Immunomodulatory Constituents from an Ascomycete, *Eupenicillium crustaceum*, and Revised Absolute Structure of Macrophorin D

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Fractionation guided by immunomodulatory activity of the EtOAc extract of the Ascomycete *Eupenicillium crustaceum* has afforded two new naturally occurring products, 4′-oxomacrophorin D (**1**) and 4′-oxomacrophorin A (**2**), as the immunosuppressive components of this fungus [**1**: 3-hydroxy-3-methyl-glutaryl (HMG) conjugate of **2**]. The structures including the absolute configurations of **1** and **2** have been determined on the basis of chemical correlation of **1** with macrophorin D (**3**). The absolute configuration of the HMG moiety in **3** has been revised from 3*R* to 3*S*.

In our recent screening program on immunomodulatory constituents from fungi, nine 2-pyrones from *Gelasinospora multiforis*,[1,2] *G. heterospora*, and *G. longispora*,[2] a hexaketide from *G. heterospora* and *G. longispora*,[2] a 2-pyrone and two macrocyclic diesters from *Diplogelasinospora grovesii*,[3] a macrocyclic sesterterpenetriol and three 2-furanones from *Gelasinospora kobi*,[4] two anthraquinones, two octaketides, and two dioxopiperazines from *Microascus tardifaciens*,[5] and four sesterterpenes and a sesquiterpenetriol diester from *Emericella aurantio-brunnea*[6] have been isolated as immunosuppressive constituents. Herein, we report that the EtOAc extract of an Ascomycete, *Eupenicillium crustaceum* Ludwig, appreciably suppressed the proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS). Solvent partitions followed by repeated chromatographic fractionations of the extract, monitored by immunomodulatory activity, afforded two compounds, tentatively named EC-A (**1**) and B (**2**), as the immunosuppressive components of this fungus. This paper deals with the structures and immunosuppressive activities of these compounds.

The EtOAc extract of *E. crustaceum* IFM42163 (=CBS-344.61) cultivated on sterilized rice medium suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 99% at 50 μg/mL. The concentrated solution of the extract in MeOH was partitioned between *n*-hexane and water into an *n*-hexane layer and an aqueous suspension. The aqueous suspension was further partitioned with EtOAc and water into an EtOAc layer and an aqueous layer [yields (%) of the *n*-hexane, EtOAc, and aqueous layers after evaporation of the solvents from the EtOAc extract: 47.8, 46.8, and 4.5, respectively]. The *n*-hexane, EtOAc, and aqueous layers suppressed the Con A-induced proliferation by 21, 46, and −19% at 12.5 μg/mL, respectively. Repeated chromatographic fractionation of the EtOAc layer guided by the immunomodulatory activity afforded two immunosuppressive components, EC-A (**1**) and B (**2**) [yields (%) of **1** and **2** from the EtOAc extract: 2.33 and 0.764, respectively].

The molecular formula of **1** was determined to be $C_{28}H_{38}O_8$ by HRFABMS. The UV spectrum of **1** gave the absorption of a conjugated >C=O system. The IR spectrum of **1** showed the absorptions of −OH, −COOH, and α,β-unsaturated >C=O groups (3448, 1750, and 1688 cm⁻¹, respectively). The ¹H and ¹³C NMR data of **1** including two-dimensional COSY, HMQC, and HMBC data suggested that **1** was a new compound similar to a drimane-type sesquiterpenyl metabolite, macrophorin D (**3**),[7] which was isolated as a self-growth inhibitor, together with an antimicrobial and cytotoxic congener, macrophorin A (**4**),[8] from a Fungi Imperfecti *Macrophoma* sp. On oxidation with MnO_2 in $CHCl_3$, **3** was converted into a compound that was identical with **1** including optical rotation. Comparison of the ¹H NMR spectrum of **1** (in CDCl₃) with that of **3** showed that the signal of H-4′ at δ 4.62 (d, $J = 1.5$ Hz) disappeared and the signal of H-5′ at δ 3.81 (d, 1.5) shifted to δ 3.77 (s), indicating that the secondary OH at position 4′ in **3** was oxidized to a ketone group to give **1** (Table 1). For the structure of macrophorin D, the (3*R*)-3-hydroxy-3-methyl-glutaryl [(3*R*)-HMG] conjugate of the drimane-type sesquiterpenyl cyclohexenone epoxide (**3a**) has already been proposed.[7] Therefore, the structure of EC-A was considered to be expressed as **1a** including the absolute configuration of (3*R*)-HMG (Figure 1). But, the (3*R*)-HMG group is rare in naturally occurring products, because (3*S*)-HMG-CoA gives (3*R*)-mevalonate, which is the biosynthetic precursor of various isoprenoids, with the aid of HMG-CoA reductase shown in Figure 2.[9] Thus, we reinvestigated the absolute configuration of the HMG moiety of **1**.

To hydrolyze reductively the HMG ester in EC-A, lithium triethylborohydride (super-hydride, LiEt₃BH) seemed to be more suitable than borane, which was utilized formerly to hydrolyze the HMG ester in macrophorin D.[7] LiEt₃BH reduces exclusively the ester group in the presence of both ester and carboxyl groups.[10] Reduction of **1** with LiEt₃BH in dry THF followed by treatment with dilute HCl gave a lactone product (**5**), which was not identical with authentic (3*S*)-mevalonolactone, but was identical with authentic (3*R*)-mevalonolactone in chiral HPLC analysis. This identification was further confirmed by the fact that 5-*O*-acetyl-1-[(*S*)-1-phenylethyl]amide derived from **5** (**6**) was identical with authentic[11] (3*R*)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]meva-lonamide[11] in reversed-phase HPLC analysis (Figure 2). Therefore, the structure, including absolute configuration of EC-A, is not **1a** containing the (3*R*)-HMG group, but **1** containing the (3*S*)-HMG group. Accordingly, the absolute
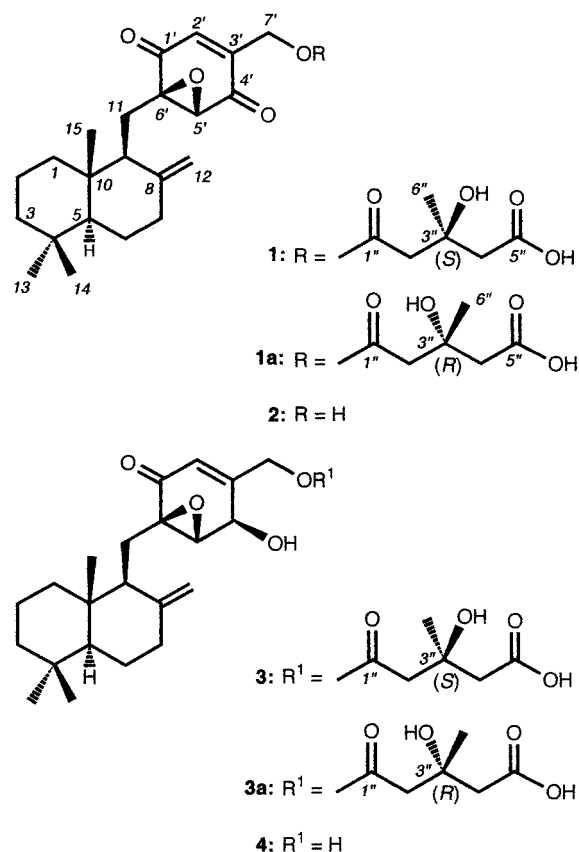
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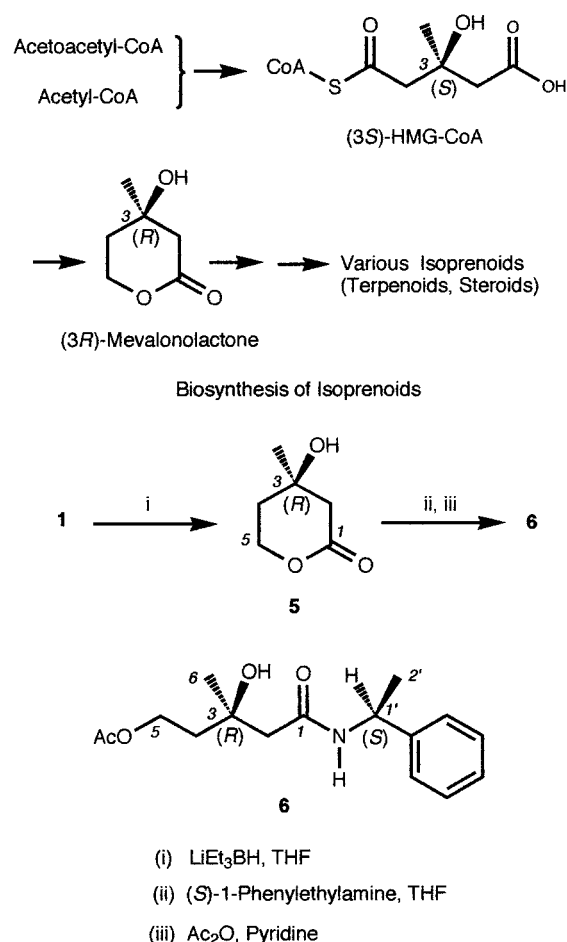† Chiba University.
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Table 1. NMR Data for 4′-Oxomacrophorin D (**1**), 4′-Oxomacrophorin A (**2**), and Macrophorin D (**3**) in CDCl₃, δ (ppm), from TMS as an Internal Standard, [coupling constants (Hz) in parentheses]

	1		2		3
position	δ_H	δ_C	δ_H	δ_C	δ_H
1	1.19 (m), 1.75 (m)	38.85 (t)	1.24 (m), 1.75 (m)	38.89 (t)	1.25 (m), 1.75 (m)
2	1.55 (2H, m)	19.27 (t)	1.53 (2H, m)	19.32 (t)	1.55 (2H, m)
3	1.19 (m), 1.41 (m)	41.94 (t)	1.15 (m), 1.38 (m)	41.99 (t)	1.18 (m), 1.40 (m)
4		33.55 (s)		33.60 (s)	
5	1.11 (dd, 12.7, 2.4)	55.44 (d)	1.10 (br d, 14.4)	55.50 (d)	1.12 (br d, 12.5)
6	1.31 (m), 1.75 (m)	24.28 (t)	1.28 (m), 1.70 (m)	24.35 (t)	1.32 (m), 1.75 (m)
7	1.95 (m), 2.36 (br d, 12.0)	37.93 (t)	1.94 (m), 2.35 (br d, 12.3)	38.02 (t)	1.88 (m), 2.36 (m)
8		148.66 (s)		148.74 (s)	
9	1.68 (d, 11.0)	51.28 (d)	1.68 (d, 11.2)	51.41 (d)	1.71 (d, 12.5)
10		39.70 (s)		39.76 (s)	
11	2.05 (dd, 14.1, 11.0), 2.46 (d, 14.1)	20.01 (t)	2.02 (dd, 14.8, 11.2), 2.49 (d,14.8)	20.24 (t)	1.97 (m) 2.38 (d, 14.3)
12	4.51 (s), 4.82 (s)	106.85 (t)	4.50 (s), 4.81 (s)	106.87 (t)	4.54 (br s), 4.82 (br s)
13	0.87 (3H, s)	33.49 (q)	0.85 (3H, s)	33.52 (q)	0.87 (3H, s)
14	0.80 (3H, s)	21.60 (q)	0.78 (3H, s)	21.64 (q)	0.80 (3H, s)
15	0.71 (3H, s)	14.42 (q)	0.69 (3H, s)	14.46 (q)	0.70 (3H, s)
1′		191.67 (s)		192.02 (s)	
2′	6.57 (s)	132.87 (d)	6.62 (s)	132.06 (d)	5.92(br s)
3′		142.11 (s)		146.84 (s)	
4′		192.12 (s)		193.55 (s)	4.62 (d, 1.5)
5′	3.77 (s)	58.80 (d)	3.72 (s)	59.06 (d)	3.81 (d, 1.5)
6′		62.56 (s)		62.50 (s)	
7′	4.83 (d, 16.3), 4.98 (d, 16.3)	59.62 (t)	4.33 (d, 16.8), 4.52 (d, 16.8)	59.17 (t)	4.74 (d, 15.7), 4.95 (d, 15.7)
1″		170.68 (s)			
2″	2.73 (d, 14.4), 2.77 (d, 14.4)	44.49 (t)			2.67 (d, 14.3), 2.74 (d, 14.3)
3″		69.81 (s)			
4″	2.67 (d, 16.4), 2.70 (d, 16.4)	44.48 (t)			2.64 (d, 15.5), 2.66(d, 15.5)
5″		170.68 (s)			
6″	1.41 (3H, s)	27.26 (q)			1.40 (3H, s)

Figure 1.

(i) LiEt₃BH, THF
(ii) (*S*)-1-Phenylethylamine, THF
(iii) Ac₂O, Pyridine

Figure 2.

structure of macrophorin D has been revised from **3a** to **3**. Thus, the structure of EC-A, a new immunosuppressant, has been verified as 4′-oxomacrophorin D (**1**) (Figure 1).

The molecular formula of **2** was determined to be C₂₂H₃₀O₄ by HRFABMS. The UV spectrum of **2** gave the absorption of a conjugated >C=O system, the same as that of **1**. The IR spectrum of **2** showed the absorptions of −OH

Table 2. Immunosuppressive Effects of 4′-Oxomacrophorin D (**1**) and 4′-Oxomacrophorin A (**2**) and Some Compounds on Con A-Induced and LPS-Induced Proliferations of Mouse Splenic Lymphocytes

	IC_{50} (μg/mL)	
compound	Con A-induced	LPS-induced
1	4.5	2.0
2	0.5	0.25
azathioprine	2.7	2.7
cyclosporin A	0.04	0.07
FK506 (tacrolimus)	1.5×10^{-5}	1.6×10^{-3}

[a] The IC_{50} value of each sample was calculated from the correlation curve between the sample concentration (horizontal axis) and the cell proliferation (vertical axis). The curve of each sample was drawn with 7 points, each of which represented the mean of 3 experiments on each correlation between 7 different concentrations and cell proliferations.

and α,β-unsaturated $>C{=}O$ groups (3448 and 1685 cm^{-1}, respectively). Comparison of the ^{1}H NMR spectrum of **2** with that of **1** showed that **2** was similar to **1** except that **2** lacked the signals due to the (3*S*)-HMG moiety. This observation was also supported from comparison of the ^{13}C NMR spectrum of **2** with that of **1** (Table 1). These spectral data, and the biosynthetic consideration that EC-B may be biosynthesized via a pathway similar to that of **1**, indicated that the structure including absolute configuration of EC-B was **2** (4′-oxomacrophorin A) (Figure 1). Ayer et al. have isolated macrophorin A (**4**) from *Penicillium brevi-compactum* and prepared the 4′-oxo derivative of **4** (**7**) from **4** with MnO$_2$ oxidation.[12] It was considered that **2** might be identical with **7**, although **2** was not directly compared with **7** described in the literature.[12] Macrophorin A (**4**) has also been isolated from *E. crustaceum* NR-RL22307 (=CBS457.72) by Wang et al.[13] However, to the best of our knowledge, this is the first time that 4′-oxomacrophorin A (**2**) has been isolated as an immunosuppressant from a natural source.

The immunosuppressive activities (IC_{50} values) of **1** and **2** were calculated against Con A- (T-cells) and LPS-induced (B-cells) proliferation of mouse splenic lymphocytes, as shown in Table 2. Both **1** and **2** suppressed about two times higher the proliferation of B-cells than that of T-cells. Compound **2** displayed about eight times higher immunosuppressive activity than **1**, indicating that the presence of a free $-CH_2OH$ group at position 3′ in **2** might be very important for the immunosuppressive activity of **2**. The immunosuppressive activity of **2** was about five to 10 times higher than that of azathioprine,[2] although lower than those of cyclosporin A[2] and FK506 (taclolimus).[2]

Experimental Section

General Experimental Procedures. Optical rotations and CD spectra were measured with a JASCO DIP 140 digital polarimeter and a JASCO J-500 spectropolarimeter, respectively. UV and IR spectra were recorded on Hitachi U-3200 and JASCO FT/IR-230 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were measured with JEOL JNM-A400 (^{1}H, 399.65; ^{13}C, 100.40 MHz) and -A500 (^{1}H, 500.00; ^{13}C, 125.65 MHz) spectrometers using chemical shift, δ (ppm), values from TMS as an internal standard. EIMS, FABMS, and HRFABMS spectra were recorded on a JEOL AUTO MS-20, a JEOL JMS-AX505 HA using *m*-nitrobenzyl alcohol (*m*-NBA) as a matrix, and a JEOL JMS-HX110A spectrometer using *m*-NBA as a matrix, respectively. Column chromatography was performed with Wako Si gel C-200. HPLC was performed using Senshu SSC-3100, Waters 600-E, and JASCO PU-980 flow systems equipped with Senshu SSC-5200, Waters 486 Tunable

UV/VIS, and JASCO 970 UV detectors at 220 nm. Evaluation of suppressive activity (IC_{50} values) of samples against the proliferation of mouse (BALB/c, male, 7−11 weeks) splenic lymphocytes stimulated with Con A and LPS was executed with the same method as that described in our previous report [this method is based on the formation ratio of MTT-formazan from exogenous 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) in lymphocytes].[2]

Fungal Material. *Eupenicillium crustaceum* IFM42163 (=CBS344.61) was deposited earlier at Research Institute for Chemobiodynamics, Chiba University (present name: Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University). The voucher specimen was also deposited in Laboratory of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University. This fungus was cultivated on sterilized moistened rice in Roux flasks (200 g/flask × 26) at 25 °C for 24 days to give reddish brown moldy rice.

Isolation of EC-A (1) and B (2) from *E. crustaceum*. The moldy rice was extracted with EtOAc (7.8 L) with shaking at room temperature for 6 h two times to give an EtOAc solution (15.6 L), which gave, after evaporation in vacuo, a wine-red EtOAc extract (43.6 g). A portion of the EtOAc extract (31.2 g) was dissolved in MeOH (60 mL). The MeOH solution was suspended in H$_2$O (1.2 L). The suspension was partitioned with *n*-hexane (1.2 L) two times into an *n*-hexane layer (after evaporation in vacuo, 14.9 g) and an aqueous suspension. The aqueous suspension was further partitioned with EtOAc (1.2 L) two times into an EtOAc layer (14.6 g) and an aqueous layer (1.4 g). A part of the EtOAc layer (7.80 g) was subjected to chromatography on a Si gel column (Wako, C-200) with CHCl$_3$, CHCl$_3$−MeOH (100:1, v/v, 50:1, 50:1, 50:1), and MeOH to give fractions 1−6 (0.49, 1.61, 1.66, 1.15, 0.58, 3.14 g), respectively. The IC_{50} values of the fractions 1−6 against the Con A-induced proliferation of the lymphocytes were >50, <10, <10, 25−35, 35−45, and 25−35 μg/mL, respectively. A portion of fraction 3 (620 mg) was chromatographed on an ODS column (Waters C$_{18}$ Sep-Pak cartridge, 16 × 50 mm) with 90% MeOH to give a fraction that was further chromatographed on an HPLC ODS column (Senshu Pegasil, 20 × 250 mm) with 70% CH$_3$CN containing 0.1% TFA at a flow rate of 8.0 mL/min to afford EC-A (**1**) (145 mg). A part of fraction 2 (101 mg) was chromatographed on a Si gel column (Wako, C-200) with *n*-hexane−EtOAc (8:1) to give EC-B (**2**) (8.0 mg).

EC-A (4′-Oxomacrophorin D) (1): yellow viscous oil; $[\alpha]_D^{25}$ +35.5° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (3.97), 260 (sh, 3.47) nm; IR ν_{max} (KBr) 3448, 2939, 2860, 1750, 1688, 1647, 1446, 1388, 1201, 971, 896 cm^{-1}; positive FABMS *m/z* 525 [(M + Na)$^+$]; negative FABMS *m/z* 501 [(M − H)$^-$]; HRFABMS *m/z* 525.2464 (calcd for C$_{28}$H$_{38}$O$_8$Na [(M + Na)$^+$], 525.2464); CD (0.797 mM, MeOH) $\Delta\epsilon$(nm) −1.05 (336), 0.00 (297), −2.65 (266), +5.01 (237), +3.69 (228), +6.79 (213); ^{1}H NMR and ^{13}C NMR, see Table 1.

EC-B (4′-Oxomacrophorin A) (2): yellow viscous oil; $[\alpha]_D^{25}$ +10.2° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (3.87), 268 (sh, 3.33) nm; IR ν_{max} (KBr) 3448, 2927, 2858, 1685, 1647, 1459, 1389, 1203, 1093, 892 cm^{-1} (lit.[12] 3440, 2960, 1710 (sh), 1687, 1460, 1440, 1385, 1200, 885); EIMS *m/z* (%) 358 [M$^+$, 29] (lit.[12] *m/z* (%) 358 [M$^+$, 4]); HRFABMS *m/z* 359.2225 (calcd for C$_{22}$H$_{31}$O$_4$ [(M + H)$^+$], 359.2223) (lit.[12] HREIMS *m/z* 358.2155 [M$^+$]); CD (1.12 mM, MeOH) $\Delta\epsilon$(nm) −0.30 (341), 0.01 (297), −2.41 (263), +7.88 (220); ^{1}H NMR and ^{13}C NMR, see Table 1 (the ^{1}H NMR data were very similar to those of compound **7** described in the literature[12]).

Conversion of Macrophorin D (3) to EC-A (1). Activated MnO$_2$ (50 mg) (Wako) was added to a solution of authentic macrophorin D (**3**), which was isolated by one of us, T.S., et al.,[7] (2.5 mg) in CHCl$_3$ (600 μL). The reaction mixture was stirred at room temperature for 20 min and filtered to remove insoluble substances. The filtrate was evaporated in vacuo to furnish 4′-oxomacrophorin D (0.8 mg), yellow viscous oil, which was identical with EC-A (**1**) by ^{1}H NMR (CDCl$_3$) and CD spectra (MeOH) and TLC behavior [Merck Kieselgel 60F$_{254}$, CHCl$_3$−MeOH (9:1), R_f 0.42].

Reductive Hydrolysis of EC-A (1). A solution of LiEt$_3$-BH (1.0 M) in dry THF (20 μL) (Aldrich) was added to a solution of EC-A (**1**) (5.0 mg) in dry THF (80 μL) under ice-cooling in an Ar stream. The reaction mixture was stirred under ice-cooling in Ar gas for 15 min. After addition of H$_2$O (100 μL) to the reaction mixture, 0.1 N HCl (500 μL) was added to adjust its pH to 3−4. The reaction mixture was then stirred at room temperature in Ar gas for 42 h to allow lactone formation. The reaction mixture was partitioned with EtOAc (1.0 mL) three times into an EtOAc layer (yellow) and an aqueous layer (colorless). The aqueous layer which contained a compound equal to mevalonolactone on TLC analysis was evaporated in vacuo to give a resinous residue, which was extracted with EtOAc (2.0 mL) two times to furnish an EtOAc solution. The EtOAc solution was evaporated in vacuo to afford compound **5** (0.8 mg), a colorless amorphous residue, which was not identical with authentic (3S)-mevalonolactone (t_R 12.8 min), but identical with authentic (3R)-mevalonolactone (t_R 13.6 min) in HPLC analysis on a chiral CD-Ph column (4.6 × 250 mm) (Shiseido) with n-hexane−EtOH (2:3) at a flow rate of 0.5 mL/min.

Formation of 5-*O*-Acetyl-1-[(*S*)-1-Phenylethyl]mevalonamide, Part 1. (*S*)-1-Phenylethylamine (60 mg) (Aldrich) was added to a solution of (3RS)-mevalonolactone (20 mg) (Aldrich) in dry THF (1.0 mL) at room temperature in an Ar stream. After stirring at room temperature under Ar gas for 18 h, the solvent was evaporated in vacuo to give a residue, which was extracted with EtOAc (20 mL) to afford an EtOAc solution. After washing with 0.1 N HCl followed by H$_2$O, the EtOAc solution was evaporated in vacuo to give a colorless oily residue. This residue was then acetylated with Ac$_2$O (0.5 mL) in pyridine (1.0 mL) at room temperature for 8 h to afford (3RS)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamide (3.8 mg), a colorless amorphous residue, which gave two peaks (t_R 83.2 and 84.8 min, integral intensity 1:1) on an HPLC ODS column (ODS-AM, 10 × 250 mm) (YMC) with MeOH−H$_2$O (2:3) at a flow rate of 2.0 mL/min [the peak, t_R 84.8 min, was verified to be that of (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamide by co-chromatography of both samples of (3RS)- and (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamides]. The ^{1}H NMR and EIMS spectra of (3RS)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]-mevalonamide were identical with those of (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamide formed from (3R)-mevalono-lactone.

Part 2. (*S*)-1-Phenylethylamine (35 mg) was added to a solution of (3R)-mevalonolactone (26 mg) (Wako) in dry THF (1.0 mL) at room temperature in an Ar stream. After stirring at room temperature under Ar gas for 18 h, the reaction mixture was treated in the same way as described for the formation of (3RS)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalona-mide from (3RS)-mevalonolactone to give a colorless oily residue. This residue was then acetylated with Ac$_2$O (0.25 mL) in pyridine (0.5 mL) at room temperature for 8 h to afford (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamide (5.0 mg), color-

less amorphous residue, which gave one peak (t_R 84.8 min) on an HPLC ODS column (ODS-AM, 10 × 250 mm) (YMC) with MeOH−H$_2$O (2:3) at a flow rate of 2.0 mL/min.

(3R)-5-*O*-Acetyl-1-[(*S*)-1-phenylethyl]mevalonamide: ^{1}H NMR (CDCl$_3$) δ 1.23 (3H, s, H$_3$-6), 1.51 (3H, d, J = 6.8 Hz, H$_3$-2′), 1.82, 1.88 (each 1H, dd, 14.1, 6.8, H$_a$-, H$_b$-4), 2.04 (3H, s, Ac), 2.28, 2.41 (each 1H, d, 14.8, H$_a$-, H$_b$-2), 4.23 (2H, t, 6.8, H$_2$-5), 4.51 (1H, br s, OH), 5.15 (1H, m, H-1′), 6.11 (1H, br peak, NH), 7.27−7.37 (5H, m, Ph) (these ^{1}H NMR data were identical with those of (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalon-amide described in the literature[11]); EIMS m/z (%) 293 (M$^+$, 11).

Part 3. (*S*)-1-Phenylethylamine (2.0 mg) was added to a solution of compound **5** (0.7 mg) in dry THF (100 μL) at room temperature in an Ar stream. After stirring at room temperature under Ar gas for 17 h, the reaction mixture was evaporated in vacuo to give a residue, which was extracted with EtOAc (2.0 mL) two times to afford an EtOAc solution. After washing with 0.1 N HCl followed by H$_2$O, the EtOAc solution was evaporated in vacuo to furnish a colorless oily residue. This residue was then acetylated with Ac$_2$O (100 μL) in pyridine (200 μL) at room temperature for 24 h to afford compound **6** (0.2 mg), colorless amorphous residue, which was not identical with authentic (3S)-5-*O*-acetyl-1-[(*S*)-1-phenyl-ethyl]mevalonamide (t_R 83.2 min), but identical with authentic (3R)-5-*O*-acetyl-1-[(*S*)-1-phenylethyl]mevalonamide (t_R 84.8 min) in HPLC analysis using an ODS column (ODS-AM, 10 × 250 mm) (YMC) with MeOH−H$_2$O (2:3) at a flow rate of 2.0 mL/min.

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