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COMPOUNDS FROM CULTURE BROTH OF MARINE BACTERIUM *OCEANISPHAERA* sp.

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Abstract

Eight compounds were isolated and characterized from the culture broth of the marine bacterium *Oceanisphaera* sp. strain, which was isolated from the sediment collecting at Halong Bay. The structures of all isolates were determined by spectroscopic analysis including MS and 2D NMR, as well as by comparison with reported data in the literature.

Keywords. *Oceanisphaera* sp., marine bacterium, 1,5-Dideoxy-3-C-methyl-arabinitol, Cyclo-(Pro-Gly), (*2S,4S*)-4-Hydroxyproline.

1. INTRODUCTION

Bacteria of the genus *Oceanisphaera* are gram-negative aerobic, moderately halophilic. Currently the genus of *Oceanisphaera* comprises only two validly described species: *O. litoralis* and *O. donghaensis* [1-3]. An overview on the literature showed that no study on secondary metabolite was reported for this genus. In a course of our screening program, an *Oceanisphaera* sp. species isolated from marine sediment of the Halong Bay displayed an antimicrobial activity. In this paper, we reported the isolation and structural characterization of eight compounds **1-8** from the culture broth of *Oceanisphaera* sp..

2. EXPERIMENTAL

2.1. General procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Japan). The ESI-MS was measured on Agilent 6120 series single quadrupole LC/MS systems (USA). NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. Column chromatography (CC) was performed using a silica gel (Kiesel gel 60, 70-230 mesh and 230-400 mesh, Merck, Germany). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (Merck, Germany).

2.2. Bacteria isolation and fermentation

Oceanisphaera sp. was isolated from marine sediment which was collected in Halong Bay in August 2013. Strain *Oceanisphaera* sp. was cultured in high-nutrient medium (30 g of instant ocean, 10 g of starch, 4 g of yeast, 2 g of peptone, 1 g of calcium carbonate, 40 mg of iron sulfate, and 100 mg of potassium bromate) for 7 days at 25 °C while shaking at 200 rpm.

2.3. Extraction and isolation

Culture broth of *Oceanisphaera* sp. (10 L) was extracted with EtOAc (5 x 10 L). After removal of solvent under reduced pressure, a residue (0.67 g) was obtained. The water solution was concentrated to dryness and washed with methanol. The methanol solution was concentrated to obtain a residue of 54 g.

The EtOAc extract was separated by column chromatography (CC) on Sephadex LH-20, eluted with MeOH to afford 7 fractions. Fraction 3 (280 mg) was purified by CC on silica gel, eluted with *n*-hexane/EtOAc gradient to give **2** (9 mg) and **1** (8 mg).

In methanol soluble constituents (54 g) were subjected to CC eluted with CH_2Cl_2/MeOH gradient

to afford 13 fractions.

Fraction 3 (450 mg) was subjected to CC on silica gel, eluted with CH_2Cl_2/MeOH (98/2) to give **3** (25 mg).

Fraction 5 (490 mg) was separated by CC on silica gel, eluted with CH_2Cl_2/MeOH (97/3) to afford 5 subfractions. Subfraction 3 (120 mg) was recrystallized in CH_2Cl_2 to furnish **7** (35 mg).

Fraction 6 (530 mg) was subjected to CC on silica gel, eluting with CH_2Cl_2/MeOH (95/5) to afford 5 subfractions. Subfraction 4 (45 mg) was recrystallized in CH_2Cl_2 to yield **6** (3 mg).

Fraction 13 (5.4 g) was subjected to a silica gel CC, using EtOAc/MeOH/NH_3 (60/40/2) to give 10 subfractions. Subfraction 8 was purified on Sephadex LH-20 CC, eluted with MeOH to give **8** (11 mg). Subfraction 4 separated on a silica gel CC, using EtOAc/MeOH/NH_3 (40/60/2) following by preparative TLC to afford **5** (9 mg). Subfraction 5 was purified by silica gel CC using CH_2Cl_2/EtOH/NH_3 (60/40/2) to give **4** (15 mg).

2,3-Butanediol (1): Oily, colorless; $[\alpha]_D^{25}$ -13.6 (*c* 0.25, MeOH); ESI-MS: *m/z* 91 $[M+H]^+$ ($C_4H_{10}O_2$). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm): 1.16 (3H, d, *J* = 6.0 Hz); 2.77 (2H, brs. -OH); 3.49 (2H, q, *J* = 4.0; 6.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 19.3 (2 CH_3); 72.5 (2 CH).

1,5-Dideoxy-3-C-methyl-arabinitol (2): White amorphous solid, m.p 156-158^{o}C, ESI-MS: *m/z* 135 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 1.00 (3H, s ,CH_3-3); 1.16 (3H, d, *J* = 6.5 Hz, H-1); 1.23 (3H,d, *J* = 6.5 Hz, H-5); 3.74 (1H, q, *J* = 6.5; 13.5 Hz, H-2); 3.97 (1H, q, *J* = 6.5; 13.0 Hz, H-4). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C (ppm) 17.1 (C-1); 18.0 (C-5); 20.6 (CH_3 -3); 70.4 (C-2); 74.9 (C-4); 75.4 (C-3).

Cyclo-(Pro-Gly) (**3**): White powder; mp. 210-211 ^{o}C; $[\alpha]_D^{25}$ -142.5^o (*c* 0.40; MeOH), ESI-MS: *m/z* 155 $[M+H]^+$ ($C_7H_{11}N_2O_2$), ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm) 1.95-2.05 (3H, m, H-4,5a), 2.35 (1H, m, H-5b), 3.57 (2H, m, H-3), 3.75 (1H, d, *J* = 17.0 Hz, H-9a), 4.11 (1H, td, *J* = 1.0, 17.0 Hz, H-9b), 4.24 (1H, dt, *J* = 2.0, 9.0 Hz, H-6).

(*2S,4S*)-4-Hydroxyproline (4): White powder, mp. 248 ^{o}C; $[\alpha]_D^{25}$ -71.2 (c 0,125; H_2O). ESI-MS: *m/z* 132 $[M+H]^+$ ($C_5H_9NO_3$); ^{1}H-NMR (500 MHz, CD_3OD&D_2O): δ_H (ppm): 2.12 (1H, qd, *J* = 4.0; 10.0; 14.0 Hz, H-3a); 2.43 (1H, qt, *J* = 2.0; 8.0; 13.5; 2.0 Hz, H-3b); 3.29 (1H, dt, *J* = 11.0; 1.5 Hz, H-1a); 3.43 (1H, dd, *J* = 3.5; 11.0 Hz, H-1b); 4.24 (1H, dd, *J* = 8.0; 9.0 Hz, H-4); 4.58 (1H, m, H-2); ^{13}C-NMR (125 MHz, CD_3OD & D_2O): δ_C (ppm): 39.2 (C-3); 54.3 (C-1); 61.3 (C-4); 71.4 (C-2); 174.7 (C=O).

Betaine (5): White powder; ESI-MS: *m/z* 118 $[M+H]^+$ $C_5H_{11}NO_2$; ^{1}H-NMR (500 MHz, CD_3OD): δ_H (ppm): 3.85 (s, 2H); 3.33 (9H, s, $3CH_3$); ^{13}C-NMR (125 MHz, CD_3OD): δ_C (ppm): 53.8 ($3CH_3$); 67.3 (CH_2); 168.7 (C=O).

Uracil (**6**): White powder; mp. 320-323^{o}C; ESI-MS (negative): *m/z* 111 $[M-H]^-$ ($C_4H_4N_2O_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H (ppm): 5.42 (1H, d, *J* = 7.5 Hz, H-5); 7.38 (1H, d, *J* = 7.5 Hz, H-6); 10.96 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C (ppm): 100.3 (C-5), 142.3 (C-6), 151.62 (C-2), 164.5 (C-4).

Acetamide (7): white needle crystal, mp. 81-83^{o}C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H (ppm) 1.96 (3H,s, CH_3); 5.96 (brs, 1H); 6.12 (brs, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 22.5 (CH_3); 173.3 (C=O).

2-Aminopropanoic acid (8): white needle crystal, mp.76-77 ^{o}C; ^{1}H-NMR (500 MHz, D_2O): δ_H (ppm) 1,36 (3H, d, *J*=7.5 Hz, CH_3); 3.67 (1H, q, *J* =7.5 Hz, H-2); ^{13}C-NMR (125 MHz, D_2O): δ_C (ppm): 16.1 (CH_3); 50.5 (C-2); 175.8 (C=O).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as colorless oil and optically, $[\alpha]_D^{25}$ -13.6 (*c* 0.25, MeOH). Its ESI-MS presented the protonated molecular ion at *m/z* 91 $[M+H]^+$. The ^{13}C-NMR and DEPT spectra of **1** presented the signals of methyl (δ_C 19.3) and methine (δ_C 72.5) groups. The ^{1}H-NMR spectrum in $CDCl_3$ of **1** showed the presence of a doublet methyl (δ_H 1.16, *J* = 6.0 Hz), proton of a methine (δ_H 3.49) and an exchangable proton at δ_H 2.77. This observation indicated compound **1** had a symmetric structure. The chemical shifts of methine goups suggested their linkage to oxygens. The structure of **1** was then confirmed by analyses of 2D-NMR spectra which allowed establishing as 2,3-butan-diol. Comparison of optical activity of **1** with that of *meso*-butan-diol revealed their identical configuration [4, 5].

^{1}H-NMR spectrum of **2** presented signals of a singlet methyl (δ_H 1.00), two doublet methyls at δ_H 1.16 (d, *J* = 6.5 Hz, CH_3-1), 1.23 (d, *J* = 6.5 Hz, CH_3-5) and two methine protons at δ_H 3.74 (q, *J*= 6.5; 13.5 Hz, H-2), 3.97 (q, *J* = 6.5; 13.0 Hz, H-4). The ^{13}C-NMR and DEPT spectra of **2** exhibited signals of the groups observed in the ^{1}H-NMR with additional signal of an oxygenated quaternary carbon at δ_C 75.4 (C-3). Detailed analyses of NMR spectra indicated the structure of **2** as 1,5-dideoxy-3-C-methyl-arabinitol [6].

Compound **3** was obtained as white solids and optically active, $[\alpha]_D^{25}$ -142.5 (*c* 0.40, MeOH). Its

ESI-MS showed the protonated molecular ion at *m/z* 155 $[M+H]^+$. The ^{1}H-NMR presented signals of a methine at δ_H 4.24 (dt, *J* = 6.5, 9.0 Hz, H-6) and four methylene groups at δ_H from 1.95 to 4.11. Comparison of the ^{1}H-NMR spectrum and TLC of **2** with [cyclo-(Pro-Gly)] which was available in our laboratory revealed their similarity. By comparison of optical activity with *S*-[cyclic(Pro-Gly)] (negative value) indicated the *S*-configuration for **3**. Thus, **3** was determined as *S*-[cyclo-(Pro-Gly)] [7].

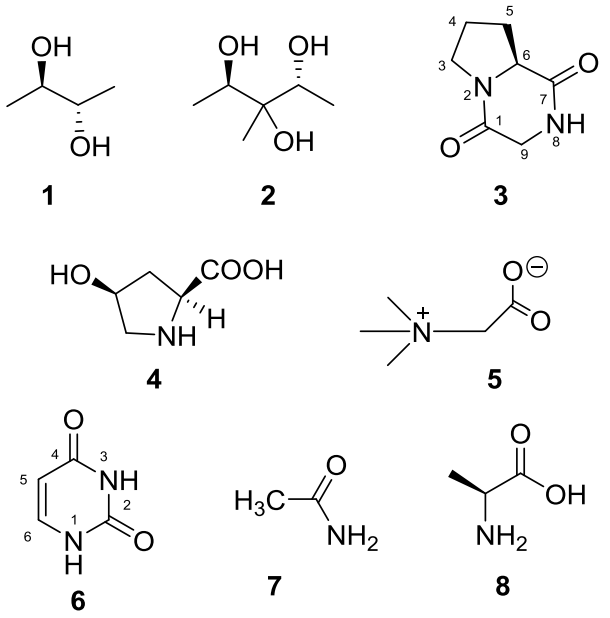

Figure 1: Isolated compounds from marine bacteria *Oceanisphaera* sp.

Compound **4** was isolated as white solids and optically active, $[\alpha]_D^{25}$ -71.2 (c 0.125, H_2O). 1D-NMR spectrum of **4** showed signals of two methine, two methylenes groups and a carboxyl group (δ_C 174.7). The chemical shifts of CH-2 (δ_C 74.8, δ_H 4.58) suggested its connection to oxygen and those of CH_2-1 (δ_C 54.3, δ_H 3.29, 3.43) and CH-4 (δ_C 61.3, δ_H 4.24) suggesting their linkages to nitrogens. Complete analyses of the NMR spectra and comparison of optical activity with the literature [8] indicated the structure of **4** as (*2S,4S*)-4-hydroxyproline.

^{1}H-NMR spectrum of **5** indicated signals of a methylene as singlet at δ_H 3.85 and three identical methyl groups at δ_H 3.33. The ^{13}C-NMR and DEPT spectra of **5** presented additional signal of a carboxyl group at δ_H 168.7. The chemical shifts of methyl and methylenes suggested their linkages to nitrogens. From the above NMR data, the structure of **5** was established as betaine. The NMR data of **5** were in agreement with those reported in the literature [9].

From analyses of NMR data and comparison with the literature, compounds **6-8** were dertermined as uracil [10-12], acetamide [13] and alanine [14], respectively.

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