CL/SMISÉC faisant appel à un monitoring de réactions multiples a été utilisé pour l'ester acétoglucose ABA dans le produit d'extraction acétylé obtenu à partir de cellules de plantes traitées par du ABA.

Mots clés : acide abscissique, esters d'acétosucres, synthèse, chromatographie, spectrométrie de masse.

[Traduit par la rédaction]

(+)-Abscisic acid (ABA, Fig. 1) is a plant growth regulator that has also been implicated in a variety of stress-related responses (1). Several metabolites of endogenous and exogenously added ABA have been isolated and identified, including the reduction products, *cis* and *trans* ABA 1', 4'-diols (2–5); the oxidized derivative, phaseic acid (6–8); and the glucose esters (β -D-glucose, C-1-linked) of ABA and the aforementioned compounds (Fig. 1) (9–12). There has also been a report of the detection of a maltose ester of ABA (13).

We were interested in following ABA metabolism, and in developing analytical methods to facilitate our studies; in particular, we sought to extend the utility of liquid chromatography–continuous flow secondary ion mass spectrometry (LC/CFSIMS), which we had earlier used to detect ABA glucose ester in a plant extract (14), to other ABA-derived conjugates, which we felt were potential metabolites. To achieve these objectives, samples of potential metabolites or suitable derivatives thereof were required as standards.

Previously, ABA glucose ester (β anomer) had been pre-

pared by reaction of ABA (cesium or triethyl ammonium salt) with α -bromoacetoglucose followed by deacetylation of the intermediate acetoglucose ester with an enzyme preparation derived from sunflower seeds (*Helianthus annuus*) (15, 16). ABA diols had been prepared by sodium borohydride reduction of ABA methyl ester, which yielded a mixture rich in both the 1', 4'-*cis* and -*trans* diols (17).

To further extend earlier synthetic efforts as well as the use of LC/CFSIMS in analysis of metabolite mixtures, we report here the preparation of various acetosugar derivatives of ABA (and deuterated ABA) and the diols, and acetylated diols of ABA, and the development of LC and LC/CFSIMS protocols using these derivatives. The acetosugar derivatives were easily prepared and possessed good stability. Some deuterated analogs were prepared as an aid in assigning fragments in the mass spectrum and for potential use as internal standards. As an example of the utility of the developed protocol, a sample plant extract was analyzed.

Results and discussion

Selective reduction of ABA

Previous preparations of ABA diols involved reduction of ABA methyl ester with sodium borohydride (2, 17). This method was not selective as the ratio of *cis/trans* 1', 4'-diols was ca. 2:1 (2). Formation of the sodium salt of ABA in THF followed by reduction with L-Selectride[®], however, rapidly led to a very clean product that consisted predominantly of the 1', 4'-*cis*-diol (*cis/trans* 10:1). Alternatively, reduction of the

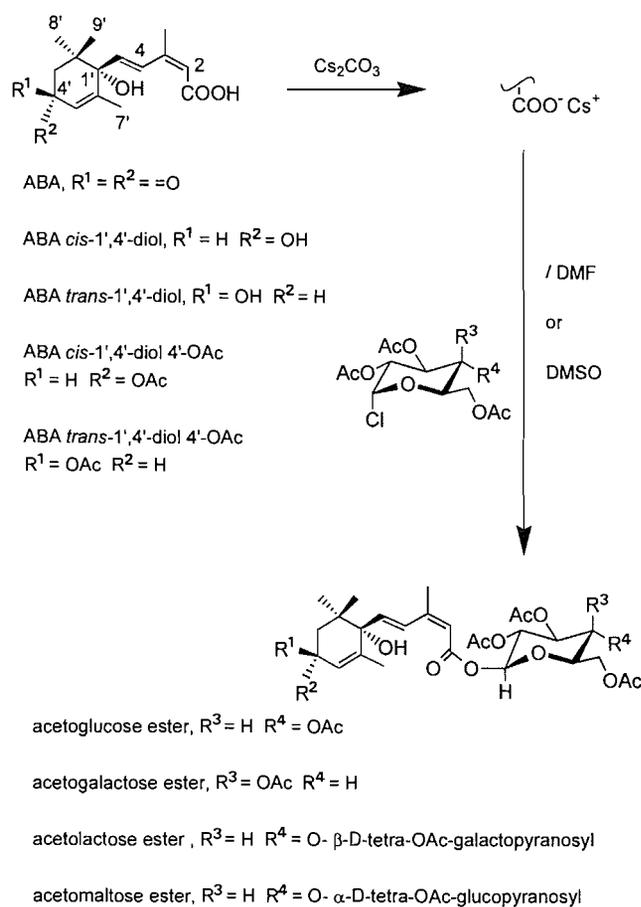
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Fig. 1. Preparation and structures of various acetosugar esters of ABA, ABA diols, and the 4'-O-acetates. Only the diastereomers derived from (+)-ABA are pictured; however, in the actual preparations racemic ABA was used.



preformed sodium salt of ABA in THF with lithium aluminum hydride led to a product mixture containing predominantly the *trans* diol (*cis/trans* <1:20), contaminated with a small amount of conjugate reduction products (< 5%). The *trans* diol could be obtained in pure form via crystallization of either the acetate or methyl ester derivative. Similarly, the *cis* diol could be obtained in pure form via crystallization of the methyl ester.

Formation of acetosugar esters

The preparation of ABA acetoglucose ester was previously achieved by reaction of α -bromoacetoglucose with the cesium salt of ABA in DMF (16). This method had been used for the preparation of esters of amino acids by reaction of their cesium salts with alkyl halides (18). As an extension of these procedures, the cesium salts of ABA, hexadeutero ABA, ABA diols, and the C-4' O-acetates of the ABA diols were prepared by treatment of the acids with Cs_2CO_3 and found to react effectively with α -chloroacetoglucose, -chloroacetogalactose, -chloroacetolactose, and -chloroacetomaltose in either DMF or DMSO (Fig. 1). β -Chloroacetoglucose did not react as well, providing ABA α -acetoglucose ester in only 21% yield. Attempted reactions with chloroacetomannose and chloroacetoglucosamine were unsuccessful. A summary of the acetosugar esters prepared is outlined in Table 1; the esters were obtained as diastereomeric mixtures, as racemic ABA and its

Table 1. Various acetosugar esters of ABA and derivatives prepared by reaction of acetochlorosugars with appropriate cesium salt.

Compound prepared	Reaction conditions	Isolated yield (%)
(\pm)-ABA β -D-acetoglucose ester	DMSO, 60°C, 5 h	53
d_6 -(\pm)-ABA α -D-acetoglucose ester	DMF, RT, 4 d	21
(\pm)-ABA <i>cis</i> diol β -D-acetoglucose ester	DMSO, RT, 4 d	59
(\pm)-ABA <i>cis</i> diol C-4'-O-acetate β -D-acetoglucose ester	DMSO, RT, 4 d	52
(\pm)-ABA <i>trans</i> diol β -D-acetoglucose ester	DMF, RT, 4 d	53
(\pm)-ABA <i>trans</i> diol C-4'-O-acetate β -D-acetoglucose ester	DMF, RT, 4 d	55
(\pm)-ABA β -acetolactose ester	DMF, RT, 4 d	65
d_6 -(\pm)-ABA β -D-acetogalactose ester	DMF, RT, 4 d	49
(\pm)-ABA β -acetomaltose ester	DMF, 60°C, 5 h	61
(\pm)-ABA <i>cis</i> diol β -acetomaltose ester	DMF, 60°C, 5 h	53
(\pm)-ABA <i>cis</i> diol C-4'-O-acetate β -acetomaltose ester	DMSO, RT, 4 d	48

diols were used in the preparations. Two sets of resonances were observed for some of the signals in the 1H NMR spectra, but the chemical shifts differed only slightly.

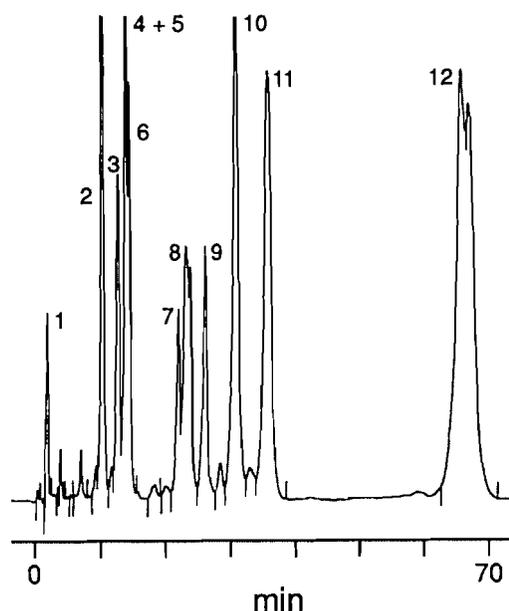
Deacetylation of acetoglucose esters

Deacetylation of the acetoglucose esters of ABA and ABA *cis* diol using a crude enzyme preparation derived from sunflower seeds (15, 16) was successful in our hands; however, the deacetylation of acetolactose and acetomaltose derivatives was problematic, being complicated by solubility problems and incomplete deacetylation. This result led us to consider the use of the acetosugar ester derivatives rather than the sugar esters for development of a general analytical protocol.

LC of acetosugar esters

The LC trace in Fig. 2 shows the separation achieved on a reverse-phase ultrasphere ODS column employing CH_3CN/H_2O (52:48, isocratic) as solvent and 24 (12 pairs of diastereomers) acetosugar derivatives. The disaccharide derivatives, e.g., ABA acetomaltose and acetolactose esters, eluted later than the corresponding monosaccharide derivatives and were well separated from each other. Separation of the α and β anomers of the acetoglucose esters of ABA was not achieved; however, separation of ABA β -acetogalactose ester from the acetoglucose esters was. The β -acetoglucose esters of the *cis* and *trans* ABA diols eluted slightly earlier than the ABA monosaccharide derivatives and were well separated from each other. The corresponding *cis* and *trans* acetate derivatives eluted much later, even later than the disaccharide esters of ABA and ABA diols; they were also well separated from

Fig. 2. HPLC separation of acetosugar esters of ABA, ABA diols, and the 4'-O-acetates, on a reverse-phase ultrasphere ODS column using isocratic acetonitrile-H₂O (52/48). Compounds: 1, ABA glucose ester; 2, ABA *trans*-1', 4'-diol acetoglucose ester; 3, ABA *cis*-1', 4'-diol acetoglucose ester; 4, ABA acetoglucose ester; 5, *d*₆-ABA α -acetoglucose ester; 6, *d*₆-ABA acetogalactose ester; 7, ABA acetolactose ester; 8, ABA *cis*-1', 4'-diol acetomaltose ester; 9, ABA acetomaltose ester; 10, ABA *trans*-1', 4'-diol 4'-OAc acetoglucose ester; 11, ABA *cis*-1', 4'-diol 4'-OAc acetoglucose ester; 12, ABA *cis* 1', 4'-diol 4'-OAc acetomaltose ester. All esters except 5 have β -D- configuration and are diastereomeric mixtures as racemic ABA was used in their preparation.



each other. The latest eluting compound was the C-4'-O-acetate of the *cis* diol of ABA acetomaltose ester. Diastereomeric pairs were not resolvable to any great extent on this column, although some separation of diastereomers was noted in the cases of the acetomaltose esters of ABA *cis* diol and the corresponding 4'-OAc.

To check on the separation of diastereomeric pairs, an LC analysis of some acetosugar esters was also performed on a Chiralcel OD column. This column, which contains a chiral stationary phase, had previously been used to resolve racemic ABA methyl ester (19). The results are summarized in Table 2. Overall, the best separation observed was between (+)- and (-) ABA- β -D-acetoglucose esters, which, under the conditions employed, were baseline resolved (R_t 11.3 and 16.9 min) with reasonable peak shapes; other ABA acetosugar esters generally eluted later, which resulted in broadened peaks and poorer resolution. The diastereomers of acetosugar esters derived from ABA diols and C-4'-O-acetates were not well separated. By comparison, (+)- and (-)-ABA methyl esters, under the same conditions, were also baseline resolved with retention times of 5.5 and 7.3 min, respectively.

MS of acetosugar esters

The continuous flow secondary ion mass spectra of the acetosugar esters contained strong ions due to the acetosugar fragments at m/z 331 for monosaccharides, and 619 and 331 for

disaccharides (Table 3). ABA acetosugar esters exhibited weak but discernible M+1 ions as well as an ion at m/z 247 (253 for *d*₆-ABA) due to the loss of the O-acetosugar. Acetosugar esters of the *cis* and *trans* 1', 4'-ABA-diols and their acetates unfortunately did not afford significant protonated parent (M+1) ions; a significant ion due to the ABA diol portion appeared at m/z 231, arising from loss of the O-sugar and either water (in the case of the diols) or acetic acid (in the case of the 4'-O-acetates).

Identification of *d*₆-ABA acetoglucose ester in an acetylated extract obtained from a maize cell suspension culture treated with *d*₆-ABA

To test the ability of LC/CFSIMS to detect an acetosugar ester in a plant extract, a maize cell suspension culture, which was known to metabolize ABA efficiently (20), was treated with *d*₆-ABA and the cells were isolated after 4 days and extracted with methanol. After acetylation, the extract was analyzed by LC/MS/MS. Figure 3 shows the reaction-monitoring chromatogram obtained for the transition m/z 601–331. The identity of the derivatized metabolite as the acetoglucose ester of ABA was established from its mass spectrum (Fig. 3) and co-chromatography with an authentic sample. No other acetosugar esters were found in this extract.

In conclusion, various acetosugar esters of ABA and ABA derivatives were made readily available for use as reference and (or) internal standards in the analysis of plant extracts. The ability of LC/CFSIMS to detect these derivatives in acetylated extracts was shown, and should prove useful both quantitatively (via use of deuterated internal standards) and qualitatively in the study of ABA metabolism. Finally, the ready formation of various acetosugar derivatives by reaction of α -chloroacetosugars with the cesium salt of an acid would appear to be quite general and may represent a means for the resolution of some racemic acids.

Experimental

General

Solvents and reagents were of reagent grade and were used without further purification. Preparative layer chromatography was performed with 1 mm thick Merck Silica gel 60 F254 20 \times 20 cm plates. Racemic abscisic acid was purchased from Aldrich Chemical Company. Deuterium-labeled abscisic acid was prepared by base-catalyzed exchange in D₂O/MeOD according to the procedure of Balsevich et al. (21). Infrared spectra (IR) were recorded using a Perkin-Elmer 257 grating infrared spectrophotometer (films on NaCl). Ultraviolet spectra (UV) were obtained (MeOH) with a Beckman DU-64 spectrophotometer. Proton nuclear magnetic resonance spectra (¹H NMR) were obtained with a Bruker AM 360 spectrometer with samples in deuteriochloroform unless otherwise noted. An asterisk (*) beside a chemical shift listing denotes that two sets of signals were distinguishable for that resonance due to the two diastereomers. LC/CFSIMS and LC/MS/MS were performed using a 0.32 \times 150 mm Spherisorb 3 μ m ODS-2 packed capillary column interfaced with a VG Analytical (Manchester, UK) 70-250 SEQ hybrid mass spectrometer equipped for continuous flow SIMS analysis according to Hogge et al. (14), except for gradient elution using a two-solvent system consisting of A: 2% glycerol and 0.1% trifluoro-

Table 2. Separation of some of the diastereomeric pairs of acetosugar esters of (\pm)-ABA and its diols by LC on a Chiralcel OD column. AcGE = acetoglucose ester, AcGalE = acetogalactose ester, AcME = acetomaltose ester, AcLE = acetolactose ester. With the exception of the second entry, all acetosugar esters were 1-O- β -D-linked.

Compounds	Retention times of diastereomers (min)	
(\pm)-ABA AcGE	11.3 [(+)-ABA AcGE] ^a	16.9 [(-)-ABA AcGE] ^a
(\pm)-ABA α -AcGE	16.3	20.3
(\pm)-ABA AcGalE	17.1	21.0
(\pm)-ABA AcME	20.2	26.3
(\pm)-ABA AcLE	17.7	21.7
(\pm)- <i>cis</i> Diol AcGE	10.8	10.8
(\pm)- <i>cis</i> Diol AcME	19.6	20.6
(\pm)- <i>trans</i> Diol 4'-OAc AcGE	9.5	9.5
(\pm)- <i>cis</i> Diol 4'-OAc AcGE	9.0	9.9
(\pm)- <i>cis</i> Diol 4'-OAc AcME	16.9	20.0

^aThe diastereomer due to (+)-ABA was found to be the faster eluting component in the case of the acetoglucose esters. This was determined by its isolation and saponification to (+)-ABA. Identification of other diastereomers was not done.

Table 3. CFSIMS data for some acetosugar derivatives of ABA and ABA diols. AcGE = acetoglucose ester, AcGalE=acetogalactose ester, AcME = acetomaltose ester, AcLE = acetolactose ester. With the exception of the fifth entry, all acetosugar esters were 1-O- β -D-linked.

Compound	CFSIMS data: <i>m/z</i> (relative intensity)				
	[M+1] ⁺	[M+1- H ₂ O] ⁺	[M+1- HOAc] ⁺	Non-sugar fragments	Sugar fragments
ABA AcGE	595(12)			247(7)	331(100)
ABA AcGalE	595(9)			247(5)	331(100)
<i>d</i> ₆ -ABA AcGE	601(8)			253(6)	331(100)
<i>d</i> ₆ -ABA AcGalE	601(7)			253(5)	331(100)
<i>d</i> ₆ -ABA α -AcGE	601(13)			253(5)	331(100)
ABA AcME	883(4)			247(6)	619(50), 331(100)
ABA AcLE	883(5)			247(7)	619(40), 331(100)
<i>d</i> ₆ -ABA AcLE	889(6)			253(6)	619(46), 331(100)
<i>trans</i> Diol AcGE	597(1)	579(2)		231(5)	331(100)
<i>cis</i> Ddiol AcGE	597(1)	579(3)		231(5)	331(100)
<i>trans</i> Diol-4'-OAc AcGE	639(1)	621(4)	579(3)	231(11)	331(100)
<i>cis</i> Diol 4'-OAc AcGE	—	621(2)	579(1)	231(7)	331(100)
<i>cis</i> Diol AcME	—	867(2)		231(6)	619(67), 331(100)
<i>cis</i> Diol-4'OAc AcME	927(1)	909(1)	867(1)	231(9)	619(45), 331(62)

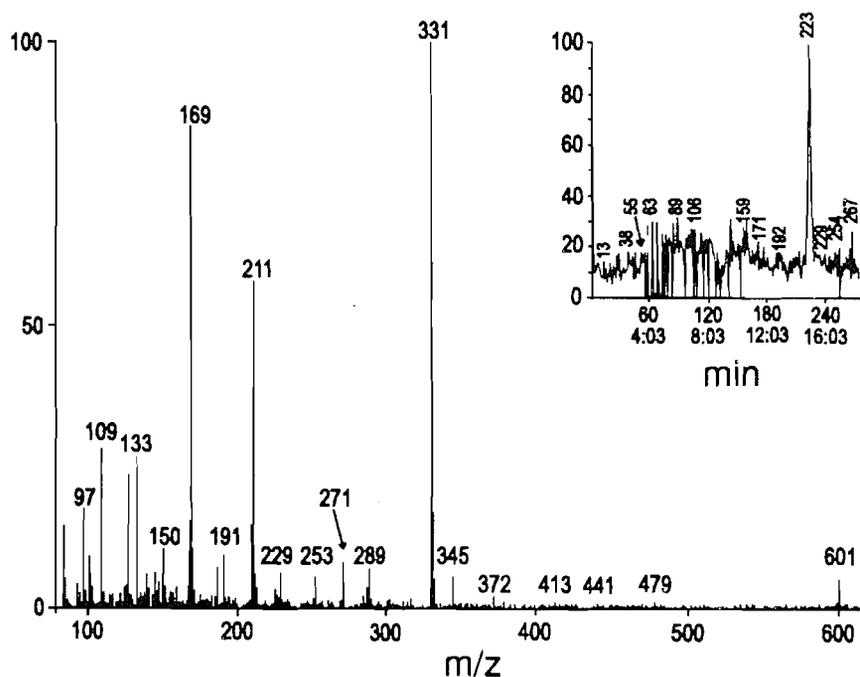
acetic acid in H₂O, and B: 80% CH₃CN, 2% glycerol, and 0.1% trifluoroacetic acid in H₂O. The gradient elution used was 50% A to 10% A in 10 min, which was held for a further 25 min. Analytical LC was performed on a Waters system equipped with a reverse-phase Ultrasphere 5 μ m ODS 4.6 mm \times 25 cm column using isocratic CH₃CN/H₂O (52:48) at a flow rate of 0.5 mL/min. UV detection at 262 nm was used. Chiral LC was performed with a Chiralcel OD 0.46 \times 25 cm coated silica column (Daicel, Los Angeles, Calif.) using isocratic

hexane/isopropanol (7:1) at a flow rate of 1 mL/min. Mass spectrometry (MS) was performed with a VG 70-250SEQ double focussing hybrid spectrometer. High-resolution mass spectrometry (HRMS) was performed using fast ion bombardment ionization with the sample in a glycerol matrix.

Reduction of ABA with L-Selectride[®] — (\pm)-ABA *cis*-1', 4'-diol

A solution of ABA (264 mg, 1 mmol) in anhydrous THF (10

Fig. 3. Reaction monitoring chromatogram for the transition m/z 601–331 (insert), and the obtained CFSIMS spectrum from analysis of an acetylated plant extract.



mL) was cooled to 0°C, treated with sodium hydride – mineral oil suspension (60%, 44 mg, 1.1 mmol), and stirred for 20 min. A 1 M solution of L-Selectride® (1.1 mL, 1.1 mmol) in THF was added at a dropwise rate over 10 min. The reaction was allowed to warm to ambient temperature and stirred for a further 3 h. Water (100 mL) was cautiously added and the aqueous solution washed with ethyl acetate (3 × 15 mL). The combined ethyl acetate washings were back-extracted with water (2 × 10 mL) and discarded. The aqueous portions were combined, made acidic by the addition of 1 N HCl (3 mL), and extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate extract was washed with water (2 × 5 mL), dried (Na₂SO₄), and concentrated in vacuo to afford a mixture consisting mainly of the *cis* diol plus ca. 9% of the *trans* diol as a white amorphous solid (260 mg, 0.98 mmol, 98%). The proportions of the two isomers were determined from the ¹H NMR spectrum of the reaction product. The diols were separated by preparative TLC on silica gel using EtOAc as solvent.

cis Diol: UV, λ_{max} (log ε): 262 (4.24); IR; ν_{max} (cm⁻¹): 3400, 2965, 1688, 1631, 1598, 1240; ¹H NMR; δ: 7.70 (1H, d, 16 Hz, C-5), 6.05 (1H, d, 16 Hz, C-4), 5.69 (1H, s, C-2), 5.64 (1H, bs, C-3'), 4.24 (1H, m, C-4'), 2.01 (3H, s, C-6), 1.79 (1H, ABX, 13 and 6 Hz, C-5'α or β), 1.71 (1H, ABX, 13 and 7 Hz, C-5'α or β), 1.65 (3H, s, C-7'), 1.00 (3H, s, C-8' or 9'), 0.92 (3H, s, C-8' or C-9'). MS (ammonia CI) m/z : 284 ([M+18]⁺, 9%), 267 ([M+1]⁺, 38%), 266 (M⁺, 69%), 248 (35%), 231 (100%); HRMS, calcd. for [M+glycerol]⁺ C₁₈H₃₁O₇: 359.2070; found: 359.2026.

trans Diol: mp 148–153°C; UV, λ_{max} (log ε): 262 (4.21); IR, ν_{max} (cm⁻¹): 3400, 2962, 1686, 1631, 1598, 1240; ¹H NMR, δ: 7.58 (1H, d, 16 Hz, C-5), 6.05 (1H, d, 16 Hz, C-4), 5.69 (1H, s, C-2), 5.67 (1H, bs, C-3'), 4.22 (1H, m, C-4'), 2.02 (3H, s, C-6), 1.78 (1H, ABX, 13 and 6.5 Hz, C-5'α), 1.64 (3H, s, C-7'), 1.61 (1H, ABX, 13 and 9.5 Hz, C-5'β), 1.02 (3H, s, C-8' or 9'), 0.89 (3H, s, C-8' or C-9'). ¹H NMR (D₂O); δ: 7.25 (1H, d, 16 Hz,

C-5), 6.11 (1H, d, 16 Hz, C-4), 5.71 (1H, s, C-2), 5.54 (1H, bs, C-3'), 4.22 (1H, m, C-4'), 1.94 (3H, s, C-6), 1.66 (1H, ABX, 13 and 6 Hz, C-5'α), 1.54 (3H, s, C-7'), 1.46 (1H, ABX, 13 and 10 Hz, C-5'β), 0.91 (3H, s, C-8' or 9'), 0.82 (3H, s, C-8' or C-9'). MS (ammonia CI) m/z : 284 ([M+18]⁺, 16%), 267 ([M+1]⁺, 10%), 266 (M⁺, 100%), 248 (30%), 231 (61%); HRMS, calcd. for [M+glycerol]⁺ C₁₈H₃₁O₇: 359.2070; found: 359.2108.

The *cis* and *trans* diols were also converted to their methyl esters by treatment with ethereal diazomethane and afforded ¹H NMR spectra comparable to published data [2].

Reduction of ABA with lithium aluminum hydride — (±)-ABA *trans*-1', 4'-diol

A solution of ABA (264 mg, 1 mmol) in anhydrous THF (10 mL) was cooled to 0°C, treated with sodium hydride–mineral oil suspension (60%, 40 mg, 1 mmol), and stirred for 20 min. LiAlH₄ (30 mg, 0.75 mmol) was added in several portions over 10 min. The reaction was allowed to warm to ambient temperature and stirred for a further 4 h. The reaction was poured into cold water (100 mL), washed with hexane (3 × 25 mL), acidified with 1 N HCl (3.5 mL), and extracted with EtOAc (3 × 30 mL). The EtOAc extract was dried (Na₂SO₄), filtered, and concentrated in vacuo to afford a mixture of the *trans* diol contaminated with ca. 5% overreduced products as a white amorphous solid (260 mg). The presence of overreduced products was determined from the ¹H NMR spectrum from signals at δ 3.6–3.9 attributable to saturated alcohols. The main product was the *trans* diol (C-4' β-OH) identical to the minor product from above; virtually no *cis* diol was observable by NMR.

(±)-ABA *trans*-1', 4'-diol C-4'-O-acetate

trans ABA diol (260 mg, ca. 0.9 mmol), obtained directly from the LiAlH₄ reduction of ABA without further purifica-

tion, was dissolved in a 1:2 (v/v) mixture of acetic anhydride and pyridine (1 mL), and let stand at ambient temperature for 17 h. The mixture was concentrated in vacuo, and the residue was dissolved in EtOAc (ca. 50 mL), washed with 1 N HCl (2×), water (3×), and concentrated in vacuo. The residue was treated with ethyl acetate (ca 1 mL) and stored at 0°C overnight. The resultant crystalline solid was isolated by filtration and the crystals dried in vacuo to afford the acetate as a white crystalline solid (170 mg), mp 185–188°C; UV, λ_{\max} (log ϵ): 258 (4.18); IR, ν_{\max} (cm⁻¹): 3400, 2960, 1720sh, 1710, 1680 sh, 1632, 1598, 1245; ¹H NMR (CDCl₃/DMSO-*d*₆, 3:1), δ : 7.61 (1H, d, 16 Hz, C-5), 6.02 (1H, d, 16 Hz, C-4), 5.55 (1H, s, C-2), 5.33 (1H, s, C-3'), 5.20 (1H, m, C-4'), 1.94 (3H, s, OAc), 1.91 (3H, s, C-6), 1.71 (1H, ABX, 6 and 13 Hz, C-5'β), 1.56 (3H, s, C-7), 0.94 (3H, s, C-8'), 0.81 (3H, s, C-9'). HRMS, calcd. for [(M-H₂O)+H]⁺ C₁₅H₂₁O₃: 249.1491; found: 249.1457.

(±)-ABA *cis*-1', 4'-diol C-4'-O-acetate

The *cis* diol (130 mg, 0.5 mmol) was treated as above except the crude product was purified by TLC on Silica gel using EtOAc to afford a colorless film (80 mg, 52%); UV, λ_{\max} (log ϵ): 258 (4.20); ¹H NMR, δ : 7.68 (1H, d, 16 Hz, C-5), 5.93 (1H, d, 16 Hz, C-4), 5.68 (1H, s, C-3'), 5.51 (1H, s, C-2) 5.26 (1H, m, C-4'), 2.01 (3H, s, OAc), 1.93 (3H, s, C-6), 1.80 (1H, ABX, 6 and 14 Hz, C-5'β), 1.72 (1H, ABX, 6 and 14 Hz, C-5'α), 1.62 (3H, s, C-7), 0.97 (3H, s, C-8'), 0.90 (3H, s, C-9'). HRMS, calcd. for [(M-H₂O)+H]⁺ C₁₅H₂₁O₃: 249.1491; found: 249.1507.

Preparation of cesium salts of ABA, ABA diols, and diol acetates

Cesium salts were prepared by treating a solution of racemic ABA, or the ABA diols, or the acetates (1 mmol) in 50% aqueous methanol (10 mL) with cesium carbonate (0.5 mmol) and concentrating the resultant solution in vacuo. The residues were treated with benzene and concentrated in vacuo (2×), and then stored in a vacuum desiccator for 24 h. The cesium salts were obtained as colorless to pale yellow glasses and were used without further purification.

Preparation of α-chloroacetosugars and β-chloroacetoglucose

The α-chloroacetosugars were prepared by reaction of the peracetylated sugars with titanium tetrachloride in refluxing chloroform as outlined in refs. 22 and 23. β-Chloroacetoglucose was prepared by reaction of glucose pentaacetate with aluminum chloride in dichloromethane as outlined in ref. 24.

General protocol for preparation of acetosugar esters

A solution of the appropriate cesium salt (1 mmol) in either DMF or DMSO (5 mL) was treated with the appropriate chloroacetosugar (1.1 mmol). After 4 days at ambient temperature or 5 h at 60°C, the reaction was treated with H₂O (30 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extract was washed with 0.1 N NaOH (2 × 10 mL), H₂O (2 × 10 mL), brine (2 × 10 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo afforded an amber oil, which was subjected to TLC on silica, gel using hexane/EtOAc (7:3) as solvent to afford the appropriate acetosugar ester as a colorless glass, generally in about 50% yield

(see Table 1). ABA acetosugar esters gave UV spectra having λ_{\max} (log ϵ): 272 (4.29), 240sh (4.07), while the acetosugar esters of ABA diols and C-4'-O-acetates gave λ_{\max} (log ϵ): 272 (4.18). ABA acetosugar esters gave IR spectra having ν_{\max} (cm⁻¹): 3500, 2962, 1748, 1663, 1630sh, 1598, 1224, 1070, 1040; acetosugar esters of ABA diols gave ν_{\max} : 3515, 2964, 1750, 1633, 1598, 1228, 1070, 1038; acetosugar esters of the C-4'-O-acetates gave ν_{\max} : 3510, 2954, 1755, 1735sh, 1633, 1598, 1225, 1070, 1040.

(±)-ABA β-*D*-acetoglucose ester: ¹H NMR (ref. 17), δ : 7.78 (1H, d, 16 Hz, C-5), 6.21, 6.20* (1H, d, 16 Hz, C-4), 5.92 (1H, bs, C-3'), 5.74 (1H, d, 8.6 Hz, sugar C-1), 5.72 (1H, s, C-2), 5.27 (1H, t, 9 Hz, sugar C-3 or 4), 5.20 (1H, t, 8.5 Hz, sugar C-2), 5.13 (1H, t, 9.5 Hz, sugar C-3 or C-4), 4.29 (1H, ABX, 12.5 and 3.4 Hz, sugar C-6), 4.11 (1H, ABX, 12.5 and 2 Hz, sugar C-6), 3.84 (1H, m, sugar C-5), 2.46, 2.44* (1H, AB, 17 Hz, C-5'α or β), 2.30 (1H, AB, 17 Hz, C-5'α or β), 2.06 (3H, s, OAc), 2.02 (3H, s, OAc), 2.01 (3H, bs, C-6), 2.00 (3H, s, OAc), 1.99 (3H, s, OAc), 1.91, 1.90* (3H, s, C-7'), 1.10 (3H, s, C-8' or 9'), 0.99* (3H, s, C-8' or 9'). HRMS, calcd. for [M+H]⁺ C₂₉H₃₉O₁₃: 595.2391; found: 595.2361.

*d*₆-(±)-ABA β-*D*-acetoglucose ester: ¹H NMR: as above except lacking resonances at δ 5.92, 2.5–2.2, and 1.9. HRMS, calcd. for [M+H]⁺ C₂₉H₃₃D₆O₁₃: 601.2767; found: 601.2787.

*d*₆-(±)-ABA α-*D*-acetoglucose ester: ¹H NMR, δ : 7.83, 7.82* (1H, d, 16 Hz, C-5), 6.39 (1H, d, 3.7 Hz, sugar C-1), 6.20, 6.19* (1H, d, 16 Hz, C-4), 5.80 (1H, s, C-2), 5.53 (1H, t, 10 Hz, sugar C-3 or 4), 5.13 (1H, t, 10 Hz, sugar C-3 or C-4), 5.10 (1H, dd, 10 and 3.7 Hz, sugar C-2), 4.2 (1H, ABX, 12.5 and 3.4 Hz, sugar C-6), 4.12 (1H, m, sugar C-5), 4.07 (1H, ABX, 12.5 and 2 Hz, sugar C-6), 2.1–1.97* (5 × 3H, 7s, 4 OAc and C-7'), 1.09, 1.08* (3H, s, C-8' or 9'), 0.99 (3H, s, C-8' or 9'). HRMS, calcd. for [M+H]⁺ C₂₉H₃₃D₆O₁₃: 601.2767; found: 601.2776.

(±)-ABA β-*D*-acetogalactose ester: ¹H NMR, δ : 7.78, 7.77* (1H, d, 16 Hz, C-5), 6.21, 6.19* (1H, 2 d, 16 Hz, C-4), 5.92 (1H, bs, C-3'), 5.73 (1H, s, C-2), 5.72 (1H, d, 8.4 Hz, sugar C-1), 5.41 (1H, t, 1.6 Hz, sugar C-4), 5.36, 5.35* (1H, 2 dd, 8 and 10 Hz, sugar C-2), 5.08 (1H, dd, 10 and 3.4 Hz, sugar C-3), 4.2–4.0 (3H, overlapping peaks, sugar C-5 and C-6), 2.45, 2.43* (1H, AB, 17 Hz, C-5'α or β), 2.29 (1H, AB, 17 Hz, C-5'α or β), 2.14, 2.10 (2 × 3H, 2s, 2 × OAc), 2.00 (6H, s, 2 × OAc), 1.97 (3H, s, C-6), 1.91, 1.89* (3H, s, C-7'), 1.09 (3H, s, C-8' or 9'), 0.99 (3H, s, C-8' or 9'). HRMS, calcd. for C₂₉H₃₉O₁₃: 595.2391; found: 595.2427.

*d*₆-(±)-ABA β-*D*-acetogalactose ester: ¹H NMR: as above except lacking resonances at δ 5.92, 2.5–2.2, and 1.9. HRMS, calcd. for [M+H]⁺ C₂₉H₃₃D₆O₁₃: 601.2767; found: 601.2795.

(±)-ABA *cis*-1', 4'-diol β-*D*-acetoglucose ester: ¹H NMR, δ : 7.73 (1H, d, 16 Hz, C-5), 6.12 (1H, d, 16 Hz, C-4), 5.76 (1H, d, 8.5 Hz, sugar C-1), 5.67 (1H, s, C-2), 5.62 (1H, bs, C-3'), 5.26 (1H, t, 9 Hz, sugar C-3 or 4), 5.18 (1H, t, 9 Hz, sugar C-3 or 4), 5.13 (1H, t, 9 Hz, sugar C-2), 4.39 (1H, ABX, 12 and 5 Hz, sugar C-6), 4.24 (1H, m, C-4'), 4.00 (1H, ABX, 12 and 2 Hz, sugar C-6), 3.85 (1H, m, sugar C-5), 2.08 (3H, s, OAc), 2.04

(3H, s, OAc), 2.02 (6H, s, OAc and C-6), 2.01 (3H, s, OAc), 1.80 (1H, ABX, 14 and 6, C-5'α or β), 1.71 (1H, ABX, 14 and 6, C-5'α or β), 1.66 (3H, s, C-7'), 1.00 (3H, s, C-8' or 9'), 0.92 (3H, s, C-8' or 9').

(±)-ABA *cis*-1', 4'-diol C-4'-O-acetate β-D-acetoglucose ester: ¹H NMR, δ: 7.71 (1H, d, 16 Hz, C-5), 6.09 (1H, d, 16 Hz, C-4), 5.74 (1H, d, 8.2 Hz, sugar C-1), 5.67 (1H, s, C-2), 5.57 (1H, bs, C-3'), 5.29 (1H, m, C-4'), 5.25 (1H, t, 9.5 Hz, sugar C-3 or 4), 5.17 (1H, t, 9 Hz, sugar C-2), 5.12 (1H, t, 9 Hz, sugar C-3 or C-4), 4.28 (1H, ABX, 12 and 5 Hz, sugar C-6), 4.10 (1H, m, sugar C-6), 3.83 (1H, m, sugar C-5), 2.05, 2.03, 2.01, 2.00, 1.99, 1.98 (6 × 3H, 6s, 5 × OAc and C-6), 1.83 (1H, m, C-5'α or β), 1.71 (1H, m, C-5'α or β), 1.66 (3H, s, C-7'), 1.01 (3H, s, C-8' or 9'), 0.93 (3H, s, C-8' or 9'). HRMS, calcd. for [(M+H)–H₂O]⁺ C₃₁H₄₁O₁₃: 621.2547; found: 621.2562.

(±)-ABA *trans*-1', 4'-diol β-D-acetoglucose ester: ¹H NMR, δ: 7.58, 7.57* (1H, 2d, 16 Hz, C-5), 6.18, 6.17* (1H, 2d, 16 Hz, C-4), 5.72, 5.71* (1H, 2d, 8.5 Hz, sugar C-1), 5.65 (1H, s, C-2), 5.63, 5.61* (1H, 2bs, C-3'), 5.22 (1H, t, 9.5 Hz, sugar C-3 or 4), 5.14 (1H, t, 9 Hz, sugar C-2), 5.10 (1H, t, 9.5 Hz, sugar C-3 or 4), 4.28–4.16 (2H, overlapping peaks, C-4' and sugar C-6), 4.07 (1H, m, sugar C-6), 3.82 (1H, m, sugar C-5), 2.04*, 2.00, 1.99, 1.98, 1.97 (5 × 3H, 5s, 4 × OAc and C-6) 1.79 (1H, m, C-5'α or β), 1.62 (3H, s, C-7'), 1.53 (1H, m, C-5'α or β), 1.01 (3H, s, C-8' or 9'), 0.87, 0.86* (3H, 2s, C-8' or 9'). HRMS, calcd. for [(M+H)]⁺ C₂₉H₄₁O₁₃: 597.2547; found: 597.2527

(±)-ABA *trans*-1', 4'-diol C-4'-O-acetate β-D-acetoglucose ester: ¹H NMR, δ: 7.70* (1H, d, 16 Hz, C-5), 6.21* (1H, d, 16 Hz, C-4), 5.74* (1H, d, 8.5 Hz, sugar C-1), 5.67 (1H, s, C-2), 5.50 (1H, bs, C-3'), 5.36 (1H, m, C-4'), 5.25 (1H, t, 9.4 Hz, sugar C-3 or 4), 5.18 (1H, t, 8.2 Hz, sugar C-2), 5.12 (1H, t, 9.4 Hz, sugar C-3 or 4), 4.27 (1H, ABX, 12.5 and 4.3 Hz, sugar C-6), 4.09 (1H, ABX, 12.5 and 2 Hz, sugar C-6), 3.82 (1H, m, sugar C-5), 2.05, 2.04, 2.01, 2.00, 1.99, 1.98 (6 × 3H, 6s, 5 × OAc and C-6), 1.87 (1H, m, C-5'α or -β), 1.68 (1H, m, C-5'α or -β), 1.65 (3H, s, C-7'), 1.06 (3H, s, C-8' or 9'), 0.89, 0.88* (3H, 2s, C-8' or 9'). HRMS, calcd. for [(M+H)]⁺ C₃₁H₄₃O₁₄: 639.2696; found: 639.2653.

(±)-ABA β-D-acetolactose ester: ¹H NMR, δ: 7.76 (1H, d, 16 Hz, C-5), 6.20, 6.19* (1H, d, 16 Hz, C-4), 5.91 (1H, s, C-3'), 5.70 (1H, d, 8 Hz, C-1 of glucose portion), 5.70 (1H, s, C-2), 5.34 (1H, m, C-4 of galactose portion), 5.26 (1H, t, 9 Hz), 5.1 (2H, overlapping peaks), 4.93 (1H, dd, 10 and 3 Hz, C-3 of galactose portion), 4.5–3.6 (8H, overlapping peaks, sugar H's), 2.45, 2.44* (1H, AB, 16 Hz, C-5'α or β), 2.29 (1H, AB, 16 Hz, C-5'α or β), 2.14, 2.09, 2.05, 2.04, 2.03, 2.02, 2.00 (7 × 3H, 7s, 7 × OAc), 2.07 (3H, s, C-6), 1.89 (3H, s, C-7'), 1.10 (3H, s, C-8' or -9'), 0.99 (3H, s, C-8' or 9'). HRMS, calcd. for [(M+H)]⁺ C₄₁H₅₅O₂₁: 883.3236; found: 883.3285

*d*₅-(±)-ABA β-D-acetolactose ester. ¹H NMR: as above, except lacking peaks at δ 5.91, 2.5–2.2, and 1.89. HRMS, calcd. for [(M+H)]⁺ C₄₁H₄₉D₆O: 889.3613; found: 889.3642.

(±)-ABA β-D-acetomaltose ester: ¹H NMR, δ: 7.76* (1H, d, 16, C-5), 6.18* (1H, d, 16, C-4), 5.89 (1H, s, C-3'), 5.74 (1H,

d, 8 Hz, sugar A C-1), 5.67 (1H, s, C-2), 5.37 (1H, d, 4 Hz, sugar B C-1), 5.35–5.25 (2H, overlapping sugar peaks), 5.04–4.94 (2H, overlapping sugar peaks), 4.82 (1H, dd, 10.5 and 4 Hz, sugar B C-2), 4.5–3.8 (6H, sugar A and B C-5's and C-6's), 2.08, 2.05, 2.01 (3 × 3H, 3s, 3 × OAc), 2.00 (3H, s, C-6), 1.98 (6H, s, 2 × OAc), 1.96, 1.95 (2 × 3H, 2s, 2 × OAc), 1.87 (3H, bs, C-7'), 1.07 (3H, s, C-8' or -9'), 0.97 (3H, s, C-8' or 9'). HRMS, calcd. for [(M+H)]⁺ C₄₁H₅₅O₂₁: 883.3236; found: 883.3227.

(±)-ABA *cis*-1', 4'-diol β-D-acetomaltose ester: ¹H NMR, δ: 7.71* (1H, d, 16 Hz, C-5), 6.10, 6.09* (1H, d, 16 Hz, C-4), 5.76 (1H, d, 8 Hz, sugar A C-1), 5.63 (2H, 2 × s, C-2, C-3'), 5.38 (1H, d, 4 Hz, sugar B C-1), 5.33 (1H, t, 10 Hz), 5.28 (1H, t, 10 Hz) 5.03 (1H, t, 10 Hz) 5.00 (1H, t, 9 Hz), 4.84 (1H, dd, 10 and 4 Hz, sugar B C-2), 4.43 (1H, m), 4.25–4.18 (3H, overlapping peaks, C-4' and sugar C-6's), 4.03 (2H, overlapping peaks), 3.92 (1H, m), 3.82 (1H, m), 1.97, 2.10, 2.07, 2.02, 2.00 (4 × 3H, 4s, 4 × OAc), 1.99 (6H, 2s, C-6 and OAc), 1.96 (2 × 3H, s, 2 × OAc), 1.64 (3H, bs, C-7'), 1.00 (3H, s, C-8' or -9'), 0.91 (3H, s, C-8' or 9'). HRMS, calcd. for [(M+H)]⁺ C₄₁H₅₇O₂₁: 885.3392; found: 885.3443

(±)-ABA *cis*-1', 4'-diol C-4'-O-acetate β-D-acetomaltose ester: ¹H NMR, δ: 7.71 (1H, d, 16 Hz, C-5), 6.09* (1H, d, 16 Hz, C-4), 5.77 (1H, d, 8 Hz, sugar A C-1), 5.64 (1H, s, C-3'), 5.57 (1H, s, C-2), 5.38 (1H, d, 4 Hz, sugar B C-1), 5.34 (1H, t, 10 Hz), 5.29 (1H, t, 9 Hz), 5.28 (1H, m, C-4') 5.03 (1H, t, 10 Hz) 5.01 (1H, t, 9 Hz), 4.84 (1H, dd, 10 and 4 Hz, sugar B C-2), 4.43 (1H, m), 4.22 (2H, overlapping peaks), 4.03 (2H, overlapping peaks), 3.93 (1H, m), 3.82 (1H, m), 2.10, 2.08, 2.04, 2.03, 2.00 (5 × 3H, 4s, 4 × OAc), 1.99 (6H, 2s, C-6 and OAc), 1.98, 1.97 (2 × 3H, 2s, 2 × OAc), 1.66 (3H, bs, C-7'), 1.01 (3H, s, C-8' or -9'), 0.93 (3H, s, C-8' or -9'). HRMS, calcd. for [(M+H)]⁺ C₄₃H₅₉O₂₂: 927.3498; found: 927.3491.

Enzymatic deacetylation of ABA *cis* diol- and ABA acetoglucose esters

The acetoglucose esters of ABA and ABA *cis*-1', 4'-diol were deacetylated using a modification of the procedure of Lehmann et al. (15) as follows. Enzyme preparation: dehusked sunflower seeds (9 g, Super Store) were added to cold (<10°C) pH 7.0 100 mM phosphate buffer (75 mL) and homogenized with an Ultra Turrax homogenizer for 2–3 min. The resulting suspension was centrifuged at 25 000g for 30 min. The middle aqueous layer was removed and re-centrifuged. The resulting clear aqueous portion, free of oil droplets, was used for deacetylations.

(±)-ABA *cis*-1', 4'-diol glucose ester

A solution of the *cis* diol β-acetoglucose ester (230 mg, 0.39 mmol) in EtOH (15 mL) was added to a vigorously stirred solution of 100 mM pH 7.0 phosphate buffer (35 mL), and the sunflower seed enzyme preparation from above (60 mL). A further portion of EtOH (3 mL) was added dropwise over a period of 20 min and the resulting mixture stirred at ambient temperature for 20 h. Isopropanol (100 mL) was added and the resulting mixture centrifuged at 10 000g for 30 min. The supernatant was concentrated in vacuo and the residue was triturated with a further portion of isopropanol (50 mL) and centrifuged again. The supernatant was concentrated in

vacuo and the residue (200 mg) subjected to thin-layer chromatography (silica gel), using $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (93:7) as the solvent system, to yield the *cis* diol glucose ester as a white amorphous solid (75 mg, 0.18 mmol, 46%). ^1H NMR (D_2O), δ : 7.46 (1H, d, 16 Hz, C-5), 6.21 (1H, d, 16 Hz, C-4), 5.76 (1H, s, C-2), 5.59 (1H, bs, C-3), 5.50 (1H, d, 8 Hz, sugar C-1), 4.20 (1H, m, C-4'), 3.9–3.3 (6H, overlapping peaks, sugar C-2 C-6), 2.01 (3H, s, C-6), 1.70 (1H, ABX, 13 and 6 Hz, C-5' α or β), 1.57 (3H, s, C-7'), 1.53 (1H, ABX, 13 and 7 Hz, C-5' α or β), 0.89 (3H, s, C-8' or 9'), 0.84 (3H, s, C-8' or C-9'); CFSIMS, m/z : 429 (2%, $\text{M}^+ + 1$), 411 (50%, $\text{M}^+ + 1 - \text{H}_2\text{O}$), 393 (28%, $\text{M}^+ + 1 - 2\text{H}_2\text{O}$), 249 (44%), 231 (100%). HRMS, calcd. for $[\text{M} + \text{H}]^+ \text{C}_{21}\text{H}_{33}\text{O}_9$: 429.2125; found: 429.2177.

(\pm)-ABA glucose ester

A solution of ABA β -acetoglucose ester (230 mg, 0.39 mmol) was treated as above to yield ABA glucose ester as a colorless film (84 mg, 0.2 mmol, 51%) having an ^1H NMR spectrum similar to that reported in the literature (17). ^1H NMR (D_2O), δ : 7.50 (1H, d, 16 Hz, C-5), 6.26 (1H, d, 16 Hz, C-4), 5.97 (1H, bs, C-3'), 5.78 (1H, s, C-2), 5.49 (1H, d, 8 Hz, sugar C-1), 3.9–3.2 (6H, overlapping peaks, sugar C-2–C-6), 2.50 (1H, AB, 17 Hz, C-5' α or β), 2.16 (1H, AB, 17 Hz, C-5' α or β), 1.99 (3H, s, C-6), 1.85 (3H, s, C-7'), 0.96 (3H, s, C-8' or 9'), 0.93 (3H, s, C-8' or C-9'); CFSIMS, m/z : 427 (27%, $\text{M}^+ + 1$), 409 (8%, $\text{M}^+ + 1 - \text{H}_2\text{O}$), 265 (base peak), 247 (77%). HRMS, calcd. for $[\text{M} + \text{H}]^+ \text{C}_{21}\text{H}_{31}\text{O}_9$: 427.1968; found: 427.1992.

Feeding of d_6 -ABA to a maize cell suspension

Suspension cultures of corn (*Zea mays* L. cv Black Mexican Sweet) were maintained on modified Murashige–Skoog medium as described previously (20, 25). d_6 -(\pm)-ABA (0.4 mg) in EtOH (30 μL) was added to the cell suspension (60 mL, ca. 2 g fresh wt. of cells) 24 h after being subcultured. After 4 d cells were separated from medium by filtration and extracted with methanol (100 mL). Cell material was removed by filtration and the methanol extract concentrated in vacuo. The residue was washed with hexane, redissolved in cold methanol/ acetonitrile (1:1, 20 mL), filtered, and concentrated in vacuo. The residue was treated with Ac_2O /pyridine (1 mL of a 1:2 mixture, v/v). After 4 h, volatiles were removed in vacuo and the residue dissolved in 50% acetonitrile (aq) and used for the LC/CFSIMS analysis described earlier.

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