A New Spongilipid from the Freshwater Sponge *Spongilla lacustris*

Jiang-Miao Hu, You-Xing Zhao, Ji-Jun Chen, Ze-Hong Miao,† and Jun Zhou*

*State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Science, Kunming 650204, P. R. China. *E-mail: jzhou@mail.kib.ac.cn*
†Division of Anti-Tumor Pharmacology, Shanghai Institute of Materia Medica, The Chinese Academy of Sciences, Shanghai 201203, P. R. China
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Sponge is an immemorial species appeared on the earth in the early Cambrian period,[1] it outspread very widely in the Jurassic and the Cretaceous period from marine to freshwater.[2] Many novel and biologically active compounds have frequently been isolated from the marine sponges these years, while research papers about freshwater sponges were lesser. Freshwater sponge *Spongilla lacustris* has been used as a traditional Chinese medicine[3,4] for near upon 500 years in China for reinforcing the kidney and supporting yang (aphrodisiac), nevertheless the pharmacy research of this species is fewer.[5] To make clear the chemical components of *Spongilla lacustris* further, the cosmopolitan species[6] were selected as our material and leading to the isolation of one new spongilipid, tetracosan-1-ol-1-*O*-*β*-*D*-gluco-pyranoside (**1**), together with ten known compounds, 1-palmitoyl-3-*β*-*D*-galactosyl-*sn*-glycerol (**2**),[7] cholesterol (**3**),[5] 5*α*-cholest-7-ene-3*β*, 6*α*-diol (**4**),[8-10] cholest-5-ene-3*β*, 7*β*-diol (**5**),[11] cholest-5-ene-3*β*, 7*α*-diol (**6**),[11] (22*E*)-cholest-5, 22-diene-3*β*, 7*α*-diol (**7**),[11] 24*ξ*-ethylcholest-5-ene-3*β*, 7*α*-diol (**8**),[12] cholest-7-ene-3*β*, 5*α*, 6*β*-triol (**9**),[13] (24*S*)-24-ethyl-cholest-7, 22-ene-3*β*, 5*α*, 6*β*-triol (**10**)[13] and (24*S*)-24-ethyl-cholest-7-ene-3*β*, 5*α*, 6*β*-triol (**11**).[13-15] The cytotoxic activity of compound **1** against two human tumor cell lines (A549 and HL-60) was also assessed and showed no activities at concentrations up to 10^{-4} mol/L.

Compound **1** was obtained as a white powder, its molecular was assigned as $C_{30}H_{59}O_6$ from the negative HR-FABMS (*m/z* 515.4298 $[M]^-$) and the NMR data, indicating one degree of unsaturation. IR spectrum exhibited absorption bands (3406 cm^{-1}) for hydroxyl. From the ^{13}C-NMR (DEPT) and MS spectrum of **1**, thirty carbons were observed as a *D*-glucopyranose moiety (*δ* 104.8, 78.7, 78.6, 75.3, 71.8, 62.9), and twenty three methylenes (*δ* 69.9, 32.2, 30.4-29.7, 26.5, 23.0) and one methyl (*δ* 14.3). The proton signal (*δ* 4.85, d, *J* = 7.7 Hz, H-1') crossed with carbon (*δ* 104.8, C-1') in the HSQC spectrum confirm the moiety to be a *β*-*D*-glucopyranose. The residual twenty four carbons with no unsaturation hint a tetracosanol group, which was proved by GC-MS spectrum of hydrolysate of **1** and ion peak (*m/z* 336 $[\mathbf{1} - Glc - H_2O]^+$) in the

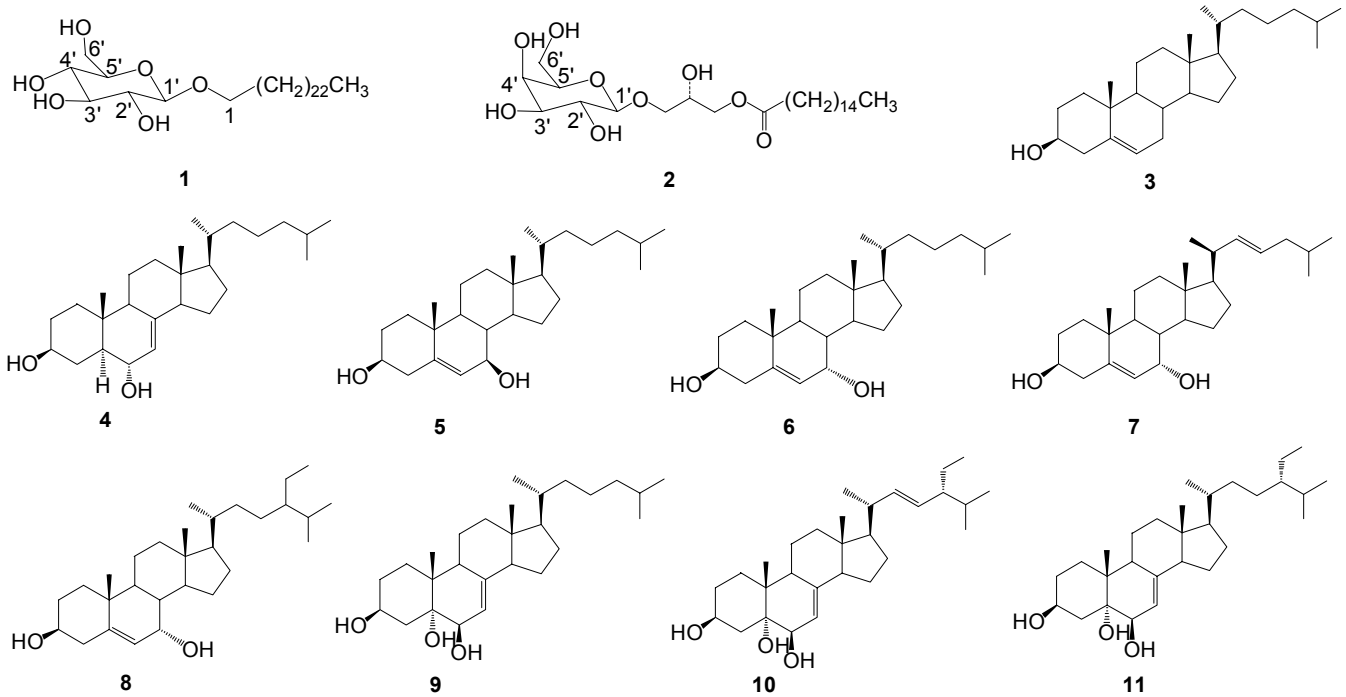

Figure 1. The structure of compounds **1**-**11**.

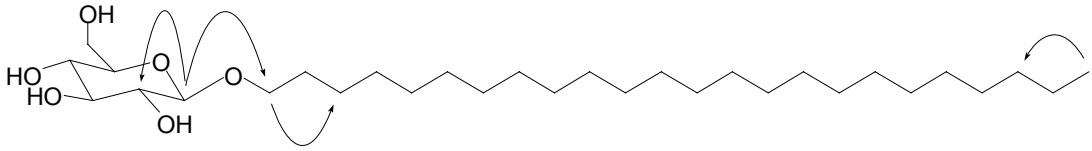

Figure 2. Key HMBC correlations of compound **1**.

EIMS spectrum of acetifying of hydrolysate of **1**. The ion peak (*m/z* 397 [**1** – Glc + AcO]$^+$) in the FABMS spectrum after hydrolyzing and acetifying of **1** proved it further. The crosspeak of atom at δ 4.85 (H-1') with carbon at δ 69.9 (C-1) in the HMBC spectrum of **1** (Fig. 2) indicated that the β-*D*-glucopyranose was linked to C-1 of the tetracosanol group. Compound **1** with four hydroxyls was further confirmed by ion peak (*m/z* 707 [**1** + 4AcO – 4H + Na]$^+$) in ESIMS after its acetification. Thus, compound **1** was determined as tetracosan-1-ol-1-*O*-β-*D*-glucopyranoside.

The ten known compounds were identified on the basis of spectroscopic analysis and comparing spectra data with literature or R_f values with authentic samples.

Experimental

General procedures. Melting points were measured on a XRC-1 micro-melting point apparatus and are uncorrected. MS spectra were obtained on a VG Auto Spec-3000 mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers, with chemical shifts (δ) in ppm relative to TMS as internal standard and coupling constants in hertz (Hz). IR spectra were measured with a Bio-Rad FTS-135 IR spectrometer with KBr pellets. UV spectra were measured on a Hitachi UV-3210 spectrophotometer. Silica gel (200-300 mesh) for column chromatography and preparative thin-layer chromatography were the products of the Qindao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Reversed-phase chromatography was with RP-18 (LiChroprep, 40-63 μm, Merck, Darmstadt, Germany).

Animal material. The dried green sponge *Spongilla lacustris* (4.5 kg) was collected from Tenchong, Yunnan Province, P. R. China, in November 2004 and identified by Prof. Li-zhen Wang, Yunnan University, Kunming, Yunnan, P. R. China. A voucher specimen (Zhai-1) is deposited at the Kunming Institute of Botany, Chinese Academy of Science, Kunming, Yunnan, P. R. China.

Extraction and isolation. The air-dried sponge (4.0 kg) was powdered and extracted three times with 80 % ethanol aq. (30 L × 3) at room temperature for 24 hours each time and filtered. The filtrate was evaporated under reduced pressure to give a residue, which was suspended in water and partitioned successively with petroleum ether, EtOAc and *n*-BuOH. The EtOAc-soluble fraction was concentrated to give a deep green gum (180.0 g) and subjected to column chromatography (CC) (silica gel, $CHCl_3$-CH_3OH, 10 : 0 → 8 : 2) to give 5 fractions A-E. Fraction B (10 g) was further purified by CC (silica gel, $CHCl_3$-CH_3OH, 92 : 8) and CC (Sephadex LH-20, $CHCl_3$-CH_3OH, 7 : 3) to afford compound **3** (1.0 g) and **4** (15 mg). Fraction C was treated same as fraction B to produce compound **5** (8 mg) and a white crystal (40 mg), the crystal was further separate by semi preparative HPLC (Agilent ODS-C18, CH_3OH-H_2O, 91 : 9) to produce compound **6** (5 mg), **7** (6 mg) and **8** (4 mg). Fraction D was treated same as fraction C and afford compound **9** (4 mg), **10** (5 mg) and **11** (4 mg). The *n*-BuOH soluble fraction was concentrated to give a deep brown gum (100.0 g), part of which (95.0 g) was subjected to CC (silica gel, $CHCl_3$-CH_3OH, 9 : 1 → 7 : 3) to give eight fractions (I-VIII). Fraction III (9.2 g) was further chromatographed on silica gel by $CHCl_3$-CH_3OH-H_2O (80 : 20 : 5) to afford nine fractions III-1–III-9. Fraction III-5 (230 mg) was purified by CC (Sephadex LH-20, MeOH) and CC (RP-18, MeOH-H_2O, 4 : 6) to produce compound **1** (20 mg). Fraction III-7 (150 mg) was treated same as III-5 to yield compound **2** (6 mg).

Lacustrisglycoside A (**1**): White powder, m.p. 82-84 ºC; $[\alpha]_D^{22} = -4.3$ (*c* 1.04, pyridine); UV (MeOH) λ_{max} (log ε): 202.0 (6.11) nm; IR (KBr) ν_{max}: 3406 (OH), 2919, 2850, 1471, 1374, 1255, 1170, 1098, 1074, 1038, 719, 652 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) δ 4.85 (d, J = 7.7 Hz, 1H, H-1'), 4.57 (brd, J = 10.4 Hz, 1H, H-6'α), 4.40 (dd, J = 10.4, 5.3 Hz, 1H, H-6'β), 4.26 (m, 1H, H-5'), 4.25 (m, 1H, H-4'), 4.08 (dd, J = 9.0, 7.2 Hz, 1H, H-1α), 4.05 (dd, J = 7.7, 8.0 Hz, 1H, H-2'), 3.97 (m, 1H, H-3'), 3.66 (dd, J = 9.0, 7.2 Hz, 1H, H-1β), 1.65 (m, 2H, H-2), 1.30-1.19 (m, 42H, H-3-23), 0.85 (t, J = 6.0 Hz, 3H, H-24); ^{13}C NMR (100 MHz, C_5D_5N) δ: 104.8 (C-1'), 78.7 (C-5'), 78.6 (C-3'), 75.3 (C-2'), 71.8 (C-4'), 69.9 (C-1), 62.9 (C-6'), 32.2 (C-22), 30.4-29.7 (C-4-21), 29.9 (C-2), 26.5 (C-3), 23.0 (C-23), 14.3 (C-24); FABMS (negative ion) *m/z* 515 (59) [M-H]$^+$, 473 (100); HR-FABMS (negative ion) *m/z* 515.4298 [M]$^-$ (calcd. for $C_{30}H_{59}O_6$, 515.4311).

Acetylate and hydrolysis. The sample (compound **1**, 2 mg) was dissolved in MeOH/H_2O (8 : 2) in a 50 mL rockered flask and 5 drops of HCl (36%) were added. After refluxed at 80 ºC for 4 hours, the hydrolysate was allowed to cool and separated successfully between petroleum ether and MeOH/H_2O (8 : 2). The petroleum ether layer was concentrated and sent for EIMS and GC-MS. The residual petroleum ether layer dissolved in Ac_2O/pyridine (6 : 1) in a sealed micro-flask and reacted at 60 ºC for 4 hours, and then the acetic reactant was subjected to positive FABMS analysis.

The sample (compound **1**, 1 mg) was dissolved in Ac_2O/pyridine (6:1) in a sealed micro-flask and reacting at 60 ºC for 4 hours, then the reactant was subjected to positive ESIMS analysis.

Cytotoxic assay. Compound **1** was tested for its cytotoxic effects against human lung carcinoma A549 and human leukemia HL-60 cell lines using the sulforhodamine B (SRB)

assay and the methyl-thiazol-tetrozolium (MTT) assay, respectively.

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