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 silica gel (Kiesel gel 60, 70-230 mesh and 230-400 mesh, Merck, Germany). Thin layer chromatography (TLC) used pre-coated silica gel 60 F₂₅₄ (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 CH₂Cl₂/MeOH gradient.
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3. RESULTS AND DISCUSSION

Compound 1 was isolated as colorless oil and optically, [α]D
-13.6 (c 0.25, MeOH). Its ESI-MS presented the protonated molecular ion at m/z 91 [M+H]+. The 13C-NMR and DEPT spectra of 1 presented the signals of methyl (δc 19.3) and methine (δc 72.5) groups. The 1H-NMR spectrum in CDCl3 of 1 showed the presence of a doublet methyl (δH 1.16, J = 6.0 Hz), proton of a methine (δH 3.49) and an exchangeable proton at δH 2.77. This observation indicated compound 1 had a symmetric structure. The chemical shifts of methine groups 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].

1H-NMR spectrum of 2 presented signals of a singlet methyl (δH 1.00), two doublet methyls at δH 1.16 (d, J = 6.5 Hz, CH2-1), 1.23 (d, J = 6.5 Hz, CH2-5) and two methine protons at δH 3.74 (q, J = 6.5; 13.5 Hz, H-2), 3.97 (q, J = 6.5; 13.0 Hz, H-4). The 13C-NMR and DEPT spectra of 2 exhibited signals of the groups observed in the 1H-NMR with additional signal of an oxygenated quaternary carbon at δc 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, [α]D
-142.5 (c 0.40, MeOH). Its...
ESI-MS showed the protonated molecular ion at m/z 155 [M+H]⁺. The ¹H-NMR presented signals of a methine at δH 4.24 (dt, J = 6.5, 9.0 Hz, H-6) and four methylene groups at δH from 1.95 to 4.11. Comparison of the ¹H-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-[cyclic-(Pro-Gly)] [7].

![Compounds from culture broth](image)

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

Compound 4 was isolated as white solids and optically active, [α]D 25 -71.2 (c 0.125, H₂O). 1D-NMR spectrum of 4 showed signals of two methine, two methylene groups and a carboxyl group (δC 174.7). The chemical shifts of CH-2 (δC 74.8, δH 4.58) suggested its connection to oxygen and those of CH₂-1 (δC 54.3, δH 3.29, 3.43) and CH-4 (δC 61.3, δH 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.

¹H-NMR spectrum of 5 indicated signals of a methylene as singlet at δH 3.85 and three identical methyl groups at δH 3.33. The ¹³C-NMR and DEPT spectra of 5 presented additional signal of a carboxyl group at δH 168.7. The chemical shifts of methyl and methylene 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 determined as uracil [10-12], acetamide [13] and alanine [14], respectively.

REFERENCES


5. Meso-2,3-butanediol was purchased from Aldrich.


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14. Comparison with the commercial sample.

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