STRUCTURE OF AUREOBASIDIN A

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Aureobasidin A, a new antifungal antibiotic, was isolated from the culture medium of *Aureobasidium pullulans* R106. Aureobasidin A was a cyclic depsipeptide consisting of eight α-amino acid units and one hydroxy acid unit. The structures of the units were found by acid hydrolysis of the antibiotic to be 2(*R*)-hydroxy-3(*R*)-methylpentanoic acid, β-hydroxy-*N*-methyl-L-valine, *N*-methyl-L-valine, L-proline, allo-L-isoleucine, *N*-methyl-L-phenylalanine, L-leucine, and L-phenylalanine. The sequence of the units was identified by NMR and FAB-MS of the products from the alkaline hydrolysis of aureobasidin A.

Aureobasidins are complexes of antifungal antibiotics produced by *Aureobasidium pullulans* R106. The production, isolation, characterization, and antifungal activity of aureobasidins have been described in the preceding paper.[1] In this paper, we report on the structure of aureobasidin A.

Aureobasidin A (**1**, Fig. 1) was obtained as colorless rods, mp 155~157°C; $[\alpha]_D^{20}$ −254.3° (*c* 1.0, MeOH).

The MW of **1** was deduced to be 1,100 by FAB-MS. From positive-ion FAB-MS with use of a *m*-nitrobenzyl alcohol matrix, $(M+H)^+$ and $(M+Na)^+$ peaks appeared at *m/z* 1,101 and 1,123, respectively. From negative-ion FAB-MS with the same matrix, $(M-H)^-$ and (M + *m*-nitrobenzyl alcohol)$^-$ peaks appeared at *m/z* 1,099 and 1,253, respectively.

The molecular formula of **1** was established to be $C_{60}H_{92}N_8O_{11}$ by elemental analysis and HRFAB-MS, *Anal* Calcd for $C_{60}H_{92}N_8O_{11}$: C 65.43, H 8.42, N 10.17. Found: C 65.17, H 8.72, N 9.93. HRFAB-MS *m/z* 1,101.694 $(M+H)^+$ (Calcd for $C_{60}H_{93}N_8O_{11}$ 1,101.696).

Alkaline Hydrolysis of **1**

The IR spectrum of **1** showed the presence of ester (1750 cm^{-1}) and amido carbonyls (1640 cm^{-1}). Mild alkaline hydrolysis (0.25 N NaOH in aqueous MeOH, room temperature, 25 hours) of **1** gave **2** and **3**. Compounds **1** and **3** were negative in the ninhydrin color reaction and did not react with diazomethane or acetic anhydride in pyridine at room temperature. Compound **2** was also negative in the ninhydrin color reaction, but gave the methyl ester derivative **2a** by treatment with diazomethane, and *O*-acetyl derivative **2b** by treatment with acetic anhydride in pyridine. Further, **2a** gave derivative **2c** with *O*-acetyl and methyl ester groups when treated with acetic anhydride in pyridine.

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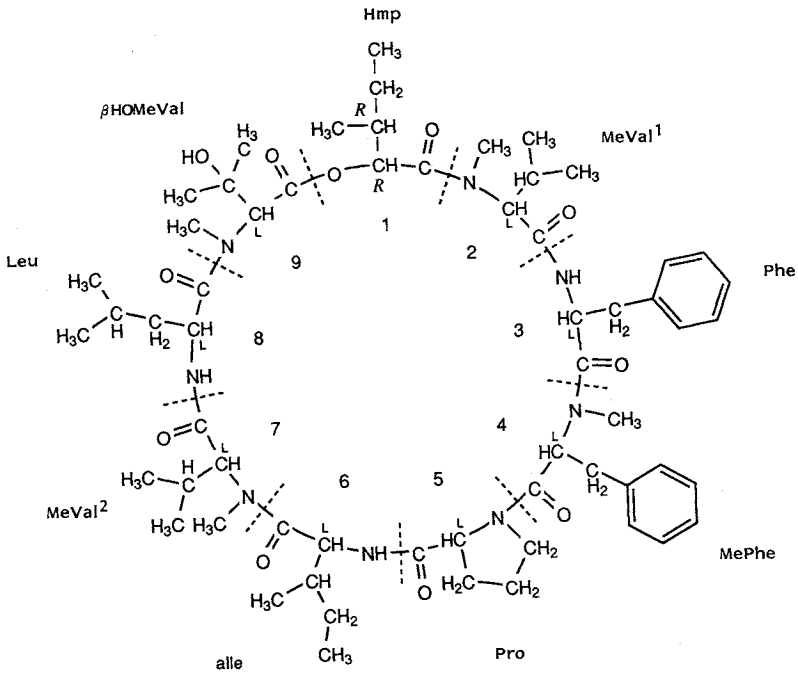
Fig. 1. Structure of aureobasidin A (**1**).



The amino acid compositions of the acid hydrolysates (6N HCl, 110°C, 24 hours) of **1**, **2**, and **3** are shown in Table 1. The results were obtained by amino acid autoanalysis and HPLC analysis with a strong cation-exchange resin (detection by fluorescence; color development with *o*-phthalaldehyde-NaClO).[2]

Table 1. The amino acid compositions of the acid hydrolysates of **1**, **2**, and **3**.

	1	**2**	**3**
β-Hydroxy-*N*-methylvaline	0.37	—	—
Sarcosine	—	1.15 (1)	—
N-Methylvaline	1.96 (2)	2.30 (2)	2.03 (2)
Proline	1.14 (1)	1.24 (1)	1.06 (1)
Alloisoleucine	0.99 (1)	1.08 (1)	0.98 (1)
Leucine	1.11 (1)	1.19 (1)	0.85 (1)
N-Methylphenylalanine	1.07 (1)	0.92 (1)	0.96 (1)
Phenylalanine	1.00 (1)	1.00 (1)	1.00 (1)
Methylamine	0.31	—	0.86 (1)

Structure of 2

Compound **2a**, the methyl ester of **2**, was hydrolyzed with 6N HCl at 110°C for 23 hours in a sealed tube. The acid hydrolysate was chromatographed on a Dowex 50W column to give seven amino acids and one hydroxy acid. The amino acids were identified as *N*-methylvaline (MeVal), sarcosine (Sar), proline, alloisoleucine (aIle), *N*-methylphenylalanine (MePhe), leucine, and phenylalanine by comparison of the ^{1}H NMR spectra and Rt's in HPLC with those of authentic samples. The hydroxy acid was identified to be 2-hydroxy-3-methylpentanoic acid (Hmp) from its ^{1}H and ^{13}C NMR spectra. The molecular formula of **2** was deduced to be $C_{57}H_{88}N_8O_{11}$ by HRFAB-MS. The ^{1}H and ^{13}C NMR spectral data of **2c**, the *O*-acetyl and methyl ester derivative of **2**, are given in Table 2. The assignment of ^{1}H and ^{13}C signals was decided with ^{1}H-^{1}H double quantum filtered (DQF) COSY, ^{1}H-^{13}C COSY, and heteronuclear multiple-bond correlation (HMBC) spectra. The ^{1}H-^{13}C correlation map of **2c** found in the HMBC experiment is shown in Fig. 2. This sequence was consistent with the results obtained by FAB-MS fragmentation of **2a** (Fig. 3).

From these results, the structure of **2** was found to be 2-hydroxy-3-methylpentanoyl-*N*-methylvalyl-

Table 2. ^{1}H and ^{13}C NMR data for **2c** in $CDCl_3$.

Assignment	^{1}H[a]	^{13}C[b]	Assignment	^{1}H	^{13}C
Hmp			Pro		
CO		171.5 s[c]	CO		171.2 s
Ac-CO		171.0 s	α-CH	4.47 br d	60.0 d
2-CH	4.99 d (7.5)	74.7 d	β-CH_2	1.95 m[d], 1.7 m[d]	28.4 t
3-CH	1.9 m[d]	36.1 d	γ-CH_2	1.9 m[d], 1.65 m[d]	24.8 t
4-CH_2	1.6 m[d], 1.26 m	24.4 t	δ-CH_2	3.33 m, 2.87 m[d]	46.9 t
4-CH_3	0.95 d (7.5)	14.8 q	aIle		
5-CH_3	0.9 m[d]	11.1 q	CO		172.9 s[g]
Ac-CH_3	2.10 s	20.6 q	α-CH	4.83 dd (7.0, 9.0)	52.7 d
MeVal[1]			β-CH	1.65 m[d]	37.7 d
CO		168.3 s	γ-CH_2	1.35 m, 1.10 m	26.4 t
α-CH	4.42 d (11.0)	62.4 d	γ-CH_3	0.8 m[d]	14.3 q
β-CH	2.23 m[d]	25.5 d	δ-CH_3	0.9 m[d]	11.9 q
γ-CH_3	0.9 m[d]	19.8 q	NH	7.37 br d	
γ-CH_3	0.8 m[d]	18.7 q	MeVal[2]		
N-CH_3	2.91 s	30.5 q	CO		170.0 s
Phe			α-CH	4.77 d (12.0)	62.1 d
CO		170.5 s	β-CH	2.23 m[d]	26.2 d
α-CH	5.07 m[d]	50.0 d	γ-CH_3	0.9 m[d]	19.4 q
β-CH_2	2.95 m[d], 2.75 dd (7.0, 13.5)	38.4 t	γ-CH_3	0.8 m[d]	18.5 q
			N-CH_3	3.11 s	30.6 q
C_γ (ar.)		136.9 s	Leu		
C_δ (ar.)	7.1～7.3 m[d]	129.4 d	CO		172.8 s[g]
C_ε (ar.)	7.1～7.3 m[d]	128.4 d[e]	α-CH	5.05 m[d]	46.8 d
C_ζ (ar.)	7.1～7.3 m[d]	126.7 d[f]	β-CH_2	1.50 m	41.5 t
NH	6.38 d (9.5)		γ-CH	1.6 m[d]	24.8 d
MePhe			δ-CH_3	0.95 d (7.5)	21.6 q
CO		168.8 s	δ-CH_3	0.9 m[d]	23.3 q
α-CH	5.99 dd (7.5, 9.5)	55.1 d	NH	7.1～7.3 m[d]	
β-CH_2	3.1 m[d], 2.95 m[d]	34.8 t	Sar		
C_γ (ar.)		136.7 s	CO		169.3 s
C_δ (ar.)	7.1～7.3 m[d]	129.1 d	α-CH_2	4.37 d (17.0), 3.86 d (17.0)	49.6 t
C_ε (ar.)	7.1～7.3 m[d]	128.2 d[e]	N-CH_3	3.17 s	36.4 q
C_ζ (ar.)	7.1～7.3 m[d]	126.6 d[f]	O-CH_3	3.70 s	52.1 q
N-CH_3	2.85 s	30.5 q			

[a] 400 MHz; δ in ppm, J in Hz.
[b] 100 MHz; δ in ppm.
[c] Assigned by DEPT experiments.
[d] Overlapping signals.
[e～g] Assignment could be interchanged.

Fig. 2. ^{1}H-^{13}C correlation map of **2c** obtained in the HMBC experiment.

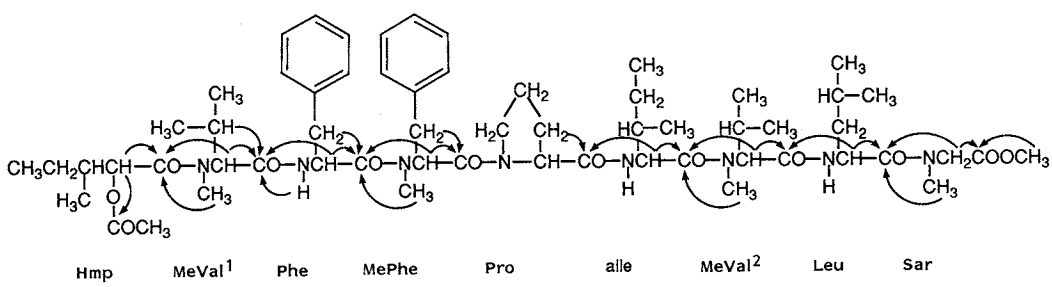
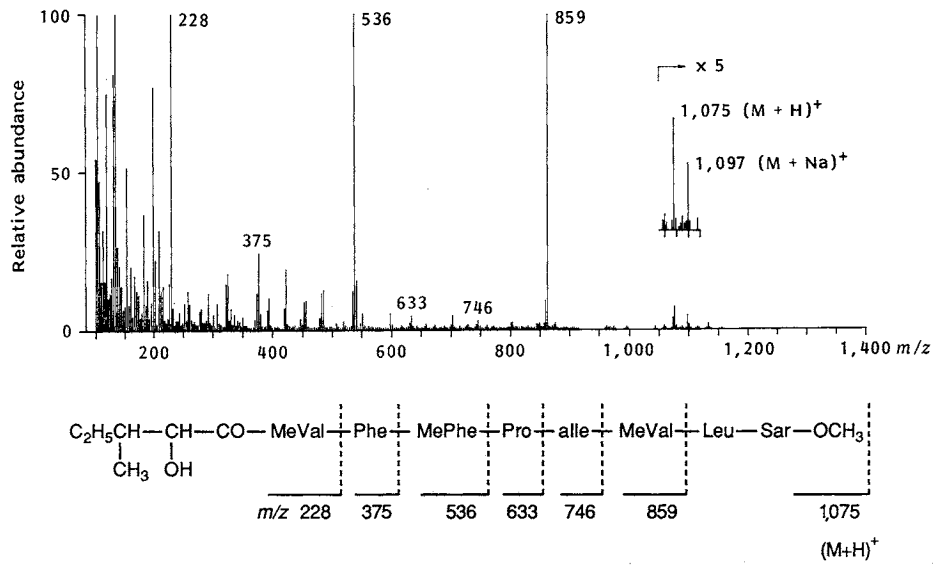
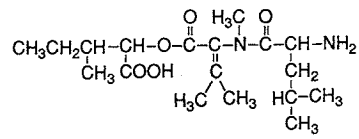


Fig. 3. FAB-MS spectrum of **2a**.



phenylalanyl-*N*-methylphenylalanyl-prolyl-alloisoleucyl-*N*-methylvalyl-leucyl-sarcosine.

Fig. 4. Structure of **3a**.



Structure of 3

Acid hydrolysis (6 N HCl, reflux, 3 hours) of **3** gave **3a**. Compound **3a** gave two peaks on HPLC and was assumed to be an equilibrium mixture because the two compounds separated by HPLC again gave two peaks when HPLC was repeated. The molecular formula of **3a** was deduced to be $C_{18}H_{32}N_2O_5$ by HRFAB-MS. HPLC of the acid hydrolysate (6 N HCl, 110°C, 24 hours) of **3a** showed the presence of 1 mol each of leucine and methylamine. The ^1H and ^{13}C NMR spectra of **3a** indicated that there were six $-CH_3$, one $>NCH_3$, two $>CH_2$, three $>CH-$, one $>CH-O-$, one $>C=C<$, and three $>C=O$ groups. The *N*-terminal amino acid of **3a** was found to be leucine by Edman degradation, but the degradation did not proceed further. These results suggested that the structure of **3a** was as shown in Fig. 4.

The molecular formula of **3** was deduced to be $C_{60}H_{90}N_8O_{10}$ by HRFAB-MS. The molecular formula of **3** lacks two hydrogens and one oxygen compared with of **1**. From these results, together with the amino acid composition of the acid hydrolysate of **3** and the structure of **2**, the structure of **3** was decided to be that shown in Fig. 5.

Structure of 1

The acid hydrolysate of **1** was chromatographed on a Dowex 50W column to isolate *β*-hydroxy-*N*-methylvaline (*β*HOMeVal). The structure of *β*HOMeVal was established by its ^1H and ^{13}C NMR spectra and FAB-MS, and by comparison with results from synthesized samples.[3] The reaction giving **2** and **3** with mild alkaline hydrolysis of **1** seemed to be that shown in Fig. 6. The *β*HOMeVal in **1** is converted to Sar by a retro-aldol reaction, and then the ester linkage is hydrolyzed to give **2**. On the other hand, the *β*HOMeVal in **1** is changed in **3** to 2,3-didehydro-*N*-methylvaline ($DH_{2,3}$MeVal) by dehydration; therefore, the ester linkage is not hydrolyzed owing to its conjugated ester carbonyl.

Fig. 5. Structure of **3**.

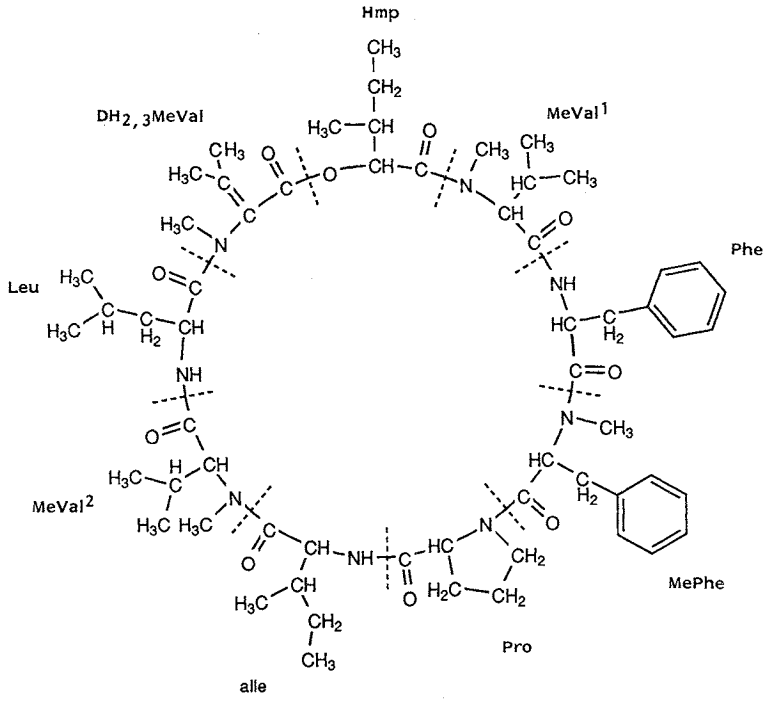
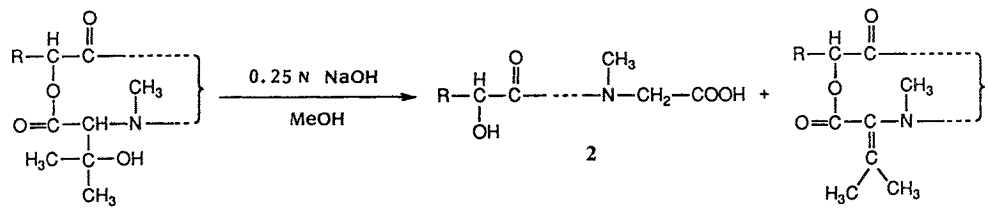


Fig. 6. Alkaline hydrolysis of aureobasidin A (**1**).



$R = C_2H_5CH(CH_3)-$

The absolute configuration of Hmp obtained by the acid hydrolysis of **2** was found to be 2*R*,3*R* by comparison of the ^1H NMR spectrum, CD curve,[4] and Rt in HPLC with a Chiralpak WH column (Daicel Chemical Industries, Ltd.) with those of the synthesized samples.[5] The HPLC Rt's of the Hmp are listed in Table 3. Next, the Rt in HPLC with a Chiralpak WH column and a Crownpak CR (+) column (Daicel) of each amino acid obtained by the acid hydrolysis of **2** was compared with that of authentic samples (Table 4). The absolute configurations of all amino acids were L. The absolute configuration of *β*HOMeVal obtained by the acid hydrolysis of **1** was L, because the CD curve was positive at 210 nm.[6,7] From these results, the complete structure of **1** was as shown in Fig. 1.

Discussion

Aureobasidin A is a cyclic depsipeptide with an octapeptide cyclized through Hmp. There are other

Table 3. HPLC data for authentic Hmp and Hmp in **2** on a Chiralpak WH column.

Hmp	Elution time (minutes)	
	Authentic Hmp	Hmp in **2**
2*R*,3*R*	17.5	17.8
2*R*,3*S*	17.1	—
2*S*,3*S*	14.9	—
2*S*,3*R*	14.2	—

—: Not detected.
Mobile phase: 0.25 mM $CuSO_4$, flow rate: 1 ml/minute, column temperature: 40°C, detection: UV absorption at 210 nm.

Table 4. HPLC data for amino acids in **2** on a Chiralpak WH or Crownpak CR (+) column.

Amino acids	Column	Elution time (minutes)	
		Authentic amino acids	Amino acids in **2**
N-Methyl-L-valine	a	23.9	24.1
N-Methyl-D-valine	a	6.6	—
L-Proline	a	10.4	10.3
D-Proline	a	16.7	—
allo-L-Isoleucine	a	17.0	17.1
allo-D-Isoleucine	a	12.5	—
N-Methyl-L-phenylalanine	a	54.2	57.3
N-Methyl-D-phenylalanine	a	13.3	—
L-Leucine	b	5.4	5.6
D-Leucine	b	3.9	—
L-Phenylalanine	a	33.0	33.9
D-Phenylalanine	a	16.7	—

—: Not detected.
Column: a, Chiralpak WH; b, Crownpak CR (+).

cyclic depsipeptides with antifungal activity: jasplakinolide,[8] geodiamolides,[9] verlamelin,[10] the aculeacin/echinocandin family,[11~14] and lipopeptin.[15] Jasplakinolide is composed of three amino acids and an oxy-trimethyl-nonanoyl group. Geodiamolides are composed of a tripeptide unit (two (*S*)-alanines and a (*R*)-3-halotyrosine) joined to a 11-carbon polypropionate unit in an 18-membered ring. The others are all lipopeptide antibiotics with a fatty acid side chain. Therefore, aureobasidin A is a new cyclic depsipeptide antibiotic.

Experimental

The mp was measured with a Yanagimoto micro-mp apparatus and is uncorrected. FAB-MS and HRFAB-MS were obtained on a Jeol JMS-DX302 spectrometer. NMR spectra were recorded on a Jeol JNM-GX400 or JNM-FX200 spectrometer, IR spectra on a Hitachi 270-30 spectrophotometer, and CD curves on a Jasco J-600 spectropolarimeter. Optical rotation was measured with a Jasco DIP-181 digital polarimeter. Amino acid autoanalysis was carried out with a Jeol JCL-300 amino acid autoanalyzer. Edman degradation was done with a Applied Biosystems 477A protein sequencer.

The *N*-methyl amino acids were analyzed by HPLC with the method used for post-column derivatization described below. Equipment—The HPLC system consisted of a Shimadzu LC-3A apparatus, a Shimadzu SGR-1A step gradient elution unit, a Shimadzu CTO-6A column oven, and a Shimadzu RF-530 fluorescence HPLC monitor. A Sanuki SSP-DM3N pump was used to supply reaction reagents. Separation conditions—Column: Shimadzu LC column ISC-07/S1504, strong cation-exchange resin, 7.5 μm, 150 × 4.0 mm; mobile phases: A, 7% EtOH in 0.2 N sodium citrate (pH 3.25); B, 0.2 N sodium citrate (pH 4.25); C, 0.6 N sodium citrate (pH 9.00); step gradient program of mobile phases: A, 5 minutes; B, 15minutes; C, 40 minutes; flow rate, 0.5 ml/minute; column temperature, 50°C. Reaction conditions—Reagents: A, 0.1% sodium hypochlorite in buffer,† flow rate, 0.5 ml/minute; B, 800 mg of *o*-phthalaldehyde and 1 g of *N*-acetyl-L-cysteine in 1,000 ml of buffer,† flow rate, 0.5 ml/minute; reaction temperature, 50°C. Detection—Fluorescence: emission, 455 nm, excitation, 340 nm.

The stereochemistry of the amino acids were examined by HPLC with chiral columns. Equipment—The HPLC system consisted of an Altex model 100A pump, a Shimadzu CTO-6A column oven, and a Soma S-310A UV detector. Analytical conditions—Column: Chiralpak WH; mobile phase, 1 mM $CuSO_4$; column temperature, 50°C; flow rate, 1.5 ml/minute; detection, UV absorption at 220 nm. Column: Crownpak CR (+); mobile phase, aq $HClO_4$, pH 2.0; column temperature, 25°C; flow rate, 0.8 ml/minute; detection, UV absorption at 220 nm.

Mild Alkaline Hydrolysis of **1**—Isolation of **2** and **3**

To a solution of **1** (105 mg) in MeOH (9 ml) was added 1 N NaOH (3 ml), and the mixture was stirred

† Buffer: Na_2CO_3 (0.348 M), H_3BO_3 (0.216 M), and K_2SO_4 (0.108 M).

for 25 hours at room temperature. The reaction mixture was neutralized with 1 N HCl and concentrated under reduced pressure. The concentrate was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with H_2O and concentrated under reduced pressure. The residue was chromatographed on a Nucleosil $5C_{18}$ column (Macherey Nagel Co.) with 85% CH_3CN, giving **2** (55 mg) and **3** (31 mg) as colorless powders.

2: ^1H NMR ($CDCl_3$) δ 2.47 (3H, s, NCH_3), 2.99 (3H, s, NCH_3), 3.24 (6H, s, $NCH_3 \times 2$). FAB-MS *m/z* 1,061 $(M+H)^+$, 1,083 $(M+Na)^+$, 1,099 $(M+K)^+$. HRFAB-MS *m/z* 1,061.662 $(M+H)^+$ (Calcd for $C_{57}H_{89}N_8O_{11}$ 1,061.665). IR (KBr) cm^{-1} 3450, 3330, 2980, 1740, 1720, 1640, 1420, 1090, 705.

3: FAB-MS *m/z* 1,083 $(M+H)^+$, 1,105 $(M+Na)^+$. HRFAB-MS *m/z* 1,083.685 $(M+H)^+$ (Calcd for $C_{60}H_{91}N_8O_{10}$ 1,083.686). IR (KBr) cm^{-1} 3330, 2970, 1735, 1635, 1460, 1410, 1090, 705.

2-Methyl Ester (**2a**)

In a solution of **2** (55 mg) in a 1 : 1 mixture of MeOH and ether (10 ml), diazomethane was bubbled until the solution turned yellow. After the reaction mixture was concentrated under reduced pressure, the residue was chromatographed on a Nucleosil $5C_{18}$ column with 70% CH_3CN, giving **2a** (46 mg) as a colorless powder.

^1H NMR ($CDCl_3$) δ 2.49 (3H, s, NCH_3), 3.04 (3H, s, NCH_3), 3.19 (6H, s, $NCH_3 \times 2$), 3.69 (3H, s, OCH_3). IR (KBr) cm^{-1} 3470, 2970, 1760, 1640, 1460, 1415, 1215, 1090, 705. FAB-MS *m/z* 1,075 $(M+H)^+$, 1,097 $(M+Na)^+$

O-Acetyl-**2** (**2b**)

To a solution of **2** (61 mg) in pyridine (3 ml) was added acetic anhydride (0.1 ml), and the mixture was stirred for 18 hours at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with 1 N HCl, saturated $NaHCO_3$ solution and brine, and concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column (Shiseido Co., Ltd.) with 60% CH_3CN containing 0.05% TFA, giving **2b** (19 mg) as a colorless powder.

^1H NMR ($CDCl_3$) δ 2.10 (3H, s, $OCOCH_3$), 2.74 (3H, s, NCH_3), 2.85 (3H, s, NCH_3), 3.23 (3H, s, NCH_3), 3.26 (3H, s, NCH_3). IR (KBr) cm^{-1} 3450, 2980, 1750, 1640, 1210, 705. FAB-MS *m/z* 1,103 $(M+H)^+$, 1,125 $(M+Na)^+$.

O-Acetyl-**2**-methyl Ester (**2c**)

To a solution of **2a** (32 mg) in pyridine (2 ml) was added acetic anhydride (0.2 ml), and the mixture was stirred for 21 hours at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with 1 N HCl, saturated $NaHCO_3$ solution and brine, and concentrated under reduced pressure. The residue was chromatographed on a Nucleosil $5C_{18}$ column with 70% CH_3CN, giving **2c** (23 mg) as a colorless powder.

FAB-MS *m/z* 1,117 $(M+H)^+$, 1,139 $(M+Na)^+$. IR (KBr) cm^{-1} 3460, 3345, 2980, 1750, 1640, 1460, 1420, 1250, 1095, 705.

Acid Hydrolysis of **2a**

Compound **2a** (101 mg) was hydrolyzed with 6 N HCl (5 ml) at 110°C for 23 hours in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Dowex 50W (140 ml) column equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). The column was developed with the same buffer solution followed by a 0.2 M pyridine-formic acid buffer (pH 3.1) and 0.2 M pyridine-acetic acid buffer (pH 4.4), in that order. Appropriate fractions were combined and concentrated under reduced pressure. The amino acids were desalted on a Dowex 50W (H^+) column with 1 N NH_4OH. Hmp was desalted on a Dowex 50W (H^+) column with H_2O. Hmp (9 mg), MeVal (9 mg), Sar (5 mg), and crude proline (7 mg) were eluted with 0.1 M pyridine-formic acid buffer (pH 3.1). aIle (3 mg), crude MePhe (13 mg), and crude leucine (4 mg) were eluted with 0.2 M pyridine-formic acid buffer (pH 3.1). Phenylalanine (13 mg) was eluted with 0.2 M pyridine-acetic acid buffer (pH 4.4).

Hmp: ^1H NMR ($CDCl_3$) δ 0.93 (3H, t, J=7.2 Hz), 1.03 (3H, d, J=6.8 Hz), 1.2~1.6 (2H, m), 1.9 (1H, br s), 4.19 (1H, d, J=2.9 Hz). ^{13}C NMR ($CDCl_3$) δ 11.6 (q), 15.3 (q), 23.7 (t), 38.9 (d), 74.6 (d), 178.7 (s). CD $[\theta]_{207}$ −2,397° (*c* 0.007, aq HCl, pH 2.5).

MeVal: ^{1}H NMR (D_2O) δ 1.04 (3H, d, J=5.4 Hz), 1.07 (3H, d, J=5.4 Hz), 2.24 (1H, m), 2.74 (3H, s, NCH_3), 3.44 (1H, d, J=4.4 Hz). FAB-MS m/z 132 $(M+H)^+$.

Sar: ^{1}H NMR (D_2O) δ 2.75 (3H, s, NCH_3), 3.63 (2H, s). FAB-MS m/z 90 $(M+H)^+$.

Proline: ^{1}H NMR (D_2O) δ 2.05 (3H, m), 2.35 (1H, m), 3.40 (2H, m), 4.14 (1H, m). FAB-MS m/z 116 $(M+H)^+$.

aIle: ^{1}H NMR (D_2O) δ 0.95 (3H, d, J=7.1 Hz), 0.96 (3H, t, J=8.6 Hz), 1.40 (2H, m), 2.09 (1H, m), 3.74 (1H, d, J=3.4 Hz). FAB-MS m/z 132 $(M+H)^+$.

MePhe: ^{1}H NMR (D_2O) δ 2.70 (3H, s, NCH_3), 3.24 (2H, d, J=6.4 Hz), 3.87 (1H, t, J=6.4 Hz), 7.2~7.5 (5H, m). FAB-MS m/z 180 $(M+H)^+$.

Leucine: ^{1}H NMR (D_2O) δ 0.97 (6H, d, J=3.2 Hz), 1.73 (3H, m), 3.74 (1H, m). FAB-MS m/z 132 $(M+H)^+$.

Phenylalanine: ^{1}H NMR (D_2O) δ 3.13 (1H, dd, J=16.0 and 8.1 Hz), 3.32 (1H, dd, J=16.0 and 5.1 Hz), 4.00 (1H, dd, J=8.1 and 5.1 Hz), 7.3~7.6 (5H, m). FAB-MS m/z 166 $(M+H)^+$.

Synthesis of Hmp

Four Hmps corresponding to L- and D-isoleucine and also allo-L- and allo-D-isoleucine were prepared from optically pure parent amino acids by a procedure described elsewhere.[5]

D-Isoleucinic acid (2*R*,3*R*): ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, J=7.4 Hz), 1.03 (3H, d, J=6.8 Hz), 1.2~1.6 (2H, m), 1.9 (1H, m), 4.18 (1H, d, J=3.7 Hz). CD $[\theta]_{207}$ −3,383° (*c* 0.006, aq HCl, pH 2.5).

L-Isoleucinic acid (2*S*,3*S*): ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, t, J=7.3 Hz), 1.03 (3H, d, J=6.8 Hz), 1.2~1.6 (2H, m), 1.9 (1H, m), 4.18 (1H, d, J=3.4 Hz). CD $[\theta]_{208}$ +2,676° (*c* 0.005, aq HCl, pH 2.5).

allo-D-Isoleucinic acid (2*R*,3*S*): ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, J=6.8 Hz), 0.97 (3H, t, J=7.5 Hz), 1.2~1.6 (2H, m), 1.9 (1H, m), 4.30 (1H, d, J=2.7 Hz). CD $[\theta]_{209}$ −5,167° (*c* 0.006, aq HCl, pH 2.5).

allo-L-Isoleucinic acid (2*S*,3*R*): ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, J=7.1 Hz), 0.97 (3H, t, J=7.5 Hz), 1.2~1.6 (2H, m), 1.9 (1H, m), 4.29 (1H, d, J=2.7 Hz). CD $[\theta]_{207}$ +4,284° (*c* 0.006, aq HCl, pH 2.5).

Acid Hydrolysis of **1**

Compound **1** (100 mg) was hydrolyzed with 6 N HCl (5 ml) at 110°C for 20 hours in a sealed tube. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Dowex 50W (50 ml) column equilibrated with 0.1 M pyridine-formic acid buffer (pH 3.1). The column was developed with the same buffer solution. The ninhydrin-positive fractions that eluted first were combined and concentrated under reduced pressure. The residue was desalted on a Dowex 50W (H^+) column with 1 N NH_4OH, giving βHOMeVal (5 mg).

^{1}H NMR (D_2O) δ 1.24 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.74 (3H, s, NCH_3), 3.45 (1H, s). ^{13}C NMR (D_2O) δ 24.0 (q), 28.4 (q), 34.1 (q), 70.7 (s), 74.0 (d), 171.6 (s). FAB-MS m/z 148 $(M+H)^+$. CD $[\theta]_{210}$ +2,894° (*c* 0.004, 0.5 N HCl). The literature[7] reports $[\theta]_{208}$ +3,503° (*c* 0.05, 0.5 N HCl).

Synthesis of Racemic βHOMeVal

Racemic βHOMeVal was synthesized by a method described previously.[3]

^{1}H NMR (D_2O) δ 1.22 (3H, s, CH_3), 1.46 (3H, s, CH_3), 2.73 (3H, s, NCH_3), 3.43 (1H, s). FAB-MS m/z 148 $(M+H)^+$.

Acid Hydrolysis of **3**

Compound **3** (81 mg) was hydrolyzed under reflux in 6 N HCl (5 ml) for 3 hours. The reaction mixture was concentrated under reduced pressure. The residue was chromatographed on a Capcell Pak C_{18} column with 30% CH_3CN containing 0.05% TFA, giving **3a** (28 mg) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 0.88 (6H, br s, $CH_3 \times 2$), 0.95 (3H, t, J=3.0 Hz, CH_3), 1.06 (3H, d, J=4.0 Hz, CH_3), 2.01 (3H, s, CH_3), 2.28 (3H, s, CH_3), 3.04 (3H, s, NCH_3), 4.13 (1H, br s), 5.02 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 11.6 (q), 15.8 (q), 21.1 (q), 22.0 (q), 22.8 (q), 23.7 (q), 24.2 (d), 24.6 (t), 36.26 (d), 36.35 (q), 40.2 (t), 50.8 (d), 79.7 (d), 128.0 (s), 153.1 (s), 161.6 (s), 166.0 (s), 171.6 (s). IR (KBr) cm^{-1} 3450, 2985, 1735, 1705, 1680, 1210, 1150, 805, 730. HRFAB-MS m/z 357.2381 $(M+H)^+$ (Calcd for $C_{18}H_{33}N_2O_5$ 357.2390).

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References

1) TAKESAKO, K.; K. IKAI, F. HARUNA, M. ENDO, K. SHIMANAKA, E. SONO, T. NAKAMURA, I. KATO & H. YAMAGUCHI: Aureobasidins, new antifungal antibiotics. Taxonomy, fermentation, isolation, and properties. J. Antibiotics 44: 919~924, 1991
2) ISHIDA, Y.; T. FUJITA & K. ASAI: New detection and separation method for amino acids by high-performance liquid chromatography. J. Chromatogr. 204: 143~148, 1981
3) IZUMIYA, N. & A. NAGAMATSU: Synthesis of hydroxyamino acid and their N-methyl derivatives. VI. Synthesis of β-hydroxy-N-methylvaline. J. Chem. Soc. Jpn. 72: 336~338, 1951
4) LISTOWSKY, I.; S. ENGLARD & G. AVIGAD: Conformational aspects of acidic sugars: circular dichroism studies. Trans. N.Y. Acad. Sci. 34: 218~226, 1972
5) WINITZ, M.; L. BLOCH-FRANKENTHAL, N. IZUMIYA, S. M. BIRNBAUM, C. G. BAKER & J. P. GREENSTEIN: Studies on diastereoisomeric α-amino acids and corresponding α-hydroxy acids. VII. Influence of β-configuration on enzymic susceptibility. J. Am. Chem. Soc. 78: 2423~2430, 1956
6) SHOJI, J.: On the configuration of the N-methylalloisoleucine contained in quinoxaline antibiotics. J. Antibiotics 26: 302~303, 1973
7) KONISHI, M.; H. OHKUMA, F. SAKAI, T. TSUNO, H. KOSHIYAMA, T. NAITO & H. KAWAGUCHI: BBM-928, a new antitumor antibiotic complex. III. Structure determination of BBM-928 A, B and C. J. Antibiotics 34: 148~159, 1981
8) CREWS, P.; L. V. MANES & M. BOEHLER: Jasplakinolide, a cyclodepsipeptide from the marine sponge, *Jaspis* sp. Tetrahedron Lett. 27: 2797~2800, 1986
9) CHAN, W. R.; W. F. TINTO, P. S. MANCHAND & L. J. TODARO: Stereostructures of geodiamolides A and B, novel cyclodepsipeptides from the marine sponge *Geodia* sp. J. Org. Chem. 52: 3091~3093, 1987
10) ROWIN, G. L.; J. E. MILLER, G. ALBERS-SCHÖNBERG, J. C. ONISHI, D. DAVIS & E. L. DULANEY: Verlamelin, a new antifungal agent. J. Antibiotics 39: 1772~1775, 1986
11) MIZUNO, K.; A. YAGI, S. SATOI, M. TAKADA, M. HAYASHI, K. ASANO & T. MATSUDA: Studies on aculeasin. I. Isolation and characterization of aculeacin A. J. Antibiotics 30: 297~302, 1977
12) KELLER-JUSLÉN, C.; M. KUHN, H. R. LOOSLI, T. J. PETCHER, H. P. WEBER & A. VON WARTBURG: Struktur des cyclopeptid-antibiotikums SL 7810 (=Echinocandin B). Tetrahedron Lett. 1976: 4147~4150, 1976
13) ROY, K.; T. MUKHOPADHYAY, G. C. S. REDDY, K. R. DESIKAN & B. N. GANGULI: Mulundocandin, a new lipopeptide antibiotic. I. Taxonomy, fermentation, isolation and characterization. J. Antibiotics 40: 275~280, 1987
14) SCHWARTZ, R. E.; R. A. GIACOBBE, J. A. BLAND & R. L. MONAGHAN: L-671,329, a new antifungal agent. I. Fermentation and isolation. J. Antibiotics 42: 163~167, 1989
15) NISHII, M.; T. KIHARA, K. ISONO, T. HIGASHIJIMA, T. MIYAZAWA, S. K. SETHI & J. A. MCCLOSKEY: The structure of lipopeptin A. Tetrahedron Lett. 21: 4627~4630, 1980