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Mechercharmycins A and B, Cytotoxic Substances from Marine-derived *Thermoactinomyces* sp. YM3-251

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Abstract A new cytotoxic substance named mechercharmycin A was isolated from marine-derived *Thermoactinomyces* sp. YM3-251. The structure of mechercharmycin A was determined by an X-ray crystallographic analysis to be cyclic peptide-like and bearing four oxazoles and a thiazole. Mechercharmycin B, a linear congener of mechercharmycin A, was also isolated from the same bacterium. Mechercharmycin A exhibited relatively strong antitumor activity, whereas mechercharmycin B exhibited almost no such activity.

Keywords cytotoxic, marine-derived *Thermoactinomyces*, cyclic peptide

Marine microorganisms have been recognized as a promising source for the development of new pharmaceuticals [1]. In the course of screening for antitumor substances from marine-derived microorganisms, we found the cyclic peptide-like compound bearing four oxazoles and a thiazol, mechercharmycin A (**1**) [2], and its linear congener, mechercharmycin B (**2**). The strain producing **1** and **2**, *Thermoactinomyces* sp. YM3-251 [3], was isolated from sea mud collected at Mecherchar in the Republic of Palau (North Pacific Ocean). The producing strain was cultured in a B2 medium [4] at 30°C with rotary shaking at 100 rpm for 7 days. The cultured broth was centrifuged. The resulting precipitate was extracted by chloroform/methanol (9 : 1), and the supernatant was extracted by ethyl acetate. Both these extracts were

Table 1 Physico-chemical properties of **1** and **2**

	1	**2**
Appearance	White powder	White powder
Molecular formula	$C_{35}H_{32}N_8O_7S$	$C_{35}H_{36}N_8O_{10}$
Molecular weight	708	728
HRFAB-MS (*m/z*)		
Found	709.2104 $(M+H)^+$	729.2612 $(M+H)^+$
Calcd.	709.2193	729.2633
$[\alpha]_D^{25}$	+110° (*c* 0.038, DMSO)	+56° (*c* 0.056, DMSO)
UV λ_{max} nm (log ε) in MeOH	223 (4.71), 260 (4.73), 300 (sh)	220 (4.65), 261 (4.62), 310 (sh)
IR ν_{max} (KBr) cm^{-1}	3400, 2962, 2925, 1655, 1543, 1509	3400, 2964, 2927, 1743, 1655, 1543, 1509

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combined, and the mixture was chromatographed on a silica gel column by step-wise elution with chloroform/methanol (1～6% methanol). Antitumor activity was observed in the 4% methanol-chloroform fraction, and this fraction was further separated by HPLC. Finally, 7 mg of **1** was obtained from a 200-liter culture, and 3 mg of **2** was also obtained from the same fraction. The physico-chemical properties of **1** and **2** are summarized in Table 1, and the NMR data are presented in Table 2.

Table 2 ^{1}H and ^{13}C NMR data of **1** and **2** in DMSO-d_6 (750 MHz for ^{1}H and 125 MHz for ^{13}C)

Position	Mechercharmycin A (**1**)		Mechercharmycin B (**2**)	
	^{13}C	^{1}H mult., *J* (Hz)	^{13}C	^{1}H mult., *J* (Hz)
1	157.14		161.22	
2	135.50		137.12	
3	139.32	9.06 s	141.95	8.65 s
4	154.63		153.79	
5	129.53*		129.83	
6	140.27	8.95 s	140.70	8.85 s
7	158.78		158.86	
8	128.54		128.79	
9	109.31	5.79 s, 5.92 s	108.22	5.71 s, 5.94 s
10	170.71		170.89	
11	58.14	4.78 t, 8.6	58.12	4.48 dd, 8.1, 7.1
12	32.05	2.05 m	30.32	2.13 m
13	19.13	0.95 d, 6.8	18.00	0.94 d, 6.8
14	18.55	1.00 d, 6.8	19.18	0.96 d, 6.8
15	170.08		170.76	
16	56.92	4.65 dd, 6.6, 3.9	55.53	4.75 dd, 9.1, 5.3
17	38.66	2.07 m	37.90	1.96 m
18	14.35	0.89 d, 6.8	14.29	0.90 d, 6.8
19	26.07	1.13 m, 1.64 m	25.99	1.18 m, 1.48 m
20	12.01	0.93 t, 7.5	11.46	0.93 t, 7.5
21	159.90		159.67	
22	130.59		129.70	
23	150.35		151.25	
24	126.41		126.16	
25	127.15	8.31 d, 7.5	127.76	8.25 d, 7.5
26	128.48	7.55 t, 7.5	128.30	7.51 m
27	129.90	7.50 t, 7.5	130.07	7.51 m
28	128.48	7.55 t, 7.5	128.30	7.51 m
29	127.15	8.31 d, 7.5	127.76	8.25 d, 7.5
30	152.00		151.11	
31	129.57*		130.16	
32	140.22	9.09 s	143.62	9.28 s
33	157.35		152.39	
34	141.31		154.86	
35	121.37	8.50 s	53.24	3.96 s
N1 (NH_2)				7.51 bs, 7.63 bs
N4		9.95 s		9.81 s
N5		8.77 d, 8.6		8.53d, 8.1
N6		8.49 d, 6.6		7.99 d, 9.1

* Interchangeable

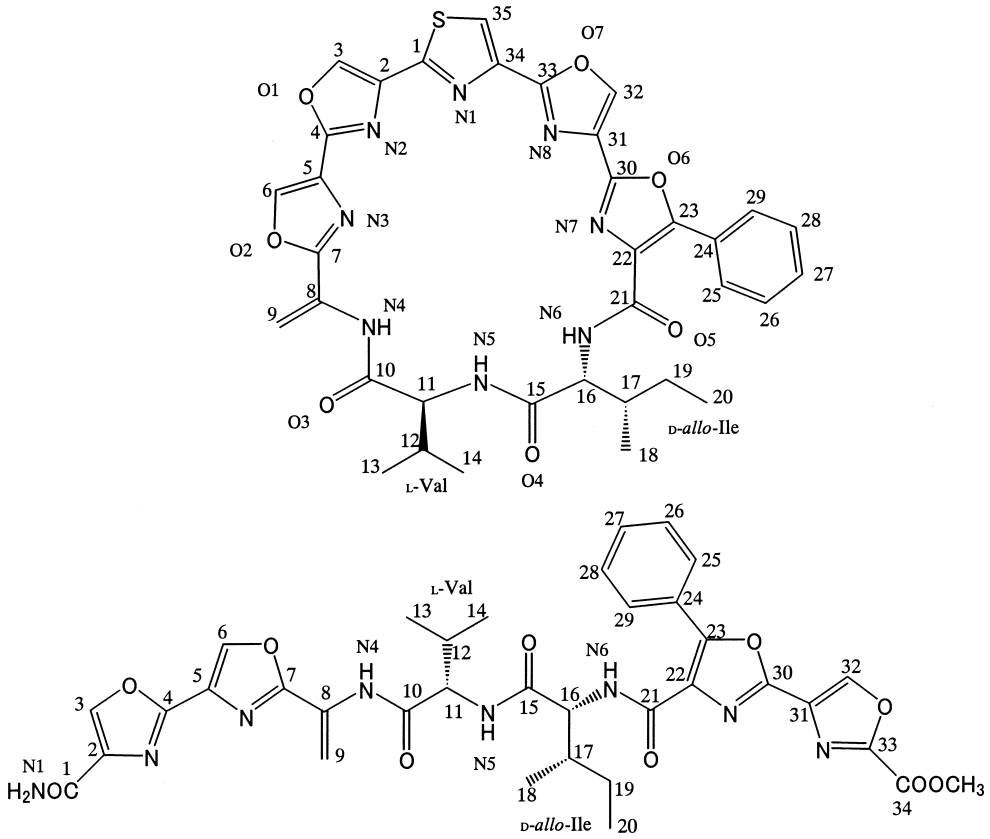

Fig. 1 Structures of **1** (upper) and **2** (lower).

The molecular formula of **1** was determined to be $C_{35}H_{32}N_8O_7S$ from its HRFAB-MS data (Table 1). The peptide moiety was composed of a dehydroalanine fragment, valine and isoleucine as determined from the 2D NMR data, including COSY, TOCSY, HSQC and HMBC. The remaining structure, which was presumed to contain four consecutive oxazoles and a thiazole from an analysis of the chemical shift of ^{1}H and ^{13}C NMR and HMBC signals from aromatic protons to quaternary aromatic carbons, could not be fully determined by the NMR studies, because there was no correlation signal by HMBC between the oxazoles, as in the case of telomestatin [5]. Consequently, the structure of **1** was determined by an X-ray crystallographic analysis. The applicable crystal was obtained by crystallization from dichloromethane/methanol (1 : 1). The crystal data and measurement conditions are summarized later [6], and the ORTEP drawing shown in Figure 2. The absolute configuration of **1** was determined from an X-ray anomalous dispersion of the S atom as shown in Figure 1.

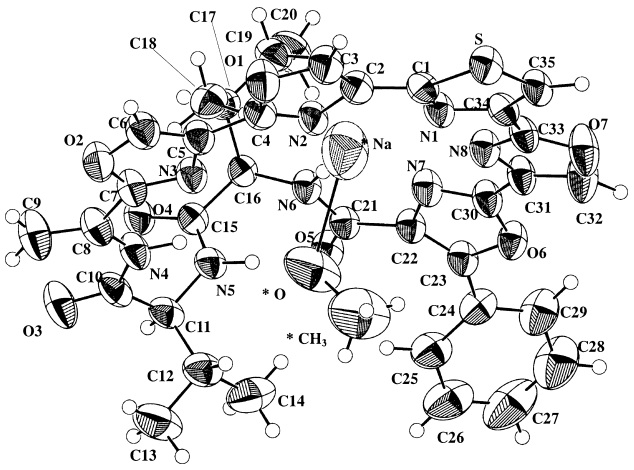

Fig. 2 Crystal structure of **1**. * $NaOCH_3$ in the crystal.

The molecular formula of **2** was determined to be $C_{35}H_{36}N_8O_{10}$ from the HRFAB-MS data (Table 1). The peptide moiety of **2** was determined by NMR studies, this partial structure being the same as that of **1**. The remaining partial structures of **2**, involving four oxazole ring structures: an oxazole, a 5-phenyloxazole, an oxazole-2-carboxylic acid methyl ester, and an oxazole-4-carboxylic acid amide, were determined from a detailed analysis of the NMR data. The respective connectivity of the oxazole and

5-phenyloxazole was determined by the HMBC signals from H9 to C7 and from N6-H to C22. The connectivity of the oxazole-2-carboxylic acid methyl ester and oxazole-4-carboxylic acid amide was not determined by the NMR studies, but instead by a fragment analysis of the LC-MS/MS data, enabling the complete structure of **2** to be determined as shown in Figure 1. The absolute stereochemistry of **2** was determined by the Marfey's method [7] and a chiral HPLC analysis.

The cytotoxic activity of **1** was relatively strong, the IC_{50} value for A549 cells (human lung cancer) being 4.0×10^{-8} M and for Jurkat cells (human leukemia) being 4.6×10^{-8} M under our assay conditions [8], while **2** did not exhibit any inhibitory activity toward either cells even at 1×10^{-6} M. The cyclic structure of **1** must have been essential for its strong antitumor activity. A further investigation of the antitumor potential of **1** is in progress.

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3. The strain producing **1** and **2**, YM3-251, has been identified as *Thermoactinomyces* sp. by taxonomic studies, including a 16S rDNA sequence analysis. The strain has been deposited in the NITE Patent Microorganisms Depositary (NPMD) in Japan as NITE P-2.
4. The B2 medium (1 liter) contained 5 g of peptone, 1 g of yeast extract, 0.1 g of iron (III) citrate *n*-hydrate, 750 ml of filtered natural sea water, and 250 ml of distilled water (pH 7.6, before sterilization).
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6. The crystal used for the X-ray crystallographic analysis contained sodium methoxide. Crystal data for **1**: $C_{36}H_{35}N_8NaO_8S$, colorless cube, M_r=762.768, triclinic $P\bar{1}$, a=11.7380(8) Å, b=12.9590(11) Å, c=13.1900(10) Å, α=89.999(6)°, β=90.062(7)°, γ=110.351(3)°, V=1881.1(2) Å^3, Z=2, ρ_{calcd}=1.322 mg m^{-3}, μ=0.158 mm^{-1}, T=298 K, 9879 measured reflections, 9879 independent reflections, 973 parameters, GOF=1.005, $R1(wR2)$=0.0495(0.1367). The measurements were carried out on Mac Science (Bruker Nonius) dip image plate diffractometer using graphite-monochromated MoK_α radiation (λ=071073Å). The crystal structure was solved by the direct method with SIR-97. Refinement was performed by a full matrix least squares refinement on F^2 with SHELXL-97. CCDC 262340 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.
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8. A549 cells were cultured in a DMEM medium containing 10% fetal bovine serum. The cells were seeded in a 96-well microplate (4,000 cells/200 ml/well) and then cultured in a CO_2 incubator (5% CO_2 - air, 37°C) for 14 hours. Serially diluted samples were added to each well, and the cells were cultured for a further 48 hours. The cell number was counted by the Alamar Blue^{TM} method, and the IC_{50} value was determined from three independent experiments. Jurkat cells were cultured in an RPMI 1640 medium containing 10% fetal bovine serum. The IC_{50} value for the Jurkat cells was determined by the same procedure as that used for the A549 cells. The IC_{50} value of staurosporine for the A549 cells was 3.5×10^{-8} M, and for the Jurkat cells was 1.5×10^{-8} M.