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Arenastatin A, a Potent Cytotoxic Depsipeptide from the Okinawan Marine Sponge *Dysidea arenaria*

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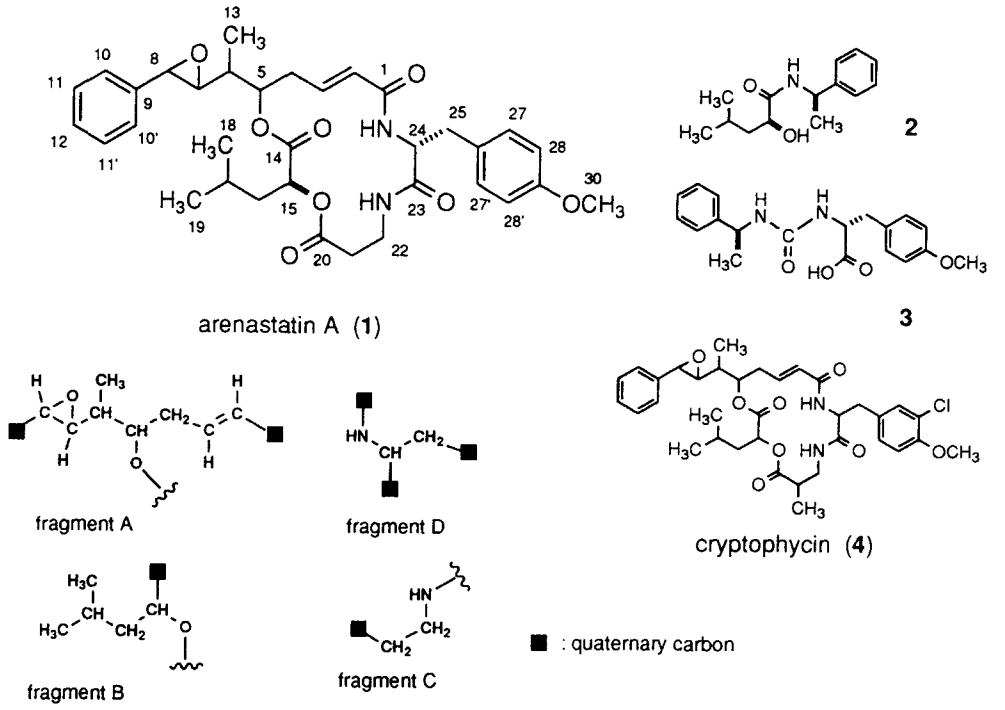
Abstract: Arenastatin A (1) has been isolated from the Okinawan marine sponge *Dysidea arenaria* and the chemical structure including parts of the absolute configurations elucidated. Arenastatin A (1) is a cyclic didepsipeptide and exhibited extremely potent cytotoxicity against KB cells at IC_{50} 5 pg/ml.

In our continuing studies of searching for new bioactive substances from marine organisms,[1] we have isolated a very potent cytotoxic didepsipeptide named arenastatin A from the Okinawan marine sponge *Dysidea arenaria*. This paper describes the structure elucidation.

An acetone extract of the titled fresh sponge (6.5 kg collected in July at Iriomote-jima, Okinawa Prefecture), which exhibited cytotoxicity of IC_{50} 3.7 μg/ml against KB cells, was subjected to bioassay-guided separation (cytotoxicities against KB cells). The extract was partitioned into a water-AcOEt mixture to provide the cytotoxic AcOEt soluble portion (68 g). Repeated SiO_2 column chromatography ($CHCl_3$-MeOH, *n*-hexane-AcOEt, *n*-hexane-acetone) of the AcOEt soluble portion furnished the active fraction (1.1 g)[93% inhibition at 0.01 μg/ml (KB)]. The active fraction was further separated by HPLC (Cosmosil $5C_{18}$-AR, MeOH-H_2O, CH_3CN-H_2O-CH_2Cl_2) to provide arenastatin A (1)(1 mg)(1.4×10^{-3}% from the AcOEt soluble portion), as the most potent cytotoxic compound (IC_{50} 5 pg/ml against KB cell line).

Arenastatin A (1) was obtained as an amorphous solid: $[\alpha]_D$ +19° (*c*=0.15, MeOH); UV λ_{max} (MeOH): 285 nm (ε=1900), 270 (2400), 230 (12500); IR (KBr): 3308, 1736, 1671, 1248 cm^{-1}. The FAB MS of 1 showed a quasi-molecular ion at *m/z* 607 $(M+H)^+$ and the molecular formula was determined as $C_{34}H_{42}N_2O_8$ by HR-FABMS and NMR analysis.

The ^1H- and ^{13}C-NMR data (Table I) showed the presence of three secondary methyls, one methoxyl, one *para*-substituted phenyl, one phenyl, one *trans*-disubstituted olefin, and four oxymethine groups. The ^1H-^1H COSY spectrum of 1 revealed the presence of four partial structures (fragment A: C-2∼C-8 with C-13, fragment B: C-15∼C-19, fragment C: C-21∼C-22-NH, and fragment D: NH-C-24∼C-25 together with a



phenyl and a *para*-substituted phenyl groups. The presence of these partial structures have also been substantiated by the HOHAHA experiment of **1**. The connectivity of these partial structures has been figured out on the basis of following HMBC correlations: 1) adjacency of fragment A and phenyl group: cross peaks between C-8 and H-10; C-9, C-10 and H-8, 2) adjacency of fragments A and B (2-hydroxy-4-methylpentanoyl moiety): cross peaks between C-14 and H-5, H-15, H_2-16, 3) adjacency of fragments B and C: cross peaks between C-20 and H-15, H_a-21, 4) adjacency of fragments C and D: cross peaks between C-23 and NH-22, H-24, H_2-25, 5) adjacency of fragments D and A: cross peaks between C-1 and H-2, H-3, NH-24, H-24, 6) *O*-methyltyrosine moiety: cross peaks between C-26 and H-24, H_2-25, H-27, H-28; C-29 and H-27, H-28, H_3-30. Based on the accumulated evidence, the plane structure of arenastatin A has been elucidated to be a cyclic didepsipeptide shown as **1**.

The absolute configuration of the 2-hydroxy-4-methylpentanoyl moiety has been determined by HPLC analysis of its α-phenylethylamide derivative **2**. Thus, arenastatin A (**1**) was treated with 1*N* aqueous hydrochloric acid at 40°C for 1 h to liberate 2-hydroxy-4-methylpentanoic acid, which was then treated with D(+)-α-phenylethylamine, triethylamine, and diethyl phosphorocyanidate[2] to give D-α-phenylethylamide **2**. The D-α-phenylethylamide **2** was then analyzed by HPLC using a chiral column [CHIRALCEL OF (DAICEL), *n*-hexane-sec-PrOH] to identify with 2(*S*)-hydroxy-4-methylpentanoyl D-α-phenylethylamide.[3]

Table 1 ^{1}H- and ^{13}C- NMR Data for Arenastatin A (**1**) in DMSO-d_6

atom	^{13}C	^{1}H (mult., J (Hz))	HMBC (^{13}C)[a]	atom	^{13}C	^{1}H (mult., J (Hz))	HMBC (^{13}C)[a]
1	164.6			17	23.6	1.56 (m)	16, 18, 19
2	125.5	5.77 (d, 15)	1, 4	18	21.1	0.78 (d, 7)	16, 17, 19
3	139.3	6.42 (ddd, 15,	1, 4, 5	19	22.3	0.76 (d, 6.5)	16, 17, 18
		10, 3.5)		20	171.6		
4a	36.2	2.27 (m)	2, 3, 5	21a	32.4	2.27 (m)	20
b		2.68 (m)		b		2.61 (m)	
5	75.6	5.12 (dd-like,	3, 6, 7, 13,14	22a	33.1	3.24 (m)	
		10.5, 5)		b		3.37 (m)	
6	39.8	1.83 (m)	5, 7, 8,13	23	170.5		
7	62.6	2.99 (dd, 7.5, 1.5)		24	55.2	4.27 (m)	1, 23, 25, 26
8	58.0	3.88 (d, 1.5)	6, 7, 9, 10	25a	34.8	2.65 (m)	23, 24, 26
9	137.0			b		2.97 (dd, 12, 4.5)	23, 24, 26
10, 10'	125.8	7.30 (d, 7)	8, 10, 11	26	130.0		
11, 11'	128.4	7.38 (m)	9, 11	27, 27'	129.7	7.12 (d, 8.5)	26, 29
12	128.1	7.35 (m)	10	28, 28'	113.6	6.82 (d, 8.5)	26, 29
13	13.4	1.05 (d, 7)	5, 6, 7	29	157.7		
14	169.8			30	54.8	3.70 (s)	29
15	70.0	4.97 (dd, 9.5, 4)	14, 16, 17, 20	22-NH		7.21 (m)	23
16a	39.2	1.24 (m)	14, 17, 18, 19	24-NH		8.20 (d, 8.5)	1
b		1.50 (m)	14, 15, 17, 18, 19				

a) C coupled with H.

Furthermore, to determine the absolute configuration of the *O*-methyltyrosine moiety, arenastatin A (**1**) was treated with 6*N* aqueous hydrochloric acid at 110°C for 4 h to furnish *O*-methyltyrosine, which was then treated with succinimido L(-)-α-phenylethylcarbamate[4] to give L-α-phenylethylcarbamoyl *O*-methyltyrosine **3**. The urethane derivative **3** was analyzed by HPLC (CAPCELL PAK C_{18} AG120, MeOH-H_2O-TFA) to identify with L-α-phenylethylcarbamoyl D-*O*-methyltyrosine **3**.[5]

In 1990, Merck group has isolated an antifungal depsipeptide cryptophycin (**4**) from a cultured cyanobacterium of *Nostoc* sp. and elucidated its plane structure.[7,8] The chemical structure of our arenastatin A (**1**) is very alike to that reported for cryptophycin (**4**). This fact may indicate a participation of a presumable symbiotic cyanobacterium in the biosynthesis of arenastatin A (**1**) in the marine sponge *Dysidea arenaria*. The stereostructure of the fragment A is currently under investigation.[9]

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References and Notes

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3) The four diastereoisomers of 2-hydroxy-4-methylpentanoyl α-phenylethylamide were prepared from D(+)- and L(-)-α-phenylethylamines and 2(*R*)- and 2(*S*)-hydroxy-4-methylpentanoic acids, the latters being prepared from D- and L-leucines by treatment with sodium nitrate and 1 *N* aqueous sulfuric acid, respectively.

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8) We have heard that Prof. R. Moore and his group, the Univ. of Hawaii, U.S.A., have also isolated related depsipeptides exhibiting potent cytotoxic activities from a cyanobacterium of *Nostoc* sp.

9) Geometry of the 7,8-epoxide in **1** has been assigned *trans* from the coupling constant ($J_{7,8}$=1.5 Hz).

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