

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 IC₅₀ 5 pg/ml.

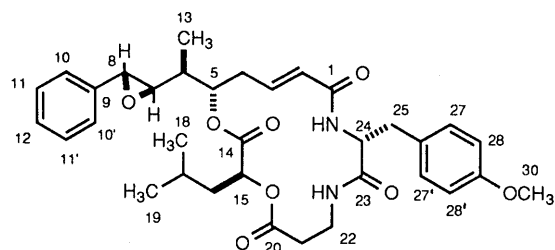
KEYWORDS marine sponge; *Dysidea arenaria*; depsipeptide; arenastatin A; cytotoxic

In the course of searching for bioactive substances from marine organisms,¹⁾ we isolated an extremely potent cytotoxic (IC₅₀ 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*-methyl-tyrosine 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*, 7*R*, and 8*R*.

Methanolysis of arenastatin A (**1**) with K₂CO₃-MeOH furnished a mixture of unstable products,⁴⁾ which was further treated with imidazole in CH₂Cl₂ to provide a tetrahydrofuranoid **2** as a single product. The compound **2**⁵⁾ showed characteristic CD maxima (MeOH): [Θ]₂₆₀ -4000 (neg.max.), [Θ]₂₄₀ +13500 (pos. max.), [Θ]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 epoxidation⁷⁾ followed by Red-Al reduction and NaIO₄ 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 AcBr⁸⁾ and then with K₂CO₃-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 analysis¹⁰⁾ 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; H₂-2 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 DEPC¹²⁾ to furnish **17**. Phosphonoacetylation of **17** in the presence of WSCI¹³⁾ provided **18**, which was then subjected to Wittig-Horner reaction with the aldehyde **13** to give **19**. K₂CO₃-MeOH treatment of **19** furnished **2**, which was found identical with that obtained above from arenastatin A (**1**) by means of HPLC, ¹H-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)

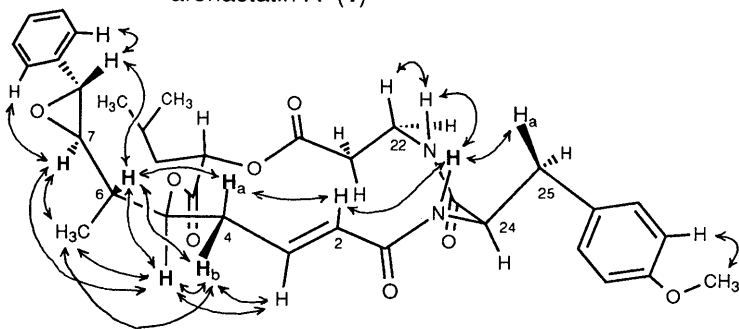
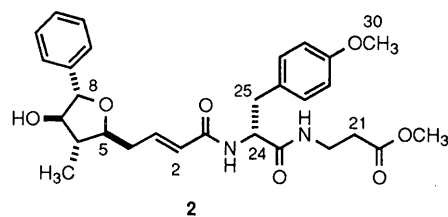


Fig. 1. ROESY Data for Arenastatin A (1)



2

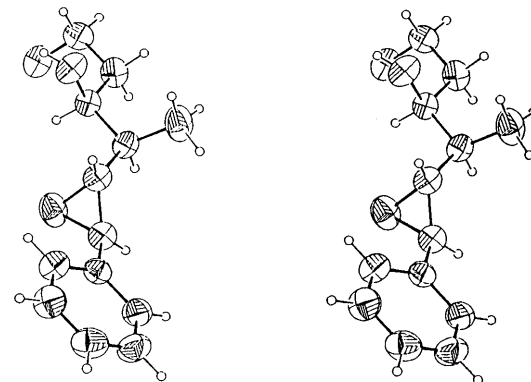


Fig. 2. ORTEP Drawing of 14

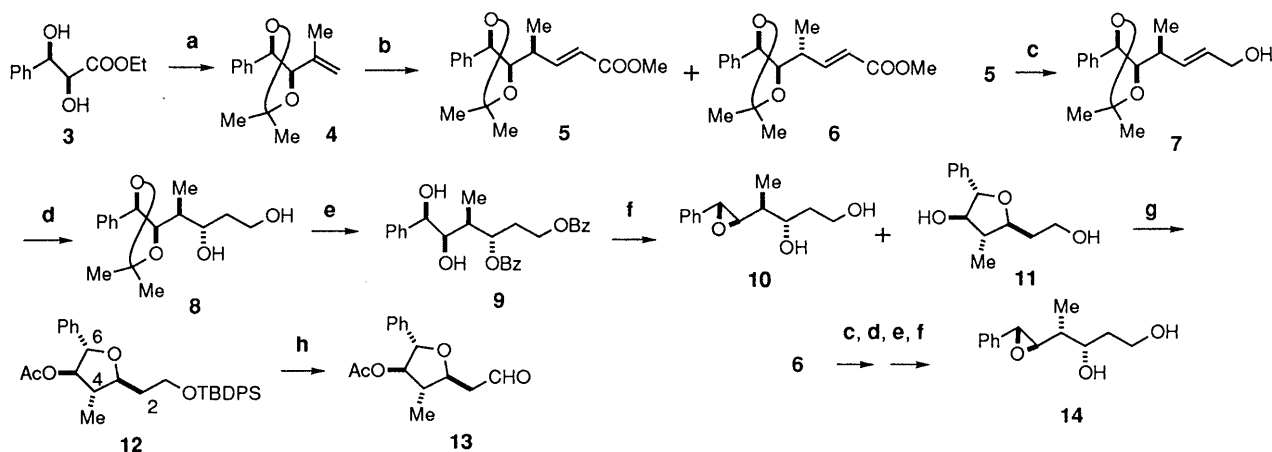


Chart 1. Reagents and Conditions : a) i) 2,2-dimethoxypropane, CSA, ii) TMSCl, MeLi, THF, iii) $\text{PPh}_3\text{CH}_3\text{Br}$, $n\text{BuLi}$, 3 steps 90%; b) i) $\text{BH}_3\cdot\text{SMe}_2$, then H_2O_2 , aqNaOH, ii) Dess-Martin periodinane, iii) $(\text{MeO})_2\text{P}(\text{O})\text{CH}_2\text{COOMe}$, NaH, THF, 3 steps 70%; c) DIBAL, CH_2Cl_2 , 99%; d) i) $D\text{-DET}$, $\text{Ti}(\text{O}^i\text{Pr})_4$, TBHP, ii) $\text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OMe})_2$, THF, iii) NaIO_4 , ether-water (1:1), 3 steps 87%; e) i) BzCl , pyridine, DMAP, ii) 80% AcOH, 2 steps 85%; f) i) $\text{CH}(\text{OMe})_3$, PPTS, CH_2Cl_2 , ii) AcBr , CH_2Cl_2 , iii) $\text{K}_2\text{CO}_3\text{-MeOH}$, 3 steps 89%; g) i) TBPDPSCl , imidazole, CH_2Cl_2 , ii) Ac_2O , pyridine, 2 steps 90%; h) i) $n\text{Bu}_4\text{NF}$, THF, ii) Dess-Martin periodinane, 2 steps 80%.

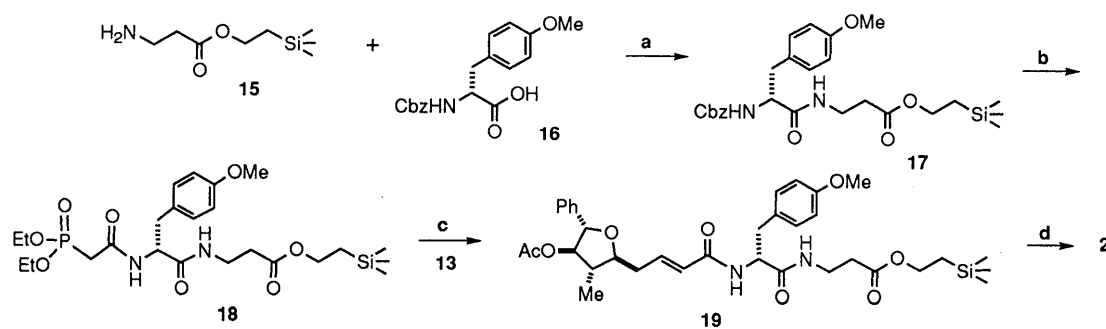


Chart 2. Reagents and Conditions: a) DEPC, Et_3N , DMF, 82%; b) i) $\text{H}_2/\text{Pd-C}$, HCOOH , MeOH, then aqNaHCO₃, ii) $(\text{EtO})_2\text{P}(\text{O})\text{CH}_2\text{COOH}$, WSCI-HCl, DMAP, THF, 2 steps 99%; c) 13, NaH, THF, 70%; d) K_2CO_3 , MeOH, 85%.

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 group¹⁵⁾ 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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- 2) M. Kobayashi, S. Aoki, N. Ohya, M. Kurosu, W. Wang, I. Kitagawa, *Tetrahedron Lett.*, **35**, in press.
- 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**: ¹H-NMR (500 MHz, CDCl₃) 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)⁺ (C₂₉H₃₇O₇N₂ by HR FAB-MS).
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- 9) **12**: ¹H-NMR (270MHz, CDCl₃) 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)⁺ (C₃₁H₃₈O₄SiNa by HR FAB-MS).
- 10) Crystallographic data for the crystalline [mp 93°C (ether)] prepared from **14**: C₁₃H₁₈O₃, $M = 222.27$. Orthorhombic, $a = 9.598$ (3), $b = 20.441$ (5), $c = 6.313$ (2) Å, $V = 1238.6$ (6) Å³. Space group P2₁2₁2₁, $z = 4$, $D_x = 1.192$ g·cm⁻³, $\mu(\text{Cu-K}\alpha) = 1.5418$ 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-K α radiation on a Rigaku AFC-5R diffractometer. By means of the ω -2 θ 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 $F_o > 4d(F_o)$.
- 11) The compound **14** was stable under the condition of K₂CO₃-MeOH or imidazole treatment.
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