Chirality of the 3-Hydroxy-3-methylglutaric Acid Moiety of Fasciculic Acid A, a Calmodulin Antagonist Isolated from *Naematoloma fasciculare*

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The chirality of the 3-hydroxy-3-methylglutaric acid (HMGA) moiety of fasciculic acid A (1), a calmodulin antagonist isolated from *Naematoloma fasciculare*, was established to be *S* by comparing the ^{1}H-NMR spectrum of the L-alanylamide of HMGA derived from 1 with those of its diastereomer and the diastereomeric mixture.

Keywords fasciculic acid A; 3-hydroxy-3-methylglutaric acid; chirality; *Naematoloma fasciculare*; mushroom

Fasciculic acid A (**1**) is a fungal metabolite of the toxic mushroom, *Naematoloma fasciculare*, which displays potent and specific calmodulin antagonistic activity.[1] Although the structure of **1** was elucidated to be 2-*O*-(3-hydroxy-3-methylglutaryl)fasciculol A, the chirality of the asymmetric center of the 3-hydroxy-3-methylglutaric acid (HMGA) moiety has been left undetermined. *In vivo*, esterification of fasciculol A with HMGA should proceed enzymatically, and hence an asymmetry must be induced at C-3 of HMGA. To data, HMGA esters have been found in a number of natural products such as terpenes,[2] phenylpropanoids[3] and others.[4] Determination of the chirality of C-3 at HMGA moieties has been performed for several compounds on the basis of HPLC analysis of 5-*O*-acetyl-[(*S*)-phenylethyl]mevaloamide, and the chirality of their HMGA moieties was clarified to be *R*. In those cases, the amide was prepared from L-(−)-α-phenethylamine and a mevalonolactone which was obtained by reduction of the HMGA esters with diborane followed by hydrolysis. Recently, however, Shirahama *et al.* have reported that the HMGA moiety of gymnopilin has the *S*-configuration, and they further suggested that previous results on the chirality of the HMGA moiety need to be revised.[5] Their stereochemical elucidation was almost identical with the known method except that the reduction of the HMGA esters was carried out with lithium borohydride instead of diborane. On the other hand, we established the chirality of C′-3 of the HMGA moiety of fasciculic acid A by the ^{1}H-NMR spectral analysis of three L-alanylamides prepared from **1** and two synthetic HMGA's. This paper deals with the determination of the absolute configuration at C-3′ of the HMGA moiety in **1** as *S* by comparing the ^{1}H-NMR spectrum of the L-alanylamide (**2**) of HMGA derived from **1** with those of its diastereomer (**3**) and the diastereomeric mixture (**4**).

Preparation of L-Alanylamides of HMGA (2—4) As shown in Chart 1, condensation of fasciculic acid A (**1**) with L-alanine methyl ester hydrochloride in the presence of *N*,*N*-dicyclohexylcarbodiimide afforded the amidation product (**5**) in 55.2% yield. Then, alkaline hydrolysis of the amide (**5**) with 2% aqueous NaOH in EtOH provided fasciculol A and (*N*-L-alanyl)-3-hydroxy-3-methylglutaric acid, of which the latter was esterified with excess ethereal diazomethane to yield **2** as a colorless viscous oil, $[\alpha]_D^{23}$ +5.16° (c=0.16, $CHCl_3$).

The amides (**3** and **4**) were also obtained by condensation of L-alanine methyl ester with the (*R*)-acid (**10**) and the (*RS*)-acid (**14**), respectively, in the same manner as described for **2**. The acids (**10** and **14**) were prepared from geraniol (**6**) and 3-methyl-1,3,5-pentanetriol (**11**), respectively, and the synthetic routes are outlined in Charts 2 and 3.

(*S*)-5-Benzyloxy-3-methylpentane-1,3-diol (**8**), prepared from geraniol in six steps according to the procedure of Nozoe *et al.*,[6] was first oxidized with Jones reagent to give an acid, which was then treated with ethereal diazomethane to afford the methyl ester (**9**) in 67.7% yield. Hydrogenolysis of **9** over 10% Pd–C in EtOH gave the debenzylation product, which was then subjected to Jones oxidation to yield the acid (**10**).

The acid (**14**) was prepared from (*RS*)-5-benzyloxy-3-methylpentane-1,3-diol (**13**) in 26.2% yield according to the

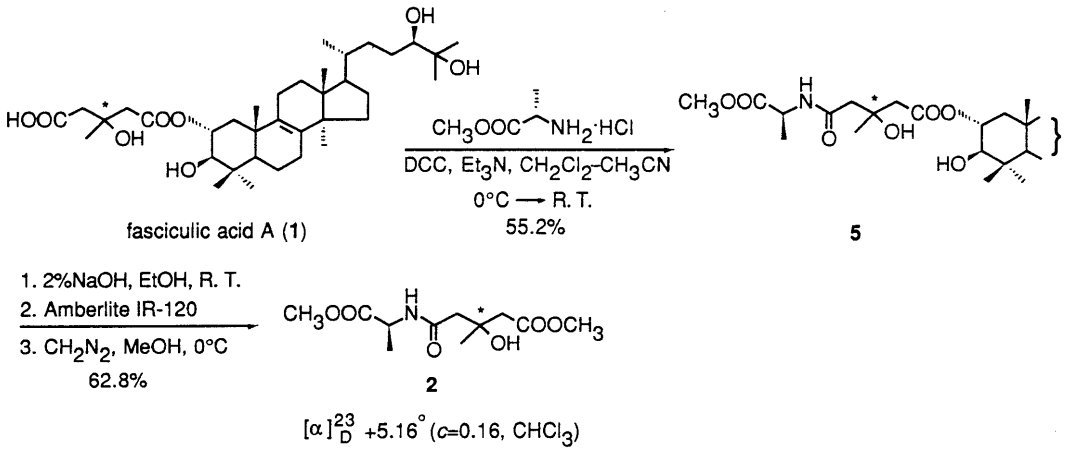


Chart 1

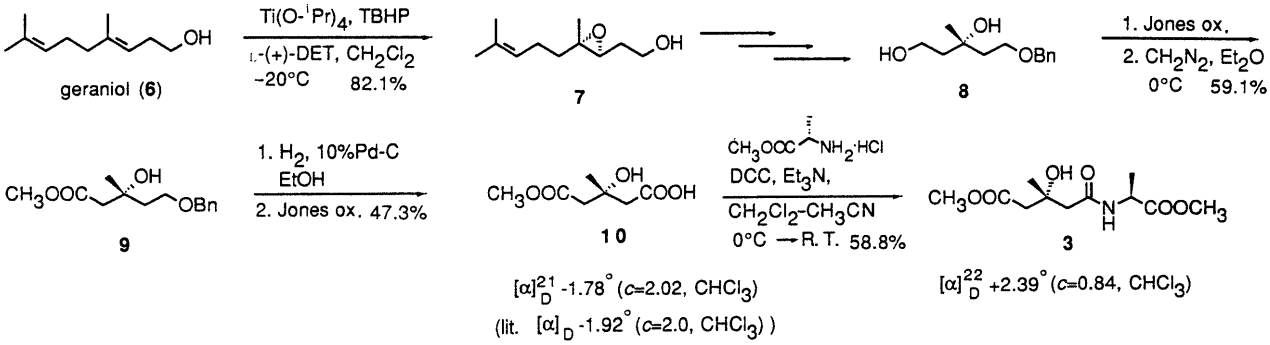


Chart 2

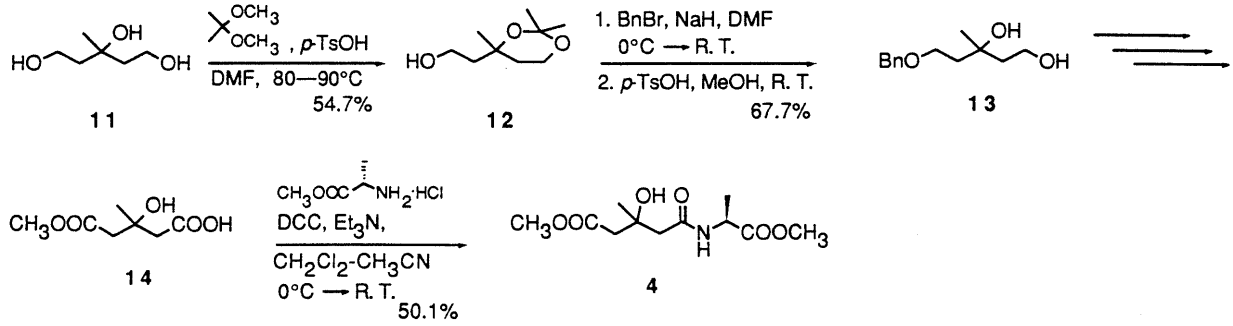
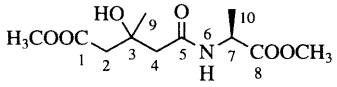


Chart 3

TABLE I. ^{1}H-NMR Spectral Data for **2**, **3** and **4** in $CDCl_3$ (δ_{ppm}, 500 MHz)



Position	2	3	4
2	2.49 (1H, d, J=14.7) 2.55 (1H, d, J=14.7)	2.43 (1H, d, J=14.7) 2.63 (1H, d, J=14.7)	2.42 (0.5H, d, J=14.7) 2.49 (0.5H, d, J=14.7) 2.55 (0.5H, d, J=14.7) 2.64 (0.5H, d, J=14.7)
4	2.55 (1H, d, J=15.1) 2.74 (1H, d, J=15.1)	2.61 (2H, s)	2.55 (0.5H, d, J=15.1) 2.61 (1H, s) 2.74 (0.5H, d, J=15.1)
7	4.59 (1H, dq, J=5.7, 7.3)	4.58 (1H, dq, J=7.1, 7.3)	4.59 (1H, qn,[a] J=7.3)
9	1.33 (3H, s)	1.36 (3H, s)	1.33 (1.5H, s) 1.36 (1.5H, s)
10	1.42 (3H, d, J=7.3)	1.42 (3H, d, J=7.3)	1.42 (3H, d, J=7.3)
$COOCH_3$	3.72 (3H, s)	3.72 (3H, s)	3.72 (3H, s)
$COOCH_3$	3.75 (3H, s)	3.75 (3H, s)	3.75 (3H, s)
OH	4.65 (1H, s)	4.60 (1H, s)	4.59 (0.5H, s) 4.65 (0.5H, s)
NH	6.88 (1H, br d, J=5.7)	6.85 (1H, br d, J=7.1)	6.83 (0.5H, br d, J=5.7) 6.88 (0.5H, br d, J=6.0)

a) Quintet.

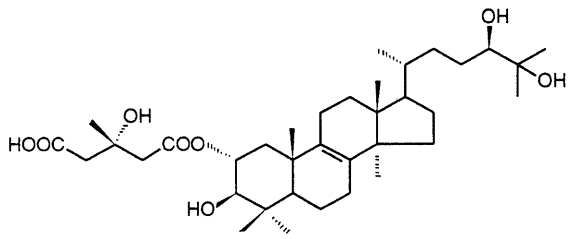


Fig. 1. Fasciculic Acid A

same procedure as used for **8**. The diol (**13**) was synthesized from 3-methyl-1,3,5-pentanetriol (**11**) in 37.0% yield by treatment with 3,3-dimethoxypropane in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) in *N*,*N*-dimethylformamide (DMF), followed by protection of the remaining hydroxyl group with a benzyl group and subsequent deblocking with *p*-TsOH in MeOH.

Determination of the Chirality of the HMGA Moiety of Fasciculic Acid A The ^{1}H-NMR spectral data for **2**, **3** and **4** are given in Table I. Figure 2 shows the 500 MHz ^{1}H-NMR spectra of **2**, **3** and **4**. As shown in Fig. 2, clear differences in NMR spectra can be seen between **2** and **3**, especially at the C-2′ and C-4′ methylene regions. The ^{1}H-NMR spectrum of **2** revealed signals assignable to H_2-2 and H_2-4 at δ 2.49 (1H, d, J=14.7 Hz), 2.55 (1H, d, J=14.7 Hz) and 2.55 (1H, d, J=15.1 Hz), 2.74 (1H, d, J=

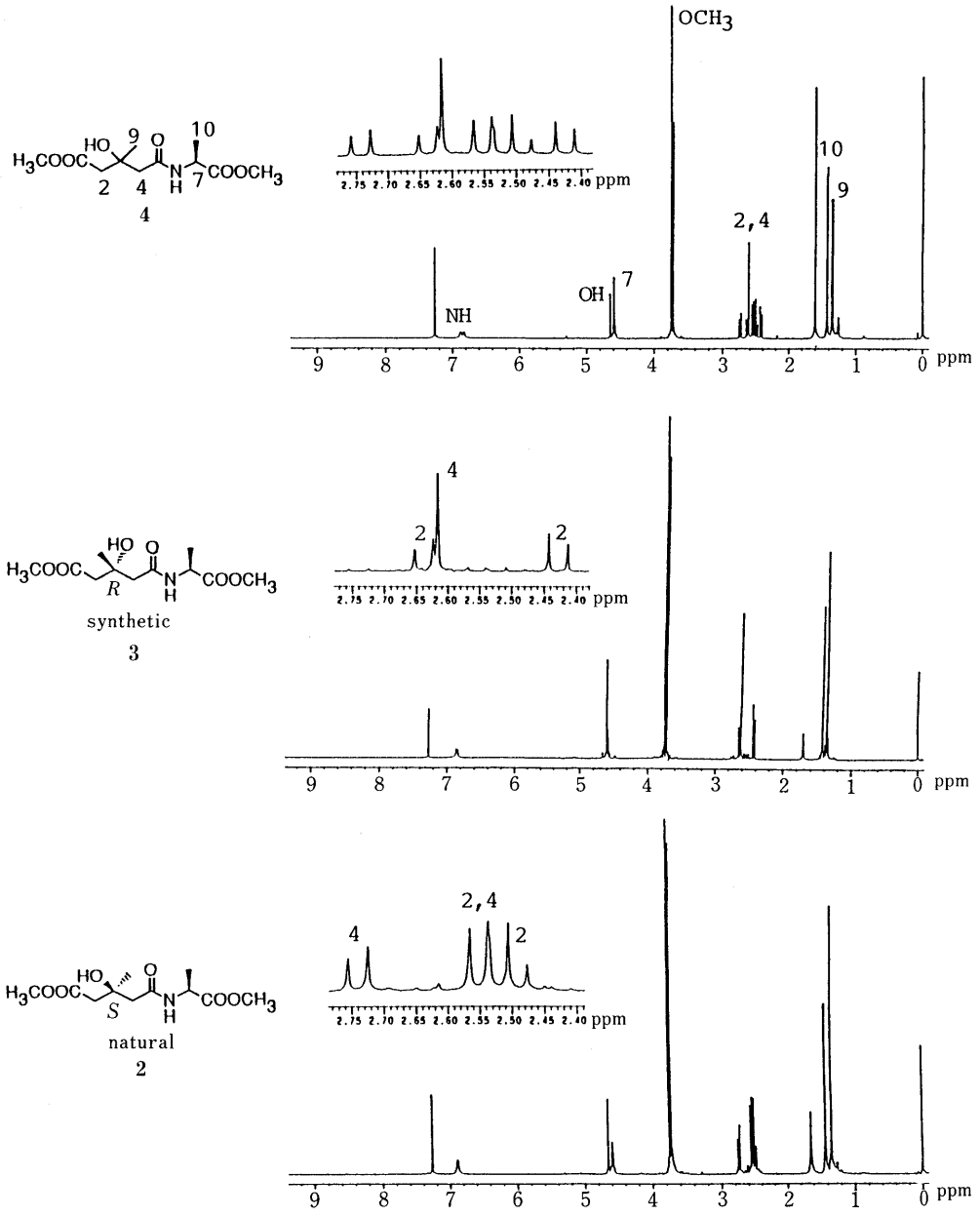


Fig. 2. ^{1}H-NMR Spectra of **2**, **3** and **4** ($CDCl_3$, 500 MHz)

15.1 Hz), respectively, while the corresponding signals in that of **3** were observed at δ 2.43 (1H, d, J=14.7 Hz), 2.63 (1H, d, J=14.7 Hz) and 2.61 (2H, s), respectively. Thus, the chirality of the HMGA moiety of fasciculic acid A was found to be *S*. The structure of fasciculic acid A, including the stereochemistry of the HMGA moiety, was also established to be as shown in Fig. 1.

Experimental

IR spectra were recorded on a JASCO A-100S infrared spectrometer with polystyrene calibration at 1601 cm^{-1}. Optical rotation values were measured on a JASCO DIP-370 polarimeter. ^{1}H-NMR spectra were recorded on a JEOL JNM GX-500 (500 MHz) and on a JEOL JNM FX-100 (100 MHz) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given δ(ppm) and multiplicities are shown as follows: singlet=s, doublet=d, triplet=t, multiplet=m and broad=br. Coupling constants (J) are given in hertz (Hz). Mass spectra were taken on a JEOL JMS DX-303 spectrometer. TLC analyses were performed on Kieselgel 60F_{254} (Merck) and spots were detected under UV irradiation and by heating on a hot plate after spraying phosphomolybdenic acid reagent.

Synthesis of Methyl (*S*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (2) 2-*O*-[5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutaryl]fasciculol A (**5**): A solution of dicyclohexylcarbodiimide (DCC, 24.8 mg, 0.12 mmol) in CH_2Cl_2 (1 ml) was added dropwise to a stirred solution of fasciculic acid A (**1**, 62.0 mg, 0.1 mmol) in CH_2Cl_2–CH_3CN (3:2, 5 ml) at 0 °C. Stirring was continued at the same temperature for 10 min, then L-alanine methyl ester hydrochloride (16.7 mg, 0.12 mmol) and Et_3N (20 μl, 0.14 mmol) were added, and the resulting mixture was stirred at room temperature for 1 h. After removal of precipitates by filtration, the filtrate was concentrated under reduced pressure and the residue obtained was chromatographed on silica gel using $CHCl_3$–MeOH mixture as the eluant to afford **5** (38.9 mg, 55.2%) as a pale yellowish amorphous powder, $[\alpha]_D^{20}$ −2.50° (c=0.16, $CHCl_3$). FD-MS m/z: 705 (M^+), 518, 476. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620—3150 (OH/NH), 2950 (br), 2870, 1725 (ester C=O), 1600 (amide C=O), 1640 (amide C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.69 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.91 (3H, d, J=6.0, 20-CH_3), 1.07 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.22 (3H, s, CH_3), 1.41 (3H, s, 3′-CH_3), 1.44 (3H, d, J=7.3, 7′-CH_3), 1.00—2.15 (21H), 2.44 (1H, d, J=14.5, H-2′), 2.60 (1H, d, J=14.5, H-2′), 2.62 (2H, s, H_2-5′), 2.88 (1H, br s, OH), 3.24 (1H, br d, J=9.8, H-3), 3.35 (1H, t, J=6.0, H-24), 3.49 (2H, s, 2×OH), 3.76 (3H, s, $COOCH_3$), 4.61 (1H, dq, J=7.7, 7.3, H-7′), 4.68 (1H, br s, OH), 5.04 (1H, ddd, J=11.5, 10.3, 4.3, H-2), 7.15 (1H, d, J=7.7, NH).

Methyl (*S*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (**2**): A stirred solution of **5** (30.1 mg, 0.04 mmol) in EtOH (3 ml) was treated with 2% NaOH aqueous solution (3 ml) at 0 °C. Stirring was continued at room temperature for 1 h, then the mixture was concentrated under reduced pressure, and the residual aqueous suspension was diluted with water and washed with EtOAc. The aqueous layer was passed through an Amberlite IR-120B column (5 ml) and concentrated to dryness. The residue was dissolved in MeOH (1 ml), and treated with ethereal diazomethane at 0 °C. After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$–MeOH as the eluant to give **2** (7.0 mg, 62.8%) as a colorless viscous oil, $[\alpha]_D^{23}$ +5.16° (c=0.16, $CHCl_3$). EI-MS *m/z*: 262 [(M+H)$^+$], 261 (M$^+$), 202, 117. HR-EIMS *m/z*: 261.1249. Calcd for $C_{11}H_{19}NO_6$: 261.1212. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3625—3200 (OH/NH), 3000, 2955, 2930, 2850, 1740 (ester C=O), 1660 (amide C=O), 1525. ^{1}H-NMR: Table I.

Synthesis of Methyl (*R*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (3) (*S*)-5-Benzyloxy-3-methylpentane-1,3-diol (**8**): The diol (**8**), a colorless oil, was obtained in 41.9% (3.94 g) yield from geraniol (**6**, 11.3 ml, 64.9 mmol) through six steps according to Nozoe's method.[6] $C_{13}H_{20}O_3$, $[\alpha]_D^{23}$ −5.12° (c=1.29, $CHCl_3$) [lit. $[\alpha]_D^{30}$ −6.4° (c=1.1, $CHCl_3$)]. FD-MS *m/z*: 225 [(M+H)$^+$]. EI-MS *m/z*: 206 (M$^+$−H_2O), 179, 107, 91. HR-EIMS *m/z*: 206.1338 (M$^+$−H_2O). Calcd for $C_{13}H_{18}O_2$: 206.1307. IR ν_{max}^{neat} cm^{-1}: 3375 (br, OH), 3070, 3040, 2975, 2950, 2875. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.28 (3H, s, CH_3), 1.40—2.16 (4H, m, H_2-2, H_2-4), 3.32 (1H, br s, OH), 3.74 (2H, t, J=5.5, H_2-5), 3.81 (2H, t, J=5.0, H_2-1), 3.88 (1H, br s, OH), 4.52 (2H, s, $C_6H_5C\underline{H}_2$), 7.32 (5H, s, $C_6\underline{H}_5CH_2$).

Methyl (*R*)-5-Benzyloxy-3-hydroxy-3-methylpentanoate (**9**): Jones reagent (8 N, 1.9 ml, 14.8 mmol) was added dropwise to a stirred solution of the diol (**8**, 1.1 g, 5.0 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 30 min. Excess oxidizing agent was decomposed with iso-PrOH, then Et_2O and Celite were added to this mixture. After removal of precipitates by filtration, the filtrate was washed with water and saturated NaCl aqueous solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in Et_2O and extracted twice with 0.5 N NH_4OH (5 ml). The aqueous layer was acidified with 0.5 N HCl and extracted twice with EtOAc (50 ml), and then the EtOAc extract was washed with water and saturated NaCl aqueous solution, and dried over anhydrous Na_2SO_4. After evaporation, the residue was chromatographed on silica gel using *n*-hexane–EtOAc as the eluant to yield (*R*)-5-benzyloxy-3-hydroxy-3-methylpentanoic acid, 754.7 mg (64.0%), as a colorless viscous oil, $[\alpha]_D^{19}$ +2.28° (c=1.58, $CHCl_3$). EI-MS *m/z*: 239 [(M+H)$^+$], 238 (M$^+$), 160, 91. HR-EIMS *m/z*: 238.1199 (M$^+$). Calcd for $C_{13}H_{18}O_4$: 238.1205. IR ν_{max}^{neat} cm^{-1}: 3700—2200 (COOH), 3400 (OH), 2980, 2930, 2875, 1715 (carboxyl/ester C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.32 (3H, s, CH_3), 1.60—2.14 (2H, m, H_2-4), 2.44 (1H, d, J=16.0, H-2), 2.60 (1H, d, J=16.0, H-2), 3.72 (2H, m, H_2-5), 4.52 (2H, s, $C_6H_5C\underline{H}_2$), 7.31 (5H, s, $C_6\underline{H}_5CH_2$). Next, excess ethereal diazomethane was added dropwise to the above acid (750.0 mg, 3.2 mmol) and the mixture was stirred for 10 min at 0 °C. After removal of the solvent, the residue was chromatographed on silica gel with *n*-hexane–EtOAc as the eluant to afford the methyl ester (**9**) as a colorless oil in the yield of 92.9% (738.1 mg), $[\alpha]_D^{21}$ −0.24° (c=1.66, $CHCl_3$). EI-MS *m/z*: 253 [(M+H)$^+$], 160, 91. HR-EIMS *m/z*: 253.1417 [(M+H)$^+$]. Calcd for $C_{14}H_{21}O_4$: 253.1440. IR ν_{max}^{neat} cm^{-1}: 3500 (br, OH), 3040, 2950, 2860, 1735 (ester C=O), 1500. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.28 (3H, s, CH_3), 1.90 (2H, t, J=6.0, H_2-4), 2.46 (1H, d, J=16.0, H-2), 2.62 (1H, d, J=16.0, H-2), 3.66 (3H, s, $COOCH_3$), 3.68 (2H, t, J=6.0, H_2-5), 3.92 (1H, s, OH), 4.48 (2H, s, $C_6H_5C\underline{H}_2$), 7.29 (5H, s, $C_6\underline{H}_5CH_2$).

Methyl (*R*)-3-Hydroxy-3-methylglutarate (**10**): The methyl ester (**9**, 305.2 mg, 1.2 mmol) in EtOH (10 ml) was hydrogenolyzed over 10% Pd–C (32 mg) at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue in acetone (30 ml) was treated with Jones reagent and the mixture was subjected to usual work-up. The product (**10**) was obtained as a colorless viscous oil after purification by silica gel chromatography using $CHCl_3$–MeOH as the eluant in 47.3% yield (100.8 mg), $[\alpha]_D^{21}$ −1.78° (c=2.02, $CHCl_3$). EI-MS *m/z*: 177 [(M+H)$^+$], 161, 143, 117. HR-EIMS *m/z*: 177.0763 [(M+H)$^+$]. Calcd for $C_7H_{13}O_5$: 177.0763. IR ν_{max}^{neat} cm^{-1}: 3700—2400 (COOH), 2980, 1720 (br, carboxyl/ester C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.40 (3H, s, CH_3), 2.57 (1H, d, J=17.0, H-2), 2.67 (2H, s, H_2-5), 2.77 (1H, d, J=17.0, H-2), 3.74 (3H, s, $COOCH_3$), 3.94 (1H, br s, OH).

Methyl (*R*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (**3**): A solution of DCC (31.6 mg, 0.15 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a solution of **10** (18.0 mg, 0.1 mmol) in CH_2Cl_2–CH_3CN (3 : 2, 3 ml) at 0 °C and the resulting mixture was stirred for 10 min. Then, L-alanine methyl ester hydrochloride (21.3 mg, 0.15 mmol) and Et_3N (30 μl, 0.22 mmol) were added, and the mixture was stirred at room temperature for 1 h. After removal of precipitates by filtration, the filtrate was concentrated under reduced pressure, then the residue was subjected to repeated column chromatographies on silica gel with $CHCl_3$–MeOH mixture to afford the amide (**3**, 15.7 mg, 58.8%) as a colorless viscous oil, $[\alpha]_D^{22}$ +2.39° (c=0.84, $CHCl_3$). EI-MS *m/z*: 262 [(M+H)$^+$], 261 (M$^+$), 202, 117. HR-EIMS *m/z*: 261.1221 (M$^+$). Calcd for $C_{11}H_{19}NO_6$: 261.1212. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3200 (OH/NH), 2995, 2950, 1740 (ester C=O), 1660 (amide C=O), 1520. ^{1}H-NMR: Table I.

Synthesis of Methyl (*RS*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (4) 4-(2-Hydroxyethyl)-2,2,4-trimethyl-1,3-dioxane (**12**): 2,2-Dimethoxypropane (7.4 ml, 59.7 mmol) and *p*-TsOH·H_2O (570.0 mg, 2.99 mmol) were added to a solution of 3-methyl-1,3,5-pentanetriol (**11**, 4.0 g, 29.9 mmol) in DMF (20 ml), and the reaction mixture was stirred at 80—90 °C for 3 h, then poured into ice water and extracted twice with EtOAc (50 ml). The extract was washed with saturated $NaHCO_3$ aqueous solution, water and saturated NaCl aqueous solution, successively, dried over anhydrous $MgSO_4$, and evaporated. The residue was chromatographed on silica gel using *n*-hexane–EtOAc (1 : 1) as the eluant to give **12** (2.52 g, 42.5%) as a colorless oil, $C_9H_{18}O_3$. FD-MS *m/z*: 175 [(M+H)$^+$], 159. IR ν_{max}^{neat} cm^{-1}: 3450 (OH), 1375. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.36 (3H, s, 4-CH_3), 1.38 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.75 (2H, t, J=6.0), 3.30 (2H, t, J=6.0), 3.70—4.08 (4H, m).

5-Benzyloxy-3-methylpentane-1,3-diol (**13**): A suspension of sodium hydride (NaH, 60% dispersion in mineral oil: 726.0 mg, 18.2 mmol) in DMF (10 ml) was cooled to 0 °C and a solution of **12** (2.1 g, 12.1 mmol) in DMF (10 ml) was added dropwise under an N_2 atmosphere with stirring. Stirring was continued at the same temperature for 1 h, then benzyl bromide (2.2 ml, 18.0 mmol) was added and the resulting mixture was stirred at room temperature for 12 h. After that, ice water was added to the above mixture and the product was extracted twice with EtOAc (50 ml). The extract was washed with water and saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with *n*-hexane–EtOAc (95 : 5 and 90 : 10) to afford the benzyl ether (2.3 g, 72.5%) as a colorless oil, $C_{16}H_{24}O_3$. FD-MS *m/z*: 264 (M$^+$), 249. IR ν_{max}^{neat} cm^{-1}: 2975, 2930, 2860. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.28 (3H, s, 4-CH_3), 1.35 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.4—2.1 (4H, m), 3.60 (2H, t, J=7.0), 3.88 (2H, m), 4.46 (2H, s, $C_6H_5C\underline{H}_2$), 7.28 (5H, s, $C_6\underline{H}_5CH_2$). Next, *p*-TsOH·H_2O (100 mg) was added to a solution of the obtained benzyl ether (1.1 g, 4.2 mmol) in MeOH (10 ml) and the reaction mixture was stirred at room temperature for 6 h. After evaporation of the solvent, the residue was chromatographed on silica gel using $CHCl_3$–MeOH (98 : 2 and 97 : 3) mixture to afford the diol (**13**, 872.0 mg, 93.4%) as a colorless oil, $C_{13}H_{20}O_3$. EI-MS *m/z*: 206 (M$^+$−H_2O), 179, 107, 91. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 3075, 3040, 2975, 2950, 2875. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.25 (3H, s, 3-CH_3), 1.48—2.13 (4H, m, H_2-2, H_2-4), 3.48—4.00 (4H, m, H_2-1, H_2-5), 4.49 (2H, s, $C_6H_5C\underline{H}_2$), 7.28 (5H, s, $C_6\underline{H}_5CH_2$).

Methyl (*RS*)-3-Hydroxy-3-methylglutarate (**14**): The acid (**14**) was obtained through four steps in 26.2% yield from the diol (**13**) according to the same procedure used for **10**. EI-MS *m/z*: 161 (M$^+$−15), 143, 117, 103. IR ν_{max}^{neat} cm^{-1}: 3440 (br, COOH/OH), 1720 (carboxyl/ester C=O). ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 1.39 (3H, s, CH_3), 2.65 (4H, s, H_2-2, H_2-4), 3.73 (3H, s, $COOCH_3$).

Methyl (*RS*)-5-[(*S*)-1-Carbomethoxyethylamino]-3-hydroxy-3-methylglutarate (**4**): A solution of DCC (19.1 mg, 0.09 mmol) in CH_2Cl_2 (1 ml) was added dropwise to a solution of **14** (13.6 mg, 0.8 mmol) in CH_2Cl_2–CH_3CN (3 : 2, 2 ml) at 0 °C and the resulting mixture was stirred at the same temperature for 10 min. To this mixture, L-alanine methyl ester hydrochloride (16.1 mg, 0.12 mmol) and Et_3N (20 μl, 0.14 mmol) were added, and the reaction mixture was stirred at room temperature for 1 h. After the same work-up as used for **3**, **4** was obtained as a colorless viscous oil in 50.1% (10.1 mg) yield. EI-MS *m/z*: 262 [(M+H)$^+$], 261 (M$^+$), 202, 117. HR-EIMS *m/z*: 261.1213 (M$^+$). Calcd for $C_{11}H_{19}NO_6$: 261.1212. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3200 (OH/NH), 3000, 2950, 1740 (ester C=O), 1660 (amide C=O), 1520. ^{1}H-NMR: Table I.

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