Spongiacysteine, a Novel Cysteine Derivative from Marine Sponge *Spongia* sp.

Keiko Kobayashi, Hiroki Shimogawa, Akira Sakakura, Toshiaki Teruya, Kiyotake Suenaga, and Hideo Kigoshi*
Department of Chemistry, University of Tsukuba, Tennoudai, Tsukuba 305-8571

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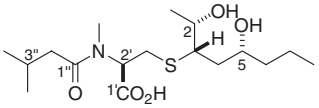
A novel cysteine derivative, spongiacysteine (**1**), was isolated from marine sponge *Spongia* sp. The gross structure was elucidated by detailed spectroscopic analysis, and the absolute stereostructure was established by its total synthesis. This compound showed moderate antimicrobial activity against rice blast fungus *Pyricularia oryzae*.

Marine sponges are a prolific source of new compounds with diverse structures that often have interesting biological activities. In our continuing search for new substances from marine sponges,[1] we investigated the constituents of marine sponge *Spongia* sp.[2] collected at Tateyama beach, Chiba Prefecture, and isolated a new cysteine derivative, spongiacysteine (**1**). In this report, we describe the isolation, structural elucidation, and synthesis of spongiacysteine.

Spongia sp. (1.9 kg, wet weight) was extracted with methanol. The methanol extracts were partitioned between H_2O and EtOAc, and the EtOAc extracts were partitioned between 90% aq methanol and hexane. The 90% aq methanol layers were concentrated and separated by a series of chromatographic processes, including column chromatography (SiO_2 and ODS) and HPLC on ODS to give spongiacysteine (**1**) (2.0 mg).



spongiacysteine (**1**)

Spongiacysteine (**1**) was isolated as a colorless oil. Its molecular formula was determined to be $C_{17}H_{33}NO_5S$ based on HRFABMS [m/z 386.1972 $(M + Na)^+$, Δ -0.5 mmu].[3] The IR spectrum showed the presence of hydroxy (3500–3300 cm^{-1}) and carbonyl (1680 and 1650 cm^{-1}) moieties. Its ^1H and ^{13}C NMR spectral data are summarized in Table 1.

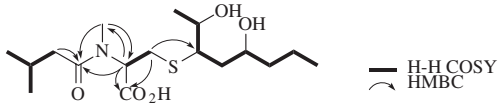


Figure 1. Partial structures of spongiacysteine (**1**) based on ^1H–^1H COSY (bold line) with important HMBC correlations (arrows).

The signals in the ^1H and ^{13}C NMR spectra were completely assigned by the analysis of the ^1H–^1H COSY, HMQC, and HMBC spectral data to suggest a planar carbon framework for spongiacysteine (**1**) as a novel cysteine derivative, as shown in Figure 1. For determination of the absolute stereochemistry, spongiacysteine (**1**) was converted into the MTPA esters (Figure 2). Chemical shift differences between the two esters gave conflicting results due to the double esterification of the hydroxy functions at C-2 and C-5. However, these data could be explained by separately considering the influence of each MTPA group on the adjacent hydrogens and referring to the examples[4–6] of the modified Mosher's method on a 1,4-diol. The absolute stereochemistry of the two hydroxy moieties was assigned as 2*S*, 5*R*. Subsequently, NMR analysis of the seven-membered lactone **2** derived from **1** (2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N)[7,8] revealed that the relative stereochemistry between C-2 and C-3 was determined to be anti stereochemistry, establishing that the absolute stereochemistry of C-3 was 3*S* (Figure 3).

Table 1. NMR spectral data of spongiacysteine (**1**) in CD_3OD

C No.	^{13}C[a] / ppm	^1H[b] / ppm (mult., J/Hz)	HMBC $^{13}C \rightarrow {}^1H$
1	19.4	1.22 d (6.3)	
2	71.6	3.91 dq (4.1, 6.3)	H-1
3	51.1	2.91 ddd (10.9, 4.1, 3.0)	H-1, 4, 3′
4	39.2	1.67 ddd (14.5, 10.9, 3.0)	H-2
		1.39 m	
5	69.7	3.83 m	H-4, 6
6	41.7	1.45 m	H-4, 7, 8
		1.38 m	
7	20.5	1.46 m	H-6, 8
		1.37 m	
8	14.9	0.92 t (6.9)	H-6, 7
1′	173.4		H-2′, 3′
2′	59.2	5.11 dd (11.1, 4.5)	H-3′, *N*-CH_3
3′	32.2	3.25 dd (14.0, 4.5)	H-2′
		3.00 dd (14.0, 11.1)	
1″	176.7		H-2′, 2″, 3″, *N*-CH_3
2″	43.7	2.34 dd (14.9, 7.3)	H-3″, 4″, 5″
		2.30 dd (14.9, 6.8)	
3″	27.3	2.13 m	H-2″, 4″, 5″
4″, 5″	23.4	1.00 d (6.6)	H-2″, 3″, 4″, 5″
N-CH_3	34.2	3.04 s	H-2′

[a] Recorded at 125 MHz, [b] Recorded at 500 MHz.

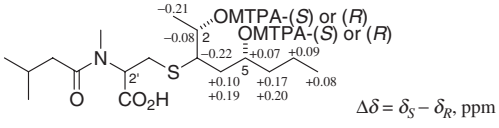


Figure 2. Chemical shift differences ($\Delta\delta$) between the MTPA derivatives of spongiacysteine (**1**) [500 MHz, $CDCl_3$].

We could not obtain any information of the stereochemistry of C-2′, however, we can postulate that the absolute stereochemistry of the cysteine moiety would be L as in the case of acyclic natural products. To confirm the absolute stereostructure, we decided to synthesize **1** stereoselectively (Scheme 1).

N-Methylcysteine (**3**)[9] reacted with isovaleroyl chloride to

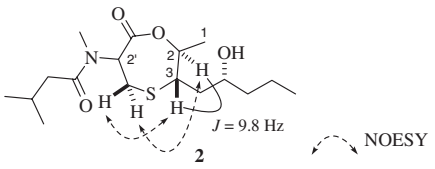
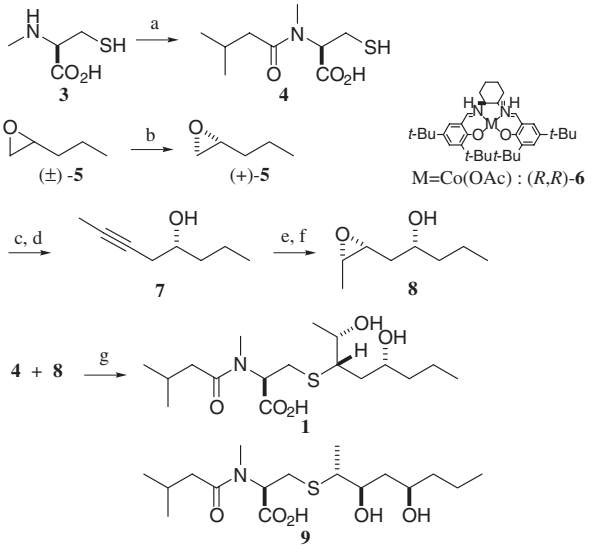


Figure 3. Selected NOESY correlations and coupling constants of the lactone **2**.

give the amide **4**. Kinetic resolution of the racemic 1,2-epoxypentane [(±)-**5**] in the presence of the asymmetric catalyst **6** provided the optically active epoxide (+)-**5**.[10] The coupling reaction of the epoxide (+)-**5** with lithium acetylide followed by methylation provided the alcohol **7**. Hydrogenation of the alcohol **7** in the presence of the Lindlar catalyst led to a homoallylic alcohol, the diastereoselective epoxidation of which provided the epoxide **8** in 99% de.[11]

Coupling of the amide **4** and the epoxide **8** was accomplished and produced spongiacysteine (**1**)[12] along with the regioisomer **9**. The ^{1}H NMR spectral data of the synthetic spongiacysteine was identical to those of the natural compound. Furthermore, to confirm the stereochemistry in such an acyclic system, synthetic **1** was converted into a seven-membered lactone **2**, whose spectral data were found to be identical with those of the authentic sample from natural **1**.



Scheme 1. Reagents and conditions: (a) isovaleroyl chloride, DMAP, pyridine, rt, 30 min, 23%; (b) (*R*,*R*)-**6**, H_2O, 0 °C → rt, 22 h, 35%, >99% ee; (c) lithium acetylide EDA complex, DMSO, rt, 2 h, 99%; (d) methyl iodide, *n*-BuLi, THF, −78 °C → rt, 4 h, 92%; (e) H_2, Lindlar catalyst, MeOH, rt, 6 h, 83%; (f) *t*-BuOOH, $VO(acac)_2$, CH_2Cl_2, 0 °C, 3 h, 99%, >99% de; (g) 1 M NaOH, THF–H_2O (1:1), 50 °C, 16 h, **1** (41%), **9** (36%).

In conclusion, we isolated a novel cysteine derivative, spongiacysteine (**1**), from marine sponge *Spongia* sp. The gross structure was elucidated by detailed spectroscopic analyses, and the absolute stereostructure was established by its total synthesis. As a result of an investigation of various biological activities of **1**, it turns out that **1** showed antimicrobial activity against rice blast fungus *Pyricularia oryzae* at IC_{90} = 100 ppm. Blasticidin, kasugamycin, etc.,[13] are known to possess the same activity and used in crop field. Spongiacysteine (**1**) has a different type of structure from them and was expected to be a lead for new types of agricultural chemicals. In our laboratory, we plan to synthesize its analogues and investigate their biological activities.

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References and Notes

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8 **2**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.79 (dd, J = 2.6, 7.3 Hz, H-2′, 1H), 4.69 (dq, J = 9.8, 6.3 Hz, H-2, 1H), 3.90 (m, H-5, 1H), 3.26 (dd, J = 7.3, 15.3 Hz, H-3′α, 1H), 3.17 (ddd, J = 2.4, 9.8, 13.8 Hz, H-3, 1H), 3.12 (s, *N*-C$\underline{H}_3$, 3H), 2.72 (dd, J = 2.6, 15.3 Hz, H-3′β, 1H), 2.28 (dd, J = 6.9, 14.8 Hz, H-2″α, 1H), 2.22 (dd, J = 6.8, 14.8 Hz, H-2″β, 1H), 2.17 (m, H-3″, 1H), 1.65–1.20 (m, H-4, H-6, H-7, 6H), 1.45 (d, J = 6.3 Hz, H-1, 3H), 0.98 (d, J = 6.4 Hz, H-4″, 3H), 0.97 (d, J = 6.4 Hz, H-5″, 3H), 0.93 (t, J = 7.1 Hz, H-8, 3H).
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