SHORT REPORT

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A New Diketopiperazine from the Marine Sponge *Callyspongia* Species

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Abstract: Chemical investigation of the sponge *Callyspongia* sp. from the South China Sea afforded one new diketopiperazine, cyclo-(*R*-Pro-6-hydroxyl-*S*-Ile) (**1**), along with six known diketopiperazines: staphyloamide A (**2**), cyclo- (*S*-Pro-*S*-Phe) (**3**), cyclo-(*R*-Pro-*R*-Phe) (**4**), cyclo-(*S*-Pro-*R*-Leu) (**5**), cyclo-(*S*-Pro-*R*-Ala) (**6**), cyclo-(*R*-Tyr-*R*-Phe) (**7**), and three known tryptophan-derived alkaloids: C^2-*α*-D-mannosylpyranosyl-tryptophan (**8**), (1*R*, 3*S*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**9**), and (1*R*,3*R*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**10**). The structures were determined on the basis of NMR and MS analysis, and the absolute configuration was determined by comparison of the optical rotation with the known compounds. This is the first report of compounds **1**, **2** , **8**–**10** from the sponge *Callyspongia*. Cyclo-(*S*-Pro-*R*-Leu) (**5**), and cyclo-(*S*-Pro-*R*-Ala) (**6**) exhibited antifouling activity against cyprid larvae of the barnacle with the LC_{50} values of 3.5 μg/cm^2 and 6.0 μg/cm^2, respectively.

Keywords: *Callyspongia* sp.; Diketopiperazine; Chemical constituents; Antifouling activity.

1. Animal Source

Callyspongia sp. belongs to the order Haplosclerida family Callyspongiidae. The genus *Callyspongia* have been found to contain a variety of structurally unique metabolites with interesting biological activities, and a number of alkaloids have been isolated from this genus so far [1-4].

The sponge was collected by hand in July 2005, off the coast of Hainan Island, P. R. China. The specimen was identified by Dr. Kyung Jin Lee, Invertebrate Research Division, National Institute of Biological Resources, Environmental Research Complex, Incheon, Korea. A voucher specimen

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2. Previous Studies

Diketopiperazines (DKPs) are a large and structurally varied group of natural products isolated from both terrestrial and marine organisms [5]. A number of DKPs were isolated from various marine sponges: *Calyx cf podatypa* [6], *Dysidea herbacea* [7], *Geodia baretti* [8], *Tedania ignis* [9], *Axinella* sp.[10], and *Dysidea fragilis*[11]. Many of these constituents are reported to have a broad range of bioactivities, such as antitumour, antibacterial, antifungal, antifouling, plant-growth regulatory, and other activities.[5, 12]. In our previous study, a new proline-containing dipeptide, named callyspongidipeptide A, and two known DKPs were isolated from *Callyspongia* sp.[13].

3. Present Study

The sponge (10 kg) was extracted three times with 95% EtOH (50 L) for 3 days. The extract was concentrated under reduced pressure, and partitioned between H_2O (4 L) and $CHCl_3$ (4 L); the $CHCl_3$ layer (405 g) was further partitioned between 85% EtOH (4 L) and petroleum ether (PE; 4 L) to yield 85% EtOH (98 g) and PE (270 g) fractions. The H_2O layer was partitioned between n-BuOH (4 L) and H_2O (4 L) to yield n-BuOH (71 g) fractions. Chromatographic purification of the 85% EtOH portion and n-BuOH portion yielded compounds **1**–**10** (Figure 1). Detailed isolation procedures for compounds **1**–**10** are shown in the supplementary material. Structure characterization was aided by various spectroscopic experiments such as MS, and NMR. NMR spectra were measured on Bruker AVANCE-500 spectrometer. ESI-MS was obtained from Thremo LCQ-DECA-XP LC-MS spectrometer. HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer.

*cyclo-(R-Pro-6-hydroxyl-R-Ile) (**1**):* $C_{11}H_{18}N_2O_3$, White amorphous powder. $[\alpha]_D^{25}$: + 27.4 (*c* 0.04, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 3.64-3.61 (2H, m, H-3), 3.60 (1H, d, *J* = 7.5 Hz, H-9), 2.29-2.26 (1H, m, H-5a), 2.18-2.16 (1H, m, H-4a), 2.13-2.09 (1H, m, H-5b), 2.09-2.05 (1H, m, H-10), 2.01-1.97 (1H, m, H-4b), 1.78-1.67 (1H, m, H-11a), 1.28-1.19 (1H, m, H-11b), 1.03 (3H, d, *J* = 7.0 Hz, H-13), 0.98 (3H, t, *J* = 7.5 Hz, H-12). ^{13}C NMR (125 MHz, CD_3OD): δ 170.5 (C-1, C), 169.5 (C-7, C), 88.1 (C-6, C), 63.6 (C-9, CH), 46.6 (C-3, CH_2), 41.2 (C-10, CH), 38.1 (C-5, CH_2), 26.6 (C-11, CH_2), 20.3 (C-4, CH_2), 15.9 (C-13, CH_3), 11.4 (C-12, CH_3). ESI-MS (neg.): *m/z* 225.1 ($[M - H]^-$). HR-ESI-MS m/z 241.1209 (calcd for C11H18N2O3Na, 241.1210).

Antifouling Assay: Antifouling efficacies of the eleven alkaloids isolated from *Callyspongia* sp. were evaluated by the settlement inhibition assay with cyprid larvae of *Balanus reticulatus*. *B. reticulatus* adults were collected from intertidal rocks in Shenzhen, China. Antifouling efficacies of the ten alkaloids were investigated according to the method of Chen *et al*. with a minor modification [14]. The tested compounds were introduced to the glass dishes using DMSO as carrier solvent.

Antimicrobial Activity: Antimicrobial assays against three bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*) were carried out using the disk diffusion method [15]. Chloramphenicol was used as positive controls against bacteria.

Compound **1** was obtained as white amorphous powder. Compound **1** exhibited a $[M - H]^-$ ion peak at *m/z* 225.1 in the negative-ion ESI-MS. The HR-ESI-MS of 1 established its molecular formula as C11H18N2O3. Comparison of **1** with staphyloamide A (**2**) revealed that it not only exhibit the same molecular mass, but almost the same physical and NMR spectroscopic data [16], and differed mainly in the shift of the C-9 proton signals from δ_H 4.20 to 3.60. The HMBC correlations of H-9 to C-1, and C-10 and H_3-13 to C-9, C-10, and C-11 were observed. This fact described that **1** was diastereoisomer to **2** at position C-9. Such change was also observed in staphyloamide B [16] and bacillusamide B [7]. Furthermore, applying the empirical trend of the proline-containing DKPs, the optical rotation of **1** ($[\alpha]_D^{25}$ = + 27.4) was allowed to propose the diketopiperazine structure cyclo-(*R*-Pro-6-hydroxyl-*S*-Ile) for compound **1** [6, 16, 17].

The identification of the other known compounds, staphyloamide A (**2**), cyclo-(*S*-Pro-*S*-Phe) (**3**) [18], cyclo-(*R*-Pro-*R*-Phe) (**4**) [6], cyclo-(*S*-Pro-*R*-Leu) (**5**) [6], cyclo-(*S*-Pro-*R*-Ala) (**6**) [19], cyclo-(*R*-Tyr-*R*-Phe) (**7**) [20], C^2-*α*-D-mannosylpyranosyl-tryptophan (**8**) [21, 22], (1*R*, 3*S*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**9**) [23, 24], and (1*R*,3*R*)-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylic acid (**10**) [23, 24], was confirmed by comparison of the ^{1}H and ^{13}C NMR data with those reported. This is the first report of compounds **1**, **2**, **8**–**10** from the sponge *Callyspongia*.

The antifouling activities of compounds **1**–**10** against larval settlement of the barnacle *Balanus reticulatus* were evaluated. Comparison of means using ANOVA and Dunnet's test showed that compounds **5** and **6** significantly inhibited settlement compared with the negative control ($p < 0.001$) without significant toxicity ($p > 0.05$). From Dunnet's test, cyclo-(*S*-Pro-*R*-Leu) (**5**), and cyclo-(*S*-Pro-*R*-Ala) (**6**) significantly reduced larval settlement with the LC_{50} values of 3.5 μg/cm^2 and 6.0 μg/cm^2, respectively.

MIC values for compounds **1**–**10** were measured against three bacteria strains of *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. All the compounds (**1**–**10**) did not show antimicrobial activities (MIC > 100 μg/mL).

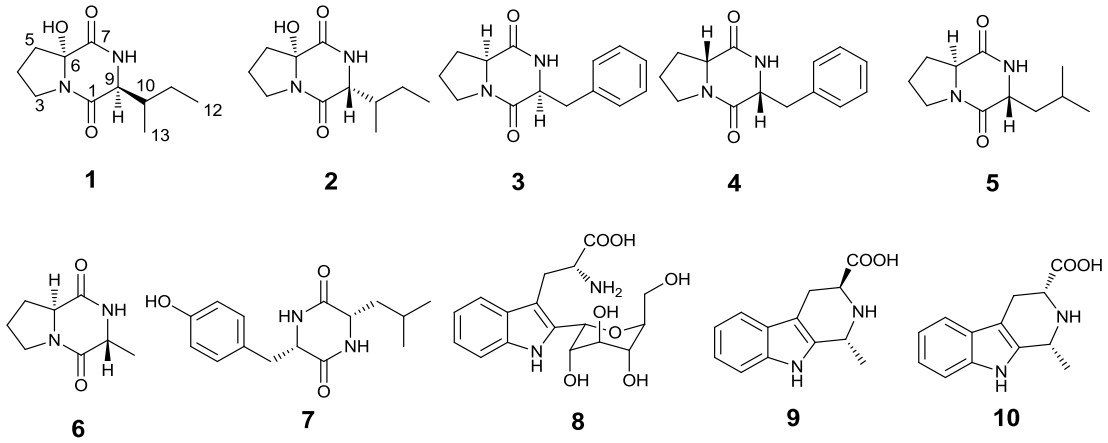

Figure 1. Structures of isolated compounds (**1**–**10)** from *Callyspongia* sp.

Our study revealed the chemical constituents of sponge *Callyspongia* sp., which is rich in the South China Sea. Ten compounds were isolated and purified including seven diketopiperazines and three tryptophan-derived alkaloids. Compound **1** is a new secondary metabolite. In order to detect whether these compounds play a role for ecological functions, the tests for antifouling and antibacterial activity were performed. The results showed significant toxicities against *Balanus reticulatus* larvae for compounds **5** and **6**, which suggest that they contribute to a chemical ecological function.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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