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Revised chirality of the acyl group of 8′-*O*-(3-hydroxy-3-methylglutaryl)-8′-hydroxyabscisic acid

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Abstract

8′-*O*-(3-Hydroxy-3-methylglutaryl)-8′-hydroxyabscisic acid is a stable conjugate of the first metabolite of abscisic acid, 8′-hydroxyabscisic acid, that is spontaneously isomerized to phaseic acid. The chirality of the 3-hydroxy-3-methylglutaryl group of the conjugate was revised to *S* based on an HPLC analysis of the diastereomer derived from mevalonolactone obtained by reduction of the conjugate with lithium borohydride.

Keywords: *Robinia pseudo-acacia*; Leguminosae; 3-hydroxy-3-methylglutaryl (HMG); Abscisic acid; Chirality; Lithium borohydride; Diborane; Mevalonolactone

1. Introduction

The plant hormone, abscisic acid (ABA, **1**) is metabolized to 8′-hydroxy-ABA (**2**) by ABA 8′-hydroxylase in plants (Fig. 1) (Crozier et al., 2000; Kushiro et al., 2004). 8′-Hydroxy-ABA (**2**) spontaneously isomerizes to phaseic acid (**3**), although the isomerization in vivo seems to be enzyme catalyzed (Milborrow et al., 1988; Todoroki et al., 1995). The instability of 8′-hydroxy-ABA (**2**) limits confirmation of its occurrence in plants, but isolation of a stable conjugate of 8′-hydroxy-ABA, 8′-*O*-(3-hydroxy-3-methylglutaryl)-8′-hydroxy-ABA (**4**), from *Robinia pseudo-acacia* has established 8′-hydroxy-ABA (**2**) as a metabolite of ABA (**1**) (Hirai et al., 1978). One of the authors previously reported the chirality of 3-hydroxy-3-methylglutaryl (HMG) group of **4** to be *R* (Hirai and Koshimizu, 1981). This identification was based on the chirality of mevalonolactone obtained by the reduction of **4** with diborane that is regarded to reduce the carboxyl group and not the ester group (Yoon et al., 1973). However, Tanaka et al. (1992) reported that the HMG group of gympolilins, bitter terpenoids from *Gymnopilus spectabilis*, had the *S*-configuration. They used lithium borohydride, which reduces the ester group and not the carboxyl group, to obtain mevalonolactone from the gympolilins. Naturally occurring HMG esters would be formed via the acylation of alcohols by (*S*)-HMG-CoA, meaning that they should possess the (*S*)-HMG group (Bergot et al., 1979). Thus, we reinvestigated the chirality of the HMG group of **4** by reduction using lithium borohydride.

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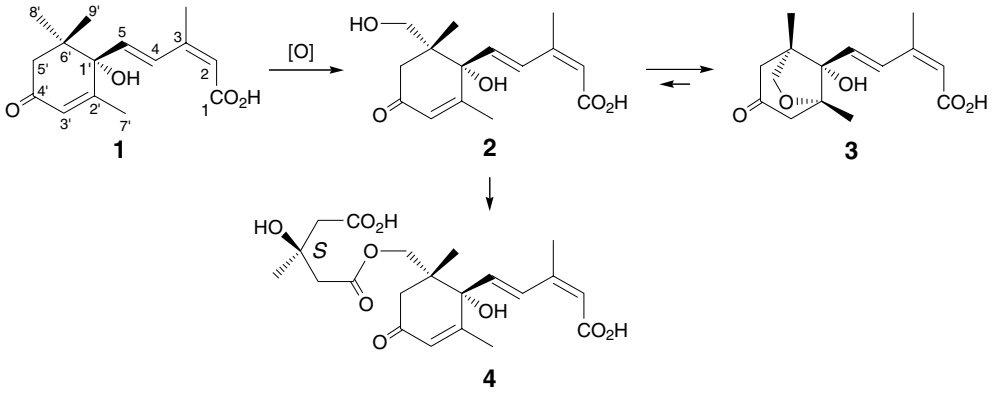
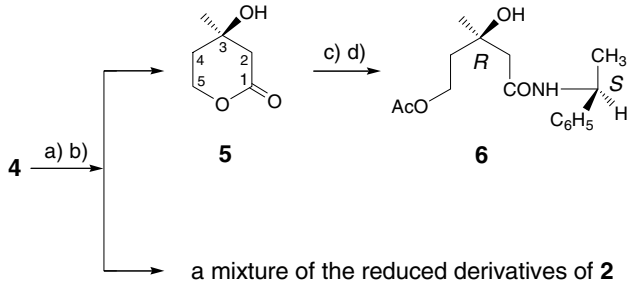


Fig. 1. Early metabolic pathway of ABA (**1**) in plants

2. Results and discussion

Compound **4** was reduced by lithium borohydride to give mevalonolactone (**5**) from the HMG group, and the reduced derivatives from 8′-hydroxy-ABA (**2**) (Scheme 1). The ^{1}H NMR spectrum suggested that the latter consisted of several components structurally related to 8′-hydroxy-ABA (**2**) but their structures could not be determined due to the small amounts of the individual compounds. Compound **5** was converted to a diastereomer, 5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide (**6**), by treatment with (*S*)-(−)-1-phenylethylamine followed by acetylation. The chirality at C-3 of **6** was determined by comparison of its retention time during HPLC with those of authentic diastereomers, (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide and its (3*S*)-diastereomer (Hirai and Koshimizu, 1981). The HPLC analysis showed that **6** was identical to the (3*R*)-diastereomer, indicating that the chirality of **5** is *R*. Thus, the absolute configuration of **4** should be revised to 8′-*O*-[(*S*)-3-hydroxy-3-methylglutaryl]-8′-hydroxy-ABA. This configuration corresponds well to the presumed in vivo formation of **4** via the acylation of 8′-hydroxy-ABA (**2**) by (*S*)-HMG-CoA.

Compound **6**, previously obtained by reduction of **4** with diborane, also had the 3*R*-configuration, by which the absolute configuration of the HMG group of **4** was assigned as *R* (Hirai and Koshimizu, 1981). This incorrect assignment was derived from the preconception for the reducing characteristic of diborane. Other researchers also determined the absolute configuration of the HMG groups in natural products using diborane. Macrophorin D (Sassa and Nukita, 1984) and viscumneoside IV (Kong et al., 1990) were suggested to possess the (*R*)-HMG group, the former of which has been revised as *S* by the reduction using lithium triethylborohydride (Fujimoto et al., 2001), while the HMG groups of tubeimoside I (Kasai et al., 1986) and tangshenosides I and IV (Mizutani et al., 1988; Yuda et al., 1990) were revealed to have the *S*-configuration. Although the reason remains to be elucidated, the results of these experiments using diborane are not consistent. This situation would be attributed to revealing the nature of the reducing characteristic of diborane, not to the co-occurrence of the (*R*)- and (*S*)-HMG groups in natural products. We should keep in mind that diborane sometimes seems to reduce the ester group rather than the carboxyl group. On the other hand, reduction using lithium borohydride always give an identical and reasonable result, the *S*-configuration, for the HMG groups (Tanaka et al., 1992, 1993; Mimaki et al., 1993; Kamo et al., 2003). All of these results strongly recommend the use of lithium borohydride or lithium triethylborohydride to confirm the absolute configuration of the HMG groups.



Scheme 1. (a) $LiBH_4$/THF, (b) H^+ (10%), (c) (*S*)-1-(−)-phenylethylamine, (d) Ac_2O/pyridine (ca. 100%).

3. Experimental

3.1. General

The ^{1}H NMR spectra were recorded by a Bruker DRX-500 FT-NMR spectrometer operating at 500.1 MHz for protons with TMS as the internal standard.

3.2. Reduction of 8′-O-(3-hydroxy-3-methylglutaryl)-8′-hydroxy-ABA (4) and derivatization of mevalonolactone

Compound **4** (30 mg) was isolated from fresh immature fruits (6.4 kg) of *Robinia pseudo-acacia* L. (Hirai et al., 1978). Compound **4** (15 mg) in lithium borohydride solution (0.5 mL, 2.0 M in THF; Aldrich) was stirred at room temperature for 12 h. The reaction was quenched with H_2O and then 1 N HCl. The solution was extracted three times with EtOAc at pH 3, and the organic layers were combined and concentrated to give a mixture of the reduced derivatives of 8′-hydroxy-ABA (10 mg). The aqueous layer was acidified to pH 1 with 1 N HCl, stirred for 3 days at room temperature, and then extracted three times with EtOAc. The organic layers were combined, and purified by HPLC with an ODS column (YMC RS-323, 250 × 10 mm) eluting with MeOH–H_2O (1:1) at a flow rate of 3.0 mL/min with detection at 200 nm to give **5** (0.5 mg). The ^{1}H NMR spectrum of **5** was identical to that of authentic mevalonolactone. (*S*)-1-(−)-Phenylethylamine (2 μL) was added to **5** in $CHCl_3$, then stored overnight at room temperature after removing the solvent in vacuo. The resulting material was acetylated with Ac_2O in pyridine, and purified by HPLC using an ODS column (YMC RS-323) eluting with MeOH–H_2O (4:1) at a flow rate of 3.0 mL/min with detection at 254 nm to give **6** (1.0 mg). Its ^{1}H NMR spectrum was consistent with that of the authentic (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl] mevalonamide.

3.3. Preparation and identification of authentic (3R)-5-O-acetyl-1-[(S)-phenylethyl]mevalonamide and (3S)-5-O-acetyl-1-[(S)-phenylethyl]mevalonamide

These diastereomers were synthesized by the reported procedure except for reacting without solvent (Hirai and Koshimizu, 1981). The diastereomers (60 mg) were subjected to preparative HPLC using a silica gel column (YMC SH-043-5, 250 × 20 mm) eluting with CH_2Cl_2-*i*-PrOH (20:1) at a flow rate of 8.0 mL/min with detection at 254 nm. Materials eluted at t_Rs 18.3 and 19.3 min were separately collected, and concentrated to give (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide (20.9 mg) and (3*S*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide (23.9 mg), respectively. These diastereomers were identified by their ^{1}H NMR spectra and by hydrolysis with 6 N NaOH (Hirai and Koshimizu, 1981). The chemical shift values of the diastereomers are different in the ^{1}H NMR spectra. These differences are reproducible, and able to be observed even when the diastereomers are mixed together.

3.3.1. (3R)-5-O-acetyl-1-[(S)-phenylethyl]mevalonamide $[\alpha]_D^{25}$ −48° (*c* 2.09, EtOH). ^{1}H NMR ($CDCl_3$): δ 7.25–7.36 (5H, *m*, 1′-C_6H_5), 6.28 (1H, *d*, *J* = 7.4 Hz, N*H*), 5.13 (1H, quintet, *J* = 6.9 Hz, H-1′), 4.22 (2H, *m*, H-5), 2.40, 2.28 (each 1H, *d*, *J* = 14.8 Hz, H-2), 2.03 (3H, *s*, 5-OCOCH_3), 1.85 (2H, *m*, H-4), 1.49 (3H, *d*, *J* = 6.9 Hz, H-2′), 1.23 (3H, *s*, 3-CH_3). Hydrolysis of this diastereomer (16.5 mg) gave (*R*)-(−)-mevalonolactone (1.8 mg), $[\alpha]_D^{21}$ −14° (*c* 0.18, EtOH). These data were identical with those already reported (Hirai and Koshimizu, 1981; Fujimoto et al., 2001).

3.3.2. (3S)-5-O-acetyl-1-[(S)-phenylethyl]mevalonamide $[\alpha]_D^{25}$ −53° (*c* 2.39, EtOH). ^{1}H NMR ($CDCl_3$): δ 7.25–7.36 (5H, *m*, 1′-C_6H_5), 6.34 (1H, *d*, *J* = 7.4 Hz, N*H*), 5.13 (1H, quintet, *J* = 6.9 Hz, H-1′), 4.20 (2H, *m*, H-5), 2.40, 2.28 (each 1H, *d*, *J* = 14.8 Hz, H-2), 2.00 (3H, *s*, 5-OCOCH_3), 1.82 (2H, *m*, H-4), 1.49 (3H, *d*, *J* = 6.9 Hz, H-2′), 1.24 (3H, *s*, 3-CH_3). Hydrolysis of this diastereomer (17.4 mg) gave (*S*)-(+)-mevalonolactone (1.8 mg), $[\alpha]_D^{21}$ +13° (*c* 0.18 EtOH). These data were identical to those already reported (Hirai and Koshimizu, 1981).

3.4. HPLC analysis of 6 and the authentic diastereomers

Compound **6** was subjected to HPLC with a silica gel column (YMC A-004, 300 × 4.6 mm) eluting with *n*-hexane–CH_2Cl_2-*i*-PrOH (20:20:1) at a flow rate of 2.0 mL/min with detection at 254 nm. The t_Rs of **6** and authentic (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide, and authentic (3*S*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide were 14.0 and 14.9 min, respectively.

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