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Tripropeptins, Novel Antimicrobial Agents Produced by *Lysobacter* sp.

II. Structure Elucidation

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Planar structures of tripropeptins (TPPs) were elucidated by spectroscopic studies including various NMR measurements. Stereochemistry of constituent amino acids of tripropeptin C (TPPC) (**3**) was identified by marfey's method except hydroxyproline which was determined by studies of NMR and CD spectra. The absolute structure of **3** was determined by analyses of the fragments obtained by Birch reduction and $LiBH_4$ reduction of **3**. The configuration of the fatty acid, isolated from acid hydrolysate of **3**, was determined to be (3*R*)-hydroxy-13-methyltetradecanoic acid from MS, NMR spectra and negative sign of the optical rotation.

We have isolated tripropetin A (**1**), B (**2**), C (**3**), D (**4**) and Z (**5**) (Fig. 1), as antimicrobial agents, from cultured cells and broth of *Lysobacter* sp. designated BMK333-48F3. In the preceding paper, the taxonomy, isolation and biological activities were reported[1]. In this paper, we describe the physico-chemical properties and structure determination of TPPs.

Result and Discussion

Tripropeptin C (**3**), a main component of TPPs, was isolated as colorless powder and its UV spectrum showed end absorption. **3** gave positive color reaction with iodide vapor, Rydon-Smith and Sakaguchi reagent. IR spectrum of **3** showed characteristic absorption of peptide bonds (1635 and 1537 cm^{-1}) and of lactone linkage (1737 cm^{-1}). Molecular formula for **3** was determined by HRFAB-MS as $C_{51}H_{83}N_{11}O_{19}$ (calcd. 1154.5927 for $(M+H)^+$, found 1154.5945), which was supported by the ^1H and ^{13}C NMR spectral data.

Other tripropeptins showed similar results, as summarized in Table 1 and these properties suggested that every tripropeptin belongs to depsipeptide antibiotics.

Planar structure of **3** was determined as follows. All bond connections between ^1H and ^{13}C signals were interpreted by DEPT and heteronuclear multiple quantum coherence (HMQC) experiments. The DEPT and HMQC experiments revealed the presence of three methyl, twentytwo methylene, fourteen methine, one sp^2 quaternary and eleven carbonyl carbons in **3**. The ^1H and ^{13}C NMR spectral data of **3** are shown in Table 2. The ^1H-^1H COSY and HMBC spectra of **3** indicated the presence of β-hydroxy fatty acid and eight amino acids, threonine (Thr), serine (Ser), arginine (Arg) and hydroxyproline (OHPro), one residue each, and 2 residues of proline (Pro) and β-hydroxyaspartic acid (β-OHAsp) in Fig. 2.

The sequence of **3** was determined by HMBC spectrum as follows. The correlation from H-2 (δ 4.62) of β-OHAsp (II) to carbonyl carbon C-5 (δ 169.9) of OHPro, from H-6

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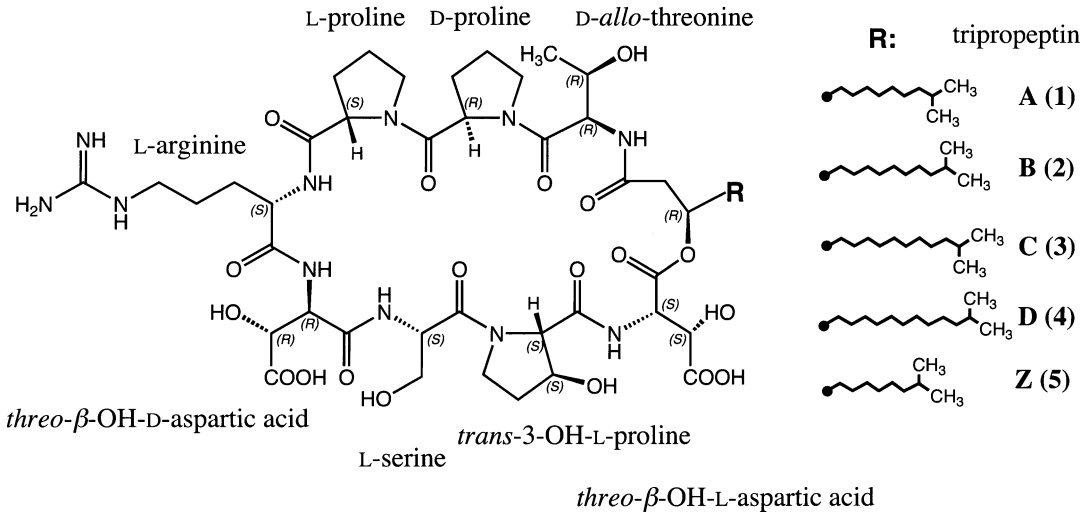
Table 1. Physicochemical properties of tripropeptin A, B, C, D and Z.

	A	B	C	D	Z
$[\alpha]_D^{24}$ (MeOH)	-7.8° (*c* 1)	-7.9° (*c* 1)	-8.4° (*c* 1)	-10.8° (*c* 1)	-14.0° (*c* 1)
HRFAB-MS(*m/z*)					
found	1126.5657(M+H)$^+$	1140.5788(M+H)$^+$	1154.5945(M+H)$^+$	1168.6101(M+H)$^+$	1112.5475(M+H)$^+$
Calcd.	1126.5632	1140.5776	1154.5927	1168.6074	1112.5491
Molecular formula	$C_{49}H_{79}N_{11}O_{19}$	$C_{50}H_{81}N_{11}O_{19}$	$C_{51}H_{83}N_{11}O_{19}$	$C_{52}H_{85}N_{11}O_{19}$	$C_{48}H_{77}N_{11}O_{19}$
IR υ_{max}(KBr)cm^{-1}	3375, 2923, 1737, 1635, 1538, 1450, 1263, 1203, 1097	3345, 2931, 1737, 1635, 1537, 1450, 1263, 1201, 1097	3372, 2927, 1737, 1635, 1537, 1452, 1263, 1203, 1097	3282, 2931, 1739, 1633,1537, 1452, 1263,1203,1099	3388, 2923, 1725, 1635, 1536, 1450, 1265, 1205,1095
TLC, Rf value[a]					
BuOH-MeOH-H_2O(4:1:2)	0.45	0.45	0.45	0.45	0.45
$CHCl_3$-MeOH-H_2O(10:5:1)	0.25	0.25	0.25	0.25	0.25
Color Reaction					
positive	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi	Rydon-Smith, Sakaguchi
Soluble	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O	MeOH,DMSO,H_2O
Insoluble	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc	$CHCl_3$,acetone,EtOAc

[a] Merck Kieselgel 60F_{254} Art. 5715

Fig. 1. Structure of tripropeptins.



(δ 4.23) of OHPro to carbonyl carbon C-10 (δ 167.9) of Ser, from an amide proton (δ 7.25) of Ser to carbonyl carbon C-13 (δ 168.5) of β-OHAsp (I), from an amide proton (δ 8.49) of β-OHAsp (I) to carbonyl carbon C-17 (δ 171.2) of Arg, from an amide proton (δ 7.77) of Arg to carbonyl carbon C-23 (δ 172.6) of Pro (I), from H-27 (δ 3.35) of Pro (I) to carbonyl carbon C-28 (δ 172.0) of Pro (II), from H-29 (δ 4.18) of Pro (II) to carbonyl carbon C-33 (δ 169.0) of Thr, from methine protons H-34 (δ 4.52) and H-39 (δ 5.06) to carbonyl carbon C-37 (δ 169.5) of 3-hydroxy-13-methyltetradecanoic acid indicated that the sequence of **3** to be 3-hydroxy-13-methyltetradecanoyl-Thr-Pro-Pro-Arg-β-OHAsp-Ser-OHPro-β-OHAsp.

A long-range coupling between C-1 (δ 168.6) and H-39

Table 2. ^{13}C and ^{1}H NMR data of tripropeptin C in DMSO-d_6.

position	type	δ C[a]	δ H[b](multiplicity, J (Hz))
1	>C=O	168.6	
2	>CH-N	54.8	H:4.62(1H, m), NH:7.80(1H, d, 10.0)
3	>CH-O	70.0	4.55(1H, d, 2.4)
4	>C=O	171.8	
5	>C=O	169.9	
6	>CH-N	68.8	4.23(1H, s)
7	>CH-O	72.5	4.26(1H, d, 3.8)
8	-CH_2-	32.3	1.75(2H, m)
9	-CH_2N<	45.0	3.53(1H, m), 3.66(1H, m)
10	>C=O	167.9	
11	>CHNH-	53.1	H:4.58(1H, m), NH:7.25(1H, d, 8.0)
12	-CH_2O-	61.3	3.53(2H, m)
13	>C=O	168.5	
14	>CHNH-	56.3	H:4.64(1H, m), NH:8.49(1H, d, 8.4)
15	>CH-O	70.0	4.5(1H, d, 2.0)
16	>C=O	172.9	
17	>C=O	171.2	
18	>CHNH-	51.7	H:4.55(1H, m), NH:7.77(1H, d, 8.6)
19	-CH_2-	28.9	1.56(1H, m), 1.63(1H, m)
20	-CH_2-	24.7	1.35(2H, m)
21	-CH_2NH-	40.3	H:3.06(2H, m), NH:7.62(m)
22	-N=C(N-)N-	156.9	
23	>C=O	172.6	
24	>CH-N	60.6	4.72(1H, m)
25	-CH_2-	31.7	1.93(1H, m), 2.13(1H, m)
26	-CH_2-	22.2	1.78(2H, m)
27	-CH_2-N	46.9	3.35(1H, m), 3.49(1H, m)
28	>C=O	172.0	
29	>CH-N	57.9	4.18(1H, t, 12.4)
30	-CH_2-	29.0	1.63(2H, m)
31	-CH_2-	24.4	1.75(1H, m), 1.85(1H, m)
32	-CH_2-N	47.3	3.54(1H, m), 3.61(1H, m)
33	>C=O	169.0	
34	>CHNH-	56.0	H:4.52(1H, d, 7.0), NH:8.04(1H, d, 8.4)
35	>CH-O	67.2	3.74(1H, m)
36	-CH_3	19.0	0.97(3H, d, 6.6)
37	>C=O	169.5	
38	-CH_2-	40.1	2.28(1H, d, 12.0), 2.66(1H,m)
39	>CH-O	72.8	5.06(1H, m)
40	-CH_2-	33.7	1.50(2H, m)
41	-CH_2-	24.1	1.21(2H, m)
42	-CH_2-	29.1	1.21(2H, m)
43	-CH_2-	29.1	1.21(2H, m)
44	-CH_2-	29.1	1.21(2H, m)
45	-CH_2-	29.1	1.21(2H, m)
46	-CH_2-	29.1	1.21(2H, m)
47	-CH_2-	26.8	1.13(1H, m), 1.21(1H, m)
48	-CH_2-	38.5	1.13(1H, m), 1.21(1H, m)
49	>CH-	27.4	1.49(1H, m)
50	-CH_3	22.5	0.83(3H, d, 7.0)
51	-CH_3	22.5	0.83(3H, d, 7.0)

[a] 125 MHz, chemical shift in ppm.
[b] 500 MHz, chemical shift in ppm.

(δ 5.06) was observed by decoupled HMBC[2]. This clearly indicated the lactone linkage forming between acyl chain and β-OHAsp (II). According to these data, planar structure of **3** was determined as shown in Fig. 2. Planar structures of other compounds, **1**, **2**, **4** and **5** were determined likewise. The ^{13}C NMR spectral data of **1**, **2**, **3**, **4**, **5** are shown in Table 3.

The stereochemistry of constituent amino acids were determined using Marfey's method[3] except hydroxyproline. Hydrolysis of **3** and its degradation products **6** and **7** gave the corresponding amino acids. The acid hydrolysates were converted to Marfey's derivatives by treating with 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA), and analyzed by HPLC. Each amino acid derivatives was identified by comparing the retention time with that of the Marfey's derivatives of authentic amino acid. The Marfey's derivatives of amino acids liberated from **3** showed peaks matching L-arginine (L-Arg), L-serine (L-Ser), D-*allo*-threonine (D-*a*Thr), *threo*-β-hydroxy-L-aspartic acid (*threo*-β-L-OHAsp), *threo*-β-hydroxy-D-aspartic acid (*threo*-β-D-OHAsp), L-proline (L-Pro) and D-proline (D-Pro).

The positions of D,L-proline and D,L-hydroxyaspartic acid were determined as follows. Amino acid analysis of the **6**, obtained by Birch reduction[4] of **3**, showed **6** comprising L-Arg, L-Ser, *threo*-β-L-OHAsp, *threo*-β-D-OHAsp, L-Pro (Fig. 3), indicated partial amino acid sequence of L-Pro-L-Arg. The amino acid analysis of **7**, obtained by $LiBH_4$ reduction[5] of **3**, showed **7** comprising L-Arg, L-Ser, D-*a*Thr, *threo*-β-D-OHAsp, L-Pro and D-Pro (Fig. 3), indicated hydroxyaspartic acid forming lactone linkage was *threo*-β-L-OHAsp.

The absolute structure of hydroxyproline (**8**) was identified to be L-*trans*-3-hydroxyproline[6] by the plus cotton effect at 220 nm in the CD spectrum [CD; $[\theta]_{240}$ +80, $[\theta]_{220}$ +1980, $[\theta]_{210}$ +2980 (c 0.033, 0.5 M HCl)] and the small coupling constant between H-2 and H-3 in ^{1}H NMR spectrum ($J_{2,3}$=1.60 Hz), in the literature [*trans* configuration, $J_{2,3}$=1.2 Hz and *cis* configuration, $J_{2,3}$=4.2 Hz].

The absolute configuration of the fatty acid (**9**) was determined to be (3*R*)-hydroxy-13-methyltetradecanoic acid (Fig. 4) from MS, NMR and negative sign of the optical rotation[5,7] $[\alpha]_D^{24}$ −7.7° (c 0.13, $CHCl_3$), in the literature $[\alpha]_D^{20}$ −12.7° (c 0.14, $CDCl_3$)[5].

According to these data, the absolute structure of **3** is determined as shown in Fig. 1.

Experimental

General

Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. UV spectra were determined on a Hitachi 557 spectrophotometer. IR spectra were recorded

Fig. 2. ^{1}H-^{1}H COSY and HMBC experiments of tripropeptin C in DMSO-d_6.

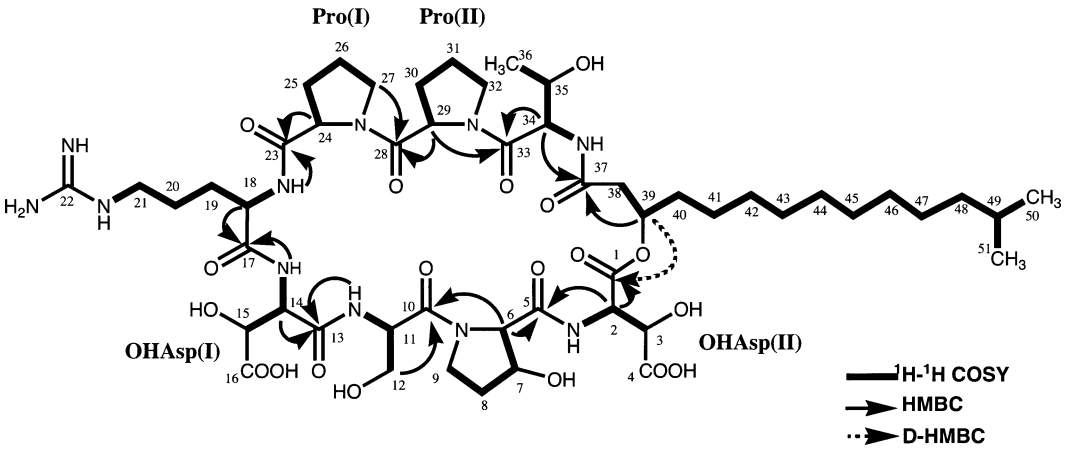


Table 3. ^{13}C NMR data of tripropeptins.

	δ C[a] (multiplicity) DMSO-d_6				
position	A	B	C	D	Z
1	169.7 (s)	169.8 (s)	168.6 (s)	168.6 (s)	170.2 (s)
2	53.0 (d)	54.9 (d)	54.8 (d)	54.8 (d)	55.1 (d)
3	69.9 (d)	70.3 (d)	70.0 (d)	70.0 (d)	70.6 (d)
4	172.7 (s)	172.6 (s)	171.8 (s)	171.9 (s)	172.7 (s)
5	171.3 (s)	171.5 (s)	169.9 (s)	169.9 (s)	171.5 (s)
6	66.4 (d)	67.2 (d)	68.8 (d)	68.8 (d)	67.2 (d)
7	70.0 (d)	70.4 (d)	72.5 (d)	72.5 (d)	70.8 (d)
8	32.4 (t)	31.8 (t)	32.3 (t)	32.3 (t)	32.8 (t)
9	44.8 (t)	44.1 (t)	45.0 (t)	45.0 (t)	44.1 (t)
10	168.8 (s)	168.4 (s)	167.9 (s)	167.9 (s)	168.4 (s)
11	52.9 (d)	52.5 (d)	53.1 (d)	53.1 (d)	52.5 (d)
12	60.5 (t)	60.1 (t)	61.3 (t)	61.3 (t)	60.5 (t)
13	169.0 (s)	169.5 (s)	168.5 (s)	168.5 (s)	169.9 (s)
14	56.1 (d)	57.3 (d)	56.3 (d)	56.3 (d)	57.7 (d)
15	66.9 (d)	70.0 (d)	70.0 (d)	70.0 (d)	69.4 (d)
16	172.9 (s)	174.3 (s)	172.9 (s)	172.9 (s)	174.9 (s)
17	172.0 (s)	171.6 (s)	171.2 (s)	171.2 (s)	170.2 (s)
18	51.8 (d)	52.1 (d)	51.7 (d)	51.7 (d)	52.1 (d)
19	28.6 (t)	29.1 (t)	28.9 (t)	28.9 (t)	29.0 (t)
20	24.6 (t)	25.2 (t)	24.7 (t)	24.7 (t)	24.9 (t)
21	39.5 (t)	39.5 (t)	40.3 (t)	40.3 (t)	40.4 (t)
22	156.7 (s)	156.6 (s)	156.9 (s)	156.9 (s)	156.7 (s)
23	172.9 (s)	174.2 (s)	172.6 (s)	172.6 (s)	174.3 (s)
24	58.0 (d)	59.9 (d)	60.6 (d)	60.6 (d)	60.1 (d)
25	31.7 (t)	31.4 (t)	31.7 (t)	31.7 (t)	31.9 (t)
26	22.3 (t)	22.2 (t)	22.2 (t)	22.2 (t)	22.3 (t)
27	44.8 (t)	47.0 (t)	46.9 (t)	46.9 (t)	47.1 (t)
28	172.2 (s)	172.9 (s)	172.0 (s)	172.0 (s)	173.1 (s)
29	56.4 (d)	57.8 (d)	57.9 (d)	57.9 (d)	57.9 (d)
30	27.5 (t)	28.8 (t)	29.0 (t)	28.4 (t)	28.9 (t)
31	24.5 (t)	24.8 (t)	24.4 (t)	24.4 (t)	24.7 (t)
32	47.0 (t)	47.3 (t)	47.3 (t)	47.3 (t)	47.4 (t)
33	169.7 (s)	169.9 (s)	169.0 (s)	169.0 (s)	170.4 (s)
34	54.8 (d)	56.0 (d)	56.0 (d)	56.0 (d)	56.0 (d)
35	65.9 (d)	65.8 (d)	67.2 (d)	67.2 (d)	65.9 (d)
36	18.9 (q)	18.6 (q)	19.0 (q)	19.0 (q)	18.6 (q)
37	169.7 (s)	170.3 (s)	169.5 (s)	169.6 (s)	170.5 (s)
38	38.6 (t)	38.1 (t)	40.1 (t)	39.5 (t)	39.5 (t)
39	73.0 (d)	73.1 (d)	72.8 (d)	72.8 (d)	73.1 (d)
40	33.6 (t)	33.0 (t)	33.7 (t)	33.7 (t)	33.0 (t)
41	24.0 (t)	23.6 (t)	24.1 (t)	24.1 (t)	23.4 (t)
42	29.0 (t)#	29.1 (t)#	29.1 (t)#	29.1 (t)#	29.2 (t)#
43	29.0 (t)#	29.2 (t)#	29.1 (t)#	29.1 (t)#	29.3 (t)#
44	29.1 (t)#	29.4 (t)#	29.1 (t)#	29.0 (t)#	26.8 (t)
45	24.7 (t)	29.2 (t)#	29.1 (t)#	28.7 (t)#	38.7 (t)
46	38.6 (t)	26.9 (t)	29.1 (t)#	29.2 (t)#	27.6 (d)
47	26.9 (d)	38.1 (t)	26.8 (t)	29.3 (t)#	22.7 (q)
48	22.6 (q)	27.5 (d)	38.5 (t)	26.8 (t)	22.7 (q)
49	22.6 (q)	22.7 (q)	27.4 (d)	38.5 (t)	-
50	-	22.7 (q)	22.5 (q)	27.4 (d)	-
51	-	-	22.5 (q)	22.5 (q)	-
52	-	-	-	22.5 (q)	-

[a] 125 MHz, chemical shift in ppm.
undistinguishable

Fig. 3. Amino acid analysis of the fragments **6** and **7**.

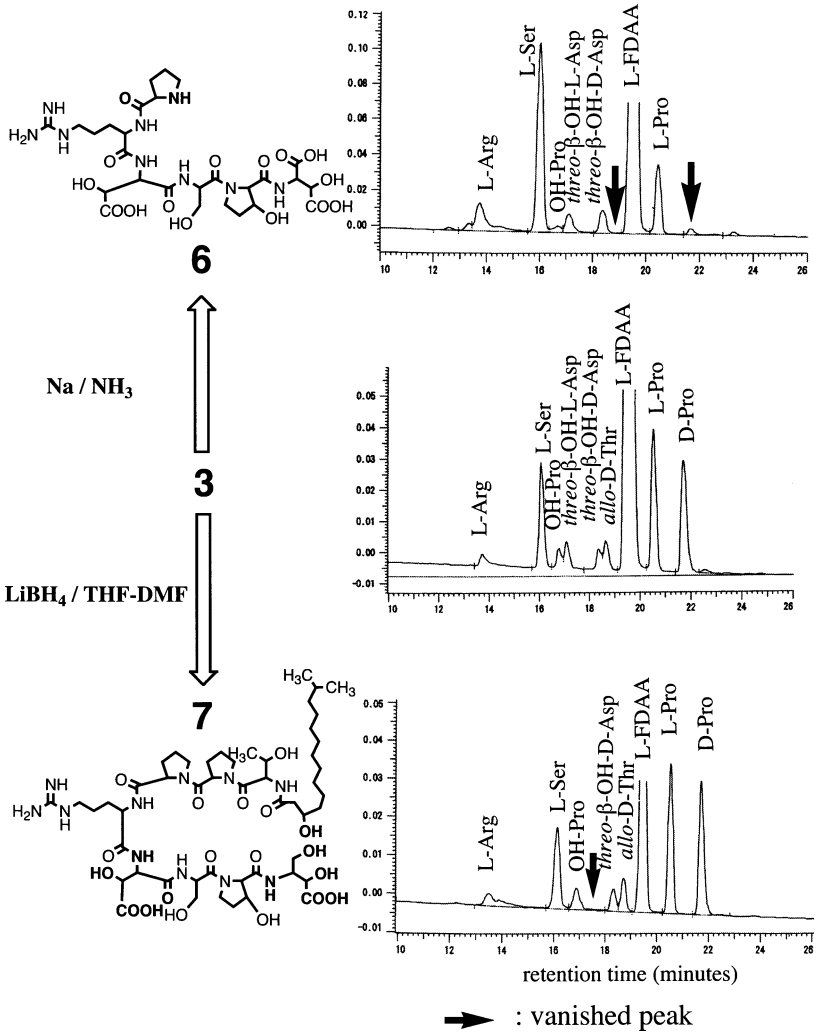
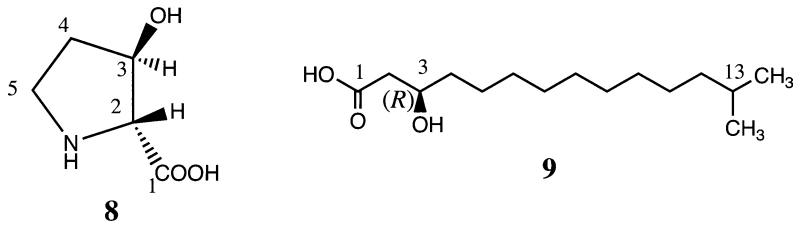


Fig. 4. L-*trans*-3-Hydroxyproline (**8**) and 3-hydroxy-13-Me-tetradecanoic acid (**9**).



using a Horiba FT-210 fourier transform infrared spectrometer. Mass spectra were recorded using a HITACHI M1200H LC/MS (APCI), JEOL JMS-SX102 (HRFAB) and JEOL JMS-T100LC (HRESI) mass spectrometer. The NMR spectra were measured using a JEOL JNM-A500 spectrometer. CD spectrum was recorded using a JASCO J-720W spectropolarimeter.

Materials

L-Arginine (L-Arg), L-serine (L-Ser), L-threonine (L-Thr),

L-proline (L-Pro), D-serine (D-Ser), D-threonine (D-Thr), D-proline (D-Pro) and *erythro*-β-hydroxy-L-aspartic acid (*erythro*-β-L-OHAsp) were purchased from WAKO Pure Chemical Industries, Ltd. D-Arginine (D-Arg) was purchased from SIGMA. L-3-Hydroxyproline (L-OHPro), *threo*-β-hydroxy-aspartic acid (*threo*-β-OHAsp), D-*threo*-β-hydroxyaspartic acid (*threo*-β-D-OHAsp), DL-*allo*-threonine (DL-*a*Thr), L-*allo*-threonine (L-*a*Thr) and 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (L-FDAA) were purchased from TOKYO KASEI.

Amino Acid Analysis using Marfey's Method

In a micro test tube, 0.5 mg of amino acid or acid hydrolysate of tripropeptin, was dissolved in 50 μl of H_2O, then, 20 μl of 1 M $NaHCO_3$ aqueous solution and 20 μl of 1% L-FDAA acetone solution were added. This reaction mixture was capped and incubated at 37°C for 60 minutes. After the addition of 20 μl of 1 M HCl aqueous solution into the tube to stop the reaction, the reaction mixture was evaporated to dryness. The residue, Marfey derivative, was dissolved in 1 ml of methanol and then 10 μl aliquot of the solution was injected into HPLC system. The analyses were performed on a ODS column (Capcell Pak, UG120, Shiseido 5 μm, 150×4.6 mm i.d.) using acetonitrile-0.01 M TFA aqueous solution as the mobile phase in the gradient elution mode (acetonitrile, 10%~40%, 30 minutes). The flow rate of the mobile phase was 2.0 ml/minute and the monitoring wavelength was set at 340 nm.

Purification of Constituent Amino Acids

40 mg of acid hydrolysate of **3** was dissolved in 1 ml of H_2O then added equal volume of ethylacetate. Ethylacetate layer was evaporated to dryness and was used for fatty acid analysis. Water layer was dried up, then subjected to column chromatography using 8 ml wet volume of AMBERLITE CG50I (NH_4^+ type resin, ROAM AND HAAS), eluted successively with 25 ml each of H_2O and 1 M NH_4OH. Amino acid, eluent and their dry weight (in parentheses) are as follows: OHAsp and Thr (H_2O, 3.8 mg), Ser (H_2O, 2.4 mg), Thr, OHPro and Pro (H_2O, 3.0 mg), Pro (H_2O, 4.0 mg), Arg (1 M NH_4OH, 1.1 mg). The former mixture was further chromatographed by using 20 ml wet volume of AMBERLITE CG50I (NH_4^+ type resin, ROAM AND HAAS) eluted with H_2O gave 2.1 mg of OHAsp and 0.8 mg of Thr. The latter mixture was also further chromatographed by using 20 ml wet volume of microcrystalline cellulose (FUNACEL, Funakoshi, Ltd.), eluted with stepwise gradient of acetone : H_2O (60 ml each of 16 : 4, 15 : 5, 14 : 6, 13 : 7). Fractions, eluted with acetone : H_2O (15 : 5), were dried up, then chromatographed by using 15 ml wet volume of microcrystalline cellulose, eluted with acetonitrile : H_2O (100 ml each of 88 : 12, 85 : 15). Eluted with latter solvent gave 1.3 mg of OHPro.

Stereochemistry of Hydroxyproline (**8**)

Hydroxyproline was obtained as colorless powder. FAB-MS; *m/z* 132.09 $(M+H)^+$, CD; $[\theta]_{240}$ +80, $[\theta]_{220}$ +1980, $[\theta]_{210}$ +2980 (*c* 0.033, 0.5 M HCl). ^1H NMR in D_2O at 10°C; δ 2.06 (m, 2H, H-4), 3.52 (m, 1H, H-5), 3.63 (m, 1H, H-5), 4.14 (d, J=1.60 Hz, 1H, H-2), 4.72 (m, 1H, H-3). ^{13}C NMR in D_2O at 10°C; δ 32.9 (C-4), 45.0 (C-5), 69.3 (C-2), 74.5 (C-3), 172.0 (C-1).

HPLC Analysis of the Marfey's Derivatives

Retention time (minutes) of standard amino acids-Marfey's derivatives were as follows: L-Arg (13.89), D-Arg (14.37), L-Ser (16.10), D-Ser (16.91), L-Thr (17.01), D-Thr (19.89), L-*a*Thr (16.75), D-*a*Thr (18.51), *threo*-β-L-OHAsp (17.20), *threo*-β-D-OHAsp (18.46), *erythro*-β-L-OHAsp (18.02), *erythro*-β-D-OHAsp (18.86), L-Pro (20.53), D-Pro (21.60). Retention time (minute) of the amino acids, isolated from acid hydrolysate of **3**, were as follows: Arg (13.89), Ser (16.08), Thr (18.51) , OHAsp (17.12, 18.40), Pro (20.48, 21.63).

Birch Reduction of **3**

The reduction was performed on 37.0 mg of tripropeptin C in 30 ml of liquid ammonia using 450 mg of sodium at −30°C. After 5 minutes, the reaction was terminated by the addition of 2.5 g of ammonium acetate, then concentrated. The residue was diluted with 30 ml of H_2O, then subjected to column chromatography using 80 ml wet volume of Dowex (50w×2, H^+ type, THE DOW CHEMICAL COMPANY) washed with 240 ml of H_2O and eluted with 240 ml of 1 M NH_4OH. The eluent was concentrated *in vacuo* then dissolved in small volume of H_2O and applied to HP20 column (Mitsubishi Chemical Co., 10 ml wet volume). The column was washed with 30 ml of deionized water, 30 ml of 50% aqueous methanol and acetone. Fractions eluted with H_2O gave 21.7 mg of **6** as colorless powder. HRESI-MS *m/z* 732.2844 $(M-H)^-$ (calcd. 732.2800 for $C_{27}H_{42}N_9O_{15}$).

Reduction of **3** with $LiBH_4$

2 mg of $LiBH_4$ was added to a solution of 10 mg of **3** in 50 μl of DMF and 2 ml of THF. The reaction mixture was refluxed for 4 hours. After the reaction mixture was cooled to room temperature, the solution was neutralized by 1 M HCl, then evaporated *in vacuo*. The residue was subjected to column chromatography using 20 ml wet volume of

Sephadex LH-20 (Pharmacia) eluted with methanol. Further purification by HPLC (Capcell Pak, UG120, Shiseido 5 μm, 150×4.6 mm i.d., the flow rate of the mobile phase was 2.0 ml/minute and the monitoring wavelength was set at 210 nm) using 35% acetonitrile aqueous solution as the mobile phase gave 7.1 mg of **7** as colorless powder. HRESI-MS *m/z* 1158.6422 $(M+H)^+$ (calcd. 1158.6362 for $C_{51}H_{87}N_{11}O_{19}$). IR (KBr); 3438, 2927, 1677, 1635, 1384, 1207, 1182, 1133 cm^{-1}.

Isolation and Configuration of Fatty Acid (**9**)

6.2 mg of ethylacetate extract of **3**-acid hydrolysate was chromatographed using silica gel column (10 ml wet volume) developed with stepwise gradient of hexane : ethylacetate (30 ml each of 3 : 1, 2 : 1, 1 : 1, 1 : 2). Fractions, eluted with hexane : ethylacetate (1 : 2), were collected and concentrated *in vacuo* to give 4.0 mg of 3-hydroxy-13-methyltetradecanoic acid (**9**). $[\alpha]_D^{24}$ −7.7° (*c* 0.13, $CHCl_3$). APCI-MS; *m/z* 257 $(M-H)^-$. ^1H NMR in $CDCl_3$; δ 0.78 (6H, d, *J*=6.6 Hz), 1.04～1.52 (19H, m), 2.31 (1H, dd, *J*=16.6 and 9.0 Hz 2-H_a), 2.40 (1H, dd, *J*=16.6 and 3.2Hz 2-H_b) and 3.96 (1H, m 3-H).

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