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Isolation, Characterization, and Antioxidant Activity of Bromophenols of the Marine Red Alga *Rhodomela confervoides*

Ke Li,[†] Xiao-Ming Li,[†] James B. Gloer,[‡] and Bin-Gui Wang*[,†]

[†]Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266071, People's Republic of China

[‡]Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States

Supporting Information

ABSTRACT: A total of 19 naturally occurring bromophenols, with six new and 13 known structures, were isolated and identified from the methanolic extract of the marine red alga *Rhodomela confervoides*. The new compounds were identified by spectroscopic methods as 3,4-dibromo-5-((methylsulfonyl)methyl)benzene-1,2-diol (**1**), 3,4-dibromo-5-((2,3-dihydroxypropoxy)methyl)-benzene-1,2-diol (**2**), 5-(aminomethyl)-3,4-dibromobenzene-1,2-diol (**3**), 2-(2,3-dibromo-4,5-dihydroxyphenyl)acetic acid (**4**), 2-methoxy-3-bromo-5-hydroxymethylphenol (**5**), and (*E*)-4-(2-bromo-4,5-dihydroxyphenyl)but-3-en-2-one (**6**). Each compound was evaluated for free radical scavenging activity against DPPH (α,α-diphenyl-β-dipicrylhydrazyl) and ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt] radicals. Most of them exhibited potent activities stronger than or comparable to the positive controls butylated hydroxytoluene (BHT) and ascorbic acid. The results from this study suggest that *R. confervoides* is an excellent source of natural antioxidants, and inclusion of these antioxidant-rich algal components would likely help prevent the oxidative deterioration of food.

KEYWORDS: Marine alga, *Rhodomela confervoides*, bromophenol, radical scavenging activity, DPPH, ABTS

INTRODUCTION

Marine algae have long been used directly as foodstuffs in Asian countries, especially in China, Korea, and Japan,[1] whereas in western countries, algae are mainly used as sources of food additives (agar, alginates, and carrageenans) and minerals.[2,3] Because of worldwide public interest in natural health foods, the consumption of edible algae as well as the development of algae-based industries have increased significantly in the last few decades.[4] In recent years, the continuous discovery of natural products with antioxidant activity from marine algae has attracted considerable attention. Antioxidants from natural sources are preferred by consumers due to concerns about the toxic and carcinogenic effects of synthetic antioxidants.[5,6]

Marine red algae of the family Rhodomelaceae (order Ceramiales) have proven to be rich sources of structurally novel and biologically active secondary metabolites.[7−18] Some of the isolated compounds from this family display significant radical scavenging,[7−11] α-glucosidase inhibition,[12] and feeding deterrent[13] anti-inflammatory activities.[14] *Rhodomela confervoides* is a member of the algal family Rhodomelaceae. This species is commonly distributed along the northern coastlines of China. Although it is not widely consumed in daily diets at present, this alga is used by local people to make homemade food as well as for aquaculture-based foodstuffs in northern China.[19] Shi and co-workers[15−18] have investigated the chemical constituents of samples of this species collected from the Qingdao coastline of Shandong Province. Recently, we reported that the crude extract and semipurified fractions of this alga possess strong α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity.[19] This encouraged us to investigate the chemical constituents of *R. confervoides* responsible for the radical scavenging activity. As a result, 19 naturally occurring bromophenols, **1**−**19** (Figure 1), were isolated and identified from a sample that was collected from another locality, the Dalian coastline of Liaoning Province. Most of these compounds contain 2,3-dibromo-4,5-dihydroxybenzyl, **1**−**4** and **7**−**13**, or 3-bromo-4-methoxy-5-hydroxybenzyl moieties, **5** and **14**−**18**, with only two exceptions, compounds **6** and **19**, which have 2-bromo-4,5-dihydroxybenzyl and 3-bromo-4,5-dihydroxybenzyl units, respectively. All of these compounds were evaluated for in vitro free radical scavenging ability using DPPH and Trolox equivalent antioxidant capacity (TEAC) assay systems.

MATERIALS AND METHODS

Plant Material. A sample of the marine red alga *R. confervoides* was freshly collected from the coastline of Dalian, Liaoning Province, People's Republic of China, in April, 2007. It was identified by Prof. B.-M. Xia at the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). A voucher specimen (no. HZ0407R) was deposited in the Key Laboratory of Experimental Marine Biology of the IOCAS.

General Apparatus and Chemicals. Optical rotations were determined on an Optical Activity AA-55 polarimeter. UV spectra were measured on a Varian Cary 50 UV/vis-NIR spectrophotometer. IR spectra were recorded on a Nicolet NEXUE 470 infrared spectrophotometer. One- and two-dimensional (1D and 2D) NMR spectra were recorded on a Bruker Avance 500-MHz spectrometer. Mass spectra were acquired on a VG Autospec 3000 mass spectrometer. Si gel (Qingdao Haiyang Chemical Co., Qingdao, China), RP-18 reverse-phase Si gel, and Sephadex

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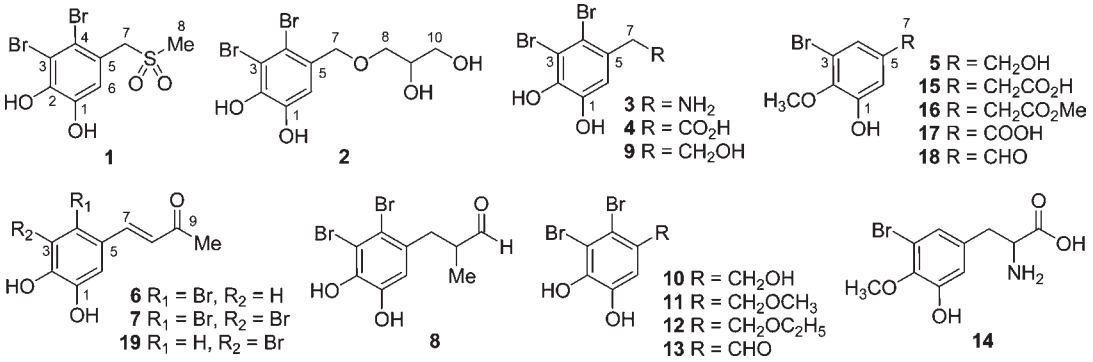

Figure 1. Chemical structures of compounds **1**−**19**.

LH-20 (Merck, Darmstadt, Germany) were used for open column chromatography. Thin-layer chromatography (TLC) was carried out on glass plates precoated with GF_{254} Si gel, and spots were visualized under UV light at 254 nm and detected by spraying with 1% $FeCl_3$ solution. Butylated hydroxytoluene (BHT), DPPH, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), and Trolox reagent (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (St. Louis, MO). Ascorbic acid was purchased from Shanghai Chemical Reagents Co. (Shanghai, China). Other chemicals used were of analytical grade.

Extraction and Isolation of Active Compounds. The air-dried and ground sample of *R. confervoides* (33 kg) was extracted with 95% EtOH by stirring 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 H_2O and then partitioned with petroleum ether (PE), EtOAc, and *n*-butanol, successively. The EtOAc extract (360 g) was chromatographed over Si gel (1500 g) eluting with petroleum ether (PE)−acetone and $CHCl_3$−MeOH to give 37 fractions on the basis of TLC analysis. Fr. 10 (5.2 g) was chromatographed over Si gel eluting with a gradient of increasing amounts of acetone (5−30%) in PE and further purified by column chromatography (CC) on Sephadex LH-20 (60 g, 15 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) to yield compounds **7** (20.1 mg), **9** (13.2 mg), and **14** (10.6 mg). Fr. 11 and Fr. 12 were combined (8.9 g) and purified by CC over Si gel eluting with a gradient of increasing amounts of acetone (10−50%) in PE and further purified by CC on Sephadex LH-20 (60 g, 15 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) to yield compounds **8** (15.3 mg), **10** (20.3 mg), **11** (15.2 mg), **18** (10.2 mg), and **19** (8.7 mg). Fr. 13−15 were also combined (6.0 g) and chromatographed over Si gel eluting with a gradient of increasing amounts of acetone (10−100%) in PE and further purified by CC on Sephadex LH-20 (100 g, 20 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) to yield compounds **4** (11.4 mg), **15** (10.5 mg), **16** (7.6 mg), and **17** (6.9 mg). Fr. 16 (2.0 g) was further separated by CC on Si gel eluted with PE/EtOAc 10:1 to afford compound **6** (6.5 mg). Fr. 17 (5.8 g) was further purified by CC on Sephadex LH-20 (25 g, 10 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) to yield compounds **12** (15.3 mg) and **13** (12.0 mg). Fr. 20 and Fr. 21 were combined (5.0 g) and further purified by Si gel eluting with PE/EtOAc 10:1 to afford **3** (15.4 mg). Fr. 26 (4.2 g) was chromatographed over Sephadex LH-20 (60 g, 15 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) and then MeOH−H_2O (1:1) to yield compound **1** (15.7 mg). Fr. 28 (6.7 g) was chromatographed over Si gel eluting with a gradient of increasing amounts of acetone (50−100%) in PE and further purified by CC on Sephadex LH-20 (60 g, 15 mm × 50 mm, i.d.) eluting with $CHCl_3$−MeOH (1:1) to yield compound **2** (12.8 mg). Fr. 34 (8.6 g) was further fractionated by CC on Si gel eluting with a gradient of increasing MeOH (20−100%) in $CHCl_3$ to yield two subfractions. The first subfraction was chromatographed over Sephadex LH-20 eluting (25 g, 10 mm × 50 mm, i.d.) with MeOH−H_2O (1:1) and then MeOH to yield compound **5** (14.7 mg).

3,4-Dibromo-5-(methylsulfonylmethyl)benzene-1,2-diol (1). Colorless powder. IR (KBr) ν_{max} 3315, 2928, 1653, 1472, 1402, 1299, 1278, 1183, 1116 cm^{-1}. UV λ_{max} (EtOH) (log ε): 221 (5.24), 296 (3.59) nm. ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.24 (1H, br.s, OH), 8.69 (1H, br.s, OH), 7.14 (1H, s, H-6), 4.55 (2H, s, H_2-7), 2.90 (3H, s, H_3-8). ^{13}C NMR (acetone-d_6, 125 MHz): δ 146.1 (C-1, qC), 145.6 (C-2, qC), 122.4 (C-5, qC), 118.9 (C-6, CH), 118.3 (C-4, qC), 114.1 (C-3, qC), 61.7 (C-7, CH_2), 40.6 (C-8, CH_3). EIMS *m*/*z* 362 (3)/360(5)/358 (2) $[M]^+$, 283 (50)/281 (97)/279 (55) $[M - CH_3SO_2]^+$, 203 (6)/201(8), $[M - CH_3SO_2 - Br]^+$, 173 (6), 133 (15), 131 (17). High-resolution electrospray ionization mass spectrometry (HRESIMS) *m*/*z* 380.8410 $[M + Na]^+$ (calcd for $C_8H_8{}^{79}Br_2O_4SNa^+$, 380.8407).

3,4-Dibromo-5-((2,3-dihydroxypropoxy)methyl)benzene-1,2-diol (2). Yellow oil. $[\alpha]_D{}^{20}$ − 25° (*c* 0.40, MeOH). IR (KBr) ν_{max} 3609, 2950, 1640, 1555, 1520, 1452, 1090, 678 cm^{-1}. UV λ_{max} (EtOH) (log ε): 218 (3.92), 293 (3.43) nm. ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.10 (1H, s, H-6), 4.50 (2H, s, H_2-7), 3.82 (1H, m, H-9), 3.60 (2H, m, H-8a/10a), 3.53 (2H, m, H-8b/10b). ^{13}C NMR (acetone-d_6, 125 MHz): δ 145.9 (C-1, qC), 144.9 (C-2, qC), 131.5 (C-5, qC), 115.8 (C-6, CH), 114.4 (C-4, qC), 113.8 (C-3, qC), 74.1 (C-7, CH_2), 73.2 (C-8, CH_2), 71.8 (C-9, CH), 64.5 (C-10, CH_2). EIMS *m*/*z* 374 (9)/372 (24)/370 (13) $[M]^+$, 283 (52)/281 (98)/279 (48) $[M - C_3H_7O_3]^+$, 252 (16), 250 (10), 217 (8), 201 (9). HRESIMS *m*/*z* 394.8923 $[M + Na]^+$ (calcd for $C_{10}H_{12}{}^{79}Br^{81}BrO_5Na^+$, 394.8929).

5-(Aminomethyl)-3,4-dibromobenzene-1,2-diol (3). Colorless crystals; mp 161−162 °C. IR (KBr): ν_{max} 3129 (br), 1595, 1505, 1473, 1404, 1278, 1184, 1021, 986, 864 cm^{-1}. UV λ_{max} (EtOH) (log ε): 221 (4.70), 293 (4.04) nm. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.05 (1H, s, H-6), 4.01(2H, s, H_2-7). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 145.2 (C-1 and C-2, qC), 124.8 (C-5, qC), 116.7 (C-6, CH), 115.2 (C-4, qC), 113.5 (C-3, qC), 43.4 (C-7, CH_2). EIMS *m*/*z* 300 (30)/298 (91)/296 (37) $[M + H]^+$, 283 (23)/281 (60)/279 (27) $[M - NH_2]^+$, 203 (2), 201 (2), 169 (3), 138 (5), 85 (50). HRESIMS *m*/*z* 297.8902 $[M + H]^+$ (calcd for $C_7H_8{}^{79}Br^{81}BrNO_2^+$, 297.8901).

2-(2,3-Dibromo-4,5-dihydroxyphenyl)acetic Acid (4). Colorless crystals; mp 156−157 °C. IR (KBr): ν_{max} 3600, 2925, 1735, 1642, 1539, 1456, 1376, 1197, 1165 cm^{-1}. UV λ_{max} (EtOH) (log ε): 215 (3.72), 230 (3.66), 293 (3.22) nm. ^{1}H NMR (acetone-d_6, 500 MHz): δ 6.97 (1H, s, H-6), 3.73 (2H, s, H_2-7);. ^{13}C NMR (acetone-d_6, 125 MHz): δ 171.8 (C-8, qC), 145.6 (C-1, qC), 144.5 (C-2, qC), 128.7 (C-5, qC), 118.1 (C-6, CH), 117.4 (C-4, qC), 113.7 (C-3, qC), 42.9 (C-7, CH_2). EIMS *m*/*z* 328 (24)/326 (48)/324 (25) $[M]^+$, 283 (48)/281 (99)/279 (52) $[M - COOH]^+$, 247 (67)/245 (70) $[M - Br]^+$, 203 (32)/201 (39) $[M - COOH - Br]^+$, 200 (11), 174 (8), 172 (9), 166 (19), 133 (15), 131 (17), 121 (11). HRESIMS *m*/*z* 348.8683 $[M + Na]^+$ (calcd for $C_8H_6{}^{79}Br^{81}BrO_4Na^+$, 348.8687).

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3-Bromo-5-(hydroxymethyl)-2-methoxyphenol (5). Colorless crystals; mp 164−165 °C. IR (KBr): ν_{max} 3355, 2931, 1570, 1487, 1426, 1235, 1233, 1182, 1118, 1001, 850 cm^{-1};. UV λ_{max} (EtOH) (log ε): 212 (3.29), 227 (3.52), 284 (3.11) nm. ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.03 (1H, d, J = 1.3 Hz, H-4), 6.91 (1H, d, J = 1.3 Hz, H-6), 4.51 (2H, s, H_2-7), 3.79 (3H, s, OCH_3). ^{13}C NMR (acetone-d_6, 125 MHz): δ 152.1 (C-1, qC), 144.8 (C-2, qC), 141.3 (C-5, qC), 122.3 (C-4, CH), 117.2 (C-3, qC), 115.2 (C-6, CH), 63.7 (C-7, CH_2), 60.7 (OCH_3). EIMS m/z 234 (98)/232 (99) $[M]^+$, 217 (15)/215 (19) $[M - OH]^+$, 213 (6), 202 (7), 189 (7), 124 (13), 110 (50). HRESIMS m/z 254.9631 $[M + Na]^+$ (calcd for $C_8H_9{}^{79}BrO_3Na^+$, 254.9632).

(*E*)-4-(2-Bromo-4,5-dihydroxyphenyl)but-3-en-2-one (6). Colorless crystals; mp 145−147 °C. IR (KBr): ν_{max} 3383, 2913, 2846, 1651, 1596, 1499, 1414, 1233, 1168, 957, 818 cm^{-1}. UV λ_{max} (EtOH) (log ε): 233 (4.13), 257 (4.14), 305 (4.22), 347 (4.24) nm. ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.77 (1H, d, 16.0 Hz, H-7), 7.31 (1H, s, H-6), 7.12 (1H, s, H-3), 6.58 (1H, d, J = 16.0 Hz, H-8), 2.31 (3H, s, H_3-10). ^{13}C NMR (acetone-d_6, 125 MHz): δ 197.4 (C-9, qC), 149.7 (C-2, qC), 146.3 (C-1, qC), 141.4 (C-7, CH), 127.6 (C-8, CH), 126.4 (C-5, qC), 120.3 (C-3, CH), 116.2 (C-4, qC), 114.5 (C-6, CH), 27.6 (C-10, CH_3). EIMS m/z 258 (4)/256 (5) $[M]^+$, 178 (10)/177 (98) $[M - Br]^+$, 162 (28), 134 (15). HRESIMS m/z 256.9818 $[M + H]^+$ (calcd for $C_{10}H_{10}{}^{79}BrO_3{}^+$, 256.9813).

Antioxidant Assay for DPPH Free Radical Scavenging Activity. The scavenging effects of samples for DPPH radical were monitored according to the method of Yen and Chen.[20] Synthetic antioxidants, BHT and ascorbic acid, were used as positive controls. A 2.0 mL aliquot of test sample (in MeOH) was added to 2.0 mL of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then kept at room temperature for 30 min in the dark, and the absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the follow equation:

$$\text{scavenging effect } (\%) = [1 - (A_{\text{sample}} - A_{\textit{sample blank}})/A_{\text{control}}] \times 100$$

where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample), and the $A_{sample\ blank}$ is the absorbance of the sample (sample without DPPH solution). A curve of the concentration plotted against the percent inhibition was used to calculate the half-maximal inhibitory concentration (IC_{50}).

TEAC Assay. The method used was adapted from Re and coworkers.[21] Briefly, the ABTS radical cation was prepared by filtering a solution of ABTS in phosphate-buffered saline (PBS) through MnO_2 powder. This solution was diluted in 5 mM PBS (pH 7.4), adjusted to an absorbance of 0.700 at 734 nm, and preincubated at room temperature prior to use for 12 h. Samples were diluted with MeOH to produce solutions of 0.3, 0.5, 1.0, 1.5, and 2 mM concentration. The sample solution was mixed with the ABTS solution. After 6 min of incubation at ambient temperature, the absorbance value of the mixture was measured at 730 nm in a UV/vis spectrophotometer. Ascorbic acid was used as a positive control. Trolox was used as a positive control. A standard calibration curve was constructed for Trolox at 0, 2, 5, 10, 50, 100, and 150 mM concentrations. Three replicates were made for each test sample. TEAC values were calculated from the Trolox standard curve and expressed as Trolox equivalents (in mM of sample).

Statistics. Values expressed are means of three replicate determinations ± standard deviations. All statistical analyses were carried out using SPSS 11.01 for Windows. Values of $p < 0.05$ were considered as significantly different (α = 0.05).

RESULTS AND DISCUSSION

Identification of Active Components. Compounds **1**−**19** were identified on the basis of detailed interpretation of the spectroscopic data as well as by comparison with literature reports. The presence of a phenolic group in each compound structure was indicated by characteristic color reactions with $FeCl_3$ solution (violet).

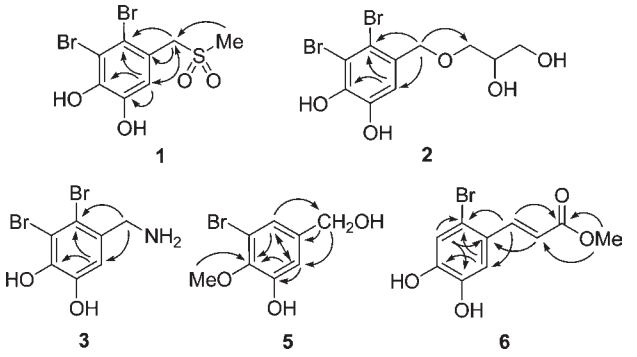

Figure 2. Key HMBC correlations of compounds **1**−**3**, **5**, and **6**.

Compound **1** was obtained as a colorless powder. The IR spectrum showed absorption bands for hydroxyl group (3315 cm^{-1}), aromatic rings (1653 and 1472 cm^{-1}), and sulfone unit (1299 and 1183 cm^{-1}). The EIMS gave a characteristic dibrominated molecular ion cluster at m/z 358/360/362 (1:2:1) $[M]^+$, and the molecular formula $C_8H_8Br_2O_4S$ was determined by HRESIMS. The ^{1}H NMR spectrum showed resonances for two phenolic hydroxyl protons at δ 9.24 and 8.69, one aromatic proton singlet at δ 7.14, a two-proton singlet for a methylene unit at δ 4.55, and a methyl singlet at δ 2.90. Consistently, eight carbon signals attributable to one methyl, one methylene, one aromatic methine, and five nonprotonated aromatic carbons were observed in the ^{13}C and DEPT NMR spectra. The oxygenated (C-1 and C-2) and brominated (C-3 and C-4) aromatic carbons were recognized by their chemical shifts at lower (δ > 140) and higher (δ < 120) fields,[11] respectively. The general features of the NMR spectroscopic data of **1** closely resembled those of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl sulfone, a sulfone-containing bromophenol identified from the marine red alga *Symphyocladia latiuscula*.[11] However, one of the brominated quaternary carbon signals was missing in the ^{13}C NMR spectrum of **1**. Instead, a signal for a methine carbon resonating at δ 118.9 was observed in the ^{13}C NMR spectrum. Accordingly, an aromatic proton at δ 7.14 (s) was detected in the ^{1}H NMR spectrm of **1**. These observations indicated that **1** was a dibrominated, *ortho*-hydroxyl sulfone derivative. The methine signal (C-6) was detected at relatively higher field (δ 118.9) in the ^{13}C NMR spectrum, which was affected by the *ortho*-hydroxyl group at C-1. The observed HMBC correlations from H-6 to C-1, C-2, C-4, and C-7 and from H_2-7 to C-4 and C-6 indicated the presence of a 2,3-dibromo-4,5-dihydroxybenzyl moiety (Figure 2), which is a typical substructure for bromophenols isolated from this algal species.[15−18] On the basis of the above evidence, the structure of **1** was assigned as 3,4-dibromo-5-(methylsulfonylmethyl)benzene-1,2-diol. Compound **1** represents the first example of a bromophenol possessing a sulfonyl moiety isolated from *R. confervoides*.

Compound **2** was obtained as yellowish oil. The EIMS gave a characteristic dibrominated molecular ion cluster at m/z 370/372/374 (1:2:1) $[M]^+$, and the molecular formula $C_{10}H_{12}Br_2O_5$ was determined by HRESIMS. The ^{1}H NMR spectrum showed the presence of one aromatic singlet at δ 7.10, one oxymethine proton multiplet at δ 3.82, and three methylenes appearing as a two-proton singlet at δ 4.50 and a pair of two-proton multiplets at δ 3.60 and 3.53. Ten carbon signals due to one aromatic

methine, five nonprotonated aromatic carbons (two oxygenated and two brominated), one oxymethine, and three oxygen-bearing methylene units were observed in the ^{13}C and DEPT NMR spectra. As was the case for **1**, a 2,3-dibromo-4,5-dihydroxybenzyl moiety was deduced to be present in **2**. The glycerol moiety was deduced on the basis of the chemical shifts at δ 73.2 (CH_2), 71.8 (CH), and 64.5 (CH_2) and the $^1H-^1H$ COSY correlations of H-9 with H-8a/10a and H-8b/10b. HMBC cross-peaks from H-6 to C-2, C-4, and C-7, from H_2-7 to C-4, C-6, and C-8 (Figure 2) resulted in the assignment of the structure of **2** as 3,4-dibromo-5-((2,3-dihydroxypropoxy)methyl)benzene-1,2-diol.

Compound **3** gave ^1H and ^{13}C NMR spectra that were very similar to those of **1**, except for the absence of the methyl singlet, suggesting that **3** also possesses a 2,3-dibromo-4,5-dihydroxybenzyl moiety. The ESIMS gave a characteristic dibrominated $[M + H]^+$ ion cluster at m/z 296/298/300 (1:2:1), and the molecular formula $C_7H_7Br_2NO_2$ was determined by HRESIMS measurement. Given this formula and the acquisition of NMR data that were otherwise nearly the same as those of **1**, the structure of **3** was assigned as 5-(aminomethyl)-3,4-dibromobenzene-1,2-diol, and the HMBC correlations agreed with this deduction (Figure 2).

Compound **4** was identified as 2-(2,3-dibromo-4,5-dihydroxyphenyl)acetic acid. The only difference in the structures of **4** and **3** was that the NH_2 group in **3** was replaced by a COOH group in **4**. This was in agreement with the molecular formula (obtained by HRESIMS analysis) and was further supported by the fact that a carboxy carbonyl signal was observed at δ 171.8 in the ^{13}C NMR spectrum of **4**.

Compound **5** was obtained as a colorless crystalline solid. The EIMS gave a characteristic monobrominated molecular ion cluster at m/z 232/234 (1:1) $[M]^+$, and the molecular formula $C_8H_9BrO_3$ was determined by HRESIMS. The ^1H NMR spectrum showed resonances for two *meta*-coupled aromatic doublets at δ 7.03 (d, J = 1.3 Hz) and 6.91 (d, J = 1.3 Hz), one *O*-methyl singlet at δ 3.79, and an isolated oxymethylene unit appearing as a two-proton singlet at δ 4.51. Eight carbon signals attributable to one methoxy group, one oxygen-bearing methylene, two aromatic methines, and four nonprotonated aromatic carbons (two oxygenated and one brominated) were evident in the ^{13}C and DEPT NMR spectra. The presence of a 3-bromo-4-methoxy-5-hydroxybenzyl moiety was supported by HMBC correlations from H-4, H-6, and OCH_3 to C-2 and from H_2-7 to C-4, C-5, and C-6 (Figure 2). The structure of **5** was therefore assigned as 3-bromo-5-(hydroxymethyl)-2-methoxyphenol. It should be mentioned that this compound was recently used as a starting material in organocatalyzed Friedel−Crafts arylation of benzylic alcohols. However, no spectroscopic data or other related information were described for this compound in the literature report.[22]

Compound **6** was obtained as a colorless crystalline solid. The EIMS gave a characteristic monobrominated molecular ion cluster at m/z 256/258 (1:1) $[M]^+$, and the molecular formula $C_{10}H_9BrO_3$ was determined by HRESIMS. The ^1H NMR spectrum showed resonances for two *para*-aromatic protons at δ 7.31 (s, H-6) and 7.12 (s, H-3), two *trans*-coupled olefinic protons at δ 7.77 (d, J = 16.0 Hz, H-7) and 6.58 (d, J = 16.0 Hz, H-8), and an acetyl methyl group at δ 2.31 (s, H_3-10). Ten signals due to an acetyl unit, two olefinic carbons, two aromatic methines, and four nonprotonated aromatic carbons (two oxygenated and one brominated) were observed in the ^{13}C and DEPT NMR spectra. HMBC correlations from H-3 to C-1 and C-5, from H-6 to C-2, C-4, and C-7, from H-7 to C-4, C-6, and C-9, from H-8 to C-5 and C-10, and from H-10 to C-8 were observed (Figure 2), leading to assignment of the structure of **6** as (*E*)-4-(2-bromo-4,5-dihydroxyphenyl)but-3-en-2-one. This is the first bromophenol metabolite containing a 2-bromo-4,5-dihydroxybenzyl unit to be reported from a member of the Rhodomelaceae family of marine red algae.

In addition to the above six new bromophenols, 13 known compounds were also isolated. By comparison with literature data, their structures were determined to be (*E*)-4-(2,3-dibromo-4,5-dihydroxyphenyl)but-3-en-2-one (**7**),[16] 3-(2,3-dibromo-4,5-dihydroxyphenyl)-2-methylpropanal (**8**),[17] 3,4-dibromo-5-(2-hydroxyethyl)benzene-1,2-diol (**9**),[18] 3,4-dibromo-5-(hydroxymethyl)benzene-1,2-diol (**10**),[13] 3,4-dibromo-5-(methoxymethyl)-benzene-1,2-diol (**11**),[13] 3,4-dibromo-5-(ethoxymethyl)benzene-1,2-diol (**12**),[13] 2,3-dibromo-4,5-dihydroxybenzaldehyde (**13**),[23] 2-amino-3-(3-bromo-5-hydroxy-4-methoxyphenyl)propanoic acid (**14**),[15] 2-(3-bromo-5-hydroxy-4-methoxyphenyl)acetic acid (**15**),[16] methyl 2-(3-bromo-5-hydroxy-4-methoxyphenyl)acetate (**16**),[16] 3-bromo-5-hydroxy-4-methoxybenzoic acid (**17**),[16] 3-bromo-5-hydroxy-4-methoxybenzaldehyde (**18**),[24] and (*E*)-4-(3-bromo-4,5-dihydroxyphenyl)-but-3-en-2-one (**19**).[9]

Antioxidant Activity in the DPPH Free Radical Scavenging Assay. Free radicals, which are involved in the process of lipid peroxidation, are considered to play key roles in numerous chronic pathologies such as cancer and cardiovascular diseases and have been implicated in the aging process. The isolated bromophenol compounds were evaluated for DPPH radical scavenging properties. As shown in Table 1, all compounds possessed the ability to scavenge DPPH radicals to varying degrees. Compound **2** was found to be the most potent DPPH scavenger (IC_{50} = 7.43 μM), followed by those of **7**, **6**, **19**, and **1**, which gave IC_{50} values of 7.62, 8.72, 9.40, and 9.52 μM, respectively. The activities of these bromophenols were significantly more potent than that of the positive control BHT (IC_{50} = 82.11 μM) ($p < 0.05$).

Several studies that look at the relationship between molecular structure and antioxidant activity of bromophenols from marine red algae have been reported.[11,25] Analysis of the DPPH radical scavenging activity of compounds **1**−**19** demonstrated that the metabolites with *ortho*-dihydroxy groups on the aromatic ring generally display higher activity than the compounds having a single free hydroxy group on the ring. This result is in accordance with the conclusions described by Chaillou and Nazareno.[26] Compound **7** showed higher activity than that of **6** and **19**, suggesting that the number and position of the bromine atom(s) are also important factors contributing to the variation in activity observed for the compounds in this assay. Compounds **5** and **14**−**18**, all possessing a 3-bromo-4-methoxy-5-hydroxybenzyl group, exhibited lower activity, with IC_{50} values ranging from 26.3 to 58.2 μM. These results indicated that replacement of a hydroxy with a methoxy group on the phenol ring significantly decreased the activity. Thus, both *ortho*-dihydroxylation and bromine atom substitution play important roles in DPPH radical scavenging activity in this class of compounds.

Antioxidant Activity in the TEAC Assay. It is generally accepted that ABTS scavenging by antioxidants is attributable to hydrogen-donating ability. The isolated bromophenols were examined for their free radical-scavenging activity against ABTS cation radicals. The results are presented in Table 1. All of the compounds possessed the ability to scavenge ABTS radicals more strongly than the positive control, ascorbic acid, with compound **6** being the most active, followed by compounds **7**, **19**, **4**, and **15**. Except for **15**, compounds with high DPPH scavenging activity also possessed strong activity in the TEAC assay.

Table 1. Antioxidant Activity of Compounds 1−19 from *R. confervoides*[a]

	DPPH radical scavenging activity	ABTS radical scavenging activity
compd	IC_{50} (μM)	TEAC (mM)
1	9.52 ± 0.04	2.06 ± 0.08
2	7.43 ± 0.10	2.11 ± 0.04
3	20.47 ± 0.07	1.87 ± 0.02
4	19.84 ± 0.06	2.87 ± 0.11
5	50.58 ± 0.23	1.60 ± 0.04
6	8.72 ± 0.05	3.68 ± 0.12
7	7.62 ± 0.01	3.45 ± 0.12
8	18.62 ± 0.08	2.11 ± 0.11
9	30.91 ± 0.12	1.98 ± 0.01
10	42.33 ± 0.25	1.56 ± 0.02
11	40.50 ± 0.20	1.62 ± 0.03
12	38.42 ± 0.23	1.36 ± 0.01
13	32.01 ± 0.12	1.09 ± 0.01
14	50.87 ± 0.32	1.63 ± 0.01
15	26.28 ± 0.21	2.35 ± 0.02
16	30.24 ± 0.20	2.07 ± 0.12
17	50.31 ± 0.34	1.86 ± 0.02
18	58.15 ± 0.39	1.32 ± 0.02
19	9.40 ± 0.05	3.10 ± 0.13
BHT	82.11 ± 0.20	
ascorbic acid		1.02 ± 0.01

[a] Each value is presented as the mean ± standard deviation (n = 3).

In conclusion, 19 bromophenols were isolated from the marine red alga *R. confervoides*, six of which are new naturally occurring secondary metabolites, constituting new additions to the molecular diversity of the Rhodomelaceae family. Most of the isolated compounds displayed significant antioxidant activity against DPPH and ABTS radicals, which indicated that these compounds are responsible for the activity observed in our previous screening tests.[19] These results suggest that consumption of marine algae as a dietary supplement or as a food ingredient has the potential to provide health benefits due to antioxidant properties. For safety considerations, however, the possible toxicity of these bromophenols should be evaluated before implementing any use as a dietary supplement or as a foodstuff.

■ ASSOCIATED CONTENT

Supporting Information. Tabulated and fully assigned NMR data for compounds **1**−**6** (Tables S1 and S2) and the ^{1}H and ^{13}C NMR spectra of **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +86-532-8289 8553. E-mail: wangbg@ms.qdio.ac.cn.

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■ REFERENCES

(1) Tseng, C. K. The past, present and future of phycology in China. *Hydrobiologia* **2004**, *512*, 11–20.

(2) Fleurence, J. Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.* **1999**, *10*, 25–28.

(3) Wong, K. H.; Cheung, P. C. K. Nutritional evaluation of some subtropical red and green seaweeds Part I-proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* **2000**, *71*, 475–482.

(4) Romarís-Hortas, V.; García-Sartal, C.; Barciela-Alonso, M. C.; Moreda-Piñeiro, A.; Bermejo-Barrera, P. Characterization of edible seaweed harvested on the Galician coast (Northwestern Spain) using pattern recognition techniques and major and trace element data. *J. Agric. Food Chem.* **2010**, *58*, 1986–1992.

(5) Ito, N.; Hirose, M.; Fukushima, S.; Tsuda, H.; Shirai, T.; Tatematsu, M. Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem. Toxicol.* **1986**, *24*, 1071–1082.

(6) Williams, G. M.; Iatropoulos, M. J.; Whysner, J. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food Chem. Toxicol.* **1999**, *37*, 1027–1038.

(7) Li, Y. X.; Li, Y.; Lee, S. H.; Qian, Z. J.; Kim, S. K. Inhibitors of oxidation and matrix metalloproteinases, floridoside, and D-isofloridoside from marine red alga *Laurencia undulata*. *J. Agric. Food Chem.* **2010**, *58*, 578–586.

(8) Li, K.; Li, X. M.; Ji, N. Y.; Gloer, J. B.; Wang, B. G. Urceolatin, a structurally unique bromophenol from *Polysiphonia urceolata*. *Org. Lett.* **2008**, *10*, 1429–1432.

(9) Li, K.; Li, X. M.; Ji, N. Y.; Wang, B. G. Natural bromophenols from the marine red alga *Polysiphonia urceolata* (Rhodomelaceae): Structural elucidation and DPPH radical-scavenging activity. *Bioorg. Med. Chem.* **2007**, *15*, 6627–6631.

(10) Li, K.; Li, X. M.; Ji, N. Y.; Wang, B. G. Bromophenols from the marine red alga *Polysiphonia urceolata* with DPPH radical scavenging activity. *J. Nat. Prod.* **2008**, *71*, 28–30.

(11) Duan, X. J.; Li, X. M.; Wang, B. G. Highly brominated mono- and bis-phenols from the marine red alga *Symphyocladia latiuscula* with radical-scavenging activity. *J. Nat. Prod.* **2007**, *70*, 1210–1213.

(12) Kurihara, H.; Mitani, T.; Kawabata, J.; Takahashi, K. Two new bromophenols from the red alga *Odonthalia corymbifera*. *J. Nat. Prod.* **1999**, *62*, 882–884.

(13) Kurata, K.; Taniguchii, K.; Takashima, K.; Hayashi, I.; Suzuki, M. Feeding-deterrent bromophenols from *Odonthalia corymbifera*. *Phytochemistry* **1997**, *45*, 485–487.

(14) Wiemer, D. F.; Idler, D. D.; Fenical, W. Vidalols A and B, new anti-inflammatory bromophenols from the Caribbean marine red alga *Vidalia obtusaloba*. *Experientia* **1991**, *47*, 851–853.

(15) Ma, M.; Zhao, J. L.; Wang, S. J.; Li, S.; Yang, Y. C.; Shi, J. G.; Fan, X.; He, L. Bromophenols coupled with nucleoside bases and brominated tetrahydroisoquinolines from red alga *Rhodomela confervoides*. *J. Nat. Prod.* **2007**, *70*, 337–341.

(16) Zhao, J.; Fan, X.; Wang, S.; Li, S.; Shang, S.; Yang, Y.; Xu, N.; Lu, Y.; Shi, J. Bromophenol derivatives from the red alga *Rhodomela confervoides*. *J. Nat. Prod.* **2004**, *67*, 1032–1035.

(17) Fan, X.; Xu, N. J.; Shi, J. G. Bromophenols from the red alga *Rhodomela confervoides*. *J. Nat. Prod.* **2003**, *66*, 455–458.

(18) Ma, M.; Zhao, J. L; Wang, S. J.; Li, S.; Yang, Y. C.; Shi, J. G.; Fan, X.; He, L. Bromophenols coupled with methyl γ-ureidobutyrate and bromophenol sulfates from the red alga *Rhodomela confervoides*. *J. Nat. Prod.* **2006**, *69*, 206–210.

(19) Wang, B. G.; Zhang, W. W.; Duan, X. J.; Li, X. M. In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). *Food Chem.* **2009**, *113*, 1101–1105.

(20) Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27–32.

(21) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. A. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1996**, *26*, 1231–1237.

(22) McCubbin, J. A.; Krokhin, O. V. Organocatalyzed Friedel−Crafts arylation of benzylic alcohols. *Tetrahedron Lett.* **2010**, *51*, 2447–2449.

(23) Katsui, N.; Suzuki, Y.; Kitamura, S.; Irie, T. 5,6-Dibromoprotocatechualdehyde and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether: New dibromophenols from *Rhodomela larix*. *Tetrahedron* **1967**, *23*, 1185–1188.

(24) Borchardt, R. T.; Huber, J. A.; Houston, M. Catechol *O*-methyltransferase. 10. 5-Substituted 3-hydroxy-4-methoxybenzoic Acids (isovanillic acids) and 5-substituted 3-hydroxy-4-methoxybenzaldehydes (isovanillins) as potential inhibitors. *J. Med. Chem.* **1982**, *25*, 258–263.

(25) Lee, J. H.; Lee, T. K.; Kang, R. S.; Shin, H. J.; Lee, H. S. The *in vitro* antioxidant activities of the bromophenols from the red alga *Tichocarpus crinitus* and phenolic derivatives. *J. Korean Magn. Reson. Soc.* **2007**, *11*, 56–63.

(26) Chaillou, L. L.; Nazareno, M. A. New method to determine antioxidant activity of polyphenols. *J. Agric. Food Chem.* **2006**, *54*, 8397–8402.