Notes

Two New Bromophenols from the Red Alga *Odonthalia corymbifera*

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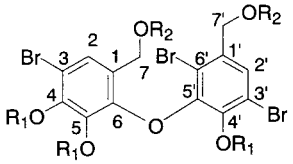
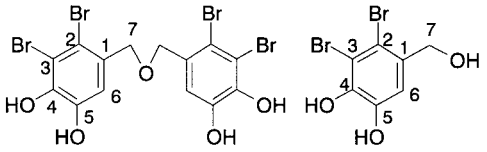
Received July 24, 1998

Two novel bromophenols (**1** and **2**) were isolated from an extract of a red alga, *Odonthalia corymbifera*, together with a known bromophenol (**3**). The novel bromophenols were determined as 4-bromo-2,3-dihydroxy-6-hydroxymethylphenyl 2,5-dibromo-6-hydroxy-3-hydroxymethylphenyl ether (**1**) and bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (**2**), from spectroscopic evidence. Compounds **1**−**3** were found to be inactivators of α-glucosidase.

In the course of our search for α-glucosidase inhibitors from marine organisms and seafoods,[1,2] we have elucidated two yeast α-glucosidase inhibitors from a brown alga.[3,4] Through further investigation, it was disclosed that red algae of Rhodomelaceae are good sources of yeast α-glucosidase inhibitors. The algae of Rhodomelaceae are known to contain large amounts of bromophenols.[5−11] For example, *Odonthalia corymbifera* (Gmelin) Greville (Rhodomelaceae) accumulates bromophenols with benzyl[12] and diarylmethane[11] skeletons. In the present paper, we report the isolation and structural elucidation of novel diaryl ether- and dibenzyl ether-type bromophenols **1** and **2** from *O. corymbifera* together with a known bromophenol **3**.[5] These compounds were evaluated for their activity against yeast α-glucosidase.



1 R_1=R_2=H
4 R_1=H, R_2=CH_3
5 R_1=R_2=CH_3



2

3

Guided by α-glucosidase-inhibitory activity, compounds **1**−**3** were obtained from an aqueous acetone extract of the fresh alga of *O. corymbifera*. The purity of the compounds was confirmed by HPLC. Compounds **1**−**3** were positive in an $FeCl_3$ test, and they gave absorption maxima at 292−293 nm in UV spectra. These results strongly suggested that **1**−**3** are phenolic compounds.

Compound **1** was determined to contain three Br atoms from the molecular ion peak cluster at *m*/*z* 512/514/516/518 in the FDMS. Compound **1** was analyzed for the molecular formula $C_{14}H_{11}O_6Br_3$ by HRFDMS. The ^{13}C NMR spectrum of **1** contained twelve aromatic and two benzylic carbon signals (Table 1). These results suggested that **1** consists of two aromatic rings. From the HMBC experiment on **1** (Table 2), the benzylic carbons at C-7 and C-7′ were coupled with the aromatic protons at H-2 and H-2′, respectively, while the benzylic protons at H-7 and H-7′ were coupled with the aromatic carbons at C-2 and C-2′. These couplings revealed that individual hydroxymethyl groups are located in ortho-positions to two different aromatic protons. The respective aromatic protons were coupled with different sets of aromatic carbons. Thus, **1** was revealed to consist of two 2,3,4,5-tetrasubstituted benzyl alcohol moieties. The aromatic proton at H-2 was coupled to one brominated and two oxygenated aromatic carbons, while the proton at H-2′ was coupled to two brominated and one oxygenated aromatic carbons. However, complete assignment of proton and carbon signals to **1** was impossible because of a limited number of the HMBC correlations and a failure to derivatize the phenolic OH in **1** with diazomethane (data not shown).

Fortunately, phenolic compound **4** was obtained from the MeOH extract of the alga. The difference in the masses between **1** and **4** in the FDMS data was 28 mass units, corresponding to the presence of two methyl groups in **1**. The NMR data of **4** were similar to those of **1** except for the presence of two methoxyl signals in **4**. Correlations observed in the HMBC experiment (Table 2) of **4** were also close to those of **1** except for the presence of correlations between the methoxyl and benzylic signals in **4**. Thus, two methoxyl groups of **4** were replaced by hydroxyl groups at the C-7 and C-7′ positions in **1**. Compound **4** was determined as an artifact because **4** was only obtained when a MeOH extraction process was carried out.

Distinction between $^2J_{CH}$ and $^3J_{CH}$ couplings of **4** was determined by the HMBC experiment with different long-range J_{CH} (LR J_{CH}) evolution times. In general, an HMBC experiment with a long LR J_{CH} evolution time (more than

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10.1021/np980324p CCC: $18.00
Published on Web 05/01/1999

Table 1. ^{13}C NMR Data of Compounds **1**−**5**

position	1	2	3	4	5
C-1	128.2	131.3	135.0	124.6	129.7
C-2	114.2	114.7[a]	113.3	115.2	113.0
C-3	105.7	113.8[a]	113.5	105.7	104.5
C-4	144.0	144.7[b]	144.0	144.8	148.0
C-5	144.1[a]	145.6[b]	145.7	144.2[a]	152.5
C-6	141.5	115.6	114.6	142.4	142.4
C-7	59.4	73.2	65.1	69.5	69.7
C-1′	134.7			131.0	136.5
C-2′	112.3			113.5	113.8
C-3′	114.2			114.1	104.5
C-4′	144.7			145.2	146.9
C-5′	146.1[a]			146.2[a]	151.6
C-6′	116.1			117.6	116.7
C-7′	64.9			75.0	74.8
CH_3O				58.2	61.0
				58.2	60.8
					58.4
					58.4
					56.7

[a,b] Data are interchangeable in the same column.

Table 2. HMBC Correlations for **1**, **2**, **4**, and **5**

compound	H	C
1[a]	H-2	C-1, C-3, C-4, C-6, C-7
	H-7	C-1, C-2, C-6
	H-2′	C-1′, C-3′, C-4′, C-6′, C-7′
	H-7′	C-1′, C-2′, C-6′
2[b]	H-6	C-2, C-4, C-5, C-7
	H-7	C-2, C-6, C-7[c]
4[b]	H-2	C-1,[d] C-3,[d] C-4, C-6, C-7
	H-7	C-1, C-2, C-6, CH_3O-7
	CH_3O-7	C-7
	H-2′	C-1′,[d] C-3′, C-4′, C-6′,[d] C-7′
	H-7′	C-1′, C-2′, C-6′, CH_3O-7′
	CH_3O-7′	C-7′
5[a]	H-2	C-4, C-6, C-7
	H-7	C-1, C-2, C-6, CH_3O-7
	CH_3O-4	C-4
	CH_3O-5	C-5
	CH_3O-7	C-7
	H-2′	C-4′, C-6′, C-7′
	H-7′	C-1′, C-2′, C-6′, CH_3O-7′
	CH_3O-4′	C-4′
	CH_3O-7′	C-7′

[a] The HMBC experiment was only performed with a long-range J_{CH} (LR J_{CH}) evolution time of 150 ms. [b] The HMBC experiments were performed with LR J_{CH} evolution times of 70 and 150 ms. [c] Inter-unit $^3J_{CH}$ coupling. [d] Correlation only detected with a LR J_{CH} evolution time of 150 ms.

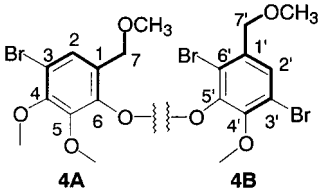

Figure 1. Partial Structures of **4**. Thick lines represent connectivities disclosed from the HMBC experiment.

100 ms) is suitable to detect $^2J_{CH}$ coupling because $^2J_{CH}$ values are smaller than $^3J_{CH}$ values in an aromatic ring.[13] The four $^2J_{CH}$ couplings of C-1/H-2, C-3/H-2, C-1′/H-2′, and C-3′/H-2′ were only seen in the HMBC experiment of **4** with a long LR J_{CH} evolution time of 150 ms. Thus, the partial structures **4A** and **4B** were determined as shown in Figure 1.

To elucidate the connectivity between the two partial structures in **4**, we determined the locations of the three free hydroxyl groups by methylation. Three methoxyl proton signals δ_H 4.01, 3.95, and 3.84 appeared in the ^1H NMR spectrum of a tri-*O*-methyl derivative (**5**). The HMBC spectrum of **5** showed three pairs of couplings between the aromatic carbons and the methoxyl protons, C-4/δ_H 3.84, C-5/δ_H 3.95, and C-4′/δ_H 4.01 (Table 2). The aromatic proton at H-2 of **5** was coupled with the aromatic carbon at C-4 and another oxygenated aromatic carbon at C-6 with $^3J_{CH}$ coupling. Thus, the C-6 position of the partial structure **4A** should be connected with the partial structure **4B**. The aromatic proton at H-2′ was coupled with the aromatic carbon at C-4′ and the brominated aromatic carbon at C-6′ with $^3J_{CH}$ coupling. Therefore, the C-5′ position of the partial structure **4B** is connected with the partial structure **4A**. The structure of **4** was assigned as 4-bromo-2,3-dihydroxy-6-methoxymethylphenyl 2,5-dibromo-6-hydroxy-3-methoxymethylphenyl ether. From these results, we determined the structure of **1** as 4-bromo-2,3-dihydroxy-6-hydroxymethylphenyl 2,5-dibromo-6-hydroxy-3-hydroxymethylphenyl ether.

Compound **2** was found to have four Br atoms from the molecular ion peak cluster at *m*/*z* 573 $[M - 1]^+$/575/577/579/581 in the FDMS. The ^{13}C NMR spectrum of **2** (Table 1) showed six aromatic and one benzylic carbon signals. The NMR spectra of **2** showed simpler patterns than expected from its molecular weight, indicating its symmetrical nature. The HMBC experiment (Table 2) showed that the benzylic carbon (C-7) and proton (H-7) were coupled with the aromatic proton (H-6) and carbon (C-6), respectively. These couplings revealed that the benzylic methylene group is located in an ortho-position to the aromatic proton. The brominated aromatic carbon at C-2 was coupled with the protons at H-6 and H-7, suggesting the presence of a Br atom at the other ortho-position of the benzylic methylene group. The aromatic proton at H-6 was coupled with two oxygenated aromatic carbons at C-4 and C-5. Thus, it was suggested that **2** contains a 2,3-dibromo-4,5-dihydroxybenzyl moiety (R). The EIMS of **2** showed two clusters of fragmentation ions at *m*/*z* 278/280/282 and 293/295/297, whereas the molecular ion peak cluster was not observed. Since these clusters correspond to $[R - H]^+$ and $[RO - 2H]^+$, respectively, compound **2** has a symmetrical R−O−R structure. The fragmentation peak, $[(RO) - 2H]^+$, was analyzed for the formula $C_7H_3O_3{}^{79}Br_2$ by HREIMS. Accordingly, the molecular formula of **2** was established as $C_{14}H_{10}O_5Br_4$. A symmetrical structure was also confirmed by the HMBC correlations of **2** since both long range $^3J_{CH}$ coupling and $^1J_{CH}$ coupling were observed between the benzylic C-7 and H-7. Thus, the structure of **2** was determined to be bis(2,3-dibromo-4,5-dihydroxybenzyl) ether.

Compound **3** was identified as 2,3-dibromo-4,5-dihydroxybenzyl alcohol (lanosol) by comparison with the literature data.[12]

Compounds **1**−**3** showed inhibition of yeast α-glucosidase reaction, with IC_{50} values of 25, 0.098, and 89 μM, respectively. The symmetrical dimer **2** was 10^3-fold more active when compared with corresponding monomer **3**. The reason **2** showed strong activity is not understood. Compounds **1**−**3** are expected to be inactivators of α-glucosidase by interaction between phenol and protein,[14,15] since **1**−**3** showed irreversible inhibition of the α-glucosidase (data not shown). Inhibition might stem from the interaction of *o*-quinones which are oxidative products of *o*-diphenols such as **1**−**3**, to enzyme protein.[14,15] Elucidation of the inhibition mechanism is now under investigation.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer System 2000 spectrometer. UV

spectra were recorded on a Hitachi U2000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 instrument. Mass spectra were recorded on a JEOL JMS-SX102A spectrometer for FDMS and a JEOL JMS-AX500 spectrometer for EIMS. Yeast α-glucosidase from *Saccharomyces* sp. was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *p*-Nitrophenyl α-D-glucopyranoside was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Algal Material. *O. corymbifera* was collected at Usujiri Experimental Station, Faculty of Fisheries, Hokkaido University, in southern Hokkaido, Japan, in July 1997, and authenticated by Dr. Hajime Yasui, Faculty of Fisheries, Hokkaido University.

Extraction and Isolation. The fresh alga (2.80 kg), washed with tap water, was cut and extracted with aqueous acetone. An aqueous acetone extract was partitioned into hexane-, EtOAc-, *n*-BuOH-, and H_2O-soluble fractions. The EtOAc-soluble fraction was further divided into acidic, phenolic, neutral, and basic fractions. The phenolic fraction showed α-glucosidase-inhibitory activity. The phenolic fraction was chromatographed on silica gel, then rechromatographed on Sephadex LH-20, and finally purified using TLC to afford bromophenols **1** (76.0 mg), **2** (86.2 mg), and **3** (1.10 g). Purity of the isolated bromophenols was confirmed by HPLC [column, Mightysil RP-18, i.d. 4.6 mm × length 250 mm, Kanto Chemical Co., Inc., Japan; mobile phase, MeCN−MeOH (3:1); chromatograph, Hitachi 655-A11 liquid chromatograph, Hitachi, Japan; detection, UV at 285 nm Hitachi 655A variable wavelength UV monitor, Hitachi, Japan].

A MeOH extract of the fresh alga (1.60 kg) was consecutively fractionated in the same manner as the aqueous acetone extract, with silica gel, Sephadex LH-20, and by TLC to afford an artifact **4** (123 mg).

4-Bromo-2,3-dihydroxy-6-hydroxymethylphenyl 2,5-dibromo-6-hydroxy-3-hydroxymethylphenyl ether (1): obtained as pale yellow solid. IR (KBr) ν_{max} 3325, 1601, 1575, 1484, 1440, 1194, 1054, 915, 865 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 292 (4.08) nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.12 (1H, s, H-2), 6.74 (1H, s, H-2′), 4.51 (2H, s, H-7′), 4.44 (2H, s, H-7); ^{13}C NMR (acetone-d_6, 126 MHz), see Table 1; FDMS *m*/*z* 518 (31), 516 (90), 514 (100), 512 [M]$^+$ (40). HRFDMS *m*/*z* 511.8087 [M]$^+$ (calcd for $C_{14}H_{11}O_6{}^{79}Br_3$, 511.8104).

Bis(2,3-dibromo-4,5-dihydroxybenzyl) ether (2): obtained as pale yellow solid. IR (KBr) ν_{max} 3421, 1584, 1474, 1074, 856 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 293 (3.85) nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.14 (2H, s, H-6), 4.59 (4H, s, H-7); ^{13}C NMR (acetone-d_6, 126 MHz), see Table 1; FDMS *m*/*z* 581 (17), 579 (67), 577 (100), 575 (71), 573 [M − H]$^+$ (19); EIMS *m*/*z* 300 (29), 298 (90), 297 (46), 296 (100), 295 (73), 294 (36), 293 (35), 282 (56), 280 (83), 278 (37); HREIMS *m*/*z* 292.8437 [M − 2H]$^+$ (calcd for $C_7H_3O_3{}^{79}Br_2$, 292.8449).

2,3-Dibromo-4,5-dihydroxybenzyl alcohol (3): obtained as pale yellow solid. UV (EtOH) λ_{max} (log ϵ) 292 (3.61) nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.18 (1H, br s, H-6), 4.57 (2H, d, J = 0.5 Hz, H-7); ^{13}C NMR (acetone-d_6, 126 MHz), see Table 1; EIMS *m*/*z* 300 (49), 298 (100), 296 [M]$^+$ (52), 219 (57), 217 (66).

4-Bromo-2,3-dihydroxy-6-methoxymethylphenyl 2,5-dibromo-6-hydroxy-3-methoxymethylphenyl ether (4): obtained as pale yellow solid: IR (KBr) ν_{max} 3347, 2934, 1587, 1480, 1436, 1188, 1079, 926, 863 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 292 (3.85) nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.02 (1H, s, H-2), 6.58 (1H, s, H-2′), 4.33 (2H, s, H-7′), 4.25 (2H, s, H-7), 3.23 (3H, s, CH_3O), 3.21 (3H, s, CH_3O); ^{13}C NMR (acetone-d_6, 126 MHz), see Table 1; FDMS *m*/*z* 546 (37), 544 (100), 542 (98), 540 [M]$^+$ (35); EIMS *m*/*z* 546 (1), 544 (4), 542 (5), 540 [M]$^+$ (4), 538 [M − 2H]$^+$ (2), 514 (34), 512 (100), 510 (100), 508 (36), 433 (44), 431 (86), 429 (47); HREIMS *m*/*z* 537.8250 [M − 2H]$^+$ (calcd for $C_{16}H_{13}O_5{}^{79}Br_3$, 537.8262).

Methylation of 4 with Diazomethane. Compound **4** was dissolved in diethyl ether and cooled on an ice bath. Diazomethane in diethyl ether was added into the solution. The resulting solution was concentrated then chromatographed by TLC. 4-Bromo-2,3-dimethoxy-6-methoxymethylphenyl 2,5-dibromo-6-methoxy-3-methoxymethylphenyl ether (**5**) was obtained as colorless oil; IR (KBr) ν_{max} 2933, 1558, 1483, 1467, 1423, 1122, 1043, 860 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 285 (3.57) nm; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.23 (1H, s, H-2), 6.58 (1H, s, H-2′), 4.35 (2H, s, H-7), 4.34 (2H, s, H-7′), 4.02 (3H, s, CH_3O), 3.95 (3H, s, CH_3O), 3.84 (3H, s, CH_3O), 3.26 (3H, s, CH_3O), 3.25 (3H, s, CH_3O); ^{13}C NMR (acetone-d_6, 126 MHz), see Table 1; FDMS *m*/*z* 588 (37), 586 (97), 584 (100), 582 [M]$^+$ (37); EIMS *m*/*z* 588 (30), 586 (89), 584 (90), 582 [M]$^+$ (31), 525 (33), 523 (100), 521 (100), 519 (34), 261 (51), 259 (52); HREIMS *m*/*z* 581.8906 [M]$^+$ (calcd for $C_{19}H_{21}O_6{}^{79}Br_3$, 581.8889).

Inhibition Assay of Yeast α-Glucosidase. An inhibition assay of yeast α-glucosidase was carried out by an agar plate method.[1] The IC_{50} values of bromophenols were determined by a colorimetric method using *p*-nitrophenyl α-D-glucopyranoside as a substrate.[3] The final substrate concentration was 0.4 mM.

Acknowledgment. We thank Dr. Hajime Yasui, Faculty of Fisheries, Hokkaido University, for identification of the alga. We are also grateful to Dr. Eri Fukushi and Mr. Kenji Watanabe, of the GC−MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University, for their help in obtaining MS data. Part of this work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan.

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NP980324P