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Reticulatins A and B and hyrtioreticulin F from the marine sponge *Hyrtios reticulatus*

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Abstract

Three new alkaloids, reticulatins A (**1**) and B (**2**) and hyrtioreticulin F (**3**), were isolated from the water-soluble fraction of an EtOH extract of the marine sponge *Hyrtios reticulatus*. Compounds **1** and **2** were found to be novel 1,3-dimethyl-5-(methylthio)imidazolium alkaloids. The structure of **3** was determined to be an indole alkaloid related to hyrtioreticulin E (**8**) and hyrtioerectine B (**9**), which were isolated previously from the *n*-BuOH-soluble fraction of the sponge extract. The presence of three NH units in **3** was indicated by the ^{1}H-^{15}N HSQC spectrum in D_2O-H_2O (5:95) containing 0.05% TFA measured at 5 ˚C. Compound **3** is likely biosynthesized from L-tryptophan, two units of L-alanine, and glycine by the Pictet-Spengler reaction. The absolute configurations of **1**-**3** were determined by the methods based on ECD spectra. In addition, the result for **1** was further confirmed by the PGME method.

Keywords: Imidazole alkaloid, Indole alkaloid, *Hyrtios reticulatus*, Marine sponge

1. Introduction

Marine sponges of the genus *Hyrtios* (family Thorectidae) are known to be a rich source of unusual secondary metabolites such as alkaloids,[1-3] sesterterpenes,[4-6] and macrolide,[7] with significant cytotoxic[3-5,7] and antimicrobial[2,6] activities. In the search for ubiquitin-activating enzyme (E1) inhibitors as leads for treatment of cancer, we previously reported the isolation of new alkaloids, hyrtioreticulins A-E (**4**-**8**),[1] and hyrtioerectine B[8] (**9**) from the *n*-BuOH-soluble fraction of an extract of the marine sponge *Hyrtios reticulatus* collected in Indonesia (Fig. 1). While searching for metabolites from the water-soluble fraction of the extract, we isolated three new alkaloids, reticulatins A (**1**) and B (**2**) and hyrtioreticulin F (**3**) (Fig. 1). Here, we report the isolation and structural elucidation of **1**-**3**.

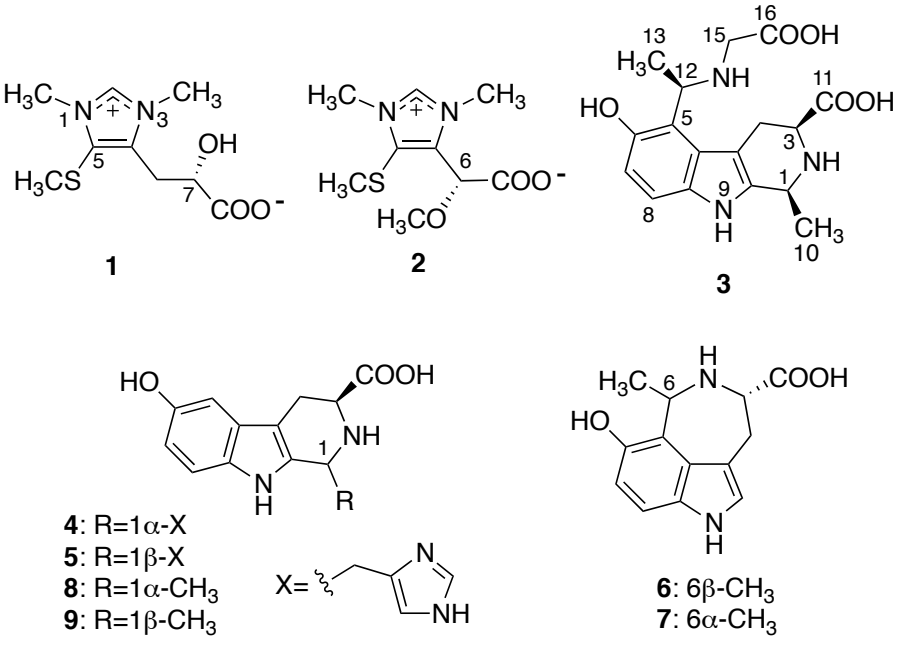


Fig. 1. Structures of reticulatins A (**1**) and B (**2**), hyrtioreticulin F (**3**), and alkaloids **4**-**9**.

2. Results

2.1. Isolation of 1-3

The EtOH extract of the sponge (400 g, wet weight) was evaporated, and the aqueous residue was extracted with EtOAc and then *n*-BuOH.[1] The MeOH-soluble portion of the remaining aqueous fraction was subjected to ODS column chromatography followed by ODS HPLC yielding three new alkaloids, reticulatins A (**1**, 1.6 mg) and B (**2**, 0.85 mg) and hyrtioreticulin F (**3**, 6.9 mg).

2.2. Structural elucidation of 1-3

The ESIMS of reticulatin A (**1**) showed a pseudomolecular ion peak at *m/z* 231 $[M+H]^+$ and the molecular formula was indicated to be $C_9H_{14}N_2O_3S$. The ^1H NMR spectrum of **1** in D_2O (Table 1) showed three singlet methyl signals at δ 2.21, 3.77, and 3.85, three methylene/methine signals at δ 3.04, 3.13, and 4.15, and a singlet deshielded signal at δ 8.66. The HSQC spectrum showed the presence of two *N*-methyl groups (δ_H 3.77, δ_C 33.4 (3-CH_3)); δ_H 3.85, δ_C 37.6 (1-CH_3)), as judged from their chemical shifts. The deshielded hydrogen (δ 8.66) was directly bound to a carbon at δ 137.5 (C-2) and showed HMBC correlations to carbons at δ 33.4 (3-CH_3), 37.6 (1-CH_3), 127.1 (qC, C-5), and 136.7 (qC, C-4) (Fig. 2). These results were reminiscent of a 1,3-dimethylimidazolium structure. Another methyl group (δ_H 2.21, δ_C 18.4) was indicated to be a *S*-methyl group by its chemical shifts, and a HMBC correlation between δ_H 2.21 and δ_C 127.1 (C-5) showed that the *S*-methyl group was attached to C-5 (Fig. 2). The remaining portion was composed of $C_3H_4O_3$. The COSY spectrum showed that a methylene (δ_H 3.04 and 3.13, δ_C 28.8 (C-6)) was coupled with a

methine (δ_H 4.15, δ_C 70.9 (C-7)), and the methine was suggested to be an oxygenated carbon by its chemical shift. HMBC cross peaks from the methylene (H_2-6) to carbons at δ 70.9 (C-7), 127.1 (C-5), 136.7 (C-4), and 178.9 (qC, C-8) showed that the methylene (C-6) was bound to C-4 (Fig. 2). The molecular formula and the chemical shift of C-8 (δ 178.9) indicated the planar structure of **1** as shown in Fig. 1. Since **1** showed a positive Cotton effect in the 210-225 nm region of its ECD spectrum (Fig. 3), the absolute configuration was indicated to be 7*S* based on the moiety of a α-hydroxy carboxylic acid.[9] In order to confirm the 7*S*-configuration, (*S*)- and (*R*)-phenylglycine methyl ester (PGME) amides of **1** were prepared.[10] The absolute stereochemistry was determined based on the chemical shift differences at H-7 and H_2-6. The Δδ (*S*-*R*) values were shown in Fig. 4. According to the paper by Yabuuchi and Kusumi,[10] the absolute configuration of PGME amides should be analyzed by taking their conformations into account. Since **1** is classified into the "α-oxy-α-monosubstituted acetic acid" group, it should be analyzed by utilizing the "model D" in the paper. Thus, 7*S*-configuration was supported.

Table 1. NMR data (400 MHz, D_2O) for **1**

Position	δ_C	δ_H (*J* in Hz)	HMBC[a]
2	137.5 CH	8.66 s	4, 5, 1-Me, 3-Me
4	136.7 qC		
5	127.1 qC		
6	28.8 CH_2	3.04 dd 15.1, 7.8	4, 5, 7, 8
		3.13 dd 15.1, 5.5	4, 5, 7, 8
7	70.9 CH	4.15 dd 7.8, 5.5	4, 6, 8
8	178.9 qC		
1-Me	37.6 CH_3	3.85 (3H) s	2, 5
3-Me	33.4 CH_3	3.77 (3H) s	2, 4

5-SMe	18.4 CH_3	2.21 (3H) s	5
6-OMe			

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

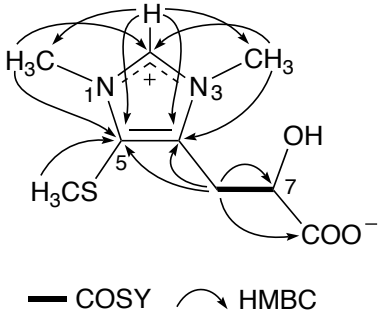


Fig. 2. COSY and key HMBC correlations for **1** in DMSO-d_6.

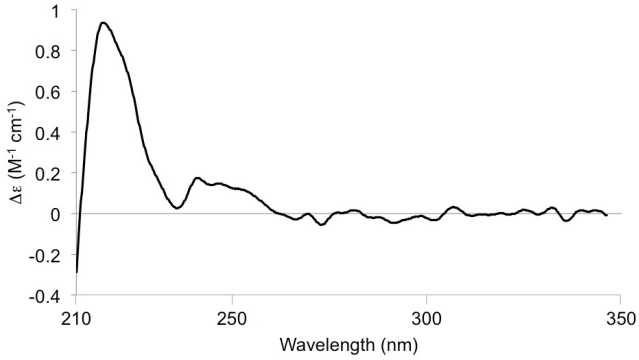


Fig. 3. ECD spectrum for **1** in MeOH.

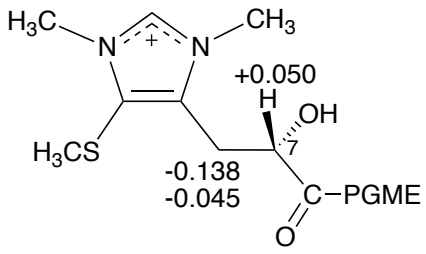


Fig. 4. Δδ (*S*-*R*) values (in ppm) for PGME amide derivatives of **1**.

The molecular formula of reticulatin B (**2**) was established by HRESIMS as $C_9H_{14}N_2O_3S$. The ^1H and ^{13}C NMR spectra (Table 2) in CD_3OD showed that **2** also contained the 1,3-dimethyl-5-(methylthio)imidazolium nucleus. Analysis of the 2D NMR spectra showed that a methoxy (δ_H 3.44, δ_C 58.6 (6-OMe)) and a carboxyl (δ_C 171.4 (C-7)) group connected to a methine (δ_H 5.24, δ_C 75.4 (C-6)) (Fig. 5), and the HMBC correlation from the methine hydrogen to δ_C 130.8 (C-5) and 137.0 (C-4) indicated that the methine was attached to C-5. The absolute configuration of **2** was elucidated by applying a quantum mechanical method to calculate the ECD spectrum (Fig. 6). A conformational analysis for 6*R*-configuration was performed based on a molecular mechanical method by CONFLEX7 with the MMFF94S force field. The obtained two outstandingly stable conformers were further optimized with the density functional theory (DFT) method at the B3LYP/6-31G(d) level in MeOH. The rotational strengths of the lowest 24 excited states for each conformer were calculated with the time-dependent density functional theory (TDDFT) method at the level of triple-zeta split-valence quality with BHandHLYP/TZVP in MeOH. The given rotational strengths were converted into Gaussian-type curves and were summed to afford the calculated ECD curve for each conformer, revealing that both conformers had the identical ECD and UV spectra. The resultant negative Cotton effect at 210 nm agreed well with the experimental ECD peaks, suggesting the absolute configuration of **2** is 6*R*.

Table 2. NMR data (500 MHz, CD_3OD) for **2**

Position	δ_C	δ_H (*J* in Hz)	HMBC[a]
2	140.7 CH	9.00 s	4, 5, 1-Me, 3-Me
4	137.0 qC		
5	130.8 qC		

6	75.4 CH	5.24 s	4, 5, 7, 6-OMe
7	171.4 qC		
1-Me	34.4 CH_3	3.93 (3H) s	2, 5
3-Me	35.8 CH_3	3.87 (3H) s	2, 4
5-SMe	19.7 CH_3	2.42 (3H) s	5
6-OMe	58.6 CH_3	3.44 (3H) s	6

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

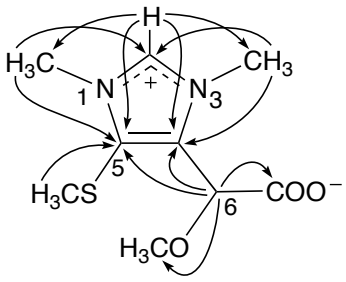


Fig. 5. Key HMBC correlations for **2** in DMSO-d_6.

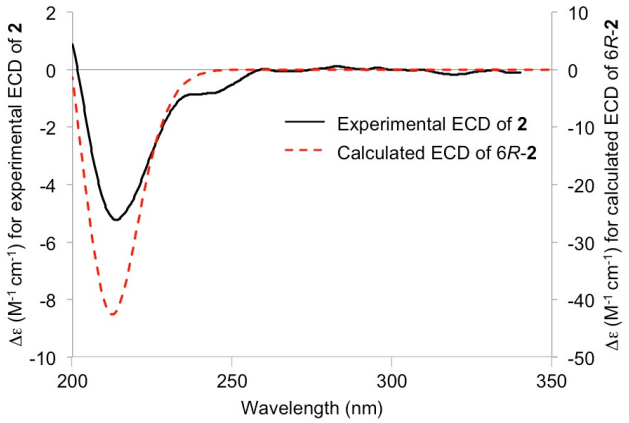


Fig. 6. Experimental and calculated ECD spectra of **2** in MeOH.

The ESIMS of hyrtioreticulin F (**3**) showed prominent ion peaks at *m/z* 346 $[M-H]^-$ and 271 $[M-H-75]^-$. The molecular formula $C_{17}H_{21}N_3O_5$ was established by HRESIMS. The ^1H NMR spectrum of **3** in DMSO-d_6 (Table 3) showed two doublet methyl groups at δ 1.63 (d, *J*

= 6.3 Hz) and 1.64 (d, *J* = 6.3 Hz), five methylene or methine signals at δ 3.19 (2H, m), 3.67 (2H, br s), 4.32 (m), 4.67 (q, *J* = 6.3 Hz), and 4.83 (m), two doublet aromatic hydrogens at δ 6.80 (d, *J* = 8.4 Hz) and 7.24 (d, *J* = 8.4 Hz), and an exchangeable hydrogen at δ 11.28 (s). The ^{13}C NMR spectrum showed two methyl carbons at δ 16.4 and 18.0, two methylene carbons at δ 24.4 and 45.6, three methine carbons at δ 49.2, 53.3, and 55.3, and ten deshielded carbons at δ 103.4 (qC), 111.6 (qC), 112.3 (CH), 112.6 (CH), 124.2 (qC), 131.3 (qC), 132.4 (qC) and 148.6 (qC), 168.3 (qC), and 170.3 (qC). The analysis of 2D NMR spectra indicated **3** to have a very similar structure to hyrtioreticulin E (**8**) and hyrtioerectine B (**9**) except for the presence of a substituent at C-5. Although the presence of a carboxyl group (δ 170.3, C-11) at C-3 was not supported by the HMBC spectrum in DMSO-d_6, correlations, H-3 and H-4/C-11 and H-3/C-1, were observed in the HMBC spectrum in D_2O-H_2O (5:95) containing 0.05% TFA measured at 5 ˚C (Fig. 7). In addition, the ^{1}H-^{15}N HSQC spectrum in the solvent showed cross peaks between δ_H 9.06/8.62 and δ_N 49.5 (N-2), δ_H 8.61 (2H) and δ_N 49.7 (N-14), and δ_H 10.41 and δ_N 125.3 (N-9), which confirmed the presence of three NH units. Under this condition, N-12 and N-14 were protonated and thus two hydrogens were attached to individual nitrogens. Although the hydrogens (δ_H 8.61 (2H)) attached to N-14 were equivalent, those on N-2 were nonequivalent (δ_H 9.06/8.62) by the effect of two substituents at C-1 and C-3. The molecular formula of **3** indicated that the substituent was composed of $C_4H_8NO_2$. The COSY spectrum showed that a remaining methyl (δ_H 1.64, δ_C 18.0 (C-13)) connected to a methine (δ_H 4.83, δ_C 53.3 (C-12)). The HMBC correlations (DMSO-d_6) from an isolated methylene (δ 3.67 (2H, br s, H_2-15)) to carbons at δ 53.3 (C-12) and 168.3 (C-16) clearly showed the structure of the substituent (Fig. 7), and the HMBC correlations from H-12 to

C-4b, C-5, and C-6 unambiguously confirmed the planar structure of **3**. The prominent ion peak at *m/z* 271 $[M-H-75]^-$ in the ESIMS was diagnostic for a terminal glycine moiety. The NOESY spectrum in DMSO-d_6 showed correlation between δ 4.32 (H-3) and δ 4.67 (H-1), which strongly indicated that **3** was the *cis*-configured derivative. Biogenetic considerations with hyrtioreticulins A-E (**4**-**8**) indicated an 3*S*-configuration for **3**. The absolute configuration of C-12 was established based on the comparison of the experimental ECD spectrum with the theoretical ECD spectra for both 12*R*- and 12*S*-configurations (Fig. 8). After conformational searches by CONFLEX7 with the MMFF94S force field, all the obtained stable conformers were further optimized by the DFT method at B3LYP/6-31G(d) level in MeOH. The most stable four conformers, which covered 90.0 % and 97.1 % of populations for 12*R*- and 12*S*-configurations, respectively, were subjected to the TDDFT calculations at B3LYP/TZVP level in MeOH. The resultant rotational strengths were converted into Gaussian-type curves and summed to give the theoretical ECD spectrum for each conformer. The calculated ECD spectra were obtained by considering the Boltzmann distributions of the conformers, implying that **3** has 12*R*-configuration.

Table 3. NMR data (500 MHz, DMSO-d_6) for **3**

Position	δ_C	δ_H (*J* in Hz)	HMBC[a]
1	49.2 CH	4.67 q 6.3	9a
3	55.3 CH	4.32 m	4
4	24.4 CH_2	3.19 (2H) m	3, 4a, 9a
4a	103.4 qC		
4b	124.2 qC		
5	111.6 qC		
6	148.6 qC		

7	112.3 CH	6.80 d 8.4	5, 8a
8	112.6 CH	7.24 d 8.4	4b, 6
8a	131.3 qC		
9		11.28 s	4a, 4b, 8a, 9a
9a	132.4 qC		
10	16.4 CH_3	1.63 (3H) d 6.3	1, 9a
11	170.3 qC		
12	53.3 CH	4.83 m	4b, 5, 6, 13
13	18.0 CH_3	1.64 (3H) d 6.3	5, 12
15	45.6 CH_2	3.67 (2H) br s	12, 16
16	168.3 qC		

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

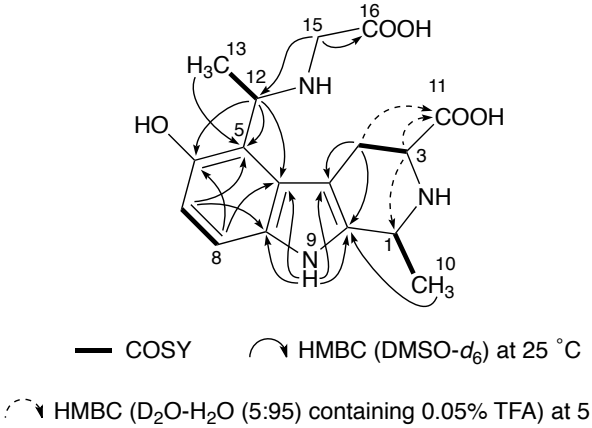


Fig. 7. COSY and key HMBC correlations for **3** in DMSO-d_6 and in D_2O-H_2O (5:95) containing 0.05% TFA.

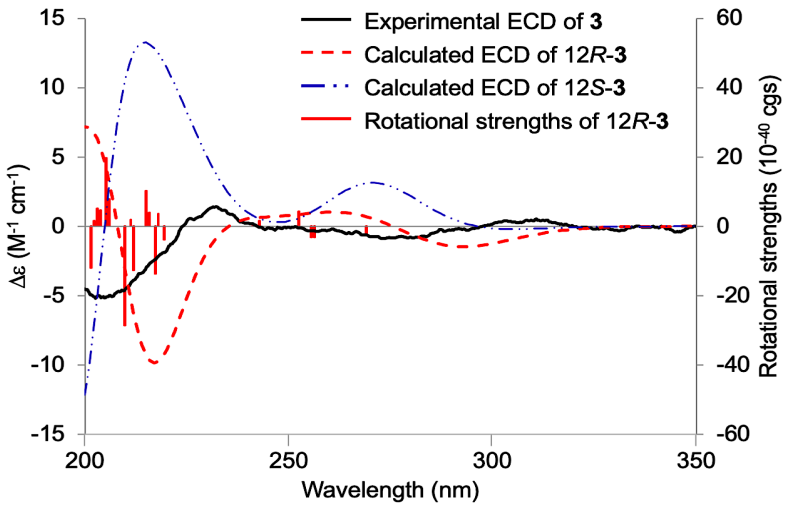


Fig. 8. Experimental and calculated ECD spectra of **3** in MeOH.

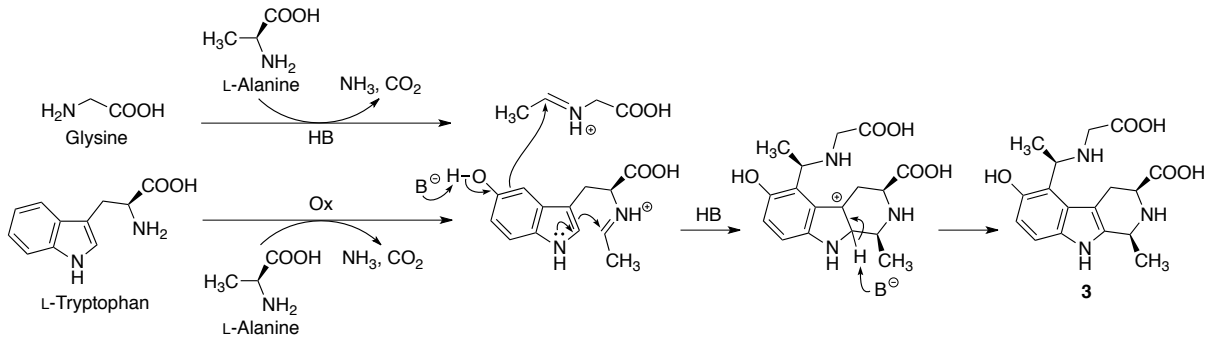
2.2. Biological activities of 1-3

Previously, we isolated hyrtioreticulins A (**4**) and B (**5**) as inhibitors of the ubiquitin-activating enzyme (E1) with IC_{50} values of 2.4 and 35 μM, respectively.[1] However, hyrtioreticulins C-E (**6**-**8**) and hyrtioerectine B (**9**) were unable to inhibit E1 even at 100 μM. To the best of our knowledge, **4** is the most potent E1 inhibitor reported so far.[1] The structurally related indole alkaloid, hyrtioreticulin F (**3**), and imidazole alkaloids, reticulatins A (**1**) and B (**2**), were tested for E1 activity and found to be inactive even at 200 μM.[11] The compounds were neither cytotoxic against HeLa cells at 5 μg/mL nor microbial against *Bacillus subtilis*, *Candida albicans*, and *Escherichia coli* at 10 μg/disk.[12]

3. Discussion

Three new alkaloids, reticulatins A (**1**) and B (**2**) and hyrtioreticulin F (**3**), were isolated from the water-soluble fraction of an extract of the Indonesian marine sponge, *Hyrtios*

reticulatus. Structural elucidation showed that **1** and **2** were 1,3-dimethyl-5-(methylthio)imidazolium derivatives. So far, only five compounds containing the 1,3-dimethyl-5-(methylthio)imidazolium moiety have been isolated as natural products.[13-16] Four of them are sponge-derived β-carboline alkaloids connected with the moiety via a CH_2 or C=O unit, and the other is an indole alkaloid containing the moiety isolated from an ascidian. Although **1** and **2** are the sixth and seventh natural products containing the moiety, they are simple 1,3-dimethyl-5-(methylthio)imidazolium derivatives without a β-carboline or indole core. Hyrtioreticulin F (**3**) was found to be an indole alkaloid related to **8** and **9**. Similarly, **3** would be biosynthesized by the Pictet-Spengler reaction with L-tryptophan, two units of L-alanine, and glycine (Scheme 1).[17] Since more *cis* isomers than *trans* isomers were isolated for **4**-**9**,[1] a small amount of the *trans* isomer of **3** also may exist in the sponge.



Scheme 1. A possible biogenetic pathway for **3**.

4. Experimental

4.1. General experimental procedure

Optical rotations were determined with a JASCO DIP-1000 polarimeter in MeOH. UV spectra were measured on a JASCO V-550 spectrophotometer in MeOH. ECD spectra were

measured on a JASCO J-820 spectropolarimeter in MeOH at 24 ˚C. IR spectra were measured on a JEOL JIR-6500W spectrophotometer. NMR spectra were recorded on a Bruker Avance 500 or JEOL JNM- ECX-400 NMR spectrometer. Chemical shifts were referenced to the residual solvent peaks (δ_H 2.49 and δ_C 39.5 for DMSO-d_6; δ_H 3.30 and δ_C 49.0 for CD_3OD), and multiplicities of carbon resonances were determined from HMQC spectra. NMR spectra in D_2O-H_2O (5:95) containing 0.05% TFA were measured at 5 ˚C, and chemical shifts of ^1H, ^{13}C, and ^{15}N NMR spectra were referenced indirectly to 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) according to IUPAC recommendations.[18] Mass spectra were measured on a JEOL JMS-700 or BRUKER esquire 3000plus-K1 mass spectrometer.

4.2. Isolation

The collection and extraction process for the marine sponge (RMNH POR 3989) (400 g, wet weight) were reported previously.[1] After extraction of the concentrated aqueous residue of the EtOH extract with EtOAc and then *n*-BuOH, the MeOH-soluble portion (6.7 g) of the remaining aqueous fraction was subjected to ODS column chromatography with MeOH/H_2O to give fractions I and II containing **1**-**3**. Fraction I (108.8 mg) eluted with 5% MeOH-H_2O was purified by HPLC (Phenomenex Phenyl-hexyl column with 5% CH_3CN-H_2O (0.05% TFA) and Develosil C30-UG5 column with 5-50% CH_3CN-H_2O (0.05% TFA)) to afford reticulatins A (**1**, 1.6 mg, 0.00040%) and B (**2**, 0.85 mg, 0.00021%). Fraction II (26.5 mg) eluted with 10% MeOH-H_2O was purified by HPLC (Cosmosil 5C18-AR-II column with 5% CH_3CN-H_2O (0.05% TFA) to afford hyrtioreticulin F (**3**, 6.9 mg, 0.0017%).

4.3. Reticulatin A (1)

Amorphous solid. $[\alpha]^{21}_{D}$ -9.9° (*c* 2.8, H_2O); UV (H_2O) λ_{max} (log ε) 274 (3.19), 244 (3.72), 208 (4.23) nm; IR (film) ν_{max} 3130, 3010, 1600, 1190, 1100, 1080 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 231 $[M+H]^+$. HRESIMS *m/z* 231.0821 (Calcd for $C_9H_{15}N_2O_3S$, 231.0798).

4.4. Reticulatin B (2)

Amorphous solid. $[\alpha]^{21}_{D}$ -6.8° (*c* 0.61, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.6) nm; IR (film) ν_{max} 3440, 3280, 2930, 1680, 1200, 1130 cm^{-1}; NMR data, see Table 2; ESIMS *m/z* 253 $[M+Na]^+$. HRESIMS *m/z* 253.0641 (Calcd for $C_9H_{14}N_2NaO_3S$, 253.0617).

4.5. Hyrtioreticulin F (3)

Amorphous solid. $[\alpha]^{21}_{D}$ -43.5° (*c* 1.34, MeOH); UV (MeOH) λ_{max} (log ε) 279 (3.4), 228 (3.8), 204 (4.0) nm; IR (film) ν_{max} 3270, 3070, 2920, 2850, 1650, 1560, 1180, 1030 cm^{-1}; NMR data (DMSO-d_6), see Table 3; NMR data (D_2O-H_2O (5:95) containing 0.05% TFA) ^1H NMR δ 1.60 (3H, d, *J* = 6.7 Hz, H-10), 1.67 (3H, d, *J* = 6.8 Hz, H-13), 3.16 (1H, dd, *J* = 15.6, 12.6 Hz, H-4), 3.33 (1H, dd, *J* = 15.6, 5.1 Hz, H-4), 3.62 (1H, d, *J* = 16.7 Hz, H-15), 3.71 (1H, d, *J* = 16.7 Hz, H-15), 4.22 (1H, dd, *J* = 12.6, 5.1 Hz, H-3), 4.59 (1H, q, *J* = 6.7 Hz, H-1), 4.82 (1H, q, *J* = 6.8 Hz, H-12), 6.74 (1H, d, *J* = 8.6 Hz, H-7), 7.24 (1H, d, *J* = 8.6 Hz, H-8); ^{13}C NMR δ 18.6 (C-10), 19.8 (C-13), 26.6 (C-4), 48.2 (C-15), 52.3 (C-1), 57.1 (C-12), 58.3 (C-3), 105.5 (C-4a), 113.5 (C-5), 115.2 (C-7), 115.8 (C-8), 126.3 (C-4b), 134.4 (C-8a), 134.6 (C-9b), 150.7 (C-6), 171.6 (C-16), 173.6 (C-11); HMBC correlations H-1/C-10; H-3/C-1, C-4,

C-11; H-4 (δ 3.16)/C-3, C-4a, C-9a, C-11; H-4 (δ 3.33)/C-3, C-4a, C-9a; H-7/C-5, C-8a; H-8/C-4b, C-6; H-10/C-1, C-9a; H-12/C-4b, C-5, C-6, C-13; H-13/C-5, C-12; H-15 (δ 3.62)/C-16; H-15 (δ 3.71)/C-12, C-16; ESIMS *m/z* 346 $[M-H]^-$ and 271 $[M-H-75]^-$. HRESIMS *m/z* 346.1396 (Calcd for $C_{17}H_{20}N_3O_5$, 346.1404).

4.6. Preparation of PGME amides of 1

To a solution of **1** (0.1 mg) in DMF (300 μL) was added (*S*)-PGME (0.1 mg), PyBOP (0.1 mg), HOBT (0.1 mg), and *N*-mehtylmorpholin (2 μL) on the ice. The mixture was kept at room temperature overnight. After evaporation to dryness, the residue was partitioned between EtOAc and H_2O, and the EtOAc fraction was purified by gel filtration HPLC using an Asahipak GS-310P column (Shoko Co., Ltd., 21.5 x 500 mm) with MeOH to give an (*S*)-PGME amide of **1**. (*R*)-PGME amide of **1** was also prepared by the same method.

4.7. ECD calculations for 2 and 3

Conformational searches were performed with CONFLEX7 (Ver. 7.A.0910 by CONFLEX, Tokyo)[19,20] using a commercially available PC (operating system: Windows7 Professional SP1 64-bit, CPU: QuadCore Xeon E3-1225 processor 3.10 GHz, RAM 8 GB) and DFT calculations were conducted with Gaussian09 (Revivion A.02 by Gaussian, Wallingford, CT) [21] with a PC (Operating System: CentOS a Linux, CPU: 2 Intel Xeon 3 5550 processors 2.67 GHz, RAM 24 GB). Theoretical ECD spectra were obtained from a typical calculation procedure[22] described in the followings. The initial structure was constructed on a graphical user interface considering the absolute configuration of interest, and was subjected to a

conformational search with CONFLEX7 using MMFF94S (2010-12-04HG) as the force field, where initial stable conformers were generated for up to 50 kcal/mol. All the stable conformers with distributions over 1 % (two from 9 conformers for **2** and ten from 3074 conformers for **3**) were further optimized by the density functional theory method supposing MeOH as the solvent with B3LYP functional and 6-31G(d) basis set. The obtained conformers were analyzed their populations by considering their Boltzmann distribution at 298 K based on their internal energies. All the conformers of distributions over 1 % (two for **2**, four for 12*R*-**3**, and four for 12*S*-**3**, covering 100 %, 90.0 %, and 97.1 %, respectively) were subjected to time-dependent simulations at approximation of the triple-zeta split-valence basis set TZVP with the hybrid functional either BHandHLYP or B3LYP supposing MeOH as the solvent. For each conformer, the resultant rotational strengths were converted into Gaussian-type curves (bandwidth sigma = 3200 cm^{-1}) and summed to give the ECD spectrum. The theoretical ECD spectrum for the compound was composed after correction based on the Boltzmann distribution of the stable conformers with over 1 %.

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