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OXIDATION OF THE 8′-POSITION OF A BIOLOGICALLY ACTIVE ABSCISIC ACID ANALOGUE*

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Key Word Index—*Bromus inermis*; Gramineae; freezing tolerance; metabolism; synthesis; abscisic acid; ABA; ABA analogues; optically active analogues; 2′,3′-dihydroabscisic acid; 8′-hydroxy-2′,3′-dihydroabscisic acid; 8′-hydroxyabscisic acid; phaseic acid.

Abstract—The metabolism of a biologically active abscisic acid (ABA) analogue, (+)-(1′*S*,2′*S*)-2′,3′-dihydroabscisic acid [(+)-4-(1*E*,3*Z*)-(4*S*,5*S*)-4-(4-carboxy-3-methyl-1,3-butadienyl)-4-hydroxy-3,3,5-trimethylcyclohexanone], which cannot form phaseic acid was studied in a cell suspension culture of bromegrass (*Bromus inermis*). The analogue induced freezing tolerance in bromegrass cells similar to (*S*)-(+)-ABA and was oxidized to (1′*S*,2′*S*)-8′-hydroxy-2′,3′-dihydroabscisic acid [4-(1*E*,3*Z*)-(3*R*,4*S*,5*S*)-4-(4-carboxy-3-methyl-1,3-butadienyl)-4-hydroxy-3-hydroxymethyl-3,5-dimethylcyclohexanone]. The enantiomer (−)-(1′*R*,2′R)−2′,3′-dihydroabscisic acid, which was inactive in inducing freezing tolerance, was converted to two metabolites, viz., the corresponding enantiomeric (1′*R*,2′*R*)-8′-hydroxy-2′,3′-dihydroabscisic acid [4-(1*E*,3*Z*)-(3*S*,4*R*,5*R*)-4-(4-carboxy-3-methyl-1,3-butadienyl)-4-hydroxy-3-hydroxymethyl-3,5-dimethylcyclohexanone] and reduction product (−)-1-(1*E*,3*Z*)-(1*R*,4*S*,6*R*)-1-(4-carboxy-3-methyl-1,3-butadienyl)-2,2,6-trimethylcyclohexan-1,4-diol. Both enantiomers of the analogue were transformed to the glucose esters. The structures of the metabolites were established by both spectroscopic analyses and chemical syntheses. The metabolite 8′-hydroxy-2′,3′-hydroxyabscisic acid is a cyclohexanone analogue of 8′-hydroxyabscisic acid, the transient oxidation product in the metabolism of ABA to phaseic acid. Like phaseic acid, the racemic form of 8′-hydroxy-2′,3′-dihydroabscisic acid was inactive in inducing freezing tolerance in bromegrass cells, suggesting that ABA and (+)-(1′*S*,2′*S*)-2′,3′-dihydroabscisic acid were the active compounds.

INTRODUCTION

The plant hormone (*S*)-(+)-abscisic acid (ABA, **1**) is implicated in the regulation of many developmental phases and stress responses including inhibition of germination and growth, reduction of transpiration by stomatal closure, and induction of freezing and chilling tolerance [1, 2]. To gain insight into the mechanism by which ABA functions as a growth regulator, our laboratories are developing arrays of ABA analogues to probe the structural requirements for the perception of the natural hormone [3] as well as investigating the involvement of ABA metabolites in different physiological processes [4]. We report here a study that combines both structure–activity and metabolism approaches to investigate the role played by ABA and its oxidation products in cold tolerance induction.

Addition of racemic ABA to bromegrass (*Bromus inermis* Leyss) suspension cell cultures at non-hardening temperatures induces freezing tolerance in the cells to −40° and below [5, 6]. This culture, which was used to study biochemical changes in cold hardening [7] and as a bioassay for investigating the structure–activity relationship of ABA analogues [3], is also a convenient system for metabolism studies as the metabolites are easily isolated from the culture medium.

Compound **1** is converted by plants and plant cell cultures to (−)-phaseic acid (PA, **4**), which is believed to be formed by the spontaneous cyclization of the transient oxidation product 8′-hydroxyabscisic acid (**3**, using ABA numbering system, Fig. 1) [8–12]. In bromegrass cell cultures **4** is subsequently reduced at the C-4′ position to dihydrophaseic acid (**5**), which accumulates in the medium [13]. Another metabolite, (+)-7′-hydroxyabscisic acid (**6**), has also been isolated from the medium of bromegrass cell cultures incubated with (+)-ABA [13]. The unnatural (*R*)-(−)-isomer of ABA (**2**) is transformed by bromegrass cell cultures to give (−)-7′-hydroxyabscisic acid (**7**) [12–16] as the only acidic metabolic product identified. ABA glucose ester was isolated as a neutral metabolite when (±)-ABA was fed to this culture [17, 18].

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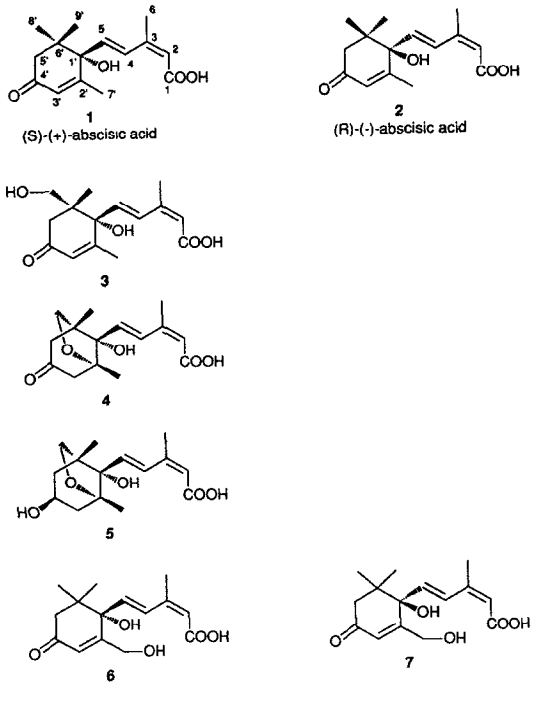


Fig. 1. Acidic metabolites of (+)- and (−)-abscisic acids (**1** and **2**) in the medium of bromegrass cell cultures.

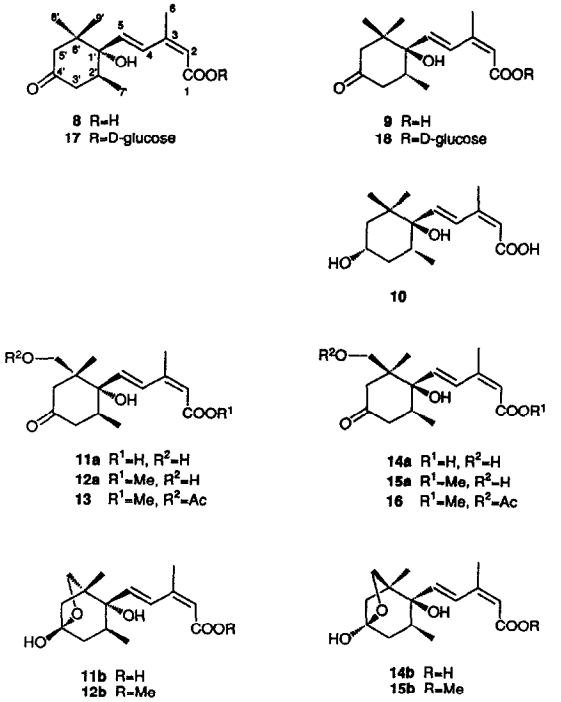


Fig. 2. Metabolites of (+)- and (−)-2′,3′-dihydroabscisic acids (**8** and **9**) in the medium of bromegrass cell cultures.

The role played by these metabolites in some of the ABA-regulated biological processes is not well understood. The synthetic racemic form of phaseic acid does not induce freezing tolerance in bromegrass cell culture [6]. Natural **4** has ABA-like activity in various bioassays including the regulation of barley germination [11, 19–21]. The putative intermediate between ABA and phaseic acid, **3**, has been isolated once [9] but has not been tested in an ABA bioassay because of its proclivity to cyclize. We therefore investigated the relative roles of ABA and its 8′-oxygenated metabolites in inducing freezing tolerance with an analogue which cannot be transformed to phaseic acid.

In the array of structural analogues of ABA screened using the bromegrass cell culture system, the known synthetic compound (±)-2′,3′-dihydroabscisic acid (Fig. 2, a 1:1 mixture of **8** and **9**) [22] had activity similar to ABA in inducing freezing tolerance [3, 6, 7]. Previous studies have shown that this analogue caused stomatal closure of *Commelina communis* [22] and abscission of cotton [23], and inhibited the germination of barley [20]. The methyl ester of (±)-2′,3′-dihydroabscisic acid inhibited the growth of rice seedlings [22].

Optically pure (+)-(1′*S*,2′*S*)-2′,3′-dihydroabscisic acid (**8**) and its (−)-(1′*R*,2′*R*)-enantiomer (**9**) have been synthesized recently [24]. The (+)-analogue **8**, which has the same absolute configuration as **1** at the C-1′ position, has activity similar to **1** in inducing freezing tolerance in bromegrass [6]; and in inhibiting the germination of cress seeds [4] and wheat embryos [25]. Compound **8** induces the expression of the ABA responsive genes *dhn* (*rab*) and wheat *lea*, but not the Em gene [25]. The analogue also cross-reacts with monoclonal antibodies raised against **1** [26]. The (−)-enantiomer **9**, which has the same configuration at C-1′ as (*R*)-(−)-ABA (**2**), was inactive in these studies [4, 6, 25, 26].

Both **8** and **9** possess a single carbon–carbon bond instead of a double bond between C-2′ and C-3′ in the ring of the ABA skeleton, and the C-7′ methyl group is *cis* to the side chain. The similarity in the biological activity observed for **8** and **1** indicates that the ring double bond at the 2′,3′ positions is not a stringent requirement for the action of the natural phytohormone in the studies mentioned above.

We have undertaken to investigate the metabolic fate of **8** and **9** in plants and plant cell cultures. Of particular interest is the possibility of forming 8′-hydroxy-2′,3′-dihydroabscisic acid (Fig. 2, **11a, 14a**) by the oxidation of **8** and **9** at the 8′-position in a fashion similar to the metabolism of **1**. These metabolites can be considered as analogues of the elusive ABA oxidation product **3**. Unlike **3**, **11a** and **14a** cannot cyclize to a phaseic acid-type structure due to the absence of the ring double bond. Investigation of the occurrence and nature of the metabolites of the analogues **8** and **9** may help to elucidate both the molecular requirements of the active sites involved in ABA metabolism, and the role played by **3** in some ABA regulated processes. This paper reports our study on the metabolism of (±)-, (+)- and (−)-2′,3′-dihydroabscisic acids in bromegrass suspension cell cultures and the activity of the oxygenated metabolite in inducing freezing tolerance.

RESULTS AND DISCUSSION

Bromegrass suspension cell cultures were treated with 0.37 or 0.75 mM (±)-2′,3′-dihydroabscisic acid, 0.37 mM **8**, or 0.37 mM (−)-(1′*R*,2′*R*)-2′,3′-dihydroabscisic acid (**9**) as described in the Experimental section of this paper. The greatest quantity of metabolites in the medium were formed after five–seven days, as determined by high performance liquid chromatography (HPLC) analysis on a Hisep^R column [27].

Metabolism of (±)-2′,3′-*dihydroabscisic acid*

Two days after the bromegrass cells were treated with (±)-2′,3′-dihydroabscisic acid, two new peaks, retention times 2.5 and 3.2 min, as well as the peak due to the unmetabolized 2′,3′-dihydroabscisic acid, retention time 3.5 min, were observed in the HPLC analysis of the medium. The change in the relative areas of the peaks during a seven day culture period is summarized in Table 1.

From the filtrate of a culture incubated for five days with 200 mg (±)-2′,3′-dihydroabscisic acid, 21 mg neutral substances and 39 mg acidic compounds were isolated after liquid-liquid extraction. Analysis by TLC (silica gel, toluene–EtOAc–HOAc 25:15:2) revealed that the acidic extract consisted of unmetabolized 2′,3′-dihydroabscisic acid (R_f *ca* 0.6) and two polar components (R_f *ca* 0.4 and 0.3). Separation by preparative TLC gave 4.4 mg dihydroabscisic acid, 12.3 mg of the metabolite with R_f 0.4, and 4.7 mg of the most polar compound.

The unmetabolized 2′,3′-dihydroabscisic acid was optically active {$[\alpha]_D = +30.7°$ (MeOH; *c* 0.44); lit. [24] **8**, $[\alpha]_D = +63.5°$ (MeOH; *c* 1.17)}. To examine the enantiomeric composition of the dihydroabscisic acid during the culture period, the unconsumed analogue was isolated from the medium three, five and seven days after inoculation. Treatment with diazomethane gave the corresponding methyl ester, which was analysed by HPLC using a Chiracel OD^R column [24, 28, 29]. The (+)- and (−)-enantiomers of the methyl ester are resolved on this chiral column. The results summarized in Table 2 showed that **9** was consumed from the culture medium faster than the (+)-enantiomer **8**. This is in contrast to our previous observation that when bromegrass cell cultures were treated with (±)-ABA, the natural (+)-isomer (**1**) was depleted from the medium more rapidly than its (−)-enantiomer (**2**) [30].

The metabolite with TLC R_f 0.4 corresponded to the component with HPLC retention time 3.2 min on the Hisep column (see above). It was a crystalline solid, mp 98–102°, with a molecular weight of 268. The ^{1}H NMR spectral data is in agreement with the molecular structure **10** (and/or its enantiomer), which was formed by reduction of the cyclohexanone group of 2′,3′-dihydroabscisic acid. The hydroxyl group at C-4′ was determined to be equatorial by decoupling studies, which showed that the C-4′ proton was coupled to the protons at C-3′ and C-5′ with axial–axial coupling constants of 11.4 Hz, and axial–equatorial coupling constants of 4.9 Hz. The metabolite isolated was optically active, with $[\alpha]_D - 63.2°$ (MeOH; *c* 0.62). Its absolute configuration and optical purity was established by comparison with the metabolite derived from **9** and with synthetic samples described later in this paper.

The more polar metabolite (R_f 0.3) corresponded to the component with HPLC retention time 2.5 min on the Hisep column. It was a crystalline solid, mp 190–203°. Although the sample appeared homogeneous by TLC and HPLC analyses, its ^{1}H NMR spectrum suggested that it was a 3:1 mixture of two compounds, both with the ABA-type side chain (Table 3), and 27 lines were observed in its ^{13}C NMR spectrum (Table 4). Treatment with diazomethane gave a solid, mp 138–148°, which was again homogeneous according to TLC analysis, and gave only one peak on a C-18 reversed phase HPLC column and a DB5^R gas chromatography (GC) column. The ^{1}H NMR data showed that it was a mixture of two methyl esters (Table 3), and their relative amount varied with the temperature at which the spectrum was recorded, while 32 lines were observed in the ^{13}C NMR spectrum (Table 4). The highest mass observed in the electron impact mass spectrum (GC-EIMS) of the mixture of esters was *m/z*

Table 1. HPLC retention times and relative peak areas of (±)-2′,3′-dihydroabscisic acid and metabolites in the medium of a bromegrass cell culture over a course of seven days

	Relative peak area (%)		
Incubation time (days)	8′-hydroxy-2′,3′-dihydroabscisic acid (**11**/**14**) (R_t 2.5)*	**10** (R_t 3.2)*	2′,3′-dihydroabscisic acid (**8**/**9**) (R_t 3.5)*
0	0	0	100
2	3	7	87
3	8	17	73
4	16	37	43
5	23	50	24
7	38	44	14

*Retention time (min).

The analysis was done on a Supelco LC-Hisep column (15 cm × 4.6 mm, 5 μm) with 1% aq. HOAc–MeCN (3:1) at 1.5 ml min^{-1} as eluent.

Table 2. Enantiomeric composition of (±)-2′,3′-dihydroabscisic acid (**8**/**9**) in the medium of a bromegrass cell culture during a period of seven days

Incubation time (days)	Relative peak area (%) (+)-**8**, methyl ester (R_t 8.5)*	(−)-**9**, methyl ester (R_t 10.2)*
0	50	50
3	53	47
5	71	29
7	75	25

*Retention time (min).

The acid was analysed as its methyl ester on a Chiracel OD HPLC column (25 cm × 4.6 mm) with i-PrOH–hexane (1:9) at 1.0 ml min^{-1} as eluent.

278. In the chemical ionization (CIMS) mode, peaks were observed at *m/z* 314, 297, 296 and 279 with ammonia as the ionizing gas; and at *m/z* 297, 279 and 249 with isobutane. The mixture of esters form a trimethylsilyl ether derivative which had CIMS (ammonia) peaks at *m/z* 386, 369, 368 and 351. The esters therefore most likely had molecular weight 296 and molecular formula $C_{16}H_{24}O_5$. Acetylation of the esters gave a single compound with the ^1H and ^{13}C NMR spectral data shown in Tables 3 and 4.

The above information strongly suggested that the metabolite isolated was 8′-hydroxy-2′,3′-dihydroabscisic acid (**11** and/or its enantiomer **14**). Compound **11**/**14** and its methyl ester (**12**/**15**) existed in both the open form **a** and the cyclized form **b** in NMR solvent, and Table 3 shows the assignment of the ^1H signals to these structures. On acetylation, the ester **12**/**15** was 'trapped' as the open form **a** to give acetate **13**/**16**.

The oxidation of 2′,3′-dihydroabscisic acid to **11**/**14** by the bromegrass cell culture is analogous to the metabolism of **1** to **4** described in the Introduction. A phaseic acid-type structure was not formed due to the absence of the ring double bond. Instead, the hydroxyl group at C-8′ in **11a**/**14a** cyclized to give the hemi-ketal **11b**/**14b**. The open and cyclized forms of both the metabolite **11**/**14** and its methyl ester **12**/**15** existed in equilibrium while in solution.

Compound **11**/**14** derived from (±)-2′,3′-dihydroabscisic acid was optically active, $[\alpha]_D$ −6.6° (MeOH; *c* 0.3). The corresponding acetate **13**/**16** had $[\alpha]_D$ +12° (MeOH; *c* 0.15) and gave two peaks (retention times 10.0 and 13.0 min) which were *ca* 3:1 in area on the Chiracel OD HPLC column. The absolute configuration of the major and minor antipodes of the 8′-hydroxy-2′,3′-dihydroabscisic acid were determined by comparison with the metabolites obtained from (+)- and (−)-2′,3′-

Table 3. ^1H NMR signals of the open and cyclized forms of 8′-hydroxy-2′,3′-dihydroabscisic acid (**11**/**14 a, b**), its methyl ester (**12**/**15 a, b**) and the acetate derivative (**13**/**16**)

Proton	Chemical shift in ppm (multiplicity, *J* in Hz) **(11/14a, b)** (CD_3OD)	**(12/15a, b)** ($CDCl_3$)	**(13/16)** ($CDCl_3$)
C-4	7.87 (*d*, 16.0)*	7.92 (*d*, 15.9)†	7.92 (*d*, 16.0)
	7.77 (*d*, 16.0)	7.79 (*d*, 15.9)	
C-5	6.51 (*d*, 16.0)	6.34 (*d*, 15.9)	6.36 (*d*, 16.0)
	6.27 (*d*, 16.0)	6.19 (*d*, 15.9)	
C-2	5.73 (*s*)	5.75 (*s*)	5.76 (*s*)
	5.71 (*s*)	5.72 (*s*)	
C-8′	3.62 (AB*q*, 8.8)	3.51, 3.88 (2*d*, 11.2)	4.14, 4.23 (2*d*, 11.6)
	4.14, 3.55 (2*d*, 8.1)	3.63, 4.18 (2*d*, 8.2)	
C-6	2.08 (*d*, 0.8)	2.04 (*s*)	2.03 (*d*, 1.2)
	2.04 (*d*, 0.8)	2.02 (*s*)	
C-9′	0.93 (*s*)	1.03 (*s*)	0.93 (*s*)
	0.95 (*s*)	0.96 (*s*)	
C-7′	0.90 (*d*, 6.6)	0.93 (*d*, 6.3)	0.89 (*d*, 6.4)
	0.85 (*d*, 6.9)	0.83 (*d*, 6.8)	
C-2′ax	2.1 (*m*)	2.6 (*m*)	2.6 (*m*)
C-5′ax	1.96 (*d*, 12.1)‡	*m*	*m*
C-3′eq	1.85 (*ddd*, 13.3, 5.8, 3.2)‡	*m*	*m*
C-5′eq	1.70 (*dd*, 12.0, 3.2)‡	*m*	*m*
C-3′ax	1.67 (*dd*, 13.3, 12.9)‡	*m*	*m*
MeO		3.69, 3.70 (2*s*)	3.70 (*s*)
MeCO			1.99 (*s*)

*The signals of the less abundant form of **11**/**14** are listed first. The two forms were in the ratio of 1:3. It has not been determined whether the open form **a** or the cyclized form **b** predominated.

†The signals of the open form **a** of methyl ester **12**/**15** are listed first. Assignment was based on the observation of variable temperature studies. The two forms **a** and **b** were in the ratio of 1:1 at 24°. The signals that increased in intensity with higher temperature were assigned to the open form **a**.

‡These signals were from the more abundant form of **11**/**14**. Multiplets were observed for the other form.

Table 4. ^{13}C NMR signals of the open and cyclized forms of 8'-hydroxy-2',3'-dihydroabscisic acid (**11/14a, b**), its methyl ester (**12/15a, b**) and the acetate derivative (**13/16**)

	Chemical shift (ppm)		
Carbon	**11/14a, b** (CD_3OD)	**12/15a, b** ($CDCl_3$)	**13/16** ($CDCl_3$)
4'	212.4 (**a**) 106.4 (**b**)	208.7 (**a**) 104.9 (**b**)	207.8
1	169.8	166.7	166.5/170.4
3	151.6	149.5, 149.8	149.2
2,4,5	118.5, 118.7, 129.9, 130.8, 136.9, 137.9	117.3, 117.8, 128.4, 129.8, 134.6, 136.5	118.0, 129.6, 134.5
1'	79.4	78.2, 79.5	78.0
8'	68.4, 75.0	69.0, 74.4	68.1
6'	49.5, 50.1	44.5, 49.0	51.2
2',3',5'	39.0, 39.5, 45.8, 46.0, 46.4, 47.8	38.0, 38.1, 44.8, 46.2, 46.7, 49.5	37.4, 44.3, 46.8
6,7',9'	15.5, 16.5, 17.8, 20.8, 21.4, 21.5	14.9, 16.1, 17.1, 20.4, 21.1, 21.2	16.0, 20.7, 21.3
MeO		51.1, 51.2	48.6
MeCO			16.0/20.7/21.3
MeCO			166.5/170.4

dihydroabscisic acids (**8** and **9**) and with synthetic samples as described below.

The neutral metabolite isolated from the bromegrass cell culture filtrate co-eluted with the forerun on the Hisep HPLC column, but gave a single peak on the C-18 reversed phase column. Data from the Plasmaspray LC-MS [17], direct probe CIMS, ^1H and ^{13}C NMR analyses indicated that it was most likely the glucose ester (**17** and/or **18**) of 2',3'-dihydroabscisic acid. The CIMS (NH_4^+) data were comparable to those of ABA glucose ester [31]. Further confirmation of the structure was achieved by hydrolysing the metabolite to its acid and sugar components. 2',3'-Dihydroabscisic acid was obtained and was determined to be a 1:1 mixture of the (+)- and (−)-enantiomers by recording the ^1H NMR spectrum of its sodium salt in the presence of γ-cyclodextrin as chiral complexing agent [24, 30, 32] and by HPLC analysis of its methyl ester on the Chiracel OD column. The sugar component gave a positive test on Chemstrip bG^R which is used for the determination of glucose level in blood [33], although other sugars such as galactose, mannose and deoxy-glucose gave positive results as well. Its TLC R_f value [34], retention time on an Aminex Carbohydrate HPLC column, and ^1H NMR spectrum agreed with those of glucose. A diastereomeric mixture of 2',3'-dihydroabscisic acid D-glucose ester (**17** and **18**) was synthesized as described later in this paper and was found to be identical to the neutral metabolite.

Metabolism of (+)-(1'S,2'S)-2',3'-*dihydroabscisic acid* (**8**)

From the filtrate of a bromegrass cell culture incubated for seven days with 24 mg **8**, 0.45 mg unmetabolized **8** and 0.30 mg 8'-hydroxy-2',3'-dihydroabscisic acid (**11**) were isolated. The recovered analogue **8** was shown to be 100% optically pure by HPLC analysis of its methyl ester on the Chiracel OD column. The quantity of the metabolite 8'-hydroxy-2',3'-dihydroabscisic acid obtained was insufficient for an accurate determination of its optical rotation. It was methylated and acetylated to **13**, which gave one peak on the Chiracel column with the same retention time as the predominant enantiomer derived from (±)-2',3'-dihydroabscisic acid (see above). The metabolite of **8** was therefore optically pure and is expected to have the absolute configuration shown in structure **11** (Fig. 2).

Metabolism of (−)-(1'R,2'R)-2',3'-*dihydroabscisic acid* (**9**)

Unmetabolized **9** (0.25 mg), reduction product **10** (0.87 mg) and 8'-hydroxy-2',3'-dihydroabscisic acid (0.37 mg) were obtained from the filtrate of a cell culture incubated for seven days with 24 mg analogue **9**. The optical purities of the three compounds isolated were determined by HPLC analyses (Chiracel OD column) of their methyl esters, or the ester-acetate in the case of 8'-hydroxy-2',3'-dihydroabscisic acid. The recovered **9** remained optically pure. The methyl esters of the reduction

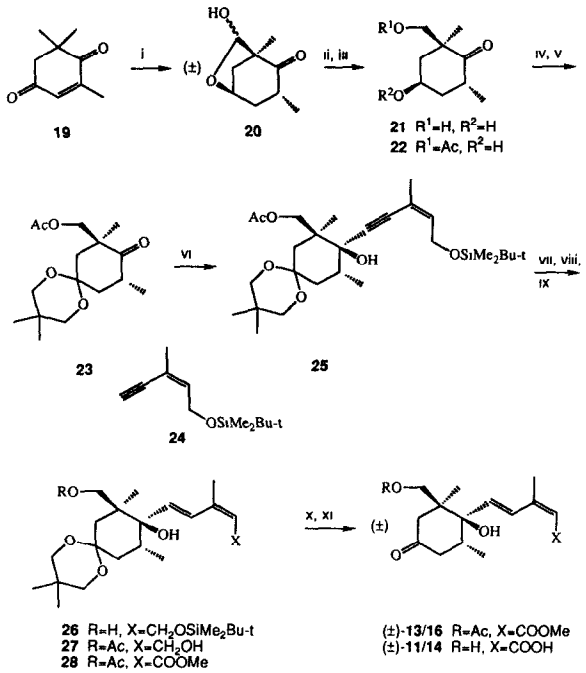
products **10** derived from both (±)- and (−)-2′,3′-dihydroabscisic acid gave a single peak on the chiral column, whereas the ester of a synthetic sample of the racemic form of **10** (see below) had two peaks under the same chromatographic conditions. The reduction products from both (±)- and (−)-2′,3′-dihydroabscisic acids were therefore optically pure and should have the absolute configuration **10**. The confirmation of the structure and absolute configuration of **10** by comparison with synthetic samples will be described in greater detail later in this paper.

The 8′-hydroxy-2′,3′-dihydroabscisic acid obtained from the oxidation of **9** is expected to have the absolute configuration **14**, which is the antipode of **11**. Its methyl ester-acetate **16** had the same HPLC retention time as the less abundant enantiomer obtained from (±)-2′,3′-dihydroabscisic acid described above.

Having identified the metabolites of (±)-, (+)- and (−)-2′,3′-dihydroabscisic acids by spectroscopic and chromatographic means, we synthesized these compounds to confirm their structures and to obtain sufficient quantities for bioassays.

Synthesis of (±)-*8′-hydroxy-2′,3′-dihydroabscisic acid*

The racemic form of the metabolite 8′-hydroxy-2′,3′-dihydroabscisic acid was synthesized by the sequence of reactions shown in Scheme 1. The preparation of the starting material **20** from oxoisophorone (**19**) has been reported previously [35]. The conversion of **20** to ketal **23** involved regioselective reduction and acetylation to **22**, followed by oxidation and regioselective ketalization. A side chain containing the desired number of carbon atoms was then introduced by treating **23** with the acetylide anion derived from the silyl ether **24** of (*Z*)-3-methylpent-2-en-4-yn-1-ol. Only one addition product **25** was obtained and was shown by NMR studies on an intermediate later in the synthesis to be the desired epimer formed by axial attack of the anion on the ketone group of **23**.



Scheme 1. Synthesis of racemic 8′-hydroxy-2′,3′-dihydroabscisic acid, (±)-**11/14**: (i) ref. [35]; (ii) LiAl(OBu-*t*)$_3$H, THF; (iii) Ac_2O, pyridine; (iv) PDC or PCC–Al_2O_3, CH_2Cl_2; (v) 2,2-dimethyl-1,3-propanediol, pyridinium tosylate, benzene; (vi) **24**, *n*-BuLi, THF; (vii) Redal^R, THF; (viii) Ac_2O, DMAP, pyridine; then *n*-Bu_4NF, THF; (ix) MnO_2, acetone; then MnO_2, NaCN, MeOH; (x) 1 M HCl, acetone; (xi) 2 M KOH, MeOH.

The acetylene group in **25** was reduced with Redal^R to give the 2-*cis*, 4-*trans*-diene arrangement in the side chain, with concomitant conversion of the acetate group to the alcohol. For ease of handling, alcohol **26** was re-acetylated before the silyl ether protecting group was removed. Compound **27** thus obtained, was used in ^{1}H NMR nuclear Overhauser enhancement (NOE) studies to establish the orientation of the side chain (Fig. 3). A NOE of *ca* 6% was observed at the C-5 vinyl proton signal (5.90 ppm) when the multiplets (1.2–1.4 ppm) due to the C-3′ and C-5′ axial protons and the C-3′ equatorial proton were irradiated. Conversely, irradiation of the C-5 vinyl proton gave rise to a NOE of *ca* 10% at 1.2–1.4 ppm, but had no effect on the signals due to the axial proton at C-2′ (multiplet, 2.18 ppm) and the methylene group at C-8′ (2 doublets, 4.33 and 4.40 ppm). Furthermore, irradiation of the C-2′ axial proton caused a NOE of *ca* 5% at the C-8′ methylene signal, and vice versa, but no change in the C-5 vinyl proton signal was observed in both cases. These spectral data strongly indicated that the side chain in **27** was in the axial position and was *cis* to the C-7′ methyl group.

The hydroxyl group in **27** was oxidized stepwise to an aldehyde and then to the methyl ester **28**. Deketalization of **28** gave a crystalline product with NMR spectral data identical to those of **13/16** derived from the methylation and acetylation of the metabolite 8′-hydroxy-2′,3′-dihydroabscisic acid. This synthetic sample of (±)-**13/16** was resolved into two peaks of the same area on the Chiracel OD HPLC column. However, large scale separation of the two enantiomers **13** and **16** by this method was laborious due to their low solubility in the eluent. (±)-8′-Hydroxy-2′,3′-dihydroabscisic acid (**11/14**) was obtained by hydrolysis of (±)-**13/16** and had NMR spectral data and HPLC (Hisep column) retention time identical to those of the metabolite isolated from the medium of bromegrass cell culture.

Synthesis of the (+)-*and* (±)-*forms of metabolite* **10**

Scheme 2 summarizes the synthetic route to **33**, the (+)-isomer of metabolite **10**. The starting material **29** was

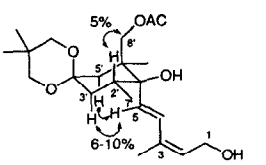
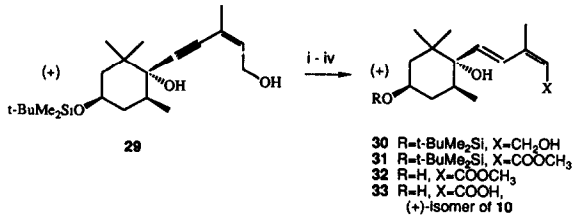


Fig. 3. ^{1}H NMR NOE of **27**.



Scheme 2. Synthesis of the optical isomer of metabolite **10**: (i) Redal[R], THF; (ii) MnO_2, acetone; then MnO_2, NaCN, MeOH; (iii) HOAc, H_2O; (iv) 2 M KOH, MeOH.

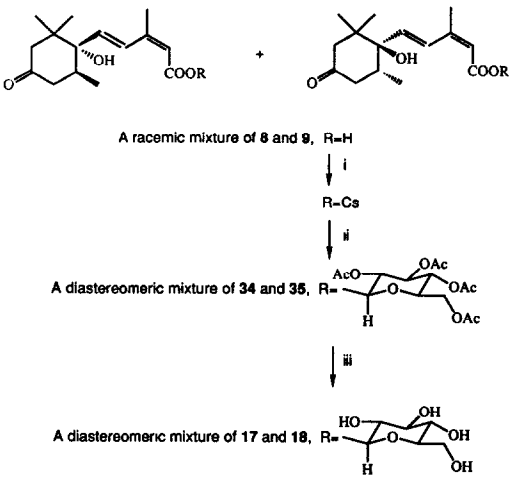
an intermediate in the synthesis of **8** [24]. The side chain in **29** was modified by the steps described above to give ester **31**. Removal of the *t*-butyldimethylsilyl ether protecting group followed by hydrolysis of the methyl ester gave crystalline product **33**, $[\alpha]_D + 58.1°$ (MeOH; *c* 0.78), which had spectral data identical to those of the metabolite **10**.

The racemic form of **10/33** was similarly synthesized from racemic starting material. The methyl ester of (±)-**10/33** gave two peaks on the Chiracel OD HPLC column (retention time 7.4 and 8.1 min). The synthetic (+)-enantiomer **33** co-eluted with the component of shorter retention time, while the methyl ester of the metabolite **10** gave a single peak at 8.1 min. We therefore conclude that the reduction product isolated from bromegrass cell culture treated with (±)- or (−)-(1′*R*,2′*R*)-2′,3′-dihydroabscisic acid was the optically pure (−)-isomer and had the absolute configuration **10**.

Synthesis of a diastereomeric mixture of the D-*glucose esters* (**17** *and* **18**) *of* (±)-2′,3′-*dihydroabscisic acid*

A procedure similar to the synthesis of the D-glucose ester of (±)-abscisic acid was followed [36]. As shown in Scheme 3, the caesium salt of (±)-dihydroabscisic acid was reacted with acetobromo-α-D-glucose (i.e. tetra-*O*-acetyl-α-D-glucopyranosyl bromide) to give a diastereomeric mixture of tetraacetates **34** and **35**. Removal of the acetate groups by treatment with a crude enzyme preparation from sunflower seeds gave a mixture of the D-glucose esters **17** and **18** of (±)-2′,3′-dihydroabscisic acid, which was identical to the neutral metabolite isolated from the medium of bromegrass cell culture fed (±)-2′,3′-dihydroabscisic acid.

In summary, both **8** and **9** fed to bromegrass cell cultures were oxidized at the 8′-position to (1′*S*,2′*S*)- and (1′*R*,2′*R*)-8′-hydroxy-2′,3′-dihydroabscisic acids **11** and **14**, respectively (Fig. 2). Compounds **11** and **14** exist as equilibrium mixtures of the open form **a** and the hemiketal **b** while in solution. Structures **11a** and **14a** are cyclohexanone analogues of **3**, the putative precursor of phaseic acid in ABA metabolism. When (±)-2′,3′-dihydroabscisic acid (a 1:1 mixture of **8** and **9**) was used in the experiment, **11** was the predominant enantiomer formed; and **9** was depleted from the medium more rapidly than its (+)-enantiomer **8**, probably due to competing reduction of **9** at the C-4′ position to metabolite **10**. The glucose esters of **8** and **9** were isolated as neutral metabolites from the culture filtrate.



Scheme 3. Synthesis of a diastereomeric mixture of the D-glucose esters (**17** and **18**) of (±)-2′,3′-dihydroabscisic acid: (i) Cs_2CO_3, MeOH, H_2O; (ii) acetobromo-α-D-glucose, DMF; (iii) enzyme preparation from sunflower seeds, pH 7.0 NaH_2PO_4 buffer

Figure 4 compares the sites of oxidation in **8** and **9** by bromegrass cell cultures observed in this study with those in **1** and **2** by the same system. Both analogues **8** and **9** were hydroxylated at the C-8′ position, whereas only **1** was oxidized at C-8′ to form **4**. The conversion of **2** to the corresponding phaseic acid (i.e. the enantiomer of **4**) has not been observed in bromegrass cell cultures, although it has been reported for other plant systems [11, 37, 38]. While both **1** and **2** are hydroxylated at C-7′ by bromegrass to give **6** and **7**, respectively, metabolites due to the oxidation of **8** and **9** at C-7′ were not detected in the culture medium in this study. The C-7′ oxidation products of **8** and **9** might have remained in the cells or have been transformed into unidentified polar material. Alternatively, the lack of oxidation at the C-7′ position of **8** and **9** could be due to structural changes caused by the absence of the ring double bond in these cyclohexanone analogues. Although the structures of ABA and 2′,3′-dihydroabscisic acid enantiomers are shown in Fig. 4 with the side chains in the axial position, they may not represent the conformations assumed by the compounds in the active site(s) of the enzyme(s) responsible for their metabolism in bromegrass cell cultures. It has been suggested that it is sterically feasible for the same enzyme to hydroxylate both the C-8′ of (+)-ABA and the C-7′ of (−)-ABA if the enantiomers assume a conformation with the side chain and the methyl group involved in the oxidation in the equatorial position [16, 39, 40]. However, this model does not fully account for the observation in this study and further investigation is required before it can be concluded whether the enantiomers of ABA and 2′,3′-dihydroabscisic acid are metabolized by the same enzyme(s) in bromegrass cell cultures.

The only C-4′ reduction observed in this study is the conversion of **9** to **10**. Unlike **4** which is reduced to **5** by

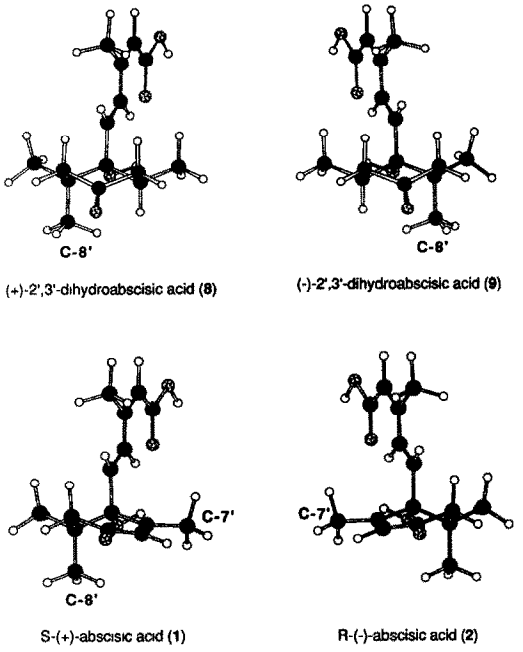


Fig. 4. Sites of oxidation in **1**, **2**, **8** and **9** fed to bromegrass cell cultures. The carbons which were oxidized are labelled in bold.

bromegrass cell cultures, the 8'-hydroxy-2',3'-dihydroabscisic acids (**11** and **14**) were not reduced at the C-4' position, possibly because the carbonyl group was protected as the hemi-ketal form **b**.

Our previous studies showed that (±)-2',3'-dihydroabscisic acid and the (+)-(1'*S*,2'*S*)-enantiomer **8** behaved similarly to (*S*)-(+)-ABA in inducing freezing tolerance in bromegrass cell culture and **9** was inactive [6]. The synthetic metabolite (±)-8'-hydroxy-2',3'-dihydroabscisic acid, like racemic phaseic acid, is ineffective in this system. We conclude that the observed biological activity of ABA and 2',3'-dihydroabscisic acid, as measured by their ability to induce freezing tolerance in bromegrass cell suspension cultures, is not due to the conversion to their 8'-hydroxylated metabolites. Studies on the effects of these compounds on bromegrass cells at the physiological and biochemical levels will be reported elsewhere.

EXPERIMENTAL

General. Melting points (mp) are uncorrected and were recorded on an Ernst Leitz Wetzlar hot stage melting point apparatus. Optical rotations were recorded at 25° in MeOH. GC sepns were carried out with a Varian 3700 instrument equipped with a DB-1701 or a DB-5 capillary column (J and W Scientific, 30 m) and a flame ionization detector. Helium at a flow rate of *ca* 2.5 cm min^{-1} was used as the carrier gas. HPLC analyses were carried out with a Gilson modular system equipped with a Supelco Hisep™ column (15 cm × 4.6 mm, 5 μm) preceded by a Supelco Hisep guard (2 cm × 4.6 mm) and eluted at 1.5 ml min^{-1} with 1% aq. HOAc–MeCN (3:1). A Supelco Supelcosil LC-18 column (3.3 cm × 4.6 mm, 5 μm) was used for some analyses and was eluted at 1.5 ml min^{-1} using a gradient prepared from 1% aq. HOAc (A) and MeCN (B), where the solvent composition was increased linearly from 10% B to 35% B over 6 min, then from 35% B to 100% B over the next 2 min. Chiral HPLC analyses were performed on an instrument consisting of a Spectra-Physics SP8700 solvent delivery system, a Spectroflow 773 variable wavelength absorbance detector (Kratos Analytical Instrument) and a Chiracel OD column (25 cm × 4.6 mm) preceded by a Whatman CSK1 HC Pellosil guard. i-PrOH–hexane (1:9) at 1.0 ml min^{-1} was used as the eluent. The detectors were set at 262 nm for all HPLC analyses. Spectra were recorded using $CHCl_3$ as solvent and matching NaCl cavity cells (0.2 mm). Proton nuclear magnetic resonance (^{1}H NMR, 360 MHz) spectra and carbon-13 (^{13}C NMR, 90.55 MHz) spectra were recorded with $CDCl_3$ as solvent and $CHCl_3$ as reference. The conventional abscisic acid numbering system is employed in the assignments of peaks. ^{1}H chemical shifts (δ) and coupling constants (J) are reported as if they are first order. The abbreviations ax and eq are used to indicate axial and equatorial protons, respectively. Low resolution mass spectra were obtained by using a DB-5 column (60 m) in a Finnigan-Mat 4000 E instrument with an Incos 2300 data system in the electron impact (EIMS) or chemical ionization (CIMS) mode. Mass spectral data are reported in mass to charge units (m/z) with the relative intensities as percentages of the base peak given in parentheses. High resolution mass spectra (HRMS) were recorded in the electron impact mode using a VG70-250SEQ instrument with a Digital PDP 11/73 data system. Unless otherwise stated, all spectroscopic analyses were performed on intermediates and products that were at least 95% pure by GC analysis. Elemental analyses (analyt.) were performed at the Department of Chemistry, the University of Saskatchewan.

Flash CC was performed using E. Merck silica gel 60 (230–400 mesh). E. Merck precoated glass plates of silica gel 60 F254 (0.25 or 1.0 mm) were used in prep. TLC. E. Merck silica gel 60 F254 plates (0.2 mm) with aluminium sheet backing were used in analytical TLC. UV active materials were detected under an UV lamp. The plates were then dipped into a soln of phosphomolybdic acid and slowly heated on a hot plate to visualize the spots. Prep. TLC was also performed on the Chromatotron (Harrison Research) with circular glass plates precoated with silica gel F254 (1, 2 or 4 mm), where the radial flow of eluent and sample were centrifugally accelerated.

The solvent tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone. *N*,*N*-Dimethyl formamide (DMF) was dried by distillation from calcium hydride and was stored over molecular sieves.

Bromegrass cell cultures. Cell suspension cultures of *B. inermis* were prepd as previously described [41]. Each flask contained 0.5 g bromegrass cells, 45 ml Erickson's medium and 5 ml soln of synthetic analogue in aq. NaOH neutralized to pH 5–7. The concns of synthetic analogues at the beginning of the culture period were: 0 mM (control), 0.37 or 0.75 mM (±)-2',3'-dihydroabscisic acid,

0.37 mM **8** and 0.37 mM **9**. Blanks containing cell free medium were prepared for each analogue as abiotic control for compound stability. The culture medium of each treatment was sampled daily under sterile conditions and the metabolite levels were determined by HPLC analysis on a Hisep column [27].

Isolation of metabolites from bromegrass cell cultures. Unmetabolized 2',3'-dihydroabscisic acid and the metabolites were isolated from the medium as previously described [13, 27]. In one experiment, approx. 2 l of medium was collected from a 5-day-old culture fed 200 mg (±)-2',3'-dihydroabscisic acid. Liquid-liquid extraction afforded 21 mg neutral substances and 39 mg acidic compounds. The acidic extract was sepd by prep. TLC (toluene–EtOAc–HOAc, 25:15:2) to give 2',3'-dihydroabscisic acid (4.4 mg, R_f 0.6), **10** (12.3 mg, R_f 0.4) and 8'-hydroxy-2',3'-dihydroabscisic acid **11/14** (4.7 mg, R_f 0.3).

The recovered 2',3'-dihydroabscisic acid had $[\alpha]_D = +30.7°$ (*c* 0.44).

Metabolite **10** had the following properties: mp 151–156°; $[\alpha]_D - 63.2°$ (*c* 0.62); IR ν_{max} cm^{-1}: 3600, 1680; ^1H NMR: δ7.71 (*d*, $J = 16.0$ Hz, 1H, H-4), 6.43 (*d*, $J = 16.0$ Hz, 1H, H-5), 5.70 (*s*, 1H, H-2), 4.02 (*dddd*, $J = 11.4$, 11.4, 4.9, 4.9 Hz, 1H, H-4'ax), 2.03 (*d*, $J = 0.9$ Hz, Me-6) and 2.04 (*m*, H-2'ax) (4H), 1.88 (*dddd*, $J = 12.2$, 4.9, 3.2, 3.2 Hz, 1H, H-3'eq), 1.71 (*ddd*, $J = 12.2$, 4.7, 2.3 Hz, 1H, H-5'eq), 1.48 (*dd*, $J = 12.2$, 11.5 Hz, 1H, H-5'ax), 1.24 (*m*, 1H, H-3'ax), 1.08 and 0.81 (2*s*, 3H each, Me-8',Me-9'), 0.79 (*d*, $J = 6.9$ Hz, 3H, Me-7'); HRMS: $[M]^+$ at *m/z* 268.1687 ($C_{15}H_{24}O_4$ requires 268.1675).

The ^1H and ^{13}C NMR spectral data of metabolite **11/14**, mp 190–203°, $[\alpha]_D - 6.6°$ (*c* 0.3), are listed in Tables 3 and 4; IR ν_{max}^{Kbr} cm^{-1}: 3500, 3255, 1705, 1682, 1628, 1598. Compound **11/14** was reacted with ethereal diazomethane to give methyl ester **12/15** mp 138–148°, which had the following spectral properties: ^1H and ^{13}C NMR (Tables 3 and 4); IR ν_{max}^{KBr} cm^{-1}: 3600, 3450, 1700; EIMS: *m/z* 278 $[M-18]^+$ (7), 248 (5), 219 (14), 191 (100); CIMS (NH_3): *m/z* 314 $[M+18]^+$ (100), 297 $[M+1]^+$ (4), 296 $[M]^+$ (8), 279$[M-18+1]^+$ (30); CIMS (isobutane): *m/z* 297$[M+1]^+$ (6), 279 $[M-18+1]^+$ (62), 249 (100); trimethylsilyl ether derivative CIMS (NH_3): *m/z* 386 $[M+18]^+$ (27), 369 $[M+1]^+$ (9), 368 $[M]^+$ (12), 351 $[M-18+1]^+$ (45). Analyt. found: C, 64.57; H, 8.43. $C_{16}H_{24}O_5$ requires: C, 64.83; H, 8.17%.

Methyl ester **12/15** was acetylated by treatment with Ac_2O, 4-dimethylaminopyridine (DMAP) and pyridine at room temp. (2 hr) to give acetate **13/16**, mp 182–185°; $[\alpha]_D + 12°$ (*c* 0.15); ^1H and ^{13}C NMR (Tables 3 and 4); IR ν_{max} cm^{-1}: 1735, 1700, 1600; EIMS: *m/z* 307 $[M-31]^+$ (2), 278 $[M-60]^+$ (10), 223 (72), 219 (14), 191 (100); CIMS (isobutane): *m/z* 339 $[M+1]^+$ (6), 321 $[M-18+1]^+$ (100).

The neutral metabolite was purified by prep. TLC (acetone) to give 2',3'-dihydroabscisic acid glucose ester (**17/18**) as a yellow oil (11 mg); ^1H NMR (acetone-d_6): δ7.96 (*d*, $J = 15.8$ Hz, 1H, H-4), 6.84 (*d*, $J = 15.8$ Hz, 1H, H-5), 5.73 (*s*, 1H, H-2), 5.54 and 5.52 (2*s* or *d*, $J = 7.8$ Hz, 1H, anomeric H), 2.15 (*d*, $J = 1.1$ Hz, 3H, Me-6), 1.02 (*s*), 0.93 (*s*), 0.94 (*s*), 0.90 (*d*, $J = 6.3$ Hz) and 0.89 (*d*, $J = 6.2$ Hz) [total 9H, Me-7',Me-8',Me-9']; ^{13}C NMR (acetone-d_6): δ213.3 (C-4'), 165.6 (C-1), 154.3, 138.3, 129.4, 116.0 (C-3, C-4, C-5, C-6), 94.4 (-O-CH-O- of glucose), 78.8 (C-1'), 77.5, 76.5, 72.8, 70.0 and 61.3 (CH_2OH and 4 CHOH of glucose), 52.8, 46.9, 42.2 and 37.7 (C-2', C-3', C-5', C-6'), 25.3, 22.9, 21.2 and 16.1 (C-6, C-7', C-8', C-9'); LC-MS (Plasmaspray, C-18 reversed phase column) [17]: *m/z* 411 $[M-17]^+$ (10), 309 (10), 291 (30), 249 $[C_{15}H_{22}O_4-17]$ (100); CIMS (direct probe, 160°, NH_3): *m/z* 446 $[M+18]^+$ (16), 284 $[C_{15}H_{22}O_4+18]^+$ (71), 266 $[C_{15}H_{22}O_4]^+$ (53), 249 $[C_{15}H_{22}O_4-17]^+$ (17), 124 (100).

Hydrolysis of 2',3'-dihydroabscisic acid glucose ester (**17/18**). A mixt. of the neutral metabolite **17/18** (4.4 mg), MeOH (2 ml) and 1 M HCl (3 drops) was stirred at room temp. under Ar for 15 hr and then heated to 60° for 6 hr. After cooling to room temp., the solvent was removed under aspirator pressure. The solid residue obtained was partitioned between H_2O and EtOAc–$CHCl_3$ (1:1). The aq. and organic layers were sepd and concd. 2',3'-Dihydroabscisic acid (3.0 mg) was obtained from the organic extract and was shown to be a racemic mixt. by ^1H NMR (D_2O, NaOD, γ-cyclodextrin) [24, 30, 32]. Treatment with CH_2N_2–ether gave methyl 2',3'-dihydroabscisate (3.7 mg), which was again racemic according to HPLC analysis on the Chiracel OD column [24, 28, 29]. A mixt. of methyl ether derivatives of sugars was obtained from the aq. extract.

To obtain the sugar residue, neutral metabolite **17/18** (1.5 mg) was hydrolysed with 1 M HCl (2 drops) at 60° for 5 hr. After cooling to room temp. and extraction with EtOAc, the aq. soln was concd. to give a yellow oil (1.1 mg) with ^1H NMR (D_2O), TLC R_f (85:15 MeCN–H_2O as eluent, aniline–diphenylamine–phosphoric acid–acetone for development [34]), and HPLC retention time (Bio-Rad Aminex Carbohydrate HPX-87C column, 300 × 7.8 mm, H_2O at 1 ml min^{-1} as eluent, refractive index detector) were very similar to those of D-glucose. An aq. soln of the yellow oil gave a positive test on Chemstrip^R bG (Boehringer Mannheim), which is used for the determination of blood glucose level.

(±)-(2R*,4S*,6S*)-2,6-*Dimethyl*-4-*hydroxy*-2-*hydroxymethylcyclohexan*-1-*one* (**21**). A soln of **20** [35] (0.51 g, 3.0 mmol) in dry THF (20 ml) was cooled to −78° under Ar. Lithium tri-*tert*-butoxyaluminohydride (1.34 g, 5.2 mmol) was added with stirring. After 30 min the reaction mixt. was hydrolysed with H_2O, acidified to pH 3 with 1 M HCl, and extracted with MeOH–$CHCl_3$ (5:95). The organic extract was dried over anhydrous Na_2SO_4 and concd under aspirator pressure to give a yellow oil (0.41 g) as crude product. Purification by flash CC (MeOH–$CHCl_3$, 5:95) gave diol **21** as an oil (0.22 g, 44% yield) which solidified on standing at room temp. Recrystallization from ether–hexane gave crystals, mp 91–93°; IR ν_{max} cm^{-1} (neat): 3450, 1700; ^1H NMR: δ4.18 (*m*, 1H, CHOH), 3.60 and 3.54 (AB*q* or 2*d*, $J = 10.6$ Hz, 2H, $-CH_2OH$), 2.97 (*m*, 1H, CHMe), 2.08–2.16 (*m*, 2H, H-3eq, H-5eq), 1.87 (*dd*, $J = 14.4$, 3.9 Hz, 1H, H-3ax), 1.70 (*ddd*, $J = 13.8$, 13.4, 2.9 Hz, 1H, H-5ax), 1.04 (*d*, $J = 6.6$ Hz,

CHMe) and 1.01 (*s*, MeC) (6H); ^{13}C NMR: δ216.2 (C=O), 70.9 and 64.6 (CHOH and CH_2OH), 49.8, 44.5, 43.7, 41.6, 22.2 and 14.8 (C, CH, $2CH_2$, 2Me); EIMS: *m/z* 172 $[M]^+$ (1), 154 $[M-18]^+$ (10), 99 (100); CIMS (NH_3): *m/z* 190 $[M+18]^+$ (100), 173 $[M+1]^+$ (60); HRMS: $[M]^+$ at *m/z* 172.1069 ($C_9H_{16}O_3$ requires 172.1039). Analyt. found: C, 62.87; H, 9.56. $C_9H_{16}O_3$ requires: C, 62.75; H, 9.37.

(±)-(2R*,4S*,6S*)-2,6-*Dimethyl*-4-*hydroxy*-2-*acetoxymethylcyclohexan*-1-*one* (**22**). A solution of diol **21** (200 mg, 1.16 mmol) and Ac_2O (290 mg, 2.84 mmol) in pyridine (10 ml) was stirred at room temp. for 2 hr. It was then diluted with H_2O and extracted with $CHCl_3$. The organic layer was dried and concd to give an oily crude product (260 mg). Purification by prep. TLC (Chromatotron, ether–hexane 9:1) gave a diacetate (R_f 0.6, 31 mg), the desired monoacetate **22** (R_f 0.4, 159 mg, 80% yield), and unreacted diol **21** (R_f 0.2, 29 mg). Compound **22** was an oil with the following spectral properties: IR ν_{max} cm^{-1}: 3620, 3500, 1720; ^{1}H NMR: δ4.72 and 4.13 (2*d*, *J*=11.2 Hz, 1H each, CH_2OAc), 4.18 (apparent *quintet*, *J*=3.3 Hz, CHOH), 3.17 (*m*, 1H, CHMe), 2.10 and 1.70 (2*m*, 2H each, $2CH_2$), 2.00 (*s*, 3H, MeCOO), 1.07 (*s*, MeC) and 1.02 (*d*, *J*=6.5 Hz, CHMe) (6H); ^{13}C NMR: δ214.1 (C=O), 171.0 (COO), 70.4 and 65.4 (CHOH and CH_2OAc), 48.9, 43.9, 42.8, 36.5, 21.2, 20.8 and 14.6 (C, CH, $2CH_2$, 3Me); EIMS: *m/z* 214 $[M]^+$ (2), 154 $[M-60]^+$ (10), 99 (100); CIMS (NH_3): *m/z* 232 $[M+18]^+$ (100), 214 $[M+1]^+$ (35); HRMS: $[M]^+$ at *m/z* 214.1210 ($C_{11}H_{18}O_4$ requires 214.1215). Analyt. found: C, 61.68; H, 8.34. $C_{11}H_{18}O_4$ requires: C, 61.65; H, 8.47.

(±)-(8R*,10S*)-8-*Acetoxymethyl*-3,3,8,10-*tetramethyl*-1,5-*dioxaspiro*[5,5] *undecan*-9-*one* (**23**). A mixt. of monoacetate **22** (150 mg, 0.70 mmol), pyridinium chlorochromate–Al_2O_3 [42] (1 mmol g^{-1}, 2.42 g, 2.42 mmol) and CH_2Cl_2 (8 ml) was stirred at room temp. for 5 hr. Filtration through a short column of Florisil and concn of the filtrate gave a diketone as an oil (135 mg) which was ketalized without purification.

To a soln of the crude oxidation product in benzene (10 ml) were added 2,2-dimethyl-1,3-propanediol (231 mg, 2.22 mmol) and pyridinium *p*-tosylate (11 mg, 0.04 mmol). The mixt. was heated at reflux under a Dean–Stark water separator and Ar atm. for 18 hr, cooled to room temp. and washed with H_2O. The aq. layer was extracted with ether. The combined benzene and ether layers were dried and concd to give an oil which was purified by prep. TLC (Chromatotron, ether–hexane 9:1) to give monoketal **23** (oil, 177 mg, 80% overall yield), IR ν_{max} cm^{-1}: 1720; ^{1}H NMR: δ4.57 and 3.99 (2*d*, *J*=11.1 Hz, 1H each, CH_2OAc), 3.50 (*m*, 4H, $2CH_2O$-), 2.89 (*m*, 1H, CHMe), 2.50 (*m*, 2H, H-7eq/11), 1.98 (*s*, 3H, MeCOO), 1.68 (*d*, *J*=14.4 Hz, H-7ax) and 1.50 (*m*, H-7eq/11) (3H), 1.08 (*s*), 1.03 (*d*, *J*=6.5 Hz), 0.97 (*s*) and 0.96 (*s*) (12H, 4Me); ^{13}C NMR: δ213.2 (C=O), 170.7 (COO), 96.3 (O-C-O), 70.6, 70.3 and 69.4 ($2CH_2O$-, CH_2OAc), 47.5, 41.9, 40.7, 37.5, 30.1, 22.5, 22.4, 21.2, 20.7 and 14.4 (2C, 1CH, $2CH_2$, 5Me); EIMS (30 eV): *m/z* 298 $[M]^+$ (0.2), 239 $[M-59]^+$ (20), 227 (51), 155 (100); CIMS (NH_3): *m/z* 316 $[M+18]^+$ (100), 299 $[M+1]^+$ (30); HRMS: $[M]^+$ at *m/z* 298.1727 ($C_{16}H_{26}O_5$ requires 298.1674). Analyt. found: C, 64.33; H, 8.83. $C_{16}H_{26}O_5$ requires: C, 64.39; H, 8.79.

(Z)-3-*Methylpent*-2-*en*-4-*yn*-1-*ol*, t-*butyldimethylsilyl ether* (**24**). A soln of (*Z*)-3-methylpent-2-en-4-yn-1-ol (5.85 g, 61 mmol) in $CHCl_3$ (40 ml) was cooled to 0°. Imidazole (5.00 g, 73 mmol) was added with stirring, followed by *t*-butyldimethylsilyl chloride (11.50 g, 76 mmol). The reaction mixt. was stirred at room temp. under a drying tube for 1.5 hr. A white ppt. was formed and was removed by suction filtration. The filtrate was concd to give a mixt. of yellow oil and solid (30 g). Purification by flash CC (hexane) followed by distillation (Kugel-Rohr, 70°, 10 Torr) gave silyl ether **24** as a liquid (7.0 g, 55% yield), ^{1}H NMR: δ5.83 (*t*, *J*=6.3 Hz, 1H, =CH), 4.35 (*dd*, *J*=6.3, 0.9 Hz, 2H, H_2COSi), 3.12 (*s*, 1H, acetylenic H), 1.85 (*d*, *J*=1.2 Hz, 3H, vinyl Me), 0.89 (*s*, 9H, 3Me), 0.06 (*s*, 6H, 2MeSi); CIMS (NH_3): *m/z* 228 $[M+18]^+$ (100), 211 $[M+1]^+$ (34).

(±)-(9Z)-(8R*,9S*,10S*)-8-*Acetoxymethyl*-9-(5-t-*butyldimethylsilyloxy*-3-*methylpent*-3-*en*-1-*ynyl*)-3,3,8,10-*tetramethyl*-1,5-*dioxaspiro* [5, 5] *undecan*-9-*ol* (**25**). A soln of silyl ether **24** (153 mg, 0.72 mmol) in dry THF was cooled to −60° under Ar. *n*-Butyllithium (1.6 M in hexane, 0.45 ml, 0.72 mmol) was added dropwise with stirring. The mixt. was then maintained at −60° for 20 min, allowed to warm up to −30° over 10 min, and again cooled to −60° before a soln of monoketal **23** (99 mg, 0.33 mmol) was added. After stirring at −60° for 1 hr, the reaction was quenched with H_2O and extracted with ether. The organic extract was dried and concd to give a pale yellow oil (272 mg), which after prep. TLC (Chromatotron, ether–hexane 3:1) afforded a mixt. (228 mg) shown by GC (DB5 column) to contain unreacted silyl ether **24**, monoketal **23** and the desired product **25**. Silyl ether **24** was removed by fractional distillation (Kugel-Rohr, 70–100°, 10 Torr) and the unreacted monoketal **23** was recovered (36 mg, 150–200°, 10 Torr). The desired product **25** was left as the residue (yellow oil, 113 mg, 100% yield) and had the following spectral properties: IR ν_{max} cm^{-1}: 3400, 1740, 1725; ^{1}H NMR: δ5.77 (*t*, *J*=6.1 Hz, 1H, =CH), 4.40 and 4.23 (2*d*, *J*=10.9 Hz, CH_2OAc) and 4.35 (*d*, *J*=6.1 Hz, CH_2OSi) (4H), 3.30–3.60 (*m*, 4H, $2CH_2O$-), 2.44 (*dd*, *J*=14.4, 2.8 Hz, 1H, H-5'eq, ABA numbering), 2.17 (*m*, CHMe), 2.05 (*s*, MeCOO), 1.85 (*s*, 3H, vinyl Me), 2.10 and 1.54 (2*m*, CH_2), 1.38 (*d*, *J*=14.4 Hz, H-5'ax, ABA numbering), 1.17 (*s*, Me), 1.06 (*d*, *J*=6.4 Hz, CHMe), 0.98 (*s*, Me), 0.88 (*s*, 3Me), 0.84 (*s*, Me), 0.05 (*s*, 6H, 2MeSi); ^{13}C NMR: δ170.8 (COO), 137.6 and 117.7 (C=C), 96.7 (O–C–O), 92.9 and 86.2 (2 acetylenic C); EIMS (30 eV): *m/z* 451 $[M-C_4H_9]^+$ (2), 393 $[M-C_6H_{15}Si]^+$ (1), 359 (45), 299 (16), 155 (100); CIMS (NH_3): *m/z* 527 $[M+19]^+$ (100), 509 $[M+1]^+$ (5), 491 $[M-17]^+$ (10). Analyt. found: C, 65.90; H, 9.75. $C_{28}H_{48}O_6Si$ requires: C, 66.10; H, 9.52.

(±)-9-(1E,3Z)-(8R*,9S*,10S*)-8-*Hydroxymethyl*-9-(5-t-*butyldimethylsilyloxy*-3-*methyl*-1,3-*pentadienyl*)-3,3,8,10-*tetramethyl*-1,5-*dioxaspiro*[5,5]*undecan*-9-*ol* (**26**). A soln of **25** (108 mg, 0.21 mmol) in dry THF (8 ml) was cooled

to 0° under Ar. Redal[R] (3.4 M in toluene, 0.2 ml, 0.68 mmol) was added with stirring. After 30 min at 0°, the reaction mixt. was added to satd NH_4Cl. The aq. layer was sepd from the organic layer, acidified to pH 3 with 1 M HCl, and extracted with ether. The combined organic layer and ether extract was washed with H_2O, satd NaCl and dried over Na_2SO_4. Evapn of solvent gave a pale yellow oil (93 mg) which was purified on the Chromatotron (ether–hexane 3:1) to give **26** as an oil (62 mg, 66% yield); IR ν_{max} cm^{-1}: 3400; ^1H NMR: δ (ABA numbering): 6.62 (*d*, $J = 15.5$ Hz, 1H, H-4), 5.80 (*d*, $J = 15.5$ Hz, 1H, H-5), 5.45 (*t*, $J = 6.5$ Hz, 1H, H-2), 4.35 (*d*, $J = 6.5$ Hz, 2H, H_2C-1), 3.99 and 3.58 (2*d*, $J = 11.8$ Hz, H_2C-8′), 3.40 (*m*, $2CH_2O$-), 1.82 (*d*, $J = 0.8$ Hz, 3H, Me-6), 0.98 (*s*, Me), 0.91 (*s*, Me), 0.88 (*s*, 3Me), 0.83 (*s*, Me), 0.79 (*d*, $J = 6.6$ Hz, Me-7′), 0.06 (*s*, 6H, 2MeSi); ^{13}C NMR: δ132.5, 129.4, 129.0 and 127.2 (4 olefinic C), 97.2 (O–C–O). Analyt. found: C, 66.67; H, 10.11. $C_{28}H_{50}O_6Si$ requires: C, 66.62; H, 10.33.

(±)-9-(1E,3Z)-(8R*,9S*,10S*)-8-*Acetoxymethyl*-9-(5-*hydroxy*-3-*methyl*-1,3-*pentadienyl*)-3,3,8,10-*tetramethyl*-1,5-*dioxaspiro*[5,5]*undecan*-9-*ol* (**27**). Compound **26** (85 mg, 0.18 mmol) was acetylated (Ac_2O, DMAP, pyridine, room temp. in the usual fashion. The crude acetate obtained was dissolved in dry THF (5 ml) and treated with tetra-*n*-butylammonium fluoride (1 M in THF, 0.35 ml, 0.35 mmol) under Ar at 0° for 1 hr, then at room temp. for 3 hr. The reaction was worked up by quenching with satd NaCl and extracting with ether. The organic extract was dried and concd to give a yellow oil (90 mg), which was purified on the Chromatotron (ether) to give the desired product **27** as a solid (50 mg, 70% overall yield). Recrystallization from ether–hexane gave crystals, mp 61–63°; IR ν_{max} cm^{-1}: 3620, 3460, 1740; ^1H NMR: δ (ABA numbering): 6.71 (*d*, $J = 15.5$ Hz, 1H, H-5), 5.90 (*d*, $J = 15.5$ Hz, 1H, H-4), 5.56 (*t*, $J = 7.1$ Hz, 1H, H-2), 4.40 and 4.33 (2*d*, $J = 11.1$ Hz, H_2C-8′) overlaps with 4.33 (*d*, $J = 7.1$ Hz, H_2C-1) (4H), 3.45 (*m*, 4H, $2CH_2O$-), 2.43 (*dd*, $J = 14.8$, 3.0 Hz, 1H, H-5′eq), 2.18 (*m*, H-2′ax), 2.06 (*s*, MeCOO), 1.86 (*s*, 3H, Me-6), 1.33 (*d*, $J = 14.9$ Hz, H-5′ax) overlaps with 1.29–1.41 (*m*, H-3′eq, ax) (3H), 0.97, 0.88, 0.84 (3*s*, 3Me) and 0.78 (*d*, $J = 6.6$ Hz, Me-7′) (12H); ^{13}C NMR: δ170.9 (COO), 134.5, 129.6, 128.2 and 127.5 (4 olefinic C), 96.8 (O–C–O), 79.4, 70.5, 70.0, 68.6, 58.4, 41.7, 40.0, 37.7, 33.9, 30.0, 22.5, 22.4, 21.3, 21.1, 20.9, 15.3; CIMS (NH_3): *m/z* 414 $[M+18]^+$ (100), 397 $[M+1]^+$ (22). Analyt. found: C, 66.70; H, 9.26. $C_{22}H_{36}O_6$ requires: C, 66.62; H, 9.16.

(±)-9-(1E,3Z)-(8R*,9S*,10S*)-8-*Acetoxymethyl*-9-(4-*carbomethoxy*-3-*methyl*-1,3-*butadienyl*)-3,3,8,10-*tetramethyl*-1,5-*dioxaspiro*[5,5]*undecan*-9-*ol* (**28**). A mixt. of **27** (23 mg, 0.06 mmol), MnO_2 (154 mg, 1.77 mmol) and acetone (5 ml) was stirred at room temp. for 30 min. The solvent was evapd under aspirator pressure. Methanol (5 ml) was added to the residue, followed by MnO_2 (164 mg, 1.88 mmol), NaCN (22 mg, 0.44 mmol) and HOAc (35 mg, 0.58 mmol). The reaction mixt. was stirred at room temp. for 4 hr and then filtered through Celite. The filtrate was concd to give a residue which was partitioned between H_2O and ether. The ether layer was dried over Na_2SO_4 and concd to give a yellow oil (31 mg) as the crude product. Purification by prep. TLC (Chromatotron, ether–hexane 9:1) gave methyl ester **28** as crystals (13 mg, 50% overall yield). Recrystallization from ether–hexane gave needles, mp 164–166°; IR ν_{max} cm^{-1}: 1720; ^1H NMR: δ (ABA numbering): 7.80 (*d*, $J = 6.0$ Hz, 1H, H-4), 6.28 (*d*, $J = 6.0$ Hz, 1H, H-5), 5.69 (*s*, 1H, H-2), 4.42 and 4.30 (2*d*, $J = 11.2$ Hz, 2H, H_2C-8′), 3.68 (*s*, 3H, MeO), 3.45 (*m*, 4H, $2CH_2O$), 2.46 (*dd*, $J = 14.8$, 3.0 Hz, 1H, H-5′eq), 2.06 (*s*, MeCOO) and 2.00 (*s*, Me-6) (6H), 0.98, 0.88, 0.85 (3*s*, 3Me) and 0.80 (*d*, $J = 6.6$ Hz, Me-7′) (12H); ^{13}C NMR: δ170.8 and 166.6 (2COO), 149.9, 135.9, 128.7 and 117.1 (4 olefinic C), 96.8 (O–C–O), 79.4, 70.5, 70.0, 68.5, 51.0, 41.7, 40.0, 37.9, 34.0, 30.1, 22.5, 22.4, 21.3, 21.2, 15.3; EIMS: *m/z* 424 $[M]^+$ (10), 393 $[M-31]^+$ (2), 364 $[M-60]^+$ (10), 309 (95), 229 (60), 155 (100); CIMS (NH_3): *m/z* 442 $[M+18]^+$ (100), 407 $[M-17]^+$ (28); HRMS $[M]^+$ at *m/z* 424.2524 ($C_{23}H_{36}O_7$ requires 424.2587). Analyt. found: C, 65.27; H, 8.48. $C_{23}H_{36}O_7$ requires: C, 65.06; H, 8.55.

In some runs of this preparation, varying amounts of a more polar crystalline product (mp 147–149°) were also obtained. Spectral analyses showed that it was the alcohol formed by the hydrolysis of the acetate group during the oxidation reaction.

(±)-4-(1E,3Z)-(3R*,4S*,5S*)-4-(4-*Carbomethoxy*-3-*methyl*-1,3-*butadienyl*)-4-*hydroxy*-3-*hydroxymethyl*-3,5-*dimethyl*-*cyclohexan*-1-*one* [(±)-**13/16**, or 8′-*acetoxy*-2′,3′-*dihydroabscisic acid, methyl ester*]. A mixt. of **28** (26 mg, 0.06 mmol), 1 M HCl (3 drops) and acetone (5 ml) was stirred at room temp. under Ar for 24 hr, neutralized with satd $NaHCO_3$, and concd under aspirator pressure. The residue was partitioned between ether and H_2O. The ether layer was dried and concd to give crystals (28 mg), which were purified by prep. TLC (Chromatotron, ether) to give (±)-**13/16** (16 mg, 80% yield) with NMR data identical to those observed for the derivative prepared from the metabolite 8′-hydroxy-2′,3′-dihydroabscisic acid (**11/14**). An analytical sample of (±)-**13/16** was obtained by recrystallization from $CHCl_3$–hexane, mp 184–186°. Analyt. found: C, 63.68; H, 7.87. $C_{18}H_{26}O_6$ requires: C, 63.87; H, 7.75%.

(±)-4-(1E,3Z)-(3R*,4S*,5S*)-4-(4-*Carboxy*-3-*methyl*-1,3-*butadienyl*)-4-*hydroxy*-3-*hydroxymethyl*-3,5-*dimethyl*-*cyclohexan*-1-*one* [(±)-**11/14**, or 8′-*hydroxy*-2′,3′-*dihydroabscisic acid*]. A soln of (±)-**13/16** (19 mg, 0.06 mmol) in 2 M KOH (5 ml) and MeOH (5 ml) was stirred under Ar at room temp. for 20 hr. Part of the solvent was removed under aspirator pressure. The remaining soln was acidified to pH 3 with 1 M HCl and extracted with EtOAc. The organic extract was washed with satd NaCl and dried over Na_2SO_4. Evapn of solvent gave crystals (17 mg) which were purified by prep. TLC (MeOH–$CHCl_3$ 1:9) to give (±)-**11/14** (12 mg, 80% yield). The synthetic (±)-**11/14** had NMR data identical to those of the metabolite 8′-hydroxy-2′,3′-dihydro-abscisic acid isolated from the medium of bromegrass cell culture.

(+)-1-(1E,3Z)-(1S,4R,6S)-4-t-*Butyldimethylsilyloxy*-1-(5-*hydroxy*-3-*methyl*-1,3-*pentadienyl*)-2,2,6-*trimethyl*-*cyclo*-*hexan*-1-*ol* (**30**). Known compound **29** [24] (460 mg,

1.26 mmol) was reduced with Redal (3.4 M in toluene, 4.0 ml, 13.6 mmol) by the procedure described above (see preparation of **26**). The crude product was chromatographed on a flash column (ether–hexane 9:1) to give the desired diene **30** as an oil (250 mg, 54% yield); $[\alpha]_D = +42.3°$ (*c* 1.20); IR ν_{max} cm^{-1}: 3600; ^1H NMR: δ (ABA numbering): 6.63 (*d*, *J* = 15.7 Hz, 1H, H-4), 6.02 (*d*, *J* = 15.7 Hz, 1H, H-5), 5.53 (*t*, *J* = 7.1 Hz, 1H, H-2), 4.30 (*d*, *J* = 7.1 Hz, 2H, H_2C-1), 3.92 (*dddd*, *J* = 11.0, 11.0, 5.1, 5.1 Hz, 1H, H-4'ax), 1.96 (*m*, 1H, H-2'ax), 1.86 (*d*, *J* = 1.0 Hz, 3H, Me-6), 1.72 (*m*, 1H, H-3'eq), 1.56 (*ddd*, *J* = 13.3, 5.1, 2.2 Hz, 1H, H-5'eq), 1.49 (*dd*, *J* = 13.3, 10.6 Hz, 1H, H-5'ax), 1.24 (*ddd*, *J* = 13.2, 13.2, 11.3 Hz, 1H, H-3'ax), 1.04 (*s*, 3H, Me), 0.88 (*s*, 9H, 3Me), 0.77 (*s*, Me) and 0.75 (*d*, *J* = 6.7 Hz, Me-7') (6H), 0.05 (*s*, 6H, 2MeSi).

(+)-(1E,3Z)-(1S,4R,6S)-1-(4-*Carbomethoxy*-3-*methyl*-1,3-*butadienyl*-2,2,6-*trimethylcyclohexan*-1,4-diol (**32**). Compound **30** (250 mg, 0.68 mmol) was oxidized with MnO_2 (2.0 g, 22.9 mmol) in acetone (30 ml), then with MnO_2 (2.0 g, 22.9 mmol), NaCN (103 mg, 2.1 mmol) and HOAc (120 mg, 2.0 mmol) in MeOH (20 ml) according to the procedure described above for the preparation of **28**. The crude methyl ester **31** obtained was used in the following desilation reaction without purification.

A solution of crude methyl ester **31** in HOAc–H_2O (3:1) was heated to 70° under Ar for 1.5 hr. It was then allowed to cool to room temp., diluted with H_2O, and extracted with ether. The ether extract was washed with satd $NaHCO_3$, satd NaCl and dried. Evapn of solvent gave a yellow oil which was purified on the Chromatotron (ether–hexane 3:1) to give the desired product **32** as a solid (107 mg, 56% overall yield), mp 105–107°; $[\alpha]_D$ +69.1° (*c* = 0.78); IR ν_{max} cm^{-1}: 3605, 1705; ^1H NMR: δ (ABA numbering): 7.75 (*d*, *J* = 16.1 Hz, 1H, H-4), 6.39 (*d*, *J* = 16.1 Hz, 1H, H-5), 5.69 (*s*, 1H, H-2), 4.01 (*dddd*, *J* = 11.4, 11.4, 5.0, 5.0 Hz, 1H, H-4'ax), 3.69 (*s*, 3H, MeO), 2.05 (*m*, H-2'ax) and 2.01 (*d*, *J* = 1.2 Hz, Me-6) (4H), 1.87 (*m*, H-3'eq), 1.71 (*ddd*, *J* = 13.1, 4.8, 2.4 Hz, 1H, H-5'eq), 1.47 (*dd*, *J* = 13.1, 11.4 Hz, H-5'ax), 1.24 (*m*, 1H, H-3'ax), 1.08 (*s*, 3H, Me-8'/9'), 0.81 (*s*, Me-8'/9') and 0.80 (*d*, *J* = 6.7 Hz, Me-7') (6H); EIMS: *m/z* 282 $[M]^+$ (2), 208 (7), 196 (16), 123 (100); HRMS: $[M]^+$ at *m/z* 282.1804 ($C_{16}H_{26}O_4$ requires 282.1831).

The racemic form of **32** was synthesized by the same sequence of reactions. The spectral properties, GC and HPLC (LC-18 reversed phase column) retention times, and TLC R_f of **32** and (±)-**32** were identical to those of the methyl ester obtained by reacting metabolite **10** with CH_2N_2–ether. HPLC (Chiracel OD column) retention times: **32**, 7.4 min; (±)-**32**, 7.4 and 8.1 min; methyl ester of metabolite **10**, 8.1 min.

(+)-1-(1E,3Z)-(1S,4R,6S)-1-(4-*Carboxy*-3-*methyl*-1,3-*butadienyl*-2,2,6-*trimethylcyclohexan*-1,4-*diol* (**33**). A mixt. of ester (+)-**32** (71 mg, 0.25 mmol), 2 M KOH (4 ml) and MeOH (4 ml) was stirred at room temp. for 6 hr. Most of the solvent was evapd, the residue was diluted with H_2O, extracted with ether, and the ether layer was discarded. The aq. layer (pH 12) was acidified to pH 2 with 1 M HCl and extracted with EtOAc. The organic extract was dried and concd to give **33** as a solid (70 mg, 100% yield). Recrystallization from $CHCl_3$ gave crystals, mp 98–103°, $[\alpha]_D = +58.1°$ (*c* 0.78). The racemic form of **33**, mp 50–58°, was similarly prepared. Both **33** and its racemic form were identical to metabolite **10** by NMR, HPLC (Hisep column) and TLC analyses.

2',3'-*Dihydroabscisic acid*, D-*glucose ester tetraacetates* (**34** *and* **35**). To a soln of (±)-2',3'-dihydroabscisic acid, (±)-**8/9** (48 mg, 0.18 mmol) in MeOH–H_2O (2:1, 7.5 ml) was added Cs_2CO_3 (33 mg, 0.10 mmol) [36]. After stirring at room temp. for 30 min the solvent was evapd at 40° under aspirator pressure. Benzene was added to the residue and evapd to azeotrope off H_2O. The residue was dried at room temp. under vacuum (10 Torr) for 3 hr to give the caesium salt as a waxy solid.

To a soln of the caesium salt in dry DMF (5 ml), acetobromo-α-D-glucose (78 mg, 0.19 mmol) was added and the mixt. was stirred at room temp. under Ar for 15 hr. The reaction was then worked up by diluting with H_2O and extracting with EtOAc. The organic layer was washed with H_2O, satd NaCl, and dried over Na_2SO_4. Evapn of solvent gave a yellow oil as the crude product (150 mg). Sepn by prep. TLC (Chromatotron, ether–hexane 9:1) gave a mixt. of tetraacetates **34** and **35** as an oily solid (60 mg, 60% yield), mp 62–72°. Further elution with EtOAc–hexane (3:1) gave unreacted (±)-**8/9** (5 mg). The mixt. of **34** and **35** has the following spectral properties: IR ν_{max} cm^{-1}: 1750; ^1H NMR: δ (ABA numbering): 7.84 and 7.83 (2*d*, *J* = 15.9 Hz, 1H, H-4), 6.54 (*d*, *J* = 16 Hz, 1H, H-5), 5.78, 5.76 and 5.74 (2H, H-2 and anomeric H); CIMS (NH_3): *m/z* 614 $[M+18]^+$ (100), 597 $[M+1]^+$ (0.6).

2',3'-*Dihydroabscisic acid*, D-*glucose ester* (**17/18**). The procedure in ref. [36] with some modification was followed. A mixt. of dehusked sunflower seeds (3 g) and pH 7 NaH_2PO_4 buffer (25 ml) was homogenized with a blender. The homogenate was centrifuged at 15000 rpm for 25 min, resulting in the formation of 3 layers. The middle layer (*ca* 23 ml) was removed by pipette. A mixt. of tetraacetates **34** and **35** (60 mg, 0.1 mmol) was dissolved in EtOH (3 ml), pH 7 NaH_2PO_4 buffer (12 ml) was added, followed by the sunflower seed enzyme soln obtained above. The mixt. was stirred at room temp. for 20 hr, EtOH (20 ml) was then added to stop the reaction, and the mixt. was centrifuged at 15000 rpm for 25 min. The supernatant was concd under aspirator pressure to give a sludge, which was extracted with MeOH–$CHCl_3$ (1:3). Evapn of solvent from the extract gave an oily solid (28 mg). Purification by prep. TLC (MeOH–$CHCl_3$ 1:3) gave an oil (10 mg, 22% yield) with spectral and chromatographic properties identical to those of the neutral metabolite **17/18** isolated from bromegrass cell culture.

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