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BROMOPHENOL COUPLED WITH DIKETOPIPERAZINE FROM MARINE RED ALGA *Symphyocladia latiuscula*

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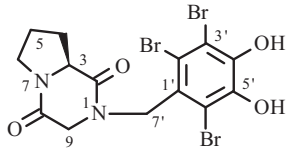
*2-(2,3,6-Tribromo-4,5-dihydroxybenzyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (**1**), a new bromophenol C-N coupled with diketopiperazine, was isolated from the ethanol extract of marine red alga* Symphyocladia latiuscula. *Its structure was elucidated by spectroscopic analysis, including high-resolution mass spectroscopy and 1 and 2-dimensional NMR techniques. The absolute configuration of **1** was assigned using Marfey's method on its acid hydrolysate.*

Keywords: *Symphyocladia latiuscula*, bromophenol, diketopiperazine.

The marine red alga *Symphyocladia latiuscula* is a rich source of bromophenols of several structural types and with various biological activities. Previous chemical studies on this species have resulted in the characterization of 15 monoaryl and diaryl bromophenols [1–7]. Some of them showed aldose reductase inhibitory activity [1], antibacterial activity [2, 7], and free-radical-scavenging activity [3–6]. As part of our recent program to assess systematically the chemical and biological diversity of seaweeds distributed along the gulf of the Yellow Sea, one new bromophenol has been isolated from *Symphyocladia latiuscula* [7]. Continuing our investigation of the same material, we report herein the isolation and structural elucidation of a new bromophenol C-N coupled with diketopiperazine (**1**). This is the first report of bromophenols coupled with diketopiperazine derivatives.



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Compound **1** was obtained as a brown amorphous powder. The positive ESI-MS spectrum exhibited a characteristic tribrominated quasi-molecular ion peak cluster at *m/z* 511/513/515/517 (1:3:3:1) $[M + H]^+$, and the molecular formula was determined as $C_{14}H_{13}{}^{79}Br_3N_2O_4$ by positive HR-ESI-MS at *m/z* 510.8509 for $[M + H]^+$. The IR spectrum showed an absorption band for hydroxyl groups at 3247 cm^{-1}, the characteristic absorption bands for an aromatic ring at 1554 and 1450 cm^{-1}, as well as the absorption bands for the carbonyl group at 1660 and 1718 cm^{-1}. The ^1H NMR spectrum of **1** in DMSO-d_6 showed two typical AB spin coupling systems attributed to two isolated methylenes at δ 5.05 (1H, d, J = 15.0 Hz) and 4.71 (1H, d, J = 15.0 Hz) for H_2-7′, at δ 3.92(1H, d, J = 15.0 Hz) and 3.17 (1H, d, J = 15.0 Hz) for H_2-9, three methylenes at δ 2.22 (1H, m) and 1.91 (1H, m) for H_2-4, 1.84 (2H, m) for H_2-5, 3.39 (1H, m) and 3.31 (1H, m) for H_2-6, as well as a methine at δ 4.20 (1H, t, J = 7.0 Hz). The protonated carbons and their corresponding protons were unambiguously assigned by the HSQC experiment.

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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	C atom	δ_C	δ_H	HMBC
2	167.0 s			1′	124.3 s		
3	58.3 d	4.20 (1H, t, J = 7.0)	4, 7	2′	117.5 s		
4	28.4 t	2.22 (1H, m)	5,	3′	114.4 s		
		1.91 (1H, m)	2, 5	4′	144.2 s		
5	22.2 t	1.84 (2H, m)		5′	146.9 s		
6	44.6 t	3.39 (1H, m)		6′	114.2 s		
		3.31 (1H, m)		7′	50.1 t	5.05 (1H, d, J = 15.0)	2, 9, 1′, 2′, 6′
8	162.6 s					4.71 (1H, d, J = 15.0)	1′, 2′, 6′
9	48.6 t	3.92 (1H, d, J = 15.0)	8				
		3.17 (1H, d, J = 15.0)	2, 8				

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

The ^{13}C NMR and DEPT spectra revealed the presence of 14 signals attributed to a diketopiperazine unit at δ_C 167.0 (s, C-2), 58.3 (d, C-3), 28.4 (t, C-4), 22.2 (t, C-5), 44.6 (t, C-6), 162.6 (s, C-8), and 48.6 (t, C-9) which was composed of glycine and proline and a 2,3,6-tribromo-4,5-dihydroxylbenzyl unit at 124.3 (s, C-1′), 117.5 (s, C-2′), 114.4 (s, C-3′), 144.2 (s, C-4′), 146.9 (s, C-5′), 114.2 (s, C-6′), and 50.1 (t, C-7′) [4]. The diketopiperazine unit was confirmed by the ^{1}H–^{1}H COSY between H-3 and H_2-4, H_2-4 and H_2-5, and H_2-5 and H_2-6 and the HMBC correlations from H-3 to C-4 and C-7, H-4b to C-2 and C-5, and H-9b to C-2 and C-8. The 2,3,6-tribromo-4,5-dihydroxylbenzyl unit was confirmed by detailed comparison of the above NMR data (Experimental section) with that of **1** in the literature [7] and the HMBC correlations from H-7′a to 1′, 2′, and 6′. The C-N bond was confirmed by the HMBC correlations from H_2-7′ to 1′, 2′ and 6′. Compound **1** was established as 2-(2,3,6-tribromo-4,5-dihydroxybenzyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione.

The configuration of proline was further confirmed by HPLC analyses of the acid hydrolysate of **1** after derivatization with the Marfey reagent (FDAA), allowing the absolute configurations at the α-carbon to be assigned as the L-configuration for proline [8].

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 DMSO-d_6 using solvent signals (DMSO, δ_H 2.50/δ_C 39.5) as reference; the coupling constants are in Hz. HR-ESI-MS 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 was identified 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 18 subfractions. The ninth subfraction was further separated by reversed-phase preparative HPLC using MeOH–H_2O–AcOH (75:20:0.1) as mobile phase to yield compound **1** (2.1 mg).

Absolute Configuration of Amino Acids [8]. Two set of compound **1** (50 μg) were dissolved in 6 N HCl (400 μL) and put in 2 mL glass vials and heated at 110°C for 24 h. Then the solvent was dried over nitrogen. The hydrolysate were

dissolved in 50 μL distilled water and treated with a 50 mmol/L solution of FDAA (50 μL) in acetone, followed by 1.0 N $NaHCO_3$ (20 μL). The reaction mixtures were heated at 40°C for 1.0 h, cooled to room temperature, and then acidified with 1.0 N HCl (20 μL). In a similar fashion, standard L-proline and D-proline were derivatized separately. The derivatives of the acid hydrolysate and the standard amino acids were subjected to Agilent reversed phase C18 column analysis (5 μm, 4.6 × 150 mm; 1.0 mL/min; UV detection at 340 nm) with a linear gradient of (A) water–0.1% formic acid and (B) CH_3CN– 0.1% formic acid from (35– 50%) over 15 min. The retention times for the FDAA derivatives of L-Pro and D-Pro were 8.3 and 9.2 min, respectively.

2-(2,3,6-Tribromo-4,5-dihydroxybenzyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (1). Brown amorphous powder; $[\alpha]_D^{25}$ –75° (*c* 0.1, MeOH). IR (ν_{max}, cm^{-1}): 3247, 1718, 1660, 1554, 1450, 1399, 1289, 1179, 1406, 1024, 9879, 823, 765, 707. ESI-MS *m/z*: 511/513/515/516.8 (1:3:3:1) $[M + H]^+$; HR-ESI-MS *m/z* 510.8509 (calcd for $C_{14}H_{13}{}^{79}Br_3N_2O_4$, 510.8504). For ^1H NMR and ^{13}C NMR data, see Table 1.

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