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MICROVIRIDINS, ELASTASE INHIBITORS FROM THE CYANOBACTERIUM *NOSTOC MINUTUM* (NIES-26)

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Key Word Index—*Nostoc minutum*; Cyanophyceae; cyclic peptide; microviridin G; microviridin H; elastase inhibitor; protease inhibitor.

Abstract—Two new microviridin-type peptides were isolated from the freshwater cyanobacterium *Nostoc minutum* (NIES-26), which we named microviridin G and H. Their structures were shown to be **1** and **2** respectively, on the basis of 2D NMR data and chemical degradation. These peptides inhibited elastase potently.

INTRODUCTION

Nostoc (Cyanophyceae) is both useful and a danger to humans. For example, *Nostoc commune* has been shown to diminish the cholesterol level in the serum of rats [1], and it therefore has the potential for a health food. As extracts from *Nostoc* also inhibited human pathogens [2], it is possible that *Nostoc* may provide biotechnologists with unique medicinal sources. But *Nostoc* also produces a variety of toxins, such as hepatotoxins [3] and cytotoxins [4, 5]. Recently, a number of unique peptides, such as microcystins [6] and cryptophycins [5] have been isolated from *Nostoc* spp. In the course of our screening program for protease inhibitors from laboratory-cultured blue-green algae, *Nostoc minutum* (NIES-26) showed strong elastase inhibitory activity. Elastase inhibitors have potential for therapeutic agents of pulmonary emphysema. Here we report the isolation and structure elucidation of the new elastase inhibitors.

RESULTS AND DISCUSSION

N. minutum (NIES-26) was obtained from the NIES-collection (Microbial Culture Collection, the National Institute for Environmental Studies, Japan) and cultured in our laboratory. The 80% methanol extract of freeze-dried algal cells was partitioned between water and diethyl ether. The aqueous layer was further extracted with *n*-butanol and subjected to ODS flash chromatography with increasing the amounts of methanol in water. The active aqueous methanol fraction was resubjected to ODS flash chromatography with increasing the amounts of methyl cyanide in water containing 0.05% TFA. The 30% methyl cyanide fraction was subjected to reversed-phase HPLC with 26% methyl cyanide containing 0.05% TFA to furnish new cyclic peptides, microviridin G (**1**, 0.003% yield, dry wt) and H (**2**, 0.007%).

Microviridin H (**2**) is a colourless amorphous powder. The molecular formula of **2** was deduced to be $C_{89}H_{115}O_{26}N_{17}$ by HRFAB-mass and NMR spectral data. The peptidic nature of **2** was suggested by the ^1H and ^{13}C NMR spectra of **2** (Table 1), and the amino acid analyses of the hydrolysate gave Asp, Thr, Ser, Glu, Leu, Tyr, Lys and Pro. The extensive NMR analyses including ^1H–^1H COSY, HOHAHA, HMQC [7] and HMBC [8] spectra could assign all the proton and carbon signals, indicating the presence of 1 mole each of Gln, Thr, Leu, Lys, Asp, Ser and Trp, 2 moles each of Glu and Pro, and 3 moles of Tyr in the molecule.

HMBC correlations from α-H or NH to C=O (Table 1) determined the partial amino acid sequences of Ac-Tyr(I)- and -Thr-Leu-Lys-Tyr(II)-Pro(II)-Ser-Asp-Trp-Glu(I)-Glu(II)-Tyr(III). HMBC correlation between Thr H-3 (the lowfield proton; δ 5.34) and Asp β-C=O suggested that Thr and Asp were esterified. The linkages between Lys ε-NH and Glu(II) γ-C=O and *O*-Me and Glu(I) were also determined by HMBC spectrum. NOESY correlations [Ac H-2/Tyr(I) NH; Thr H-2/Leu NH; Leu NH/Lys NH; Leu H-2/Lys

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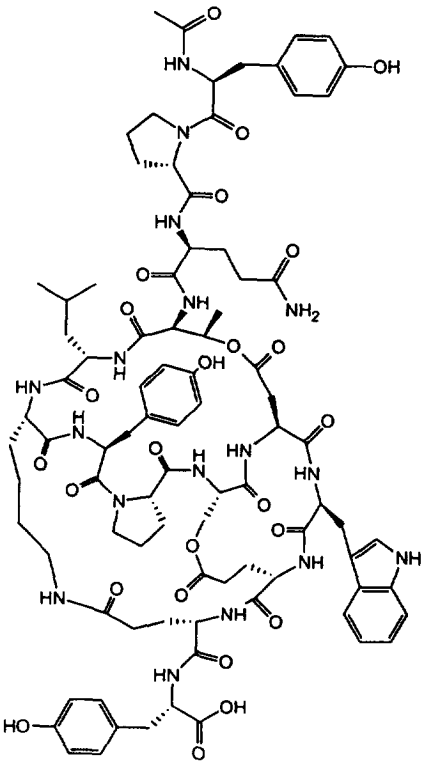

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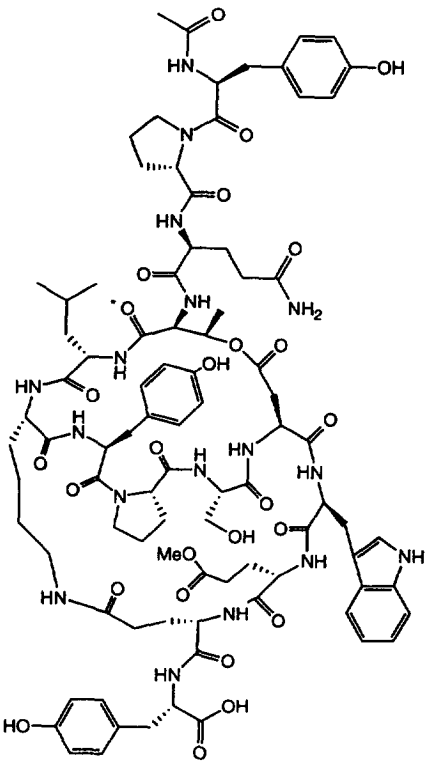

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NH; Lys H-2/Tyr(II) NH; Tyr(II) H-2/Pro(II) H-2; Pro(II) H-3/Ser NH; Ser H-2/Asp NH; Asp NH/Trp NH; Asp H-2/Trp NH; Trp NH/Glu(I) NH; Trp H-2/Glu(I) NH; Glu(I) H-2/Glu(II) NH; Glu(II) H-2/Tyr(III) NH] supported partial sequences obtained from HMBC spectrum. Finally, the partial sequence of -Tyr(I)-Pro(I)-Gln-Thr-was determined by NOESY correlations [Tyr(I) H-2/Pro(I) H-5; Pro(I) H-3/Gln NH; Gln NH/Thr NH; Gln H-2/Thr NH]. These findings suggested that **2** had a structure closely related to microviridins [A (**3**); B (**4**); C (**5**)] (Fig. 1) isolated from *Microcystis viridis* [9] and *M. aeruginosa* [10]. To confirm the stereochemistry of the amino acids, **2** was hydrolysed by 6 M HCl and 6 M HCl containing 1% formic acid. Chiral GC analyses of *N*-trifluoroacetyl methyl ester derivatives of the acid hydrolysate showed that all of the amino acid residues in **2** were L-form. These results suggested the structure of microviridin H (**2**).

Microviridin G (**1**) is a colourless amorphous powder. The molecular formula of **1** was deduced for $C_{88}H_{111}O_{25}N_{17}$ by HRFAB-mass and NMR spectral data (Table 2). ^1H and ^{13}C NMR spectra of **1** suggested that **1** was closely related to **2**. The amino acid analyses of **1** indicated that **1** had the same amino acid composition as that of **2**. The detailed analyses of ^1H–^1H COSY, HOHAHA, HMQC and HMBC spectra also supported this result. The major difference of **1** from **2** was the absence of a methoxy group (δ_H 3.55, δ_C 51.3) and one more hydroxyl group (δ_H 5.25) correlating with Ser H-2 in COSY spectrum. Furthermore, HMBC correlation between Ser H-3 (the lowfield proton; δ 4.11, 4.73) and Glu(I) γ-C=O suggested that Ser and Glu(I) were esterified. The sequence of **1** was confirmed by analyses of HMBC and NOESY spectra. HMBC correlations of **1** (Table 2) determined partial sequences of Ac-Tyr(I)-Pro(I)-, -Thr-Leu-Lys-Tyr(II)-Pro(II)-, -Ser-Asp- and -Trp-Glu(I)-Glu(II)-Tyr(III). The linkage between Thr H-3 (the lowfield proton; δ 5.36) and Asp β-C=O was also determined by HMBC spectrum, and it suggested that Thr and Asp were esterified. NOESY correlations [Ac H-2/Tyr(I) NH; Tyr(I) H-2/Pro(I) H-5; Thr H-2/Leu NH; Leu NH/Lys NH; Leu H-2/Lys NH; Lys H-2/Tyr(II) NH; Tyr(II) H-2/Pro(II) H-2; Ser H-2/Asp NH; Trp NH/Glu(I) NH; Trp H-2/Glu(I) NH; Glu(I) H-2/Glu(II) NH; Glu(II) H-2/Tyr(III) NH] supported partial sequences obtained from HMBC spectrum. Furthermore, partial sequences of -Pro(I)-Gln-Thr-, -Pro(II)-Ser-, -Asp-Trp- and -Lys ε-NH-Glu(II) γ-C=O- were determined by NOESY correlations [Pro(I) H-3/Gln NH; Gln NH/Thr NH; Gln H-2/Thr NH; Pro(II) H-3/Ser NH; Asp NH/Trp NH; Asp H-2/Trp NH; Glu(II) H-4/Lys ε-NH]. To determine the stereochemistry, **1** was also hydrolysed by 6 M HCl containing 1% formic acid. Chiral GC analyses of *N*-trifluoroacetyl methyl ester derivatives of the acid hydrolysate showed that all of the amino acid residues in **1** were L-form. The structure of microviridin G was suggested to be **1**.

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

Position	^{1}H *J* (Hz)	^{13}C	HMBC (^{1}H)	Position	^{1}H *J* (Hz)	^{13}C	HMBC (^{1}H)
Ac				Pro(II)			
1		169.0	Ac 2, Tyr 2, NH	1		170.5	Pro(II) 2, Ser NH
2	1.73 (*s*)	22.3		2	3.57 (*m*)	60.4	
Tyr(I)				3	1.44 (*m*)	31.2	Pro(II) 2
1		170.3	Tyr(I) 3		1.73 (*m*)		
2	4.56 (*m*)	52.4	Tyr(I) 3, NH	4	1.60 (*m*)	21.7	Pro(II) 2
3	2.59 (*dd*, 14.2, 10.0)	36.0	Tyr(I) 2, 5, 9, NH	5	3.26 (*m*)	46.5	
	2.82 (*dd*, 14.2, 4.2)				3.54 (*m*)		
4		128.0	Tyr(I) 2, 3, 6, 8	Ser			
5, 9	7.07 (*d*, 8.6)	130.1	Tyr(I) 3, 5, 9	1		169.6	Ser 2, Asp NH
6, 8	6.64 (*d*, 8.6)	115.0	Tyr(I) OH	2	4.32 (*m*)	54.2	
7		155.8	Tyr(I) 5, 6, 8, 9	3	3.54 (*m*)	61.7	
NH	8.16 (*d*, 8.3)				3.58 (*m*)		
Pro(I)				OH	5.25 (*br*)		
1		171.7	Pro(I) 2, 3, Gln NH	NH	7.35 (*d*, 8.5)		
2	4.34 (*dd*, 8.3, 4.0)	59.3	Pro(I) 4	Asp			
3	1.86 (*m*)	29.0	Pro(I) 4, 5	1		170.19	Asp 2, Trp NH
	1.99 (*m*)			2	4.63 (*m*)	49.1	Asp 3
4	1.82 (*m*)	24.5	Pro(I) 5	3	2.59 (*m*)	35.1	
	1.90 (*m*)			4		170.4	Asp 2, 3, Thr 3
5	3.49 (*m*)	46.8		NH	8.61 (*d*, 8.1)		
	3.62 (*m*)			Trp			
Gln				1		171.2	Trp 2, Glu(I) NH
1		171.8	Gln 2, Thr NH	2	4.38 (*m*)	54.2	Trp 3
2	4.33 (*m*)	52.0	Gln 3,4, NH	3	2.89 (*m*)	27.7	Trp 2
3	1.76 (*m*)	27.7	Gln 2, 4		3.10 (*m*)		
	1.93 (*m*)			1′	10.78 (*d*, 2.4)		
4	2.14 (*m*)	31.5	Gln 2, 3, NH_2	2′	7.15 (*d*, 2.4)	123.8	Trp 3, 1′
5		174.0	Gln 3, 4, NH_2	3′		109.5	Trp 2, 3, 1′, 2′ 4′
NH	8.18 (*d*, 7.9)			4′	7.52 (*d*, 7.9)	118.16	Trp 6′
NH_2	6.77 (*br*)			5′	6.94 (*m*)	118.24	Trp 7′
	7.18 (*br*)			6′	7.03 (*m*)	120.6	Trp 4′ 5′
Thr				7′	7.29 (*d*, 8.1)	111.3	TrP 4′ 5′
1		168.7	Thr 2, Leu 2, NH	8′		136.1	Trp 3, 2′, 4′, 5′, 7′
2	4.55 (*m*)	54.6	Thr 4	9′		127.1	Trp 1′, 2′, 4′, 6′
3	5.34 (*m*)	70.5	Thr 4	NH	7.58 (*d*, 7.3)		
4	1.08 (*d*, 6.4)	16.3	Thr 3	Glu(I)			
NH	7.67 (*d*, 8.8)			1		170.4	Glu(I) 2, Glu(II) NH
Leu				2	4.07 (*m*)	52.4	Glu(I) 3, 4
1		170.9	Leu 2, Lys NH	3	1.77 (*m*)	26.3	Glu(I) 2, 4
2	4.22 (*m*)	51.2	Leu 3, Thr NH		1.89 (*m*)		
3	1.38 (*m*)	40.2	Leu 5, 5′	4	2.18 (*t*, 7.6)	29.5	Glu(I) 2, 3
	1.57 (*m*)			5		172.8	Glu(I) 3, 4, *O*-Me
4	1.43 (*m*)	24.1	Leu 3, 5, 5′	NH	7.77 (*d*, 6.2)		
5	0.76 (*d*, 6.6)	21.3	Leu 3, 4, 5′	*O*-Me	3.55 (*s*)	51.3	
5′	0.86 (*d*, 6.4)	23.0	Leu 3, 4, 5	Glu(II)			
NH	8.37 (*d*, 8.3)			1		171.0	Tyr(III) 2, NH
Lys				2	4.23 (*m*)	51.7	Glu(II) 3, 4
1		170.28	Lys 2, Tyr(I) NH	3	1.59 (*m*)	28.3	Glu(II) 4
2	4.21 (*m*)	52.9	Lys NH		1.92 (*m*)		
3	1.54 (*m*)	32.2	Lys 2, 4, 5	4	2.06 (*m*)	31.5	
4	1.18 (*m*)	22.8	Lys 2, 3, 5, 6	5		171.5	Glu(II) 4, Lys εNH
	1.25 (*m*)			NH	7.90 (*d*, 8.4)		
5	1.38 (*m*)	29.2	Lys 6	Tyr(III)			
	1.42 (*m*)			1		172.8	Tyr(III) 2, 3
6	3.02 (*m*)	38.3	Lys 5	2	4.31 (*ddd*, 8.3, 7.7, 5.3)		
αNH	6.59 (*d*, 7.5)					53.8	Tyr(III) 3, NH
εNH	7.38 (*br*)			3	2.78 (*dd*, 14.0, 8.3)	35.9	Tyr(III) 2, 5, 9
Tyr(II)					2.90 (*dd*, 14.0, 5.3)		
1		170.5	Tyr(II) 2, Pro(II) 2	4		127.3	Tyr(III) 2, 3, 6, 8
2	4.47 (*m*)	51.5	Tyr(II) 3	5,9	6.98 (*d*, 8.6)	130.0	Tyr(III) 3
3	2.67 (*m*)	38.5	Tyr(II) 2, 5, 9	6, 9	6.63 (*d*, 8.6)	114.9	Tyr(III) OH
	2.72 (*m*)			7		155.9	Tyr(III) 5, 6, 8, 9
4		126.2	Tyr(II) 3, 6, 9	OH	9.17 (*s*)		
5, 9	6.93 (*d*, 8.6)	130.2	Tyr(II) 3	NH	7.98 (*d*, 7.7)		
6, 8	6.67 (*d*, 8.6)	115.2	Tyr(II) OH				
7		156.2	Tyr(II) 5, 6, 8, 9 OH				
OH	9.29 (*s*)						
NH	8.28 (*m*)						

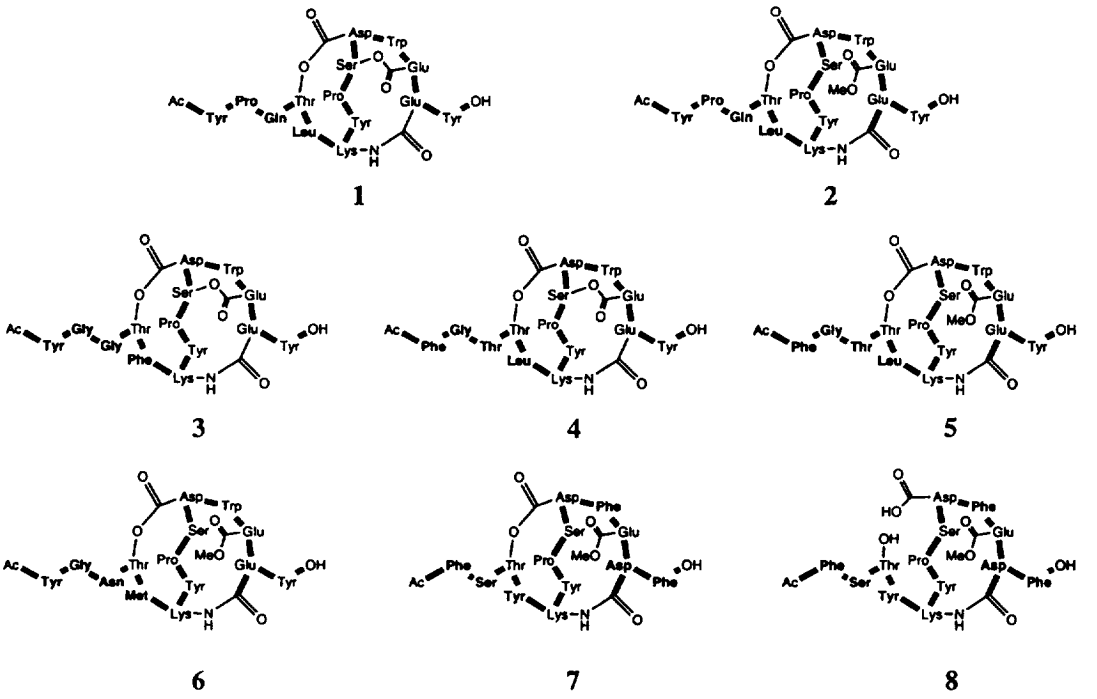

Fig. 1. Microviridins isolated from cyanobacteria.

Microviridins G and H inhibited elastase potently with an IC_{50} of 0.019 and 0.031 μg ml^{-1}, respectively. Microviridins G and H also inhibited chymotrypsin with an IC_{50} of 1.4 and 2.9 μg ml^{-1}, respectively, but did not inhibit trypsin, papain, thrombin and plasmin at 100 μg ml^{-1}.

Inhibitory activity against elastase of microviridins is summarized in Table 3. Microviridin A (**3**), which was isolated from *M. viridis* (NIES-102) [9], had no elastase inhibitory activity. B (**4**) and C (**5**), which were isolated from *M. aeruginosa* (NIES-298) [10], had almost the same IC_{50} against elastase as G (**1**) and H (**2**). D-F (**6**–**8**), which were isolated from *Oscillatoria agardhii* (NIES-204) [11], showed weak elastase inhibitory activities. In these compounds (Fig. 1), the ester linkage with the OH group of Ser is not important for elastase inhibitory activity, but the amino acid sequence of X-Thr-Y affects elastase inhibitory activity. B (**4**), C (**5**), D (**6**), E (**7**), F (**8**), G (**1**) and H (**2**) have a hydrophilic amino acid residue in the place of X while A (**3**) does not. Since B (**4**), C(**5**), G(**1**) and H(**2**) have stronger activity than D (**6**) and E(**7**), the Leu in the place of Y is important for the inhibitory activity. In addition, the absence of the ester linkage between the OH group of Thr and β-C=O of Asp(1) affects the inhibitory activity in the case of F (**8**).

EXPERIMENTAL

General methods. NMR spectra were recorded on a Bruker AM600 NMR spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent peak: δ_H 2.49 and δ_C 39.5 for DMSO-d_6. Optical rotations were determined by a Jasco DIP-371 digital polarimeter. UV spectra were measured on a Hitachi 330 spectrophotometer. FAB-MS were measured using glycerol as a matrix on a Jeol SX102 mass spectrometer. HPLC was performed on a Shimadzu LC-6A liquid chromatograph with Capcell Pak C_{18} column (250 mm × 10 mm i.d.). Amino acid analyses were carried out with a Hitachi L-8500A amino acid analyser. Chiral GC experiments were performed on a Shimadzu GC-9A gas chromatograph fitted with an Alltech Chirasil-L-Val capillary column (25 m × 0.25 mm i.d.).

Culture of Nostoc minutum. *Nostoc minutum* (NIES-26) was obtained from the NIES-collection and cultured in 10 l glass bottles containing CB medium [12] with aeration (filtered air, 0.3 l min^{-1}) at 25° under illumination of 250 μE m^{-2} s on a 12L:12D cycle. After 25 days, the algal cells were filtered by 95 μm nylon plankton nets (Swiss Silk Bolting Cloth Mfg Co., Ltd) and lyophilized. The cells yielded 0.4 g l^{-1} on average and were kept in a freezer at −20° until extracted.

Extraction and isolation. Freeze-dried algal cells (231 g from 590 l of culture) were extracted with MeOH–H_2O (8:2), concd and partitioned between Et_2O and H_2O. The H_2O soluble fr. was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was subjected to flash chromatography on ODS with 20, 30, 40, 50 and 60% MeOH, MeOH and CH_2Cl_2. The fr. eluted with 40% MeOH showed elastase inhibitory activity, and was chromatographed on ODS with 20, 30 and 50% MeCN containing 0.05% TFA. 30% MeCN fr. was finally sepd by HPLC on Capcell Pak C_{18} with 26% MeCN containing 0.05% TFA to yield microviridin H (**1**, 7.0 mg) and G (**2**, 16.0 mg).

Table 2. ^{1}H and ^{13}C NMR data of microviridin G (**1**) in DMSO-d_6

Postion	^{1}H *J* (Hz)	^{13}C	HMBC (^{1}H)	Postion	^{1}H *J* (Hz)	^{13}C	HMBC (^{1}H)
Ac				Pro(II)			
1		169.24	Ac 2, Tyr 2, NH	1			
2	1.73 (*s*)	22.2		2	3.51 (*m*)	60.5	
Tyr(I)				3	1.60 (*m*)	30.6	Pro(II) 2
1				4	1.48 (*m*)	21.7	
2	4.56 (*m*)	52.4	Tyr(I) 3, NH		1.64 (*m*)		
3	2.60 (*dd*, 14.1, 9.1)	36.0	Tyr(I) 2, 5, 9	5	3.22 (*m*)	46.1	
	2.82 (*dd*, 14.1, 4.2)				3.38 (*m*)		
4		127.9	Tyr(I) 2, 3, 6, 9	Ser			
5, 9	7.07 (*d*, 8.5)	130.1	Tyr(I) 3	1		170.3	Ser2, Asp NH
6, 8	6.64 (*d*, 8.5)	115.0	Tyr(I) OH	2	4.50 (*m*)	52.3	
7		155.7	Tyr(I) 5, 6, 8, 9, OH	3	4.11 (*m*)	61.7	
OH	9.19 (*s*)				4.73 (*m*)		
NH	8.17 (*d*, 8.3)			NH	6.65 (*d*, 8.5)		
Pro(I)				Asp			
1		171.8	Pro(I) 2, 3, Gln NH	1		172.3	Trp 2, NH
2	4.35 (*m*)	59.2	Pro(I) 4	2	4.40 (*br*)	52.0	Asp 3
3	1.86 (*m*)	29.0	Pro(I) 4, 5	3	2.63 (*m*	34.4	
	1.99 (*m*)				2.80 (*dd*, 13.5, 9.1)		
4	1.82 (*m*)	24.5	Pro(I) 5	4		169.2	Asp 3 Thr 3
	1.90 (*m*)			NH	9.00 (*br*)		
5	3.49 (*m*)	46.8		Trp			
	3.62 (*m*)			1		171.4	Trp 2, Glu(I) NH
Gln				2	4.49 (*m*)	53.9	Trp 3
1		171.75	Gln 2, 3, Thr NH	3	3.21 (*m*)	25.7	Trp 2
2	4.37 (*m*)	51.9	Gln 3, 4, NH	1′	10.90 (*s*)		
3	1.76 (*m*)	27.6	Gln 2, 4, NH	2′	7.21 (*d*, 2.6)	123.5	Trp 3, 1′
	1.93 (*m*)			3′		109.1	Trp 2, 3, 1′, 2′, 4′
4	2.17 (*m*)	31.3	Gln 2, 3, NH_2	4′	7.41 (*d*, 7.9)	118.1	Trp 6′
5		174.0	Gln 3, 4, NH_2	5′	6.97 (*d*, 8.3)	118.5	Trp 7′
NH	8.20 (*d*, 7.7)			6′	7.05 (*m*)	121.2	Trp 4′ 5′
NH_2	6.81 (*d*, 2.4)			7′	7.26 (*m*)	111.6	TrP 4′ 5′
	7.20 (*d*, 2.4)			8′		136.2	Trp 3, 2′, 4′, 5′, 7′
Thr				9′		127.3	Trp 1′, 2′, 4′, 6′
1		168.8	Thr 2, Leu 2, NH	NH	7.34 (*br*)		
2	4.56 (*dd*, 8.7, 1.8)	54.5	Thr 4	Glu(I)			
3	5.36 (*qd*, 6.6, 1.8)	71.2	Thr 4	1		169.86	Glu(II) NH
4	1.12 (*d*, 6.6)	16.9	Thr 3	2	3.96 (*m*)	53.2	
NH	7.52 (*d*, 8.7)			3	1.50 (*m*)	24.2	
Leu					1.81 (*m*)		
1		170.08	Leu 2, 3, Lys NH	4	1.05 (*m*)	29.0	Glu(I) 3
2	4.24 (*m*)	50.8	Leu 3, Thr NH		2.05 (*m*)		
3	1.32 (*m*)	40.4	Leu 4, 5, 5′	5			
	1.55 (*m*)			NH	6.50 (*d*, 6.9)		
4	1.43 (*m*)	24.1	Leu 3, 5, 5′	Glu(II)			
5	0.76 (*d*, 6.6)	21.7	Leu 3, 4, 5′	1		170.9	Tyr(III) 2, NH
5′	0.82 (*d*, 6.6)	23.0	Leu 3, 4, 5	2	4.10 (*m*)	51.9	
NH	8.41 (*d*, 8.5)			3	1.52 (*m*)	28.3	Glu(II) 4
Lys					2.03 (*m*)		
1		169.9	Lys 2, Tyr(I) NH	4	1.89 (*m*)	31.9	Glu(II) 2
2	4.10 (*m*)	52.5	Lys 3, NH		2.11 (*m*)		
3	1.54 (*m*)	32.0	Lys 2, 4, 5	5		171.2	Glu(II) 4, Lys εNH
4	1.19 (*m*)	21.2	Lys 2, 3, 5	NH	7.18 (*m*)		
5	1.28 (*m*)	28.3		Tyr(III)			
	1.40 (*m*)			1		172.7	Tyr(III) 2, 3
6	2.95 (*m*)	37.4	Lys 5	2	4.26 (*dd*, 8.2, 2.7)	53.9	Tyr(III) 3, NH
	3.20 (*m*)			3	2.79 (*dd*, 14.0, 8.2)	35.9	Tyr(III) 2, 5, 9
αNH	6.70 (*d*, 7.2)				2.90 (*dd*, 14.0, 5.2)		
εNH	6.90 (*br*)			4		127.4	Tyr(III) 2, 3, 6, 8
Tyr(II)				5, 9	7.01 (*d*, 8.5)	130.0	Tyr(III) 3
1		170.5	Tyr(II) 2, Pro(II) 2	6, 8	6.63 (*d*, 8.5)	114.9	Tyr(III) 5, 9, OH
2	4.34 (*m*)	51.7	Tyr(II) 3	7		155.9	Tyr(III) 5, 6, 8, 9
3	2.67 (*m*)	37.3	Tyr(II) 2, 5, 9	OH	9.18 (*s*)		
	2.77 (*m*)			NH	7.81 (*d*, 7.7)		
4		126.4	Tyr(II) 3, 6, 9				
5, 9	6.94 (*d*, 8.7)	130.1	Tyr(II) 3				
6, 8	6.65 (*d*, 8.7)	115.0	Tyr(II) OH				
7		156.2	Tyr(II) 5, 6, 8, 9, OH				
OH	9.28 (*s*)						
NH	8.39 (*d*, 7.5)						

Table 3. Elastase inhibitory activity of all microviridins with IC_{50} values ($\mu g\ ml^{-1}$)

Microviridin G (**1**)	0.019	Microviridin C (**5**)	0.084
Microviridin H (**2**)	0.031	Microviridin D (**6**)	0.7
Microviridin A (**3**)	—	Microviridin E (**7**)	0.6
Microviridin B (**4**)	0.044	Microviridin F (**8**)	5.8

Microviridin G (**1**). $[\alpha]_D^{20}$ −7.3° (MeOH; *c* 0.19); FAB-MS *m/z* 1806 $[M+H]^+$; HRFAB-MS *m/z* 1804.7771 $[M-H]^-$ calcd for $C_{88}H_{111}O_{25}N_{17}$ (Δ −8.8 mmu); UV λ_{max}^{MeOH} nm (log ε): 221 (4.67), 280 (3.73).

Microviridin H (**2**). $[\alpha]_D^{20}$ −20.5° (MeOH; *c* 0.36); FAB-MS *m/z* 1838 $[M+H]^+$; HRFAB-MS *m/z* 1836.8088 $[M-H]^-$ calcd for $C_{89}H_{115}O_{26}N_{17}$ (Δ −3.3 mmu); UV λ_{max}^{MeOH} nm (log ε): 221 (4.67), 280 (3.85).

Amino acid analyses. Compounds **1** and **2** (100 μg each) were hydrolysed with 0.5 ml of 6 M HCl and sealed in vials at 110° for 16 hr. The soln was evapd and redissolved in 0.02 M HCl for sepn on a Hitachi L-8500A amino acid analyser with ninhydrin detection.

Chiral GC analyses of animo acids. The hydrolysates of **1** and **2** by 6 M HCl at 110° for 16 hr were heated in 10% HCl in MeOH (0.5 ml) at 100° for 30 min and then treated with $(CF_3CO)_2O$–CH_2Cl_2 (1:1, 0.6 ml) for 5 min. The GC-MS analyses were carried out by using a Chirasil-L-Val capillary column with a flame ionization detector (FID). Column temp. was kept at 50° for 5 min and increased to 200° at a rate of 4° min^{-1}. He was used as a carrier gas. Retention times (min): D-Thr (10.0), L-Thr (11.8), D-Pro (12.6), L-Pro (12.9), D-Leu (14.2), D-Ser (14.3), L-Leu (15.9), D-Asp (17.4), L-Asp (17.8), L-Ser (18.4), D-Glu (22.5), L-Glu (23.6), D-Tyr (30.2), L-Tyr (31.1), D-Lys (35.2), L-Lys (36.5).

For Trp, the hydrolysates of **1** and **2** by 6 M HCl containing 1% HCO_2H at 110° for 16 hr were treated as described above. The GC-MS analyses also were carried out by using a Chirasil-L-Val capillary column. Column temp. was kept at 100° for 5 min and increased to 200° at a rate of 4° min^{-1}. He was used as a carrier gas. Retention times (min): D-Trp (19.4), L-Trp (21.5).

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REFERENCES

1. Hori, K., Ishibashi, G. and Okita, T., *Plant Foods and Human Nutrition*, 1994, **45**, 63.
2. de Cano, M. S., de Mulé, M. G. Z., de Caire, G. Z. and de Halperin, D. R., *Journal of Applied Phycology*, 1990, **2**, 79.
3. Sivonen, K., Carmichael, W. W., Namikoshi, M., Rinehart, K. L., Dahlem, A. M. and Niemela, S. I., *Applied Environmental Microbiology*, 1990, **59**, 2650.
4. Moore, B. S., Chen, J.-L., Patterson, G. M. L. and Moore, R. E., *Journal of the American Chemical Society*, 1990, **112**, 4061.
5. Trimurtulu, G., Ohtani, I., Patterson, G. M. L., Moore, R. E., Corbett, T. H., Valeriote, F. A. and Demchik, L., *Journal of the American Chemical Society*, 1994, **116**, 4729.
6. Namikoshi, M., Rinehart, K. L. and Sakai, R., *Journal of Organic Chemistry*, 1990, **55**, 6135.
7. Bax, A. and Subramamian, S., *Journal of Magnetic Resonance*, 1986, **67**, 565.
8. Bax, A. and Summers, M. F., *Journal of the American Chemical Society*, 1986, **108**, 2093.
9. Ishitsuka, M. O., Kusumi, T., Kakisawa, H., Kaya, K. and Watanabe, M. M., *Journal of the American Chemical Society*, 1990, **112**, 8180.
10. Okino, T., Matsuda, H., Murakami, M. and Yamaguchi, K., *Tetrahedron*, 1995, **51**, 10679.
11. Shin, H. J., Murakami, M., Matsuda, H. and Yamaguchi, K., *Tetrahedron*, 1996, **52**, 8159.
12. Watanabe, M. M. and Satake, K. N., *NIES-Collection List of Strains*. National Institute of Environmental Studies, Tsukuba, 1994, p. 30.