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Inhibitory Potencies of Bromophenols from Rhodomelaceae Algae against α-Glucosidase Activity

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Inhibitory potencies against α-glucosidase activities were compared among bromophenols obtained from extracts of three Rhodomelaceae red algae, *Symphyocladia latiuscula*, *Odonthalia corymbifera*, and *Polysiphonia morrowii*. The bromophenols from these species are characterized by a number of Br atoms per benzene ring: *S. latiuscula*, three Br atoms (**1** and **2**); *O. corymbifera*, two Br atoms (**3**–**7**); and *P. morrowii*, one Br atom (**8** and **9**). The bromophenols exhibited mixed inhibition against yeast α-glucosidase reaction. In particular, symmetric dibenzyl ethers, bis(2,3,6-tribromo-4,5-dihydroxybenzyl) ether (**2**) and bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (**5**), exhibited about 10^3 fold smaller K_i values than the other bromophenols. Their inhibition modes are disclosed to be mixed inhibition closed to competitive inhibition. Inhibitory potencies of the bromophenols increased with the increasing degree of bromo-substitution per benzene ring and the decreasing degree of methyl-substitution. In contrast to the strong inhibitory activity against yeast α-glucosidase activity, the bromophenols moderately inhibited rat-intestinal sucrase and maltase activity. The inhibitory potencies were all comparative among the bromophenols examined.

Key words: *Symphyocladia latiuscula*, *Odonthalia corymbifera*, *Polysiphonia morrowii*, Rhodomelaceae, α-glucosidase, inhibition, bromophenol

Red algae of the family Rhodomelaceae contain bromophenols.[1-7] One of the major groups of bromophenols bears a 3,4-dihydroxybenzyl skelton.[1] They are divided into three groups according to the number of Br atoms in a benzene ring: three Br atoms, **1** and **2** (*Polysiphonia lanosa*, *Rhodomela subfusca*, and *Symphyocladia latiuscula*),[1,5] two Br atoms, **3**–**7** (*Odonthalia corymbifera* and *R. larix*);[1] and one Br atom, **8** and **9** (*P. lanosa* and *O. dentata*).[1]

Bromophenols are known to have their various biological activities such as antibiotic,[1,5,8,9] anti-inflammatory,[6] feeding-deterrent,[7] and antimutagenic activities.[10] They also inhibit a variety of enzymes including phospholipase A_2,[6] 15-lipoxygenase,[11] inosine monophosphate dehydrogenase,[11,12] and guanosine monophosphate synthetase.[11] In the course of our search for α-glucosidase inhibitors from marine organisms and seafoods, we obtained novel α-glucosidase-inhibitory bromophenols[13] **5**–**7** from *O. corymbifera* along with known bromophenols[14] **3** and **4**. We report the isolation of known bromophenols **1**[15] and **2**[5] from *S. latiuscula* and **8**[16] and **9**[16] from *P. morrowii* (Fig. 1), and inhibitory potencies of these bromophenols and their derivatives against α-glucosidases derived from yeast and rat intestines.

Materials and Methods

General

EI and FD mass spectra were recorded on JEOL JMS-AX500 and JEOL JMS-SX102A spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded in acetone-d_6 on a Bruker AMX-500 spectrometer. UV spectra were recorded on a HITACHI U2000 spectrophotometer. *p*-Nitrophenyl α-D-glucopyranoside was purchased from Tokyo Kasei Kogyo. α-Glucosidase from *Saccharomyces cerevisiae*, Glucose B-Test Wako, sucrose, and maltose were obtained from Wako Pure Chemical Industries. Rat-intestinal acetone powder was purchased from Sigma Chemicals.

Algal Samples

Samples of *S. latiuscula* (wet weight, 850 g) were collected near Usujiri Experimental Station, Faculty of Fisheries, Hokkaido University, in southern Hokkaido, July 1997. While specimens of *P. morrowii* were collected in June 1996 (wet weight, 2400 g) and May 1997 (wet weight, 1200 g), in Hakodate, southern Hokkaido. These algae were identified by Drs. Hajime Yasui and Hiroyuki Mizuta, Faculty of Fisheries, Hokkaido University.

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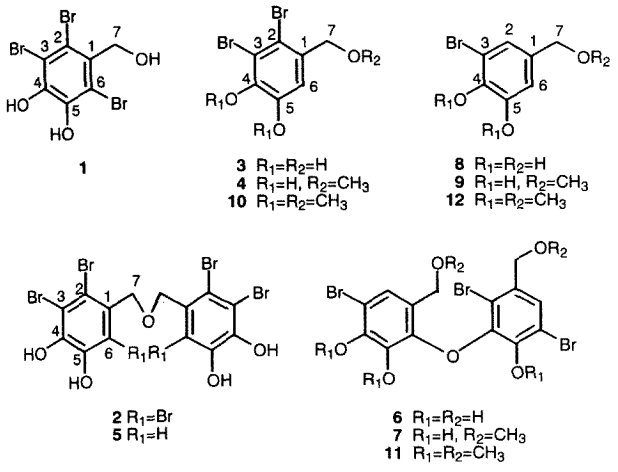

Fig. 1. Structures of bromophenols and their methyl ethers.

Extraction and Isolation

Fresh samples of *S. latiuscula* (850 g) washed with tap water were cut into small pieces and soaked into 90% aqueous acetone (v/v) for a couple of days. The extract was fractionated by a series of solvent partitions and α-glucosidase-inhibitory activity was concentrated into the EtOAc-soluble phenolic fraction. The fraction was chromatographed on silica gel ($CHCl_3$-EtOAc), and the active fraction was purified by preparative TLC on silica gel (toluene:EtOAc:acetic acid=5:7:1) to afford a mixture of bromophenols **1** and **2**. Finally bromophenols **1** (96 mg) and **2** (120 mg) was obtained by Sephadex LH-20 gel filtration (acetone).

For comparison of the constituents extracted from *P. morrowii* with different solvents, 90% aqueous acetone and methanol were employed. Fresh samples of *P. morrowii* (wet weight, 1200 g), collected in 1997, was extracted with 90% aqueous acetone (v/v). The extract was fractionated in the same manner to obtain **8** (200 mg). Fresh alga of *P. morrowii* (wet weight, 2400 g), collected in 1996, was extracted with methanol. Bromophenol **9** (170 mg) was obtained from the methanol extract through several chromatographies.

Isolation of bromophenols **3–7** from *O. corymbifera* was reported in the previous paper.[13]

2,3,6-Tribromo-4,5-dihydroxybenzyl Alcohol (**1**)

FDMS *m/z* (rel. int.): 380 (31.9), 378 (97.0), 376 (100), 374 (M^+, 37.3); ^1H NMR δ_H (multiplicity, position): 4.96 (s, H-7); ^{13}C NMR δ_C (position): 145.5 (C-5 or C-4), 143.8 (C-4 or C-5), 129.9 (C-1), 119.4 (C-6), 114.6 (C-2), 113.6 (C-3), 69.5 (C-7); HMBC correlation: H-7↔C-1, C-2, C-6.

Bis(2,3,6-tribromo-4,5-dihydroxybenzyl) Ether (**2**)

FDMS *m/z* (rel. int.): 742 (5.28), 740 (31.0), 738 (77.7), 736 (100), 734 (78.7), 732 (32.2), 730 (M^+, 5.97); ^1H NMR δ_H (multiplicity, position): 4.96 (s, H-7); ^{13}C NMR δ_C (position): 145.5 (C-5 or C-4), 143.8 (C-4 or C-5), 129.9 (C-1), 119.4 (C-6), 114.6 (C-2), 113.6 (C-3), 69.5 (C-7); HMBC correlation: H-7↔C-1, C-2, C-6, C-7 (inter-unit $^3J_{CH}$ coupling).

Identification of Bromophenols **3–7** *and an O-Methyl Derivative* **11**

Identification of bromophenols **3–7** and an *O*-methyl derivative **11** was reported in the previous paper.[13]

Methylation of **4**

Bromophenol **4** (20.0 mg) was dissolved in diethyl ether and cooled in an ice bath. Diazomethane in diethyl ether was added into the solution. The product was purified by preparative TLC to obtain 2,3-dibromo-4,5-dimethoxybenzyl methyl ether (**10**, 18.1 mg): EIMS *m/z* (rel. int.): 342 (49.1), 340 (100), 338 (M^+, 49.8), 311 (35.9), 309 (72.2), 307 (36.5), 261 (51.5), 259 (52.1); ^1H NMR δ_H (multiplicity, position): 7.22 (br. s, H-6), 4.46 (br. s, H-7), 3.92 (s, Ar-OCH_3), 3.80 (s, Ar-OCH_3), 3.43 (s, 7-OCH_3); ^{13}C NMR δ_C (position): 154.1 (C-5), 148.1 (C-4), 136.5 (C-1), 122.1 (C-2), 115.1 (C-3), 113.0 (C-6), 75.4 (C-7), 60.7 (OCH_3), 58.8 (OCH_3), 56.9 (OCH_3).

3-Bromo-4,5-dihydroxybenzyl Alcohol (**8**)

EIMS *m/z* (rel. int.): 220 (97.6), 218 (M^+, 100), 202 (62.9), 200 (61.6), 139 (24.1); ^1H NMR δ_H (multiplicity, position): 6.97 (br. s, H-2), 6.84 (br. s, H-6), 4.46 (br. s, H-7); ^{13}C NMR δ_C (position): 146.2 (C-5), 142.5 (C-4), 136.2 (C-1), 122.4 (C-2), 113.9 (C-6), 109.8 (C-3), 63.9 (C-7); HMBC correlation: H-2↔C-3, C-4, C-6, C-7; H-6↔C-2, C-4, C-5, C-7; H-7↔C-1, C-2, C-6.

3-Bromo-4,5-dihydroxybenzyl Methyl Ether (**9**)

EIMS *m/z* (rel. int.): 234 (95.0), 232 (M^+, 97.6), 203 (98.3), 201 (100), 153 (50.6); ^1H NMR δ_H (multiplicity, *J* in Hz, position): 6.95 (d, 1.9, H-2), 6.82 (d, 1.9, H-6), 4.26 (br. s, H-7), 3.23 (s, 7-OCH_3); ^{13}C NMR δ_C (position): 146.6 (C-5), 143.1 (C-4), 132.3 (C-1), 123.5 (C-2), 114.8 (C-6), 109.8 (C-3), 74.0 (C-7), 57.8 (OCH_3).

Methylation of **9**

Bromophenol **9** (17.9 mg) was methylated as in the case of **4** to obtain 3-bromo-4,5-dimethoxybenzyl methyl ether (**12**, 15.3 mg): EIMS *m/z* (rel. int.): 262 (98.9), 260 (M^+, 100), 231 (87.2), 229 (88.1); ^1H NMR δ_H (multiplicity, *J* in Hz, position): 7.09 (d, 1.8, H-2), 6.99 (d, 1.8, H-6), 4.37 (br. s, H-7), 3.87 (s, Ar-OCH_3), 3.77 (s, Ar-OCH_3), 3.32 (s, 7-OCH_3); ^{13}C NMR δ_C (position): 154.7 (C-5), 146.5 (C-4), 137.1 (C-1), 123.9 (C-2), 117.5 (C-6), 112.2 (C-3), 73.9 (C-7), 60.5 (OCH_3), 58.1 (OCH_3), 56.4 (OCH_3).

A Yeast α-Glucosidase Assay

A yeast α-Glucosidase assay was carried out by colorimetric method as described previously.[17]

Rat-intestinal Maltase and Sucrase Assays

Preparation of crude maltase and sucrase solution and measurement of their activities were carried out by a modified method of Asano *et al.*[18]

Rat-intestinal acetone powder (10 g) was dispersed into 0.9% (w/v) saline (100 m*l*), sonicated (30 s × 3), then centrifuged (3000 rpm × 30 min) to obtain supernatant as a crude enzyme solution. This solution was used directly to determine sucrase activity, while the dilute solution (25% with 0.9% saline) was used for maltase activity.

Sucrase activity was determined in a mixture of 500 mM

sucrose (0.1 m*l*), bromophenol in methanol (0.05 m*l*), and 0.1 M maleate buffer (pH 6.0, 0.75 m*l*). Methanol did not affect enzyme activity under this condition. The mixture was preincubated for five minutes at 37°C, and reaction was initiated by adding crude enzyme solution (0.1 m*l*) to the reaction mixture. The mixture was incubated for 60 minutes at 37°C. Reaction was terminated by the addition of 2.0 M maleate-Tris-NaOH buffer (pH 7.4, 1.0 m*l*). As for measurement of maltase activity, 500 mM of maltose was used instead of sucrose.

The reaction mixture was extracted with diethyl ether to remove bromophenol because it hindered the determination of glucose released (data not shown). Diethyl ether in the reaction mixture was removed under a N_2 gas stream. The glucose released in the solution was determined by Glucose B-Test Wako based on the glucose oxidase method. The reaction mixture (0.02 m*l*) and Glucose B-Test Wako (3.0 m*l*) were mixed and incubated for 20 minutes at 37°C. Absorbance of the mixture was measured at 505 nm.

Results

Bromophenols **1** and **2** were obtained from aqueous acetone extract of the red alga of *S. latiuscula*. Although separation of **1** and **2** was difficult by normal phase chromatography, Sephadex LH-20 column chromatography overcame this problem. The structures of **1** and **2** were identified by spectral data as 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol and bis(2,3,6-tribromo-4,5-dihydroxybenzyl) ether, respectively. Bromophenols **8** and **9** isolated from *P. morrowii* were identified by spectral data to be 3-bromo-4,5-dihydroxybenzyl alcohol and 3-bromo-4,5-dihydroxybenzyl methyl ether, respectively.

The bromophenols thus obtained strongly inhibited yeast α-glucosidase (Table 1). In particular, the symmetric bisbenzyl ethers **2** and **5** showed extremely strong activity with K_i values of 14 and 53 nM, respectively. Tribrominated benzenes **1** and **2** were more potent than the corresponding dibrominated and monobrominated benzenes **3**, **5**, and **8**. Lineweaver-Burk plot analysis disclosed that the mode of action of bromophenols **1**, **3**, **6**, and **8** was mixed inhibition (Table 1). The inhibition mode of symmetric bisbenzyl ethers **2** and **5** was unable to be determined as whether competitive or mixed inhibition, since lines on Lineweaver-Burk plots intersected close to the vertical axis. *O*-Methylation of bromophenols **3**, **6**, and **8** decreased their inhibitory activity (Table 2). In particular, full methylation markedly decreased inhibitory activity (derivatives **10–12**).

Inhibitory potencies of the bromophenols against rat-intestinal α-glucosidase activities as sucrase and maltase were also evaluated (Table 3). The inhibitory potencies against rat-intestinal α-glucosidase were much lower than those against yeast α-glucosidase. The IC_{50} values of the bromophenols were all comparable.

Table 1. IC_{50} and K_i values and inhibition mode of bromophenolic benzyl alcohols and bisbenzyl ethers against yeast α-glucosidase

Bromophenol	IC_{50}[a] (μM)	K_i[b] (μM)	Inhibition mode[c]
1	11	7.0	M
2	0.030	0.014	C or M
3	89	72	M
5	0.098	0.053	C or M
6	25	11	M
8	100	81	M

[a] IC values were determined by an inhibition assay with substrate concentration of 0.4 mM.
[b] K_i values were determine by Dixon plot analysis.
[c] Inhibition mode was determined by Lineweaver-Burk plot analysis: M, mixed inhibition; C, competitive inhibition.

Table 2. IC_{50} values of methylated derivatives of bromophenols against yeast α-glucosidase

Benzyl methyl ether		Permethylated derivative	
Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
4	170	**10**	>1000 (21.1)[b]
7	53	**11**	>1000 (10.5)[b]
9	>1000 (39.5)[b]	**12**	>1000 (6.7)[b]

[a] IC values were determined by an inhibition assay with substrate concentration of 0.4 mM.
[b] Inhibition (%) at 1000 μM compound.

Table 3. IC_{50} values of bromophenolic benzyl alcohols and bisbenzyl ethers against rat-intestinal sucrase and maltase

Bromophenol	IC_{50}[a] (mM)	
	Sucrase	Maltase
1	4.2	>5.0 (42.3)[b]
2	2.4	3.2
3	2.4	2.5
5	1.0	1.1
6	3.5	3.1
8	3.6	4.8

[a] IC_{50} values were determined by an inhibition assay with substrate concentration of 50 mM.
[b] Inhibition (%) at 5.0 mM compound.

Discussion

The bromophenols examined showed potent inhibitory activity against yeast α-glucosidase with K_i values ranging between 0.014 and 81 μM (Table 1). A noticeable feature is that the inhibitory potencies of the bromophenols decreased when methylated (Table 2). In particular, full methylation of phenolic hydroxyl groups markedly decreased inhibitory potencies. Thus, phenolic hydroxyl groups may play an important role in exerting inhibitory activity. Polyphenolic compounds such as tannins from terrestrial plants and phlorotannins from marine algae are known to be associated with a variety of proteins to form complex.[19,20] For example, *o*-quinones derived from catechols are covalently bound to protein amino and thiol groups.[21–23] Wiemer *et al.*[6] could not elucidate whether the inhibitory property of bromophenols against phospholipase A_2 was caused by catechols or more reactive *o*-quinones formed after oxidation. Bromophenols bearing catechol moiety might tightly bind or covalently react with

enzymes in the form of either catechols or *o*-quinones, since 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**3**) irreversibly inhibited yeast α-glucosidase (data not shown). The bromophenols exhibited mixed inhibition, which is characterized by a combination of competitive and noncompetitive inhibition, against yeast α-glucosidase, while (+)-catechin oxidation products are known to exhibit simple noncompetitive inhibition against β-glucosidase.[24] Bromophenols should bind to both active and non-active sites of the enzymes. Symmetric bisbenzyl ethers **2** and **5** showed similar competitive inhibition to each other. These symmetric ethers could strongly bind to the active site of the enzymes. For this reason, their inhibition modes must differ with the degree of oligomerization as the case of ellagitannins.[25]

Highly brominated bromophenols **1** and **2** showed stronger inhibition than the less brominated bromophenols **3**/**8** and **5**. Highly brominated bromophenols might mimic the spatial structure of α-glucoside. In addition, highly brominated catechols are susceptible to oxidation and converted into *o*-quinones. They will strongly bind to the enzymes due to debrominated aromatic nucleophilic substitution. Effect of bromo-substitution on the inhibitory potency, however, is not fully understood.

The bromophenols examined also inhibited rat-intestinal α-glucosidase (sucrase and maltase) though the effect itself was moderate. This suggests that binding of bromophenols is less specific to the enzyme because they can bind to various proteins included in the crude enzyme solution. Inhibitory potencies against rat-intestinal α-glucosidase were comparable among all bromophenols examined. In contrast, symmetric bisbenzyl ethers **2** and **5** exhibited stronger inhibition against yeast α-glucosidase than other bromophenols. The symmetric bisbenzyl ethers **2** and **5** are suggested to fit the surface shape of the active site of yeast α-glucosidase.

The search for intestinal α-glucosidase (sucrase and maltase) inhibitors in seafoods and marine organisms is important, because they are expected to suppress the postprandial hyperglycemia of diabetic patients without side effects. Though it is still not clear whether bromophenols can suppress hyperglycemia, at least the symmetric bisbenzyl ethers **2** and **5** highly inhibit yeast α-glucosidase.

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