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NOTE

Sargassumol, a novel antioxidant from the brown alga *Sargassum micracanthum*

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Free radicals have been implicated in the pathogenesis of diseases such as ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation and the initiation of cancer.[1–3] There is considerable evidence that antioxidants may help prevent illnesses caused by oxidative stress due to their capacity to quench free radicals, thereby protecting cells and tissues from oxidative damage. Thus, the demand for alternative antioxidants from natural sources is gradually growing.

The genus *Sargassum* (Sargassaceae), which belongs to the large brown algae, is distributed mainly throughout temperate Pacific coastal regions, areas of the Indian Ocean and along the Australian coast. It comprises more than 400 species. These are known to produce diverse bioactive entities including plastoquinones,[4] chromanol,[5] cyclopentenone[6] and polysaccharides.[7] *S. micracanthum* is found in Japan and South Korea, mainly on the south and east coasts. Extracts of *S. micracanthum* have been shown to exhibit antioxidant, anti-viral and selective vasodilation effects.[4,5,8] As part of our ongoing effort to find natural antioxidants, we have isolated a new free-radical scavenger, designated sargassumol (**1**, Figure 1), from the methanolic extract of *S. micracanthum*. In this paper, the isolation, structure determination and free radical scavenging activity of **1** are described.

Dried *S. micracanthum* (122 g) collected at Wando county, Jeonnam province, Korea was ground and extracted with methanol at room temperature. This methanolic extract was concentrated under reduced pressure and the aqueous resultant was consecutively partitioned with hexane, chloroform, ethyl acetate and *n*-butanol. The ethyl acetate-soluble portion, exhibiting potent radical scavenging activity, was concentrated under reduced pressure, subjected to Sephadex LH-20 column chromatography and eluted with 70% aqueous methanol. An antioxidant fraction was further separated by preparative reversed-phase HPLC eluted with a gradient of methanol concentrations increasing to 40% in water acidified with 0.04% trifluoroacetic acid to afford compound **1** (2 mg).

The molecular weight of sargassumol was determined to be 468 by FAB-mass measurements providing quasi-molecular ion peaks at *m/z* 469 $[M+H]^+$ and 491 $[M+Na]^+$ in positive mode. Its molecular formula was established to be $C_{20}H_{20}O_{13}$ by high-resolution FAB-mass measurement (*m/z* 469.0952 $[M+H]^+$, Δ - 3.0 mmu) in combination with ^1H and ^{13}C NMR data. This molecular formula requires 11 degrees of unsaturation. The ^1H NMR spectrum of sargassumol measured in DMSO (dimethyl sulfoxide)-d_6 showed signals due to hydroxyl protons at δ 9.26, 9.10, 7.48, 5.66 and 5.53, which are not observed in CD_3OD, two aromatic methines at δ 5.85, an olefinic methine at δ 4.82, a methine at δ 4.17, two methoxy methyl protons at δ 3.73 and 3.68 and two isolated non-equivalent methylene groups at δ 3.12/2.70 and 3.00/2.33. In the ^{13}C NMR spectrum, two ketone carbonyl carbons at δ 201.5 and 197.1, two ester carbonyl carbons at δ 170.5 and 169.3, five sp^2 quaternary carbons, three sp^2 methines, three oxygenated sp^3 quaternary carbons at δ 81.1, 79.9 and 75.6, two methoxy methyl carbons at δ 54.0 and 51.7, one methine carbon at δ 52.5 and two methylene carbons at δ 48.5 and 37.0 were evident. The proton-bearing carbons were established by HMQC spectrum, as shown in Table 1. In an ^1H-^1H COSY spectrum, the methine proton at δ 4.82 (H-3) showed a cross-peak with the methine proton at δ 4.17 (H-1), suggesting their allylic relationship (Figure 2). The structure of compound **1** was determined by HMBC spectrum, as shown in Figure 2. A long-range correlation of the methine proton at δ 4.82 (H-3) to the oxygenated sp^2 quaternary carbon at δ 173.7 (C-2) and the chemical shift value of the carbonyl carbon at δ 197.1 (C-4) suggested the presence of an α,β-unsaturated ketone moiety. In addition, the methine proton at δ 4.82 (H-3) revealed a long-range correlation to the oxygenated sp^3 quaternary carbon at δ 79.9 (C-5), and the hydroxyl protons at δ 5.66 and 5.53 revealed crucial HMBC correlations to the carbons at δ 79.9 (C-5), 75.6 (C-9), and 48.5 (C-6) and 79.9 (C-5), 75.6 (C-9) and 52.5 (C-1), respectively, establishing a six-membered

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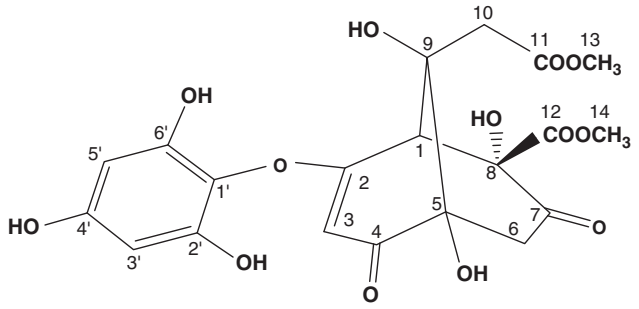

Figure 1 Structure of sargassumol.

Table 1 ^{1}H and ^{13}C NMR spectral data for sargassumol in DMSO-d_6[a]

no.	δ_C	δ_H
1	52.5	4.17 (1H, s)[b]
2	173.7	
3	102.1	4.82 (1H, s)
4	197.1	
5	79.9	
5-OH		5.66 (1H, s)
6	48.5	3.00 (1H, d, *J*=15.8)
		2.33 (1H, d, *J*=15.8)
7	201.5	
8	81.1	
8-OH		7.48 (1H, s)
9	75.6	
9-OH		5.53 (1H, s)
10	37.0	3.12 (1H, d, *J*=17.9)
		2.70 (1H, d, *J*=17.9)
11	170.5	
12	169.3	
13	51.7	3.68 (3H, s)
14	54.0	3.73 (3H, s)
1′	121.4	
2′, 6′	150.2	
2′-OH, 6′-OH		9.10 (br s)
3′, 5′	95.0	5.85 (2H, s)
4′	156.8	
4′-OH		9.26 (1H, s)

Abbreviation: DMSO, dimethyl sulfoxide.
[a]NMR data were recorded at 600 MHz for protons and at 150 MHz for carbons.
[b]Proton resonance integral, multiplicity and coupling constants (*J*=Hz) are in parentheses.

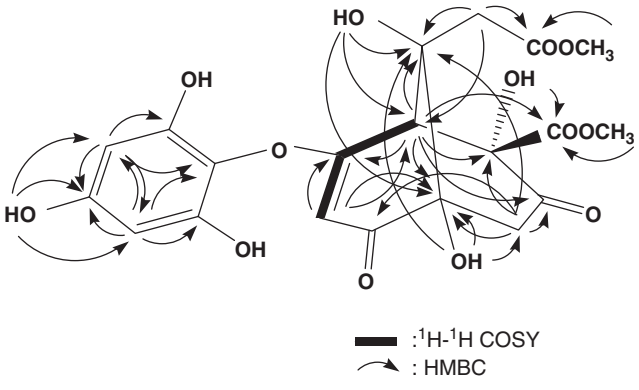

Figure 2 Two-dimensional NMR correlations for sargassumol.

ring including an α,β-unsaturated ketone. Additionally, the methine proton at δ 4.82 (H-3) and the methylene protons at δ 3.00 and 2.33 (H-6), respectively, showed long-range correlations to the same carbons, at δ 201.5 (C-7), 81.1 (C-8), 79.9 (C-5) and 75.6 (C-9), revealing that sargassumol has a bicyclo[3.3.1]nonane skeleton. Two methoxycarbonyls were assigned by HMBC correlations from the methyl protons at δ 3.73 to the carbonyl carbon at δ 169.3 and from the methyl protons at δ 3.68 to the carbonyl carbon at δ 170.5, and they were attached to the C-8 and C-10 positions, respectively, by long-range correlations from the methine proton at δ 4.17 (H-1) to the carbonyl carbon at δ 169.3 and from the methylene protons at δ 3.12 and 2.70 (H-10) to the carbonyl carbon at δ 170.5. Additional long-range correlations from the hydroxyl proton at δ 7.48 (8-OH) to the carbonyl carbon at δ 169.3 and from the methylene protons at δ 3.12 and 2.70 (H-10) to the quaternary carbon at δ 75.6 (C-9) established the presence of the sargassumketone moiety, previously reported as a chemical constituent of *Sargassum kjellmanianum*.[9] The remaining six carbons at δ 156.8, 150.5, 121.4 and 95.0 and four degrees of unsaturation suggested the presence of a symmetric benzene ring in **1**, and it was assigned as 2,4,6-trihydroxyphenoxy group on the basis of carbon chemical shifts and long-range correlations from the methine proton at δ 5.85 (H-3′ and H-5′) to the oxygenated quaternary carbons at δ 156.8 (C-4′), 150.2 (C-2′ and C-6′) and 121.4 (C-1′). A phenolic hydroxyl proton at δ 9.26 showed a long-range correlation to the carbons at δ 156.8 (C-4′) and 95.0 (C-3′ and C-5′), implying a connection of C-1′ to the sargassumketone moiety. By the interpretation process for assignments of oxygen atoms, the phloroglucinol moiety should therefore be connected to enol oxygen at C-2 of the sargassumketone moiety by ethereal linkage. Therefore, the structure of sargassumol was unambiguously determined to be a novel antioxidant with sargassumketone and hydroxyphloroglucinol moieties. Although the stereochemistry of the sargassumketone moiety remains to be investigated, the proton chemical-shift values of H-6 at δ 3.64 (1H, d, *J*=15.8 Hz) and 3.11 (1H, d, *J*=15.8 Hz) and H-10 at δ 3.81 (1H, d, *J*=17.2 Hz) and 3.33 (1H, d, *J*=17.2 Hz) in C_5D_5N were consistent with those previously reported for sargassumketone,[9] and its carbon chemical shifts in DMSO-d_6 are also in good agreement with those of sargassumketone. Thus, the relative stereochemistry of sargassumol may be the same as that of sargassumketone, as shown in Figure 1.

The antioxidant effects of sargassumol were evaluated by free radical-scavenging activity and reducing-power assays. Free radical-scavenging activities against the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)) radical cation and the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical were measured using methods described in the literature.[10] Sargassumol showed minimal scavenging activity against DPPH radical, but exhibited potent ABTS radical-scavenging activity with an IC_{50} value of approximately 47 μM, comparable to that of trolox (IC_{50}, 45 μM). Reducing power was evaluated using the potassium ferricyanide reduction method with minor modification.[11] In brief, sample (10 μl) was mixed with 25 μl of 200 mM potassium phosphate buffer (pH 6.6) and 25 μl of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After addition of 25 μl of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (50 μl) was mixed with 50 μl distilled water and 10 μl of 0.1% ferric chloride, and absorbance measured at 700 nm. When reducing power was expressed as activity relative to trolox, sargassumol was about five-fold less active than trolox.

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