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〈Note〉

Nitrite Scavenging Activity of Bromophenol Congeners from *Symphyocladia latiuscula*

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Nitrite scavenging activity of a methanol extract of *Symphyocladia latiuscula* was studied. The methanol extract scavenged the nitrite in a dose-dependent manner. The MeOH extract was then sequentially partitioned with *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH and H_2O. The scavenging activity of the fractions increased in order of CH_2Cl_2, *n*-hexane, EtOAc, *n*-BuOH, and H_2O. Especially, the activity of the CH_2Cl_2 fraction was comparable to that of L-ascorbic acid. Column chromatography of the most active CH_2Cl_2 fraction over silica gel yielded three active bromophenol congeners (1~3) which were identified as (2*R*)-2-(2,3,6-tribromo 4,5-dihydroxybenzyl) cyclohexanone (1), 2,3,6-tribromo 4,5-dihydroxybenzyl methyl ether (2), and 2,3,6-tribromo 4,5-dihydroxybenzyl alcohol (3) respectively.

Key words: *Symphyocladia latiuscula*, nitrite scavenging activity, red alga, bromophenol

Carcinogenic N-nitroso compounds are produced by the acid-catalyzed reaction of nitrite with certain nitrogen compounds (Smith and Schwinghaer, 1969). But direct evidence linking N-nitroso compounds with human cancer causation is still scant. Since the presence of nitrite is a prerequisite in the formation of N-nitroso compounds, any compound that could compete successfully with the secondary amine for the available nitrite would reduce the possibility of N-nitroso compound formation. We have previously reported screening result on the MeOH extract of different kinds of seaweed as to their nitrite scavenging activity, and the MeOH extract of a red alga, *Symphyocladia latiuscula* was shown to have strong nitrite scavenging (Choi et al., 1997). Since the MeOH extract of *S. latiuscula* scavenged nitrite, we further examined the nitrite scavenging activity of various fractions obtained from the MeOH extract. And this paper also reports the isolation of active principles, and their nitrite scavenging activity.

Seaweed tissues (580 g, dry weight) of *S. latiuscula* were extracted with MeOH under reflux. The extracts were concentrated to dryness *in vacuo* at 40℃ to render the MeOH extract (148 g, yield 25.5%), and then partitioned with *n*-hexane (14.2 g), CH_2Cl_2 (23 g), EtOAc (11.2 g), *n*-BuOH (36.8 g), and H_2O (53.6 g) in sequence to make the corresponding dried extracts. To find the nitrite scavengers from *S. latiuscula*, the nitrite scavenging activity was evaluated by measuring the percentages of nitrite remaining after sample treatment (APHA, AWWA and WPCF, 1985). The control (absence of samples) was taken as 100%, and the percent intensity was calculated by spectrophotometry at 540 nm. As shown in Figure 1, the MeOH extract of *S. latiuscula* exhibited significant nitrite scavenging activity. Its 50% scavenging concentration (SC_{50}) was 0.07 mg/mL. The MeOH extract was then sequentially partitioned with *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH and H_2O. The nitrite scavenging activities of different solvent soluble fractions obtained from the MeOH extract are also invetigated, and the results are shown in Table 1. All solvent soluble fractions were effectively scavenged except for H_2O soluble fraction of the MeOH extract; the effect of these depended on their conentration. The scavenging

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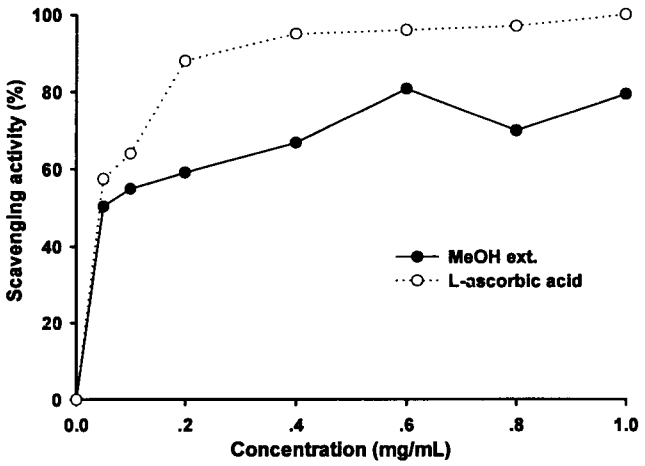

Fig. 1. The nitrite scavenging effect of the methanol extract from *S. latiuscula*.

Table 1. The nitrite scavenging effect of various fractions and isolated compounds from *S. latiuscula*

Fractions/compounds	SC_{50} (mg/mL)*
n-Hexane fraction	0.26
CH_2Cl_2 fraction	0.14
EtOAc fraction	0.42
n-BuOH fraction	0.53
H_2O fraction	1.00
Compound 1	0.16
Compound 2	0.29
Compound 3	0.25
L-ascorbic acid	0.03

* Scavenging activity was expressed as the mean of 50% scavenging concentration of triplicate determines, obtained by interpolation of concentration-scavenging curve.

activity of the fractions increased in order of CH_2Cl_2, *n*-hexane, EtOAc, *n*-BuOH, and H_2O. Especially, the activity of the CH_2Cl_2 fraction was comparable to that of L-ascorbic acid which is a well known nitrite scavenger. These results suggest that the MeOH extract and the CH_2Cl_2 soluble fraction of *S. latiuscula* are effective nitrite scavengers. The strong active CH_2Cl_2 fraction of the MeOH extract from *S. latiuscula* was further purified to isolate the nitrite scavenging compounds. Column chromatography on silica gel of the CH_2Cl_2 soluble fraction of the MeOH extract furnished compounds 1~3 in the order of increasing polarity, respectively. Compounds 1~3 (Fig. 2) were readily elucidated as (2*R*)-2-(2,3,6-tribromo 4,5-dihydroxybenzyl) cyclohexanone (1, yield 6.5 mg, 0.0011% dry weight), 2,3,6-tribromo 4,5-dihydroxybenzyl methyl ether (2, yield 2.82 g, 0.486%) and 2,3,6-tribromo 4,5-dihydroxybenzyl alcohol (3, yield 2.5 mg, 0.0004%) by comparison of reported spectroscopic data and finally confirmed by comparisons with authentic samples (Park et al., 1999; Choi et al., 2000). The activities of compounds 1~3, and L-ascorbic acid on the nitrite scavenge are also shown Table 1. Compounds 1~3 showed nitrite scavenging activities with SC_{50} values of 0.16, 0.29 and 0.25 mg /mL, respectively. These nitrite scavenging activities were comparable to that of L-ascorbic acid (SC_{50}= 0.03 mg/mL). This is the first examples of bromophenols scavenging nitrite.

Kim et al. (1987) reported that the chloroform-soluble fraction of red alga, *Polysiphonia ulceolata* and *Enteromorpha compressa* possess a marked scavenging activity. Kim et al. (1996) also screened for nitrite scavenging activity of eight algal species *Laminaria japonica, Undaria pinnatifida, Codium fragile, Polysiphonia tenera, sargassum fulvellum, Enteromorpha compressa, Ecklonia cava,* and *Ecklonia stolonifera.* We also found that the brown alga *Ecklonia stolonifera* showed a marked nitrite scavenging activity, and isolated phenolic compounds such as phloroglucinol and phlorotannin A as active principles (Choi et al., 1997). There was a report that phenolic compounds are effective in nitrite scavenging (Kang et al., 1996).

Bromophenols are known to have their various biological activities such as antibiotic (Kurata and Amiya, 1980; Pettit et al., 1996), anti-inflammatory (Wiemer et al., 1991), feeding-deterrent (Kurata and Amiya, 1977), antioxidant (Park et al., 1999; Choi et al., 2000), and antimutagenic activities (Wall et al., 1989). They also inhibit a variety of enzymes including phospholipase A_2 (Wiemer et al., 1991), 15-lipoxygenase (Fu et al., 1995), inosine monophosphate synthetase (Fu et al., 1995; Chen et al., 1994), and α-glucosidase (Kurihara et al., 1999). However, no report on the nitrite scavenging activity of bromophenols yet appeared.

The present work would tend to indicate that the methanol extract of *S. latiuscula* and their fractions and its components, bromophenol congeners, may be useful as nitrite scavengers. Investigation of further nitrite scavenging principles are now in progress.

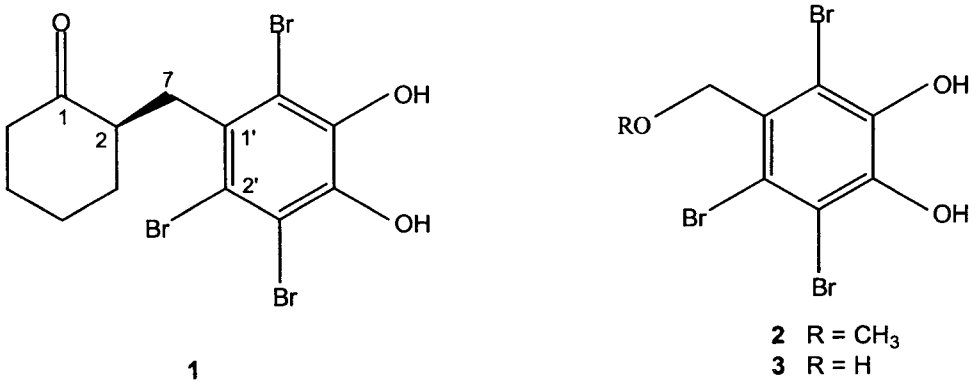

Fig. 2. Structures of compounds 1~3.

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