

Facile Aromatisation of Abscisic Acid

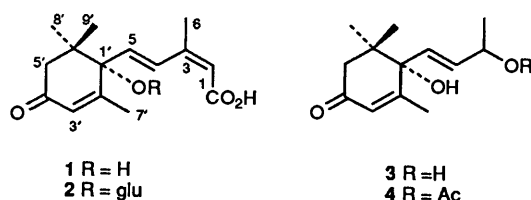
Michael H. Beale,^b Anqi Chen,^a Polly A. Harrison^b and Christine L. Willis^{*a}

^a School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

^b Department of Agricultural Sciences, University of Bristol, AFRC-IACR, Long Ashton Research Station, Bristol BS18 9AF, UK

Treatment of abscisic acid (ABA) or its methyl ester either with acetic anhydride and toluene-*p*-sulfonic acid or with lithium diisopropylamide and methyl chloroformate led to aromatisation of the cyclohexene ring. A mechanism for the reaction is proposed which involves the initial formation of the 4',5'-enol followed by a carbocationic rearrangement initiated by loss of the 1'-tertiary alcohol. Analogues of ABA with a saturated side chain do not undergo this aromatisation process.

The hormone (+)-(*S*)-abscisic acid† (ABA) **1** is widely distributed in higher plants and is involved in many developmental processes, including stomatal guard cell closure and inhibition



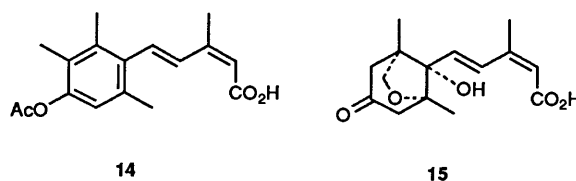
of seed germination.¹ Little definitive structure-activity data for ABA is available, mainly due to the diverse nature of the bioassays that have been used and the apparent equal activity of both natural and unnatural enantiomers in some assays. As part of a project aimed at delineating those parts (if any) of the ABA molecule that can be modified without significantly lowering biological activity, with the longer term objective of preparing affinity probes, we have examined the derivatisation of the 1'-hydroxy group and enolisation of the 4'-ketone. This paper describes a previously unseen aromatisation of the cyclohexene ring of ABA which is observed under differing reaction conditions.

Results and Discussion

It has been reported that derivatisation of the 1'-tertiary hydroxy group of ABA **1** is not easily achieved, attempted acetylation was unsuccessful² and trimethylsilylation only occurs under forcing conditions.³ In addition, acetylation of a structurally related natural product, blumenol A **3** gives solely the primary monoacetate **4**.⁴ The poor reactivity of the 1'-hydroxy group in ABA is unlikely to be due to steric hindrance since not only is it known that the alcohol adopts an equatorial position but also that the glycosyl ether **2** is a naturally-occurring derivative.¹

The reaction conditions for the attempted acetylation of ABA were not reported.² Hence our investigations on the derivatisation of the 1'-hydroxy group began by treatment of racemic ABA methyl ester **5** with acetic anhydride in the presence of toluene-*p*-sulfonic acid, conditions that normally acetylate tertiary hydroxy groups⁵ (Scheme 1). A single product was obtained which was less polar than the starting material. From the ¹H NMR spectrum it was apparent that simple acetylation had not occurred and that the product, although containing an

acetate residue (δ 2.34, 3 H, s), was the result of a significant structural change in the ring. All four methyl groups gave signals in the region δ 2.06–2.28 (*cf.* ABA methyl ester, 8'- and 9'-CH₃ resonate at δ 0.99 at 1.09) and the characteristic AB pattern at δ 2.34 assigned to the 5'-hydrogen atoms had disappeared with the appearance of a new signal at δ 6.74 integrating to one proton. The ¹³C NMR spectrum displayed six signals in the region δ 134–150 and hence the aromatic structure **6** was proposed. This structure was confirmed by mass spectrometry (M^+ 302) and the ¹³C-¹H correlation spectrum enabled complete assignment of the ¹H and ¹³C NMR spectra as reported in the Experimental section. Treatment of ABA **1** under the same reaction conditions gave the analogous aromatic acid **14**.

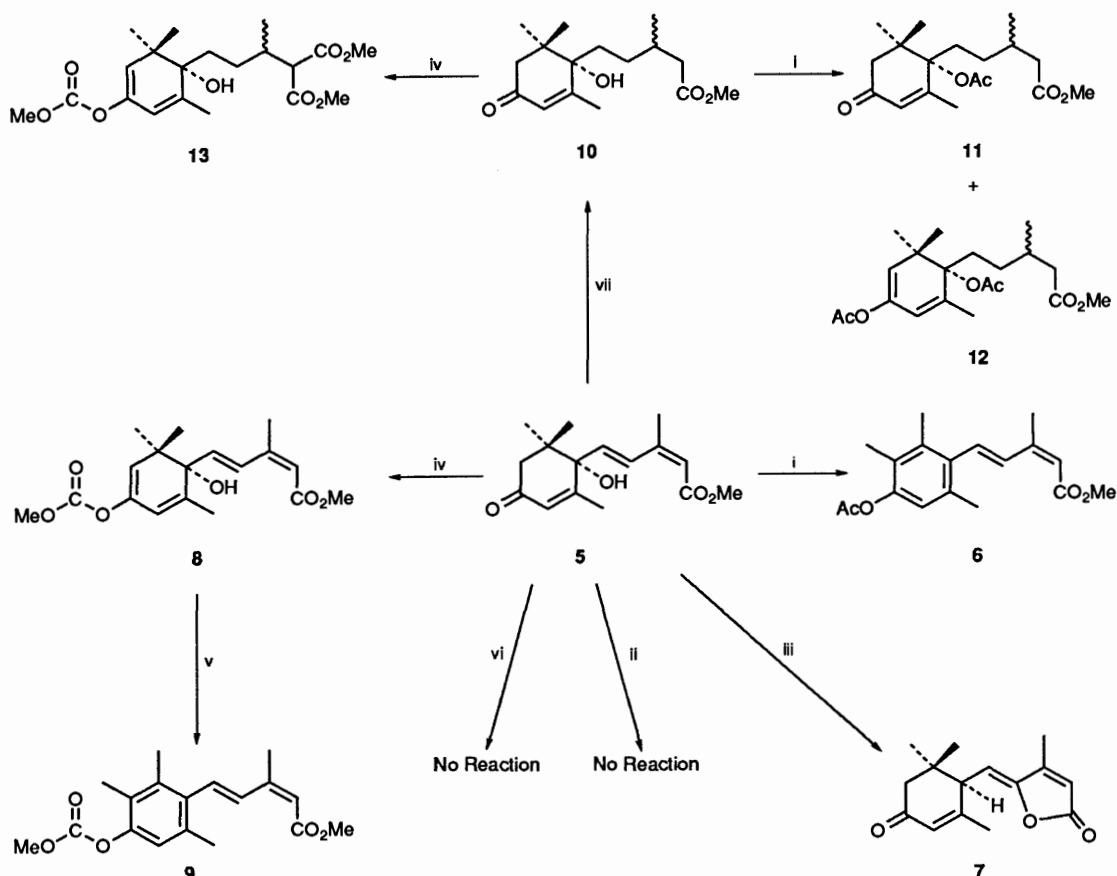


The reaction may be envisaged to proceed *via* the initially formed enol acetate which loses the 1'-hydroxy (or acetoxy) group to give the secondary carbocation (Scheme 2). Subsequent methyl migration to give the tertiary carbocation and aromatisation gives the product. The reaction was shown not to occur simply by acid catalysis, as treatment of ABA methyl ester with toluene-*p*-sulfonic acid in methanol overnight returned unchanged starting material. This is in contrast to the known instability of ABA in hydrochloric acid-formic acid which produces the unsaturated γ -lactone **7** along with other minor products (but not aromatic compounds).⁶

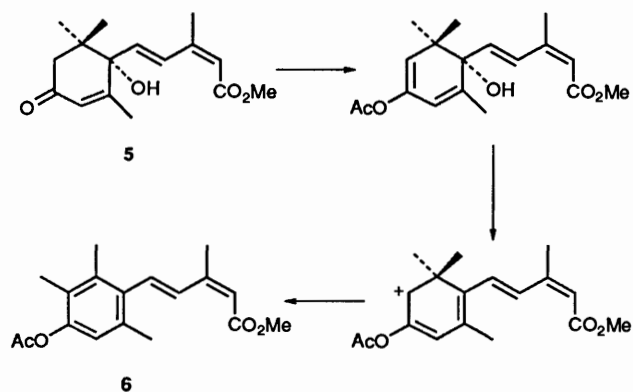
The role of the 4',5'-enol function in initiating the aromatisation was confirmed as follows. Treatment of ABA methyl ester **5** with lithium diisopropylamide (LDA) followed by methyl chloroformate gave the enol derivative **8**. The ¹H NMR spectrum of **8** displayed four methyl singlets at the expected chemical shifts (δ 1.10, 1.19, 1.79 and 2.04), two methoxy signals (δ 3.70 and 3.85) and broad olefinic signals at δ 5.54 and 5.70 assigned to 2-H and 3'-H, respectively. The ¹³C-DEPT spectrum confirmed that *O*-alkylation and not *C*-alkylation had indeed occurred [δ 121 (C-2), 125 (C-3') and 137 (C-5')]. The enol carbonate **8** was found to be unstable and on standing in deuteriochloroform at 5 °C readily aromatised to **9**, identified by comparison with the NMR data of **6** and by ¹³C-¹H correlation spectroscopy. ABA methyl ester **5** is stable in deuteriochloroform.

Phaseic acid **15** is a metabolite of (+)-(*S*)-ABA, which is

† Systematic name: (+)-(*S*)-5-(1'-hydroxy-2',6',6'-trimethyl-4'-oxocyclohex-2'-enyl)3-methylpentane-2,4-dienoic acid.



Scheme 1 Reactions of ABA methyl ester **5**. *Reagents and conditions*: i, Ac₂O, TsOH; ii, TsOH, MeOH; iii, HCl, HCO₂H; iv, LDA, ClCO₂Me; v, CDCl₃, 5 °C; vi, CDCl₃; vii, H₂, 10% Pd on CaCO₃.



Scheme 2 Proposed mechanism of aromatisation

formed *via* hydroxylation of the 9'-methyl group and cyclisation of the product *via* conjugate addition to the enone. Milborrow has reported⁷ that in contrast to ABA, acetylation of phaseic acid with acetic anhydride-pyridine proceeds smoothly to the 1'-acetate. Therefore, the resistance of the 1'-hydroxy group of ABA to derivatisation may be due to electronic effects of the neighbouring π -systems. To investigate this we attempted to saturate selectively the 2',3'-double bond of ABA. However attempted conjugate reduction with a number of reagents *e.g.* *L*-selectride, hexatriphenylcopper(0) hydride⁸ or tributyltin hydride in the presence of palladium(0)⁹ proved unsuccessful returning either starting material or giving an inseparable mixture of products. In contrast catalytic hydrogenation of ABA methyl ester **5** over palladium led to side chain saturation giving an inseparable mixture (1.5 : 1) of C-3 epimers **10**.

Treatment of hydrogenated ABA methyl ester **10** with acetic anhydride and toluene-*p*-sulfonic acid gave a complex mixture of products including the 1'-acetate **11** and a compound tentatively assigned the structure **12** from its GC-MS. No aromatic compounds were detected by either ¹H NMR or GC-MS. Treatment of **10** with lithium diisopropylamide followed by methyl chloroformate resulted in alkylation at C-2 as well as formation of the ring enol methyl carbonate giving **13** as the major product. As in the case of the acetylation reaction on **10**, no aromatic products were detected.

In conclusion it is apparent that abscisic acid fails to be acetylated at the C-1' hydroxy group because of combined electronic effect of the 4,5 and 2',3' double bonds. Formation of enol derivatives at C-4' leads to a facile aromatisation reaction which requires the presence of the side chain 4,5-olefin.

Experimental

All organic solvents were distilled prior to use, light petroleum refers to the fraction with the boiling range 40–60 °C. Mass spectra are electron impact and were recorded using a VG Micromas 3D8/RS2 MS9 double focussing mass spectrometer with an electron energy of 70 eV. ¹H and ¹³C NMR spectra were recorded on JEOL GX-270 and GX-400 spectrometers with deuteriochloroform as solvent unless otherwise stated, using tetramethylsilane as the internal standard. NMR chemical shifts are given in δ values as ppm downfield of tetramethylsilane. *J* Values are given in Hz. M.p. were recorded on a Gallenkamp apparatus. GC-MS was performed on a DANI 3800 HR G.C. system with a bonded OV-101 wall coated open tubular (WCOT) column (25 m \times 0.22 mm i.d., phase thickness 0.25 μ m) interfaced with a VG 7070 mass spectrometer using

VG 2050 data system. Flash chromatography was carried out according to the procedure outlined by Still *et al.*¹⁰ using silica gel (230–600 mesh ASTM, Merck Kieselgel 60). The columns were eluted with light petroleum–ethyl acetate mixtures increasing in polarity and the fractions analysed by TLC using Merck 60 HF₂₅₄ aluminium-backed plates. These were visualised under uv light and then by spraying with 5% sulfuric acid in ethanol, followed by heating at 120 °C for several minutes.

ABA Methyl Ester 5.—Abscisic acid **1** (0.407 g) in acetone (9 cm³) was treated dropwise with ethereal diazomethane at 0 °C until the yellow colour persisted. After a further 2 min, the excess diazomethane was blown off under a stream of nitrogen and the solvent removed under reduced pressure to give the methyl ester **5** as a yellow oil (0.430 g, 100%); δ_{H} 0.99 (s, 8'-H₃), 1.09 (s, 9'-H₃), 1.19 (s, 6-H₃), 1.99 (s, 7'-H₃), 2.29 (d, *J* 17, 5'-H), 2.48 (d, *J* 17, 5'-H), 3.36 (s, OH), 3.71 (s, CO₂CH₃), 5.73 (s, 2-H), 5.93 (s, 3'-H), 6.17 (d, *J* 16, 4-H) and 7.85 (d, *J* 16, 5-H); δ_{C} 20.9 (C-6), 22.5 (C-7), 24.5 (C-8), 25.7 (C-9), 43.0 (C-6'), 51.1 (C-5'), 52.6 (CO₂Me), 80.9 (C-1'), 119.4 (C-2), 128.3 (C-3'), 129.4 (C-4), 137.6 (C-5), 152.1 (C-2'), 163.0 (C-3), 170.2 (C-1) and 198.5 (C-4); *m/z* 278 (M⁺, 0.5%), 260 (1), 222 (4), 190 (100), 162 (27) and 134 (26).

Acetylation of ABA Methyl Ester 5.—ABA methyl ester **5** (100 mg) and toluene-*p*-sulfonic acid (8 mg) were stirred in acetic anhydride (10 cm³) for 5 h. Water (40 cm³) was added and the mixture extracted with ethyl acetate. The combined organic phase was dried with anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a yellow oil which was purified by flash chromatography. Elution with 10% ethyl acetate–light petroleum gave methyl (2Z,4E)-5-(4'-acetoxy-2',5',6'-trimethylphenyl)-3-methylpenta-2,4-dienoate **6** (20 mg) as a gum (Found: M⁺, 302.1521. C₁₈H₂₂O₄ requires *M*, 302.1518); δ_{H} 2.06 (s, 6-H₃), 2.16, 2.27 and 2.28 (3 × s, 5'-CH₃, 6'-CH₃, 7'-H₃), 2.32 (s, CH₃CO), 3.69 (s, 1-CO₂CH₃), 5.76 (s, 2-H), 6.74 (s, 3'-H), 6.98 (d, *J* 17, 4-H) and 7.72 (d, *J* 17, 5-H); δ_{C} 12.8 (C-6), 17.6, 20.8, 20.9 and 30.2 (5'-CH₃, 6'-CH₃, 7'-H₃, 4'-OCOCH₃), 51.1 (1-COOCH₃), 117.3 (C-2), 120.8 (C-3), 126.2 (C-4), 131.8 (C-5), 133.9 (C-3'), 134.6 (C-1'), 134.8 (C-6'), 136.5 (C-2'), 148.0 (C-5'), 150.5 (C-4'), 166.6 (4'-CO₂CH₃) and 169.6 (1-CO₂CH₃); *m/z* 302 (M⁺, 82%), 260 (100), 245 (62), 213 (67), 201 (66), 186 (85), 91 (41) and 43 (43).

Further elution with 10% ethyl acetate–light petroleum returned unchanged starting material **2** (45 mg).

Acetylation of ABA 1.—ABA **1** (100 mg) was stirred with toluene-*p*-sulfonic acid (20 mg) and acetic anhydride (5 cm³) for 16 h, at room temperature. Water (25 cm³) was added and partitioned with ethyl acetate (3 × 20 cm³). The combined organic extracts were dried over anhydrous sodium sulfate, concentrated under reduced pressure and the resultant brown oil purified by flash chromatography. Elution with 20% ethyl acetate–light petroleum gave (2Z,4E)-5-(4'-acetoxy-2',5',6'-trimethylphenyl)-3-methylpenta-2,4-dienoic acid **14** as a crystalline solid (55 mg); m.p. 173 °C (Found: M⁺, 288.1351. C₁₇H₂₀O₄ requires *M*, 288.1361); δ_{H} 2.04 (s, 6-H₃), 2.16, 2.24 and 2.25 (3 × s, 5'-CH₃, 6'-CH₃, 7'-H₃), 2.34 (s, 4'-COCH₃), 5.76 (s, 2-H), 6.74 (s, 3'-H), 6.93 (d, *J* 18, 4-H) and 7.74 (d, *J* 18, 5-H); *m/z* 288 (M⁺, 45%), 246 (59), 213 (24), 187 (100), 91 (26) and 43 (91).

Elution with 35% ethyl acetate–light petroleum returned unchanged ABA (**30** mg).

Treatment of ABA Methyl Ester 5 with Lithium Diisopropylamide and Methyl Chloroformate.—Diisopropylamine (130 mm³) was added to butyllithium (1.86 mol dm⁻³; 470 mm³) at room temperature in tetrahydrofuran (THF) (3 cm³) and

stirred for 10 min and then cooled to –78 °C. A solution of ABA methyl ester **5** (100 mg) in THF (2 cm³) was added dropwise, followed by methyl chloroformate (120 mm³, 2 equiv.). The reaction mixture was allowed to warm to room temperature over a period of 1 h and then stirred for 3 h before being worked up as usual. Purification by flash chromatography, eluting with 20% ethyl acetate–light petroleum gave methyl (2Z,4E)-5-(1'-hydroxy-4'-methoxycarbonyloxy-2',6',6'-trimethylcyclohexa-2',4'-dienyl)-3-methylpenta-2,4-dienoate **8** (36 mg) as an oil (Found: M⁺ 336.1559. C₁₈H₂₄O₆ requires *M*, 336.1573); δ_{H} 1.10 (s, 8'-H₃), 1.19 (s, 9'-H₃), 1.78 (br s, 7'-H₃), 2.04 (br s, 6-H₃), 3.70 (s, CO₂CH₃), 3.85 (s, OCO₂CH₃), 5.23 (s, 5'-H), 5.54 (br s, 2-H), 5.70 (br s, 3'-H), 6.56 (d, *J* 17, 4-H) and 7.84 (d, *J* 17, 5-H); δ_{C} 17.2 (C-8'), 21.4 (C-9'), 22.0 (C-6), 23.1 (C-7'), 40.7 (C-6'), 51.1 (1-CO₂CH₃), 55.0 (4'-OCO₂CH₃), 80.2 (C-1'), 117.7 (C-4), 118.7 (C-5), 121.2 (C-2), 125.1 (C-3'), 137.3 (C-5'), 142.4 (C-3), 144.9 (C-2'), 149.2 (C-4'), 151.3 (4'-OCO₂CH₃) and 167.0 (1-CO₂CH₃); *m/z* 336 (M⁺, 15%), 304 (9), 289 (23), 260 (4), 228 (28), 201 (45), 125 (51), 83 (100), 57 (30) and 43 (31).

Elution with 40% ethyl acetate–light petroleum returned starting material **2** (32 mg).

The enol derivative **9** was left to stand in deuteriochloroform in an NMR tube at 5 °C for 48 h. From the ¹H, ¹³C and ¹³C–¹H NMR correlated spectra it was evident that **8** had rearranged to the aromatic compound **9**; δ_{H} 2.12 (s, 6-H₃), 2.16, 2.27 and 2.29 (3 s, 5'-CH₃, 6'-CH₃, 7'-H₃), 3.69 (s, 1-CO₂CH₃), 3.92 (s, 4'-OCO₂CH₃), 5.76 (s, 2-H), 6.82 (s, 3'-H), 6.93 (d, *J* 17, 4 H) and 7.72 (d, *J* 17, 5-H); δ_{C} 12.9 (C-6), 17.6, 20.4 and 20.5 (5'-CH₃, 6'-CH₃, C-7'), 51.2 (1-CO₂CH₃), 56.4 (4'-OCO₂CH₃), 117.4 (C-2), 120.86 (C-3'), 126.3 (C-3) 131.9 (C-6'), 133.9 (4-C), 134.6 (C-1'), 134.8 (C-5), 136.5 (C-2'), 148.0 (C-5'), 150.5 (C-4'), 154.9 (4'-OCO₂CH₃) and 169.7 (1-CO₂CH₃).

Catalytic Hydrogenation of ABA Methyl Ester 5.—ABA methyl ester **5** (650 mg) in methanol (15 cm³) was stirred with 10% palladium on calcium carbonate (40 mg) under a hydrogen atmosphere at room temperature. The catalyst was filtered off and the solvent removed under reduced pressure. Purification by flash chromatography eluting with 20% ethyl acetate–light petroleum gave a 1.5:1 mixture of isomers of methyl 5-(1'-hydroxy-2',6',6'-trimethyl-4'-oxocyclohex-2'-enyl)-3 ξ -methylpentanoate **10** (620 mg) as a white solid (Found: M⁺, 282.1839 C₁₆H₂₆O requires *M*, 282.1857); *major isomer* δ_{H} 0.93 (d, *J* 6, 6-H₃), 1.05 (br s, 8'-H₃), 1.08 (br s, 9'-H₃), 1.43, 1.64, 1.72, 1.79 and 1.87 (4 × m, 5-H₂, 3-H, 4-H₂), 1.79 (m, 5-H), 1.87 (m, 4-H), 2.05 (d, *J* 1.5, 7'-H₃), 2.21 (t, *J* 7, 2-H), 2.25 (br d, *J* 18, 5'-H), 2.26 (dd, *J* 7, 3, 2-H), 2.52 (br d, *J* 18, 5'-H), 3.67 (s, CO₂CH₃) and 5.86 (br s, 3'-H); *minor isomer* δ_{H} 0.96 (d, *J* 6, 6-H₃), 1.05 (s, 8'-H₃), 1.08 (s, 9'-H₃), 1.18, 1.47, 1.69, 1.75 and 1.85 (5 × m, 5-H₂, 3-H, 4-H₂), 1.99 (d, *J* 1.5, 7'-H₃), 2.13 (t, *J* 7, 2-H), 2.25 (br d, *J* 18, 5-H), 2.30 (dd, *J* 7, 2.5, 2-H), 2.52 (br d, *J* 18, 5'-H), 3.66 (s, CO₂CH₃) and 5.86 (br s, 3'-H); *m/z* 282 (M⁺, 3%), 194 (38), 150 (100) and 125 (27).

Acetylation of Hydrogenated ABA Methyl Ester 10.—Hydrogenated ABA methyl ester **10** (140 mg) in acetic anhydride (10 cm³) was stirred with toluene-*p*-sulfonic acid (12 mg) for 22 h at room temperature. The usual work up gave an oil which was purified by flash chromatography. Elution with 15% ethyl acetate–light petroleum gave a 1.5:1 mixture of diastereoisomers of methyl 5-(1'-acetoxy-4'-hydroxy-2',6',6'-trimethylcyclohexa-2',4'-dienyl)-3 ξ -methylpentanoate **11** as a gum (22 mg); δ_{H} (major isomer) 0.94 (d, *J* 6.5, 6'-H₃), 1.09 (s, 8'-H₃), 1.16 (s, 9'-H₃), 2.04 (s, 7'-H₃), 2.08 (s, OAc), 2.25 (br d, *J* 18, 5'-H), 2.52 (br d, *J* 18, 5'-H), 3.66 (s, CO₂CH₃) and 5.87 (br s, 3'-H); δ_{H} (minor isomer) 0.96 (d, *J* 6.5, 6'-H₃),

1.09 (s, 8'-H₃), 1.16 (s, 9'-H₃), 2.03 (s, 7'-H₃), 2.08 (s, OAc), 2.25 (d, *J* 18, 5'-H), 2.52 (d, *J* 18, 5'-H), 3.66 (s, CO₂CH₃) and 5.87 (br s, 3'-H); *m/z* 324 (M⁺, 1%), 282 (7), 264 (7), 226 (7), 194 (9), 149 (24), 91 (16) and 44 (100). Further elution with 20% ethyl acetate–light petroleum gave a complex mixture of products which were analysed by GC–MS. From the mass spectra, one of the components of the mixture was tentatively assigned as the diacetoxy derivative **12**; *m/z* 366 (M⁺, 1%), 324 (5), 306 (8), 264 (35), 249 (18), 191 (15), 135 (87) and 43 (100).

Treatment of 10 with Lithium Diisopropylamide and Methyl Chloroformate.—Diisopropylamine (300 mm³) was added dropwise to butyllithium (1.86 mol dm⁻³; 1.14 cm³) at room temperature in THF (3 cm³) and stirred for 10 min before being cooled to -78 °C. Hydrogenated ABA methyl ester **10** (200 mg) in THF (2 cm³) was added dropwise to the LDA solution, followed by methyl chloroformate (200 mm³). The reaction was allowed to warm to room temperature over 1.5 h and then worked up as usual. Purification by flash chromatography, eluting with 20% ethyl acetate–light petroleum gave a 1.5 : 1 mixture of diastereoisomers of methyl 5-(1'-hydroxy-4'-methoxycarbonyloxy-2',6',6'-trimethylcyclohexa-2',4'-dienyl)-2-methoxycarbonyl-3-methylpentanoate **13** as a gum (110 mg) (Found: M⁺, 398.1930 C₂₀H₃₀O₈ requires *M*, 398.1940); *major isomer* δ_H 0.91 (d, *J* 7, 6-H₃), 1.00 (s, 8'-H₃), 1.01 (s, 9'-H₃), 1.78 (s, 7'-H₃), 3.20 (d, *J* 7, 2-H), 3.65 and 3.66 (2 × s, 1-H₃, 2-CO₂CH₃, 4'-OCO₂CH₃), 5.09 (br s, 5'-H) and 5.57 (br s, 3'-H); *minor isomer* δ_H 0.88 (d, *J* 7, 6-H₃), 1.00 (s, 8'-H₃), 1.01 (s, 9'-H₃), 1.78 (s, 7'-H), 3.22 (d, *J* 7, 2-H), (2 × s, 1-OCH₃, 2-

CO₂CH₃, 4'-OCO₂CH₃), 5.09 (br s, 5'-H) and 5.57 (br s, 3'-H); *m/z* 398 (M⁺, 13%), 342 (7), 322 (20), 317 (5), 305 (5), 281 (5), 181 (24), 159 (43), 135 (94), 83 (67), 69 (94), 55 (83) and 43 (100).

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