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Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives

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Abstract—A series of bromophenols was obtained by isolation from red alga *Odonthalia corymbifera* and by reactions of bis(hydroxyphenyl)methanes with bromine. New bromophenols including 3,3′,5,5′-tetrabromo-2,2′,4,4′-tetrahydroxydiphenylmethane (**10**), a regioisomer of the potent antimicrobial natural product, together with known derivatives were synthesized in high yield. All of the isolated and synthesized compounds were tested for antimicrobial activity against Gram-negative, Gram-positive bacteria and fungi. The preliminary structure–activity relationship, to elucidate the essential structure requirements for antimicrobial activity, has been described. Among the isolated natural products 2,2′,3,3′-tetrabromo-4,4′,5,5′-tetrahydroxydiphenylmethane (**4**) was found to be the most active derivative against *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. The synthetic bromophenols 3,3′-dibromo-6,6′-dihydroxydiphenylmethane (**13**) and 3,3′,5,5′-tetrabromo-6,6′-dihydroxydiphenylmethane (**14**) showed potent antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Proteus vulgaris*, and *Salmonella typhimurium*.

Bromophenol compounds have been frequently encountered in various marine organisms including red algae, brown algae, ascidians, and sponges. Especially, the red algae of the family Rhodomelaceae are known as a rich source of bromophenols.[1–17] Some of these compounds previously isolated from the family exhibited a wide spectrum of pharmacological activities such as enzyme inhibition,[3,9] cytotoxic,[4] antioxidant,[8] feeding-deterrent,[10] anti-inflammatory,[11] and antimicrobial[13] activities.

In the course of our search for biologically active constituents from marine algae, we collected *Odonthalia corymbifera*, (family, Rhodomelaceae), whose crude extract exhibited moderate antimicrobial activity against various microorganisms. Bioassay-guided separation of the crude extract using a variety of chromatographic techniques afforded several bromophenol compounds.[18] Based upon the results of combined spectral analyses, the metabolites were identified to be 4-(2-aminoethyl)-2,6-dibromophenol (**1**),[19,20] 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**2**),[10] 2,3-dibromo-4,5-dihydroxybenzyl methyl ether (**3**),[17] 2,2′,3,3′-tetrabromo-4,4′,5,5′-tetrahydroxydiphenylmethane (**4**),[15] 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-hydroxymethyl diphenylmethane (**5**),[6] and 3-bromo-4-(2,3-dibromo-4,5-dihydroxybenzyl)-5-methoxymethylpyrocatechol (**6**) (Fig. 1).[15]

The in vitro antimicrobial activities of the bromophenols **1**–**6** were assessed against three representative Gram-positive bacteria viz. *Staphylococcus aureus* (ATCC6538p), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (IFC 12708), three Gram-positive bacteria viz. *Proteus vulgaris* (ATCC3851), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 25922), and four fungal organisms viz. *Candida albicans* (ATCC10231), *Aspergillus fumigatus* (HIC6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO

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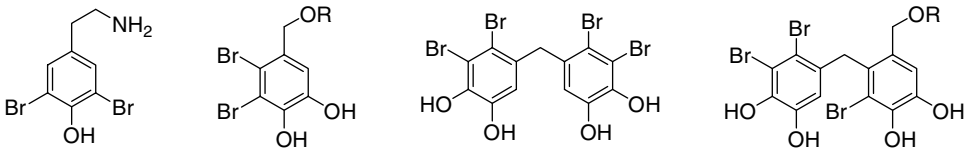


1 **2** R = H, **3** R = CH_3 **4** **5** R = H, **6** R = CH_3

Figure 1. Bromophenols from *Odonthalia corymbifera*.

40996).[21,22] Data for the metabolites are presented in Tables 1 and 2 as the minimal inhibitory concentration (MIC). Among the isolated natural products compound **4** was found to be the most active derivative against *C. albicans*, *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes*. Specially, the di-phenolic metabolites **4**–**6** display good inhibition activity against Gram-positive and Gram-negative organisms except *E. coli*, whereas the mono-phenolic metabolites **1**–**3** showed no antibacterial activities (Table 1). However, this trend does not translate exactly

Table 1. Antibacterial activity

Compound	Antibacterial activity (MIC, μg/ml)					
	S. aureus	*B. subtilis*	*M. luteus*	*P. vulgaris*	*S. typhimurium*	*E. coli*
1	100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	25	25	25	50	50	>100
5	50	100	50	100	100	>100
6	25	25	25	25	25	>100
7	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
12	25	25	25	25	25	>100
13	3.12	3.12	1.56	3.12	3.12	>100
14	1.56	1.56	1.56	1.56	3.12	>100
16	25	12.5	12.5	25	25	>100
17	12.5	6.25	6.25	6.25	6.25	>100
18	>100	>100	>100	>100	>100	>100
Ampicillin	1.56	1.56	1.56	3.12	3.12	12.5

Microorganisms: *Staphylococcus aureus* ATCC6538p; *Bacillus subtilis* ATCC 6633; *Micrococcus luteus* IFC 12708; *Proteus vulgaris* ATCC3851; *Salmonella typhimurium* ATCC 14028; *Escherichia coli* ATCC 25922.

Table 2. Antifungal Activity

Compound	Antifungal activity (MIC, μg/ml)			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
1	100	>100	>100	>100
2	>100	>100	50	50
3	100	>100	12.5	12.5
4	1.56	0.78	1.56	1.56
5	100	>100	50	50
6	25	25	25	25
7	>100	>100	>100	>100
8	>100	>100	>100	>100
10	>100	>100	>100	>100
12	>100	>100	>100	>100
13	>100	>100	>100	>100
14	>100	>100	>100	>100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	>100	>100	>100	>100
Amphotericin B	6.25	3.12	3.12	3.12

Microorganisms: *Candida albicans* ATCC10231; *Aspergillus fumigatus* HIC6094; *Trichophyton rubrum* IFO 9185; *Trichophyton mentagrophytes* IFO 40996.

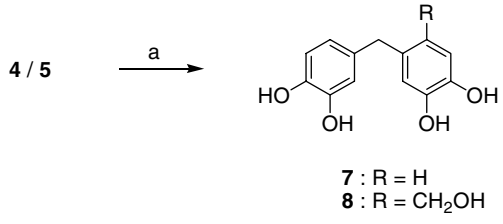
to antifungal activities. As shown in Table 2, mono-phenolic compound **3** also showed moderate levels of inhibition toward some fungi.

In order to examine modifications of the bioactive bromophenol via chemical transformations, hydrodehalogenation reactions of natural products **4** and **5** were carried out[23] as shown in Scheme 1. The reactions of the bromophenols **4** and **5** with H_2 gas in the presence of Pd/Al_2O_3 and triethylamine in methanol gave corresponding debrominated phenolic compounds **7**[24] and **8**, respectively. The transformed phenolic compounds **7** and **8** were also evaluated for in vitro antimicrobial activities toward various microorganisms. The results are added in Tables 1 and 2. According to the data shown in the Tables, compounds **7** and **8** were found to be completely inactive against Gram-negative, Gram-positive bacteria and fungi.

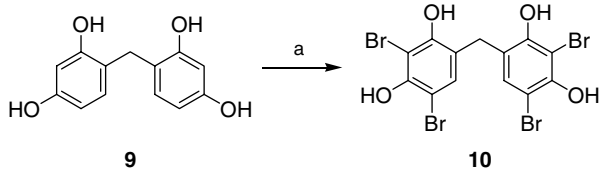
These results revealed that the di-phenolic backbone and the presence of one or more bromines on the phenol ring are important for antimicrobial activity. Therefore, the focus of the continued study was to demonstrate that the number and position of bromines in bromophenols could influence potency against microorganisms. The bromophenol analogues have been synthesized as shown in Schemes 2–4.[25]

To investigate the positional effects of bromines on the phenol ring on the antibacterial activity, the analogue 3,3′,5,5′-tetrabromo-2,2′,4,4′-tetrahydroxydiphenylmethane (**10**) was synthesized by the reaction of phenolic compound **9**,[26] prepared by the reduction from the corresponding benzophenone, with excess of bromine in acetic acid in 90% yield (Scheme 2). Similarly, treatment of bis(2-hydroxyphenyl)methane (**11**) with two equivalents of bromine in the mixture of acetic acid and CH_2Cl_2 gave the mixture of bromophenols 3-bromo-2′,6-dihydroxydiphenylmethane (**12**) and 3,3′-dibromo-6,6′-dihydroxydiphenylmethane (**13**) in 41% and 53% yields, respectively.[27] The 3,3′,5,5′-tetrabromo-6,6′-dihydroxydiphenylmethane (**14**)[28] was prepared by the reaction of the same reactant with excess of bromine in acetic acid as a solvent to increase the solubility of the intermediates in excellent yield (Scheme 3). As shown in Scheme 4, the other bromophenols, 3,3′-dibromo-4,4′-dihydroxydiphenylmethane (**16**), 3,3′,5-tribromo-4,4′-dihydroxydiphenylmethane (**17**), and 3,3′5,5′-tetrabromo-4,4′-dihydroxydiphenylmethane (**18**),[29] were also synthesized from bis(4-hydroxyphenyl)methane (**15**) in the same manner. All of the strongly activated phenols **9**, **11**, and **15** toward electrophilic aromatic substitution reacted readily with bromine to afford various bromophenols just as expected.

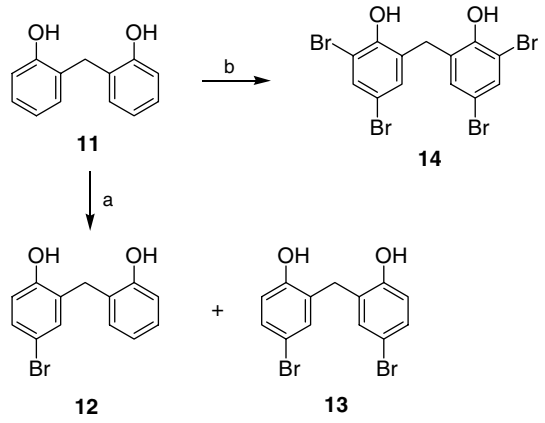
The seven analogues prepared by these bromination reactions were also tested for antibacterial and antifungal activities. The minimum inhibitory concentrations of antibacterial and antifungal activity are summarized in Tables 1 and 2, respectively. The underlying reasons for the antimicrobial properties of natural and synthetic bromophenols are not well understood. Disappointedly, we did not observe any antibacterial or antifungal activity by analogue **10**, which is only a regioisomer of the potent



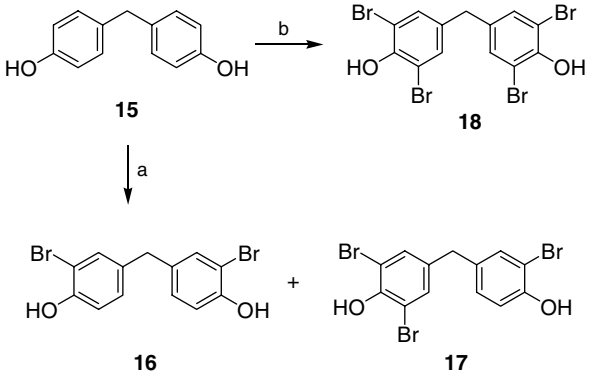
Scheme 1. Catalytic hydrodebromination. Reagents: (a) H_2, Pd/Al_2O_3/Et_3N, MeOH.



Scheme 2. Bromination of bis(2,4-dihydroxyphenyl)methane. Reagents: (a) excess Br_2, AcOH.



Scheme 3. Bromination of bis(2-hydroxyphenyl)methane. Reagents: (a) Br_2, AcOH+CH_2Cl_2, (b) excess Br_2, AcOH.



Scheme 4. Bromination of bis(4-hydroxyphenyl)methane. Reagents: (a) Br_2, AcOH+CH_2Cl_2, (b) excess Br_2, AcOH.

antifungal natural product **4**. However, some of synthetic bromophenols exhibited moderate to good antibacterial activity with degrees of variation (Table 1). Among synthetic bromophenols, compounds **13** and **14** have equally potent activities against *S. aureus*, *B. subtilis*, *M. luteus*, *P. vulgaris*, and *S. typhimurium* as ampicillin, a standard reference compound.

In terms of the antifungal activity, natural bromophenol **4** showed roughly two- to fourfold lower MIC values than the reference compound, amphotericin B. On the other hand, replacement of position and number of hydroxyl group and bromine by synthesis of bromophenols led to inactive compounds against *C. albicans*, *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes* (Table 2).

In conclusion, a series of bromophenols has been prepared. The antimicrobial activity of these compounds was evaluated against various Gram-positive, Gram-negative bacteria and fungi. Among the isolated natural products 2,2′,3,3′-tetrabromo-4,4′,5,5′-tetrahydroxydiphenylmethane (**4**) was found to be the most active derivative against *C. albicans*, *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes*. The synthetic bromophenols 3,3′-dibromo-6,6′-dihydroxydiphenylmethane (**13**) and 3,3′,5,5′-tetrabromo-6,6′-dihydroxydiphenylmethane (**14**) showed potent antibacterial effect against *S. aureus*, *B. subtilis*, *M. luteus*, *P. vulgaris*, and *S. typhimurium*. By modifying the bromophenol substituents, we were able to discover several compounds which had potency profiles in our antimicrobial assays. Further work on the modifying of these compounds in an expanded series of halogenated phenol compounds will be reported in due course.

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22. *Candida albicans* ATCC 10231, *Aspergillus fumigatus* HIC 6094, *Trichophyton rubrum* IFO 9185, and *Trichophyton mentagrophytes* IFO 40996 were used for antifungal activity tests. *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation, and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum*, *and T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for 2 weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial twofold dilutions in the range of 100 to 0.8 μg/ml. Ten microliters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for $48 \sim 72$ h at 28 °C.
23. Experimental: The 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^1H and ^{13}C, respectively, on a Varian UNITY 500 spectrometer in methanol-*d*4 with solvent peaks as references. Mass spectra were recorded on a ThermoFinnigan Surveyor MSQ spectrometer. Column chromatography was performed with silica gel (230-400 mesh), RP-18 reversed-phase silica gel (43-60 μm). Typical procedure: A stirred mixture of the bromophenol **5** (15 mg), 10% Pd/Al_2O_3 (7 mg), and triethylamine (15 mg) in methanol was hydrogenated

using H_2 balloon at room temperature for 2 hrs. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was partitioned between CH_2Cl_2 and H_2O, and the organic layer was concentrated to yield corresponding phenolic compound **8** quantitatively. *3,3′,4,4′-Tetrahydroxy-6-hydroxymethyldiphenylmethane* (**8**): ^{1}H NMR (CD_3OD, 500 MHz) δ 6.81 (1H, s), 6.65 (1H, d, J = 8.05 Hz), 6.54 (1H, s), 6.52 (1H, d, J = 1.95 Hz), 6.45 (1H, dd, J = 8.05, 1.95 Hz), 4.42 (2H, s), 3.76 (2H, s); ^{13}C NMR (CD_3OD, 125 MHz) δ 146.1, 145.1, 144.3(x2), 134.3, 132.1, 131.6, 120.9, 118.5, 117.0, 116.8, 116.2, 62.9, 37.8; APCI(-) *m/z* 261.0 (M-H).

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25. General procedure: A mixture of 0.1 mmol (24 mg) of 2,2′,4,4′-tetrahydroxydiphenylmethane (**9**) and bromine (81 mg, 0.51 mmol) in 3 ml of acetic acid was stirred at room temperature for 2 hrs. The excess of bromine was removed by blowing with N_2, and the solvent was evaporated under reduced pressure. The crude product was purified by RP-18 column chromatography with a mixture of water and methanol as an eluent to afford 49 mg (0.09 mmol, 90 %) of compound **10**. *3,3′,5,5′-Tetrabromo-2,2′,4,4′-tetrahydroxydiphenylmethane* (**10**): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.11 (1H, s), 3.81 (1H, s); ^{13}C NMR (CD_3OD, 125 MHz) δ153.0, 150.8, 133.0, 122.7, 102.1, 101.3, 30.8; APCI(-) *m/z* 547.8 (M-H). All other compounds of this series were synthesized by following the above procedure. *3-Bromo-2′,6-dihydroxydiphenylmethane* (**12**): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.09 (1H, dd, J = 8.77, 2.43 Hz), 7.06 (1H, d, J = 2.43 Hz), 7.03 (1H, m), 7.02 (1H, m), 6.77 (1H, m), 6.74 (1H, td, J = 7.31, 0.97 Hz), 6.67 (1H, d, J = 8.77 Hz), 3.84 (2H, s); ^{13}C NMR (CD_3OD, 125 MHz) δ 156.1, 155.5, 133.7, 131.6 (x2), 130.6, 128.4, 127.8, 120.7, 117.6, 116.0, 112.1, 30.5; ESI(-) *m/z* 278 (M-H). *3,3′-Dibromo-4,4′-dihydroxydiphenylmethane* (**16**): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.23 (2H, d, J = 2.19 Hz), 6.94 (2H, dd, J = 8.29, 2.19 Hz), 6.80 (2H, d, J = 8.29 Hz), 3.73 (2H, s); ^{13}C NMR (CD_3OD, 125 MHz) δ 152.4, 134.1, 132.9, 128.7, 116.0, 109.5, 38.9; ESI(-) *m/z* 358 (M-H). *3,3′,5-Tribromo-4,4′-dihydroxydiphenylmethane* (**17**): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.24 (1H, d, J = 2.39 Hz), 7.22 (2H, m), 6.93 (1H, dd, J = 8.29, 2.39 Hz), 6.81 (1H, d, J = 8.29 Hz), 3.70 (2H, s); ^{13}C NMR (CD_3OD, 125 MHz) δ 152.6, 149.4, 135.8, 133.3, 133.0, 132.2 (x2), 128.8, 116.2, 111.1 (x2), 109.7, 38.5; ESI(-) *m/z* 436 (M-H).
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