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CHIRALITY OF THE ACYL GROUP OF β-HYDROXY-β-METHYLGLUTARYLHYDROXYABSCISIC ACID

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Key Word Index—*Robinia pseudacacia*; Leguminosae; β-hydroxy-β-methylglutarylhydroxyabscisic acid; chirality; optical resolution; mevalonolactone.

Abstract—The β-carbon of the acyl group of β-hydroxy-β-methylglutarylhydroxyabscisic acid was shown to possess *R*-configuration by HPLC analysis of the reduced product.

INTRODUCTION

Abscisic acid is metabolized to phaseic acid (PA) (**1**) via a highly unstable metabolite (**2**), which was called metabolite C [1]. This metabolite, hydroxyabscisic acid (HOABA), has been shown to occur in the seeds of *Robinia pseudacacia* as a stable conjugate, β-hydroxy-β-methylglutarylhydroxyabscisic acid (HMG-HOABA) (**3**) [2]. However, the chirality at the β-carbon of the HMG group has not been established. If one of the carboxyl groups of HMG is stereospecifically esterified with HOABA *in vivo*, C-3 of the HMG residue must possess either *R*- or *S*-configuration. We now report the elucidation of the absolute configuration at C-3 of HMG by the selective reduction of the HMG moiety to give mevalonolactone. A convenient method for the optical resolution of (*R,S*)-mevalonolactone is also described.

RESULTS AND DISCUSSION

HMG-HOABA was reduced with borane so that C-3 of the HMG moiety retained its configuration after reduction and hydrolysis of the ester [3]. On TLC (Si gel, solvent system 1), the reduction product was observed as a quenched spot (R_f 0.50) under UV radiation (HMG-HOABA, R_f 0.31). It decomposed to two compounds during purification by preparative TLC. One was identified as mevalonolactone (**4**) and the other as PA (**1**) by comparison with authentic samples (^{1}H NMR and MS). This showed that the free carboxyl group of HMG was reduced and that of the HOABA moiety was not. The instability of the reduction product (**5**) can be ascribed to attack of the ester carbonyl by the primary hydroxyl group formed from the free carboxyl groups of the HMG moiety.

The enantiomeric composition of the mevalonolactone was determined by HPLC analysis. Treatment of (*R,S*)-mevalonolactone with (*S*)-(−)-1-phenylethylamine in THF gave two diastereomeric amides, the monoacetates of which were separated by HPLC (column, 30 cm × 3.9 mm i.d. μ-Porasil; flow rate, 1.5 ml solvent system 2/min; pressure, 33 kg/cm^2; detection, $UV_{254\,nm}$; R_ts of diastereomers 10.7 and 11.7 min) and then hydrolysed to give optically active mevalonolactone. By measuring the optical rotation of the mevalonolactones, the first eluted diastereomer was shown to be (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]-mevalonamide and the second one was shown to be (3*S*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]-mevalonamide [4]. The monoacetyl-(*S*)-phenylethylamide (**6**) of the mevalonolactone derived from HMG-HOABA (identified by MS comparison with the diastereomers obtained above) was similarly analysed by HPLC to give a peak which co-chromatographed with the first eluted diastereomer. The mevalonolactone (**4**) derived from HMG-HOABA was, thus, proved to have the *R*-configuration. This result indicates that the absolute configuration at the acyl group of HMG-HOABA is *R*.

If HMG-HOABA were derived *in vivo* by the reaction of HOABA with HMG-CoA, the C-3 of the acyl group must have *S*-configuration. Interestingly, however, the absolute configuration at C-3 is *R*, suggesting that HOABA is not acylated by the usual mechanism through HMG-CoA. It is possible that another acylation mechanism is involved in the acylation of HOABA.

EXPERIMENTAL

Reduction of HMG-HOABA. HMG-HOABA (50 mg) was reduced with 1 M BH_3 (1.0 ml) in dry THF (50 ml) at −18° for 30 min under a stream of dry N_2 and left at room temp. overnight. H_2O (0.6 ml) was added to the reaction mixture at 0°. The THF was removed and the products were partitioned into EtOAc at pH 3.

Purification and derivatization of mevalonolactone (**4**) *and phaseic acid* (**1**) *derived from the reduction products of HMG-HOABA.* The EtOAc extract (43 mg) was applied to Merck pre-coated Si gel plates (0.25 mm) which were then developed to 34 mm in C_6H_6–EtOAc–HOAc (11:8:1) (system 1). The zone of R_f 0.24–0.30 was scraped off and eluted with EtOAc. The EtOAc eluate (4 mg) was further purified by Si gel TLC in C_6H_6–EtOAc–HOAc (7:12:1) (system 3). The EtOAc eluates of the zones R_f 0–0.33 and 0.58–0.78 gave **4** (0.6 mg) and **1** (1.0 mg), respectively. **4** was treated with (*S*)-(−)-1-phenylethylamine (10 mg), followed by Ac_2O–C_5H_5N (1:2), to afford the monoacetate of (*S*)-1-phenylethylamide (**6**) (2.0 mg). An aliquot of **1** was methylated by ethereal CH_2N_2 for GC/MS. The GLC

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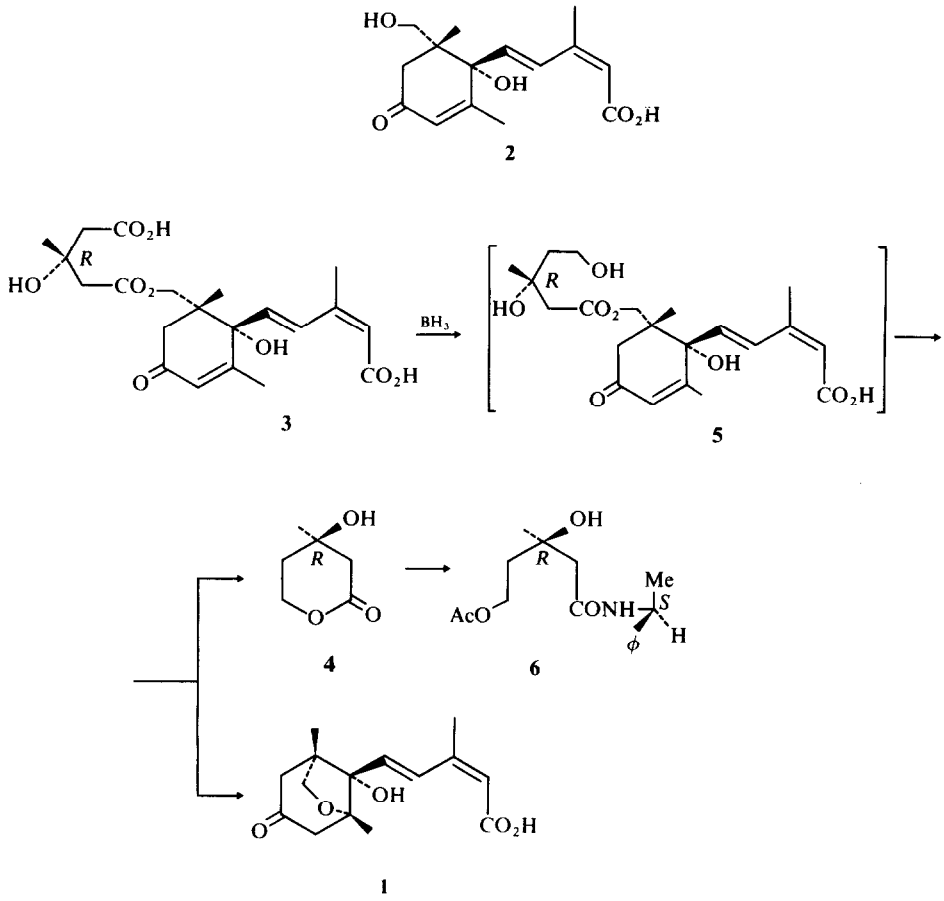

conditions were: column, 1.8 m × 2 mm glass packed with 2% OV-17 on Gaschrom Q (100–120 mesh); flow rate, 20 ml He/min; column temp, 135° for mevalonolactone and 180° for methyl ester of **1**.

Mevalonolactone (**4**). ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.35 (3 H, *s*), 1.86 (1 H, *d*, J = 5 Hz), 1.95 (1 H, *d*, J = 5 Hz), 2.50 (1 H, *d*, J = 17 Hz), 2.59 (1 H, *d*, J = 17 Hz), 4.43 (2 H, *m*); GC/MS *m/z* (rel. int.): 112 (M^+ − 18, 60), 88 (4), 82 (4), 81 (100), 72 (2), 69 (9), 68 (6), 66 (3), 60 (6), 54 (15), 53 (38), 52 (17), 50 (9).

Phaseic acid (**1**). ^{1}H NMR (90 MHz, Me_2CO-d_6): δ 1.04 (3 H, *s*), 1.22 (3 H, *s*), 2.13 (3 H, *s*), 2.33 (1 H, *dd*, J = 18 and 2.4 Hz), 2.40 (1 H, *dd*, J = 18 and 2.4 Hz), 2.77 (1 H, *dd*, J = 18 and 3.0 Hz), 2.86 (1 H, *d*, J = 18 Hz), 3.65 (1 H, *d*, J = 7.6 Hz), 3.95 (1 H, *dd*, J = 7.6 and 3.0 Hz), 5.80 (2 H, *br s*), 6.65 (1 H, *d*, J = 16 Hz), 8.23 (1 H, *d*, J = 16 Hz).

Methyl ester of **1**. GC/MS *m/z* (rel. int.): 294 (M^+, 60), 276 (22), 262 (17), 217 (16), 205 (8), 195 (6), 177 (25), 167 (40), 163 (35), 154 (38), 140 (15), 139 (55), 135 (34), 125 (100), 122 (73), 121 (50), 99 (24).

Preparation and separation of diastereomers derived from (RS)-*mevalonolactone.* (*S*)-(−)-1-Phenylethylamine (80 mg) was added to (*RS*)-mevalonolactone (60 mg) in 3 ml of THF and the mixture left for 12 hr at room temp. After evapn of THF, EtOAc (50 ml) was added to the residues and washed with 0.1 N HCl, followed by H_2O. EtOAc was removed *in vacuo* to yield an oily residue, which was acetylated with Ac_2O in C_5H_5N (1:2) at room temp. overnight. The diastereomers (120 mg) were separated by prep. HPLC: column, 30 cm × 7.8 mm, μ-Porasil; flow rate, 7 ml/min; pressure, 33 kg/cm^2; solvent, *n*-hexane–CH_2Cl_2–*iso*-PrOH–MeOH, 100:100:0.5:0.5 (system 2); detection, UV_{254nm}. GC/MS was carried out using a 1 m × 3 mm glass column containing 3% OV-1 coated on Chromosorb W (80–100 mesh) with a He flow rate 13 ml/min and a column temp. of 192°.

(3R)-5-O-*Acetyl*-1-[(S)-*phenylethyl*]-*mevalonamide*, $[\alpha]_D^{24}$ −58.8° (EtOH; *c* 1.30); UV λ_{max}^{EtOH} nm (log ε): 258 (2.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3670, 3430, 2970, 2930, 1735, 1655, 1605; ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.22 (3 H, *s*), 1.48 (3 H, *d*, J = 7 Hz), 1.83 (2 H, *t*, J = 7 Hz), 2.02 (3 H, *s*), 2.28 (1 H, *d*, J = 15 Hz), 2.36 (1 H, *d*, J = 15 Hz), 4.18 (2 H, *t*, J = 7 Hz), 5.08 (1 H, *quintet*, J = 7 Hz), 6.65 (NH, *d*, J = 7 Hz), 7.25 (5 H, *s*); GC/MS *m/z* (rel. int.): 293 (M^+, 2), 278 (2), 275 (2), 260 (2), 246 (2), 233 (3), 218 (2), 216 (2), 215 (5), 206 (6), 200 (4), 188 (2), 174 (4), 163 (21), 148 (10), 132 (5), 120 (100), 106 (77), 105 (62).

(3S)-5-O-*acetyl*-1-[(S)-*phenylethyl*]-*mevalonamide*, $[\alpha]_D^{24}$ −67.5°(EtOH; *c* 0.83); UV λ_{max}^{EtOH} nm (log ε): 258 (2.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3670, 3430, 2970, 2930, 1735, 1655, 1605; ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.24 (3 H, *s*), 1.48 (3 H, *d*, J = 7 Hz), 1.80 (2 H, *t*, J = 7 Hz), 2.00 (3 H, *s*), 2.29 (1 H, *d*, J = 15 Hz), 2.34 (1 H, *d*, J = 15 Hz), 4.16 (2 H, *t*, J = 7 Hz), 5.07 (1 H, *quintet*, J = 7 *Hz*), 6.78 (NH, *d*, J = 7 Hz), 7,27 (5 H, *s*); GC/MS *m/z* (rel. int.): 293 (M^+, 2), 278 (2), 275 (3), 260 (2), 246 (2), 233 (4), 218 (2), 216 (2), 215 (6), 206 (7), 200 (5), 188 (2), 174 (4), 163 (22), 148 (10), 132 (6), 120 (100), 106 (77), 105 (63).

Hydrolysis of diastereomers. The faster eluting diastereomer (3*R*) (29 mg) was dissolved in 6 M NaOH (3 ml), refluxed for 4 hr and partitioned with EtOAc. The aq. layer was acidified with 6 M HCl and then extracted with EtOAc. The EtOAc layer was washed with a small amount of H_2O and concd to give (*R*)-(−)-mevalonolactone (9.6 mg), $[\alpha]_D^{26}$ −15° (EtOH; *c* 0.48). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 3580, 3430, 2960, 2920, 1735, 1600. The slower

eluting diastereomer (43 mg) was hydrolyzed in the same manner to give (*S*)-(+)-mevalonolactone (16.7 mg), $[\alpha]_D^{26}$ +19° (EtOH; *c* 0.84). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 3580, 3430, 2960, 2920, 1735, 1600.

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