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SYNTHESIS OF (+)-, (-)- and (±)-7'-HYDROXYABSCISIC ACID

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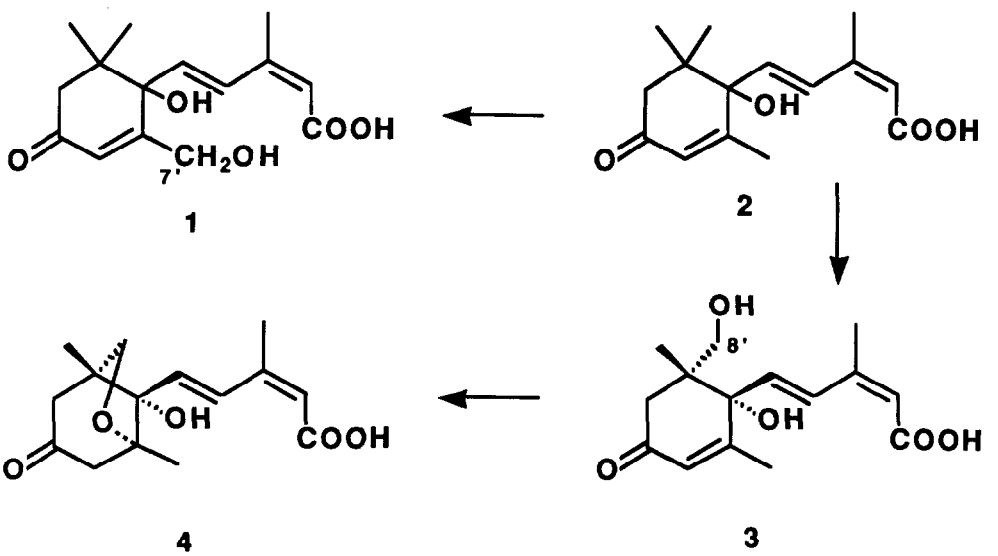
Abstract: A total synthesis of 7'-hydroxyabscisic acid (1), a putative metabolite of the plant hormone abscisic acid (ABA), is described. The key intermediate in the twelve step synthesis is 2-t-butyldimethylsiloxymethyl-4,4-ethylenedioxy-6,6-dimethylcyclohex-2-en-1-one (5) which contains the desired masked hydroxyl group at the C-7' position of ABA. The final transformation in the synthesis, the hydrolysis of the methyl ester of 1 is readily accomplished with porcine liver esterase. Optically pure (+)- and (-)-forms of 1 are obtained by resolution of the silyl ether methyl ester by HPLC on a chiral column.

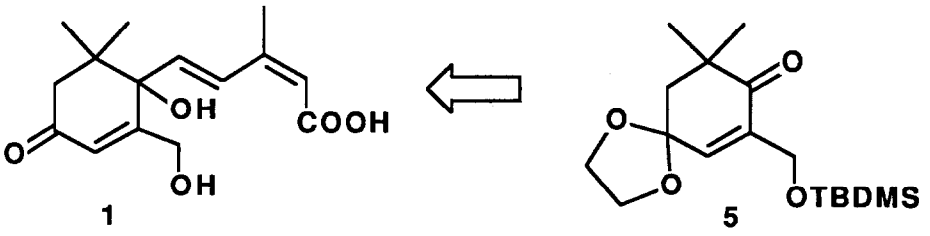
7'-Hydroxyabscisic acid (**1**) is a major product of the biotransformation of (±)-abscisic acid (**2**) fed to plants[1-3] and plant cells[4, 5] and there is mounting evidence[6, 7] that **1** is a natural metabolite of the plant hormone (S)-(+)-abscisic acid [(+)-**2**]. There is currently widespread interest[8] in the role that acidic metabolites arising from oxidation of ABA play in plant growth and development, particularly in regulation of seed germination, embryo development and response to environmental stress. A well established major pathway of ABA metabolism in plants involves enzymic oxidation of (+)-ABA to 8'-hydroxyabscisic acid (**3**) which is cyclized *in vivo* to phaseic acid[8] (**4**). Phaseic acid (**4**) induces similar biological responses as ABA in a number of bioassays [8, 9]. This metabolite has been the subject of synthetic efforts in our laboratory[10] and others[11, 12].



It has been suggested[13] that 7'-hydroxyabscisic acid (**1**) is an artifact arising from racemic ABA employed in feeding experiments, and that (R)-(-)-ABA is transformed to **1** by the enzyme system that converts ABA to phaseic acid. In these metabolic studies the chirality of the isolated 7'-hydroxyabscisic acid was not unambiguously established, and the source of the metabolite not determined. Recently however, 7'-hydroxyabscisic acid has been found as an endogenous constituent in leaves of *Vicia faba*,[6] and has also been observed as a transient biotransformation product of (+)-**2** in a plant cell culture system[7]. No reports of the synthesis or biological activity of **1** have yet appeared.

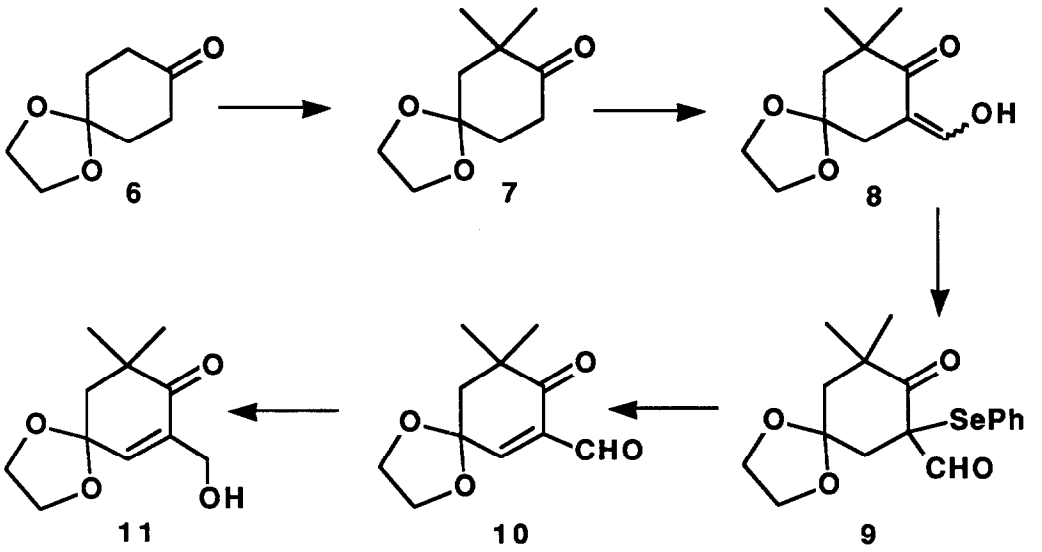
As part of our continuing studies on the chemistry and biological activity of abscisic acid and its metabolites and analogs, we undertook to synthesize racemic 7'-hydroxyabscisic acid (±)-**1**. Our goal was to prepare both (+)-**1** and (-)-**1** to permit the assignment of the absolute stereochemistry of products of (+)- and (-)-ABA metabolism in plant cells. A second important objective was to provide authentic material for evaluation of the biological activity of the putative natural product. This paper describes the total synthesis of (±)-7'-hydroxyabscisic acid and the preparation of optically pure (+)- and (-)-forms.

The strategy for the synthesis of racemic **1** employed the silyl ether **5** as the key intermediate.



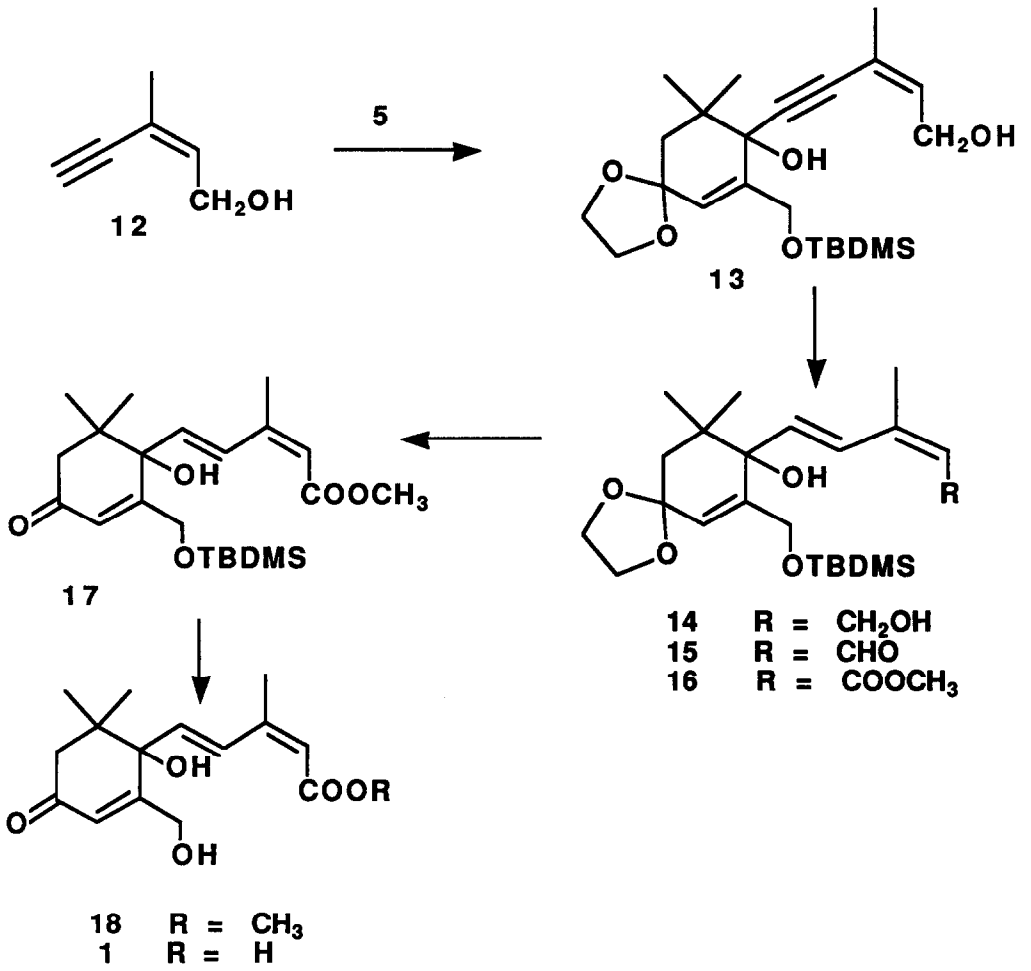
Conversion of **5** to **1** was expected to be achieved by methods previously used in the synthesis of ABA[14] and ABA analogs[15]. It was hoped that the enantiomers of methyl 7'-hydroxyabscisate or an intermediate in the synthesis could be resolved by an HPLC system that has been successfully used to separate the optical antipodes of methyl abscisate[16, 17].

The key intermediate **5** was prepared by sequential modification of commercially available 1,4-cyclohexanedione monoketal (**6**).



Methylation[18] with iodomethane using sodamide as base in tetrahydrofuran afforded a 1:2 mixture of the monomethylated and dimethylated compounds from which the desired dimethylketone **7** was obtained in 38 % overall yield. Claisen condensation[19] of **7** with ethyl formate and sodium hydride in tetrahydrofuran afforded **8** as a 7:3 mixture of the cisoid and transoid forms. The center of unsaturation in the six-membered ring was introduced in two steps by first treating **8** with sodium hydride followed by quenching the resulting enolate with phenyl selenyl bromide[20] to give **9** in 60% yield from **7**. Oxidation of **9** with 15% hydrogen peroxide resulted in the formation of the α,β-unsaturated ketoaldehyde **10** in 81% yield. Selective reduction of the aldehyde functionality of **10** to the allylic alcohol **11** was best accomplished with triisobutylaluminum[21] in toluene. Other reagents and conditions gave poor results. For example, sodium borohydride in methanol and bis (2-methoxyethoxyaluminum hydride in tetrahydrofuran gave a mixture of complex products; diisobutylaluminum hydride in both benzene (0 - 5 °C) and toluene (-78 °C) gave very low yields of desired alcohol. 9-BBN in tetrahydrofuran at room temperature gave no reaction. The protection of the hydroxyl group of **11** was achieved via treatment with tert-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole[22] to give **5** in 51% yield from **10**.

Having accomplished the synthesis of **5**, the stage was set for appending the side chain of **1**.



Alkylation of **5** with the dianion of cis 3-methyl-2-penten-4-yn-1-ol[23] **12** (obtained by addition of two equivalents of n-butyllithium) gave **13** in 70% yield. Reduction with bis(2-methyoxyethoxy)aluminum hydride[24] in tetrahydrofuran afforded **14** in 51% yield. The dienol **14** was converted to the aldehyde **15** by treatment with manganese dioxide[25] in dichloromethane, then, a Corey oxidation[26] of **15** gave the ester **16.** The ketal was removed by treatment with p-toluenesulfonic acid monohydrate in acetone giving **17** in 44% overall yield

from **14**. Methyl 7'-hydroxyabscisate (**18**) was obtained by desilylation with tetrabutylammonium fluoride[22] in 80% yield. Attempts to saponify the ester under standard conditions with base proved unsuccessful and resulted in degradation of the molecule. The hydrolysis was readily accomplished with porcine liver esterase[27] affording racemic 7'-hydroxyabscisic acid (**1**) in 91% yield.

Both methyl 7'-hydroxyabscisate (**18**) and the silyl ether **17** could be resolved by HPLC employing a chiral column, with **17** affording sharper peaks on chromatography. The TBDMS ether methyl ester **17** was resolved on a preparative scale, and the individual optically active compounds were converted to (+)-**1** and (-)-**1** by the procedure employed for the racemic mixture. In a feeding study to be reported elsewhere, in which (±)-ABA [(±)-**2**] was supplied to cultured bromegrass cells, the 7'-hydroxyabscisic acid, analyzed as the methyl ester, co-eluted with (-)-**18**. We conclude that 7'-hydroxyabscisic acid derived from natural ABA (+)-**2** is the (+)-form.

Experimental

General

Melting points were determined with a microscope hot stage apparatus and are uncorrected. Gas chromatographic (GC) separations were performed with a Varian 3700 instrument equipped with a 30 m x 0.32 mm (i.d.) DB-1701 capillary column (J & W Scientific Durabond) and a flame ionization detector. Helium at a flow rate of ca. 2.5 ml min^{-1} was used as carrier gas. Flash chromatography was performed using E. Merck silica gel 60 (230-400 mesh). Preparative TLC was performed on a Chromatotron (Harrison Research) with circular glass plates precoated with silica gel PF 254 (1, 2, or 4 mm) where the radial flow of eluant and sample were centrifugally accelerated. Infrared spectra (IR) were recorded with a Perkin-Elmer 257 Grating Infrared Spectrophotometer, and ultraviolet spectra (UV) with a Beckman DU-8 instrument. Proton nuclear magnetic resonance spectra (^{1}HNMR) were obtained at 360 MHz with a Bruker AM-360-WB spectrometer, using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts are reported in ppm downfield from tetramethylsilane, and coupling constants are given in Hz. Low resolution mass spectra were obtained with a Finnigan 4500 GC-MS instrument equipped with a 60-meter DB-5 capillary column and operated in either the electron impact (EIMS) or chemical ionization (CIMS) mode. High resolution electron impact (HREIMS), fast ion bombardment (FIBMS), and high resolution fast ion bombardment (HRFIBMS) mass spectra were recorded with a VG 70-250SEQ double-focussing hybrid spectrometer. For the FIB experiments, a beam of 35-keV cesium atoms was focussed on the sample in a glycerol matrix.

Tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone. Benzene was distilled from lithium aluminum hydride, and dimethylformamide was distilled from calcium hydride and stored over molecular sieves. Elemental analyses were performed by the Microanalytical Laboratory of the University of Alberta, Edmonton, Canada.

4,4-Ethylenedioxy-2,2-dimethylcyclohexanone (7)

To sodamide (10.0 g, 0.256 mol) in dry THF (50 mL), under argon atmosphere, was added monoketal **6** (10 g, 0.064 mol) in THF (50 mL). After 30 min, CH_3I (9.6 mL, 0.154 mol) was added in a dropwise fashion, maintaining the reaction temperature < 20 ^{0}C. The solution was stirred for 1 h and the reaction mixture was diluted with saturated aqueous NH_4Cl. The solution was extracted with Et_2O (3 x 100 mL). The combined organic layers were washed with saturated aqueous NaCl, dried over Na_2SO_4 and concentrated. Flash chromatography of the crude product on silica gel, eluting with 20% Et_2O in hexane gave the dimethylated compound **7** (4.5 g, 38% yield). IR (film) 1705 cm^{-1}; ^{1}HNMR δ 3.97-3.93 (m, 4H, -OCH_2CH_2O-), 2.51-2.55 (m, 2H, H-6), 1.93-1.98 (m, 2H, H-5), 1.84-1.85 (m, 2H, H-3), and 1.13 (s, 6H, $C(CH_3)_2$); EIMS m/z 184 (M^+, 1.4), 127 (14), 113 (20), 100 (29), and 85 (72.5).

Anal. Calcd. for $C_{10}H_{16}O_3$: C, 65.13; H, 8.82%. Found: C, 64.89; H, 8.70%.

4,4-Ethylenedioxy-2-hydroxymethylene-6,6-dimethylcyclohexanone (8)

To NaH (4.8 g, 114 mmol, 57% dispersion in oil) washed with hexane (3 x 20 mL) in a dry three-necked round-bottomed flask adapted with a thermometer and mechanical stirrer under an argon atmosphere, was added a solution of the monoketal **7** (8.4 g, 45.6 mmol) in THF (150 mL). Ethyl formate (14.7 mL, 182 mmol) was added and the reaction solution cooled in an ice bath, before ethanol (1-2 mL) was added. After the reaction had started, the ice bath was removed. After 3 h, ~20 mL of ethanol then water were added to the reaction mixture with external cooling. The THF layer was separated and the mixture was extracted with Et_2O (3 x 100 mL). The aqueous layer was acidified and extracted with Et_2O (3 x 100 mL). The combined organic phases were washed with saturated aqueous NaCl, dried over Na_2SO_4 and evaporated to afford 7.3 g of crude product which was generally used in the next reaction without further purification. In one experiment, the residue was purified by flash chromatography on silica gel eluting with 2% CH_3OH in CH_2Cl_2 to give **8**. IR (film) 3650-3100, 1680, and 1560 cm^{-1}; ^{1}HNMR δ 14.7 (bs, 7/10H, cisoid OH), 8.50 (bs, 7/10H, cisoid vinylic proton), 3.94 (s, 4H, -OCH_2CH_2O-), 2.52 (s, 7/10x2H, cisoid H-3), 1.74 (s, 2H, H-5), and 1.23, 1.19 (2 s, 7/10x6H, cisoid $(CH_3)_2$) and the transoid form showed the following 14.6 (s, 3/10H, OH) , 8.15 (bs, 3/10H, vinylic proton), 2.45 (s, 3/10x2H, H-3), and 1.19 (s, 3/10x6H,$(CH_3)_2$); EIMS m/z 212 (M^+, 3.0), 127 (49), 113 (16), 99 (16), and 87 (100).

4,4-Ethylenedioxy-2-formyl-2-phenylseleno-6,6-dimethylcyclohex-2-en-1-one (9)

To a mechanically stirred suspension of NaH (2.17 g, 51.6 mmol, 57% dispersion in mineral oil; washed free of oil with hexane (3 x 25 mL)), in THF (30 mL) under an argon atmosphere at 0 ^{0}C was added, in a dropwise fashion, the crude enol **8** (7.3 g, 34.4 mmol) in THF (40 mL). The ice bath was removed and the thickening

reaction solution was stirred for 30 min. A solution of benzeneselenyl bromide (8.9 g, 37.8 mmol) in THF (50 mL) was added and the solution was stirred for 30 min. The reaction mixture was slowly added to a stirring solution of ice, Et_2O (250 mL) and saturated aqueous $NaHCO_3$ (75 mL). The separated aqueous layer was washed two more times with Et_2O. The combined organic layers were washed with saturated aqueous NaCl solution and dried over Na_2SO_4. Concentration of the organic layer and recrystallization of residue from ether:hexane gave the selenide **9** (10.0 g, 60% yield over 2 steps). mp 140-141 °C; IR ($CHCl_3$), 1705, and 1690 cm^{-1}; ^{1}HNMR δ 9.39 (d, J = 0.6 Hz, 1H, CHO), 7.48-7.29 (m, 5H, Ar-H), 3.86-3.79 (m, 4H, -OCH_2CH_2O-), 2.35 (dd, J = 14.5, 2.7 Hz, 1H, H-3eq), 2.19 (d, J = 14.2 Hz, 1H, H-5ax), 2.13 (d, J = 14.5 Hz, 1H, H-3ax), 1.86 (dd, J = 14.2, 2.7 Hz, 1H, H-5eq), 1.35 (s, 3H, CH_3 ax), and 1.19 (s, 3H, CH_3 eq); CIMS (NH_3) m/z 369 ([M+1]$^+$, 48)

Anal. Calcd. for $C_{17}H_{20}SeO_4$: C, 55.43; H, 5.48%. Found: C, 55.55; H, 5.54%.

4,4-Ethylenedioxy-2-formal-6,6-dimethylcyclohex-2-en-1-one (10)

The seleno compound **9** (10.0 g, 27.3 mmol) in CH_2Cl_2 (80 mL) was oxidized by dropwise addition of 15% hydrogen peroxide (11.7 mL, 51.9 mmol). The mixture was stirred at room temperature for 45 min, at which time it had turned milky. The organic layer was washed twice with water and each aqueous portion was extracted with two portions of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by flash column chromatography with 3% CH_3OH in CH_2Cl_2 to give the unsaturated ketoaldehyde **10** (4.6g, 81% yield). IR ($CHCl_3$) 1709, 1685, and 1625 cm^{-1}; ^{1}HNMR δ 10.07 (s, 1H, CHO), 7.12 (t, J = 1 Hz, 1H, H-3), 4.07-4.03 (m, 4H, -OCH_2CH_2O-), 2.11 (d, J = 1 Hz, 2H, 2xH-5), and 1.23 (s, 6H, 2xCH_3); CIMS (NH_3) m/z 211 ([M+1]$^+$, 96).

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.83; H, 6.72%. Found C, 62.75; H, 6.63%.

4,4-Ethylenedioxy-2-hydromethyl-6,6-dimethylcyclohex-2-en-1-one (11)

The keto-aldehyde **10** (4.84 g, 23 mmol) in toluene (50 mL) was stirred under an argon atmosphere at -78 °C and triisobutylaluminum (27.6 mL, 1.0 M in toluene, 27.6 mmol) was slowly added. After stirring for 5 min, CH_3OH (5 mL) was added. The mixture was stirred for a further 5 min before evaporation of most of the solvent and the residue taken up in saturated aqueous NH_4Cl. The solution was then extracted with $CHCl_3$ (2 x 100 mL). The combined organic phases were dried over Na_2SO_4, and concentrated to give 3.76 g of crude product which was used in the next reaction without further purification. In one experiment the product was purified by flash chromatography eluting with 5% methanol in CH_2Cl_2 . IR (film) 3460 (br) and 1670 cm^{-1}; ^{1}HNMR δ 6.47 (m, 1H, H-3), 4.24 (m, 2H, -CH_2OH), 4.03-3.97 (m, 4H, -OCH_2CH_2O)-), 2.06 (d, J = 1.0 Hz, 2H, 2xH-5), and 1.18 (s, 6H, 2xCH_3); EIMS m/z 212 (M$^+$, 11), 194 (2), and 156 (100).

Anal. Calcd. for $C_{11}H_{16}O_4$: C, 62.23; H, 7.60%. Found: C, 62.35 ; H, 7.59%.

2-t-Butyldimethylsiloxymethyl-4,4-ethylenedioxy-6,6-dimethylcyclohex-2-en-1-one (5)

To the crude alcohol **11** (3.76 g, 17.7 mmol) in dimethylformamide (30 mL) at room temperature, was added imidazole (3.0 g, 44.3 mmol) and t-butyldimethylsilyl chloride (3.2 g, 21.2 mmol). After stirring for 1 h, the mixture was diluted with water and washed with hexane (3 x 100 mL). The combined hexane layers were dried over Na_2SO_4 and concentrated to give a residue which upon flash column chromatography with 20% Et_2O in hexane gave the silyl ether **5** (3.81 g, 51% yield over 2 steps). IR (neat) 1675 cm^{-1}; ^{1}HNMR δ 6.55 (m, 1H, H-3), 4.30 (d, J = 2 Hz, 2H, 2xH-7), 4.02-3.99 (m, 4H, -OCH_2CH_2O-), 2.07 (m, 2H, 2xH-5), 1.18 (s, 6H, -$C(CH_3)_2$, 0.89 (s, 9H, $C(CH_3)_3$), and 0.60 (s, 6H,-$Si(CH_3)_2$; EIMS m/z 326 (M^+, 1), 269 (82) and 197 (64).
HREIMS m/z calcd. for $(M-15)^+$ $C_{16}H_{27}O_4Si$: 311.1679. Found: 311.1667.

5-(2-t-butyldimethylsiloxymethyl-4,4-ethylenedioxy-1-hydroxy-6,6-dimethylcyclohex-2-enyl)-3-methylpent-2-en-4-yn-1-ol (13)

To a solution of **12** (1.4 g, 14.5 mmol) in THF (150 mL) under an argon atmosphere in a dry ice/acetone bath was added slowly n-BuLi (18.3 mL, 29.0 mmol). The solution was allowed to warm to 0 ^{0}C (20 min.), then cooled to -78 ^{0}C before addition of a solution of **5** (3.96 g, 12.1 mmol) in THF (50 mL). The reaction was allowed to warm to room temperature. After 1 h, water was added and the resulting solution extracted with Et_2O (3 x 100 mL). The combined organic phases were then washed with saturated aqueous NaCl, dried over Na_2SO_4 and evaporated to give residue which upon flash chromatography on silica gel with 3:1 ether-hexane gave **13** (3.6 g, 70% yield) along with recovered starting material **5** (900 mg). Yield based on recovered starting material was 90%. IR (neat) 3550-3060 cm^{-1}; ^{1}HNMR δ 5.85 (qt, J = 6.9, 1.4 Hz, H-2), 5.53 (s, 1H, H-3'), 4.59 (d, J = 12.4 Hz, 1H, CHOSi), 4.26 (t, J = 6.9 Hz, 2H, H-1), 4.16 (d, J = 12.4 Hz, 1H, CHOSi), 3.92-3.97 (m, 4H, -OCH_2CH_2O-), 1.98 (d, J = 14.1 Hz, 1H, H-5), 1.88 (d, J = 14.1 Hz, 1H, H-5), 1.86 (s, 3H, C-3 CH_3), 1.12, 1.10 (both s, 3H each, $C(CH_3)_3$), 0.89 (s, 9H, $SiC(CH_3)_3$), 0.09,0.08 (both s, 3H, each, $Si(CH_3)_2$); CIMS (NH_3) m/z 423 ($[M+1]^+$, 1), 405 ($[M+1 - H_2O]^+$, 100).
HRFIBMS m/z calcd. for $C_{23}H_{39}O_5Si$: 423.2568. Found: 423.2612.

5-(2-t-butyldimethylsiloxymethyl-4,4-ethylenedioxy-1-hydroxy-6,6-dimethylcyclohex-2-enyl)-3-methylpenta-2,4-dien-1-ol (14)

Bis(2-methoxyethoxy)aluminum hydride (3.6 mL, 12.2 mmol) was added in dropwise fashion to a THF (25 mL) solution of **13** (1.73 g, 4.1 mmol) under an argon atmosphere and cooled with an ethylene glycol /dry ice bath. After the addition, the reaction mixture was allowed to stir for 4 h. Water was added slowly to the reaction mixture and the solution was extracted with Et_2O (3 x 100mL), dried over Na_2SO_4 and evaporated. Flash chromatography with 1:1 ether-hexane gave the dienol **14** (880 mg, 51%). IR (film) 3400 cm^{-1}; ^{1}HNMR δ 6.68 (d, J = 15.8, 1H, H-4), 5.75 (d, J = 15.8 Hz, 1H, H-5), 5.51-5.58 (m, 2H, H-2, H-3'), 3.85-4.30 (m, 6H, -OCH_2 and OCH_2CH_2O), 1.88-1.78 (m, 5H, H-5', C-3 CH_3), 1.06 , 0.88 (both s, 3H each, $C(CH_3)_2$), 0.86 (s, 9H, $Si(CH_3)_3$), 0.04, 0.03 (both s, 3H each, $Si(CH_3)_2$); CIMS (NH_3) m/z 425 ($[M+1]^+$, 19), 407 ($[M+1 -H_2O]^+$, 100).
Anal. Calcd. for $C_{23}H_{40}SiO_5$: C, 65.05; H, 9.50%. Found C, 66.94; H, 9.64%.

5-(2-t-Butyldimethylsiloxymethyl-4,4-ethylenedioxy-1-hydroxy-6,6-dimetylcyclohex-2-enyl)-3-methylpenta-2,4-dien-1-al (15)

A mixture of MnO_2 (3.6 g, 42 mmol) and the diol **14** (900 mg, 2.1 mmol) in acetone (50 mL) was stirred at room temperature for 1 h. The suspension was filtered and the filtrate washed with Et_2O. Concentration of the filtrate and washings gave 825 mg of crude aldehyde **15** which was generally used in the next reaction without further purification. In one reaction, the crude product was purified by flash chromatography eluting with 1:1 diethyl ether-hexane. IR ($CHCl_3$) 3600-3400 and 1665 cm^{-1}; ^{1}HNMR δ 10.21 (d, J = 8.3 Hz, 1H, CHO), 7.39 (d, J=15.5 Hz, 1H, H-4), 6.16 (d, J = 15.5 Hz, H-5), 5.84 (d, J = 8.3 Hz, 1H, H-2), 5.57 (s, 1H, C-2' CH_3), 4.24 (dd, J = 12.1, 1.0 Hz, -CHOSi-), 4.05-3.88 (m, 5H, -CHOSi- and -OCH_2CH_2O-), 2.05 (d, J = 1.0 Hz, 3H, sidechain CH_3), 1.87 (d, J = 14.5 Hz, 1H, H-5'ax), 1.81 (dd, J = 14.5, 1 Hz, H-5'eq), 1.08 and 0.9 (both s, 3H each, $C(CH_3)_2$), 0.86 (s, 9H, -$SiC(CH_3)_3$), 0.05 and 0.04 (both s, 3H each, $Si(CH_3)_2$); CIMS (NH_3) m/z 423 ($[M+1]^+$, 100), 405 ($[M+1 - H_2O]^+$, 45).
Anal. Calcd. for $C_{23}H_{38}SiO_5$: C , 65.36; H, 9.07. Found: C, 65.56; H, 9.03%.

Methyl5-(2-t-butyldimethylsiloxymethyl-4,4-ethylenedioxy-1-hydroxy-6,6-dimethylcyclohex-2-enyl)-3 methylpenta-2,4-dienoate (16)

To the crude aldehyde **15** (825 mg, 2.0 mmol) in CH_3OH (20 mL) was added sequentially MnO_2 (2.8 g, 32 mmol), NaCN (235 mg, 4.8 mmol), and CH_3COOH (115 μL, 2.0 mmol) and stirred for 2 h. The mixture was filtered, and the residue washed with CH_3OH. The CH_3OH solution was evaporated at reduced pressure and the product partitioned between water and Et_2O. The aqueous phase was extracted with Et_2O (2 x 100 mL) and the combined ether phases were extracted with water (2 x 100 mL) and then with saturated aqueous NaCl (1 x 100mL), followed by drying over Na_2SO_4 and evaporation to afford 740 mg of crude product which was used in the next reaction without further purification. In one reaction the product was purified by flash chromatography eluting with 1:1 ether-hexane to afford pure ester **16**. IR ($CHCl_3$) 3560-3280, 1715, 1630, and 1605 cm^{-1}; ^{1}HNMR δ 7.72 (d, J =16.0 Hz, 1H, H-4), 6.10 (d, J=16.0 Hz, 1H, H-5), 5.66 (s, 1H, H-3'), 5.59 (d, J=1 Hz, 1H, H-2), 4.28 (dd, J = 12.6, 1.3 Hz, -CHOTBDMS), 4.03-3.89 (m, 5H, -CHOTBDMS and -OCH_2CH_2O-), 3.68 (s, 3H, -$COOCH_3$), 1.97 (d, J = 1.2 Hz, 3H, C-3 CH_3), 1.88 (d, J = 14.5 Hz, 1H, H-5'ax), 1.78 (dd, J = 14.5, 1.3 Hz, 1H, H-5'eq), 1.07, 0.89 (both s, 3H, each, $C(CH_3)_2$), 0.86 (s, 9H, -$Si(CH_3)_3$), and 0.04, 0.03 (both s, 3H, -$Si(CH_3)_2$); CIMS (NH_3) m/z 453 ($[M+1]^+$, 10), 435 ($[M+1 - H_2O]^+$, 15), 321 (100).
Anal. Calcd. for $C_{24}H_{40}SiO_6$: C, 63.68; H, 8.91%. Found C, 63.37; H, 9.05%.

Methyl 7'-t-butyldimethylsiloxyabscisate (17)

Crude **16** (740 mg, 1.6 mmol) was dissolved in acetone (30 mL) and p-toluenesulfonic acid (30 mg, 0.16 mmol) was added. After 1 h, the acetone was evaporated and the residue was taken up in saturated aqueous $NaHCO_3$ and extracted with Et_2O (3 x 100 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried

over Na_2SO_4 and concentrated. Flash chromatography with 1:1 ether hexane gave **17** (630 mg, 44% yield over 3 steps). IR ($CHCl_3$) 1705 and 1665 cm^{-1}; ^{1}HNMR δ 7.82 (d, J = 16.1 Hz, 1H, H-4), 6.15 (d, J = 16.1 Hz, 1H, H-5), 6.09 (bs, 1H, H-3'), 5.72 (bs, 1H, H-2), 4.45 (dd, J = 15.7,1.5 Hz, -CHOTBDMS), 4.24 (dd, J = 15.7, 1.4 Hz, 1H, -CHOTBDMS), 3.67 (s, 3H, $COOCH_3$), 2.43 (d, J = 17.3 Hz, 1H, H-5'), 2.29 (d, J = 17.3 Hz, 1H, H-5'), 1.98 (d, J = 1.2 Hz, 3H, C-3 CH_3), 1.09 and 0.98 (both s, 3H each, $(CH_3)_2$) 0.86 (s, 9H, $Si(CH_3)_3$), and 0.04 (s, 6H, $-Si(CH_3)_2$; CIMS (NH_3) m/z 426 ($[M+18]^+$, 100).
HREIMS Calcd for $(M-OCH_3)^+$ $C_{21}H_{33}O_4Si$: 377.2148; Found 377.2181.

Methyl 7'-hydroxyabscisic Acid (18)

The silyl ether **17** (200 mg, 0.49 mmol) was dissolved in THF (6 mL) at 0 ^{0}C under an argon atmosphere and tetrabutylammonium fluoride (1.08 mL, 1.08 mmol) was added. The mixture was then stirred for 20 min, and then diluted with water. The aqueous layer was then extracted with Et_2O (3 x 10 mL) and $CHCl_3$ (2 x 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. Chromatography over silica eluting with ether gave **18** (115 mg, 80% yield). IR ($CHCl_3$) 3600, 3400, 1710, 1670, and 1610 cm^{-1}; ^{1}HNMR δ 7.66 (d, J = 16.1 Hz, 1H, H-4), 6.15 (d, J = 16.1 Hz, 1H, H-5), 6.13 (s, 1H, H-3'), 5.71 (s, 1H, H-2), 4.43 (dd, J= 15.9, 1.3 Hz, 1H, -CHOH), 4.24 (dd, J = 15.9, 1.2 Hz, 1H, -CHOH), 3.65 (s, 3H, $-COOCH_3$), 2.40 (d, J = 17.2 Hz, 1H, H-5'), 2.27 (d, J = 17.2, 1H, H-5'), 1.98 (d, J = 1.2 Hz, 3H, sidechain CH_3), 1.08 and 0.99 (both s, 3H each, $-C(CH_3)_2$); EIMS m/z 294 (M^+, 1).
HREIMS calcd. for $C_{16}H_{22}O_5$: 294.1467. Found 294.1466.

7'-Hydroxyabscisic Acid (±1)

The ester **18** (115 mg, 0.39 mmol) was dissolved in methanol (2.5 mL) and potassium phosphate buffer (0.1M, pH 7.5, 10 mL), porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 0.5 mL), and KOH solution (1M, added dropwise to adjust the pH to 8.0) were added and the solution stirred overnight. 10% HCl was added until the pH was less than 3 and the mixture was repeatedly extracted ethyl acetate (6 x 10 mL) to obtain the product from the resultant emulsion. The combined ethyl acetate phases were extracted with saturated aqueous $NaHCO_3$ (3 x 25 mL). The aqueous phases were acidified with HCl and extracted with ethyl acetate (3 X 25 mL). The combined ethyl acetate phases were washed with saturated aqueous NaCl (1 x 100 mL), dried over Na_2SO_4 and evaporated to afford **1** (100 mg, 97% yield). mp (CH_3CN) 185-187 °C; IR ($CHCl_3$) 3400-3600, 3600, 1720, 1670 cm^{-1}; UV λ_{max} (CH_3OH) 251 E 19,530), λ_{max} (acidic methanol) 260 E 20,000; ^{1}HNMR δ $CDCl_3$ 7.64 (d, J = 16.4 Hz, 1H, H-4), 6.17 (d, J = 16.4 Hz, 1H, H-5), 6.14 (s, 1H, H-3'), 5.76 (s, 1H, H-2), 4.45 (dd, J = 15.4, 1.0 Hz, 1H, HCO, 4.27 (dd, J = 15.4, 1.0 Hz, 1H, HCO), 2.43 (d, J = 17.3 Hz, 1H, H-5'), 2.28 (d, J = 17.3 Hz, 1H, H-5'), 2.03 (d, J = 0.8 Hz, 3H, C-3 CH_3), 1.11 (s, 3H, gem CH_3), 1.03 (s, 3H, gem CH_3), identical with the literature values[5]; ^{1}HNMR CD_3 OD δ 7.76 (d, J = 16.1 Hz, 1H, H-4), 6.25 (d, J = 16.1 Hz, 1H, H-5), 6.20 (s, 1H, H-3'), 5.74 (s, 3H, H-2), 4.38 (dd, J = 18.6, 1.7 Hz, 1H, HCO, 4.17 (dd, J = 18.6, 1.5 Hz, 1H, HCO), 2.54 (d, J = 17.0 Hz, 1H, H-5'), 2.21 (d, J = 17.0 Hz, 1H, H-5'), 2.02 (br s, 3H, C-3 CH_3), 1.06 (s, 3H, gem CH_3), 1.01 (s, 3H, gem CH_3); EIMS m/z 262 ($[M-H_2O]^+$, 1), 206 (27), 188 (37), 161 (39), and 84 (100).
HRFIBMS calcd for $C_{15}H_{21}O_5$: 281.1389; found 281.1388.

Resolution of 7'-Hydroxyabscisic Acid (±1)

Racemic silyl ether methyl ester **17** was conveniently separated by HPLC using a semipreparative chiral column, and the individual isomers converted as above to (+)-1 and (-)-1. Thus, 0.09 mL of a solution of the methyl ester **17** (approx. 200 mg/mL isopropanol/ hexane (1:9)) was injected onto a Chiracel OD column (Daicel, 25 cm by 1 cm ID, preceded by a Whatman CSK1 HC Pellosil guard column) eluting with isopropanol/ hexane (1:9) at 2 mL/min, with UV detection at 262 nm. Repeated injections (approx. 15 mg/injection, 10 runs) afforded 66 mg of one isomer eluting at 9.5 min and 70 mg of the second isomer eluting at 13.8 min. Analysis by analytical HPLC (Daicel Chiralcel OD 0.46 x 25 cm) indicated each isomer to be greater than 99% optically pure. The resolved isomers of **17** were converted as above to the methyl ester (+)-**18** (36 mg, from the isomer of **17** eluting at 9.5 min, 76%, $[a]_D^{20}$ + 378 °, and (-)-**18** (35 mg, from the isomer eluting at 13.8 min, 69%, $[a]_D^{20}$ - 376 °). Hydrolysis of (+)-**18** (36 mg, 0.12 mmol) with the enzyme as above yielded (+)-1 (31 mg, 90 %). ^{1}HNMR CD_3OD δ 7.76 (d, J = 16.1 Hz, 1H, H-4), 6.25 (d, J = 16.1 Hz, 1H, H-5), 6.20 (s, 1H, H-3'), 5.74 (s, 3H, H-2), 4.38 (dd, J = 18.6, 1.7 Hz, 1H, HCO, 4.17 (dd, J = 18.6, 1.5 Hz, 1H, HCO), 2.54 (d, J = 17.0 Hz, 1H, H-5'), 2.21 (d, J = 17.0 Hz, 1H, H-5'), 2.02 (br s, 3H, C-3 CH_3), 1.06 (s, 3H, gem CH_3), 1.01 (s, 3H, gem CH_3); $[a]_D^{20}$ 342° (c = 1% in methanol); mp 140-141 °C fromCDCl$_3$. Hydrolysis of (-)-**18** (35 mg, 0.12 mmol) afforded (-)1 (30 mg, 89%) as an oil. ^{1}HNMR δ $CDCl_3$ 7.64 (d, J = 16.3 Hz, 1H, H-4), 6.16 (d, J = 16.4 Hz, 1H, H-5), 6.14 (s, 1H, H-3'), 5.75 (s, 1H, H-2), 4.45 (dd, J = 15.4, 1.0 Hz, 1H, HCO, 4.26 (d, J = 15.4 Hz, 1H, HCO), 2.43 (d, J = 17.3 Hz, 1H, H-5'), 2.28 (d, J = 17.3 Hz, 1H, H-5'), 2.03 (d, J = 0.8 Hz, 3H, C-3 CH_3), 1.11 (s, 3H, gem CH_3), 1.03 (s, 3H, gem CH_3) ; $[a]D^{20}$ - 342° (c = .7% in methanol).

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REFERENCES

1. Railton, I.D.; Cowan, A.K. *Plant Science* **1985**, *42*, 169.
2. Lehmann, H.; Schutte, H.R. *J. Plant Physiol.* **1984**, *117*, 201.
3. Railton, I.D.; Cowan, A.K. *Plant Physiol.* **1987**, *84*, 157.
4. Lehmann, H.; Bohm, H.; Schutte, H-R. *J. Plant Physiol.* **1983**, *109*, 423.
5. Lehmann, H.; Priess, A.; Schmidt, J. *Phytochem.* **1983**, *22*, 1277.
6. Lehmann, H; Schwenen, L. *Phytochem.* **1988**, *27*, 677.
7. Hampson, C.R.; Reaney, M.J.T.; Abrams, G.D.; Shibata, S.; Nelson, L.A.K.; Abrams, S.R.; Gusta, L.V. *Plant Physiol.* **1990**, *93* (1 suppl.) 5.
8. Zeevaart, J.A.D.; Creelman, R.A. *Ann. Rev. Plant Physiol. and Plant Mol. Biol.* **1988**, *39* , 439-73.
9. Nolan, R.C. ; Ho, T-H. D. *Plant Physiol.* **1988**, *88* , 588-93.
10. Abrams, G.D.; Abrams, S.R.; Nelson, L.A.K.; and Gusta, L.V.*Tetrahedron* **1990**, *46* , 5543-5554.
11. Kitahara, T.; Touhara, K.; Watanabe, H.; Mori, K. *Tetrahedron* **1989**, *45*, 6387-6400.

12. Takahashi, S.; Oritani, T.; Yamashita, K. *Agric Biol. Chem.* **1989**, *53* , 2711- 2718.
13. Boyer, G.L.; Zeevaart, J.A.D. *Phytochem.* **1986**, *25*, 1103.
14. Mayer, H.J.; Rigassi, N.; Schwieter, U.; Weedon, B.C.L. *Helv. Chim. Acta* **1976**, *59*, 1424-1427.
15. Lamb, N; Abrams, S.R. *Can. J. Chem.* **1990**, *68*, 1151-62.
16. Railton, I.D. *J. Chromatogr.* **1987**, *402*, 371-2.
17. Abrams, S.R.; Reaney, M.J.T.; Abrams, G.D.; Mazurek, T., Shaw, A.C.; Gusta, L.V. *Phytochem.* **1989**, *28*, 2885-2889.
18. Khan, N.A.; Deatherage, F.E.; Brown, J.B. *Organic Synthesis* **1963**,*Coll. Vol. 4*, 851.
19. Tanaka, A.; Yamashita, K. *Agric. Biol. Chem.* **1978**,*42*, 1585.
20. Reich, H. J.; Renga, J. M.; Ieva L. *J. Am. Chem. Soc.* **1975**, *97*, 5434.
21. Leuenberger, von Hans G. W.; Boguth, W.; Widmer, E.; Zell, R. *Helv. Chim. Acta*, **1976**, *59*, 1832.
22. Corey, E.J.; Venkateswarlu, A. *J.Am. Chem. Soc.* **1972**, *94*, 6190.
23. Derguini, F.; Balogh-Nair, V.; Nakanishi, K. *Tetrahedron Lett.* **1979**, *51*, 4899.
24. Jones, T.K.; Denmark, S.E. *Org. Synth.* **1986**, *64*, 182.
25. Attenburrow, J.; Cameron, A.F.B.; Chapman, J.H.; Evans, R.M.; Hans, B.A.; Jansen, A.B.A.; Walker, T. *J. Chem. Soc.* **1952**, 1094.
26. Corey, E.J.; Gilman, N.W.; Ganem, B.E. *J.Am. Chem. Soc.* **1968**, *90*, 5616-5617.
27. Sicsic, S.; Ikbal, M.; Le Goffic, F. *Tetrahedron Lett.* **1987**, *28* , 1887-1888; Toone, E.J., Werth, M.J.; Jones, J.B. *J. Am. Chem. Soc.* **1990**, *112*, 4946-4952.