

Article

Two New Bromophenols with Radical Scavenging Activity from Marine Red Alga *Symphycladia latiuscula*

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Abstract: Chemical investigation of a Chinese collection of marine red alga *Symphycladia latiuscula* yielded two new highly brominated phenols. The structures of the new compounds were elucidated by detailed spectroscopic analysis, including HRMS, 1D and 2D NMR and MS methods. Compounds **1** and **2** were evaluated for radical scavenging capability by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical with the IC₅₀ value of 14.5 and 20.5 µg/mL, respectively.

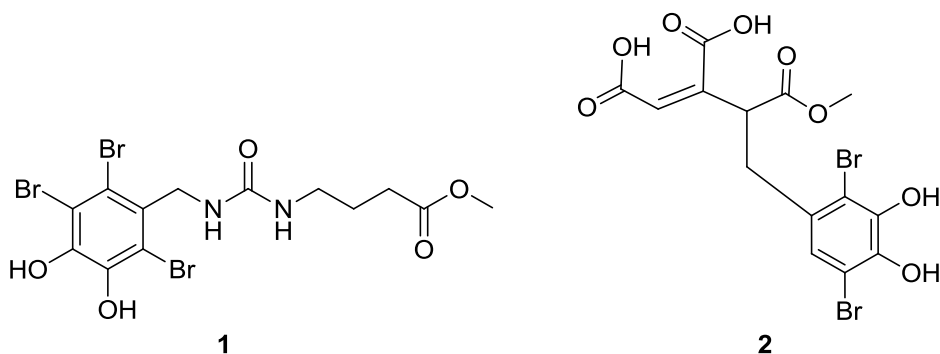
Keywords: red alga; *Symphycladia latiuscula*; bromophenol

1. Introduction

Symphycladia latiuscula (Harvey) Yamada is a marine red alga distributed along the coasts of Northern China, Korea, and Japan [1]. This red alga is a rich source of bromophenols with high chemical diversity and various biological activities. Previous chemical studies on this species have resulted in the characterization of 25 monoaryl and diaryl bromophenols with a variety of bioactivities,

such as antibacterial [2,3], antifungal [4,5], free-radical-scavenging [6,7], aldose reductase inhibitory [8], antiviral [9], anticancer [10] and Taq DNA polymerase inhibitory activities [11]. During the course of our continuing search for new biologically active bromophenols from this marine red alga, by mass spectrum guided fractionation, two new bromophenols (**1** and **2**) with radical scavenging activity were characterized (Figure 1). Herein, we report the isolation, structure elucidation and bioactivity evaluation of these bromophenols.

Figure 1. Structures of compounds **1** and **2**.



2. Results and Discussion

Compound **1** was obtained as a light brown amorphous powder. The positive ESIMS of **1** gave a pseudo molecular ion peak cluster for a tribrominated molecule at 517/519/521/523(1:3:3:1) $[M + H]^+$. The molecular formula was determined to be $C_{13}H_{15}Br_3N_2O_5$ by analysis of its HRESIMS (m/z 516.8613 $[M + H]^+$). The 1H NMR spectrum of **1** showed three multiplets assignable to a 1,3-disubstituted propane unit at δ 2.99 (2H, quartet, $J = 6.6$ Hz, H_{2-4}), 1.60 (2H, quintet, $J = 6.6$ Hz, H_{2-3}), and 2.28 (2H, t, $J = 6.6$ Hz, H_{2-2}), one doublet sp^3 methylene at δ 4.51 (2H, d, $H_{7'}$) and an ester methoxyl singlet at δ 3.58 (3H, s, OCH_3), and two exchangeable broad triplets assigned to amino protons at δ 5.85 (1H, brt, $J = 4.8$ Hz, $N'-H$) and 5.84 (1H, brt, $J = 6.6$ Hz, $N-H$). The ^{13}C NMR data for **1** revealed the carbon signals associated with the above structural units (Table 1) as well as a set of resonances for the 2,3,6-tribromo-4,5-dihydroxybenzyl unit [2–8] and two additional signals associated to ester carbonyl carbon at δ 173.2 (C-1) and one sp^2 -hybridized quaternary carbon at δ 157.4 (C-5). The NMR signals of protons and corresponding carbons were assigned by the 1H - 1H COSY and HSQC experiments (Table 1). The structure of **1** was unambiguously established by 1H - 1H COSY, HSQC, and HMBC experiments. In the HMBC spectrum, the correlations (Figure 2) from $H_{2-7'}$ to C-1', C-2', and C-6', in combination with chemical shift values of brominated quaternary carbons ($\delta < 120$) and oxygenated quaternary carbons ($\delta > 140$), demonstrated the existence of the 2,3,6-tribromo-4,5-dihydroxybenzyl unit in **1**. The HMBC correlations from H_{2-2} , H_{2-3} , and the methoxyl protons to C-1, H_{2-2} and H_{2-3} to C-4, H_{2-4} to C-5, together with the 1H - 1H COSY signals between H_{2-4} and $H-N$ and between H_{2-3} and H_{2-2} and H_{2-4} and between $H_{2-7'}$ and $H-N'$, revealed the presence of a methyl γ -ureidobutyrate moiety in **1**. In addition, HMBC correlations from $H_{2-7'}$, $H-N'$, $H-N$ and H_{2-4} to C-5 revealed that C-5 connected across N to C-7 and across N' to C-7'. Therefore, **1** was determined as methyl $N'-(2,3,6$ -tribromo-4,5-dihydroxybenzyl)- γ -ureidobutyrate. It is very interesting that the similar compound methyl $N'-(2,3$ -dibromo-4,5-dihydroxybenzyl)- γ -ureidobutyrate was isolated from the same

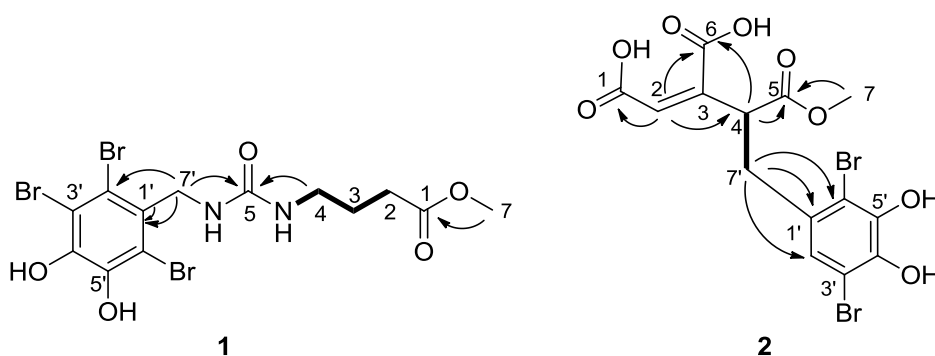
family marine red alga *Rhodomela confervoidesc* [12]. The specific halogenase may play an important role in the biosynthesis of these bromophenols.

Table 1. NMR data for compounds **1** (600 MHz, DMSO-*d*₆) and **2** (600 MHz, Methanol-*d*₄).

Compound 1				Compound 2		
pos	C mult	(<i>J</i> in Hz)	HMBC ^a	C mult	(<i>J</i> in Hz)	HMBC ^a
1	173.2, C			168.4, C		
2	30.7, CH ₂	2.28, t (6.6)	1, 3, 4	131.5, CH	6.76, s	
3	25.5, CH ₂	1.60, quint (6.6)	1, 2, 4	144.2, C		
4	38.5, CH ₂	2.99, quart (6.6)	2, 3, 5	44.2, CH ₂	5.06, dd (10.8, 4.2)	2, 3, 5, 6, 7'
N-H		5.84, brt (6.6)				
5	157.4, C			174.2, C		
6				169.1, C		
OMe	51.2, CH ₃	3.58, s	1	52.7, CH ₃	3.68, s	5
1'	129.7, C			132.0, C		
2'	116.8, C			126.3, CH	6.73, s	3', 4', 6', 7'
3'	113.8, C			109.6, C		
4'	144.7, C			143.7, C		
5'	143.7, C			145.4, C		
6'	113.7, C			113.5, C		
7'	46.7, CH ₂	4.51, d (4.8)	1', 2', 6'	36.9, CH ₂	3.60, dd (13.8, 4.2)	3, 4, 5, 1', 2', 6'
			5		2.98, dd (13.8, 10.8)	3, 4, 5, 1', 2', 6'
N-H'		5.85, brt (4.8)				

^a HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

Figure 2. Key HMBC correlations (H→C) and ¹H–¹H COSY (bold line) for compounds **1** and **2**.



Compound **2** was obtained as a light brown amorphous powder. The positive ESIMS of **1** gave a pseudo molecular ion peak cluster for a tribrominated molecule at 467/469/471(1:2:1) [$M + H$]⁺. The molecular formula was determined to be C₁₄H₁₂Br₂O₈ by analysis of its HRESIMS (m/z 466.8985 [$M + H$]⁺). The ¹H NMR spectrum of **2** displayed two alkenyl protons at δ 6.76 (H, s, H-2) and 6.73 (H, s, H-2'), one sp³ methylene at δ 3.60 (1H, dd, 13.8, 4.2, H-7'a) and 2.98 (1H, dd, 13.8, 10.8, H-7'b), one doublet doublets at δ 5.06 (1H, dd, 10.8, 4.2, H-4) and an ester methoxyl singlet at δ 3.68 (3H, s, OCH₃). The ¹³C NMR data for **2** revealed a *cis*-aconitic acid methyl ester moiety which was reported from the same species in our previous studies [4], two oxygenated quaternary at downfield ($\delta > 140$) and two brominated quaternary carbons at high field ($\delta < 120$). The *cis*-aconitic acid methyl ester

moiety was confirmed by the HMBC cross peaks from H-2 to C-1, C-4 and C-6, from H-4 to C-5 and C-6 and from H₃-7 to C-5. Meanwhile, the HMBC correlations from H-2' (δ 6.73, s) to C-3', C-4', C-6' and -7' and from H₂-7' to C-1', C-2' and C-6' revealed that this compound contains a 3,6-dibromo-4,5-dihydroxybenzyl unit. Finally, the connection between C-4 and C-7' was assigned by the COSY signals between H-4 and H-7'a and 7'b. The ROESY correlation between H-2 and H-4 established the Z configuration about $\Delta^{2,3}$. The absolute configuration of C-4 was not assigned. Although methanol was used during the isolation, only mono-methyl ester of the tri-acid compound was detected from these samples. These data indicated that compound **2** could be a natural product. Microbial reductive dechlorination of polychlorinated biphenyls can occur in aquatic sediments [13], which suggested that the 2'-debrominated analogues **2** might be derived from the reductive debromination of the hexa-substituted compound.

Compounds **1** and **2** were evaluated for radical scavenging capability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Compounds **1** and **2** exhibited moderate radical scavenging ability with IC₅₀ value of 14.5 and 20.5 μ g/mL, respectively. The IC₅₀ of positive control vitamin C is 7.82 μ g/mL.

3. Experimental Section

3.1. General Experimental Procedures

NMR spectra were recorded on a Varian Inova 600 MHz spectrometer at 600 MHz for ¹H and 125 MHz for ¹³C in DMSO-*d*₆ and Methanol-*d*₄ using solvent signals (DMSO: δ _H 2.50/ δ _C 39.51; Methanol: δ _H 3.31/ δ _C 49.15) as reference; the coupling constants were in Hz. ESIMS spectra were recorded with a ABI Mariner ESI-TOF. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory) and Sephadex LH-20 (Pharmacia Co., Sweden) columns. HPLC was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series diode array detector and performed using an Agilent Zorbax Eclipse XDB-C8 (5 μ m) semipreparative column (9.4 $\times$ 250 mm).

3.2. Algal 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.

3.3. Extraction and Isolation

The air-dried red alga *Symphyocladia latiuscula* (4.3 kg) was extracted with 95% EtOH at room temperature (3 $\times$ 72 h). After the solvent was removed under reduced pressure at <40 °C, a dark residue (610 g) was obtained. The residue was partitioned between EtOAc and H₂O and the EtOAc-soluble partition (320 g) was chromatographed over silica gel, eluting with a gradient of 0%–100% Me₂CO/petroleum ether [4]. The fraction eluted by 30% Me₂CO/petroleum ether was further fractionated over Sephadex LH-20 using petroleum ether–CHCl₃–MeOH (5:5:1) to afford

18 fractions. The ninth fraction from the LH-20 column was further fractionated by ODS column, which was eluted with a stepwise gradient of 0%–100% MeOH/H₂O to afford 11 subfractions. The fourth ODS subfraction was subjected to HPLC fractionation (Zorbax Eclipse XDB-C8 5 μ m 250 $\times$ 9.4 mm column) to yield compound **1**. The 13th fraction from the LH-20 column was further fractionated by ODS column, which was eluted with a stepwise gradient of 0%–100% MeOH/H₂O to afford 11 subfractions. The third ODS subfraction was subjected to HPLC fractionation (Zorbax Eclipse XDB-C8 5 μ m 250 $\times$ 9.4 mm column) to yield compound **2**.

Compound 1: Light brown amorphous powder; IR $\nu_{\max}$ 3335, 2953, 1702, 1627, 1548, 1452, 1398, 1365, 1168, 1117, 1067, 1031, 968, 942, 910, 882, 831, 766, 702 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 517/519/521/523(1:3:3:1) $[\text{M} + \text{H}]^+$; HRESIMS m/z by analysis of its HRESIMS (m/z 516.8613 $[\text{M} + \text{H}]^+$) (calcd for $\text{C}_{13}\text{H}_{16}^{79}\text{Br}_3\text{N}_2\text{O}_5$, 516.8604).

Compound 2: Light brown amorphous powder; $[\alpha]_D^{20} +10.0$ (c 0.05, MeOH); IR $\nu_{\max}$ 3367, 3296, 2954, 2615, 1766, 1715, 1643, 1568, 1472, 1439, 1423, 1264, 1137, 1018, 933, 910, 816, 643, cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 467/469/471(1:2:1) $[\text{M} + \text{H}]^+$; HRESIMS m/z 466.8985 $[\text{M}]^+$ (calcd for $\text{C}_{14}\text{H}_{13}^{79}\text{Br}_2\text{O}_8$, 466.8972).

3.4. Scavenging Ability on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical

Each power (0.1–20 mg/mL, 4.0 mL) in deionized water was mixed with 1.0 mL of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank [14]. The scavenging ability was calculated as follows:

$$\text{scavenging ability (\%)} = [(\text{A}_{517} \text{ of control} - \text{A}_{517} \text{ of sample}) / \text{A}_{517} \text{ of control}] \times 100 \quad (1)$$

Vitamin C was used for positive control.

4. Conclusions

Symphyclocladia latiuscula is a rich source of bromophenols with specific subunit of 2,3,6-tribromo-4,5-dihydroxybenzene. During the course of our systematic search for new biologically active bromophenols from this marine red alga, two new bromophenols (**1** and **2**) with radical scavenging activity were characterized by mass spectrum guided fractionation. Compounds **1** and **2** exhibited radical scavenging capability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical with the IC_{50} value of 14.5 and 20.5 $\mu\text{g/mL}$, respectively.

Acknowledgments

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Samples Availability: Available from the authors.