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A NOVEL BROMOPHENOL FROM MARINE RED ALGA *Symphyocladia latiuscula*

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*2,3,6-Tribromo-4,5-dihydroxybenzyl ethyl ether (**1**), a new bromophenol, was isolated from the ethanol extract of marine red alga* Symphyocladia latiuscula*, with a known compound, 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (**2**). Their structures were elucidated by spectroscopic analysis, including high-resolution mass spectroscopy, and 1 and 2-dimensional NMR techniques. Compounds **1** and **2** showed inhibitory activity against* Staphyloccocus aureus *with IC_{50} 102 and 50 μg/mL, respectively.*

Key words: *Symphyocladia latiuscula*, bromophenol, *Staphyloccocus aureus*.

Symphyocladia latiuscula is a marine red algal species belonging to the family Rhodomelaceae, order Ceramiales, that is distributed mainly in Korea, Japan, and the north part of the Chinese coast. Some bromophenols have been isolated from this species that possess aldose reductase inhibitory activity [1], antibacterial activity [2], and free-radical-scavenging activity [3–6]. In the course of our search for biologically active constituents of marine algae from Chinese coast, we have shown that the ethanol extract and EtOAc fraction of *Symphyocladia latiuscula* possess anti-*Staphyloccocus aureus* activity. Therefore, the EtOAc fraction was subject to further chemical investigation, which resulted in the isolation and identification of two bromophenols: 2,3,6,-tribromo-4,5-dihydroxybenzyl ethyl ether (**1**) and the known compound 2,3,6,-tribromo-4,5-dihydroxybenzyl methyl ether (**2**). Herein, we report the isolation, structure elucidation, and antimicrobial activity of these bromophenols.

Compound **1** was obtained as a brown gum. The IR spectrum showed absorptions at 3493, 1582, and 1548 cm^{-1}, suggesting the presence of phenolic hydroxyl and aromatic rings. The ESIMS of **1** exhibited a characteristic tribrominated pseudomolecular ion peak cluster at *m/z* 401/403/405/407 $[M–H]^-$ (1:3:3:1), and the HRESIMS at *m/z* 400.8018 $[M–H]^-$ established the molecular formula $C_9H_9Br_3O_3$. The ^{13}C NMR and DEPT spectrum showed six quaternary carbons [δ_C 130.5 (s, C-1), 113.7 (s, C-2), 114.3 (s, C-3), 143.8 (s, C-4), 145.3 (s, C-5), 118.9 (s, C-6)], two oxymethylene [δ_C 66.4 (t, C-8), δ_C 74.2 (t, C-7)], and one methyl carbon signal [δ_C 15.5 (q, C-9)]. Besides a pair of characteristic proton spin-coupling systems attributed to one ethoxyl unit at δ_H 3.58 (2H, q, J = 7.0 Hz, H-8) and 1.17 (3H, t, J = 7.0 Hz), the ^1H NMR spectrum showed another singlet at δ_H 4.82 (2H, s, H-7), which was assigned to the oxymethylene protons of the fully substituted benzene ring. The protonated carbons and their corresponding protons were unambiguously assigned by the HSQC experiment. The $^1H–^1H$ COSY showed a cross peak for H_2-8 and H_3-9, which confirmed the ethoxyl group. In the HMBC spectrum, the correlations from H-7 to C-1, C-8, and C-9 established the connections from C-7 to C-8 by an oxygen atom and the attachment of the ethyloxymethylene group to C-1. In combination with the chemical shift values of the carbons and biogenetic considerations in which the bromophenols from *Symphyocladia latiuscula* are characterized by a 2,3,6-tribromo-4,5-dehydroxy moiety. Compound **1** was established as 2,3,6-tribromo-4,5-dihydroxybenzyl ethyl ether.

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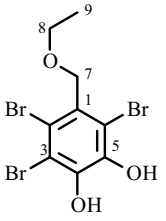
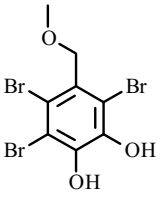
TABLE 1. ^{1}H and ^{13}C NMR Spectral Data of Compound **1**, J/Hz*

C atom	δ_C	δ_H	HMBC
1	130.5 s		
2	113.7 s		
3	114.3 s		
4	143.8 s		
5	145.3 s		
6	118.9 s		
7	74.2 t	4.82 (2H, s)	C-1, C-2, C-6
8	66.4 t	3.58 (2H, q, J = 7.0)	C-7, C-9
9	15.5 q	1.17 (3H, t, J = 7.0)	C-8

*Mutiplicities and coupling constants are in parentheses; assignment based on HMBC and HMQC experiments.



1



2

The ESIMS of **2** exhibited a characteristic tribrominated pseudomolecular ion peak cluster at *m/z* 387/389/391/393 $[M–H]^-$ (1:3:3:1). The ^{13}C NMR and DEPT spectrum showed a similar fully substituted benzene ring [δ_C 130.5 (s, C-1), 113.7 (s, C-2), 114.3 (s, C-3), 145.4 (s, C-4), 119.0 (s, C-6), 143.8 (s, C-5)], one oxymethylene [δ_C 76.0 (t, C-7)], and one methoxyl signal [δ_C 58.2 (q, C-8)]. The ^{1}H NMR spectrum showed one methoxyl singlet and one oxymethylene singlet. All these data indicated compound **2** may be 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether, which agrees with the literature [7].

EXPERIMENTAL

IR spectra were recorded by the FT-IR microscope transmission method on a Nicolet 5700 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Inova 500 MHz spectrometer at 500.103 MHz for ^{1}H and 125.762 MHz for ^{13}C in acetone-d_6 using solvent signals (acetone-d_6, δ_H 2.05/δ_C 29.8, 206.1) as reference, and the coupling constants are in Hz. HRESIMS data were measured with an APEX II FT-ICR-MS spectrometer (Bruker Daltonics, Inc. USA). Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an Agilent 1100 series and an Alltima 250 cm × 2.2 cm i.d. preparative column packed with C18 (10 μm). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light and by spraying with 2% $FeCl_3$ in 95% EtOH.

Plant Material. *Symphyocladia latiuscula* was collected on the coast of Qingdao, Shandong Province, China, in May 2004. The specimen identification was verified by Dr. Kui-Shuang Shao (Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China). A voucher specimen (No. 2004X16) was deposited at the Herbarium of the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China.

Extraction and Isolation. The air-dried red alga *Symphyocladia latiuscula* (2.3 kg) was extracted with 95% EtOH at room temperature for 3 × 72 h. After the solvent was removed under reduced pressure at <40°C, a dark residue (60 g) was obtained. The residue was suspended in water and then partitioned with EtOAc. The EtOAc-soluble fraction (12 g) was chromatographed over silica gel, eluting with a gradient of increasing Me_2CO (0–100%) in petroleum ether. The fraction eluted by 30% Me_2CO in petroleum ether was rechromatographed over Sephadex LH-20 using petroleum ether–$CHCl_3$–MeOH (5:5:1) to afford three subfractions. The second subfraction was further separated by reversed-phase preparative HPLC using MeOH–H_2O–AcOH (80:20:0.1) as mobile phase to yield compounds **1** (9.1 mg) and **2** (7.2 mg).

2,3,6-Tribromo-4,5-dihydroxybenzyl ethyl ether (1), brown gum; IR (ν_{max}, cm^{-1}): 3493, 3128, 2978, 2938, 2892, 1582, 1548, 1454, 1404, 1354, 1316, 1270, 1170, 1006, 909, 826, 704; ESIMS *m/z*: 401/403/405/407 $[M–H]^-$ (1:3:3:1); HRESIMS: *m/z* 400.8018 (calcd for $C_9H_8Br_3O_3$, 400.8024); for ^1H NMR and ^{13}C NMR data, see Table 1.

Assay for Antimicrobial Activity. Antimicrobial activities were determined using the agar diffusion test using paper disks against *Staphyloccocus aureus*. The plates were inoculated and incubated at 37°C for 48 h. The MIC (minimum inhibitory concentration) was determined from plates with the lowest concentration of compounds on which the strain would not grow. Compounds **1** and **2** showed bioactivity against *Staphyloccocus aureus* with IC_{50} 102 and 50 μg/mL, respectively.

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