A New Diketopiperazinone from the Marine Sponge
*Callyspongia* Species

Bin Yang¹, Jingxia Huang², Xiuping Lin¹, Yanying Zhang¹
Huaming Tao³ and Yonghong Liu¹∗

¹CAS Key Laboratory of Tropical Marine Bio-resources and Ecology / Guangdong Key Laboratory of Marine Materia Medica / Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, P. R. China

²Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou 510060, P. R. China

³School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, P. R. China

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**Abstract:** Chemical investigation of the sponge *Callyspongia* sp. from the South China Sea afforded one new diketopiperazinone, 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'-α-D-mannosylpyranosyl-tryptophan (8), (1R, 3S)-1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylic acid (9), and (1R,3R)-1-methyl-2,3,4,9-tetrahydro-1H-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, 5-10 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 LC₅₀ values of 3.5 μg/cm² and 6.0 μg/cm², respectively.

**Keywords:** *Callyspongia* sp.; Diketopiperazine; Chemical constituents; Antifouling activity. © 2015 ACG Publications. All rights reserved.

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

∗Corresponding author: E-mail: Yonghongliu@scsio.ac.cn (Y.Liu), Phone:+86-20-89023244
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 callyspongidiipeptide 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 H₂O (4 L) and CHCl₃ (4 L); the CHCl₃ 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 H₂O layer was partitioned between n-BuOH (4 L) and H₂O (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. HR-ESI-MS data were obtained from Thermo LC-DECA-XP LC-MS spectrometer. HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer. HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer. HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer. HR-ESI-MS data were measured on a Bruker micro TOF-QII mass spectrometer.

**Antifouling Assay:** Antifouling efficacies of the eleven alkaloids isolated from *Callyspongia* sp. were evaluated by the settlement inhibition assay with cyprid larv 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 C₁₁H₁₈N₂O₅. 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 δH 4.20 to 3.60. The HMBC correlations of H-9 to C-1, and C-10 and H-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 (α₀D = + 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], (1R, 3S)-1-methyl-2,3,4,9-tetrahydro-1\(^H\)-pyrido[3,4-b]indole-3-carboxylic acid (9) [23, 24], and (1R,3R)-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).

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

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References


