Structures and Aldose Reductase Inhibitory Effects of Bromophenols from the Red Alga *Symphyocladia latiuscula*

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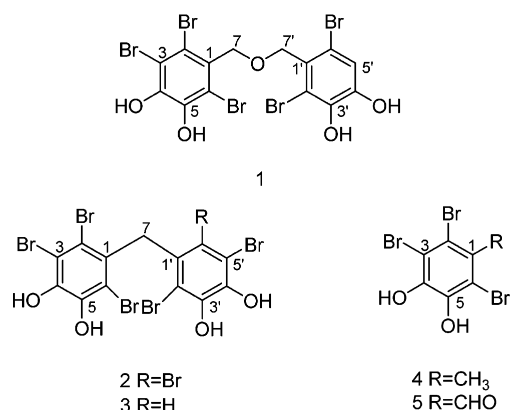
Three new bromophenols, 2,2′,3,6,6′-pentabromo-3′,4,4′,5-tetrahydroxydibenzyl ether (**1**), bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (**2**), and 2,2′,3,5′,6-pentabromo-3′,4,4′,5-tetrahydroxydiphenylmethane (**3**), were isolated from the red alga *Symphyocladia latiuscula*. Two bromophenols, 2,3,6-tribromo-4,5-dihydroxymethylbenzene (**4**) and 2,3,6-tribromo-4,5-dihydroxybenzaldehyde (**5**), were also reported for the first time as natural products. Their structures were elucidated on the basis of chemical and spectroscopic methods including HREIMS, HRFABMS, and 1D and 2D NMR spectral techniques. Compounds **1**−**5** exhibited significant aldose reductase inhibitory activity.

Red algae of the family Rhodomelaceae are known to contain high concentrations of bromophenols. Some of the bromophenols previously isolated from this family[1−17] have shown significant nitrite scavenging,[5] α-glucosidase inhibition,[7] antioxidant,[8] feeding-deterrent,[10] and anti-inflammatory activities.[11] *Symphyocladia latiuscula* (Harvey) Yamada is a member of the family Rhodomelaceae, order Ceramiales,[18] widely distributed in the Gulf of the Bo Sea, China. 2,3,6-Tribromo-4,5-dihydroxybenzyl methyl ether, 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol, (2*R*)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl)cyclohexanone, and bis(2,3,6-tribromo-4,5-dihydroxybenzyl) ether have been isolated from this alga.[6,7]

While searching for biologically active constituents from algae, we found several types of algae exhibited potential effects on diabetic complications.[19−21] During screening for aldose reductase (AR) inhibitory agents from algae, we found *S. latiuscula* extract showed an inhibitory effect on AR. Therefore, we investigated the chemical constituents of *S. latiuscula* collected from the coast of Dalian, China, in an effort to identify the AR inhibitory agents.

The air-dried and ground red alga *S. latiuscula* was extracted using 95% EtOH, and the concentrated extract was suspended in water and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc extract was chromatographed over silica gel eluting with *n*-hexane−EtOAc and EtOAc−MeOH. The subsequent fractions were further purified using a variety of chromatographic techniques to yield three new bromophenols: 2,2′,3,6,6′-pentabromo-3′,4,4′,5-tetrahydroxydibenzyl ether (**1**), bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (**2**), and 2,2′,3,5′,6-pentabromo-3′,4,4′,5-tetrahydroxydiphenylmethane (**3**). Two bromophenols, 2,3,6-tribromo-4,5-dihydroxymethylbenzene (**4**) and 2,3,6-tribromo-4,5-dihydroxybenzaldehyde (**5**), were reported for the first time as natural products. Herein, we report the isolation and structural elucidation and AR inhibitory evaluation of these bromophenols.



Compound **1** was obtained as a yellowish amorphous solid, mp 132−134 °C. The negative FABMS spectrum with glycerol as a matrix exhibited a quasi-molecular ion peak cluster at *m/z* 651/653/655/657/659/661 (1:5:10:10:5:1), which suggested the presence of five bromine atoms in **1**. The molecular formula was determined as $C_{14}H_9Br_5O_5$ by negative HRFABMS at *m/z* 650.6280 (calcd for $C_{14}H_8{}^{79}Br_5O_5$ 650.6289). The ^{1}H NMR spectrum of **1** in acetone-d_6 showed a singlet attributed to an aromatic proton at δ 7.12 (1H, s, H-5′), two singlets assigned to oxymethylene protons at δ 4.96 (2H, s, H-7) and 4.84 (2H, s, H-7′), and four exchangeable broadened singlets for the phenolic hydroxyl groups at δ 9.15 (1H, br s, OH), 8.93 (1H, br s, OH), 8.72 (1H, br s, OH), and 8.26 (1H, br s, OH). The ^{13}C NMR and DEPT spectra of **1** displayed 14 carbon signals attributed to a pentasubstituted benzene ring, a hexasubstituted benzene ring, and a pair of oxymethylene groups (see Experimental Section). The protonated carbons were assigned from the HMQC experiment for **1**, and the oxygenated quaternary carbons were recognized by their chemicals shifts (δ > 140 ppm). The above spectral data indicated that **1** was a pentabrominated tetrahydroxydibenzyl ether. In the HMBC spectrum, cross-peaks from aromatic and oxymethylene protons to their correlated long-range carbons unambiguously established the substitution pattern for the aromatic ring. Long-range correlations from H_2-7 to C-1, C-2, C-6, and C-7′; from H_2-7′ to C-1′, C-2′ C-6′, and C-7; and from H-5′ to C-1′, C-3′, C-4′, and C-6′ unequivocally demonstrated that the aromatic proton was assigned to C-5′.

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Therefore, the structure of **1** was determined as 2,2′,3,6,6′-pentabromo-3′,4,4′,5-tetrahydroxydibenzyl ether (**1**).

Compound **2** was obtained as a yellowish amorphous solid, mp 228−230 °C. Its negative FABMS spectrum with glycerol as a matrix gave characteristic hexabrominated molecular ion peaks at *m/z* 699/701/703/705/707/709/711 (1:5:12:15:12:5:1). The molecular formula $C_{13}H_6Br_6O_4$ was determined by negative HRFABMS at *m/z* 698.5280 (calcd for $C_{13}H_5{}^{79}Br_6O_4$ 698.5288). The ^{1}H NMR spectrum of **2** in acetone-d_6 showed a singlet attributed to methylene protons at δ 4.84 (2H, s, H-7) and two singlets for the phenolic hydroxyl groups at δ 8.62 (2H, s, OH) and 8.77 (2H, s, OH). The ^{13}C NMR and DEPT spectra of **2** (see Experimental Section) exhibited seven carbons: one benzylic methylene carbon and six sp^2 carbons including one nonoxygenated quaternary carbon, two oxygenated quaternary carbons, and three brominated quaternary carbons. The NMR spectra of **2** showed simpler patterns than expected from its molecular weight, which indicated its symmetrical nature. The substituted patterns for the aromatic rings in the HMBC spectrum were unambiguously established by correlations from H-7 to C-1, C-2, and C-6. Therefore, the structure of **2** was determined to be bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (**2**).

Compound **3** was obtained as a yellowish amorphous solid, mp 168−172 °C. The negative FABMS spectrum with glycerol as a matrix showed the pentabrominated molecular ion peak cluster at *m/z* 621/623/625/627/629/631 with a ratio of abundances 1:5:10:10:5:1. The molecular formula was determined as $C_{13}H_7Br_5O_4$ by negative HRFABMS at *m/z* 620.6179 (calcd for $C_{13}H_6{}^{79}Br_5O_4$ 620.6183). The ^{1}H NMR spectrum of **3** in acetone-d_6 showed a singlet attributed to an aromatic proton at δ 6.25 (1H, s, H-6′) and a doublet assigned to a methylene proton at δ 4.19 (2H, d, J = 0.8 Hz). The ^{13}C NMR and DEPT spectra (see Experimental Section) displayed a methylene carbon signal and 12 sp^2 carbon signals including a methine and 11 quaternary carbons (four oxygenated, δ > 140 ppm). The above spectral data indicates that **3** possesses a pentabrominated diarylmethane structure with four hydroxyls. In the HMBC spectrum, the methylene proton showed long-range correlations with C-1, C-2, C-6, C-1′, C-2′, and C-6′, while the aromatic proton showed long-range correlations with C-2′, C-4′, and C-5′. Accordingly, the structure of **3** was determined as 2,2′,3,5′,6-pentabromo-3′,4,4′,5-tetrahydroxydiphenylmethane (**3**).

Compound **4** was obtained as a yellowish amorphous solid, mp 126−129 °C. The HREIMS of **4** showed an [M] ion at *m/z* 357.7842, corresponding to the molecular formula $C_7H_5Br_3O_2$ (calcd for $C_7H_5{}^{79}Br_3O_2$ 357.7840). The EIMS spectrum gave characteristic tribrominated molecular ion peaks at *m/z* 358/360/362/364 with a ratio of abundance 1:3:3:1. The ^{1}H NMR spectrum of **4** in acetone-d_6 showed only a singlet attributed to methyl protons at δ 2.58 (3H, s, H-7). The ^{13}C NMR and DEPT spectra of **4** (see Experimental Section) displayed seven carbons including a methyl and six quaternary carbons (two oxygenated, δ > 140 ppm). These spectral data revealed a tribromominated dihydroxy methylbenzene structure for **4**. The substituted patterns were established by long-range correlations from methyl protons to C-1, C-2, and C-6. Thus, the structure of **4** could be determined as 2,3,6-tribromo-4,5-dihydroxymethylbenzene (**4**).

Compound **5** was obtained as a yellowish amorphous solid, mp 134−135 °C. The negative FABMS spectrum with glycerol as a matrix showed the tribrominated molecular ion peaks at *m/z* 371/373/375/377 (1:3:3:1). The molecular formula was determined as $C_7H_3Br_3O_3$ by negative HRFABMS at *m/z* 370.7557 (calcd for $C_7H_2{}^{79}Br_3O_3$ 370.7554). The ^{1}H NMR spectrum of **5** was very similar to that of **4** (see Experimental Section), with the exception of the loss of signal attributed to the methyl protons and the appearance of a proton signal at δ 10.08 (1H, s, H-7). In addition, the ^{13}C NMR spectra showed a signal at δ 191.14, which was assigned to an aldehyde. Therefore, the structure of **5** was identified as 2,3,6-tribromo-4,5-dihydroxybenzaldehyde (**5**).

Table 1. Aldose Reductase Inhibitory Activity of Isolated Compounds **1**−**5**

compound	IC_{50} (μg/mL)
1	0.11
2	0.40
3	0.40
4	1.15
5	0.25
epalrestat	0.03
quercetin	1.05

Under hyperglycemia, because of the saturation of hexokinase with ambient glucose, the increased flux of glucose through the polyol pathway accounts for as much as one-third of the total glucose turnover.[22] This leads to overflow of products of the polyol pathway along with depletion in reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the oxidized form of nicotinamide adenine dinucleotide (NAD^+), the cofactor used in the pathway. AR (alditol/ oxidoreductase, E.C.1.1.1.21) is the first enzyme of the polyol pathway that reduces excess D-glucose into D-sorbitol with concomitant conversion of NADPH into $NADP^+$.[23] The polyol pathway seems to play an important role in the development of degenerative complications of diabetes, such as neuropathy, nephropathy, retinopathy, cataracts, and cardiovascular disease. The inhibitory effects of the isolated compounds (**1**−**5**) on AR were tested using the human muscle AR recombinant. The inhibitory effects of compounds **1**−**3** and **5** were much higher than that of quercetin, which was used as the positive control. The effect of **4** was similar to that of quercetin (Table 1). These compounds are likely to be responsible for the AR inhibitory effect of *S. latiuscula*.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanako micromelting apparatus and were used uncorrected. EIMS and FABMS (negative ion mode, glycerol) were obtained using a JEOL JMS-700 mass spectrometer. ^{1}H and ^{13}C NMR spectra were measured on JEOL AL-400 and LA-500 spectrometers using tetramethylsilane as the internal standard. All chemical shifts (δ) were given in ppm, and the samples were solubilized in acetone-d_6. Column chromatography was performed with silica gel 60 N (100−210 μm), RP-18 reverse-phase silica gel, and Sephadex LH-20. TLC was carried out on precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm) and silica gel RP-18 60 F_{254} (Merck, 0.25 mm). Detection was achieved by spraying with 10% H_2SO_4 in MeOH followed by heating. HPLC was performed using a COSMOSIL silica preparative column (10 × 250 mm, normal-phase HPLC) and a COSMOSIL ODS preparative column (20 × 250 mm, reversed-phase HPLC).

Material. The red alga *Symphyocladia latiuscula* was collected at the coast of Dalian, China, in April 2003 and identified by Dr. Z.-A. Yao. A voucher specimen (20030401) was deposited at the Department of Natural Products, Institute of Biological Engineering, Dalian University, China.

Extraction and Isolation. The air-dried and ground red alga *S. latiuscula* (9.4 kg) was reflux extracted twice with 95%

EtOH for 4 h. After the solvent was removed under reduced pressure, a brown residue was obtained. The residue was suspended in water and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc extract (80 g) was chromatographed over silica gel (3000 g) eluting with *n*-hexane−EtOAc and EtOAc−MeOH and separated into 20 fractions (I-XX) on the basis of TLC analyses. Fraction IX (500 mg) was purified by normal-phase preparative HPLC using *n*-hexane−EtOAc (80:20) as the mobile phase to give **4** (5.7 mg). Fraction XV (3 g) was chromatographed over Sephadex LH-20 eluting with acetone and separated into two subfractions. The first subfraction was further separated by ODS column chromatography eluting with a gradient of increasing MeOH (70−100%) in water and reverse-phase preparative HPLC using MeOH−H_2O (80:20) as the mobile phase to yield **1** (7.5 mg) and **2** (40.7 mg). The second fraction was chromatographed on an ODS column and eluted using a gradient of increasing MeOH (70−100%) in water and reversed-phase preparative HPLC using MeOH−H_2O (65:35) as the mobile phase to yield **3** (6.4 mg) and **5** (22.6 mg).

2,2′,3,6,6′-Pentabromo-3′,4,4′,5-tetrahydroxydibenzyl ether (1): yellowish white amorphous solid; mp 132−134 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.15 (1H, br s, OH), 8.93 (1H, br s, OH), 8.72 (1H, br s, OH), 8.26 (1H, br s, OH), 7.12 (1H, s, H-5′), 4.96 (2H, s, H-7), 4.84 (2H, s, H-7′); ^{13}C NMR (acetone-d_6, 100 MHz) δ 146.81 (s, C-4′), 145.14 (s, C-4), 143.55 (s, C-5), 143.45 (s, C-3′), 129.75 (s, C-1), 127.97 (s, C-1′), 119.04 (s, C-6), 118.54 (d, C-5′), 115.31 (s, C-6′), 114.81 (s, C-2′), 114.45 (s, C-2), 113.44 (s, C-3), 73.84 (t, C-7), 72.29 (t, C-7′); FABMS *m/z* 651, 653, 655, 657, 659, 661 [M − H]$^-$ (3, 14, 27, 27, 15, 4); HRFABMS *m/z* 650.6280 (calcd for $C_{14}H_8{}^{79}Br_5O_5$, 650.6289).

Bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (2): yellowish white amorphous solid; mp 228−230 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.77 (2H, br s, OH), 8.62 (2H, br s, OH), 4.84 (2H, s, H-7); ^{13}C NMR (acetone-d_6, 100 MHz) δ 143.60 (s, C-4, 5, 3′, 4′), 131.45 (s, C-1, 1′), 118.16 (s, C-6, 2′), 114.19 (s, C-2, 6′), 114.03 (s, C-3, 5′), 47.49 (t, C-7); FABMS *m/z* 699, 701, 703, 705, 707, 709, 711 [M − H]$^-$ (2, 10, 24, 32, 24, 10, 2); HRFABMS *m/z* 698.5280 (calcd for $C_{13}H_5{}^{79}Br_6O_4$, 698.5288).

2,2′3,5′,6-Pentabromo-3′,4,4′,5-tetrahydroxydiphenyl methane (3): yellowish white amorphous solid; mp 168−172 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.25 (1H, s, H-6′), 4.42 (2H, d, J = 0.8 Hz, H-7); ^{13}C NMR (acetone-d_6, 100 MHz) δ 144.65 (s, C-4), 144.37 (s, C-3′), 144.03 (s, C-5), 142.43 (s, C-4′), 130.97 (s, C-1), 130.48 (s, C-1′), 121.91 (d, C-6′), 118.08 (s, C-6), 113.93 (s, C-2, 3), 112.08 (s, C-2′), 109.46 (s, C-5′), 44.63 (t, C-7); FABMS *m/z* 621, 623, 625, 627, 629, 631 [M − H]$^-$ (4, 15, 29, 29, 15, 4); HRFABMS *m/z* 620.6179 (calcd for $C_{13}H_6{}^{79}Br_5O_4$, 620.6183).

2,3,6-Tribromo-4,5-dihydroxymethylbenzene (4): yellowish white amorphous solid; mp 126−129 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 2.58 (3H, s, H-7); ^{13}C NMR (acetone-d_6, 100 MHz) δ 144.30 (s, C-4), 143.70 (s, C-5), 130.37 (s, C-1), 116.81 (s, C-6), 113.76 (s, C-2), 113.02 (s, C-3), 25.49 (s, C-7); EIMS *m/z* 358, 360, 362, 364 [M]$^+$ (33, 100, 98, 31); HREIMS *m/z* 357.7842 (calcd for $C_7H_5{}^{79}Br_3O_2$, 357.7840).

2,3,6-Tribromo-4,5-dihydroxybenzaldehyde (5): yellowish white amorphous solid; mp 134−135 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.08 (1H, s, H-7); ^{13}C NMR (acetone-d_6, 100 MHz) δ 190.63 (d, C-7), 148.74 (s, C-4), 143.91 (s, C-5), 127.43 (s, C-1), 118.55 (s, C-6), 114.60 (s, C-4), 111.40 (s, C-3); FABMS *m/z* 371, 373, 375, 377 [M − H]$^-$ (11, 33, 33, 12); HRFABMS *m/z* 370.7557 (calcd for $C_7H_2{}^{79}Br_3O_3$, 370.7554).

Enzyme Inhibition Assay. The inhibitory activity of the compounds was determined against AR recombinant from human muscle cell purchased from Wako Pure Chemical Industries Ltd. (Code No. 547-00581). The inhibitory activity has been measured spectrophotometrically at pH 6.2 and at 25 °C using 0.15 mM NADPH with 10 mM DL-glyceraldehyde as a substrate and 3×10^{-3} unit/mL enzyme, in 0.2 M sodium phosphate buffer. The decrement in absorption of NADPH at 340 nm due to the oxidation of NADPH by AR was monitored continuously with a spectrophotometer (Shimadzu UV 160). The concentrations of test compounds showing 50% inhibitions of AR (IC_{50}) were estimated from the least-squares regression line of four plots of the logarithmic concentrations versus the remaining activity.

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