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The in vitro antioxidant activities of the bromophenols from the red alga *Tichocarpus crinitus* and phenolic derivatives

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Abstract : A series of bromophenols and corresponding debrominated phenolic compounds was prepared by isolation from red alga *Tichocarpus crinitus* and by structural modification of natural bromophenols. Their structures were determined by extensive NMR analysis and the antioxidant activities were established through the determination of their abilities to inhibit free radicals using DPPH· as the stable radical. The most active free radical scavengers were the debrominated phenolic derivatives 3,4-dihydroxybenzyl alcohol and 3,3',4,4'-tetrahydroxydiphenylmethane.
Keywords*:* bromophenols, red alga, NMR analysis, antioxidant

INTRODUCTION

Currently, the research interest has been focused on the potential role of antioxidants in the prevention and treatment of aging, cancer, diabetes, hypertension and several other diseases.[1] Antioxidants provide protection to living organisms from damage caused by free radicals, which are generated in many bioorganic redox processes. These free radicals may induce oxidative damage in various components of the body by way of lipid peroxidation, protein damage and DNA strand breaking.[2] Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions or oxygen

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scavengers. Among various determining methods of free radical scavenging activity, the assay employing the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) has been the most widely used in vitro test has received most attention owing to its ease of use and its convenience.[3] In the DPPH assay, the antioxidant activity of each compounds are evaluated by monitoring the decrease in absorbance at 515nm as DPPH· (purple) is transformed to the reduced form DPPH-H (yellow).

In the course of our search for biologically active constituents from algae, we found the red alga *Tichocarpus crinitus* collected from the coast of Sokcho, Korea, exhibited potential effects on antioxidant activity. Red algae are known to contain high concentrations of bromophenols, which have shown significant antioxidant,[4] nitrite scavenging,[5] α-glucosidase inhibition,[6] anti-inflammatory activities,[7] aldose reductase inhibitory activity,[8] and cytotoxicities.[9]

In this report, the identification, structural modification, and antioxidant activities of the major bromophenols from the *Tichocarpus crinitus* were investigated.

EXPERIMENTAL

General Experimental Procedures

The 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Varian UNITY 500 spectrometer in methanol-*d*4 with solvent peaks as references. Column chromatography was performed with silica gel (230-400 mesh), RP-18 reversed-phase silica gel (43-60 μm).

Algal Material

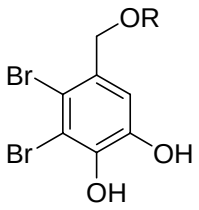
The red alga *T. crinitus* was collected at the coast of Sokcho, Korea, in April 2006 and identified by Dr. Rae-Seon Kang in KORDI. A voucher specimen (No. 06SC-4) was deposited at the Marine Resources Researches Department, Korea Ocean Research and Development Institute.

Extraction and Isolation

The fresh collection was immediately frozen using dry ice and kept at -25°C until investigated chemically. Air-dried alga *T. crinitus* (174 g) was extracted with MeOH (1 L x 2) and dichloromethane (1 L x 1) at room temperature. After the solvent was removed under reduced pressure at <40 °C, the residue (16.5 g) was partitioned with H_2O and CH_2Cl_2. The H_2O layer was evaporated to dryness in vacuo and the residue (13.4 g) was partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer (4.2 g) was subjected to C_{18} reversed-phase vacuum flash chromatography using gradient mixture of MeOH and H_2O as eluents (elution order 50, 40, 30, 20, 10% aqueous MeOH, 100% MeOH). The fraction eluted with 40% aqueous MeOH was dried and separated by reversed-phase HPLC (YMC-ODS-A column, 50% aqueous MeOH) to give compound **1** and **2**. The dichloromethane fraction (2.6 g) was chromatographed over silica gel, eluting with a gradient increasing MeOH (0-100%) in CH_2Cl_2, and separated into eight fractions. The fraction (390mg) elude with 10% methanol was dried and separated by reversed-phase HPLC (YMC-ODS-A column, 50% aqueous CH_3CN) to afford compound **3**. The names of each compound were revealed as follows: 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**1**), 2,3-dibromo-4,5-dihydroxybenzylmethyl ether (**2**), and 2,2',3,3'-tetrabromo-4,4',5,5'-tetrahydroxydiphenylmethane (**3**).[10,11] And the purified metabolites were isolated in following amounts: 20.4, 38.7, 15.2 mg for **1**-**3**, respectively.

Catalytic Hydrodehalogenation [12]

A vigorously stirred mixture of the bromophenols (10-20 mg), 10% Pd/C (50% of the weight of the bromophenols) and triethylamine (1.2 equiv. for each mol of the bromophenols) in methanol was hydrogenated using H_2 balloon at room temperature for 1hr. The reaction mixture was filtered using a membrane filter and the filtrate was evaporated to dryness. The residue was partitioned betweenCH_2Cl_2 and H_2O, and the organic layer was concentrated to give corresponding debrominated phenolic compounds quantitatively: 3,4-dihydroxybenzyl alcohol (**4**), 3,4-dihydroxybenzyl-methyl ether (**5**), 3,3',4,4'-tetrahydroxydiphenylmethane (**6**).



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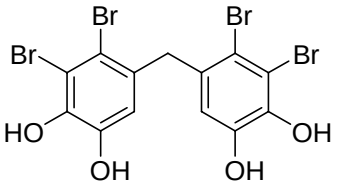
2 R = CH_3



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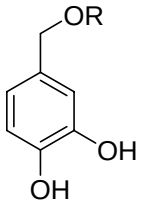

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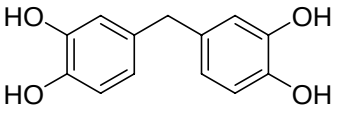

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Antioxidant activity (DPPH assay)

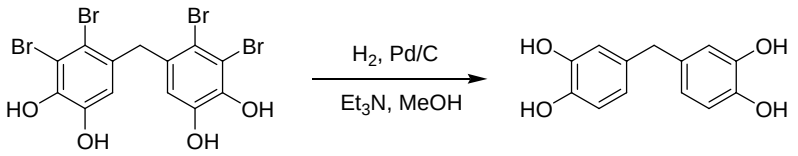
The free radical scanvenging effect of the samples was measured from the bleaching of purple colored methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as previously reported.[13] Ten microliter of each phenolic compounds (100μg/ml) in methanol was added to 200 μl of DPPH in methanol solution. After incubation at 37 ˚C for 30 min, the absorbance was read against a blank at 517 nm using ELISA micro plate reader. The radical scavenging activity of the tested samples was measured as a decrease in the absorbance of $DPPH^{\cdot}$ and was calculated in following way:

$$\text{scavenging capacity (\%)} = (1 - A_{sample}/A_{control}) \times 100.$$

RESULTS AND DISCUSSION

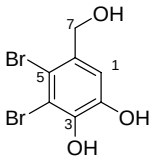
Guided by antioxidant activity, three bromophenols 1-3 were obtained from an extract of the fresh alga of *Tichocarpus crinitus*. Compounds **1**, **2**, **3** were isolated from the *T. crinitus* for the first time, and had previously been reported from other natural sources. The structures were determined by comparison of their spectral data with the literature values. The antioxidant activities of the three isolated bromophenol metabolites were tested against DPPH free radical. Among three bromophenol, compound **1** showed the most potent radical scavenger activity.

To investigate the influence of bromine in phenol ring toward antioxidant activity, chemical transformation was carried out. The debrominated derivatives of each natural bromophenols were prepared from chemical modifications using catalytic hydrodehalogenation according to the literature.



3 **6**

The natural bromophenols **1**, **2**, **3** were reacted with hydrogen gas in the presence of 10% Pd/C and Et_3N in methanol at room temperature to afford compound **4**, **5**, **6** in excellent yield, respectively. The NMR data of natural bromophenol and its derivative were compared in Table 1 and 2.



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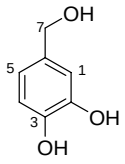

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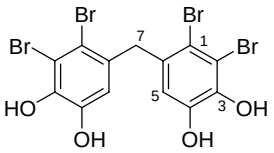

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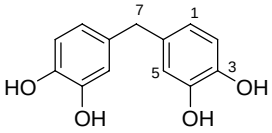

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Table 1. Comparison of NMR data for compound **1** and **4** in CD_3OD

	Compound **1**		Compound **4**	
position	^1H	^{13}C	^1H	^{13}C
1	7.00 (1H, s)	114.9	6.79 (1H, d, 2.2)	115.7
2		144.7		146.2
3		146.4		145.6
4		114.2	6.72 (1H, d, 8.3)	116.1
5		114.1	6.65 (1H, dd, 8.3, 2.2)	119.9
6		134.2		134.3
7	4.55 (2H, s)	65.7	4.42 (2H, s)	65.1

Table 2. Comparison of NMR data for compound **3** and **6** in CD_3OD

	Compound **3**		Compound **6**	
position	^1H	^{13}C	^1H	^{13}C
1		116.8	6.48 (2H, dd, 8.1, 2.0)	121.0
2		114.4	6.64 (2H, d, 8.1)	116.6
3		144.4		144.3
4		146.4		146.1
5	6.46 (2H, s)	116.7	6.56 (2H, d, 2.0)	117.0
6		132.6		135.0
7	4.01 (2H, s)	45.4	3.64 (2H, s)	41.5

Table 3. The percentage of DPPH scavenging capacity of phenolic compounds

Compound	**1**	**2**	**3**	**4**	**5**	**6**
scavenging capacity (%)	67	30	27	95	54	93

The antioxidant activity of six phenolic compounds was assessed on the basis of radical scavenging effect of the stable DPPH free radical. The scavenging capacities of phenolic compounds were compared with 2,6-di-*tert*-butyl-4-methylphenol (BHT) at 100μg/ml.

As summarized in Table 3, the most active free radical scavengers were compounds **4** and **6**. The activity of each compound was about 27% to 95% compared with BHT against the free radical DPPH, which increased in the following order: 2,2',3,3'-tetrabromo-4,4',5,5'-tetrahydroxydiphenylmethane (**3**), 2,3-dibromo-4,5-dihydroxybenzylmethyl ether (**2**), 3,4-dihydroxybenzyl-methyl ether (**5**), 2,3-dibromo-4,5-dihydroxybenzyl alcohol (**1**), 3,3',4,4'-tetrahydroxydiphenyl-methane (**6**), and 3,4-dihydroxybenzyl alcohol (**4**). Further, a general trend showed that the presence of deactivating group (Br) toward aromatic ring leads to decrease in the antioxidant activity in comparison to the presence of hydrogen atom. To the best of our knowledge, this is the first report on the comparison of antioxidant activity between natural bromophenols and their debrominated derivatives.

Acknowledgement

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