A New Spongil lipid from the Freshwater Sponge *Spongilla lacustris*

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Sponge is an innumerable 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 medicine3,4 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 species6 were selected as our material and leading to the isolation of one new spongil lipid, tetracosan-1-ol-1-2-2-D-glucopyranoside(1), together with ten known compounds. 1-palmitoyl-3-β-D-galactosyl-

[Diagram of compounds 1-11]

Figure 1. The structure of compounds 1-11.
EIMS spectrum of acetylyzing of hydrolysate of 1. The ion peak ($m/z$ 397 [1 - Glc + AcO] ) in the FABMS spectrum after hydrolyzing and acetylyzing of 1 proved it as correct. 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 acetylyzing. 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 $	ext{R}^2$ 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 FT-S-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 Qundao 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, CHCl₃-CH₂OH, 10 : 0 → 8 : 2) to give 5 fractions A-E. Fraction B (10 g) was further purified by CC (silica gel, CHCl₃-CH₂OH, 92 : 8) and CC (Sephadex LH-20. CHCl₃-CH₂OH, 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. CH₃OH-H₂O, 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, CHCl₃-CH₂OH, 9 : 1 → 7 : 3) to give eight fractions (I-VIII). Fraction III (9.2 g) was further chromatographed on silica gel with CHCl₃-CH₂OH-H₂O (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-H₂O, 4 : 6) to produce compound 1 (20 mg). Fraction III-7 (150 mg) was treated same as III-5 to yield compound 6 (6 mg).

Lacustrisglycoside A (1): White powder, m.p. 82-84 °C, [α]D$_{20}^{\text{p}}$ = -4.3 (c 1.04, pyridine); UV (MeOH) λ$_{max}$ (log ε) 202.0 (6.11) nm; IR (KBr) 3460 (OH), 2919, 2850, 1471, 1374, 1255, 1170, 1098, 1074, 1038, 719, 652 cm$^{-1}$; $^1$H NMR (400 MHz, CD$_3$N$_2$) δ 4.85 (d, $J$ = 7.7 Hz, 1H, H-1'), 4.57 (br, $J$ = 10.4 Hz, 1H, H-6'a), 4.40 (dd, $J$ = 10.4, 5.3 Hz, 1H, H-6'b), 4.26 (m, 1H, H-5'), 4.25 (m, 1H, H-4'), 4.05 (dd, $J$ = 9.0, 7.2 Hz, 1H, H-1'a), 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-1'b), 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, CD$_3$N$_2$) δ 104.8 (C-1'), 78.7 (C-5'), 75.3 (C-2'), 37.1 (C-4'), 49.9 (C-1), 62.9 (C-6), 32.2 (C-22), 30.4 (C-9), 29.7 (C-21), 29.9 (C-2), 26.5 (C-23), 23.0 (C-24), 14.3 (C-25). FABMS (negative ion) $m/z$ 515 [M-H]$^-$. 473 (100). HR-FABMS (negative ion) $m/z$ 515.4289 [M]$^-$ (calcld. for C$_{25}$H$_{34}$O$_{5}$, 515.4311).

Acetylate and hydrolysis. The sample (compound 1, 2 mg) was dissolved in MeOH-H$_2$O (8 : 2) in a 50 mL round flask and 5 drops of HCl(36%) were added. After refluxed at 80 °C for 4 hours, the hydrolysate were allowed to cool and separated successively between petroleum ether and MeOH-H$_2$O (8 : 2). The petroleum ether layer was concentrated and sent for EIMS and GC-MS. The residual petroleum ether layer dissolved in Ac$_2$O/pyridine (6 : 1) in a sealed micro-flask and reacting at 60 °C for 4 hours, and then the acetic reagent was subjected to positive FABMS analysis.

The sample (compound 1, 1 mg) was dissolved in Ac$_2$O/pyridine (6 : 1) in a sealed micro-flask and reacting at 60 °C 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-tetrazolium (MTT) assay, respectively.

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References