Preparation of (±)-[1,2-$^{13}C_2$]Abscisic Acid for Use as a Stable and Pure Internal Standard†

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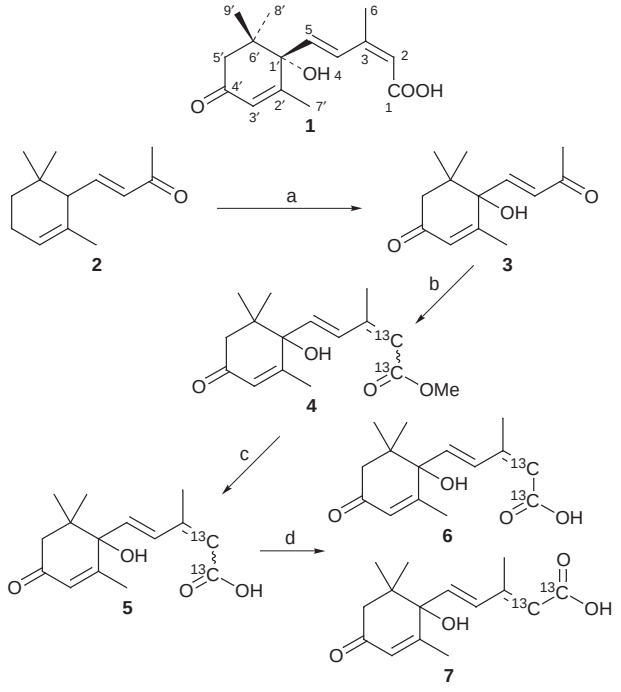
[1,2-$^{13}C_2$]ABA, which is designed as a stable and pure internal standard for GC/MS analysis, is synthesized through the Witting reaction of 1-hydroxy-4-keto-α-ionone with carbomethoxymethylenetriphenylphosphorane prepared from [1,2-$^{13}C_2$]bromoacetic acid followed by saponification of the product methyl [1,2-$^{13}C_2$]ABA.

Abscisic acid (ABA) **1** is involved in the control of many processes in plants; such as the acceleration of abscission, induction of dormancy, inhibition of rooting, and stimulation of stomatal closure.[1] The determination of endogenous concentrations of ABA is essential for elucidating the biosynthesis, metabolism, and mode of action of ABA at the molecular level, and isotopically labeled ABA is a powerful tool for determining such endogenous concentrations of ABA by GC/MS analysis. The syntheses of [$^2H_6$]ABA[2] and [3-Me-$^2H_3$]ABA[3] have been reported. [$^2H_6$]ABA is readily synthesized from ABA by an exchange of protons, but its use is restricted since extractions must be carried out below pH 8. [3-Me-$^2H_3$]ABA is more stable than [$^2H_6$]ABA to saponification conditions using alcoholic KOH but its deuterium content is 73% and includes both [$^2H_1$] and [$^2H_2$]. Since there has been no report on labeled ABA which is stable and isotopically pure, we report here the synthesis of ^{13}C-labeled ABA which meets our requirements as an internal standard for use in GC/MS analysis.



Scheme 1 Reagents: (a) *tert*-butylchomrate; (b) $Ph_3P{=}{}^{13}CHCOOMe$; (c) EtOH, KOH; (d) ODS–HPLC

To prepare pure isotopically labeled ABA, we adopted a preparative procedure using [1,2-$^{13}C_2$]bromoacetic acid (purchased from Nippon Sanso Co. Ltd., Tokyo, Japan) as a starting material based on that reported by Roberts *et al.* (Scheme 1).[4] [1,2-$^{13}C_2$]Bromoacetic acid was reacted with diazomethane to give methyl [1,2-$^{13}C_2$]bromoacetate in almost quantitative yield. Carboxymethyltriphenylphosphonium bromide was synthesized in quantitative yield from triphenylphosphine and methyl [1,2-$^{13}C_2$]bromoacetate. Carbomethoxymethylenetriphenylphosphorane was then prepared [mp (recryst) 123–125 °C] by treatment with aqueous NaOH. 1-Hydroxy-4-keto-α-ionone **3**, a common intermediate in the synthesis of ABA, was obtained by the oxidation of α-ionone (**2**) with *tert*-butyl chromate in refluxing *tert*-butyl alcohol.[4] Wittig reaction of **3** and phosphorane gave a mixture **4** of methyl [1,2-$^{13}C_2$]ABA and methyl [1,2-$^{13}C_2$]*t*-ABA, which were then subjected to saponification to give a mixture **5** of [1,2-$^{13}C_2$]ABA and [1,2-$^{13}C_2$]*t*-ABA. [1,2-$^{13}C_2$]ABA **6** was separated from [1,2-$^{13}C_2$]*t*-ABA **7** by reversed phase HPLC. It has been reported that the storage of [3-Me-$^2H_3$]ABA for 6 months in MeOH at −2 °C led to some chemical degradation and some randomization of the label,[3] however no detectable change was detected in [1,2-$^{13}C_2$]ABA even under the conditions described above.

In conclusion, [1,2-$^{13}C_2$]ABA was readily prepared from α-ionone and was found to be suitable for use as an internal standard. It should play an important role in the quantification of endogenous levels of ABA.

Experimental

[$^{13}C_2$]Carbomethoxymethylenetriphenylphosphorane (60 mg) was added to 4-keto-1-hydroxy-α-ionone (40 mg)[4] in 2 ml toluene, and the mixture was refluxed for 5 h. After evaporation of the solvent, the products were separated by column chromatography (hexane–ethyl acetate 3 : 1) to give a mixture of methyl [1,2-$^{13}C_2$]ABA and methyl [1,2-$^{13}C_2$]*t*-ABA (yield 42%). The mixture (14 mg) was added to a solution of KOH (3 mg) in dry EtOH and refluxed for 30 min. The reaction mixture was poured into 10 ml 1M HCl and extracted three times with ethyl acetate. The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting mixture was separated by HPLC (Senshu Pak Pegasil ODS column, 20 mm × 250 mm, MeOH–H_2O 65 : 35) to give 5.8 mg [1,2-$^{13}C_2$]ABA. ^{1}H NMR (300 MHz, CD_3OD) δ_H 1.062 (3H, s, 9′-H_3), 1.100 (3H, s, 8′-H_3),1.968 (3H, s, 7′-H_3), 2.064 (3H, d, J_{CH} = 5.64 Hz, 6-H_3), 2.217 (1H, d, J_{HH}16.77 Hz, 5′*R*-H), 2.561 (1H, d, J_{HH} = 16.77 Hz, 5′S-H), 5.782 (1H, d, J_{CH} = 159.03 Hz, 2-H), 5.955 (1H, s, 3′-H), 6.238 (1H, d, J_{HH} = 15.79 Hz, 5-H), 7.783 (1H, d, J_{HH} = 15.79 Hz, 4-H); ^{13}CNMR (300 MHz, CD_3OD) δ_C 18.3 (C-7′), 19.6 (C-6), 22.4 (C-8′), 23.7 (C-9′), 41.6 (C-6′), 49.5 (C-5′), 79.2 (C-1′), 118.8 (d, J_{CC} = 71.82 Hz, C-2),126.0 (C-3′),127.9 (C-4), 135.8 (C-5), 148.0 (C-3); 164.6 (C-2′), 167.9 (d, J_{CC} = 71.82 Hz, C-1), 199.1 (C-4′). Two peaks at δ118.8 and 167.9, which were assigned

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to C-2 and C-1, respectively, were very intense, indicating that both were labeled by ^{13}C. HR-EI-MS for [1,2-$^{13}C_2$]ABA methyl ester m/z $[M]^+$: Found, 280.3528. Calc. for $C_{14}{}^{13}C_2H_{22}O_4$, 280.3541.

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