Chinese Chemical Letters Vol. 14, No. 12, pp 1255 – 1258, 2003 1255
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Bandunamide, a Novel Cyclopeptide from the Streptomyces *Griseovariabilis bandungensis*

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Abstract: A new cyclic octapeptide, bandunamide, was isolated from the acetone extracts of streptomyces *griseovariabilis bandungensis*. This cyclic octapeptide exhibits strong antimicrobial activity against *Phytophthora drechsleri* (IC_{50}=15 ng/mL), *Colletotrchum higginsiannum* (IC_{50}=15.6 ng/mL), *Piricularia oxyzae* (IC_{50}=0.2 μg/mL), and *Fusarium oxysporum f. Sp.* (IC_{50}=100 μg/mL). The structure elucidation of bandunamide is herein reported.

Keywords: Streptomyces, *Griseovariabilis bandungensis*, Bandunamide, structure elucidation, anti-fungus.

Cyclic peptides are a chemical class well known from bacteria and fungi[1, 2]. During our screening for novel bioactive compounds of microbial origin, we have isolated a novel antibiotic named bandunamide, which was a cyclic octapeptide, from the culture broth of streptomyces *griseovariabilis bandungensis*. This compound exhibits strong anti-microbial activity against *Phytophthora drechsleri* (IC_{50}=15 ng/mL), *Colletotrchum higginsiannum* (IC_{50}=15.6 ng/mL), *Piricularia oxyzae* (IC_{50}=0.2 μg/mL), and *Fusarium oxysporum f. Sp.* (IC_{50}=100 μg/mL). We report herein the structural elucidation of bandunamide (**Figure 1**).

The acetone extract (10.0 g. wet weight) of the cultured streptomyces mycelia was subjected to silica gel chromatography, eluting with benzene and acetone. The fraction, eluted with benzene-acetone (2:1), was further chromatographed on flash silica gel column (solvent: chloroform / acetone = 4 / 1) and Sephadex LH-20 column (solvent: methanol / chloroform = 7 / 3) to give 750 mg buff powder, which was purified by preparative reverse-phase HPLC (ODS-2, 30% H_2O-MeOH, at 1.5 mL/min) to give 380 mg pure bandunamide as white powder.

Bandunamide was showed intensive C=O and N-H absorptions at 1700 and 3390 cm^{-1} in IR spectrum which were characteristic for peptide-type natural found products. HR-FABMS analysis showed the molecular formula $C_{53}H_{76}N_{10}O_{10}S_3$ [*m/z*, M^+ calcd. 1108.4417], with unsaturated value 21. The ^{13}C NMR and DEPT spectra

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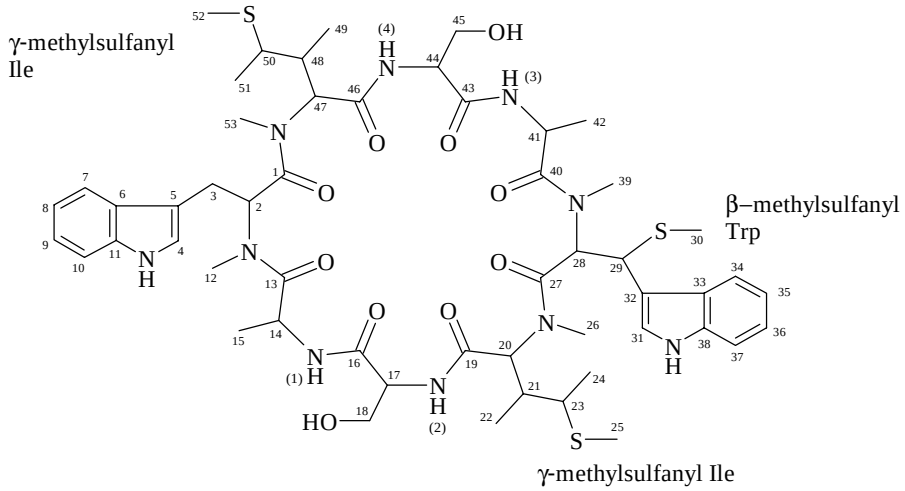
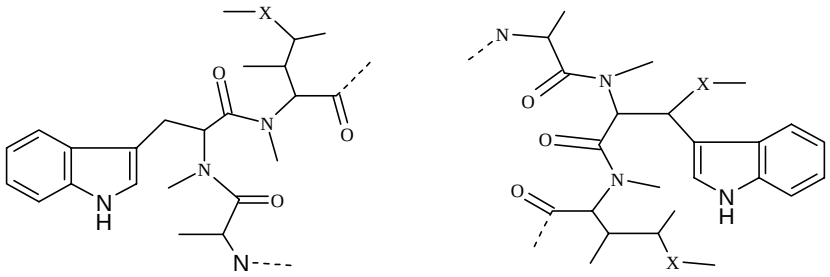
($CDCl_3$) of bandunamide showed thirteen methyls (four of which were N-methyl), one *C*-methylene, and two *O*-methylenes, thirteen sp^3 methine (eight of which are *N*-methine), ten sp^2 methine, six sp^2 quarternary and eight amide-type carbonyl carbons. Taking the molecular formula into account, bandunamide was proposed an octapeptide containing aryl amino acid residues.

Figure 1 Structure of bandunamide



The ^1H NMR spectrum displayed 74 proton signals (see **Table 1**), which suggested the presence of active protons according to the molecular formula data. Four doublet proton signals (δ 8.84, 8.63, 7.00, 6.88) indicated that four of the amino-acid residues contain NH protons. However, the other four amino-acid residues possess the N-CH_3 groups, confirmed by the four CH_3 singlet signals (δ 3.14, 3.06, 2.98 and 2.97). The direct connectivity of proton and carbon atoms was established by the ^1H-^{13}C XHCORR spectrum (**Table 1**).

Figure 2 Structures of the two segments of bandunamide



Integrative analysis of the ^1H-^1H COSY45 spectrum and the ^1H-^{13}C COLOC spectrum revealed the eight partial structures I to VIII, which were identified as residues of two alanines, two serines, one tryptophan, one substituted tryptophan, and two

substituted isoleucine, respectively. The COLOC spectrum showed following relationships: H-53 was related to C-1, C-47; H-12 to C-2, C-13 in the segment A

Table 1 NMR spectral data of bandunamide (in $CDCl_3$, δ, ppm)

No.	δ_C	δ_H	No.	δ_C	δ_H
1	170.3		30	15.2	2.05 (3H, s)
2	53.6	6.09 (1H, m)	31	143.5	9.57 (1H, s)
3	27.3	2.85 (1H, m), 3.40 (1H, m)	32	140.2	
4	143.4	9.57 (1H, s)	33	142.4	
5	140.1		34	129.6	8.14 (1H, m)
6	142.3		35	131.2	7.70 (1H, m)
7	129.5	8.14 (1H, m)	36	132.2	7.80 (1H, m)
8	131.1	7.70 (1H, m)	37	129.5	7.89 (1H, m)
9	132.1	7.80 (1H, m)	38	144.0	
10	129.3	7.89 (1H, m)	39	29.9	2.98 (3H, s)
11	144.0		40	173.3	
12	32.3	2.97 (3H, s)	41	46.3	4.92 (1H, m)
13	173.6		42	18.2	1.38 (3H, d, J=6.8Hz)
14	46.5	4.78 (1H, m)	43	167.3	
15	17.1	1.35 (3H, d, J=6.8Hz)	44	52.4	4.94 (1H, m)
16	167.5		45	64.9	4.55 (1H, m), 4.74 (1H, m)
17	53.6	4.80 (1H, m)	46	171.1	
18	65.1	4.79 (1H, m), 4.69 (1H, m)	47	59.0	5.33 (1H, d, J=10.4Hz)
19	171.5		48	37.5	2.12 (1H, m)
20	59.7	5.39 (1H, d, J=10.8Hz)	49	9.4	0.67 (3H, d, J=7.6Hz)
21	37.3	2.21 (1H, m)	50	28.6	1.75 (1H, m)
22	9.8	0.71 (3H, d, J=7.6Hz)	51	21.7	0.84 (3H, d, J=6.8Hz)
23	28.6	1.65 (1H, m)	52	15.8	0.83 (3H, s)
24	21.7	0.93 (3H, d, J=6.8Hz)	53	31.4	3.14 (3H, s)
25	15.8	0.85 (3H, s)	NH -1		8.84 (1H, d, J=6.0Hz)
26	30.8	3.06 (3H, s)	NH -2		6.88 (1H, d, J=7.6Hz)
27	168.8		NH -3		8.63 (1H, d, J=7.6Hz)
28	59.9	6.45 (1H, d, J=8.8Hz)	NH -4		7.00 (1H, d, J=7.2Hz)
29	51.9	4.89 (1H, d, J=8.4Hz)			

(Figure 2), while H-26 was related to C-20, C-27; H-39 to C-28, C-40 in the segment B.

Accounting above spectral data, molecular weight and degree of unsaturation, the structure of bandunamide was suggested as cyclic peptide with two free carboxylic groups of two serines. Furthermore, in view of the comparability of the chemical shifts

between two alanines, two serines, two substituted isoleucine, tryptophan and substituted tryptophan, we concluded that the molecular of bandunamide must have an approximative C_2 symmetry. Similar results have been found in the litereture[3, 4]. Consequently, the entire structure of the cyclic octapeptide is determined.

The cyclic peptides also can be found from other species of streptomyces[5, 6].

Acknowledgment

This work was supported by NNSFC Grant 39870903 and Guangdong Provincial Scientific Grant 980726

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Received 18 November, 2002