Stereospecific Synthesis of a Novel Farnesyl Protein Transferase Inhibitor, Valinoctin A and Its Analogues

MAKOTO TSUDA, YASUHIKO MURAOKA and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

RYUICHI SEKIZAWA and KAZUO UMEZAWA

Department of Applied Chemistry,
Faculty of Science and Technology, Keio University,
3-14-1 Hiyoshi, Kohoku-ku, Yokohama, 223, Japan

(Received for publication May 10, 1996)

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoic acid was synthesized by Curtius rearrangement of an azide derivative of (*S*)-malic acid. Total syntheses of valinoctin A and its analogues were achieved by a coupling of (2*S*,3*R*)-3-amino-2-hydroxyoctanoic acid moiety with L-valine or several other amino acids moieties. 2*S* configuration of 3-amino-2-hydroxyoctanoic acid moiety was found to be important for the inhibitory activity and the L-valine moiety of valinoctin A was exchangeable with other L-amino acids.

Valinoctin A (**1**), a novel farnesyl protein transferase inhibitor, was isolated from fermentation broth of *Streptomyces* sp. MJ858-NF3[1]. The structure was elucidated as 3-amino-2-hydroxyoctanoyl-L-valine from NMR and mass spectra and degradation studies. The sterochemistry of 3-amino-2-hydroxyoctanoyl moiety was confirmed to be 2*S*,3*R* configuration from X-ray crystallographic analysis.

In this paper, we report the stereospecific synthesis of (2*S*,3*R*)-3-amino-2-hydroxyoctanoic acid and total synthesis of valinoctin A and its analogues. Farnesyl protein transferase inhibitory activities of them were also measured.

Chemistry

SEEBACH *et al.* reported that the diesters of (*S*)-malic acid could be alkylated with good diastereoselectivity at C3 using two equivalents of lithium diisopropylamide and various alkyl halides[2]. NORMAN *et al.* reported the synthesis of bestatin and that the use of lithium bis(trimethylsilyl)amide instead of lithium diisopropylamide enhanced the diastereoselectivity[3]. We tried to synthesize homochiral 3-amino-2-hydroxy acid moiety of valinoctin A by this method.

The synthesis of valinoctin A and its amino acid analogues were shown in Scheme 1. (*S*)-malic acid was first converted diester (**2**), and then stereocontrolled alkylation at C3 was achieved using two equivalents of lithium bis(trimethylsilyl)amide and iodopentane. Diester (**3**) showed 46 : 1 selectivity (NMR analysis) for the *R* isomer at C3. Saponification of **3** with 1 N NaOH afforded diacid (**4**), which was recrystallized from ether-hexane (1 : 3). Treatment of **4** with trifluoroacetic anhydride gave an intermediate cyclic anhydride, which was subsequently opened with EtOH to give the mono-acid (**5**)[4]. Treatment of **5** with triethylamine and diphenylphosphoryl azide gave oxazolidone (**6**) *via* isocyanate intermediate[5]. Saponification of **6** with 1 N NaOH and followed by deionization by using strong acidic ion-exchange resin gave (2*S*,3*R*)-3-amino-2-hydroxyoctanoic acid (**7**). Protection of the amino group of **7** with benzyl *S*-4,6-dimethylpyrimidine-2-ylthiocarbonate and triethylamine gave *N*-benzyloxycarbonyl derivative of **7** (**8**). Coupling reaction of **8** with L-valine benzyl ester or other amino acid benzyl esters using 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) gave *N*- and *C*-terminal protected compounds (**9**~**12**). Hydrogenation of **9**~**12** with palladium-black gave valinoctin A and its analogues (**1** and **13**~**15**).

Enzyme Inhibition

Table 1 shows the inhibitory activities of valinoctin A, its stereoisomers[1] and replacement analogues in this study. The (2*S*,3*S*)-stereoisomer (**1b**) showed almost the same inhibitory activity against farnesyl protein transferase as **1**. (2*R*,3*S*)- and (2*R*,3*R*)-isomer (**1a** and **1c**) indicated no significant inhibitory activities. These data

Scheme 1. Synthesis of valinoctin A and its analogues.

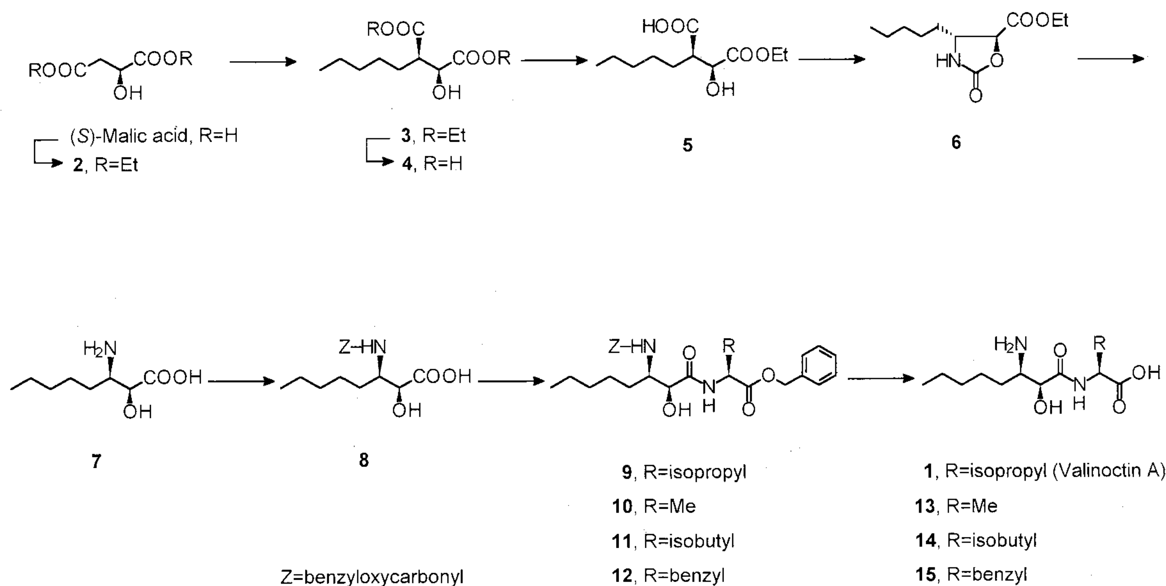

Table 1. Inhibitory activities of valinoctin A and its analogues (IC_{50}, μg/ml).

Compound[a]	Stereochemistry of 3-amino-2-hydroxy acid moiety	Farnesyl protein transferase
1	(2*S*,3*R*)	0.90
1a	(2*R*,3*S*)	>20
1b	(2*S*,3*S*)	1.0
1c	(2*R*,3*R*)	>20
13	(2*S*,3*R*)	>20
14	(2*S*,3*R*)	1.2
15	(2*S*,3*R*)	0.58

[a] **1a**~**1c** were prepared by the method reported[1].

suggests that (2*S*)-stereochemistry of the 3-amino-2-hydroxyoctanoic acid moiety is important for the inhibitory activity.

Analogue **13**, in which L-Val of valinoctin A was replaced by L-Ala, showed no significant inhibitory activity. On the other hand, the L-leucine analogue (**14**) showed essentially the same inhibitory activity as **1**. The L-phenylalanine analogue (**15**) showed slightly increased anti-farnesyl protein transferase activity.

Experimental

General

Melting points were determined on a micro melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz with a JEOL JNM-GX400 spectrometer. FAB-MS spectra were measured on a JEOL JMS-SX102 mass spectrometer. TLC was carried out on Merck precoated silica gel $60F_{254}$ plates.

(*S*)-Diethyl Malate (**2**)

A solution of (*S*)-malic acid (5.37 g, 40.0 mmol) in absolute ethanol (30 ml) was refluxed for 23 hours in the presence of phosphoryl chloride (23.7 mg). After evaporation of the solvent, ether (20 ml) was added to the residue and the solution was washed with saturated aq $NaHCO_3$ (20 ml × 2) and saturated aq NaCl (20 ml), and dried (Na_2SO_4). Evaporation of the solvent gave a coloress oil of **2**, 4.73 g (62.2%): Rf 0.34 (hexane - EtOAc, 2:1); $[\alpha]_D^{24}$ −12.0° (1 dm, neat); FAB-MS (positive) *m/z* 191 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, *J*=7.1 Hz, CH_3), 1.32 (3H, t, *J*=7.1 Hz, CH_3), 2.78 (1H, dd, J=6.1, 16.1 Hz, C*Ha*HbCO), 2.85 (1H, dd, *J*=4.4, 16.1 Hz, CHa*Hb*CO), 3.26 (1H, d, *J*=5.4 Hz, OH), 4.17 (2H, q, *J*=7.1 Hz, CH_2), 4.27 (1H, dq, *J*=7.1, 14.2 Hz, C*Ha*Hb), 4.28 (1H, dq, *J*=7.1, 14.2 Hz, CHa*Hb*), 4.48 (1H, ddd, *J*=4.4, 5.4, 6.1 Hz, CH).

(2*S*,3*R*)-Diethyl 2-Hydroxy-3-pentylsuccinate (**3**)

To a solution of freshly distilled **2** (3.81 g, 20.1 mmol) in dry THF (80 ml) was added dropwise 1 M lithium bis(trimethylsilyl)amide in hexane solution (45 ml, 45 mmol) under argon at −72°C. The temperature of the reaction mixture was allowed to rise to −20°C within 30 minutes. The solution was cooled back to −72°C, and 1-iodopentane (6.57 ml, 50.4 mmol) was added dropwise. Stirring was continued, first for 6 hours at

$-72°C$, then overnight while the temperature was allowed to rise to 21°C. The reaction mixture was quenched with a solution of AcOH (5.2 ml, 91.2 mmol) in ether (8 ml) at $-50 \sim -60°C$ and was then poured into a mixture of ether (230 ml) and water (30 ml). The organic layer was washed successively with saturated aq $NaHCO_3$ (20 ml) and saturated aq NaCl (20 ml), and the aqueous layer was extracted with ether (80 ml × 2). The combined ethereal solution was dried (Na_2SO_4), and evaporated to give an oil. The product was purified by silica gel column chromatography with hexane - EtOAc (7:2) to give **3** (0.80 g), and a mixture of **3** and *O*-trimethylsilyl product of **3** (4.63 g).

To the mixture (4.63 g) in THF (30 ml) was added 1 M HCl (30 ml), and the mixture was stirred for 30 minutes. After evaporation of THF, the residual aqueous layer was extracted with EtOAc (50 ml and 30 ml × 2). The combined organic layer was washed with saturated aq NaCl (30 ml), and dried (Na_2SO_4). The solvent was evaporated and the residual oil was purified by silica gel column chromatography with hexane - EtOAc (7:2) to give **3** as an oil, 1.70 g (total 47.8%): Rf 0.52 (hexane - EtOAc, 2:1); FAB-MS (positive) *m/z* 261 $(M+H)^+$, 215 $(M-EtO)^+$, 187 $(M-COOEt)^+$; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz, CH_3), 1.25 (3H, t, J=7.3 Hz, CH_3), 1.30 (3H, t, J=7.3 Hz, CH_3), *ca.* 1.27~1.45 (6H, m, overlappiing, $CH_2 \times 3$), 1.65 (1H, m, C*Ha*Hb), 1.84 (1H, m, CHa*Hb*), 2.84 (1H, ddd, J=3.4, 7.8, 7.8 Hz, CH), 3.19 (1H, d, J=7.8 Hz, OH), 4.15 (2H, q, J=7.3 Hz, CH_2), 4.20~4.33 (3H, m, CH_2, C*H*OH), NMR of the product indicated an 46:1 ratio of (2*S*,3*R*)/(2*S*,3*S*).

Rf value and spectral data of (2*S*,3*R*)-diethyl 3-pentyl-2-trimethylsiloxysuccinate: Rf 0.69 (hexane - EtOAc, 4:1); FAB-MS (positive) *m/z* 333 $(M+H)^+$, 259 $(M-(CH_3)_3Si)^+$, 73 $((CH_3)_3Si)^+$; ^1H NMR ($CDCl_3$) δ 0.11 (9H, s, $(CH_3)_3Si$), 0.87 (3H, t, J=6.8 Hz, CH_3), 1.19~1.47 (13H, m, $CH_3 \times 2$, $CH_2 \times 3$, C*Ha*Hb), 1.64 (1H, m, CHa*Hb*), 2.81 (1H, ddd, J=4.4, 7.3, 7.8 Hz, CH), 4.07~4.28 (4H, m, $CH_2 \times 2$), 4.30 (1H, d, J=7.3 Hz, $(CH_3)_3SiOCH$).

(2*S*,3*R*)-2-Hydroxy-3-pentylsuccinic Acid (**4**)

A solution of **3** (2.71 g, 10.4 mmol) in dioxane (30 ml) and 2 M NaOH (30 ml) was refluxed for 1 hour. After acidification with 6 M HCl, the solution was concentrated. The residue was diluted with water (30 ml) and extracted with EtOAc (40 ml and 20 ml × 2), and dried (Na_2SO_4). Evaporation of the solvent gave a solid of **4** (2.03 g, 95.4%). Recrystallization of **4** from ether - hexane (1:3) gave pure **4**, 1.84 g (86.6%): Rf 0.62 (BuOH - AcOH - H_2O, 4:1:1); mp 102~104°C; FAB-MS (negative) *m/z* 203 $(M-H)^-$; ^1H NMR (CD_3OD) δ 0.91 (3H, t, J=6.8 Hz, CH_3), 1.23~1.46 (6H, m, $CH_2 \times 3$), 1.55 (1H, m, C*Ha*Hb), 1.70 (1H, m, CHa*Hb*), 2.76 (1H, ddd, J=5.9, 5.9, 8.9 Hz, CH), 4.23 (1H, d, J=5.9 Hz, C*H*OH), 4.90 (3H, br s, OH, COOH × 2).

(2*S*,3*R*)-Ethyl Hydrogen 2-Hydroxy-3-pentylsuccinate (**5**)

To an ice-cold solution of **4** (1.84 g, 9.01 mmol) was added trifluoroacetic anhydride (3.0 ml, 21.7 mmol), and the suspension was stirred. Within 1 hour the mixture became homogeneous. After stirring was continued for additional 2 hours in an ice bath, the TFA and trifluoroacetic anhydride were removed by vacuum evaporation while the flask was kept at 0°C. The resulting residue was dissolved in dry EtOH (6 ml). After the mixture was stirred for 3.5 hours, the solvent was evaporated to give a syrup. The product was purified by silica gel column chromatography with $CHCl_3$ - MeOH - AcOH (140:5:1) to give **5** as coloress syrup, 2.05 g (98.0%): Rf 0.35 ($CHCl_3$ - MeOH - AcOH, 65:5:1); $[\alpha]_D^{26}$ +7.2° (*c* 7.9, $CHCl_3$); FAB-MS (negative) *m/z* 231 $(M-H)^-$; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz, CH_3), 1.30 (3H, t, J=7.3 Hz, CH_3), *ca.* 1.24~1.48 (6H, m, overlapping, $CH_2 \times 3$), 1.68 (1H, m, C*Ha*Hb), 1.83 (1H, m, CHa*Hb*), 2.90 (1H, ddd, J=3.4, 7.3, 7.3 Hz, CH), 4.26 (2H, q, J=7.3 Hz, CH_2), 4.31 (1H, d, J=3.4 Hz, C*H*OH), 9.60 (br, OH, COOH).

(4*R*,5*S*)-Ethyl 2-Oxooxazolidine-4-pentyl-5-carboxylate (**6**)

A solution of **5** (2.05 g, 8.83 mmol) in toluene (30 ml) and triethylamine (1.42 ml, 10.1 mmol) was heated to 90°C, and added diphenylphosphoryl azide (2.09 ml, 9.70 mmol). After the solution was heated for 4 hours at 90°C, the solution was concentrated. The residue was diluted with water (10 ml) and extracted with EtOAc (10 ml × 3), and dried (Na_2SO_4). Evaporation of the solvent gave a crude oil. The product was purified by silica gel column chromatography with hexane - EtOAc (4:1~3:1) to give **6** as colorless syrup, 1.62 g (80.1%): Rf 0.50 (hexane - EtOAc, 1:1); $[\alpha]_D^{26}$ +35.3° (*c* 6.0, $CHCl_3$); FAB-MS (positive) *m/z* 230 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz, CH_3), 1.32 (3H, t, J=7.3 Hz, CH_3), *ca.* 1.24~1.47 (6H, m, overlapping, $CH_2 \times 3$), 1.67 (2H, m, CH_2), 3.84 (1H, br q, CH), 4.29 (2H, q, J=7.3 Hz, CH_2), 4.57 (1H, d, J=4.9 Hz, CHCO), 7.05 (1H, s, NH).

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoic Acid (**7**)

A solution of **6** (1.62 g, 7.07 mmol) in EtOH (15 ml) and 2 M NaOH (15 ml) was refluxed for 9 hours. Then, EtOH was evaporated under reduced pressure. The residual aqueous solution was charged on a column of Dowex 50W-X4 (H^+, 60 ml) and the column was washed with H_2O (200 ml). Elution with 5% aq NH_3 afforded a fraction containing **7**. Evaporation of the fraction gave a solid of **7**, (1.21 g, 97.7%). This solid was crystallized from PrOH - H_2O (1:1) to give pure **7**: Rf 0.45 (BuOH - AcOH - H_2O, 4:1:1); mp 215~217°C (dec); $[\alpha]_D^{26}$ −8.8° (*c* 0.50, H_2O - MeOH (1:1)) [ref, $[\alpha]_D^{25}$ −5.0° (*c* 0.85, H_2O - MeOH (1:1)][6]; FAB-MS (positive) *m/z* 176 $(M+H)^+$; ^1H NMR (CD_3COOD) δ 0.91 (3H, t,

$J=6.8$ Hz, CH_3), 1.26~1.51 (6H, m, $CH_2 \times 3$), 1.71 (1H, m, C*Ha*Hb), 1.84 (1H, m, CHa*Hb*), 3.79 (1H, ddd, $J=2.4$, 7.3, 7.3 Hz, CH), 4.39 (1H, d, $J=2.4$ Hz, C*H*OH).

(2*S*,3*R*)-3-Benzyloxycarbonylamino-2-hydroxyoctanoic Acid (**8**)

A mixture of **7** (1.18 g, 6.73 mmol), benzyl *S*-4,6-dimethyl pyrimidin-2-ylthiocarbonate (2.23 g, 8.13 mmol), water (5 ml), dioxane (5 ml) and triethylamine (1.42 ml, 10.1 mmol) was stirred for 23 hours at room temperature. To the mixture was added water (25 ml) and acidified to pH 2 by addition of 1 M HCl. The aqueous layer was extracted with EtOAc (30 ml and 20 ml × 2), and the combined organic layer was washed with 1 M HCl (20 ml × 2) and saturated aq NaCl (20 ml), and dried (Na_2SO_4). Evaporation of the solvent gave a crude syrup. The product was purified by silica gel column chromatography with $CHCl_3$ - MeOH - AcOH (300 : 2 : 1 ~ 300 : 12 : 1) to give **8** as colorless syrup, 2.05 g (98.5%). This syrup was crystallized from hexane - EtOAc (2 : 1) to give pure **8**: Rf 0.50 ($CHCl_3$ - MeOH - AcOH, 18 : 2 : 1); mp 90~91°C; $[\alpha]_D^{26}$ +37.6° (*c* 1.0, MeOH); FAB-MS (negative) *m*/*z* 308 $(M-H)^-$; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.4$ Hz, CH_3), 1.18~1.45 (6H, m, $CH_2 \times 3$), 1.50~1.72 (2H, m, CH_2), 4.12 (1H, brq, N-CH), 4.19 (1H, d, $J=1.5$ Hz, C*H*OH), 5.06, 5.18 (2H, ABq, $J=$ 12.0 Hz, PhCH_2), 5.19 (1H, d, $J=9.8$ Hz, NH), 7.22~7.40 (5H, m, Ph).

(2*S*,3*R*)-3-Benzyloxycarbonylamino-2-hydroxyoctanoyl-L-valine Benzyl Ester (**9**) and its Analogues (**10**~**12**)

To an ice-cold solution of **8** (312.0 mg, 1.01 mmol), L-valine benzyl ester *p*-toluenesulfonate (398.6 mg, 1.05 mmol), and HOBt (276.0 mg, 2.04 mmol) in DMF (3 ml) was added triethylamine (0.147 ml, 1.05 mmol) and EDC·HCl (249.7 mg, 1.30 mmol), and the resulting mixture was chilled in an ice bath for 2 hours. Stirring was continued for 21 hours at room temperature, and the mixture was diluted with EtOAc (30 ml). The mixture was washed with saturated aq $NaHCO_3$ (15 ml), 10% aq citric acid (15 ml) and saturated aq NaCl (15 ml), and dried (Na_2SO_4). Evaporation of the solvent gave a crude product. The product was purified by silica gel column chromatography with hexane - EtOAc (5 : 1 ~ 2 : 1) to give **9** as colorless crystals, 443.5 mg (88.0%): Rf 0.37 (hexane - EtOAc, 3 : 2); mp 81°C; $[\alpha]_D^{24}$ −17.5° (*c* 1.3, MeOH); FAB-MS (positive) *m*/*z* 499 $(M+H)^+$, 455 $(M-CO_2+H)^+$, 365 $(M-\text{benzyloxycarbonyl}+2H)^+$, 91 $(C_7H_7)^+$; ^1H NMR ($CDCl_3$) δ 0.80, 0.85 (3H, 3H, two d, each $J=6.8$ Hz, $CH_3 \times 2$ (Val)), 0.87 (3H, t, overlapping, CH_3), 1.16~1.47 (6H, m, $CH_2 \times 3$), 1.59~1.80 (2H, m, CH_2), 2.16 (1H, m, β-CH (Val)), 3.84 (1H, N-CH), 4.19 (1H, dd, $J=3.2$, 6.4 Hz, C*H*OH), 4.56 (1H, dd, $J=4.9$, 8.8 Hz, α-CH (Val)), 4.87 (1H, d, $J=6.4$ Hz, OH), 5.04 (2H, s, PhCH_2), 5.13, 5.19 (2H, ABq, $J=12.2$ Hz, PhCH_2), 5.51 (1H, d, $J=8.3$ Hz, NH), 7.21~7.42 (11H, m, Ph × 2, NH(Val)).

Compounds **10**~**12** were prepared by a similar procedure.

Crude (2*S*,3*R*)-3-benzyloxycarbonylamino-2-hydroxyoctanoyl-L-alanine benzyl ester (**10**) prepared from **8** (149.4 mg) and L-alanine benzyl ester *p*-toluenesulfonate (177.7 mg) was recrystallized from EtOAc - hexane to give **10** (196.4 mg, 86.4%) as colorless crystals: Rf 0.36 (hexane - EtOAc, 3 : 2); mp 116~117°C; $[\alpha]_D^{24}$ −20.1° (*c* 1.4, MeOH); FAB-MS (positive) *m*/*z* 471 $(M+H)^+$, 427 $(M-CO_2+H)^+$, 91 $(C_7H_7)^+$; ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, $J=6.4$ Hz, CH_3), 1.17~1.45 (6H, m, $CH_2 \times 3$), 1.34 (3H, d, $J=7.3$ Hz, CH_3 (Ala)), 1.51~1.76 (2H, m, CH_2), 3.85 (1H, N-CH), 4.16 (1H, brs, C*H*OH), 4.57 (1H, quinted, $J=7.3$ Hz, α-CH (Ala)), *ca.* 4.64 (1H, br, overlapping, OH), 5.01, 5.08 (2H, ABq, $J=12.2$ Hz, PhCH_2), 5.14, 5.18 (2H, ABq, $J=12.5$ Hz, PhCH_2), 5.44 (1H, d, $J=8.8$ Hz, NH), 7.20~7.41 (11H, m, Ph × 2, NH (Ala)).

Crude (2*S*,3*R*)-3-benzyloxycarbonylamino-2-hydroxyoctanoyl-L-leucine benzyl ester (**11**) prepared from **8** (137.4 mg) and L-leucine benzyl ester *p*-toluenesulfonate (183.2 mg) was recrystallized from EtOAc-hexane to give **11** (195.2 mg, 85.7%) as colorless crystals: Rf 0.40 (hexane - EtOAc, 3 : 2); mp 98~99°C; $[\alpha]_D^{24}$ −20.0° (*c* 1.0, MeOH); FAB-MS (positive) *m*/*z* 513 $(M+H)^+$, 469 $(M-CO_2+H)^+$, 91 $(C_7H_7)^+$; ^1H NMR ($CDCl_3$) δ 0.80~0.95 (9H, m, $CH_3 \times 3$), 1.18~1.43 (6H, m, $CH_2 \times 3$), 1.50~1.68 (5H, m, CH_2, β-CH_2 (Leu), γ-CH (Leu)), 3.85 (1H, N-CH), 4.18 (1H, brs, C*H*OH), 4.65 (1H, m, α-CH (Leu)), 4.70 (1H, br, OH), 5.03, 5.05 (2H, ABq, $J=10.3$ Hz, PhCH_2), 5.14, 5.16 (2H, ABq, $J=13.0$ Hz, PhCH_2), 5.47 (1H, d, $J=8.8$ Hz, NH), 7.16 (1H, d, $J=8.3$ Hz, NH(Leu)), 7.25~7.40 (10H, m, Ph × 2).

Crude (2*S*,3*R*)-3-benzyloxycarbonylamino-2-hydrxyo-octanoyl-L-phenylalanine benzyl ester (**12**) prepared from **8** (123.0 mg) and L-phenylalanine benzyl ester *p*-toluenesulfonate (177.9 mg) was recrystallized from EtOAc - hexane to give **12** (193.1 mg, 88.8%) as colorless crystals: Rf 0.42 (hexane - EtOAc, 3 : 2); mp 96.5~97.5°C; $[\alpha]_D^{24}$ +1.1° (*c* 1.0, MeOH); FAB-MS (positive) *m*/*z* 547 $(M+H)^+$, 503 $(M-CO_2+H)^+$, 91 $(C_7H_7)^+$; ^1H NMR ($CDCl_3$) δ 0.86 (3H, t, $J=6.8$ Hz, CH_3), 1.15~1.80 (8H, m, $CH_2 \times 4$), 3.03 (2H, d, $J=6.2$ Hz, β-CH_2 (Phe)), 3.80 (1H, N-CH), 4.12 (1H, brs, C*H*OH), 4.39 (1H, br, OH), 4.87 (1H, dt, $J=6.2$, 7.8 Hz, α-CH (Phe)), 5.04, 5.06 (2H, ABq, $J=13.7$ Hz, PhCH_2), 5.09, 5.13 (2H, ABq, $J=12.2$ Hz, PhCH_2), 5.35 (1H, d, $J=8.3$ Hz, NH), 6.94~7.41 (16H, m, Ph × 3, NH(Phe)).

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoyl-L-valine (Valinoctin A, **1**) and its Analogues (**13**~**15**)

To a solution of **9** (294.4 mg, 0.590 mmol) in MeOH - AcOH - H_2O (5 : 2 : 3, v/v (ml)) was added palladium-black catalyst (10.3 mg). The mixture was hydrogenated at room temperature in a hydrogen atmosphere for 18 hours. The catalyst was filtered off, evaporation of the solvent gave a solid. The product was purified by

Sephadex LH-20 column chromatography with 5% AcOH-MeOH to give **1** as a solid, 162.0 mg (100%): Rf 0.54 (BuOH - MeOH - H_2O, 4 : 1 : 2), 0.25 ($CHCl_3$ - MeOH - AcOH, 65 : 35 : 3); mp 212~215°C (dec); $[\alpha]_D^{26}$ −22.9° (*c* 0.44, MeOH); High-resolution FAB-MS (positive) *m/z* 275.1980 [calcd for $C_{13}H_{27}N_2O_4$ $(M+H)^+$; 275.1971]; ^1H NMR (CD_3OD) δ 0.93 (3H, t, *J*=7.1 Hz, CH_3), 0.95, 0.98 (3H, 3H, two d, each *J*=6.8 Hz, $CH_3\times2$ (Val)), 1.24~1.54 (6H, m, $CH_2\times3$), 1.58 (1H, m, C*Ha*Hb), 1.81 (1H, m, CHa*Hb*), 2.23 (1H, m, β-CH (Val)), 3.42 (1H, ddd, *J*=3.4, 6.8, 6.8 Hz, N-CH), 4.17 (1H, d, *J*=4.9 Hz, α-CH (Val)), 4.23 (1H, d, *J*=3.4 Hz, C*H*OH).

Synthetic **1** was identical with the natural valinoctin A in every respects including farnesyl protein transferase inhibitory activity.

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoyl-L-alanine (**13**, 100.0 mg (98.0%)) was prepared from **10** (194.9 mg) by a similar procedure using MeOH - AcOH - H_2O (6 : 2 : 2, v/v (ml)) as a solvent: Rf 0.22 ($CHCl_3$ - MeOH - AcOH, 65 : 35 : 3); mp 204~205°C (dec); $[\alpha]_D^{23}$ −33.2° (*c* 0.42, MeOH); High-resolution FAB-MS (positive) *m/z* 247.1675 [calcd for $C_{11}H_{23}N_2O_4$ $(M+H)^+$; 247.1658]; ^1H NMR (CD_3OD) δ 0.94 (3H, t, *J*=6.8 Hz, CH_3), 1.31~1.52 (6H, m, $CH_2\times3$), 1.42 (3H, d, *J*=3.7 Hz, CH_3(Ala)), 1.62, 1.80 (1H, 1H, two m, CHaHb), 3.48 (1H, ddd, *J*=2.9, 6.8, 7.8 Hz, N-CH), 4.17 (1H, q, *J*=7.3 Hz, α-CH(Ala)), 4.19 (1H, d, *J*=2.9 Hz, C*H*OH).

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoyl-L-leucine (**14**, 100.9 mg (100%)) was prepared from **11** (193.7 mg) by a similar procedure using MeOH - AcOH - H_2O (6 : 2 : 2, v/v (ml)) as a solvent and purified by Sephadex LH-20 column chromatography with 10% AcOH-MeOH: Rf 0.32 ($CHCl_3$ - MeOH - AcOH, 65 : 35 : 3); mp 193~198°C (dec); $[\alpha]_D^{24}$ −41.5° (*c* 0.40, MeOH); High-resolution FAB-MS (positive) *m/z* 289.2133 [calcd for $C_{14}H_{29}N_2O_4$ $(M+H)^+$; 289.2127]; ^1H NMR (CD_3OD) δ 0.94 (3H, t, *J*=6.8 Hz, CH_3), 0.94, 0.96 (3H, 3H, two d, each *J*=5.8 Hz, $CH_3\times2$ (Leu)), 1.28~1.52 (6H, m, $CH_2\times3$), 1.61 (1H, m, C*Ha*Hb), 1.65~1.75 (3H, m, β-CH_2 (Leu), γ-CH (Leu)), 1.81 (1H, m, CHa*Hb*), 3.45 (1H, ddd, *J*=3.4, 6.8, 6.8 Hz, N-CH), 4.21 (1H, d, *J*=2.9 Hz, C*H*OH), 4.23 (1H, t, *J*=6.8 Hz, α-CH (Leu)).

(2*S*,3*R*)-3-Amino-2-hydroxyoctanoyl-L-phenylalanine (**15**, 105.3 mg (100%)) was prepared from **12** (178.6 mg) by a similar procedure using MeOH - AcOH - H_2O (6 : 2 : 2, v/v (ml)) as a solvent: Rf 0.36 ($CHCl_3$ - MeOH - AcOH, 65 : 35 : 3); mp 207~208°C (dec); $[\alpha]_D^{24}$ −43.8° (*c* 0.40, MeOH); High-resolution FAB-MS (positive) *m/z* 323.1961 [calcd for $C_{17}H_{27}N_2O_4$ $(M+H)^+$; 323.1971]; ^1H NMR (CD_3OD) δ 0.93 (3H, t, *J*=6.8 Hz, CH_3), 1.23~1.50 (7H, m, $CH_2\times3$, C*Ha*Hb), 1.64 (1H, m, CHa*Hb*), 3.09 (1H, dd, *J*=9.3, 13.9 Hz, β-C*Ha*Hb (Phe)), *ca.* 3.30 (2H, m, obscured by solvent, β-CHa*Hb* (Phe), N-CH), 4.09 (1H, d, *J*=3.9 Hz, C*H*OH), 4.42 (1H, dd, *J*=4.4, 9.3 Hz, α-CH (Phe)), 7.12~7.31 (5H, m, Ph).

Enzyme Assay

Farnesyl protein transferase inhibitory activity was assayed as described before[1].

References

1) SEKIZAWA, R.; H. IINUMA, Y. MURAOKA, H. NAGANAWA, N. KINOSHITA, H. NAKAMURA, M. HAMADA, T. TAKEUCHI & K. UMEZAWA: Isolation and synthesis of novel farnesyl protein transferase inhibitors, valinoctins A and B, from *Streptomyces* strain MJ858-NF3. J. Nat. Prod. 59: 232~236, 1996
2) SEEBACH, D.; J. AEBI & D. WASMUTH: Diastereoselective α-alkylation of β-hydroxylic esters through alkoxide enolates. (+)-Diethyl (2*S*,3*R*)-3-allyl-2-hydroxysuccinate from (−)-diethyl *S*-malate. *In* Organic Syntheses. Vol. 63. *Ed.*, G. SAUCY, pp. 109~120, John Wiley & Sons, 1985
3) NORMAN, B. H. & M. L. MORRIS: A stereospecific synthesis of (−)-bestatin from L-malic acid. Tetrahedron Lett. 33: 6803~6806, 1992
4) MILLER, M. J.; J. S. BAJWA, P. G. MATTINGLY & K. PETERSON: Enantioselective synthesis of 3-substituted 4-(alkoxycarbonyl)-2-azetidinones from malic acid. J. Org. Chem. 47: 4928~4933, 1982
5) MAEDA, H.; M. SUZUKI, H. SUGANO & K. MATSUMOTO: A facile synthesis of (*S*)-isoserine from (*S*)-malic acid. Synthesis 1988: 401~402, 1988
6) JEFFORD, C. W.; J. B. WANG & Z-. H. LU: A concise diastereospecific synthesis of 3-amino-2-hydroxy acids. Tetrahedron Lett. 34: 7557~7560, 1993