Supporting Information

Rec. Nat. Prod. **7:2 (2013) 119-123**

Chemical Constituents of the sponge *Mycale* species from South China Sea

Xuefeng Zhou[1], Xiuping Lin[1], Xieyang Guo[2], Bin Yang[1], Xian-Wen Yang[1] and Yonghong Liu[1*]

[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

Table of Contents **Page**

S1: Identification of compounds 1–11 2
S2: Brine shrimp larvae toxicity test 4
S3: Acetylcholinesterase inhibitory activity test 4

[*] Corrosponding author: E-mail: yonghongliu@scsio.ac.cn (Liu Y)

Identification of compounds 1–11

Henicosanoic acid methyl ester (**1**). Colorless oil; ESI(+)-MS: [*m/z* 341 $[M+H]^+$]; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.65 (3H, s, OCH_3), 2.28 (2H, t, *J* = 6.5 Hz, H-2), 1.62 (2H, m, H-3), 1.22~1.27(34H, H-4~20), 0.88 (3H, t, *J* = 7.0 Hz, H-21). The structure was confirmed by comparison with literature data [13].

Hexadecyl ethers of glycerol (**2**). Colorless oil; ESI(+)-MS: [*m/z* 317 $[M+H]^+$]; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.87 (1H, m, H-2), 3.69 (2H, m, H-3), 3.45~3.55 (4H, m, H-1, H-1′), 1.57 (2H, m, H-2′) 1.22~1.27 (26H, H-3′~15′), 0.88 (3H, t, *J* = 7.5 Hz, H-16′). The structure was confirmed by comparison with literature data [14].

N-Docosanoyl-D-erythro-(2S,3R)-16-methyl-heptadecasphing-4(E)-enine (C_{22}-ceramide) (**3**). White amorphous powder ($CHCl_3$); ESI(+)-MS: [*m/z* 623 $[M+H]^+$]; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.67 (1H, dd, *J* = 3.0, 11.0 Hz, H-1a), 3.93 (dd, *J* = 3.5, 11.0 Hz, H-1b), 3.88 (1H, m, H-2), 4.30 (1H, dd, *J* = 3.5, 6.5 Hz, H-3), 5.50 (1H, dd, *J* = 6.5, 15.5, H-4), 5.75 (1H, dt, *J* = 7.5, 15.5 Hz, H-5), 2.02 (2H, m, H-6); 1.82 (2H, m, H-7), 1.20~1.26 (14H, brs, H-8~14), 2.50 (2H, m, H-15), 1.56 (m, H-16), 0.85 (6H, d, *J* = 7.0 Hz, H-17, H-18), 2.26 (2H, t, *J* = 7.5 Hz, H-2′) 1.63 (2H, m, H-3′), 1.20~1.26 (36H, brs, H-4′~21′), 0.89 (3H, t, *J* = 7.0 Hz, H-22′), 6.20 (1H, d, *J* = 7.5 Hz, NH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 62.56 (C-1), 54.56 (C-2), 74.75 (C-3), 128.85 (C-4), 134.33 (C-5), 32.27 (C-6), 31.93 (C-7), 29.71 (C-8~13), 29.96 (C-14), 39.10 (C-15), 27.97 (C-16), 22.66 (C-17, C-18), 173.80 (C-1′), 36.86 (C-2′), 25.76 (C-3′), 29.72 (C-4′~20′), 22.70 (C-21′), 14.10 (C-22′). The structure was confirmed by comparison with literature data [15].

Dibutyl phthalate (**4**). Colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.48 (2H, dd, *J* = 9.0, 2.0 Hz, H-3, H-6), 7.26 (2H, dd, *J* = 9.0, 2.5 Hz, H-4, H-5), 4.07 (4H, t, *J* = 6.5 Hz, H-1′, H-1″), 1.47 (4H, m, H-2′, H-2″), 1.19 (4H, m, H-3′, H-3″), 0.72 (6H, t, *J* = 7.5 Hz, H-4′, H-4″); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.5 (C-1, C-8), 131.7 (C-2, C-7), 127.9 (C-3, C-6), 130.1 (C-4, C-5), 64.5 (C-1′, C-1″), 29.8 (C-2′, C-2″), 18.3 (C-3′, C-3″), 12.7 (C-4′, C-4″). The structure was confirmed by comparison with literature data [16].

Cholesterol (**5**). Colorless needle crystal ($CHCl_3$); the compound was identified by co-TLC

comparison with cholesterol we obtained and reported previously [17].

5α,8α-Epidioxycholest-6,22-dien-3β-ol (**6**). Colorless needle crystal ($CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.47 (1H, d, J = 8.5 Hz, H-7), 6.20 (1H, d, J = 8.5 Hz, H-6), 5.28 (1H, dd, J = 15.0, 7.0 Hz, H-22), 5.17 (1H, dt, J = 15.0, 8.5 Hz, H-23), 3.92 (H, m, H-3), 0.98 (3H, d, J = 6.5 Hz, H-21), 0.84 (3H, s, H-19), 0.82 (3H, d, J = 6.5 Hz, H-27), 0.80 (3H, d, J = 6.5 Hz, H-26), 0.76 (3H, s, H-18); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 34.88 (C-1), 30.30 (C-2), 66.54 (C-3), 37.11 (C-4), 82.24 (C-5), 135.60 (C-6), 130.94 (C-7), 79.51 (C-8), 51.26 (C-9), 37.12 (C-10), 23.60 (C-11), 39.52 (C-12), 44.64 (C-13), 51.87 (C-14), 20.82 (C-15), 28.94 (C-16), 56.30 (C-17), 13.04 (C-18), 18.38 (C-19), 39.86 (C-20), 20.94 (C-21), 137.65 (C-22), 127.02 (C-23), 42.10 (C-24), 28.73 (C-25), 22.47 (C-26), 22.52 (C-27). The structure was confirmed by comparison with literature data [18].

5-Hexadecyl-pyrrole-2-carboxaldehyde (**7**). Pale white flakes. ESI(+)-MS: [*m/z* 320 $[M+H]^+$]; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 10.50 (1H, brs, NH), 9.35 (1H, s, CHO), 6.91 (1H, m, H-3), 6.08(1H, m, H-3), 2.68 (2H, t, J = 7 Hz, H-6), 1.65 (2H, m, H-7), 1.22~1.27 (26H, brs, H-8~20), 0.86 (3H, t, J = 7.0 Hz, H-21). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 131.82 (C-2), 123.18 (C-3), 109.41 (C-4), 144.09 (C-5), 31.89 (C-6), 29.92 (C-7), 29.63~27.80 (C-8~19), 22.64 (C-20), 14.04 (C-21), 178.05 (CHO). The ^{1}H-NMR and ^{13}C-NMR data were similar with those of 5-alkylpyrrole-2-carboxaldehyde derivatives reported in the literatures [5][12], and the ESI(+)-MS: [*m/z* 320 $[M+H]^+$] analysis suggest the compound was 5-hexadecyl-pyrrole-2-carboxaldehyde.

Benzoic acid (**8**). Colorless needle crystal (MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 11.7 (1H, brs, COOH), 8.13 (2H, d, J = 8.0 Hz, H-3, H-7), 7.62 (1H, dd, J = 7.5 Hz, H-5), 7.47 (2H, dd, J = 7.5, 8.0 Hz, H-4, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 172.3 (C-1), 129.1 (C-2), 130.1 (C-3, C-7), 128.4 (C-4, C-6), 133.7 (C-5). The structure was confirmed by comparison with literature data [19].

4-Hydroxybenzoic acid (**9**). Colorless needle crystal ($CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.78 (2H, d, J = 8.5 Hz, H-2, H-6), 6.78 (2H, d, J = 8.5 Hz, H-3, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.4 (COOH), 162.5 (C-4), 132.5(C-2, C-6) , 122.7 (C-1), 116.0 (C-3, C-5). The

structure was confirmed by comparison with literature data [20].

Thymine (**10**). White amorphous powder (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.00 (1H, brs, 3-NH), 10.56 (1H, brs, 1-NH), 7.23 (1H, s, H-4) , 1.72 (3H, s, 5-CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 163.6 (C-6), 151.0 (C-2), 140.2 (C-4), 107.1 (C-5) , 18.1 (5-CH_3). The structure was confirmed by comparison with literature data [21].

Uracil (**11**). White amorphous powder (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.00 (1H, brs, 3-NH), 10.56 (1H, brs, 1-NH), 7.38 (1H, d, J = 7.6 Hz, H-4) , 5.44 (1H, d, J =7.6 Hz, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 164.1 (C-6), 151.3 (C-2) , 142.0 (C-4) , 100.1 (C-5). The structure was confirmed by comparison with literature data [21].

Brine shrimp larvae toxicity test

The test samples (pure compounds) were dissolved in DMSO and added to test tubes containing 10 brine shrimps in simulated brine water (5 mL), with the final test concentrations as 25, 12.5, 6.25, 3.125, 1.563, and 0.781 μg/mL serially. Simulated brine water with same amount of DMSO was used as negative control and tacrine as positive control. After incubated at room temperature for 24 h, median lethal concentrations (LC_{50}) of the test samples were obtained by plotting the percentage of dead shrimp against the logarithm of the sample concentrations.

Acetylcholinesterase inhibitory activity test

The AChE inhibitory activities were measured according to Ellman's coupled enzyme assay with some modifications as described. In the experiment, 0.2 units of AChE dissolved in 0.1 M potassium phosphate buffer (pH 7.4) and test compounds dissolved in DMSO were added to each well of a 96-well plate, with the final test concentrations as 25, 12.5, 6.25, 3.125, 1.563, and 0.781 μg/mL serially.. Then, 50 μM acetylthiocholine iodide and 50 μM 5,5'-dithiobis(2-nitrobenzoic acid) dissolved in 0.1 M potassium phosphate buffer (pH 7.4) were added to each well. Potassium phosphate buffer with same amount of DMSO was used as negative control and tacrine as positive control. The reaction was carried out at 30° C for 30 min. The absorbance was measured at 410 nm using a spectrophotometer and the median inhibitory concentration (IC_{50}) was calculated.