

Chemical Constituents of the Sponge *Mycale* Species from South China Sea

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(Received April 29, 2011; Revised September 12, 2012; Accepted February 5, 2013)

Abstract: Chemical investigation of the sponge *Mycale* species from the South China Sea afforded eleven known compounds, hencosanoic acid methyl ester (**1**), hexadecyl ethers of glycerol (**2**), *N*-docosanoyl-*D*-erythro-(2*S*,3*R*)-16-methyl-heptadecasping-4(*E*)-enine (**3**), dibutyl phthalate (**4**), cholesterol (**5**), 5 α ,8 α -epidioxycholest-6,22-dien-3 β -ol (**6**), 5-hexadecyl-pyrrole-2-carboxaldehyde (**7**), benzoic acid (**8**), 4-hydroxybenzoic acid (**9**), thymine (**10**), and uracil (**11**). Compounds **1–4**, **6–9** were obtained from the sponge of the genus *Mycale* for the first time, and **4** and **6** showed toxicity in the brine shrimp lethality test with the LD₅₀ values at 2.9 μ g/mL and 4.7 μ g/mL, respectively.

Keywords: Sponge *Mycale* species; Chemical constituents; Brine shrimp lethality test.

1. Animal Source

Sponges of the genus *Mycale* are among the richest sources of pharmacologically active chemicals isolated from marine organisms. Many bioactive constituents have been reported from the sponge *Mycale* from California [1], New Zealand [2], Kenyan [3], Japan [4], India [5], and so on. Some of their components even exhibit strong bioactivities [6]. However, there are no any bioactive constituents reported from the sponge *Mycale* from the China Sea.

During the course of our search for bioactive constituents from the South China Sea marine sponges, the sponge *Mycale* species from the South China Sea were investigated. The sponge was

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collected off the coast of Sanya (the South China Sea), Hainan province of China, in May, 2010. Animal material was stored in a $-20\text{ }^{\circ}\text{C}$ freezer prior to extraction. The specimen was identified as *Mycale* sp. by Dr. Kyung Jin Lee, Wildlife Genetic Resources Center, National Institute of Biological Resources, Environmental Research Complex, Incheon, Korea, and the voucher (Ms201005) of *Mycale* sp. was deposited in Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology.

2. Previous Studies

Several compounds were isolated from the genus *Mycale* from all over the world, such as sterols [7], terpenoids [8,9], macrolides [2], cyclic diamines [3], and cyclic tetrapeptides [10]. In the sponge *Mycale* species from the South China Sea, only three compounds, 1-(4-hydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione, uracil, and pentacosanoic acid (2-hydroxy-1-hydroxymethyl-heptadec-3-enyl)-amide were reported previously [11].

3. Present Study

The sponge *Mycale* sp. (2 kg, wet wt) were crushed and extracted with 75% alcohol ($3 \times 4\text{ L}$) at room temperature. The combined alcohol extracts were concentrated *in vacuo*. The residue was partitioned between H_2O (2 L) and CHCl_3 ($3 \times 2\text{ L}$), followed by partitioning of CHCl_3 layer between 90% EtOH and petroleum ether (PE), to yield 90% EtOH fraction (9.3 g) and PE fraction (21 g). The 90% EtOH fraction was chromatographed on silica gel column using $\text{CHCl}_3/\text{MeOH}$ gradiently to obtain subfractions 1–8 (pure CHCl_3 , $\text{CHCl}_3/\text{MeOH}$ 100:1, 50:1, 20:1, 10:1, 4:1, 1:1, and pure MeOH). Subfraction 1 was chromatographed on repeated silica gel column [PE / EtOAc (50:1)] to obtain **1** (19 mg) and **2** (36 mg). Compound **3** (41 mg), **4** (43 mg), and **5** (214 mg) were obtained from subfraction 2 by repeated Sephadex LH-20 column [$\text{CHCl}_3/\text{MeOH}$ (1:1)] and silica gel column [PE / EtOAc (20:1)] chromatographic purification. Subfraction 3 was chromatographed on repeated Sephadex LH-20 column [$\text{CHCl}_3/\text{MeOH}$ (1:1)] to obtain **6** (29 mg). Subfraction 4 were subjected to silica gel column eluted with PE / Acetone (50:1 to 10:1) yielding **7** (16 mg), while subfraction 5 gave **8** (12 mg) and **9** (15 mg). Compound **10** (41 mg) and **11** (43 mg) were yielded from subfraction 6 by recrystallisation. NMR spectra were measured on Bruker AVANCE-500 spectrometer. ESI-MS was obtained from Thermo LCQ-DECA-XP LC-MS spectrometer.

Compound **7** was obtained as white flakes, and was identified as 5-hexadecyl-pyrrole-2-carboxaldehyde by comparison of ESI(+)-MS: [m/z 320 [M+H]⁺] analysis and ¹H NMR and ¹³C NMR data with the 5-alkylpyrrole-2-carboxaldehyde derivatives reported in the literatures [5][12]. Ten other compounds were identified as henicosoic acid methyl ester (**1**)[13], hexadecyl ethers of glycerol (**2**)[14], *N*-docosanoyl-*D*-*erythro*- (2*S*,3*R*)-16-methyl -heptadecasping-4(*E*)-enine (C₂₂-ceramide) (**3**) [15], dibutyl phthalate (**4**)[16], cholesterol (**5**)[17], 5 α ,8 α -epidioxycholest-6,22-dien-3 β -ol (**6**)[18], benzoic acid (**8**)[19], 4-hydroxybenzoic acid (**9**)[20], thymine (**10**)[21], and uracil (**11**)[21] by comparison of ¹H NMR and ¹³C NMR data with those reported in the literatures or their behaviors on TLC with those compounds we reported previously

[17][22] (Fig. 1). These isolated compounds were found in the sponge *Mycale* species from the South China Sea for the first time, except uracil (11). Otherwise, this is the first report of compounds 1–4, 6–9 from the sponge *Mycale* from all over the world.

Compounds 3–9 were evaluated for their toxicity against brine shrimp (*Artemia salina*) larvae and anti-acetylcholinesterase activity, by the method described previously [22,23], and given as supporting information. All the compounds showed no anti-acetylcholinesterase activity. Compounds 4 and 6 showed toxicity against brine shrimp larvae with the LD₅₀ values at 2.9 µg/mL and 4.7 µg/mL, respectively, with 0.28 µg/mL of tacrine as the positive control [22].

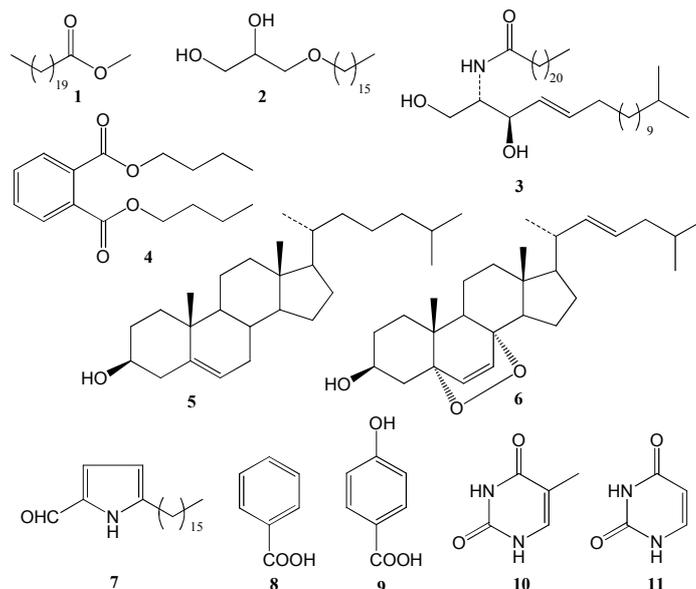


Figure 1. Isolated compounds from sponge *Mycale* sp.

Our study revealed the chemical constituents of sponge *Mycale* sp., which is rich in the South China Sea. The results showed significant toxicities against *Artemia salina* larvae for compounds 4 and 6, which suggest that these may be involved in chemical defence of the sponge.

Acknowledgment

This work was supported by the Knowledge Innovation Program of Chinese Academy of Sciences (KSCX2-YW-G-073, SQ201019, KSCX2-EW-G-12B), National Basic Research Program of China (973)'s Project (2011CB915503 and 2010CB833800), National Natural Science Foundation of China (No. 20902094 and 30973679), Open Funds of Key Laboratory of Marine and Estuarine Fisheries Resources and Ecology, Ministry of Agriculture, and Open Funds of Hubei Key Laboratory of Natural Medicinal Chemistry and Evaluation of Resources.

Supporting Information

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

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