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Research Article

Deuterated abscisic acid analogs for mass spectrometry and metabolism studies

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Summary

Four analogs of abscisic acid (ABA) with deuterium atoms at non-exchangeable positions have been synthesized to be used as standards for quantitation of the plant hormone ABA by mass spectrometry and also to be employed as substrates for metabolism studies. Deuterium atoms were introduced in the side chain of the molecule, at C-4 and/or C-5, by deuteride or hydride reduction of a propargylic alcohol, an intermediate in the synthesis. As well, deuterium labels were introduced at the C-8′ position by conjugate addition of a Grignard reagent containing the label to a cyclohexadienone intermediate, affording specific isotopically labeled ABA molecules with one to five deuterium atoms. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: abscisic acid; ^{2}H labeled standard; hydride reduction of propargylic alcohol; 1,4-conjugate addition of Grignard

Introduction

Abscisic acid, ABA, **1**, a monocyclic sesquiterpenoid natural product, is a signaling molecule present at low concentrations in all higher plants. ABA has been found to play important roles in plant growth and development in diverse processes including regulation of stomatal

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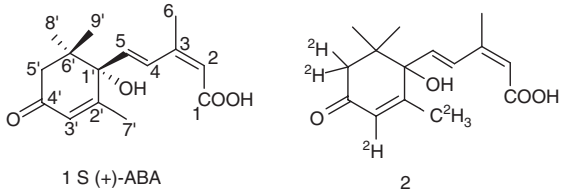


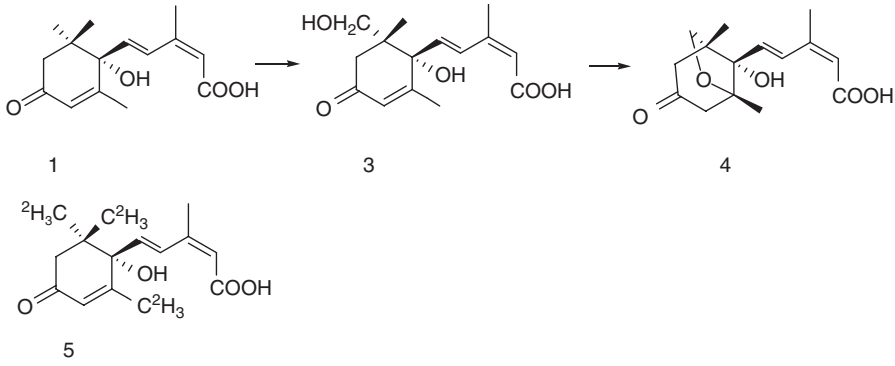
Figure 1. ABA 1 and [$^2H_6$]ABA 2

aperture, seed maturation and germination, root and shoot growth, transpiration and stress tolerance.[1,2] For example, ABA regulates genes involved in the synthesis of storage reserves in developing seeds and prevents immature seeds from germinating prematurely.[3] In response to abiotic stresses such as heat, freezing or drought, ABA levels in plants elevate, triggering specific biochemical responses.[4] The biosynthesis of ABA and some catabolic pathways have been elucidated, and a number of genes involved in ABA biosynthesis have been cloned.[5, 6] There is currently much research into the molecular basis of ABA action and a growing need for improved tools for studying ABA levels and turnover.[7]

The most generally employed methods to measure ABA levels in plant tissues are gas chromatography combined with mass spectrometry (GC/MS) of ABA methyl ester, and RIA or ELISA immunoassay of the free acid using a monoclonal antibody raised against a conjugated derivative of ABA, verified by GC/MS(Figure 1).[8]

[$^2H_6$]ABA **2** has been most commonly employed as the internal standard in GC/MS quantitation of ABA. This internal standard, synthesized by base exchange,[9] works well for analytical procedures in which base exchange is minimal. Efforts have been made to develop an ABA deuterated at C-6 of the side chain, to provide an internal standard that would be stable to exchange, with limited success.[10] The synthesis of a ^{13}C labeled ABA has been reported.[11]

Deuterated ABA analogs have also been employed in plant metabolism studies to assess the relative roles of ABA and its catabolites in biological processes affected by ABA.[12] The principal oxidative degradation pathway of ABA (Scheme 1) is through hydroxylation at the 8′-carbon atom, thought to be mediated by a P450 monooxygenase.[5] The initial oxidation product, 8′-hydroxy ABA, **3** , exists in equilibrium with the bicyclic metabolite phaseic acid **4**. In metabolism studies using corn cells, the rate of oxidation of the deuterated ABA **5** has been found to be significantly slower than that of the natural hormone **1**. This



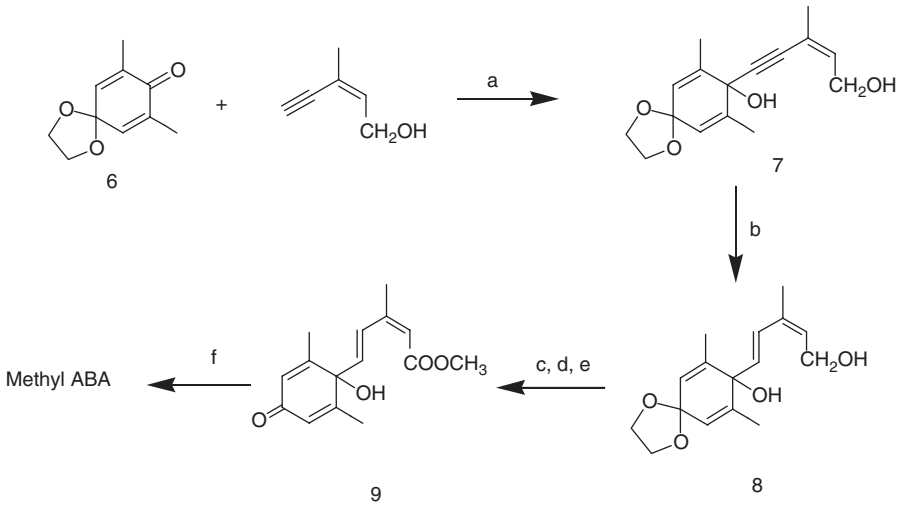
Scheme 1. Principal oxidative pathway of metabolism of ABA

reduced rate was attributed to a primary isotope effect, arising from cleavage of the stronger C–D versus C–H bond at the 8′-carbon atom.[9] The ABA (+)-**5** was employed as a probe to assess the role of the hormone in cress seed germination. Nonadeutero (+)-**5** was significantly more effective than the natural hormone (+)-**1**, consistent with the hormone itself being the biologically active species, and that the oxidation process through the 8′-carbon atom is the key degradation pathway.

In this paper, we report the synthesis of a series of deuterated ABA molecules, designed as internal standards for GC/MS and HPLC coupled to electrospray mass spectrometry (HPLCMS/MS). The molecules were also designed to be starting materials for standards of ABA metabolites through biotransformation of labeled ABA molecules, and as probes for studying ABA metabolism pathways *in planta*.

Results and discussion

The general scheme employed for synthesizing ABA with deuterium atoms in the side chain and the ring was based on a previous syntheses of ABA and analogs modified at the 8′-carbon atom.[13,14] The basic skeleton of the molecule is constructed by alkylation of the monoprotected cyclohexadienone **6** with the dilithium salt of *cis*-3-methylpent-2-en-4-yn-1-ol (Scheme 2). Reduction of the triple bond of the propargylic alcohol of the product **7** affords the dienol **8**. Oxidation of the alcohol to the ester followed by deprotection of the ketone affords the cyclohexadienone **9**. The 8′-methyl group of ABA is introduced by conjugate addition of methyl magnesium bromide to the enone of **9**. It



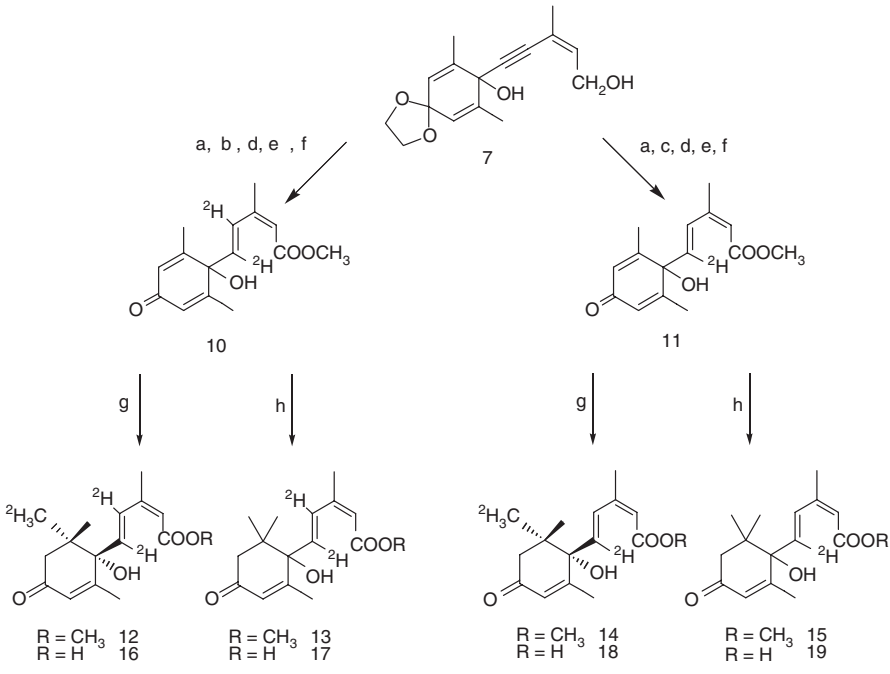
Scheme 2. Reagents (a) n-BuLi, THF; (b) Red-Al®; (c) MnO_2, acetone; (d) MnO_2, NaCN, AcOH, MeOH; (e) HCl, THF; (f) CH_3MgBr

had earlier been shown that the conjugate addition afforded the product with alkyl group on the same face of the molecule as the hydroxyl group. The reaction performed with trideuterated methyl magnesium bromide afforded 8′-trideuteromethyl ABA.[13]

The scheme was modified to incorporate deuterium specifically at either the C-4, C-5 or both positions by deuteride reduction of the triple bond of the propargylic alcohol.[15] The deuteride addition takes place on the carbon alpha to the hydroxyl with the postulated formation of a cyclic intermediate. The proton or deuteron on the beta position arises from quenching of the reaction with water or deuterium oxide. Thus, reduction of **7** with lithium aluminum deuteride followed by quenching with deuterium oxide afforded the dideuterated dienol that was converted to the methyl ester **10** (Scheme 3). Reduction of the triple bond of **7** with deuteride reagent, followed by quenching with water, led to incorporation of deuterium at the C-5 position, which on further oxidation afforded **11**.

The mono and dideuterated cyclohexadienones **10** and **11** were converted to the labeled ABA by reaction with methyl magnesium bromide or trideuteromethyl magnesium bromide, followed by hydrolysis of the methyl ester, affording **16** – **19** (Scheme 3).

Analysis of the deuterium incorporation achieved in the procedure was estimated by examination of the molecular ion clusters in LC/MS/MS spectra of the acids **16** – **19**. The ion for ABA $^2H_0$ (m/z 263) in the



Scheme 3. Reagents (a) $LiAl^2H_4$, 2 $CH_3O(CH_2)_2OH$; (b) ^2H_2O; (c) H_2O; (d) MnO_2, acetone; (e) MnO_2, NaCN, AcOH, MeOH; (f) HCl, THF; (g) C^2H_3MgI (h) CH_3MgBr

preparation of the acid **19** was found to be 0.5% of the ion for the monodeuterated analog, indicating the introduction of the deuteride at C-5 gave 99.5% incorporation of the label. The sample of acid **18** contained 0.8% ABA $^2H_3$ indicating that the Grignard addition with labeled reagent proceeded with essentially quantitative isotopic incorporation. The samples of acids **16** and **17** contained 2 and 1.7% of the ABA with 1 Da lower than the expected value, consistent with a slightly lower incorporation of label in the quenching with deuterium oxide.

Experimental section

The synthesis of the unlabeled ABA using this procedure has been published.[14] The procedures and spectra for synthesis of the isotopically labeled ABA molecules are presented below. LCMS/MS spectra were obtained in negative ion electrospray mode using a capillary voltage of–2.75 KV, counter electrode 35 V, collision energy (E_{LAB}) of 14 V, and cell pressure of 1.0×10^{-3} mBar with argon.

*Methyl-(2Z,4E)-[4,5-$^2H_2$]-5-(1-hydroxy-2,6-dimethyl-4-oxo-2,5-cyclohexadien-1-yl)-3-methylpenta-2,4-dienoate (***10***).*

To a solution of $LiAl^2H_4$ (96 atom % ^2H) (22.7 ml 1.0 M in THF, 22.7 mmol) in THF (50 ml) at 0°C was added dropwise 2-methoxyethanol (3.57 ml, 45.3 mmol). After 30 min., the solution was cannulated to a stirred solution of ketal alcohol **7** (4.17 g, 15.1 mmol) in THF (80 ml) at -78°C. The solution was warmed to 0°C. After 2 h, the reaction was quenched with ^2H_2O (99.9 atom % ^2H) (10 ml) and allowed to stir for 1 h. Saturated $NaHCO_3$ (50 ml) was added and the solution was extracted with ether (3 × 150 ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. The crude product was dissolved in acetone (100 ml) and stirred with MnO_2 (26.0 g, 300 mmol) at room temperature. After 2 h, the solution was filtered through a pad of Celite® and concentrated under vacuum. The crude ketal aldehyde was dissolved in methanol (150 ml) and stirred with NaCN (1.78 g, 36.2 mmol), AcOH (0.86 ml, 15.1 mmol) and MnO_2 (19.7 g, 226 mmol) at room temperature. After 1.5 h, the solution was filtered through a pad of Celite® and concentrated under vacuum. The residue was brought up in saturated $NaHCO_3$ (50 ml) and extracted with ether (3 × 150 ml). The combined organic layers were dried ($MgSO_4$), filtered through a pad a silica gel and concentrated under vacuum. To a solution of the ketal ester (3.76 g, 12.2 mmol) in THF (100 ml) was added HCl (10%, 1 ml) and water (5 ml). After 15 min., the acid was quenched with saturated. $NaHCO_3$ (20 ml) and diluted with ether (200 ml). The organic layer was washed with brine (20 ml), dried ($MgSO_4$) and concentrated under vacuum. Flash chromatography (EtOAc-hexane, 1:3) gave methyl ester **10** (2.05 g, 51%). Mp: 136.5–137.5°. IR λ_{max}: 3366, 2954, 1715, 1669, 1619, 1438, 1380, 1238, 1162 cm^{-1}. ^1H NMR: δ 6.03 (s, 2 H), 5.73 (s, 1 H), 3.69 (s, 3 H), 1.97 (s, 6 H), 1.94 (s, 3 H). ^{13}C NMR: 185.6, 166.4, 160.3, 149.1, 136.0 (m), 128.5 (m), 126.4, 118.3, 75.0, 51.2, 20.9, 18.1. HRMS: calcd for $C_{15}H_{16}{}^2H_2O_4$ $[M]^+$ m/z 264.1329, found 264.1324.

*Methyl-(2Z,4E)-[5-^2H]-5-(1-hydroxy-2,6-dimethyl-4-oxo-2,5-cyclohexadien-1-yl)-3-methylpenta-2,4-dienoate (***11***).*

Ketal alcohol **7** (13.6 g, 48.0 mmol) was transformed into methyl ester **11** (6.44 g, 51%) following the same procedure for methyl ester **10**, with the following change. After reduction of the alkyne the reaction was quenched with H_2O (10 ml). Mp: 136.6–136.7°. IR λ_{max}: 3358, 2953,

1715, 1668, 1622, 1436, 1380, 1238, 1161 cm^{-1}. ^1H NMR: δ 8.03 (s, 1 H), 6.06 (s, 2 H), 5.74 (s, 1 H), 3.71 (s, 3 H), 1.97 (s, 6 H), 1.95 (s, 3 H). ^{13}C NMR: 185.7, 166.4, 160.5, 149.2, 136.0 (m), 128.6, 126.4, 118.3, 75.0, 51.2, 21.0, 18.1. HRMS: calcd for $C_{15}H_{17}{}^2H_2O_4$ $[M]^+$ m/z 263.1267, found 263.1266.

*Methyl-(2Z,4E)-[4,5-$^2H_2$]-5-[(1R,6R)/(1S,6S)-1-hydroxy -2,6-dimethyl-6-(methyl-$^2H_3$)-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoate (***12***).*

To a stirred solution of methyl ester **10** (2.00 g, 7.58 mmol) in THF (50 ml) at -78°C was added dropwise C^2H_3MgI (99+ atom % ^2H) (37.9 ml, 1.0 M solution in diethyl ether) using a syringe pump (rate = $0.33ml^{-1}min$). After 6 h, the temperature was raised to 0°C and the reaction was quenched with saturated NH_4Cl (25 ml). The solution was extracted with ether (3 × 150 ml) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. Flash chromatography (EtOAc–hexane 1:4) gave methyl $^2H_5$-ABA **12** (1.06 g, 49%). IR λ_{max}: 3478, 2955, 1711, 1660, 1435, 1377, 1238, 1160 cm^{-1}. ^1H NMR: δ 5.92 (q, J = 1 Hz, 1 H), 5.72 (q, J = 1 Hz, 1 H), 3.67 (s, 3 H), 2.44 (d, J = 17 Hz, 1 H), 2.26 (d, J = 17 Hz, 1 H), 1.98 (d, J = 1 Hz, 3 H), 1.90 (d, J = 1 Hz, 3 H), 0.98 (s, 3 H). ^{13}C NMR: δ 197.8, 166.4, 162.4, 149.3, 135.9 (m), 127.9 (m), 127.0, 118.2, 79.6, 51.2, 49.7, 41.3, 24.2, 22.2 (m), 21.1, 18.9. GCMS (EI) m/z: 192 (65), 191 (52), 163 (39), 136 (71), 127 (100), 113 (39), 109 (26), 93 (35).

*Methyl-(2Z,4E)-[4,5-$^2H_2$]-5-[(1RS)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoate (***13***).*

Methyl ester **10** (2.01 g, 7.6 mmol) was transformed into methyl $^2H_2$-ABA **13** (1.07 g, 49%) following the same procedure for methyl $^2H_5$-ABA **12**, with the following change. The Grignard reagent used was CH_3MgBr (12.7 ml, 3.0 M solution in diethyl ether). IR λ_{max}: 3478, 2953, 1714, 1660, 1621, 1435, 1375, 1238, 1161 cm^{-1}. ^1H NMR: δ 5.92 (q, J = 1 Hz, 1 H), 5.72 (q, J = 1 Hz, 1 H), 3.67 (s, 3 H), 2.44 (d, J = 17 Hz, 1 H), 2.26 (d, J = 17 Hz, 1 H), 1.98 (d, J = 1 Hz, 3 H), 1.90 (d, J = 1 Hz, 3 H), 1.08 (s, 3 H), 0.99 (s, 3 H). ^{13}C NMR: δ 197.7, 166.4, 162.4, 149.3, 135.9 (m), 127.7 (m), 127.0, 118.2, 79.6, 51.2, 49.7, 41.5, 24.2, 23.0, 21.1, 18.9. GC/MS (EI): m/z 192 (92), 191 (69), 163 (46), 136 (84), 127 (100), 113 (44), 109 (52), 93 (51).

Methyl-(2Z,4E)-[5-^{2}H]-5-[(1R,6R)/(1S,6S)-1-hydroxy–2,6-dimethyl-6-(methyl-$^2H_3$)-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoate (**14**).

Methyl ester **11** (5.17 g, 19.7 mmol) was transformed into methyl $^2H_4$-ABA **14** (2.85 g, 51%) following the same procedure for methyl $^2H_5$-ABA **12**. IR λ_{max}: 3471, 2957, 1714, 1660, 1625, 1436, 1377, 1240, 1161. ^{1}H NMR: δ 7.84 (s, 1 H), 5.92 (q, J = 1 Hz, 1 H), 5.73 (q, J = 1 Hz, 1 H), 3.68 (s, 3 H), 2.44 (d, J = 17 Hz, 1 H), 2.26 (d, J = 17 Hz, 1 H), 1.98 (d, J = 1 Hz, 3 H), 1.90 (d, J = 1 Hz, 3 H), 0.99 (s, 3 H). ^{13}C NMR: δ 197.7, 166.4, 162.4, 149.3, 135.9 (m), 128.0, 127.1, 118.2, 79.6, 51.2, 49.7, 41.3, 24.2, 21.2, 18.9 . GC/MS (EI) m/z: 191 (100), 163 (39), 135 (71), 126 (84), 112 (45), 92 (37).

Methyl-(2Z,4E)-[5-^{2}H]-5-[(1RS)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoate (**15**).

Methyl ester **11** (98.0 mg, 0.37 mmol) was transformed into methyl ^{2}H-ABA **15** (56 mg, 54%) following the same procedure for methyl $^2H_5$-ABA **12**, with the following change. The Grignard reagent used was CH_3MgBr (0.62 ml, 3.0 M solution in diethyl ether). IR λ_{max}: 3477, 2951, 1714, 1655, 1625, 1438, 1376, 1239, 1161 cm^{-1}. ^{1}H NMR: δ 7.80 (s, 1 H), 5.90 (q, J = 1 Hz, 1 H), 5.71 (q, J = 1 Hz, 1 H), 3.65 (s, 3 H), 2.43 (d, J = 17 Hz, 1 H), 2.25 (d, J = 17 Hz, 1 H), 1.97 (d, J = 1 Hz, 3 H), 1.89 (d, J = 1 Hz, 3 H), 1.07 (s, 3 H), 0.98 (s, 3 H). ^{13}C NMR: δ 197.8, 166.4, 162.6, 149.4, 136.1 (m), 127.9, 127.0, 118.1, 79.6, 51.2, 49.7, 41.5, 24.3, 23.0, 21.2, 18.9. GCMS (EI) m/z: 191 (100), 163 (41), 135 (75), 126 (84), 112 (36), 92 (54).

(2Z,4E)-[4,5-$^2H_2$]-5-[(1R,6R)/(1S,6S)-1-hydroxy-2,6-dimethyl-6-(methyl-$^2H_3$)-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoic acid (**16**).

Methyl ester **12** (1.06 g, 3.75 mmol) was dissolved in ethanol (15 ml) and water (50 ml). A solution of KOH (300 mg) in water (1 ml) was added and the solution was heated to 50°C. Water (100 ml) was slowly added over the course of the reaction so that the starting material would remain in solution. After 3 h, the solution was cooled to room temperature, acidified with 10% HCl, extracted into EtOAc (3×100 ml), dried ($MgSO_4$) and concentrated under vacuum. The residue was brought up in EtOAc (150 ml) and washed with saturated $NaHCO_3$ (3×25 ml). The aqueous phase was acidified with 10% HCl,

extracted into EtOAc (3×100 ml), dried ($MgSO_4$) and concentrated under vacuum to give $^2H_5$-ABA **16** (0.91 g, 90%). IR λ_{max}: 3600–2400, 3450, 2961, 1651, 1374, 1258, 1160 cm^{-1}. ^1H NMR: δ 5.95 (q, J = 1 Hz, 1 H), 5.75 (q, J = 1 Hz, 1 H), 2.47 (d, J = 17 Hz, 1 H), 2.27 (d, J = 17 Hz, 1 H), 2.03 (d, J = 1 Hz, 3H0, 1.91 (d, J = 1 Hz, 3H), 1.01 (s, 3H). ^{13}C NMR: δ 197.9, 170.3, 162.5, 151.5, 136.4 (m), 127.9 (m), 127.1, 117.8, 79.8, 49.6, 41.4, 24.2, 23.0 (m), 21.4, 19.0. LCMS/MS m/z: 268 $(M\text{-}H)^-$ (13), 224 (55), 209 (14), 206 (18), 205 (6), 157 (100), 156 (93), 155 (16), 114 (5).

Methyl esters **13**, **14** and **15** were hydrolyzed following the procedure for compound **16** to give $^2H_2$-ABA **17**, $^2H_4$-ABA **18** and ^2H-ABA **19**, respectively.

*(2Z,4E)-[4,5-$^2H_2$]-5-[(1RS)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoic acid (***17***).*

IR λ_{max}: 3600–2400, 3450, 2968, 1658, 1375, 1255, 1161. ^1H NMR ($C^2H_3O^2H$): δ 5.91 (q, J = 1 Hz, 1H), 5.73 (q, J = 1 Hz, 1H), 2.52 (d, J = 17 Hz, 1 H), 2.18 (d, J = 17 Hz, 1H), 2.03 (d, J = 1 Hz, 3H), 1.92 (d, J = 1 Hz, 3H), 1.06 (s, 3H), 1.02 (s, 3H). ^{13}C NMR ($C^2H_3O^2H$): δ 201.0, 169.4, 166.5, 151.0, 137.5 (m), 129.0 (m), 127.6, 119.5, 80.5, 50.7, 42.8, 24.7, 23.5, 21.2, 19.6. LCMS/MS m/z: 265 $(M\text{-}H)^-$ (10), 221 (56), 206 (23), 203 (11), 202 (6), 154 (100), 153 (91), 152 (16), 111 (6).

*(2Z,4E)-[5-^2H]-5-[(1R,6R)/(1S,6S)-1-hydroxy-2,6-dimethyl-6-(methyl-$^2H_3$)-4-oxo-2-cyclohexen-1-yl]3-methylpenta-2,4-dienoic acid (***18***).*

IR λ_{max}: 3600–2400, 3444, 2962, 1660, 1377, 1257, 1162 cm^{-1}. ^1H NMR: δ 7.79 (s, 1H), 5.95 (q, J = 1 Hz, 1H), 5.75 (q, J = 1 Hz, 1H), 2.47 (d, J = 17 Hz, 1H), 2.27 (d, J = 1 Hz, 1H), 2.03 (d, J = 1 Hz, 3H), 1.91 (d, J = 1 Hz, 3H), 1.01 (s, 3H). ^{13}C NMR: δ 198.1, 170.9, 162.7, 151.6, 136.5 (m), 128.2, 127.1, 117.9, 79.8, 49.6, 41.4, 24.2, 23.0 (m), 21.4, 19.0. LCMS/MS m/z: 267 $(M\text{-}H)^-$ (6), 223 (30), 208 (8), 205 (12), 204 (2), 156 (100), 155 (10), 114 (3).

*(2Z,4E)-[5-^2H]-5-[(1RS)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methylpenta-2,4-dienoic acid (***19***).*

IR λ_{max}: 3600–2400, 3450, 2967, 1655, 1255, 1160 cm^{-1}. ^1H NMR ($C^2H_3O^2H$): δ 7.76 (s, 1H), 5.91 (q, J = 1 Hz, 1H), 5.73 (q, J = 1 Hz, 1H), 2.52 (d, J = 17 Hz, 1H), 2.18 (d, J = 1 Hz, 1H), 2.03 (d, J = 1 Hz,

3H), 1.92 (d, J = 1 Hz, 3H), 1.05 (s, 3H), 1.02 (s, 3H). ^{13}C NMR ($C^2H_3O^2H$): δ 201.0, 169.4, 166.5, 151.1, 137.6 (m), 129.3, 127.6, 119.5, 80.5, 50.6, 42.8, 24.7, 23.5, 21.3, 19.6. LCMS/MS m/z: 264 $(M\text{-}H)^-$ (5), 220 (34), 205 (15), 202 (9), 153 (100), 152 (10), 111 (3).

Conclusions

The synthetic scheme yielded ABA molecules with deuterium incorporation of high regiochemical and isotopic purity. The isotopic labels at C-4, -5 and -8′ are stable under acid or base conditions. The procedure would also be amenable for synthesis of tritiated ABA analogs. The labeled analogs are useful standards for mass spectrometry analysis of ABA using LCMS/MS but not for GC/MS. In GC/MS, the ion used for quantitation for unlabeled methyl ABA has m/z 190, which arises from loss of methanol and 2-butene from the molecular ion. The loss of methanol arises from the methoxyl group of the ester and a proton from either the hydroxyl group or C-4.[16,17] For the deuterated analogs with one deuterium in the side-chain at C-5 the base peak has m/z 191 and for those with two deuterium atoms in the sidechain the base peak has m/z 192 with m/z 191 about 80% of the intensity of the base peak. The compounds **16 – 19** prepared in this work can be used for negative ion mode electrospray LC/MS/MS as the base peak corresponds to the molecular ion. The complete series of isotopically labeled ABA analogs have been employed in studies on the fragmentation of ABA by electrospray mass spectrometry to be reported elsewhere. The (+)-enantiomer of **17** is a particularly useful molecule for tracking ABA catabolism as it is labeled at carbons not susceptible to oxidation by plant enzymes. As the molecular weight of the ABA analog **17** is 2 Da higher than the natural substance it and the analogous catabolites can clearly be distinguished from endogenous ABA and metabolites by mass spectrometry. The ABA analogs **16** and **18** have utility as precursors through biotransformation by plant cells[13] to labeled 7′-hydroxyABA, a mass spectrometry standard for the ABA catabolite.

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