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

Xuefeng Zhou¹, Xiuping Lin¹, Xieyang Guo², Bin Yang¹,

Xian-Wen Yang¹ and Yonghong Liu¹*

¹ Key Laboratory of Marine Bio-resources Sustainable Utilization / Guangdong Key Laboratory of Marine Materia Medica / Research Center for Marine Microbes, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

² School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China

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**Abstract:** Chemical investigation of the sponge *Mycale* species from the South China Sea afforded eleven known compounds, henicosanoic acid methyl ester (1), hexadecyl ethers of glycerol (2), N-docosanoyl-D-erythro-(2S,3R)-16-methyl-heptadecasphing-4(E)-enine (3), dibutyl phthalate (4), cholesterol (5), 5α,8α-epidioxycholest-6,22-dien-3β-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 µg/mL and 4.7 µg/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

* Corresponding author: E-mail: yonghongliu@scsio.ac.cn
collected off the coast of Sanya (the South China Sea), Hainan province of China, in May, 2010. Animal material was stored in a –20 °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-1H-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 × 4 L) at room temperature. The combined alcohol extracts were concentrated *in vacuo*. The residue was partitioned between H₂O (2 L) and CHCl₃ (3 × 2 L), followed by partitioning of CHCl₃ 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 CHCl₃/MeOH gradiently to obtain subfractions 1–8 (pure CHCl₃, CHCl₃/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 [CHCl₃/MeOH (1:1)] and silica gel column [PE / EtOAc (20:1)] chromatographic purification. Subfraction 3 was chromatographed on repeated Sephadex LH-20 column [CHCl₃/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 Thremo 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 henicosanoic acid methyl ester (1)[13], hexadecyl ethers of glycerol (2)[14], N-docosanoyl-D-erythro- (2S,3R)-16-methyl-heptadecasphing-4(E)-ene (C₂₂-ceramide) (3) [15], dibutyl phthalate (4)[16], cholesterol (5)[17], 5α,8α-epidioxycholest-6,22-dien-3β-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.
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 LD$_{50}$ 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](http://www.acgpubs.org/RNP)

**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.

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**Supporting Information**

Supporting Information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)
Chemical constituents of the sponge *Mycale* species

References


