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Natural bromophenols from the marine red alga *Polysiphonia urceolata* (Rhodomelaceae): Structural elucidation and DPPH radical-scavenging activity

Ke Li,[a,b] Xiao-Ming Li,[a,*] Nai-Yun Ji[a,b] and Bin-Gui Wang[a,*]

[a]*Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China*
[b]*Graduate School of Chinese Academy of Sciences, Beijing 100049, PR China*

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Abstract—Three new natural occurring bromophenols, 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid (**1**), (*E*)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (**2**), and (3,5-dibromo-4-hydroxyphenyl) acetic acid butyl ester (**3**), together with one known bromophenol, 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane (**4**), were isolated and identified from the marine red alga *Polysiphonia urceolata*. The structures of these compounds were elucidated by extensive analysis of 1D and 2D NMR and IR spectra and MS data. Each of the isolated compounds was evaluated for scavenging α,α-diphenyl-β-picrylhydrazyl (DPPH) radical activity and all of them exhibited significant activity with IC_{50} values ranging from 9.67 to 21.90 μM, compared to the positive control, a well-known antioxidant butylated hydroxytoluene (BHT), with IC_{50} 83.84 μM.

1. Introduction

Free radical attacks biological molecules such as lipids, proteins, enzymes, DNA, and RNA, leading to cell or tissue injury associated with many diseases including aging, atherosclerosis, and carcinogenesis.[1–4] In addition, free radical-mediated oxidative-damage plays an important role as an underlying factor in the initiation and progression of some neurodegenerative disorders such as Parkinson's and Alzheimer's diseases.[5–7] Phenolic compounds have recently received significant attention among various antioxidants and many studies have been performed to identify natural antioxidative phenols with pharmacological activity.[8] Similar to the study of other marine organisms, the investigation of biological active metabolites of marine algal origin has significantly increased in the last three decades.[9] The marine red algae of the family Rhodomelaceae are known to contain high concentrations of bromophenols with diversity of molecular structures.[10–15] Some of the bromophenols previously isolated from this family have shown significant nitrite scavenging,[11] α-glucosidase inhibition,[12] antioxidant,[13] feeding-deterrent,[14] and anti-inflammatory activities.[15] *Polysiphonia urceolata* is a member of the algal family Rhodomelaceae, belonging to the order Ceramiales.[16] This species is widely distributed in northern coastalines of China. Two bromophenols of monoaryl and diaryl structure types have been previously reported from *P. urceolata* that was collected at the coast of Qingdao, China.[17] Our recent publication demonstrated that the extract, fractions, and semi-purified subfractions of *P. urceolata* possess potent scavenging activity on α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals.[18] As part of our investigation on the chemical constituents of this species, we wish to report herein the isolation and structural elucidation of three new bromophenols, 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid (**1**), (*E*)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (**2**), and (3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester (**3**), together with a known compound, 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane (**4**).[19]

Compounds **1**–**4** were evaluated for the ability of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging activity and all of them exhibited potent activity with IC_{50} values ranging from 9.67 to 21.90 μM. Compound

Keywords: DPPH radical-scavenging activity; Marine red alga; *Polysiphonia urceolata*; Bromophenols.
* Corresponding authors. Tel.: +86 532 8289 8579; fax: +86 532 8288 0645 (X.-M.L.), tel.: +86 532 8289 8553; fax: +86 532 8288 0645 (B.-G.W.); e-mail addresses: lixmqd@yahoo.com.cn; wangbg@ms.qdio.ac.cn

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2 showed strong activity, and its activity was higher than the positive control, butylated hydroxytoluene (BHT), a well-known synthetic antioxidant.

2. Structures of compounds 1–4

The dried marine red algal material of *P. urceolata* was extracted with EtOH. The ethanolic extract was dissolved in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH, respectively. Repeated chromatographic separations of the EtOAc-soluble fraction led to the purification of pure substances **1**–**4** Figure 1.

Compound **1** was obtained as brown needles. The IR spectrum (KBr) showed absorption bands for hydroxyl (3412 cm^{-1}) and carbonyl (1708 cm^{-1}) groups and aromatic rings (1592, 1560, and 1494 cm^{-1}). The EIMS of **1** gave a characteristic tribrominated molecular ion cluster at *m*/*z* 514/512/510/508 (1:3:3:1) $[M]^+$, and the molecular formula $C_{15}H_{11}Br_3O_5$ was determined by HRESIMS at *m*/*z* 532.8036 $[M + Na]^+$ (calcd for $C_{15}H_{11}{}^{79}Br_2{}^{81}BrO_5Na$, 532.8034). In the NMR spectra, proton signals at δ_H 6.90 (1H, d, J = 2.0, H-2′) and 6.72 (1H, d, J = 2.0, H-6′) and carbon signals at δ_C 134.6 (s, C-1′), 124.7 (d, C-2′), 110.0 (s, C-3′), 142.3 (s, C-4′), 146.5 (s, C-5′), and 116.4 (d, C-6′) indicated the presence of a 3-bromo-4,5-dihydroxyphenyl moiety in the molecules. Meanwhile, a two-proton broad singlet at δ_H 7.56 (2H, s, H-2″/6″) and carbon signals at δ_C 132.6 (s, C-1″), 132.8 (d, C-2″/6″), 111.4 (s, C-3″/5″), and 150.8 (s, C-4″) revealed the presence of a 3,5-dibromo-4-hydroxyphenyl moiety in **1**. In addition, the signals at δ_H 3.86 (1H, dd, J = 6.7, 8.9 Hz, H-2), 3.18 (1H, dd, J = 8.9, 13.8 Hz, H-3a), and 2.88 (1H, dd, J = 6.7, 13.8 Hz, H-3b) in the ^1H NMR spectrum and three carbon signals at δ_C174.1 (s, C-1), 52.3 (d, C-2), and 39.3 (t, C-3) in the ^{13}C NMR and DEPT spectra demonstrated the presence of a 2,3-disubstituted propionic acid unit in **1**. The unambiguous assignments of the signals were based on the HSQC and HMBC (Fig. 2) experiments. In combination with the chemical shift values of the two benzene rings, long-range correlations from H-2, H-3a, and H-3b to the carboxyl carbon (C-1) confirmed the presence of the 2,3-disubstituted propionic acid unit, while correlations from H-2 to C-1″, C-2″, and C-6″ and from H-3a/H-3b to C-1′, C-2′, and C-6′ clearly located the 3,5-dibromo-4-hydroxyphenyl and 3-bromo-4,5-dihydroxyphenyl to C-2 and C-3 of the propionic acid unit, respectively. Therefore, the structure of **1** was determined as 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)propionic acid.

Compound **2** was obtained as brown needles. The IR spectrum (KBr) showed absorption bands for hydroxyl (3410 cm^{-1}) and conjugated carbonyl (1672 cm^{-1}) groups and aromatic rings (1623, 1595, 1520 cm^{-1}). Its EIMS exhibited monobrominated molecular ion peak cluster at *m*/*z* 258/256 (1:1) $[M]^+$, and the molecular formula $C_{10}H_9BrO_3$ was determined by HRESIMS at *m*/*z* 256.9806 $[M]^+$ (calcd for $C_{10}H_{10}{}^{79}BrO_3$, 256.9813). Two aromatic signals at δ_H 7.23 (1H, d, J = 1.8 Hz, H-2) and 7.02 (1H, d, J = 1.8 Hz, H-6) in the ^1H NMR spectrum indicated the presence of a phenyl group with 1,3,4,5-tetrasubstitution. Meanwhile, the ^1H NMR spectrum of **2** showed two *trans*-coupled olefinic doublets at δ_H 7.42 (1H, d, J = 16.2 Hz, H-7) and 6.50 (1H, d, J = 16.2 Hz, H-8) and an acetyl singlet at δ_H 2.32 (3H, s, H-10). Besides the acetyl carbons (δ_C 201.3 and 27.1) and the two olefinic carbons (δ_C 145.0 and 126.0), the ^{13}C NMR spectrum of **2** displayed six additional carbon signals attributed to a 3-bromo-4,5-dihydroxyphenyl moiety. In the HMBC spectrum, cross-peaks from aromatic protons to their correlated long-range carbons unambiguously established the substitution patterns of the aromatic rings (Fig. 2). Long-range correlations from H-7 to C-2, C-6, and C-9, and from H-8 to C-1 and C-10 confirmed the structure of **2** to be (*E*)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one.

Compound **3** was obtained as colorless crystals. The IR spectrum showed absorption bands for hydroxyl (3422 cm^{-1}) and carbonyl (1718 cm^{-1}) groups and aromatic ring (1557 and 1481 cm^{-1}). The EIMS of **3** gave a characteristic dibrominated molecular ion peak cluster at *m*/*z* 368/366/364 (1:2:1) $[M]^+$, and the molecular formula $C_{12}H_{14}Br_2O_3$ was determined by HRESIMS at *m*/*z* 388.9184 $[M+Na]^+$ (calcd for $C_{12}H_{14}{}^{79}Br^{81}BrO_3Na$, 388.9187). The ^1H NMR spectrum of **3** showed signals attributed to two aromatic protons at δ_H 7.50 (2H, s, H-2, and H-6), a methyl group at δ_H 0.90 (3H, t, J = 7.4 Hz, H-4′), and four methylene groups at δ_H 4.07 (2H, t, J = 6.6 Hz, H-1′), 3.61 (2H, s, H-7), 1.58 (2H, m, H-2′), and 1.36 (2H, m, H-3′). Two aromatic protons at δ_H 7.50 (2H, s, H-2/6) in ^1H NMR spectrum and six carbon signals at δ 130.2 (s, C-1), 134.2 (d, C-2/6), 111.3 (s, C-3/5), and 150.6 (s, C-4) in ^{13}C NMR and DEPT spectra indicated the presence of a 3,5-dibromo-4-hydroxyphenyl unit in **3** (the oxygenated and brominated quaternary carbons were identified by the chemical shifts at $\delta_C > 140$ and $\delta_C < 120$ ppm, respectively). The ^1H-^1H COSY spectrum of **3** revealed the presence of a butyl ester unit in the structure (Fig. 2). In the HMBC spectrum (Fig. 2), long-range correlations from the methyene proton H-7 (δ_H 3.61) to C-1, C-2, C-6, and C-8, from H-2/6 (δ_H 7.50) to C-4 and C-7, and from H-1′ (δ_H 4.07) to C-8 unambiguously established the structure of the compound **3**. Therefore, the structure of **3** was determined as (3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester.

3. DPPH radical-scavenging activity

The radical-scavenging activity of compounds **1**–**4** was evaluated by using the DPPH radical scavenging assay as reported previously.[18] While compounds **1**, **3**, and **4** showed strong activities with IC_{50} values of 21.90, 16.11, and 19.64 μM (Table 1), respectively, compound**2** showed the strongest activity with IC_{50} value of 9.67 μM, which was 9-fold more potent than that of the well-known synthetic antioxidant, butylated hydroxytoluene (BHT, IC_{50} = 83.84 μM, Table 1).

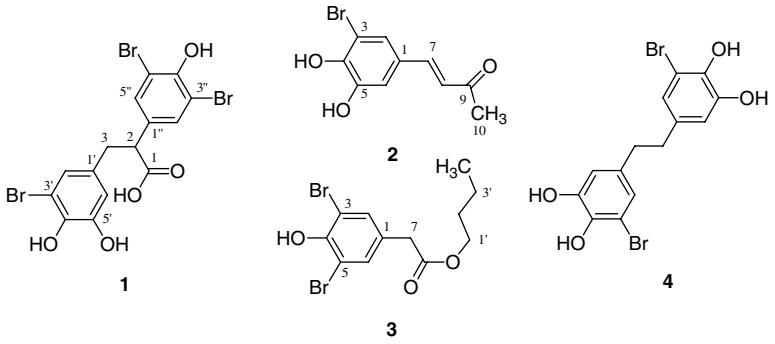

Figure 1. Structures of compounds **1**–**4**.

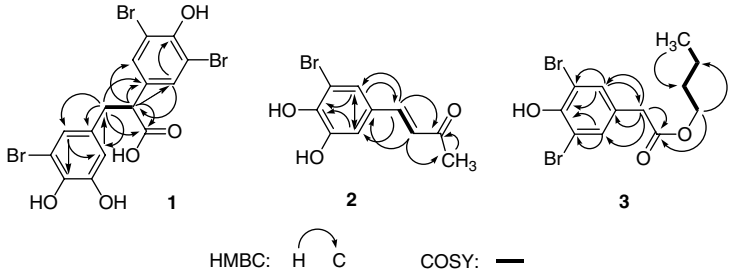

Figure 2. Selected HMBC and ^{1}H-^{1}H COSY correlations of **1**–**3**.

Table 1. DPPH radical-scavenging activity of compounds **1**–**4**[a]

Compound	$IC_{50} \pm SD$ (μM)
1	21.90 ± 0.1
2	9.67 ± 0.04
3	16.11 ± 0.06
4	19.64 ± 0.09
BHT	83.84 ± 0.3

[a] Each value is presented as the means ± SD ($n = 3$).

The isolated bromophenols **1**–**4** were shown to possess potent DPPH radical-scavenging activity in the low micromolar range, and the activity is stronger than the well-known antioxidant, BHT. This result is in good correlation with our earlier report in which we demonstrated that the crude extract, the EtOAc-soluble fraction, and the semi-purified sub-fractions of *P. urceolata* possessed high DPPH radical-scavenging activity and phenolic compounds were proposed to responsible for the activity.[18]

The capacity of radical scavenging is widely used as a parameter for in vitro evaluation of medicinal bioactive compounds since radical scavengers are closely related to their biofunctionalities such as the reduction of chronic diseases like DNA damage, mutagenesis, and carcinogenesis which are often associated with the termination of free radical propagation in biological systems.[20] The potent DPPH radical-scavenging activity of the isolated metabolites **1**–**4** demonstrated in this study indicates the potential application value of the marine red alga *P. urceolata*. However, further in vitro and in vivo bioassays for the individual bromophenols as well as in vivo safety are warranted.

4. Conclusion

The isolation of bromophenols **1**–**4** was a new addition to the molecular diversity of the marine red algal Rhodomelaceae family. Antioxidant analysis of isolated metabolites suggested an important role of this kind of substances as DPPH radical-scavenging factors and supports the research of antioxidative secondary metabolites from marine red algae of Rhodomelaceae family. In conclusion, compounds **1**–**4**, which proved to be effective micromolar in vitro scavenger of DPPH radicals, can be used as good antioxidant and might be a starting point for a drug discovery program aimed at enhancing their potencies.

5. Experimental

5.1. General methods

IR spectra were recorded with a Nicolet NEXUE 470 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance 500 spectrometer. COSY, HSQC, and HMBC experiments were performed using standard Bruker pulse sequences. Low and high resolution mass spectra were recorded on a VG Autospec 3000 spectrometer. Column chromatography was carried out using commercial silica gel (Qingdao Haiyang Chemical Group Co.; 200–300 and 300–400 mesh) and Sephadex

LH-20 (Pharmacia Biosciences). All solvents were of spectral grade or distilled prior to use.

5.2. Algal material

The marine red alga *P. urceolata* Grev. was collected off the coast of Qingdao, P. R. China, in April, 2006. It was identified by Prof. B.-M. Xia and Dr. L.-P. Ding at Institute of Oceanology, Chinese Academy of Sciences (IOCAS). A voucher specimen (No. HZ06041) was deposited in the Hebarium of Marine Organisms at IOCAS.

5.3. Extraction and isolation

The air-dried and ground red alga *P. urceolata* (30.5 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 was obtained. The residue was suspended in water and then partitioned with petroleum ether, EtOAc, and *n*-butanol, successively. The EtOAc-soluble extract (300 g) was subjected to column chromatography (CC) over Si gel (1200 g) eluting with petroleum ether–acetone (0–100%) and $CHCl_3$–MeOH (10–100%) to afford 36 fractions on the basis of TLC analyses. Fraction 2 (5.6 g) eluted with PE/EtOAc 10:1 were further purified by reversed-phase semi-preparative HPLC (MeOH-H_2O, 30:70) to afford **3** (19.6 mg). Fraction 5 (12.9 g) was further chromatographed over Si gel eluting with a gradient of increasing acetone (20–100%) in petroleum ether to yield five subfractions, and the third and fourth subfractions were combined and was further purified by reversed-phase semi-preparative HPLC using MeOH–H_2O (30:70) as the mobile phase to yield compounds **1** (21.3 mg) and **2** (40.2 mg). Fraction 16 (16.3 g) was further fractionated by CC on Si gel eluting with a gradient of increasing amount of acetone (10–100%) in petroleum ether and further purified by CC on Sephadex LH-20 eluting with $CHCl_3$-MeOH (1:1) to yield compound **4** (20.3 mg).

5.3.1. 3-(3-bromo-4,5-dihydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl) propionic acid (1). Brown needles; M.p. 103–105 °C; UV (EtOH) λ_{max} 214 (1.89), 273 (1.20) nm; IR (KBr) ν_{max} 3412, 2954, 2919, 1708, 1592, 1560, 1494, 1475, 1431, 1412, 1322, 1284, 1168, 1119, 989, 875, 842 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ_H 3.86 (1H, dd, J = 6.7, 8.9 Hz, H-2), 3.18 (1H, dd, J = 8.9, 13.8 Hz, H-3a), 2.88 (1H, dd, J = 6.7, 13.8 Hz, H-3b), 6.90 (1H, d, J = 2.0 Hz, H-2′), 6.72 (1H, d, J = 2.0 Hz, H-6′), 7.56 (2H, s, H-2″, H-6″); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 174.1 (s, C-1), 52.3 (d, C-2), 39.3 (t, C-3), 134.6 (s, C-1′), 124.7 (d, C-2′), 110.0 (s, C-3′), 142.3 (s, C-4′), 146.5 (s, C-5′), 116.4 (d, C-6′), 132.6 (s, C-1″), 132.8 (d, C-2″, C-6″), 111.4 (s, C-3″, C-5″), 150.8 (s, C-4″); EIMS *m/z* 514 (2), 512 (6), 510 (6), 508 (2) $[M]^+$, 203 (91), 201 (100), 123 (17); HRESIMS *m/z* 532.8036 $[M+Na]^+$ (calcd for $C_{15}H_{11}{}^{79}Br_2{}^{81}BrO_5Na$, 532.8034).

5.3.2. (*E*)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (2). Brown needles; M.p. 184–185 °C; UV (EtOH) λ_{max} 245 (1.31), 337 (1.38); IR (KBr) ν_{max} 3410, 3294, 3008, 2922, 1672, 1623, 1595, 1520, 1430, 1362, 1296, 1270, 1184, 1134, 1019, 993, 972, 848, 829, 806 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ_H 7.23 (1H, d, J = 1.8 Hz, H-2), 7.02 (1H, d, J = 1.8 Hz, H-6), 7.42 (1H, d, J = 16.2 Hz, H-7), 6.50 (1H, d, J = 16.2 Hz, H-8), 2.32 (3H, s, H-10); ^{13}C NMR (CD_3OD, 125 MHz) δ_C 128.4 (s, C-1), 126.2 (d, C-2), 110.9 (s, C-3), 147.5 (s, C-4), 147.2 (s, C-5), 114.0 (d, C-6), 145.0 (d, C-7), 126.0 (d, C-8), 201.3 (s, C-9), 27.1 (q, C-10); EIMS *m/z* 258 (30), 256 (31) $[M]^+$, 243 (26), 241 (32), 162 (100), 134 (23); HRESIMS *m/z* 256.9806 $[M+H]^+$ (calcd for $C_{10}H_{10}{}^{79}BrO_3$, 256.9813).

5.3.3. (3,5-dibromo-4-hydroxyphenyl)acetic acid butyl ester (3). Colorless crystal; M.p. 150–152 °C; UV (EtOH) λ_{max} 208 (1.59), 274 (1.24) nm; IR (KBr) ν_{max} 3422, 2959, 2931, 1718, 1636, 1557, 1481, 1411, 1339, 1285, 1233, 1163, 1132, 1063, 1022, 937, 870, 800 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ_H 7.50 (2H, s, H-2, H-6), 3.61 (2H, s, H-7), 4.07 (2H, t, J = 6.6 Hz, H-1′), 1.58 (2H, m, H-2′), 1.36 (2H, m, H-3′), 0.90 (3H, t, J = 7.4 Hz, H-4′); ^{13}C NMR (acetone-d_6, 125 MHz) δ_C 130.2 (s, C-1), 134.2 (d, C-2, C-6), 111.3 (s, C-3, C-5), 150.6 (s, C-4), 39.6 (t, C-7), 171.4 (s, C-8), 65.2 (t, C-1′), 31.5 (t, C-2′), 19.8 (t, C-3′), 13.9 (q, C-4′); EIMS *m/z* 368 (13), 366 (26), 364 (13) $[M]^+$, 267 (50), 265 (100), 263 (51), 187 (40), 185(50); HRESIMS *m/z* 388.9184 $[M+Na]^+$ (calcd for $C_{12}H_{14}{}^{79}Br{}^{81}BrO_3Na$, 388.9187).

5.4. Determination of the DPPH radical-scavenging activity

DPPH radical-scavenging activity of compounds **1**–**4** was evaluated by the method as previously reported.[18]

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