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A refined method for determining the absolute configuration of the 3-hydroxy-3-methylglutaryl group

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Abstract—A refined method for the determination of the absolute configuration of the 3-hydroxy-3-methylglutaryl group in natural products was developed. An interchange between the order of reduction with lithium borohydride and amidation with a chiral amine described in the previous procedure resulted in a high yield of the desired derivative. The refined method is applicable to various natural products with the 3-hydroxy-3-methylglutaryl group, even if their available amounts are 0.1 mg. The absolute configuration of the group in the lanostane-type triterpene acid, which had been isolated in a small amount, was established as *S*.

1. Introduction

The 3-hydroxy-3-methylglutaryl (HMG) group is occasionally found in natural products, such as terpenoids,[1–16] flavonoids,[17,18] and phenylpropanoids.[19–24] We have previously isolated a known lanostane-type triterpene acid with HMG group **1** and a new one **2** from the fruiting bodies of *Piptoporus betulinus* and found their anti-inflammatory activities (Fig. 1).[1]

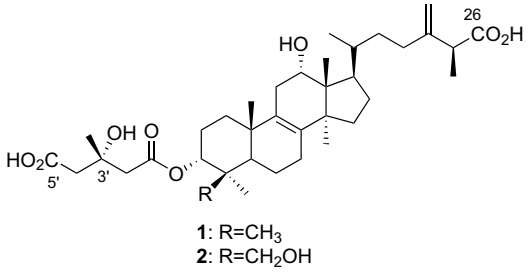

Figure 1. Lanostane-type triterpene acids from *Piptoporus betulinus*.

Naturally occurring HMG esters, which are formed via the acylation of the hydroxy group with (*S*)-HMG-CoA, possess an (*S*)-configuration at the C-3 stereogenic carbon.[25] The absolute configuration of the HMG groups in natural products has been confirmed by amidation with L-alanine methyl ester[6] or reduction with $LiBH_4$ to mevalonolactone.[2–5] We used the $LiBH_4$ reduction to establish the configuration of **1** as (*S*).[1] This reagent is more suitable for the selective reduction of the ester when compared to other hydride donors such as $LiAlH_4$ and $NaBH_4$: the former reduces not only esters but also carboxylic acids, while the latter requires high temperature to reduce esters. Although the $LiBH_4$ reduction is a proper method for the chemical determination of the absolute configuration of the HMG group, the reduction and following acid-catalyzed cyclization gave mevalonolactone in low yields (10–30%), requiring large amounts of natural products (11–300 mg, 11–250 μmol).[1–5] We were unable to determine

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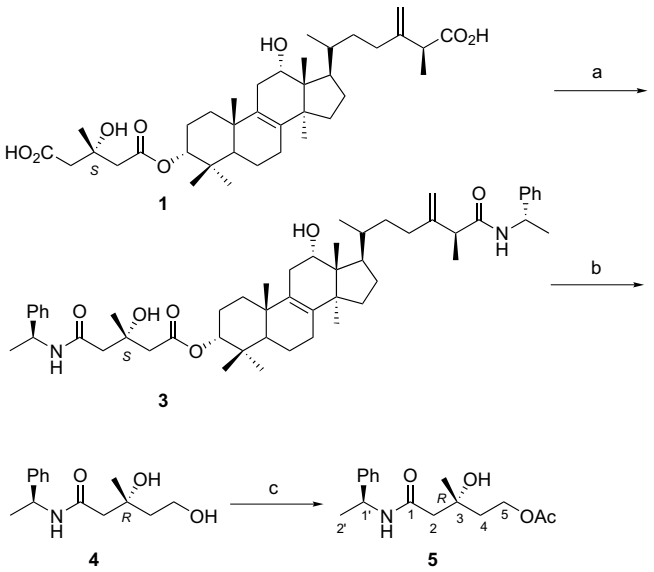
the absolute configuration of **2** due to the small amount available (4.3 mg).[1] Thus, the absolute configuration of the HMG groups in natural products has been sometimes left unknown.[12–21]

Herein we report a refined method for the determination of the absolute configuration of the HMG group, which is applicable to a small amount of natural products. By using this method, we have determined the absolute configuration of the HMG group of **2**.

2. Results and discussion

We interchanged the order of reduction and amidation described in the previous method (Scheme 1). As a model compound, we used **1**, the absolute configuration of which has already been established as (*S*). Amidation of **1** (23 mg) with (*S*)-(−)-phenylethylamine gave ester **3**. Reduction of the ester group of **3** with $LiBH_4$ gave alcohol **4** in a yield of 70%. Acylation of **4** using Ac_2O and pyridine afforded amide **5**. It is noteworthy that reduction of **3** with $LiBH_4$ gave a high yield of **4**, which was crucial for the total yield. In the previous scheme, $LiBH_4$ is probably inactivated by carboxylic acids at C-26 and C-5′, giving a low yield, while in the present scheme, the carboxylic acids are protected by (*S*)-(−)-phenylethylamine, giving a high yield.



Scheme 1. Preparation of amide **5** from **1**. Reagents and conditions: (a) (*S*)-(−)-phenylethylamine, PyBOP®, HOBt, Et_3N, DMF, quant.; (b) $LiBH_4$, THF, 70%; (c) Ac_2O, pyridine, CH_2Cl_2, quant.

The chirality at the C-3 position of **5** was assigned by comparing its retention time on HPLC with those of authentic diastereomers, (3*R*)-**5** and (3*S*)-**5**.[5] Injection of 5 μg of **5** gave the chromatogram that was satisfactory for identification. HPLC analysis showed that **5** was identical to the (3*R*)-diastereomer, meaning that the absolute configuration of the HMG group of **1** is (*S*), as proven previously.[1]

A scaled down method was also successful. Ester **3** prepared from 0.1 mg (0.16 μmol) of **1** was usable for the next step without purification after amidation, indicating that the method was applicable to a wide variety of natural products with the HMG group. Although alcohol **4** needed to be purified before acetylation in the case of a small-scaled derivatization, (3*R*)-**4** and (3*S*)-**4** were eluted at the same retention time on an ODS column, which was confirmed by purification of the diastereomers derived from commercial racemic mevalonolactone (data not shown). In total, amide **5** was prepared from **1** through three steps in a 35% yield. Thus, 0.1 mg (0.16 μmol) of **1** gave enough sample to be analyzed by HPLC for determining the absolute configuration of the HMG group (Fig. 2). Compared to the determination process previously reported, the scale down of the starting ester amount to smaller than 1% was achieved by the present scheme. For HPLC analysis, ca. 10 μg of amide **5** including co-chromatography is required. Although the required amount of the starting material depends on the yield of the $LiBH_4$ reduction, the minimal amount would be ca. 0.1 mg in many cases.

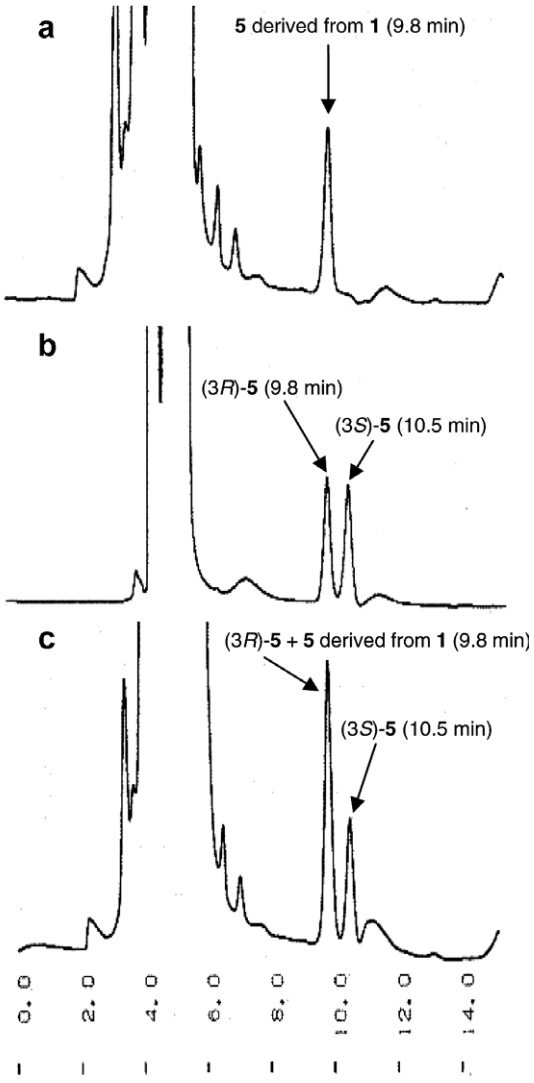
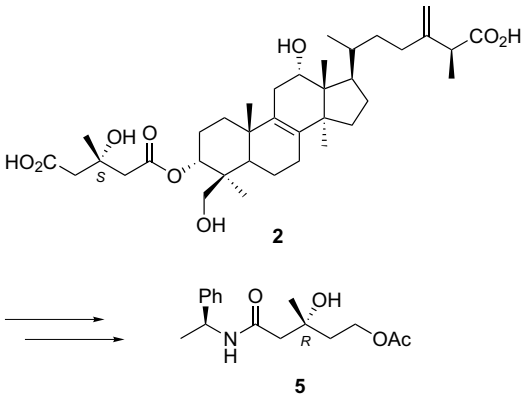


Figure 2. HPLC analysis of amide **5** derived from **1**. (a) Chromatogram of amide **5** derived from 0.1 mg of **1**. (b) Chromatogram of authentic (3*R*)- and (3*S*)-**5**. (c) Co-chromatogram of amide **5** (a) and the authentic diastereomers (b).

Using the refined method, we successfully determined the absolute configuration of the HMG group from 0.3 mg (0.46 μmol) of **2** (Scheme 2). The chirality of **5** at the C-3 position was established as (*R*) on the basis of HPLC analysis. Thus, the absolute configuration of the HMG group of **2** was determined to be (*S*).



Scheme 2. Preparation of amide **5** from **2**.

As far as we have investigated, normal phase chromatography is appropriate for separating (3*R*)-**5** and (3*S*)-**5**. If analysis by HPLC is performed under different conditions, (3*R*)- and (3*S*)-diastereomers should be distinguished from each other. For this purpose, it would be convenient to isolate small amounts of them and compare the chemical shift values of the acetyl groups, 2.03 ppm for the former and 2.00 ppm for the latter, in the ^{1}H NMR spectra.[2]

3. Conclusion

In conclusion, we have refined the previous procedure to achieve an improvement in the total yield of **5** derived from a natural product with the HMG group. This method would be useful for determining the absolute configuration of the HMG group occurring in small amounts in natural products.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX 500 FT-NMR spectrometer in $CDCl_3$ at 500 and 125 MHz, respectively. Chemical shifts were relative to tetramethylsilane as an internal standard. Mass spectra were obtained in a Jeol JMS 700 mass spectrometer. Optical rotations were determined with a Jasco DIP-1000 polarimeter.

4.2. (25*S*,3′*S*)-(−)-12α-Hydroxy-3α-{3′-hydroxy-3′-methyl-[(*S*)-phenylethyl]glutarylamide}-24-methyllanosta-8,24(31)-dien-26-[(*S*)-phenylethyl]amide 3

To a solution of **1** (23 mg, 37 μmol), (*S*)-(−)-phenylethylamine [19 μL, 150 μmol, ee (GLC) 98%; Aldrich], and Et_3N (31 μL, 220 μmol) in DMF (0.5 mL) were added PyBOP® (76 mg, 150 μmol) and HOBt (20 mg, 150 μmol) at 0 °C under Ar. The mixture was stirred for 30 min at room temperature, and then the reaction was quenched with dilute aqueous HCl. The organic materials were extracted with ether and concentrated. The residue was purified by ODS cartridge (10 g, Bond Elut C18; Varian) column chromatography eluting with H_2O, H_2O–MeOH (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9), and MeOH (30 mL of each). Concentration of the material eluted with MeOH gave **3** (31 mg, quant.) as a colorless oil. $[\alpha]_D^{20} = -14.5$ (*c* 2.15, $CHCl_3$). ^{1}H NMR δ: 0.58 (3H, s), 0.86 (3H, s), 0.93 (3H, s), 0.94 (3H, d, *J* = 6.6 Hz), 0.98 (3H, s), 1.10 (3H, s), 1.16 (2H, m), 1.28–1.49 (6H, m), 1.29 (3H, d, *J* = 7.2 Hz), 1.32 (3H, s), 1.46 (3H, d, *J* = 3.2 Hz), 1.49 (3H, d, *J* = 6.9 Hz), 1.59–1.65 (6H, m), 1.90–2.08 (9H, m), 2.42 (1H, d, *J* = 14.6 Hz), 2.53 (1H, d, *J* = 14.6 Hz), 2.55 (1H, s), 2.61 (1H, m), 3.05 (1H, q, *J* = 7.2 Hz), 3.96 (1H, d, *J* = 8.1 Hz), 4.72 (1H, s), 4.96 (1H, s), 4.98 (1H, s), 5.12 (2H, m), 5.87 (1H, d, *J* = 8.1 Hz), 6.75 (1H, d, *J* = 8.1 Hz), 7.23–7.34 (10H, m); ^{13}C NMR δ: 16.0, 16.3, 17.8, 17.9, 18.7, 21.7, 21.8, 22.1, 23.3, 24.5, 25.9, 27.1, 27.7, 30.5, 31.3, 31.9, 32.7, 34.3, 36.1, 36.7, 36.7, 43.1, 44.8, 45.4, 47.1, 48.2, 48.4, 48.7, 49.6, 49.6, 70.2 (C × 2), 73.2, 78.7, 111.6, 126.0 (C × 2), 126.1 (C × 2), 127.3, 127.3, 128.6 (C × 2), 128.7 (C × 2), 132.7, 134.8, 143.3 (C × 2), 150.4, 170.2, 172.3, 172.7; HRFABMS calcd for $C_{53}H_{77}N_2O_6$ [$(M+H)^+$] 837.5782, found 837.5845.

4.3. (3*R*)-1-[(*S*)-Phenylethyl]mevalonamide 4

To a solution of **3** (31 mg, 37 μmol) in THF (0.5 mL) was added $LiBH_4$ (180 μL, 370 μmol, 2.0 M in THF; Aldrich) at 0 °C under Ar. The mixture was stirred for 12 h at room temperature, and then the reaction was quenched with aqueous HCl. The organic materials were extracted with EtOAc and concentrated. The residue was purified by ODS cartridge (10 g, Bond Elut C18) column chromatography eluting with H_2O, H_2O–MeOH (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9), and MeOH (30 mL of each). Concentration of the material eluted with H_2O–MeOH (6:4 and 5:5) gave **4** (6.4 mg, 70%) as a colorless oil. $[\alpha]_D^{18} = -73.8$ (*c* 3.40, $CHCl_3$). ^{1}H NMR δ: 1.27 (3H, s, C*H*$_3$-3), 1.50 (3H, d, *J* = 6.9 Hz, H-2′), 1.71, 1.78 (each 1H, m, H-4), 2.25, 2.52 (each 1H, d, *J* = 14.3 Hz, H-2), 3.81, 3.91 (each 1H, m, H-5), 5.15 (1H, t, *J* = 6.9 Hz, H-1′), 6.41 (1H, br s, N*H*), 7.25–7.36 (5H, m, C$_6$*H*$_5$-1′); ^{13}C NMR δ: 21.8 (C-2′), 27.0 (*C*H$_3$-3), 42.1 (C-4), 46.8 (C-2), 48.8 (C-1′), 59.6 (C-5), 72.5 (C-3), 126.1 (*C*$_6$H$_5$), 127.5 (*C*$_6$H$_5$), 128.8 (*C*$_6$H$_5$), 143.0 (*C*$_6$H$_5$), 171.4 (C-1); EIMS (probe) 70 eV *m*/*z* (%) 251 $[M]^+$ (16), 206 (12), 120 (100), 106 (40), 105 (91); HREIMS calcd for $C_{14}H_{21}NO_3$ (M^+) 251.1521, found 251.1507.

4.4. (3*R*)-5-*O*-Acetyl-1-[(*S*)-phenylethyl]mevalonamide 5

To a solution of **4** (6.4 mg, 26 μmol) in CH_2Cl_2 (0.5 mL) were added Ac_2O (11 μL, 100 μmol) and pyridine (7.4 μL, 100 μmol). The mixture was stirred for 13 h, and then the reaction was diluted with water. The organic materials were extracted with EtOAc and concentrated to give **5**

(7.5 mg, quant.) as a colorless oil. $[\alpha]_D^{18} = -59.0$ (*c* 3.75, $CHCl_3$). For the other spectral data, see Refs. 2,8.

4.5. Preparation of amide 5 from 1 on a small scale

To a solution of **1** (0.1 mg, 0.16 μmol), (*S*)-(−)-phenylethylamine (1 drop from a micro syringe), and Et_3N (1 drop from a micro syringe) in DMF (0.1 mL) were added PyBOP® (0.3 mg, 0.6 μmol) and HOBt (0.1 mg, 0.7 μmol) at 0 °C under Ar. The mixture was stirred for 1 h at room temperature, and then the reaction was quenched with diluted aqueous HCl. The organic materials were extracted with ether. Evaporation of the solvent gave crude **3**, which was immediately used for the next step without purification. To a solution of crude **3** in THF (0.1 mL) was added $LiBH_4$ (1 drop from a micro syringe, 2.0 M in THF; Aldrich) at 0 °C under Ar. The mixture was stirred for 12 h at room temperature, and then the reaction was quenched with aqueous HCl. The organic materials were extracted with EtOAc. Evaporation of the solvent gave an oil, which was purified by ODS cartridge (500 mg, Bond Elut C18) column chromatography eluting with H_2O, H_2O–MeOH (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9), and MeOH (3 mL of each). The material eluted with H_2O–MeOH (8:2, 7:3, 6:4, 5:5, and 4:6) was concentrated and then subjected to HPLC using an ODS column (Shodex C18-5B, 250 × 4.6 mm) eluting with H_2O–MeOH (1:1) at a flow rate of 1.0 mL/min with detection at 212 nm. The material eluted at t_R 8.4 min was separately collected and concentrated to give **4**. To a solution of **4** in CH_2Cl_2 (0.1 mL) were added Ac_2O (1 drop from a micro syringe) and pyridine (2 drops from a micro syringe). The mixture was stirred for 6 h, and then the reaction was diluted with water. The organic materials were extracted with EtOAc, and evaporation of the solvent gave **5** (16 μg, 35% from **1**).

4.6. Calibration curve of 5

A linear relationship between the amount of **5** and the peak area was observed in the range of 5–250 ng by using an ODS column (Shodex C18-5B 250 × 4.6 mm) eluting with H_2O–MeOH (1:1) at a flow rate of 1.0 mL/min with detection at 212 nm. The t_R of **5** was 16.5 min.

4.7. HPLC analysis of (3*R*)-5 and (3*S*)-5

Amide **5** (5 μg, 17 nmol) in CH_2Cl_2-*i*-PrOH (20:1) was subjected to HPLC with a silica gel column (YMC A-004, 300 × 4.6 mm) eluting with CH_2Cl_2-*i*-PrOH (20:1) at a flow rate of 1.0 mL/min with detection at 254 nm. The t_Rs of (3*R*)-**5** and (3*S*)-**5** were 9.8 and 10.5 min, respectively.

4.8. Determination of the absolute configuration of 2

Amide **5** (24 μg, 80 nmol) was prepared from **2** (0.3 mg, 0.46 μmol) by the same procedure as described in Section 4.5. Amide **5** (5 μg, 17 nmol) was subjected to HPLC with a silica gel column (YMC A-004, 300 × 4.6 mm) eluting with CH_2Cl_2-*i*-PrOH (20:1) at a flow rate of 1.0 mL/min with detection at 254 nm.

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