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PROBING THE ROLE OF THE HYDROXYL GROUP OF ABA: ANALOGUES WITH A METHYL ETHER AT C-1′*

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Key Word Index—*Triticum aestivum* L.; Gramineac; Clark's Cream; Katepwa; wheat; abscisic acid analogues; antitranspirant; germination inhibition; C-1′ methyl ether; acetylenic analogues.

Abstract—(+)-(*S*)- and (−)-(*R*)-C-1′-*O*-methyl abscisic acids and their methyl esters, as well as the methyl ethers of the acetylenic analogue of methyl ABA, were synthesized through an enantioselective route, giving a series of optically active, new C-1′ substituted analogues with known stereochemistry. In a wheat embryo germination inhibition assay, (−)-C-1′-*O*-methyl ABA shows high activity, comparable with (+)- and (−)-ABA, whereas (+)-C-1′-*O*-methyl ABA is less active. In a wheat seedling transpiration assay, both analogues exhibit weak activity although the (+)-ABA-like analogue is more potent than its enantiomer. The anti-transpirant response increases over time, which may indicate that the analogue is being metabolized to ABA *in vivo*.

INTRODUCTION

The plant hormone abscisic acid [(+)-ABA, **1**] regulates many aspects of plant growth, including germination inhibition, transpiration and responses to stress [1–4]. We are interested in determining the structural features of the ABA molecule that are needed for initiating ABA responses in these specific physiological processes [5–7]. Identification of regions of the ABA molecule which can be altered without affecting biological activity is important for the development of probes for ABA-binding proteins, as well as for development of plant growth regulators targetted towards specific biological activities.

In this study we are investigating the importance of the 1′-hydroxyl group of ABA in two ABA-responsive physiological processes—germination inhibition and transpiration. We have chosen to use wheat for our studies to characterize differences in structural requirements for different processes in the same species. We chose two physiological assays in which the enantiomers of ABA have shown different responses. In wheat embryo germination inhibition, both natural (+)-(*S*)- and unnatural (−)-(*R*)-ABA (**2**) are equally active [5], while in transpiration reduction in barley seedlings the natural form is much more effective [8]. It has been postulated that there are separate ABA receptors for germination inhibition and transpiration [9].

Others have previously investigated the role of the 1′-hydroxyl group of ABA, using racemic 1′-desoxyABA (**3**), an analogue with the hydroxyl group replaced with a hydrogen. This analogue was weakly active in triggering the expression of the ABA-inducible RAB-16 mRNA in barley aleurone protoplasts [10], suggesting that the ABA hydroxyl group is important, but not absolutely required. Earlier studies showed that strong activity observed with 1′-desoxyABA in a wheat coleoptile growth assay [11] could be explained by the observation that the compound was converted into ABA in plants [12].

We have synthesized the 1′-*O*-methyl ether of ABA (**4**) to probe the importance of the 1′-hydroxyl group of ABA. Replacement of the alcohol hydrogen with a methyl group will change the hydrogen bonding capabilities of the C-1′ oxygen in the receptor binding site. The replacement also increases the steric bulk at the C-1′ centre, which is shown in Fig. 1. The dotted areas represent the size, at the van der Waals' radius, of the hydrogen on the C-1′-oxygen of ABA (the molecule on the left) versus the methyl group on the C-1′-oxygen of the 1′-*O*-methyl ether of ABA (the molecule on the right). The synthesis and biological testing in wheat of ABA analogues bearing a 1′-oxygen methyl group is presented here.

RESULTS AND DISCUSSION

We synthesized a series of C-1′-*O*-methyl ether ABA derivatives (**4–7**) from ABA analogues containing a free C-1′ hydroxyl group and protected C-4′ and C-1 oxygens.

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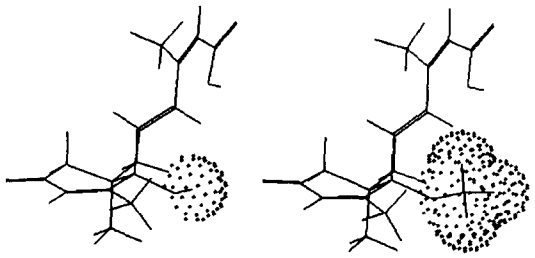
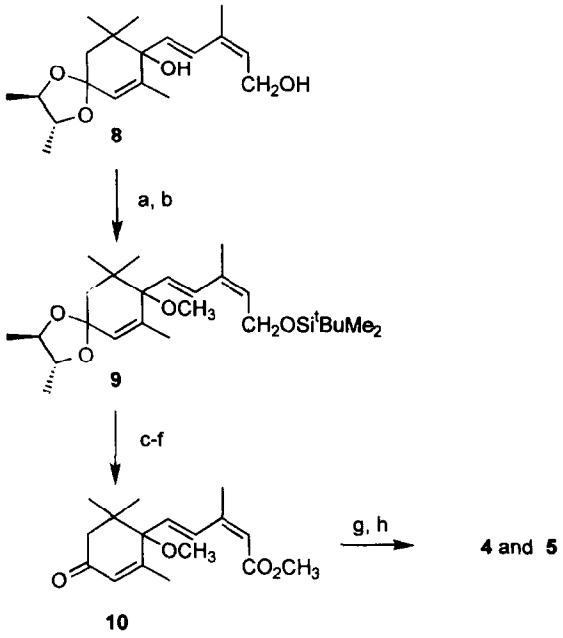


Fig. 1. Comparison of the van der Waals' radius of the hydrogen on the C-1′-hydroxyl group of ABA and the methyl group on the C-1′-*O*-methyl group of C-1′-*O*-methyl ABA. Generated in SYBYL® software by TRIPOS Inc.

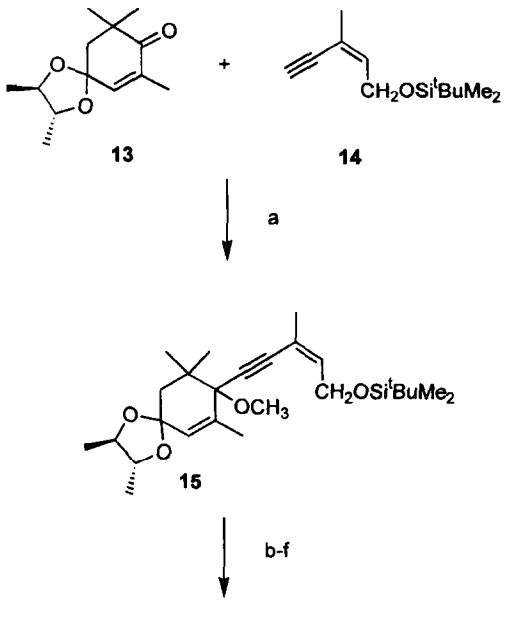
We found that the conditions necessary for forming the methyl ether were quite stringent. We developed a procedure involving formation of the lithium salt of the alcohol with methyllithium followed by alkylation with methyl iodide. Attempts to form alkoxides with other bases and different cations proved unsuccessful. Completion of the synthesis of the C-1′-*O*-methyl ether of ABA was accomplished by standard functional group manipulations of the C-4′ and C-1 positions, as described below.

Specifically, we were interested in synthesizing optically pure forms of the C-1′-*O*-methyl ABA, **4** and **5**, and the C-4 C-5 acetylenic analogues, **6** and **7**. We wanted to test **4** and **5** versus **1** and **2**. In the acetylenic series, we were restricted to comparing esters as the acetylenic acids of ABA were chemically unstable. The method for obtaining the optically pure forms of the starting materials for these compounds has been described by us earlier [13]. For the ABA analogues **4** and **5**, the ketal diol **8** was used (Scheme 1). The ketal was composed of a mixture of known diastereomers with the C-1′-(*S*)-form predominating. Protection of the terminal hydroxyl as the *tert*-butyldimethyl silyl ether, and then methylation of the more hindered C-1′ alcohol by conversion into the lithium alkoxide, followed by treatment with methyl iodide, gave **9**. Removal of the C-1 protecting group with tetra-*n*-butyl ammonium fluoride, oxidation in two steps to the ester and hydrolysis of the ketal gave **10**, a 7:3 mixture in which the (*S*)-form of the C-1′-*O*-methyl ABA methyl ester predominated. The mixture was readily resolved using HPLC with a chiral column, giving the optically pure products **11** and **12**. Each ester was hydrolysed to the C-1′-*O*-methyl ABA, **4** and **5**. These experiments using the chiral ketal and correlation with previous syntheses established the absolute stereochemistry of the methyl ethers **4** and **5**. In subsequent preparations, the scheme was modified using the inexpensive ethylene ketal protecting the 4′-carbonyl group.

The optically pure acetylenic *O*-methyl derivatives were synthesized by a similar route. Alkylation of the chiral ketal of oxoisophorone (**13**) with the lithium salt of **14**, followed by quenching of the reaction with methyl iodide gave a mixture (7:3) of two acetylenic methyl ethers (**15**) (Scheme 2). Deprotection and oxidation



Scheme 1. Outline of synthesis of **4** and **5**. a: *Tert*-butyldimethylsilylchloride, imidazole; b: (i) methyllithium, (ii) methyliodide; c: tetrabutylammonium fluoride; d: MnO_2; e: MnO_2, NaCN, acetic acid, methanol; f: 10% HCl; g: HPLC resolution; h: porcine liver esterase.



Scheme 2. Outline of synthesis of **6** and **7**. a: (i) *n*-Butyllithium, (ii) methyliodide; b: tetrabutylammonium fluoride; c: MnO_2; d: MnO_2, NaCN, acetic acid, methanol; e: 10% HCl; f: HPLC resolution.

afforded **6** and **7**, which could be separated by HPLC using a chiral column. As before, the absolute stereochemistry was established in the alkylation step.

We tested the methyl ethers in two assays, one which measured their effect on the transpiration rate of Katepwa wheat seedings and a second which measured their ability to inhibit the germination of Clark's Cream (*Triticum aestivum* L.) wheat embryos.

At 3 hr after application, the transpiration rate of wheat seedlings is reduced by 70% when natural ABA (**1**) is applied as a root drench at a concentration of 10 μM or greater (Fig. 2). In contrast, the corresponding optically pure methyl ether derivative **4** is only weakly active over all concentrations applied. After 8 hr of imbibing **1** at concentrations of 10 μM or greater, transpiration is reduced by 70–80%. Interestingly, high concentrations of the C-1′-*O*-methyl derivative **4** also causes a reduction in transpiration rate, by *ca* 65% at 8 hr. This result suggests that the methyl ether may be metabolized to ABA or another active compound. Cleavage of the ether in the plant could be taking place at a rate which produces sufficient ABA-like compound after 3 hr of imbibition to account for the weak activity. Alternate explanations of the slightly higher activity at 8 hr are that the analogue could be stimulating ABA synthesis in the plant, or that the uptake of the methyl ether is slower than that of ABA.

We find that (*R*)-ABA (**2**) slightly reduces transpiration in wheat seedlings (Fig. 3) as compared to (*S*)-ABA. At 3 hr, application of 100 μM **2** only reduces transpiration by 20%. At 1000 μM, a 60% reduction is seen. The

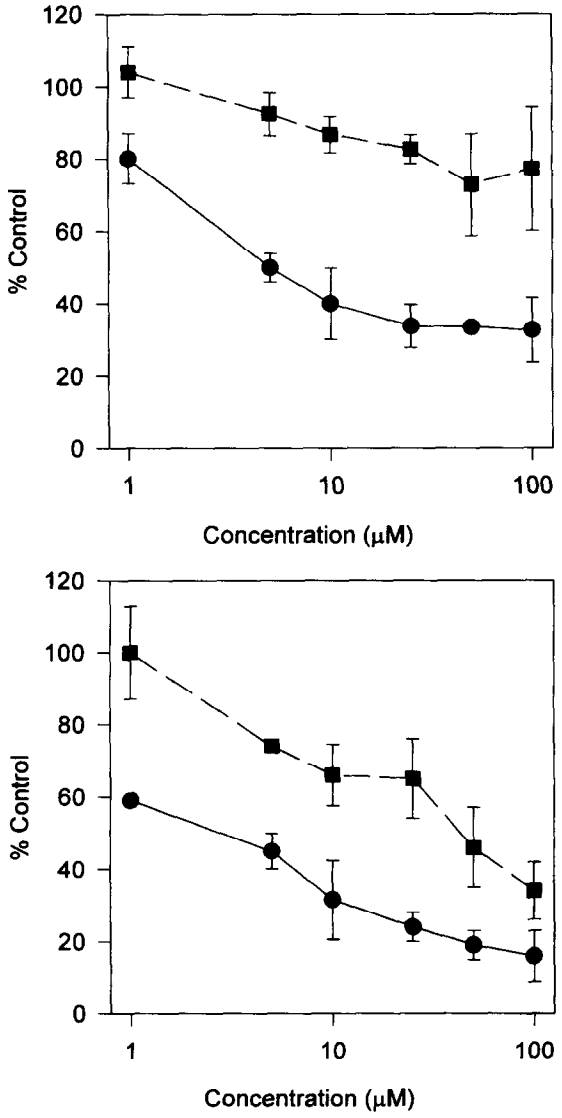


Fig. 2. Effect on transpiration rate of wheat seedlings of (+)-ABA (**1**, ●) and (+)-C-1′-*O*-methyl ABA (**4**, ■). At 3 hr, upper graph; at 8 hr, lower graph. Error bars represent the standard deviation of three replicates.

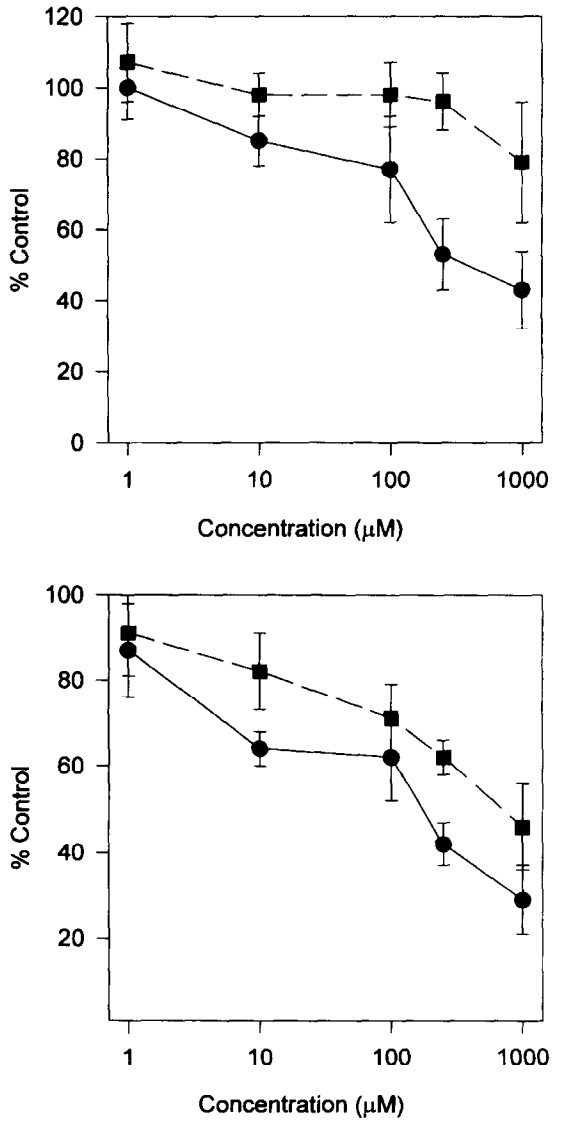


Fig. 3. Effect on transpiration rate of wheat seedlings of (−)-ABA (**2**, ●) and (−)-C-1′-*O*-methyl ABA (**5**, ■). At 3 hr, upper graph; at 8 hr, lower graph. Error bars represent the standard deviation of three replicates.

corresponding methyl ether **5** is inactive at 3 hr. As in the comparison of natural ABA (**1**) with its methyl ether (**4**), at 8 hr, the observed transpiration rates caused by the **2** and the ether **5** are closer.

In germination of wheat embryos, both ABA enantiomers are equally effective inhibitors, and addition of (*R*)-ABA does not result in elevated levels of endogenous (*S*)-ABA [5]. In the present series of experiments, the (*R*)- and (*S*)-methyl ether analogues **4** and **5** were compared to **1** in inhibition of Clark's Cream embryos' germination (Fig. 4) over nine days. The results are presented using a weighted germination index, as previously reported [5]. Both ethers exhibit much greater activity in this assay relative to (*S*)-ABA than in the transpiration assay. Surprisingly, the (*R*)-methyl ether **5** is the more potent isomer, nearly as active as (*S*)-ABA, with 20 μM giving a 50% inhibition of germination (Gl_{50}). The (*S*)-methyl ether **4** is weaker over all concentrations tested, with 50% inhibition observed between 50 and 100 μM. The differences in activity of the enantiomers may reflect differences in metabolism over the time of the assays or a separate receptor with distinct structural requirements.

Analogues of ABA with the acetylenic ester side chain (**16** and **17**) are as active as methyl esters of ABA in germination inhibition of excised wheat embryos [5]. The corresponding methyl ether analogues **6** and **7** are comparatively much weaker inhibitors of germination, showing about one-tenth the activity. The concentration of **16** which inhibits germination by 50% is between 1 and 10 μM; however, for methyl ether analogue **6** the Gl_{50} is 100 μM. In this case, both enantiomers showed similar activity. These results confirm that the ABA hydroxyl group is important in the recognition of ABA and its analogues in germination inhibition.

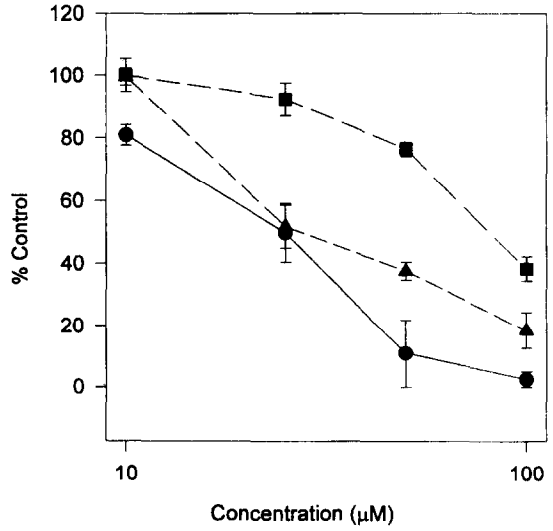
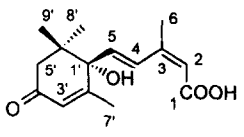
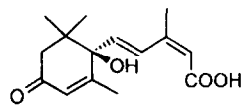


Fig. 4. Germination inhibition index of wheat as a percentage of control for (+)-ABA (**1**, ●), (+)-C-1′-*O*-methyl ABA (**4**, ■) and (−)-C-1′-*O*-methyl ABA (**5**, ▲). Error bars represent the standard error of three replicates.



1



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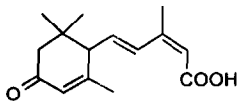

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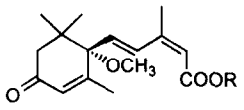
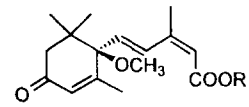
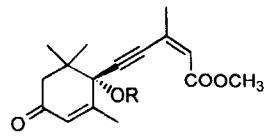


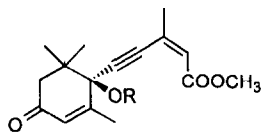
4: R = H
11: R = CH_3



5: R = H
12: R = CH_3



6: R = CH_3
16: R = H



7: R = CH_3
17: R = H

Our findings show the importance of testing ABA analogues as optically pure enantiomers. In the transpiration assay, the difference in activity between (*R*)- and (*S*)-ABA has long been recognized, and was reflected again in the activity of the methyl ethers. The inactivity of these compounds at 3 hr shows that the change to the molecule at the C-1′-hydroxyl group affects perception at some stage of the pathway, be it transport or recognition at a receptor. In germination, this is the first time we have observed higher activity in an analogue related to the unnatural form of ABA. Again, the lowered activity of both enantiomers suggests that the 1′-hydroxyl group is necessary for perception. In this case, differences in uptake from ABA can be ruled out as being the only cause for low activity as the enantiomers themselves showed differing activity. Because the ethers tended to exhibit higher activity in longer term assays, we have undertaken to determine the metabolic fate of the optically pure methyl ether compounds using maize (*Zea mays* L. cv. Black Mexican Sweet) suspension-cultured cells, a system that is convenient for monitoring ABA metabolism and for isolating and identifying metabolites [14]. The results of this investigation will be reported at a later date.

EXPERIMENTAL

Wheat seedling transpiration assay

Seedlings. Wheat seedlings of the variety Katepwa were germinated and grown at 23°C beside a north facing window. Additional lighting, a 100 W incandescent bulb,

was used during the winter months to create a 16-hr day. Seeds (certified, 1993) were sown 2.5 cm below the surface in culture tubes containing vermiculite, watered with nutrient soln as required (Plant Prod 20-20-20) and used in the assay between the 1st and 2nd leaf stage (6–10 days after planting).

Measuring system. The gas measuring system has been described previously [15]. The seedling is partially sealed into a Plexiglass measuring chamber exposing 2.5 cm of the leaf length to the cell environment. Light is provided by a 150 W Sylvania spotlight. Temps of the leaf surface are monitored with Cu–constanan thermocouples. Filtered air of constant humidity is passed through the cells (0.1 $l min^{-1}$) and the dew point temps of the inlet air and outlet air of each cell are recorded with a humidity sensor (General Eastern-Condensation Dew Point Hygrometer, 1200 APS and Optical Dew Point Sensor, Model 1211). The outlet air from 4 cells plus the background humidity of the inlet air are alternately sampled via a Scanivalve switch. The plant is allowed to equilibrate 2–4 hr in the chamber before introduction of 2 ml analogue soln to the culture tube (an aq. soln containing 1% EtOH). Prior to the assay each culture tube is fitted with a small syphon (glass capillary tube and plastic tubing) attached to a reservoir tube containing 3 ml nutrient water soln. Dew point temps are recorded for 16 hr after addition of the analogue and the transpiration rate (μMol $H_2O\ cm^{-2}\ s^{-1}$) calculated, and then given as a percentage of the initial transpiration rate of the plant (typically 0.28–0.35 μMol $H_2O\ cm^{-2}\ s^{-1}$), corrected for the effect of the control.

Wheat embryo germination assay

Dormant grains of the soft, white wheat (*T. aestivum* L., cvs Clark's Cream) were obtained as previously described [16]. For bioassay, embryos with some adhering endosperm and pericarp attached were cut from the grains with a razor blade. These embryos germinated readily in H_2O [16]. Inhibitory activity was tested for each analogue at 0, 10, 25, 50 and 100 μM. For each analogue concn 3 replicate germination assays were conducted on 10 embryos each at 30°. The number of germinated embryos was counted daily for 9 days and a weighted germination index [17] calculated. Solns of (*S*)- and (*R*)-ABA, Me esters and analogues were prepd by dissolving samples in a minimal amount of DMSO (100 μl) and then diluting into 10 mM Mes, pH 5.8, containing 0.5% (w/v) tetramethylthiuram disulphide (Aldrich).

Synthesis of methyl ethers

Mps (uncorr.) were determined with a microscope hot stage apparatus. ^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer, with $CDCl_3$ as solvent and $CHCl_3$ as reference, unless otherwise specified. For clarity, the conventional ABA numbering system is employed in assignments of peaks in the ^{1}H NMR spectra. In the case of inseparable diastereoisomers, the NMR spectral data is quoted for the major diastereoisomer and signals marked with an asterisk indicate and overlap with the minor diastereoisomer. IR spectra were obtained with a Perkin Elmer 237B instrument. Optical rotations were obtained from a Perkin-Elmer 141 Polarimeter and were carried out in MeOH. Flash CC was performed using E. Merck Silica gel 60 (230–400 mesh). HREIMS were recorded with a VG 70–250SEQ double-focusing hybrid spectrometer. GC-MS were obtained with a DB-5 column (60 m) in a Finnigan 4000 E instrument operated in the EIMS mode. Commercially available compounds were used in this work without further purification. THF was distilled over Na and benzophenone.

(+)-S- and (−)-R-C-1′-methyl ethers of ABA, 4 *and* 5

8-(2Z,4E)-(2S,3S,8S)-8-(5-tert-*Butyldimethylsiloxy*-3-*methyl*-2,4-*pentadienyl*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro* [4.5]*dec*-6-*en*-8-*ol.* To an ice cooled soln of diol **8** (6.6 g, 20.5 mmol) in 100 ml dry CH_2Cl_2 was added imidazole (1.6 g, 24.6 mmol) and *tert*-butyldimethylsilyl chloride (3.7 g, 24.6 mmol). The mixt. was warmed to room temp., stirred for 1 hr and filtered. The filtrate was washed with CH_2Cl_2 and the combined organics were washed with H_2O and brine, dried over Na_2SO_4 and concd to give 8.2 g (92% yield) of pure product. IR (neat $\nu_{max}\ cm^{-1}$: 3490 (*br*, O–H); HREIMS: $[M]^+$ at *m/z* 436.3007 ($C_{25}H_{44}O_4Si$ requires 436.3309); ^{1}H NMR (C_6D_6): δ6.84 (*d*, 1H-4, J = 15.7 Hz), 5.77 (*d*, 1H-5, J = 15.7 Hz), 5.53 (*m*, 2H-2, 3′), 4.43* (*m*, 2H-1), 3.50–3.35* (*m*, 2H, $OCHCH_3$), 2.12 (*d*, 1H-5′, J = 14.4 Hz), 1.75 (*dd*, 1H-5′, J = 14.3, 1.4 Hz), 1.69 (*d*, 3H-6/7′, J = 0.8 Hz), 1.66 (*d*, 3H-6/7′, J = 1.1 Hz), 1.22 (*s*, 3H-8′/9′), 1.03 (*s*, 3H-8′/9′), 0.97 (*s*, 9H, $SiC(CH_3)_3$ overlapping *m*, 6H, $OCHCH_3$), 0.09 [*s*, 6H, $Si(CH_3)_2$].

8-(2Z,4E)-(2S,3S,8S)-8-(5-tert-*Butyldimethylsiloxy*-3-*methyl*-2,4-*pentadienyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro*[4.5]*dec*-6-*ene* (9). The above alcohol (6 g, 13.8 mmol) was dissolved in THF and cooled to − 78° (dry ice/Me_2CO bath). MeLi (14.7 ml, 20.7 mmol) was slowly added, and the mixt. warmed to 0°. After 20 min, an excess of MeI (5 ml, 80 mmol) was added and the reaction mixt. was warmed to room temp. and left overnight. After quenching with H_2O, the THF layer was removed and the aq. layer extracted (× 2) with Et_2O. The combined organics were washed with brine, dried over Na_2SO_4 and concd. Flash CC eluting with Et_2O–hexane (3:7) gave 3.7 g product and a mixed fr. (1.7 g) of starting material and product (4:1). This mixt. could be resubmitted to the reaction conditions to give a further 1.3 g product, giving a combined yield of 81% of desired Me ether. IR (neat) $\nu_{max}\ cm^{-1}$: 2950 (C–H), 1100 (C–O); HREIMS: $[M]^+$ at *m/z* 450.3123 ($C_{26}H_{46}O_4$ Si requires 450.3165); ^{1}H NMR (C_6D_6): δ6.84 (*d*, 1H-4, J = 15.7 Hz), 5.74 (*s*, 1H-2/3′), 5.72 (*d*, 1H-5, J = 15.7 Hz), 5.63* (*m*, 1H-2/3′), 4.45* (*m*, 2H-1), 3.53–3.41* (*m*, 2H, $OCHCH_3$), 3.23 (*s*, 3H-1′), 2.25 (*d*, 1H-5′, J = 13.9 Hz), 1.91 (*d*, 1H-5′,

J = 13.9 Hz), 1.75* (*d*, 3H-6/7′, J = 1.0 Hz), 1.69 (*d*, 3H-6/7′, J = 1.4 Hz), 1.29 (*s*, 3H-8′/9′), 1.13 (*s*, 3H-8′/9′), 1.05* (*m*, 6H, $OCHCH_3$), 0.99 (*s*, 9H, $SiC(CH_3)_3$), 0.10 [*s*, 6H, $Si(CH_3)_2$].

8-(2Z,4E)-(2S,3S,8S)-8-(5-*Hydroxy*-3-*methyl*-2,4-*pentadienyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro* [4.5]*dec*-6-*ene*. To an ice-cooled soln of **9** (5 g, 11.1 mmol) in dry THF (100 ml) under Ar, was added dropwise Bu_4NF (1.0 M in THF, 14.4 ml, 14.4 mmol). The reaction soln was allowed to warm to room temp. After 1 hr, H_2O was added, the THF was sepd and the aq. layer extracted with Et_2O. The pooled organics were washed with brine soln and then dried over Na_2SO_4 and concd. Flash CC eluting with Et_2O–hexane (3:1) gave 3.4 g (92% yield) of the deprotected alcohol. IR (neat) ν_{max} cm^{-1}: 3420 (O–H), 1095 (C–O); HREIMS: $[M]^+$ at *m/z* 336.2307 ($C_{20}H_{32}O_4$ requires 336.2301); ^1H NMR (C_6D_6): δ6.82 (*d*, 1H-4, J = 15.8 Hz), 5.74 (*d*, 1H-5, J = 16.3 Hz overlapping *s*, 1H-2/3′), 5.54* (*m*, 1H-2/3′), 4.29–4.13* (*m*, 2H-1), 3.50–3.36* (*m*, 2H, $OCHCH_3$), 3.22 (*s*, 3H-1′), 2.16 (*bd*, 1H-5′, J = 14.1 Hz), 1.90 (*d*, 1H-5′, J = 13.9 Hz), 1.74* (*d*, 3H-6/7′, J = 1.0 Hz), 1.68 (*d*, 3H-6/7′, J = 1.5 Hz), 1.29 (*s*, 3H-8′/9′), 1.11 (*s*, 3H-8′/9′), 1.03* (*m*, 6H, $OCHCH_3$).

8-(2Z,4E)-(2S,3S,8S)-8-(3-*Methyl*-5-*oxo*-2,4-*pentadienyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro* [4.5] *dec*-6-*ene*. The above alcohol (2.67 g, 3.0 mmol), MnO_2 (4.9 g, 60 mmol) and Me_2CO (50 ml) were stirred at room temp. in a round bottom flask fitted with a drying tube. The reaction mixt. was left overnight, then filtered and the residual cake of MnO_2 washed with Et_2O. After concn, the crude material was purified by flash CC eluting with Et_2O–hexane (3:7) to give 1.95 g (74% yield) of the desired aldehyde. IR (neat) ν_{max} cm^{-1}: 1670 (C=O), 1095 (C–O); HREIMS: $[M]^+$ at *m/z* 334.2129 ($C_{20}H_{30}O_4$ requires 334.2144); ^1H NMR (C_6D_6): δ10.30 (*d*, 1H-1, J = 7.9 Hz), 7.31 (*d*, 1H-4, J = 15.8 Hz), 5.86 (*d*, 1H-5, J = 15.7 Hz), 5.75* (*m*, 2H-2/3′), 3.51–3.38* (*m*, 2H, $OCHCH_3$), 3.09 (*s*, 3H-1′), 2.28 (*d*, 1H-5′, J = 14.0 Hz), 1.82 (*d*, 1H-5′, J = 14 Hz), 1.55* (*d*, 3H-6/7′, J = 1.1 Hz), 1.52 (*s*, 3H-6/7′), 1.05* (*m*, 12H-8′/9′ and $OCHCH_3$).

8-(2Z,4E)-(2S,3S,8S)-8-(4-*Carbomethoxy*-3- *methyl*-2,4-*butadienyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro*[4.5] *dec*-6-*ene*. To the above aldehyde (1.22 g, 3.7 mmol) in MeOH (30 ml) was added sequentially MnO_2 (4.8 g, 55.5 mmol), NaCN (435 mg, 8.9 mmol) and HOAc (212 μl, 3.7 mmol). The reaction mixt. was stirred for 1.5 hr at room temp. The suspension was filtered and washed with MeOH. After concn, the residue was partitioned between Et_2O and H_2O and the organic layer was washed with satd NaCl soln, dried over Na_2SO_4 and concd. The crude product was purified by flash CC eluting with Et_2O–hexane (3:7) giving 832 mg (63% yield). IR (neat) ν_{max} cm^{-1}: 1720 (C=O), 1095 (C–O); HREIMS: $[M]^+$ at *m/z* 364.2248 ($C_{21}H_{32}O_5$ requires 364.2250); ^1H NMR (C_6D_6): δ8.18 (*d*, 1H-4, J = 16.2 Hz), 5.95 (*d*, 1H-5, J = 16.2 Hz), 5.76 (*s*, 1H-2/3′), 5.70 (*s*, 1H-2/3′), 3.59–3.39* (*m*, 2H, $OCHCH_3$), 3.39 (*s*, 3H-1′), 3.27 (*s*, 3H, CO_2CH_3), 2.43 (*d*, 1H-5′, J = 13.7 Hz), 1.86 (*d*, 1H-5′, J = 13.8 Hz), 1.70 (*d*, 3H-6/7′, J = 1.0 Hz), 1.63 (*d*, 3H-6/7′, J = 1.3 Hz), 1.23 (*s*, 3H-8′/9′), 1.13 (*s*, 3H-8′/9′), 1.04* (*m*, 6H, $OCHCH_3$).

(+)-(2E,4Z)-(4S)-4-(4-*Carbomethoxy*-3-*methylbutyl*-2,4-*dienyl*)-4-*methoxy*-3,5,5-*trimethylcyclohex*-2-*enone* (**10**). To the above ketal (830 mg dissolved in 20 ml THF) was added 20 ml 10% HCl. The reaction mixt. was allowed to stir at room temp. for 1 hr at which time H_2O was added and the mixt. extracted (×3) with Et_2O. The combined organics were washed with brine soln, dried over Na_2SO_4 and concd. The crude product was purified by flash CC eluting with Et_2O–hexane (1:1) to give 566 mg (85% yield) of the Me ester. The mixt. of enantiomers could be analysed and purified at this point by HPLC using a Chiralpak OD column. The ratio of enantiomers was determined to be 7:3, with the major enantiomers (**11**) having the same relative configuration as (+)-ABA [13]. The major enantiomer showed the following spectral data: $[\alpha]_D^{20}$ + 86.6° (4.40% in $CHCl_3$): m.p. 82–83° (recrystallized from Et_2O/hexane); IR ($CHCl_3$) ν_{max} cm^{-1}: 1710 (C=O, ester), 1665 (C=O, enone), HREIMS: $[M]^+$ at *m/z* 292.1667 ($C_{17}H_{24}O_4$ requires 292.1675); ^1H NMR: δ7.74 (*d*, 1H-4, J = 16.4 Hz), 6.03 (*d*, 1H-5, J = 16.7 Hz, overlapping *s*, 1H-3′), 5.75 (*s*, 1H-2), 3.69 (*s*, 3H, CO_2CH_3), 3.35 (*s*, 3H-1′), 2.62 (*bd*, 1H-5′, J = 14.1 Hz), 2.10 (*d*, 1H-5′, J = 15.8 Hz), 2.04 (*d*, 3H-6/7′, J = 1.0 Hz), 1.99 (*d*, 3H-6/7′, J = 1.2 Hz), 1.01 (*s*, 3H-8′/9′), 0.99 (*s*, 3H-8′/9′). ^{13}C NMR: δ198.96, 166.365, 149.19, 132.87, 130.37, 129.37, 129.28, 118.15, 53.81, 51.16, 49.02, 42.94, 25.01, 23.55, 21.16. The minor enantiomer **12** showed identical spectral properties, with the following changes: $[\alpha]_D^{20}$ − 93.1° (2.22% in $CHCl_3$).

(+)-(2E,4Z)-(4S)-4-(1-*Carboxy*-3-*methylbutyl*-2,4-*dienyl*)-4-*methoxy*-3,5,5-*trimethylcyclohex*-2-*enone* (**4**). Optically pure ester **11** (25.7 mg) was dissolved in MeOH (12 drops) and K-Pi buffer (0.1 M, pH 7.5, 2 ml), porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 200 μl) and KOH soln (1 M, added dropwise to adjust the pH to 8.0) were added and the soln stirred overnight. 10% HCl was added until the pH was < 3 and the mixt. was extracted with EtOAc (6 × 10 ml) to obtain the product from the resulting emulsion. The combined EtOAc phases were extracted with satd aq. $NaHCO_3$ (3 × 25 ml). The aq. phases were acidified with HCl and extracted with EtOAc (3 × 25 ml). The EtOAc extract was washed with brine and dried over Na_2SO_4. Evapn gave 19 mg (78%) of the desired acid. $[\alpha]_D^{20}$ + 94.7° (2.64% in $CHCl_3$); mp 131–132.5°; IR ($CHCl_3$) ν_{max} cm^{-1}: 2500–3200 (*br*, OH), 1650 (*br*, C=O); HREIMS: $[M]^+$ at *m/z* 278.1513 ($C_{16}H_{22}O_4$ requires 278.1518); ^1H NMR: δ 7.71 (*d*, 1H-4, J = 16.3 Hz), 6.06 (*d*, 1H-5, J = 16.2 Hz, overlapping *s*, 1H-3′), 5.79 (*s*, 1H-2), 3.33 (*s*, 3H-1′), 2.62 (*bd*, 1H-5′, J = 15.5 Hz), 2.07 (*d*, 3H-6/7′, J = 0.8 Hz overlapping 1H-5′), 1.98 (*d*, 3H-6/7′, J = 1.2 Hz), 1.0 (*s*, 3H-8′/9′), 0.99 (*s*, 3H-8′/9′). ^{13}C NMR: δ199.04, 170.68, 151.47, 133.51, 130.34, 129.31, 117.76, 53.76, 48.94, 42.95, 25.02, 23.55, 22.79, 21.43. The minor enantiomer **12** could be hydrolysed to **5** under the same conditions and showed identical spectral properties, with the following changes: $[\alpha]_D^{20}$ − 88.4° (1.82% in $CHCl_3$).

(+)-R- and (−)-S-C-1′-methyl ethers of acetylenic ABA, 6 and 7

(+)-8(Z)-(2S,3S,8R)-8-(5-tert-*Butyldimethylsiloxy*-3-*methylpent*-3-*en*-1-*ynyl*)-8-*methoxy*- 2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro*[4.5]*dec*-6-*ene* (**15**). A soln of known alkyne **14** [18] (1.22 g, 5.85 mmol) in dry THF (25 ml) was cooled in a dry ice/Me_2CO bath under an Ar atmosphere. *n*-BuLi (Aldrich; 1.6 M in hexane, 3.7 ml, 5.85 mmol) was added dropwise with stirring. The reaction soln was kept at − 78° for 30 min. A soln of chiral ketal **13** [13] (880 mg, 3.9 mmol) in dry THF (25 ml) was added dropwise. After the addition was complete, the reaction soln was warmed to room temp. over 45 min. When analysis by TLC showed that all starting material had reacted, the soln was cooled down to − 78° and 5 ml of MeI was added. The mixt. was allowed to warm to room temp. again and was left for 2 hr. The reaction was quenched with H_2O, the THF layer removed and the aq. layer extracted (×3) with Et_2O. The pooled organics were washed with brine and dried over Na_2SO_4. Purification by flash CC eluting with Et_2O–hexane (1:9) gave 1.33 g of an inseparable mixt. of diastereoisomers (7:3 ratio, 76% yield). IR (neat) ν_{max} cm^{-1}: 1100 (C–O); GC-MS (M-44) at *m/z* 392 (14.7%); ^1H NMR (C_6D_6): δ5.80* (*m*, 1H-2′), 5.58 (*s*, 1H-3′), 4.54* (*m*, 2H-5), 3.46* (*m*, 2H, $OCHCH_3$), 3.43 (*s*, 3H-1′), 2.36 (*d*, 1H-5′, J = 13.8 Hz), 2.18 (*d*, 1H-5′, J = 13.8 Hz), 1.96 (*d*, 3H-7′, J = 1.3 Hz), 1.67 (*d*, 3H-6, J = 1.4 Hz), 1.42 (*s*, 3H-8′/9′), 1.38 (*s*, 3H-8′/9′), 0.99 (*s*, 9H, $SiC(CH_3)_3$), 0.98 (*s*, 6H, J = 4.3 Hz, $OCHCH_3$), 0.12 [*s*, 6H, $Si(CH_3)_2$].

(+)-8(Z)-(2S,3S,8R)-8-(5-*Hydroxy*-3-*methylpent*-3-*en*-1-*ynyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro*[4.5]*dec*-6-*ene*. To an ice-cooled soln of **15** (1.28 g, 2.9 mmol) in dry THF (40 ml) under Ar, was added dropwise tetrabutylammonium fluoride (1.0 M in THF, 3.75 ml). The reaction soln was allowed to warm to room temp. After 2 hr, H_2O was added and the aq. layer extracted with Et_2O. The pooled organics were washed with brine soln, and then dried over Na_2SO_4 and concd. No further purification was required, giving a quantitative yield of the deprotected alcohol. IR (neat) ν_{max} cm^{-1}: 3400 (*br*, O–H), 1090 (C–O); GC-MS (M-44) at *m/z* 278 (67.4%); ^1H NMR (C_6D_6): δ5.63* (*m*, 1H-2), 5.56* (*s*, 1H-3′), 4.22* (*m*, 2H-1), 3.44* (*m*, 2H, $OCHCH_3$), 3.41 (*s*, 3H-1′), 2.35 (*d*, 1H-5′, J = 13.7 Hz), 2.21 (*d*, 1H-5′, J = 13.8 Hz), 1.93 (*d*, 3H-7′, J = 1.2 Hz), 1.66 (*d*, 3H-6, J = 1.4 Hz), 1.40 (*s*, 3H-8′/9′), 1.36 (*s*, 3H-8′/9′), 1.02* (*m*, 6H, $OCHCH_3$).

(+)-8(Z)-(2S,3S,8R)-8-(4-*Carbomethoxy*-3-*methylbut*-3-*en*-1-*ynyl*)-8-*methoxy*-2,3,7,9,9-*pentamethyl*-1,4-*dioxaspiro*[4.5]*dec*-6-*ene*. The above alcohol (1.1 g, 3.3 mmol), MnO_2 (5.4 g, 66 mmol) and Me_2CO (75 ml) were stirred at room temp. in a round bottom flask fitted with a drying tube. After 1 hr, the reaction mixt. was filtered and the residual cake of MnO_2 washed with Et_2O. The combined organics were evapd, giving 1 g (92% yield) of crude product which was pure enough to carry on to the next reaction. To the crude aldehyde (1.0 g, 3.0 mmol) in MeOH (75 ml) was added sequentially MnO_2 (3.7 g, 45 mmol), NaCN (353 mg, 7.3 mmol) and HOAc (170 μl, 3 mmol). The reaction mixt. was stirred for 1.5 hr at room temp. The suspension was filtered and washed with MeOH. After concn, the residue was partitioned between Et_2O and H_2O and the organic layer washed with satd NaCl soln, dried over Na_2SO_4 and concd. The crude product was purified by flash CC eluting with Et_2O–hexane (3:7), giving 605 mg (56% yield). IR (neat) ν_{max} cm^{-1}: 1720 (C=O); GC-MS (M-44) at *m/z* 306 (54.6%); ^1H NMR (C_6D_6): δ5.78* (*m*, 1H-2), 5.60 (*s*, 1H-3′), 3.61 (*s*, 3H, CO_2CH_3), 3.45* (*m*, 2H, $OCHCH_3$), 3.38 (*s*, 3H-1′), 2.42 (*m*, 1H-5′), 2.25 (*m*, 1H-5′), 2.08 (*d*, 3H-7′, J = 1.5 Hz), 1.57 (*d*, 3H-6, J = 1.5 Hz), 1.46 (*s*, 6H-8′/9′), 1.03 (*m*, 6H, $OCHCH_3$).

(+)-4(Z)-(4R)-4-(4-*Carbomethoxy*-3-*methylbut*-3-*en*-1-*ynyl*)-4-*methoxy*-3,5,5-*trimethylcyclohex*-2-*enone* (**6**, **7**). To the diastereotopic mixt. of ketal esters from above (390 mg dissolved in 15 ml THF) was added 15 ml 10% HCl. The reaction mixt. was allowed to stir at room temp. for 1 hr at which time H_2O was added and the mixt. was extracted (×3) with Et_2O. The combined organics were washed with brine soln, dried over Na_2SO_4 and concd. The crude product was purified on a 4 mm Chromatotron plate, eluting with Et_2O–hexane (3:7) to give 205 mg (52% yield) of the Me ester. The mixt. of enantiomers could be analysed and purified at this point by HPLC using a semi-prep. Chiralpak AS column (30% IPA in hexane, flow rate 2 ml min^{-1}). The ratio of enantiomers was determined to be 7:3, with the major enantiomer **6** having the same relative configuration as (+)-ABA [13]. The major enantiomer **6** showed the following spectral data. $[\alpha]_D^{20}$ + 196.7° (0.61% in $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 1710 (C=O, ester), 1660 (C=O, enone), HREIMS: $[M]^+$ at *m/z* 290.1520 ($C_{17}H_{22}O_4$ requires 290.1518); ^1H NMR: δ 6.03 (*d*, 1H-2, J = 1.5 Hz), 5.87 (*s*, 1H-3′), 3.69 (*s*, 3H, CO_2CH_3), 3.54 (*s*, 3H-1′), 2.57 (*br d*, 1H-5′), 2.32 (*brd*, 1H-5′), 2.15 (*s*, 3H-7′), 2.07 (*d*, 3H-6, J = 1.3 Hz), 1.15 (*s*, 6H-8′/9′). The minor enantiomer **7** showed identical spectral properties, with the following changes: $[\alpha]_D^{20}$ − 196.3° (0.43% in $CHCl_3$).

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