Heptaibin, a Novel Antifungal Peptaibol Antibiotic from *Emericellopsis* sp. BAUA8289

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In the course of our antifungal screening program, a novel antifungal substance, heptaibin (**1**, Fig. 1) was isolated from the culture of *Emericellopsis* sp. BAUA8289 together with emerimicin IV[1] (**2**, Fig. 1), a peptaibol antibiotic. The structure of **1** was determined by spectroscopic studies. Compound **1** was an analog of emerimicin IV, in which the isovaline residue is replaced with an α-amino-isobutyric acid. In this paper, we report the fermentation, isolation, structure determination and biological activities of **1**.

Heptaibin is produced by *Emericellopsis* sp. BAUA8289, a fungus isolated from the root of a rice plant growing in Akita, Japan.

A slant culture of fungal strain BAUA8289 was inoculated into 5 test tubes (25 mm) containing 10 ml of a culture medium consisting of soluble starch 2.0%, glucose 1%, soybean flour 1.5%, malt extract 0.5%, Mg_2SO_4 0.05%, KH_2PO_4 0.05%, V8 vegetable juice 10% (v/v), potato dextrose 10% (v/v). The test tubes were shaken on a reciprocal shaker (250 rpm) at 25°C for 72 hours. Half ml of this seed culture was transferred into each of 100 test tubes (30 mm) each contained 10 g of a pressed barley medium containing 0.5% malt extract. The inoculated test tubes were incubated stationary for 10 days at 25°C.

The solid culture mass (1 kg) was extracted with MeOH (1000 ml). After filtration, the extract was concentrated. The residue (50 ml) was extracted with EtOAc (50 ml) three times. The EtOAc layer was concentrated. The residue (652 mg) was dissolved in MeOH, applied to preparative HPLC (CAPCELL PAK C18 UG120 4.6×100 mm, 43% MeCN), and two colorless crystalline materials, **1** (14.1 mg) and **2** (28.6 mg) were obtained.

The structure determination of **1** was performed by comparison with **2**. In the FAB-MS spectrum of **1**, the peak of *m/z* 1581 $(M+Na)^+$ was observed. The molecular weight of **2** was suggested to be 1558. This molecular weight was 14 mass less than that of **2**. The fragmentation pattern of FAB-MS spectrum of **1** was similar to that of **2**. Peaks of *m/z* 884, 799, 714, 601, 544, 445, 360, 275, 190 were observed in both spectra. However, a peak of *m/z* 1210 was observed in **1** instead of *m/z* 1224 in **2**. The amino acid sequence from b9 to b12 in **2** was Hyp (4-hydroxyproline)-Gln-Iva (isovaline). These results indicated that one of the amino acid between b9 and b12 in **2** is replaced with another amino acid in **1**.

In the ESI-MS spectra of **1** and **2**, the fragment peaks was obtained at *m/z* 676 and 690, respectively. Since the peak of *m/z* 690 in **2** was assigned to fragment y-6, MS/MS measurement targeting these fragments were performed. In the spectrum of **2**, the fragments were observed at *m/z* 539, 454, 341, 242, 350 and 152, which were assigned to b14, b13, b12, b11, y3, y1, respectively. The mass difference from b12 to b11 was 99 indicating Iva. Others, in the spectrum of **1**, the fragments were observed at *m/z* 525, 440, 327, 242, 350 and 152. The mass difference 85 from b12 to b11 indicates Aib (α-aminoisobutyric acid).

Fig. 1. Structure of heptaibin (**1**) and emerimicin IV (**2**).

1 Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Aib-Hyp-Aib-Phol

2 Ac-Phe-Aib-Aib-Aib-Val-Gly-Leu-Aib-Aib-Hyp-Gln-Iva-Hyp-Aib-Phol

Ac : acetyl, Aib : α-aminoisobutyric acid, Iva : isovaline, Phol : phenylalaninol
Hyp : 4-hydroxyproline,

Fig. 2. FAB-MS spectra of **1** and **2**.

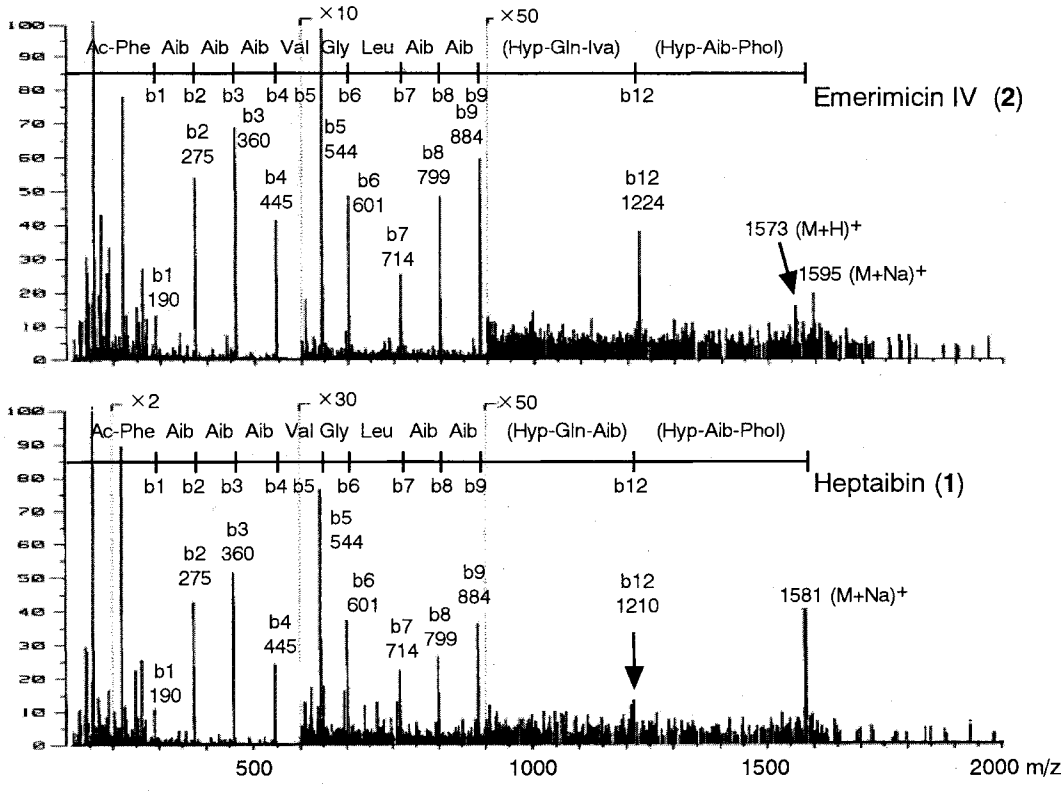

Fig. 3. ESI-MS/MS spectra for fragments of **1** and **2**.

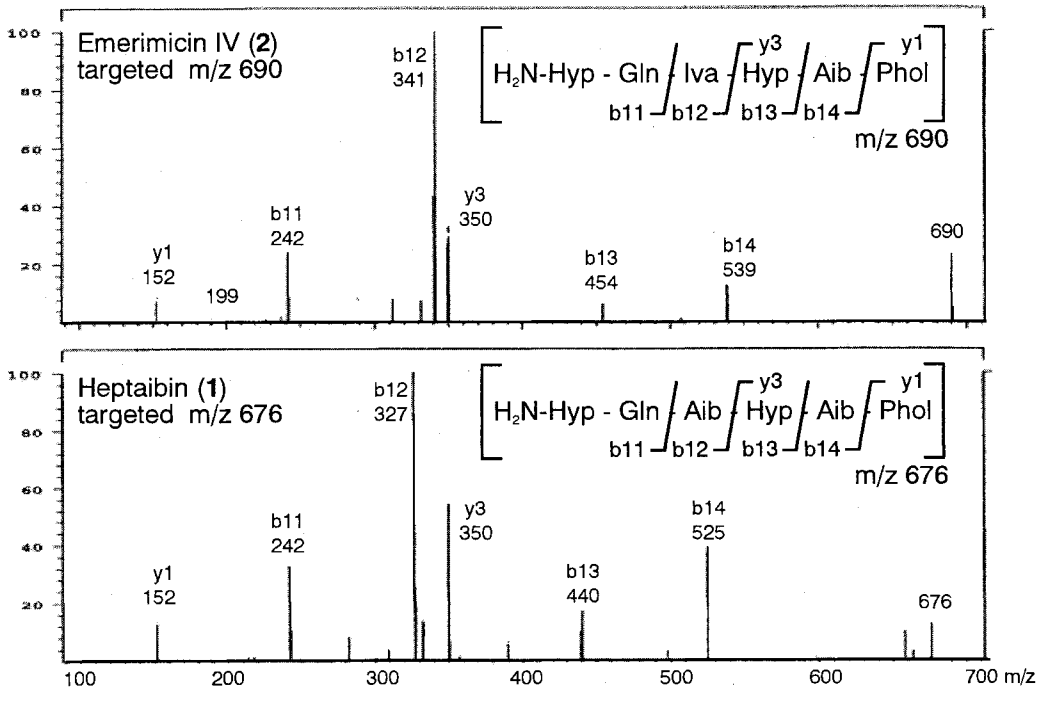

Table 1. ^{1}H and ^{13}C-NMR data of emerimicin IV and heptaibin in CD_3OH.

Position		Emerimicin IV* δ ^{13}C ppm	Emerimicin IV* δ ^{1}H ppm (multiplicity, J	Heptaibin δ ^{13}C ppm	Heptaibin δ ^{1}H ppm (multiplicity, J)
Ac	C=O	174.01		173.99 [a]	
	Me	22.56	1.99 (s)	22.51	1.99 (s)
Phe	NH		8.23 (brs)		8.33 (brs)[a]
	C=O	174.01		173.98 [a]	
	α	57.59	4.44 (m)	57.61	4.43 (m)
	β	38.11 [a]	3.11 (dd, 13.7, 7.6)	38.02 [b]	3.10 (dd, 14.0, 7.9)
			3.01 (dd, 13.7, 7.6)		3.00 (dd, 14.0, 7.9)
	Ph	127-141	7.2-7.4 (m)	127-141	7.2-7.4 (m)
Aib-1	NH		8.19 (s)		8.31 (brs)[a]
	C=O	177.26		177.40 [c]	
	α	57.95 [b]		57.91	
	Me	26.04	1.36 (s)	24.57	1.37 (s)
	Me	24.59	1.38 (s)	25.95	1.37 (s)
Aib-2	NH		7.651 (s)		7.71 (s)
	C=O	177.47		177.28 [c]	
	α	57.76		57.69	
	Me	23.51	1.41 (s)	26.86 [e]	1.39 (s)
	Me	26.91	1.39 (s)	23.45 [d]	1.40 (s)
Aib-3	NH		7.89 (s)		7.93 (s)
	C=O	179.22		179.20	
	α	57.89 [b]		57.81	
	Me	27.48	1.51 (s)[a]	23.62 [d]	1.50 (s)
	Me	23.82 [c]	1.51 (s)[a]	27.42	1.51 (s)
Val	NH		7.68 (d, 6.10)		7.70 (d, 8.6)
	C=O	176.09		176.10	
	α	64.39	3.71 (dd, 8.9, 6.1)	64.38	3.69 (dd, 8.6, 6.1)
	β	30.70	2.22 (m)	30.65	2.22 (m)
	γ	19.73	1.01 (d, 6.7)	20.69	0.99 (d, 6.71)
		20.66	1.01 (d, 6.7)	19.73	0.995 (d, 6.7)
Gly	NH		8.13 (t, 5.8)		8.17 (t, 5.7)
	C=O	173.43		173.43	
	α	45.50	3.88 (dd, 16.2, 5.8)	45.46	3.86 (dd, 15.9, 5.7)
			3.76 (dd, 16.2, 5.8)		3.76 (dd, 15.9, 5.7)
Leu	NH		7.804 (d, 5.5)		7.85 (d, 5.5)
	C=O	175.41 [d]		175.40 [f]	
	α	56.01	4.25 (m)	55.91	4.24 (m)
	β	41.19	1.75 (m), 1.64 (m)	41.11	1.64 (m), 1.75 (m)
	γ	25.94	1.77 (m)	25.88	1.76 (m)
	δ	23.11	0.92 (d, 6.10)	21.93	0.91 (d, 6.1)
		21.95	0.94 (d, 6.10)	23.09	0.94 (d, 6.1)
Aib-4	NH		7.57 (s)		7.62 (s)
	C=O	178.14		178.09	
	α	58.29		58.25	
	Me	23.66	1.55 (s)	27.88 [e]	1.53 (s)
	Me	27.92	1.51 (s)[a]	23.71 [d]	1.55 (s)

* Measured at 40°C.

a-f) May be interchangeable in column.

TMS was used as internal refernce.

Table 1. (Continued)

		Emerimicin IV*		Heptaibin	
Position		δ ^{13}C ppm	δ ^{1}H ppm (multiplicity, *J*	δ ^{13}C ppm	δ ^{1}H ppm (multiplicity, *J*)
Aib-5	NH		7.88 (d)		7.92 (s)
	C=O	176.19		176.24	
	α	58.43		58.35	
	Me	24.22	1.61 (s)	24.17	1.60 (s)
	Me	26.60	1.51 (s)[a]	26.31	1.50 (s)
Hyp-1	C=O	175.44 [d]		175.44 [f]	
	α	62.87	4.60 (t, 8.9)	62.93	4.59 (t, 9.0)
	β	37.99 [a]	1.94 (m), 2.38 (m)	37.95 [b]	2.35 (m), 1.94(m)
	γ	71.55 [e]	4.50 (brs)	71.48	4.51 (brs)
	δ	58.36	3.97 (d, 12.8)	58.39	3.95 (d, 12.5)
			3.78 (d, 12.8)		3.76 (d, 12.5)
Gln	NH		8.26 (d, 8.9)		8.23 (d, 8.9)
	C=O	173.83		173.79	
	α	54.73	4.33 (m)	54.21	4.36 (m)
	β	28.34	2.14 (m), 2.38 (m)	27.84	2.12 (m), 2.4 (m)
	γ	33.06	2.4-2.3 (m)	32.80	2.4-2.3 (m)
	δ	177.26		177.43	
	ε		6.64 (brs)		6.74 (s)
			7.39 (brs)		7.49 (s)
	NH		7.60 (s)		7.95 (s)
Iva	C=O	176.37		175.81	
or	α	60.51		57.87	
Aib	β	20.83	1.53 (s)	26.79 [e]	1.52 (s)
		29.84	1.90 (m), 2.27 (m)	24.36	1.61 (s)
	γ	7.95	0.87 (t, 7.5)		
Hyp-2	C=O	174.87		174.86	
	α	63.91	4.42 (dd, 11.0, 7.6)	63.83	4.40 (dd, 10.7, 7.6)
	β	37.85	1.82 (m), 2.28 (m)	37.88 [b]	1.82 (m), 2.27 (m)
	γ	71.47 [e]	4.42 (brs)	71.48	4.45 (brs)
	δ	58.67	3.58 (dd, 12.3, 3.4)	58.56	3.56 (dd, 12.2, 3.1)
			3.85 (d, 12.3)		3.84 (d, 12.2)
Aib-6	NH		7.80 (s)		7.84 (s)
	C=O	177.34		177.28	
	α	58.48		58.45	
	Me	23.78 [d]	1.45 (s)	23.77 [d]	1.45 (s)
	Me	27.92	1.30 (s)	27.84 [e]	1.31 (s)
Phol	NH		7.2-7.3		7.32 (d, 9.5)
	α	54.53	4.15 (m)	54.65	4.12 (m)
	β	38.04 [a]	2.73 (dd, 14.0, 9.2)	38.02 [b]	2.98 (dd, 13.7, 5.2)
			2.98 (dd, 14.0, 5.5)		2.71 (dd, 13.7, 9.5)
	CH_2OH	65.26	3.61 (brs)	65.25	3.61 (t, 5.3)
	Ph	127-141	7.2-7.4 (m)	127-141	7.2-7.4 (m)

* Measured at 40°C.

a-f) May be interchangeable in column.

TMS was used as internal refernce.

Table 2. Antimicrobial activity of heptaibin.

		MIC (μg/ml)	
		1	**2**
Bacteria	*Staphylococcus aureus* FDA209P JC-1	8	4
	Escherichia coli NIH JC-2	>64	>64
	Pseudomonas aeruginosa PAO-1	>64	>64
Fungi	*Aspergillus fumigatus* TIMM 0069	13	>64
	Candida albicans ATCC 90018	32	16
	Cryptococcus neoformans ATCC 10259	32	16

Therefore, it was suggested that the structure of **1** is that of **2** where Iva is replaced with Aib.

This structure was confirmed by NMR measurement (Table 1). In addition, Val, Leu, Glu, Phe and Phol in **1** were determined to be L and Hyp was determined to be L-4-*trans* by MARFEY's method[2].

The antimicrobial activity of **1** is summarized in Table 2. **1** showed the growth inhibition against Gram-positive bacteria and fungi, however, did not inhibit Gram-negative bacteria at 64 μg/ml. These activities were comparable to that of **2**. Moreover, **1** showed a moderate activity against the protozoa (*Rhabditella pseudoelongata*) at 50 μg/ml.

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