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# HYMENAMIDES G, H, J, AND K, FOUR NEW CYCLIC OCTAPEPTIDES FROM THE OKINAWAN MARINE SPONGE *HYMENIACIDON* SP.

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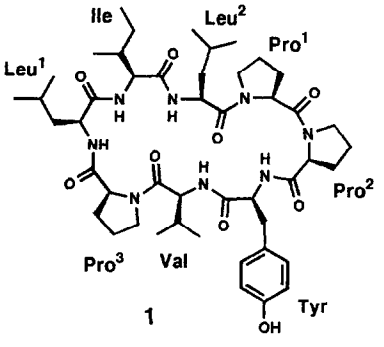
**Abstract:** Four new cyclic octapeptides, hymenamides G, H, J, and K, (**1** ~ **4**), have been isolated from the Okinawan marine sponge *Hymeniacidon* sp. and the structures elucidated on the basis of 2D NMR data and Edman degradation experiments of their partial hydrolysis products.

In our continuing studies on bioactive substances from marine organisms[1], we isolated previously five new cyclic heptapeptides, hymenamides A ~ E[2], from the Okinawan marine sponge *Hymeniacidon* sp. Further investigation of extracts of this sponge resulted in isolation of four new cyclic octapeptides, designated hymenamides G, H, J, and K (**1** ~ **4**). In this paper we describe the isolation and structure elucidation of **1** ~ **4**.

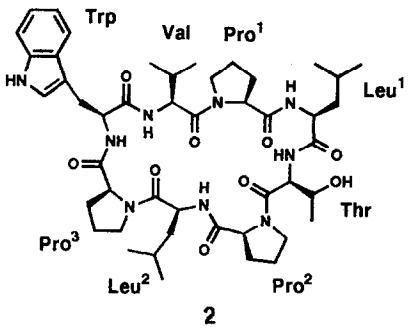
The sponge of the genus *Hymeniacidon* was collected off Manza, Okinawa Island, and kept frozen until used. The MeOH extracts of the sponge were partitioned between EtOAc and $H_2O$, and the aqueous layer was subsequently extracted with *n*-BuOH. The EtOAc soluble material was subjected to a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/$H_2O$) followed by repeated separation on a Sepadex LH-20 column (MeOH and $CHCl_3$/MeOH). Further purification using a silica gel column ($CHCl_3$/MeOH), a Sep-Pak $C_{18}$ cartridge, and/or $C_{18}$ HPLC ($CH_3CN/H_2O/CF_3CO_2H$) afforded hymenamides G (**1**, 0.0016%, wet weight) and H (**2**, 0.0007%) as colorless amorphous solids. The *n*-BuOH soluble fraction was chromagraphed on a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/$H_2O$), a Sephadex LH-20 column (MeOH), and $C_{18}$ HPLC ($CH_3CN/H_2O/CF_3CO_2H$) to give hymenamides J (**3**, 0.0027%) and K (**4**, 0.0003%) as colorless amorphous solids.

Hymenamide G {**1**, $[\alpha]_D^{17}$ -127° (*c* 0.97, MeOH)} showed the pseudomolecular ion peak at *m/z* 893 ($M^+$+H) in the FABMS spectrum. The molecular formula, $C_{47}H_{72}N_8O_9$, of **1** was established by the HRFABMS (*m/z* 893.5481, $M^+$+H, Δ -1.9 mmu). The IR absorptions at 3400 and 1650 $cm^{-1}$ were attributed to hydroxy and amide carbonyl groups, respectively. Its peptide nature was suggested by five doublet amide

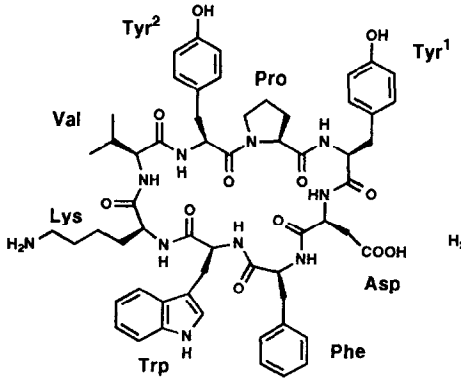
NH proton signals and eight amide carbonyl carbon resonances observed in the $^{1}H$ and $^{13}C$ NMR (Table 1) spectra in DMSO-$d_6$. Standard amino acid analysis of the acid hydrolysate of **1** revealed 1 mol each of tyrosine (Tyr), valine (Val), isoleucine (Ile), two mol of leucine (Leu), and three mol of proline (Pro) residues. The absolute stereochemistry of each amino acid in **1** was determined to be L-configuration by HPLC analyses of (1-fluoro-2,4-dinitro-phen-5-yl)-L-alaninamide (FDAA) derivatives[3] of the acid hydrolysate of **1**. Assignments of the proton chemical shifts of eight amino acid residues were provided by detailed analyses of the NMR data including $^{1}H$-$^{1}H$ COSY, HSQC, and HMBC spectra in DMSO-$d_6$. The presence of a segment of Ile–Leu$^{2}$–Pro$^{1}$–Pro$^{2}$–Tyr–Val–Pro$^{3}$–Leu$^{1}$ in **1** was deduced from following ROESY correlations: NH-Ile/NH-Leu$^{2}$, αH-Leu$^{2}$/δH-Pro$^{1}$, αH-Pro$^{1}$/αH-Pro$^{2}$, δH-Pro$^{2}$/H2-Tyr, NH-Tyr/NH-Val, αH-Val/δH-Pro$^{3}$, αH-Pro$^{3}$/NH-Leu$^{1}$. Connection between CO-Pro$^{3}$ and NH-Leu$^{1}$ suggested by the HMBC correlation for NH-Leu$^{1}$/CO-Pro$^{3}$. Thus the structure of hymenamide G (**1**) was elucidated to be cyclo-(Pro$^{1}$–Pro$^{2}$–Tyr–Val–Pro$^{3}$–Leu$^{1}$–Ile–Leu$^{2}$).



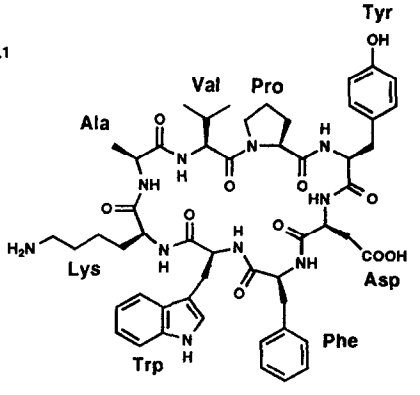

1




2




3




4

Hymenamide H {**2**, $[\alpha]_D^{20}$ -88° (*c* 1.02, MeOH)} showed pseudomolecular ion peaks at *m/z* 904 ($M^+$+H), 926 ($M^+$+Na), and 942 ($M^+$+K) in the FABMS spectrum, and the molecular formula, $C_{47}H_{69}N_9O_9$, of **2** was established by the HRFABMS (*m/z* 904.5281, $M^+$+H, Δ -1.5 mmu). The $^1H$ and $^{13}C$ NMR (Table 2) data implied that **2** was a peptide. Five aromatic {δ 7.46 (d), 7.33 (d), 7.06 (t), 6.98 (br.s), and 6.97 (t)} and an amine {δ 10.75 (br.s)} proton signals suggested the presence of an indole ring. Standard amino acid analysis of the hydrolysate of **2** with methanesulfonic acid revealed 1 mol each of threonine (Thr), Val, and tryptophan (Trp), two mol of Leu, and three mole of Pro residues. $C_{18}$ HPLC analyses of FDAA derivatives of the acid hydrolysate

Table 1. $^1H$ and $^{13}C$ NMR Data of Hymenamide G (**1**) in DMSO-$d_6$.

| position | $^1H$[a] | | *J*(Hz) | $^{13}C$[a] | | position | $^1H$[a] | | *J*(Hz) | $^{13}C$[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pro[1] | | | | | | Pro[3] | | | | | |
| α | 4.01 | dl | 3.4, 10.3 | 59.02 | d | α | 3.90 | t | 8.3 | 61.36 | d |
| β | 2.26 | ddt | 3.4, 8.8, 13.0 | 27.74 | t | β | 2.16 | m | | 29.10 | t |
| | 1.73 | m | | | | | 1.79 | m | | | |
| γ | 1.92 | m | | 24.47 | t | γ | 2.01 | m | | 24.47 | t |
| | 1.89 | m | | | | | 1.85 | m | | | |
| δ | 3.46 | ddl | 4.4, 6.8, 10.3 | 46.63 | t | δ | 3.62 | m | | 47.75 | t |
| | 3.30 | dt | 7.3, 10.3 | | | | 3.58 | m | | | |
| CO | | | | 169.79 | s | CO | | | | 172.22 | s |
| Pro[2] | | | | | | Leu[1] | | | | | |
| α | 4.37 | d | 7.8 | 59.94 | d | α | 3.60 | m | | 55.77 | d |
| β | 1.97 | m | | 30.76 | t | β | 2.13 | m | | 37.08 | t |
| | 1.94 | m | | | | | 1.55 | m | | | |
| γ | 1.54 | m | | 20.83 | t | γ | 1.51 | m | | 24.70 | d |
| | 0.67 | m | | | | $CH_3$ | 0.87 | d | 6.4 | 23.20 | q |
| δ | 3.16 | ddl | 7.8, 11.2, 18.6 | 46.05 | t | $CH_3$ | 0.86 | d | 6.4 | 20.97 | q |
| | 2.96 | brt | 9.7 | | | CO | | | | 171.84 | s |
| CO | | | | 170.04 | s | NH | 8.66 | d | 6.8 | | |
| Tyr | | | | | | Ile | | | | | |
| α | 4.13 | ddl | 3.9, 6.8, 11.7 | 57.34 | d | α | 4.04 | dl | 8.8, 9.3 | 59.02 | d |
| β | 3.10 | dl | 3.9, 13.7 | 36.33 | t | β | 1.48 | m | | 37.21 | d |
| | 2.85 | dl | 11.7, 13.7 | | | γ | 1.39 | m | | 24.32 | t |
| 1 | | | | 126.90 | s | | 1.06 | m | | | |
| 2, 6 | 6.95[b] | d | 8.8 | 129.39[c] | d | βCH$_3$ | 0.82 | d | 6.8 | 15.05 | q |
| 3, 5 | 6.68[b] | d | 8.8 | 115.18[c] | d | γCH$_3$ | 0.78 | t | 7.3 | 10.27 | q |
| 4 | | | | 156.14 | s | CO | | | | 169.96 | s |
| OH | 9.31 | br | | | | NH | 7.94 | d | 9.3 | | |
| CO | | | | 171.01 | s | | | | | | |
| NH | 7.62 | d | 3.9 | | | Leu[2] | | | | | |
| | | | | | | α | 4.54 | brdt | 4.4, 7.3 | 49.20 | d |
| Val | | | | | | β | 1.84 | m | | 40.62 | t |
| α | 4.43 | dl | 9.3, 10.3 | 55.25 | d | | 1.20 | m | | | |
| β | 1.84 | m | | 31.48 | d | γ | 1.62 | m | | 24.64 | d |
| $CH_3$ | 0.90 | d | 7.5 | 18.97 | q | $CH_3$ | 0.93 | d | 6.4 | 21.95 | q |
| $CH_3$ | 0.87 | d | 7.5 | 18.00 | q | $CH_3$ | 0.88 | d | 6.4 | 22.81 | q |
| CO | | | | 171.04 | s | CO | | | | 169.10 | s |
| NH | 7.42 | d | 9.3 | | | NH | 7.84 | d | 7.3 | | |

a) δ in ppm. b) 2H. c) 2C.

of **2** showed that all amino acid residues were L-forms. The $^{1}H$ and $^{13}C$ chemical shifts of each amino acid residue (Table 2) were assigned by detailed analyses of $^{1}H$-$^{1}H$ COSY, HOHAHA, HSQC, and HMBC spectra. The presence of three segments of $Pro^{1}$–$Leu^{1}$–Thr, $Pro^{2}$–$Leu^{2}$, and $Pro^{3}$–Trp–Val was elucidated by the following HMBC correlations: CO-$Pro^{1}$/NH-$Leu^{1}$, CO-$Leu^{1}$/NH-Thr, CO-$Pro^{2}$/NH-$Leu^{2}$, CO-$Pro^{3}$/NH-Trp, and CO-Trp/NH-Val. The ROESY cross-peak for αH-$Leu^{2}$/δH-$Pro^{3}$ revealed that the carbonyl of $Leu^{2}$ was attached to the nitrogen of $Pro^{3}$. The connectivities between Thr and $Pro^{2}$ and between Val and $Pro^{1}$ were deduced from the ROESY correlations for αH-Thr/δH-$Pro^{2}$ and αH-Val/δH-$Pro^{1}$. Thus the structure of hymenamide H (**2**)

Table 2. $^{1}H$ and $^{13}C$ NMR Data of Hymenamide H (**2**) in DMSO-$d_6$.

| position | $^{1}H^{a}$ | | *J*(Hz) | $^{13}C^{a}$ | | position | $^{1}H^{a}$ | | *J*(Hz) | $^{13}C^{a}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $Pro^{1}$ | | | | | | $Leu^{2}$ | | | | | |
| α | 4.09 | t | 7.8 | 60.45 | d | γ | 1.56 | m | | 24.14 | d |
| β | 2.15 | m | | 29.61 | t | $CH_3$ | 0.88 | d | | 23.35 | q |
| | 1.73 | m | | | | $CH_3$ | 0.86 | d | | 20.97 | q |
| γ | 2.01 | m | | 24.64 | t | CO | | | | 170.99 | s |
| | 1.88 | m | | | | NH | 8.13 | d | | | |
| δ | 3.96 | m | | 47.84 | t | | | | | | |
| | 3.30 | m | | | | $Pro^{3}$ | | | | | |
| CO | | | | 170.57 | s | α | 3.88 | t | 7.8 | 60.45 | d |
| | | | | | | β | 1.84 | m | | 28.41 | t |
| $Leu^{1}$ | | | | | | | 1.64 | m | | | |
| α | 3.49 | m | | 52.96 | d | γ | 2.01 | m | | 24.64 | t |
| β | 2.20 | m | | 35.84 | t | | 1.84 | m | | | |
| | 1.69 | m | | | | δ | 3.78 | m | | 46.67 | t |
| γ | 1.47 | m | | 24.60 | d | | 3.48 | m | | | |
| $CH_3$ | 0.85 | d | 6.8 | 22.91 | q | CO | | | | 171.13 | s |
| $CH_3$ | 0.81 | d | 6.8 | 20.87 | q | | | | | | |
| CO | | | | 169.79 | s | Trp | | | | | |
| NH | 8.13 | d | 8.3 | | | α | 3.94 | m | | 55.63 | d |
| | | | | | | β | 3.46 | m | | 24.50 | t |
| Thr | | | | | | | 3.39 | dl | 3.9, 14.2 | | |
| α | 4.89 | dl | 3.4, 9.3 | 56.17 | d | 1 | 10.75 | brs | | | |
| β | 4.15 | m | | 67.74 | d | 2 | 6.98 | brs | | 123.34 | d |
| $CH_3$ | 1.04 | d | 6.9 | 18.95 | q | 3 | | | | 111.15 | s |
| OH | 5.21 | d | 12.7 | 126.90 | s | 4 | | | | 136.04 | s |
| CO | | | | 168.80 | s | 5 | 7.46 | d | 7.8 | 118.01 | d |
| NH | 7.51 | d | 9.3 | | | 6 | 6.97 | t | 7.8 | 118.12 | d |
| | | | | | | 7 | 7.06 | t | 7.8 | 120.79 | d |
| $Pro^{2}$ | | | | | | 8 | 7.33 | d | 7.8 | 111.30 | d |
| α | 4.46 | dl | 3.4, 9.3 | 59.11 | d | 9 | | | | 127.10 | s |
| β | 2.08 | m | | 28.67 | t | CO | | | | 170.08 | s |
| | 1.92 | m | | | | NH | 8.01 | d | 7.3 | | |
| γ | 1.81 | m | | 24.64 | t | | | | | | |
| | 1.75 | m | | | | Val | | | | | |
| δ | 3.70 | dt | 7.3, 17.1 | 47.02 | t | α | 4.56 | t | 9.3 | 56.03 | d |
| | 3.55 | m | | | | β | 2.17 | m | | 27.81 | d |
| CO | | | | 171.29 | s | $CH_3$ | 0.82 | d | 6.3 | 19.41 | q |
| | | | | | | $CH_3$ | 0.75 | d | 6.3 | 18.19 | q |
| $Leu^{2}$ | | | | | | CO | | | | 170.32 | s |
| α | 4.41 | m | | 48.31 | d | NH | 7.44 | d | 9.3 | | |
| β | 1.69 | m | | 37.84 | t | | | | | | |
| | 1.13 | m | | | | | | | | | |

a) δ in ppm.

was concluded to be cyclo-(Pro[1]-Leu[1]-Thr-Pro[2]-Leu[2]-Pro[3]-Trp-Val).

Hymenamide J {**3**, $[\alpha]_D^{20}$ -69° (*c* 0.17, MeOH)} showed pseudomolecular ion peaks at 1099 ($M^+$+H) and 1121 ($M^+$+Na) in the FABMS spectrum, and the molecular formula, $C_{58}H_{70}N_{10}O_{12}$, of **3** was determined by the HRFABMS (*m/z* 1099.5280, $M^+$+H, Δ +2.7 mmu). The IR absorptions at 3400, 1680, and 1640 $cm^{-1}$ were attributed to NH/OH, carboxyl, and amide carbonyl groups, respectively, and combination with $^1H$

Table 3. $^1H$ and $^{13}C$ NMR Data of Major Conformer of Hymenamide J (**3**) in DMSO-$d_6$.

| position | $^1H$[a] | | *J*(Hz) | $^{13}C$[a] | | position | $^1H$[a] | | *J*(Hz) | $^{13}C$[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pro[1] | | | | | | Trp | | | | | |
| α | 4.16 | m | | 63.06 | d | 1 | 10.85 | br.s | | | |
| β | 1.91 | m | | 28.53 | t | 2 | 7.12 | br.s | | 123.22 | d |
| | 1.72 | m | | | | 3 | | | | 110.29 | s |
| γ | 1.81 | m | | 24.65 | t | 4 | | | | 137.41 | s |
| | 1.78 | m | | | | 5 | 7.53 | d | 8.0 | 118.19 | d |
| δ | 3.42 | m | | 47.03 | t | 6 | 6.95 | t | 8.0 | 118.29 | d |
| | 3.40 | m | | | | 7 | 7.06 | t | 8.0 | 120.96 | d |
| CO | | | | 172.00[d] | s | 8 | 7.34 | d | 8.0 | 111.35 | d |
| | | | | | | 9 | | | | 125.73 | s |
| Tyr[1] | | | | | | CO | | | | 170.46[d] | s |
| α | 4.69 | m | | 49.49 | d | NH | 7.61 | m | | | |
| β | 2.93 | m | | 36.43 | t | | | | | | |
| | 2.74 | m | | | | Lys | | | | | |
| 1 | | | | 127.76[e] | d | α | 3.64 | m | | 60.09 | d |
| 2, 6 | 6.94[b] | d | 8.3 | 130.19[c,f] | d | β | 1.80 | m | | 29.04 | t |
| 3, 5 | 6.63[b] | d | 8.3 | 114.87[c,g] | q | | 1.74 | m | | | |
| 4 | | | | 156.34[h] | s | γ | 1.29[b] | m | | 22.35 | t |
| OH | 9.16[i] | br. | | | | δ | 1.52[b] | m | | 26.48 | t |
| CO | | | | 171.63[d] | s | ε | 2.74[b] | m | | 38.71 | t |
| NH | 8.64 | m | | | | $\varepsilon NH_2$ | 7.68[b] | m | | | |
| | | | | | | CO | | | | 170.26[d] | s |
| Asp | | | | | | NH | 7.98 | m | | | |
| α | 4.33 | m | | 51.17 | d | | | | | | |
| β | 2.60 | m | | 35.92 | d | Val | | | | | |
| | 2.58 | m | | | | α | 4.31 | m | | 56.80 | d |
| βCO | | | | 172.49 | s | β | 2.04 | sep. | 6.8 | 31.22 | d |
| CO | | | | 171.23[d] | s | $CH_3$ | 0.93 | d | 6.8 | 18.91 | q |
| NH | 7.34 | m | | | | $CH_3$ | 0.92 | d | 6.8 | 18.32 | q |
| | | | | | | CO | | | | 170.13[d] | s |
| Phe | | | | | | NH | 8.13 | m | | | |
| α | 4.37 | m | | 54.72 | d | | | | | | |
| β | 2.85 | m | | 36.43 | t | $Tyr^2$ | | | | | |
| | 2.79 | m | | | | α | 4.12 | m | | 56.16 | d |
| 1 | | | | 136.15 | s | β | 2.80 | m | | 35.50 | t |
| 2, 6 | 7.04[b] | d | 6.8 | 128.67[c] | d | | 2.69 | m | | | |
| 3, 5 | 7.15[b] | t | 6.8 | 128.05[c] | d | 1 | | | | 127.06[e] | s |
| 4 | 7.11 | t | 6.8 | 126.20 | d | 2, 6 | 6.86 | d | 8.3 | 129.61[c,f] | d |
| CO | | | | 170.90[d] | s | 3, 5 | 6.60 | d | 8.3 | 114.87[c,g] | d |
| NH | 8.42 | m | | | | 4 | | | | 155.70[h] | s |
| | | | | | | OH | 9.24[i] | br. | | | |
| Trp | | | | | | CO | | | | 169.89[d] | s |
| α | 4.44 | m | | 54.00 | d | NH | 8.37 | m | | | |
| β | 3.34 | m | | 27.35 | t | | | | | | |
| | 3.07 | m | | | | | | | | | |

a) δ in ppm. b) 2H. c) 2C. d) ~ i) These proton and carbon signals were interchangeable.

and $^{13}C$ NMR profiles of **3** was indicative of a peptide. Standard amino acid analysis of the acid hydrolysate of **3** with methanesulfonic acid revealed 1 mol each of aspartic acid (Asp), Val, phenylalanine (Phe), lysine (Lys), Trp, and Pro, and two mol of Tyr residues. Each amino acid residue was assigned to be L-configuration by Marfey's procedure. It was difficult to obtain further structural information from NMR data, since the $^{1}H$ and $^{13}C$ NMR spectra of **3** showed two sets of signals (Table 3 and Experimental) due to conformational isomers[4]. The sequence of **3** was declared by Edman degradation[5] of the partial hydrolysates as followed. Hydrolysis of **3** with formic acid[6] afforded two partial hydrolysis products, **5** and **6**, which showed a pseudomolecular ion at *m/z* 1002 (**5**) and 1117 (**6**), respectively. Constituent amino acid residues in **5** were revealed as 1 mol of Val, Phe, Lys, Trp, and Pro, and two mol of Tyr residues, while constituent amino acid residues in **6** were the same as those of **3**. Edman degradation experiments revealed the sequences of Phe–Trp–Lys–Val–$Tyr^{2}$–Pro–$Tyr^{1}$ for **5** and Phe–Trp–Lys–Val–$Tyr^{2}$–Pro–$Tyr^{1}$–Asp for **6**. Treatment of the methyl ester of **3** with $LiBH_4$ followed by amino acid analysis of the acid hydrolysate gave homoserine in place of Asp, demonstrating that αCO-Asp but not γCO-Asp was attached to NH-Phe. Thus the structure of hymenamide J (**3**) was concluded to be cyclo-(Pro–$Tyr^{1}$–Asp–Phe–Trp–Lys–Val–$Tyr^{2}$). The amide bond of $Tyr^{2}$–Pro in the major conformer of **3** was elucidated to be trans on the basis of the carbon chemical shift (δ 24.65)[7] of γC-Pro, while the carbon chemical shift (δ 20.88) of γC-Pro of the minor conformer of **3** suggested that it possessed a cis amide bond[8] at $Tyr^{2}$–Pro.

Hymenamide K {**4**, $[\alpha]_D^{20}$ -36° (*c* 0.45, MeOH)} showed the pseudomolecular ion peak at *m/z* 1007 in the FABMS spectrum, and the molecular formula, $C_{52}H_{66}N_{10}O_{11}$, of **4** was established by the HRFABMS (*m/z* 1007.4940, $M^{+}$+H, Δ -5.1 mmu). The IR absorptions at 3400, 1670, and 1640 $cm^{-1}$ were attributed to amino, carboxyl, and amide carbonyl groups, respectively. The $^{1}H$ and $^{13}C$ NMR data (Table 4) indicated that **4** was a peptide. Standard amino acid analysis of the hydrolysate of **4** with methanesulfonic acid revealed 1 mol each of alanine (Ala), Asp, Val, Phe, Tyr, Lys, Trp, and Pro residues, all of which were determined to be L-configuration by HPLC analyses of

$H_2N$—Phe–Trp–Lys–Val–Tyr–Pro–Tyr—COOH

**5**

$H_2N$—Phe–Trp–Lys–Val–Tyr–Pro–Tyr–Asp—COOH

**6**

$H_2N$—Phe–Trp–Lys–Ala–Val–Pro–Tyr—COOH

**7**

FDAA derivatives of the hydrolysate. The $^{1}H$ and $^{13}C$ NMR chemical shifts of each amino acid residue in **4** were assigned by detailed analyses of the $^{1}H$-$^{1}H$ COSY and HSQC spectra. Although **4** existed as a single conformation, it was difficult to obtain enough informations for determining the sequence of **4** from the HMBC and ROESY data, owing to broadening of the proton signals[9]. The sequence of **4** was determined by Edman degradation of a partial hydrolysis product of **4** as followed. Treatment of **4** with dilute formic acid followed by HPLC separation afforded a linear peptide (**7**) with a molecular weight of 909. Edman degradation experiments of **7** demonstrated that **7**

Table 4. $^{1}H$ and $^{13}C$ NMR Data of Hymenamide K (**4**) in DMSO-$d_6$.

| position | $^{1}H^{a}$ | | $J$(Hz) | $^{13}C^{a}$ | | position | $^{1}H^{a}$ | | $J$(Hz) | $^{13}C^{a}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pro | | | | | | Trp | | | | | |
| α | 4.06 | dl | 7.8, 8.8 | 62.27 | d | α | 4.20 | m | | 56.38 | d |
| β | 2.11 | m | | 28.75 | t | β | 3.35 | m | | 26.48 | t |
| | 1.66 | m | | | | | 3.17 | m | | | |
| γ | 1.98 | m | | 24.89 | t | 1 | 10.95 | brs | | | |
| | 1.86 | m | | | | 2 | 7.26 | brs | | 123.64 | d |
| δ | 3.63 | m | | 47.40 | t | 3 | | | | 108.14 | s |
| | 3.61 | m | | | | 4 | | | | 136.32 | s |
| CO | | | | 171.58[d] | s | 5 | 7.62 | d | 8.3 | 118.03 | d |
| | | | | | | 6 | 7.07 | t | 8.3 | 118.56 | d |
| Tyr | | | | | | 7 | 7.12 | t | 8.3 | 121.10 | d |
| α | 4.11 | m | 7.8 | 59.94 | d | 8 | 7.40 | d | 8.3 | 111.54 | d |
| β | 3.14[b] | m | | 33.92 | t | 9 | | | | 126.45 | s |
| 1 | | | | 128.01 | s | CO | | | | 170.63[d] | s |
| 2, 6 | 6.95[b] | d | 8.3 | 129.59[c] | d | NH | 7.28 | m | | | |
| 3, 5 | 6.67[b] | d | 8.3 | 115.03[c] | d | | | | | | |
| 4 | | | | 155.92 | s | Lys | | | | | |
| OH | 9.21 | s | | | | α | 4.26 | m | | 56.38 | t |
| CO | | | | 171.43[d] | s | β | 2.07 | m | | 30.69 | t |
| NH | 7.55 | m | | | | | 1.40 | m | | | |
| | | | | | | γ | 1.36 | m | | 22.40 | t |
| Asp | | | | | | | 1.33 | m | | | |
| α | 4.66 | m | | 49.72 | d | δ | 1.53[b] | m | | 26.48 | t |
| β | 2.71 | m | | 35.22 | t | ε | 2.75[b] | m | | 38.20 | t |
| | 2.61 | m | | | | $NH_2$ | 7.66[b] | br | | | |
| βCO | | | | 173.46 | s | CO | | | | 170.15[d] | s |
| CO | | | | 171.27[d] | s | NH | 7.30 | m | | | |
| NH | 7.65 | m | | | | | | | | | |
| Phe | | | | | | Ala | | | | | |
| α | 4.13 | m | | 56.22 | d | α | 3.59 | m | | 49.61 | d |
| β | 2.85 | m | | 33.92 | t | $CH_3$ | 1.28 | d | 6.8 | 15.03 | q |
| | 2.76 | m | | | | NH | 7.78 | m | | | |
| 1 | | | | 136.32 | s | CO | | | | 170.15[d] | s |
| 2, 6 | 7.14[b] | d | 6.8 | 128.20[c] | d | | | | | | |
| 3, 5 | 6.92[b] | t | 6.8 | 128.01[c] | d | Val | | | | | |
| 4 | 7.06 | t | 6.8 | 126.04 | d | α | 4.72 | m | | 54.58 | d |
| CO | | | | 171.27[d] | s | β | 2.16 | m | | 30.03 | d |
| NH | 7.32 | m | | | | $CH_3$ | 0.88 | d | 6.8 | 19.77 | q |
| | | | | | | $CH_3$ | 0.62 | d | 6.8 | 16.35 | q |
| | | | | | | CO | | | | 169.97[d] | s |
| | | | | | | NH | 7.09 | m | | | |

a) δ in ppm. b) 2H. c) 2C. d) These carbon signals were interchangeable.

was Phe-Trp-Lys-Ala-Val-Pro-Tyr, a ring-opening and Asp-lacking derivative of **4**. The ROESY cross-peak for αH-Asp/NH-Phe suggested that αH-Asp was attached to NH-Phe. Thus the structure of hymenamide K (**4**) was concluded to be cyclo-(Pro-Tyr-Asp-Phe-Trp-Lys-Ala-Val). The amide bond of Val-Pro of **4** was elucidated to be trans from the carbon chemical shift (δ 24.89)[7] of γC-Pro.

Solution conformations of hymenamides G (**1**) and H (**2**) in DMSO-$d_6$ (Fig. 1) were deduced from detailed analyses of the ROESY spectra as well as temperature coefficients of the amide proton chemical shifts. Hymenamide G (**1**) existed as a single conformation in DMSO solution. The amide bond at CO(Pro$^1$)/N(Pro$^2$) was indicated as cis geometry {type VI(a) β-turn}[10] by the ROESY cross-peak for αH-Pro$^1$/αH-Pro$^2$, while all the other amide bonds were assigned as trans geometries by the ROESY correlations. ROESY cross-peaks were observed for αH-Pro$^1$/NH-Tyr and αH-Pro$^1$/NH-Val, implying that NH-Tyr and NH-Val were oriented inside the peptide ring. Temperature coefficients {Δδ/ΔT: +0.1 (Val), -1.6 (Ile), -1.7 (Leu$^2$), and -2.6 (Tyr)} of the chemical shifts of α-amide protons were less than that of Leu$^1$ (Δδ/ΔT: -4.6), indicating that the former four amide protons participated in intramolecular hydrogen

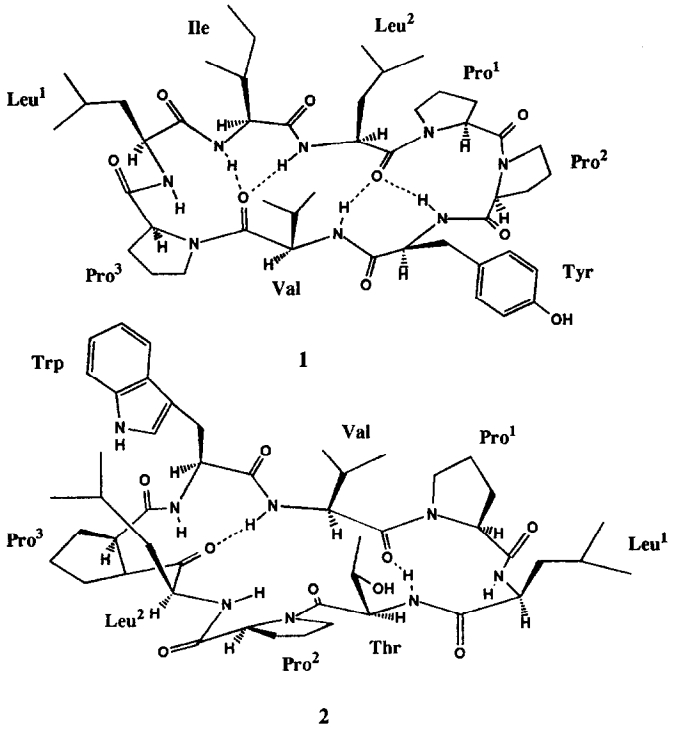



**Fig. 1 Plausible Conformations of Hymenamides G and H (1 and 2) Proposed on Basis of NMR Data.**
Hash lines showed transannular hydrogen bonds.

bonds[11]. Model consideration of **1** was suggestive of the presence of four transannular hydrogen bonds at CO(Leu$^2$)/NH(Tyr), CO(Leu$^2$)/NH(Val), CO(Val)/NH(Ile), and CO(Val)/NH(Leu$^2$), which were attributed to two β-bulge motifs in the molecule[12]. The ROESY correlations for NH-Leu$^1$/αH-Pro$^3$ and NH-Leu$^1$/αH-Leu$^1$ indicated that NH-Leu$^1$ was oriented to be parallel to Cα-H bonds of Pro$^3$ and Leu$^1$ and that the amide bond at CO(Pro$^3$)/NH(Leu$^1$) was implied to have type II β-turn[10]. Thus the backbone of hymenamide G (**1**) was elucidated to have two types of β-turn, namely type II at CO(Pro$^3$)/NH(Leu$^1$) and type VI(a) at CO(Pro1)/N(Pro2), and four transannular hydrogen bonds at CO(Leu$^2$)/NH(Tyr), CO(Leu$^2$)/NH(Val), CO(Val)/NH(Ile), and CO(Val)/NH(Leu$^2$), incorporating β-bulge motifs.

Hymenamide H (**2**) also existed as a single conformation in DMSO solution. The ROESY correlations for αH-Val/δH-Pro$^1$, αH-Thr/δH-Pro$^2$, and αH-Leu$^2$/δH-Pro$^3$ suggested that the three X−Pro amide bonds in **2** were all trans geometries. Temperature coefficients of the α-amide proton chemical shifts of Thr and Val (Δδ/ΔT: +0.1 and -1.6, repectively) were less than those of Leu$^1$, Leu$^2$, and Trp (Δδ/ΔT: -3.2, -3.0, and -6.2, repectively), indicating the presence of two intramolecular hydrogen bonds at CO(Val)/NH(Thr) and CO(Leu$^2$)/NH(Val). Model consideration incorporating large temperature coefficients of the amide proton chemical shifts of Leu$^1$ and Trp and the ROESY correlations observed for αH-Pro$^3$/NH-Trp, NH-Trp/NH-Val, and NH-Leu$^1$/αH-Pro$^1$ indicated that the amide bonds at CO(Pro$^1$)/NH(Leu$^1$) and CO(Pro$^3$)/NH(Trp) were type II β-turns[10]. The ROESY correlations of βH-Thr/NH-Leu$^2$ and OH-Thr/βH-Leu$^1$ indicated that the side-chain of Thr and the amide proton of Leu$^2$ were oriented inside the peptide ring. Thus the solution conformation of hymenamide H (**2**) was implied to have two sets of type II β-turns at CO(Pro$^1$)/NH(Leu$^1$) and CO(Pro$^3$)/NH(Trp), and two intramolecular hydrogen bonds at CO(Val)/NH(Thr) and CO(Leu$^2$)/NH(Val).

Hymenamides G, H, J, and K (**1** ~ **4**) are new proline-containing cyclic octapeptides from the marine sponge *Hymeniacidon* sp. Since a cyclic octapeptide hymenistatin-1[13], cyclo-(Pro$^1$−Pro$^2$−Tyr−Val−Pro$^3$−Leu−Ile$^1$−Ile$^2$), has been isolated from a sponge of the same genus, hymenamides G, H, J, and K (**1** ~ **4**) are the second example of cyclic octapeptides from marine sponges. Hymenamides G and H (**1** and **2**) are neutral peptides containing three Pro residues and an aromatic amino acid residue. Solution conformation of hymenamide G (**1**) is considered to have two β-turns and four transannular hydrogen bonds, while that of hymenamide H (**2**) may involve two β-turn and two transannular hydrogen bonds, suggesting that position and number of Pro residues may play important roles for solution conformations of these cyclic peptides[2]. Hymenamides J (**3**) and K (**4**) contain four or three aromatic amino acid residues, respectively, in addition to both an acidic and a basic residues. Hymenamide J (**3**) exists as a mixture of two conformers having trans/cis amide bond at Tyr$^2$−Pro in DMSO, while hymenamide K (**4**) exists as a single conformation.

Hymenamide J (3) was cytotoxic against L1210 murine leukemia cells and KB human epidermoid carcinoma cells ($IC_{50}$, 2.6 and 0.76 μg/mL, respectively), while hymenamide H (2) exhibited cytotoxicity only against L1210 cells ($IC_{50}$, 6.3 μg/mL). On the other hands, hymenamides G (1) and K (4) showed no cytotoxicity. Hymenamides G (1) and K (4) showed inhibitory activity against protein tyrosine kinase *c-erbB*-2[14] ($IC_{50}$, 63 and 73 μg/mL, respectively).

## EXPERIMENTAL

**General Methods.** The optical rotation was observed using a JASCO DIP-370 polarimeter. UV and IR spectra were taken on a Shimadzu UV-220 and a JASCO IR Report-100 spectrometer, respectively. $^1H$ and $^{13}C$ NMR spectra were conducted with a JEOL EX-400 spectrometer in DMSO-$d_6$ at 24.5, 30.0, 40.0, 50.0, and 60.0 °C. The resonances of residual DMSO at $\delta_H$ 2.5 and $\delta_C$ 39.5 were using as internal references for $^1H$ and $^{13}C$ NMR spectra, respectively. Standard amino acid analyses were performed with Hitachi amino acid autoanalyzer (Model 835) by using a column (4.0 x 250 mm, #2617) at flow rate of 0.275 mL/min with 0.2N Na buffer and detected at 570 and 440 nm for Pro. Edman degradation experiments were performed by an Applied Biosystems 477A protein sequencer and 120A PTH analyzer. FAB mass spectra were recorded employing a JEOL HX-110 spectrometer.

**Collection, Extraction, and Separation.** The sponge *Hymeniacidon* sp. was collected off Manza, Okinawa Island and kept frozen until used. The sponge (1.5 kg, wet weight) was extracted with MeOH (3 L x 2). Evaporation of the extract afforded a residue (69.5 g), which was dissolved in a mixture of EtOAc (1 L) and $H_2O$ (1 L). The aqueous layer was extracted with EtOAc (900 mL x 3) and subsequently with *n*-BuOH (900 mL x 3). The ethyl acetate soluble fraction (6.20 g) was subjected to a silica gel column (Wako gel C-300, Wako Pure Chemical, 4.8 x 45 cm) with $CHCl_3$/*n*-BuOH/AcOH/$H_2O$ (1.5:6:1:1). The fraction (1.86 g, 440 ~ 800 mL) was chromatographed on a Sephadex LH-20 column (Pharmacia Fine Chemical, 2 x 100 cm) with MeOH to afford two fractions containing peptides. The fraction (240 mg) eluting with 160 ~ 220 mL was rechromatographed with a LH-20 column (2 x 100 cm) with $CHCl_3$/MeOH (1:1) followed by a silica gel column (5 x 130 mm) with $CHCl_3$/MeOH (94:6) to afford a crude peptide (26.1 mg), which was further purified by a Sep-Pak $C_{18}$ cartridge (Waters) with $CH_3CN/H_2O$ (60:40) containing 0.1% $CF_3CO_2H$ to give hymenamide G (1, 24.6 mg, 0.0016 %, wet weight). The other fraction (185 mg) eluting with 120 ~ 160 mL in the first LH-20 column was rechromatographed on a LH-20 column (2 x 100 cm; $CHCl_3$/MeOH, 1:1) and then a Sep-Pak $C_{18}$ cartridge ($CH_3CN/H_2O/CF_3CO_2H$, 55:45:0.1) followed by $C_{18}$ HPLC (Asahipak ODP-50, Asahi Chemical Industry, 10 x 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 55:45:0.1; flow rate, 2.0 mL/min; UV detection at 254 nm) to afford hymenamide H (2, 11.0 mg, 0.0007 %,

$t_R$ 11.4 min) as a colorless amorphous solid. The *n*-BuOH soluble fraction (15.1 g) was subjected to a silica gel column (Wako gel C-300, 4.8 x 45 cm) with $CHCl_3$/*n*-BuOH/AcOH/$H_2O$ (1.5:6:1:1). The fraction (1250 mg) eluting with 700 ~ 860 mL was passed through a Sephadex LH-20 column (2.0 x 100 cm) with MeOH followed by $C_{18}$ HPLC (Asahipak ODP-50, 10 x 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 35:65:0.05; flow rate, 2.5 mL/min; UV detection at 254 nm) to yield hymenamides J (**3**, 40 mg, 0.0027 %, $t_R$ 12.7 min) and K (**4**, 4.5 mg, 0.0003 %, $t_R$ 10.8 min).

**Hymenamide G (1).** A colorless amorphous solid; $[\alpha]_D^{17}$ -127° (*c* 0.97, MeOH); IR (KBr) $\nu_{max}$ 3400, 2940, 1650, 1610, 1510, 1445, and 1020 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 226 (sh.) and 280 nm ($\varepsilon$ 1700); $^1H$ and $^{13}C$ NMR (see Table 1); FABMS (Pos., glycerol matrix) *m/z* 893 ($M^+$+H); HRFABMS (Pos., glycerol matrix) *m/z* 893.5481 ($M^+$+H, calcd for $C_{47}H_{73}N_8O_9$, 893.5500).

**Hymenamide H (2).** A colorless amorphous solid; $[\alpha]_D^{20}$ -88° (*c* 1.02, MeOH); IR (KBr) $\nu_{max}$ 3400, 2950, 2920, 1630, 1510, 1440, 1380, 1200, 1170, and 1020 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 229 (sh.) and 279 nm ($\varepsilon$ 1400); $^1H$ and $^{13}C$ NMR (see Table 2); FABMS (Pos., glycerol matrix) *m/z* 904 ($M^+$+H), 926 ($M^+$+Na), and 942 ($M^+$+K); HRFABMS (Pos., glycerol matrix) *m/z* 904.5281 ($M^+$+H, calcd for $C_{47}H_{70}N_9O_9$, 904.5296).

**Hymenamide J (3).** A colorless amorphous solid; $[\alpha]_D^{20}$ -69° (*c* 0.17, MeOH); IR (KBr) $\nu_{max}$ 3400, 2950, 2920, 2845, 1680, 1640, 1510, 1450, 1380, 1255, 1200, 1180, 1130, and 1010 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 222 ($\varepsilon$ 38000) and 280 (7000); $^1H$ and $^{13}C$ NMR of a major conformer (see Table 3); $^{13}C$ NMR of a minor conformer (DMSO-$d_6$) $\delta$ 172.49 (s), 172.34 (s), 172.11 (s), 172.02 (s), 172.02 (s), 171.92(s), 171.85(s), 170.90 (s), 169.90 (s), 155.89 (s), 155.74 (s), 137.42, (s), 135.15 (s), 130.34 (d, 2C), 129.68 (d, 2C), 128.67 (d, 2C), 128.25 (s), 128.11 (d, 2C), 127.06 (s), 126.15 (d), 125.73 (s), 123.86 (d), 120.88 (d), 118.29 (d), 118.19 (d), 115.24 (d, 2C), 114.94 (d, 2C), 111.35 (d), 109.91 (s), 63.06 (d), 61.59 (d), 59.36 (d), 56.45 (d), 54.08 (d), 54.02 (d), 53.80 (d), 53.51 (d), 45.43 (t), 38.67 (t), 36.43 (t), 35.92 (t), 34.80 (t), 31.22 (d), 30.21 (t), 29.12 (t), 28.15 (t), 27.35 (t), 26.48 (t), 22.35 (t), 20.88 (t), 19.55 (q), and 18.12 (q); FABMS (Pos. glycerol matrix) *m/z* 1099 ($M^+$+H) and 1121 ($M^+$+Na); FABMS (Pos., diethanolamine matrix) *m/z* 1099 ($M^+$+H), 1121 ($M^+$+Na), and 1204 ($M^+$+diethanolamine+H); FABMS (Neg., glycerol matrix) *m/z* 1097 ($M^-$-H); HRFABMS (Pos., glycerol matrix) *m/z* 1099.5280 ($M^+$+H, calcd for $C_{58}H_{71}N_{10}O_{12}$, 1099.5253).

**Hymenamide K (4).** A colorless amorphous solid; $[\alpha]_D^{20}$ -36° (*c* 0.45, MeOH); IR (KBr) $\nu_{max}$ 3400, 2920, 1670, 1640, 1510, 1450, 1380, 1205, 1180, 1130, and 1015 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 221 ($\varepsilon$ 25000) and 280 nm (5000); $^1H$ and $^{13}C$ NMR (see Table 4); FABMS (Pos., glycerol matrix) *m/z* 1007 ($M^+$+H) and 1029 ($M^+$+Na); HRFABMS (Pos., glycerol matrix) *m/z* 1007.4940 ($M^+$+H, calcd for $C_{52}H_{67}N_{10}O_{11}$, 1007.4991).

**Acid Hydrolysis of 1 ~ 4.** Hymenamide G (**1**, 0.1 mg) was dissolved in 6N HCl (100 μL) and heated in a sealed tube at 110 °C for 24 h. The acidic solution was prepared for pH 2 with 1N NaOH aq. and subjected to automatic amino acid analyzer. Three mol of Pro, two mol of Leu, and 1 mol each of Val, Ile, and Tyr were found in the hydrolysate of **1**. Hymenamides H, J, and K, (**2** ~ **4**) (each 0.1 mg) in 4N methanesulfonic acid (100 μL) was hydrolyzed in a sealed tube at 115 °C for 24 h, and each solution was prepared for pH 2 with 1N NaOH aq., and subjected to automatic amino acid analyzer. Three mol of Pro, two mol of Leu, and 1 mol each of Thr, Val, and Trp were found in the hydrolysate of **2**. Two mol of Tyr and 1 mol each of Asp, Val, Phe, Trp, Lys, and Pro were found in the hydrolysate of **3**. 1 mol each of Asp, Ala, Val, Tyr, Phe, Trp, Lys, and Pro was found in the hydrolysate of **4**.

**1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA) Derivatization of the Hydrolysate of 1 ~ 4.** Hymenamide G (**1**, 0.1 mg) was treated with 6N HCl (100 μL) at 110 °C for 24 h. The excess HCl was removed by $N_2$ gas, and aqueous solution (20 μL) of the hydrolysate was reacted with 1% FDAA/acetone (5 μL) and 1 M $NaHCO_3$ (10 μL) at 40 °C for 1 h. After cooling to room temperature, the reaction mixture was neutralized with 2N HCl (5 μL). The solvent was evaporated, and the residue was added DMSO (50 μL). Hymenamides H, J, and K (**2** ~ **4**, each 0.1 mg) were treated with 4N methanesulfonic acid (100 μL) at 115 °C for 24 h. The solution was subjected on Amberlite CG-120/Celite (1:9) with 1N HCl and then 2N $NH_3$. The fraction eluting with 2N $NH_3$ was evaporated under reduced pressure. Each hydrolysate was carried out by the same procedure as described above.

**$C_{18}$ HPLC Analyses of the FDAA Derivatives of Amino Acids.** The FDAA derivatives of standard amino acids were prepared by the same procedure as described above. The FDAA derivatives of hydrolysates of **1** ~ **4** and standard amino acids were subjected to $C_{18}$ HPLC analyses using Inatosil ODS-2 column (GL Sciences Inc., 5μm, 4.6 x 150 mm) and following gradient program; solvent A, acetonitrile; solvent B, 50 mM triethylamine phosphate buffer (pH 3.0); time (min)/A(%)/B(%), 0/10/90, 60/60/40; flow rate, 1 mL/min; detection at 340 nm; column temperature, 40 °C. The retention times (min) of FDAA derivatives of authentic L and D amino acids were as follows; L-Lys (11.3 and 32.5), D-Lys (12.5 and 35.9), L-Thr (15.2), D-Thr (20.4), L-Asp (15.4), D-Asp (17.8), L-Ala (19.1), D-Ala (23.6), L-Pro (20.9), D-Pro (23.7), L-Tyr (21.5 and 39.0), D-Tyr (25.8 and 44.1), L-Val (26.5), D-Val (32.5), L-Trp (29.4), D-Trp (33.0), L-Ile (29.8), D-Ile (35.7), L-Phe (30.2), D-Phe (35.2), L-Leu (31.3), and D-Leu (37.1). The retention times (min) of FDAA derivatives of the hydrolysates of **1** ~ **4** were as follows; L-Pro (20.9), L-Tyr (21.5 and 39.0), L-Val (26.5), L-Ile (29.8), and L-Leu (31.3) in **1**; L-Thr (15.2), L-Pro (20.9), L-Val (26.5), L-Trp (29.4), and L-Leu (31.3) in **2**; L-Lys (11.3 and 32.5), L-Asp (15.4), L-Pro (20.9), L-Tyr (21.5 and 39.0), L-Val (26.5), L-Trp (29.4), and L-Phe (30.2) in **3**; L-Lys (11.3 and

32.5), L-Asp (15.4), L-Ala (19.1), L-Pro (20.9), L-Tyr (21.5 and 39.0), L-Val (26.5), L-Trp (29.4), and L-Phe (30.2) in **4**.

**Partial Hydrolysis of 3 and 4 with 2% Formic Acid.** 2% formic acid solution (2 mL) of hymenamide J (**3**, 2.7 mg) was treated in a sealed tube at 110 °C for 5 h. After evaporation, the residue was subjected to $C_{18}$ HPLC (Asahipak ODP-50, 10 x 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$, 30:70:0.05; flow rate, 2.0 mL/min; UV detection at 230 nm) to give linear peptides **5** (1.0 mg, $t_R$ 8.0 min) and **6** (0.6 mg, $t_R$ 5.0 min) as colorless solids; FABMS of **5** and **6** (Pos., glycerol matrix) *m/z* 1002 ($M^+$+H) and 1117 ($M^+$+H), respectively. Hymenamide K (**4**, 1.0 mg) was treated by the same procedure as described above to give a linear peptide **7** (0.3 mg, $t_R$ 4.4 min) as a colorless solid; FABMS of **7** (Pos., glycerol matrix) *m/z* 910 ($M^+$+H). Linear peptides **5** ~ **7** (each 0.1 mg) were hydrolyzed with 4N methanesulfonic acid in a sealed tube at 115 °C for 24 h, and the residues were subjected to automatic amino acid analyzer. Two mol of Tyr and 1 mol each of Val, Phe, Trp, Lys, and Pro were found in the hydrolysate of **5**. Two mol of Tyr and 1 mol each of Asp, Val, Phe, Trp, Lys, and Pro were found in the hydrolysate of **6**. 1 mol each of Ala, Val, Tyr, Phe, Trp, Lys, and Pro were found in the hydrolysate of **7**. **5** ~ **7** were subjected to an automatic amino acid sequencer to elucidate as the sequence of Phe-Trp-Lys-Val-$Tyr^2$-Pro-$Tyr^1$, Phe-Trp-Lys-Val-$Tyr^2$-Pro-$Tyr^1$-Asp, and Phe-Trp-Lys-Ala-Val-Pro-Tyr, respectively, from *N*-terminus.

**Reduction of Methyl Ester of 3 with $LiBH_4$.** Hymenamide J (**3**, 1.0 mg) in $CH_3CN$/MeOH (9:1, 0.5 mL) was treated with trimethylsilyldiazomethane (10 μL) and *N*-ethyldiisopropylamine (5 μL) at room temperature for 3 h. After evaporation the residue was passed through a Sephadex LH-20 column (0.5 x 40 cm) to give the methyl ester of **3** (1.3 mg). Treatment of the methyl ester in THF (0.3 mL) with $LiBH_4$ (5 mg) and MeOH (3 mL) followed by Diaion HP-20 coloumn chromatography (Mitsubishi Kasei, 0.5 x 10 cm, MeOH) afforded the reductive product of **3** (0.4 mg). The acid hydrolysate of the reductive product was subjected to automatic amino acid analyzer; two mol of Tyr and 1 mol each of Val, Phe, Trp, Lys, Pro, and homoserine.

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