THE ABSOLUTE STEREOSTRUCTURE OF ARENASTATIN A, A POTENT CYTOTOXIC DEPSIPEPTIDE FROM THE OKINAWAN MARINE SPONGE *DYSIDEA ARENARIA*

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The absolute stereostructure of arenastatin A (**1**), which was isolated from the Okinawan marine sponge *Dysidea arenaria*, has been determined on the bases of NMR and synthetic studies. Arenastatin A (**1**) is a cyclic depsipeptide exhibiting extremely potent cytotoxicity against KB cells with IC_{50} 5 pg/ml.

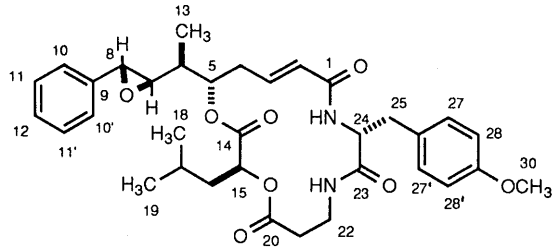
KEYWORDS marine sponge; *Dysidea arenaria*; depsipeptide; arenastatin A; cytotoxic

In the course of searching for bioactive substances from marine organisms,[1] we isolated an extremely potent cytotoxic (IC_{50} 5 pg/ml for KB cells) depsipeptide named arenastatin A (**1**) from the Okinawan marine sponge *Dysidea arenaria* and have elucidated the plane structure including the absolute configurations of the 2-hydroxy-4-methylpentanoyl and *O*-methyltyrosine moieties.[2] In this paper, we report the absolute stereostructure elucidation of arenastatin A (**1**) on the bases of NMR and synthetic studies.

The relative stereostructure of the C-5~C-8 part in arenastatin A (**1**) has been figured out from the ROESY experiment of **1** (in DMSO-d_6) as shown in Fig.1.[3] Thus, the following correlations were substantial for characterizing the stereostructure: between HN-24 and H-2, HN-22, H_a-25; H_a-4 and H-2, H-6; H_b-4 and H-3, H-5, H-6, H-13; H-5 and H-3, H-6, H-7, H-13; H-6 and H-8; H-7 and H-13. Consequently, the absolute configurations of the C-5~C-8 part in arenastatin A (**1**) has been presumed as 5*S*, 6*S*, 7R, and 8*R*.

Methanolysis of arenastatin A (**1**) with K_2CO_3-MeOH furnished a mixture of unstable products,[4] which was further treated with imidazole in CH_2Cl_2 to provide a tetrahydrofuranoid **2** as a single product. The compound **2**[5] showed characteristic CD maxima (MeOH): $[\Theta]_{260}$ -4000 (neg.max.), $[\Theta]_{240}$ +13500 (pos. max.), $[\Theta]_{222}$ +46000 (pos.max.). For the purpose of elucidating the absolute stereostructure of arenastatin A (**1**), we then synthesized **2**. In this process, a 2*S*,3*R*-diol ester **3**, which was prepared from ethyl cinnamate by osmium-catalyzed asymmetric dihydroxylation,[6] was converted to an acetonide and then to a compound **4** by TMSCl-MeLi treatment followed by Wittig reaction. Hydroboration of **4** and subsequent oxidation and Wittig-Horner reaction furnished a diastereomeric mixture of two α,β-unsaturated carboxylates **5** and **6** in 1.2:1 ratio. An allyl alcohol **7**, obtained by DIBAL reduction of **5**, was subjected to Sharpless epoxidation[7] followed by Red-Al reduction and $NaIO_4$ oxidation to furnish a 1,3*S*-diol **8**. After exchange of the protective groups in **8**, an ortho ester of the resulting 5,6-diol **9** was treated with AcBr[8] and then with K_2CO_3-MeOH to give an epoxide **10** and a tetrahydrofuranoid **11** in 1.4:1 ratio. Silylation in the presence of imidazole of the mixture of **10** and **11** and subsequent acetylation furnished another tetrahydrofuranoid **12** as a sole product, which was then converted to an aldehyde **13**. The relative stereostructure of **13** was confirmed by the NOE experiment of **12**[9] and by the X-ray crystallographic analysis[10] of **14**[11] (Fig.2), which was synthesized from **6** through the same procedure as from **5** to **10**. In the NOE experiment, nuclear-Overhauser enhancements were observed for the proton signals between 4-Me and H-3, H-5; H_2-2 and H-4, H-6. Thus, 3*S*,4*R*,5*R*,6*R* configurations in **14** as well as 3*S*,4*S*,5*R*,6*S* configurations in **12** have been clarified.

Next, β-alanine 2-(trimethylsilyl)ethyl ester (**15**) was coupled with a D-*O*-methyltyrosine derivative **16** using DEPC[12] to furnish **17**. Phosphonoacetylation of **17** in the presence of WSCI[13] provided **18**, which was then subjected to Wittig-Horner reaction with the aldehyde **13** to give **19**. K_2CO_3-MeOH treatment of **19** furnished **2**, which was found identical with that obtained above from arenastatin A (**1**) by means of HPLC, ^{1}H-NMR, and CD comparisons. Based on the accumulated foregoing evidence, the absolute stereostructure of arenastatin A (**1**) has been determined as **1**.



arenastatin A (**1**)

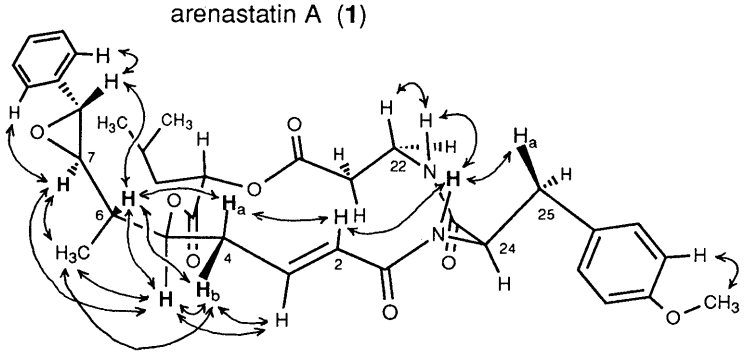
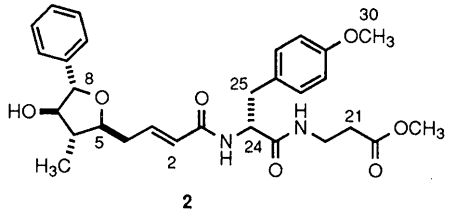


Fig. 1. ROESY Data for Arenastatin A (**1**)



2

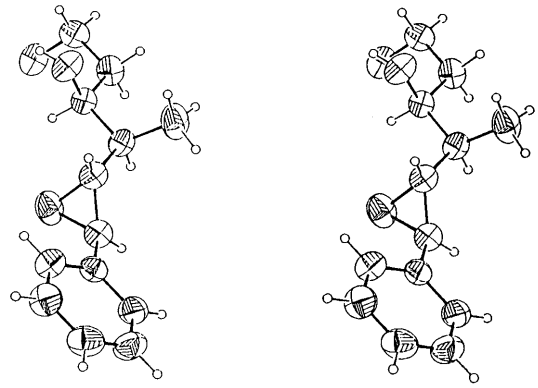


Fig. 2. ORTEP Drawing of **14**

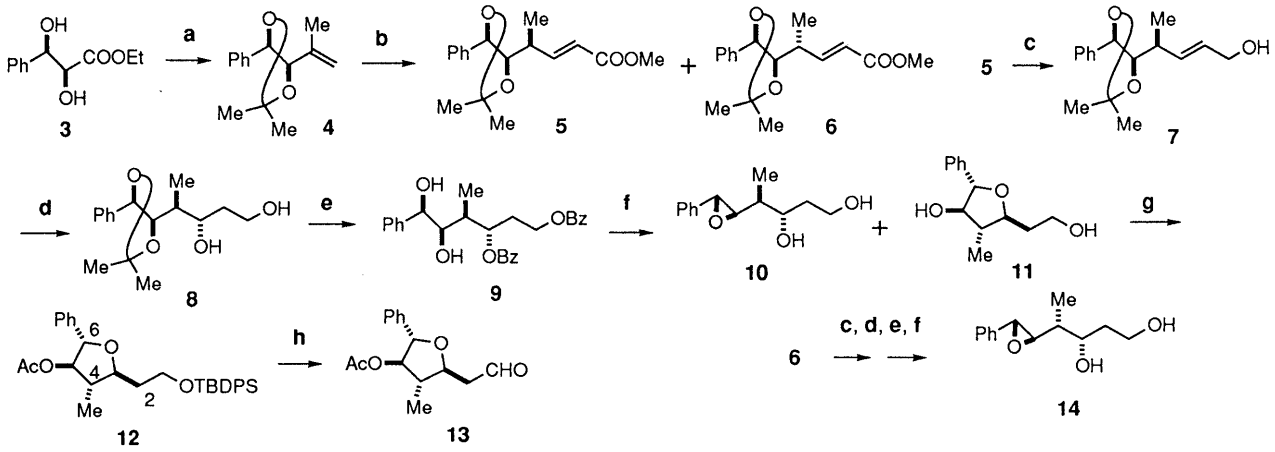


Chart 1. Reagents and Conditions : **a**) i) 2,2-dimethoxypropane, CSA, ii) TMSCl, MeLi, THF, iii) PPh_3CH_3Br, ^nBuLi, 3 steps 90%; **b**) i) $BH_3{\cdot}SMe_2$, then H_2O_2, aqNaOH, ii) Dess-Martin periodinane, iii) $(MeO)_2P(O)CH_2COOMe$, NaH, THF, 3 steps 70%; **c**) DIBAL, CH_2Cl_2, 99%; **d**) i) *D*-DET, $Ti(O^iPr)_4$, TBHP, ii) $NaAlH_2(OCH_2CH_2OMe)_2$, THF, iii) $NaIO_4$, ether-water (1:1), 3 steps 87%; **e**) i) BzCl, pyridine, DMAP, ii) 80% AcOH, 2 steps 85%; **f**) i) $CH(OMe)_3$, PPTS, CH_2Cl_2, ii) AcBr, CH_2Cl_2, iii) K_2CO_3-MeOH, 3 steps 89%; **g**) i) TBDPSCl, imidazole, CH_2Cl_2, ii) Ac_2O, pyridine, 2 steps 90%; **h**) i) $^nBu_4NF$, THF, ii) Dess-Martin periodinane, 2 steps 80%.

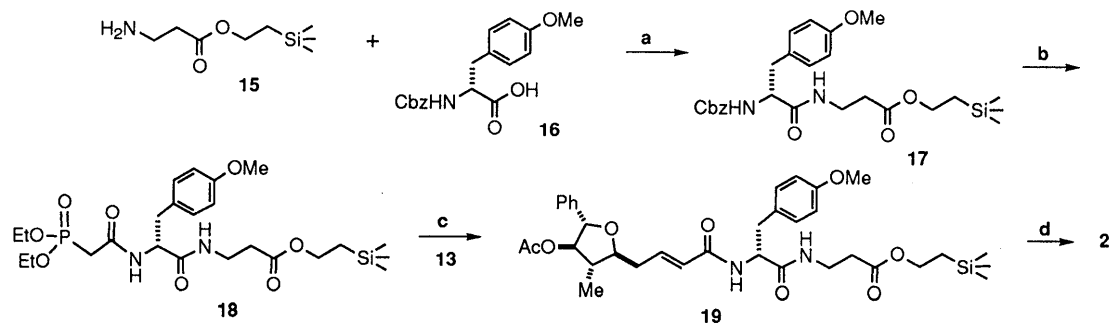


Chart 2. Reagents and Conditions: **a**) DEPC, Et_3N, DMF, 82%; **b**) i) H_2/Pd-C, HCOOH, MeOH, then aq$NaHCO_3$, ii) $(EtO)_2P(O)CH_2COOH$, WSCI·HCl, DMAP, THF, 2 steps 99%; **c**) **13**, NaH, THF, 70%; **d**) K_2CO_3, MeOH, 85%.

In 1990, a Merck group isolated an antifungal depsipeptide named cryptophycin from a cultured cyanobacterium *Nostoc* sp. and reported the plane structure.[14] Very recently, Prof. Moore and his group[15] have also isolated cryptophycin and allied compounds (named cryptophycins A~G) as antitumor substances again from a cultured cyanobacterium *Nostoc* sp. and elucidated their absolute stereostructures. Very interestingly, arenastatin A (**1**), isolated by us from a marine sponge, corresponds to a β-alanine analog of cryptophycin B.

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3) All proton signals of arenastatin A (**1**) have been assigned on the bases of COSY, HOHAHA, HMBC, and HMQC experiments of **1**[2] ($J_{4a,5}$=10.5 Hz, $J_{4b,5}$=1.5 Hz, $J_{6,5}$=5.5 Hz).

4) From the spectral data, the mixture was presumed to contain des-(2-hydroxy-4-methylpentanoyl)-arenastatin A (unstable) and **2** (stable). To convert the former to the latter (**2**), the imidazole treatment was carried out.

5) **2**: ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 7.3-7.4 (5H), 7.11 (2H, d, *J*=8.5 Hz, H-27,27'), 6.93 (1H, dt, *J*=15.5,7.5, H-3), 6.81 (2H, d, *J*=8.5, H-28,28'), 6.12 (2H, m, HN-22,24), 5.92 (1H, d, *J*=15.5, H-2), 4.64 (1H, d, *J*=7.5, H-8), 4.58 (1H, m, H-24), 3.96 (1H, m, H-5), 3.77 (3H, s, H-30), 3.71 (1H, m, H-7), 3.63 (3H, s, COOMe), 3.47, 3.35 (both 1H, m, H-22), 3.08 (1H, dd, *J*=13.5,5.5, H_a-25), 2.91 (1H, dd, *J*=13.5,8.5, H_b-25), 2.61, 2.50 (both 1H, m, H-4), 2.42, 2.33 (both 1H, m, H-21), 2.04 (1H, m, H-6), 1.10 (3H, d, *J*=6.5, H-13). FAB-MS: *m/z* 525 $(M+H)^+$ ($C_{29}H_{37}O_7N_2$ by HR FAB-MS).

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9) **12**:^{1}H-NMR (270MHz, $CDCl_3$)δ: 7.2-7.7 (15H), 5.05 (1H, t, *J*=4.5 Hz, H-5), 4.93 (1H, d, *J*=4.5, H-6), 4.08 (1H, m, H-3), 3.90 (2H, m, H-1), 2.12 (1H, m, H-4), 2.08 (3H, s, OAc), 1.91 (2H, m, H-2), 1.06 (9H, s), 1.03 (3H, d, *J*=7.5, 4-Me). FAB-MS: *m/z* 525 $(M+Na)^+$ ($C_{31}H_{38}O_4SiNa$ by HR FAB-MS).

10) Crystallographic data for the crystalline [mp 93°C (ether)] prepared from **14**: $C_{13}H_{18}O_3$, M = 222.27. Orthorhombic, *a* = 9.598 (3), *b* = 20.441 (5), *c* = 6.313 (2) Å, V= 1238.6 (6) Å^3. Space group $P2_12_12_1$, z = 4, D_X = 1.192 g·cm^{-3}, μ(Cu-Kα) = 1.5418 cm^{-1}. Crystal size 0.4 x 0.2 x 0.4 mm. The X-ray analysis: Intensity data were measured at 293 K with graphite monochromated Cu-Kα radiation on a Rigaku AFC-5R diffractometer. By means of the ω-2θ scanning mode, intensities of 1198 independent reflections with $\sin\theta/\lambda < 0.54$ Å^{-1} were obtained. The structure was solved by direct and difference Fourier methods and refined by full matrix least-squares with anisotropic temperature factors for non-H atoms of **14**. The final *R* value was 0.0370 for 948 reflections with *Fo*>4d(*Fo*).

11) The compound **14** was stable under the condition of K_2CO_3-MeOH or imidazole treatment.

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