A Cyclohexanonyl Bromophenol from the Red Alga *Symphyocladia latiuscula*

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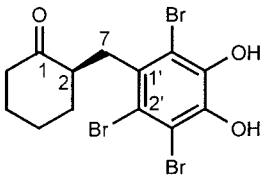
From an extract of the red alga *Symphyocladia latiuscula*, a bromophenol (**1**) was isolated and characterized as (2*R*)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl)-cyclohexanone based on the spectroscopic evidence. The bromophenol was found to be a scavenger of 1,1-diphenyl-2-picrylhydrazyl radical.

Symphyocladia latiuscula (Harvey) Yamada is a member of the family Rhodomelaceae, belonging to the order Ceramiales.[1] A previous phytochemical investigation performed on this species resulted in the isolation of bromophenols.[2−4] Previously we reported that the methanolic extract of the red alga *S. latiuscula* exerts antioxidant activity on 1,1-diphenyl-2-picrylhyrdazyl (DPPH) radicals.[5] From this methanolic extract, 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (**3**) was isolated as one of the active principles, together with the inactive component, cholesterol.[6] In the course of our continuous study on the active principles of this alga, a new bromophenol named symphyoketone (**1**) was isolated along with the known 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol (**2**). These compounds were individually evaluated for scavenging on DPPH radicals.



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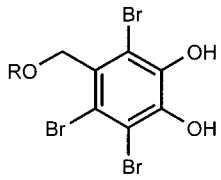

2 R = H
3 R = CH_3

Column chromatography of the CH_2Cl_2-soluble part of the methanol extract of the alga yielded two bromophenols, compounds **1** and **2**, in order of increasing polarity. The structure of **2** was identified by comparison with published spectral data as 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol.[3,7]

Compound **1** was obtained as an amorphous white powder. The molecular formula of **1** was determined as $C_{13}H_{13}O_3Br_3$, based on the NMR and MS data [$(M + H)^+$ *m/z* 454.8476 for $C_{13}H_{14}O_3{}^{79}Br_3$, Δ −1.7 mmu; *m/z* 456.8484 for $C_{13}H_{14}O_3{}^{79}Br_2{}^{81}Br$, Δ +1.1 mmu; *m/z* 458.8459 for $C_{13}H_{14}O_3{}^{79}Br^{81}Br_2$, Δ +0.7 mmu]. The IR spectrum of **1** showed absorption bands at 3485 (OH), 1702 (CO) and 1664 (aromatic C=C) cm^{-1}. The NMR spectra of **1** in $CDCl_3$ showed signals similar to those of bromophenols **2** and **3**.[3,6] In addition to the tribromo-dihydroxybenzyl signals, additional signals attributable to a cyclohexanonyl group were recognized. These data indicated that **1** is a tribromo-dihydroxybenzyl cyclohexanone derivative. COSY, HMQC, and HMBC data analysis also provided evidence for the cyclohexanonyl group and established all of the expected connectivities for **1**. Benzylic methylene protons (H-7, δ_H 3.50, 3.19) showed correlations with the carbonyl carbon at δ_C 211.7 and the quaternary carbons at δ_C 132.5 (C-1′), 112.1 (C-2′), and 117.5 (C-6′) of the aromatic ring. A methine proton at δ_H 2.80 (m, H-2) showed correlations with the carbonyl carbon and the benzylic methylene protons, indicating that the cyclohexanonyl group was connected at C-7. The absolute configuration of C-2 was established from its ORD spectrum. The ORD spectrum of **1** showed a positive Cotton effect at 228 nm, indicating that **1** possesses an 2*R* configuration according to the octant rule.[8] Consequently, the structure of **1** was established as (2*R*)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl)-cyclohexanone, named symphyoketone.

Compounds **1** and **2** showed scavenging activities of DPPH radical, with IC_{50} values of 8.5 and 7.5 μM, respectively. These radical scavenging activities were 2-fold more potent than that of L-ascorbic acid (IC_{50} = 15.3 μM).

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Mitamura-Riken polarimeter. UV and IR spectra were recorded on a Varian Carey UV−vis spectrophotometer and a Perkin-Elmer FT-IR spectrometer, respectively. LRFABMS and HRFABMS data were recorded on a JEOL JMS-HX110/110A spectrometer. ^1H and ^{13}C NMR spectra were measured by Bruker DMX 600 and Varian UNITY-Plus 300 spectrometers. Chemical shifts were referenced to the respective residual solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 3.30 and δ_C 49.0 for CD_3OD). COSY, HMQC, and HMBC spectra were recorded on a Bruker DMX 600 using pulsed field gradients. Column chromatography was carried out using Si gel (Merck, 70−230 mesh). TLC was performed on the precoated Merck Kieselgel 60 F_{254} plates (0.25 mm), and 50% H_2SO_4 was used as spray reagent. L-Ascorbic acid and DPPH were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO).

Plant Material. Leafy thalli of *S. latiuscula* were collected at Chungsapo, Pusan, in January 1998, and authenticated by Prof. K. W. Nam of the Department of Marine Biology, Pukyong National University. A voucher specimen (no. 980128) has been deposited in the author's laboratory (J.S.C.).

Extraction and Isolation. Seaweed tissues (580 g, dry wt) were refluxed with MeOH for 3 h (9 L × 3). The total filtrate was concentrated to dryness in vacuo at 40 °C to render the MeOH extract (148 g), and this extract was suspended in distilled water and partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 fraction showed strong scavenging activity against DPPH radicals.[5] Thus, the CH_2Cl_2 fraction (21 g) was applied to a Si gel column, which was eluted with CH_2Cl_2−MeOH (gradient) to yield 22 subfractions. Fraction 4 (1.2 g) was further chromatographed on

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a Si gel column eluting with CH_2Cl_2−MeOH (10:1) to give compound **1** (6.5 mg). Fraction 12 (0.8 g) was further chromatographed on a Si gel column eluting with CH_2Cl_2−MeOH (5:1) to give compound **2** (2.5 mg).

(2*R*)-2-(2,3,6-Tribromo-4,5-dihydroxybenzyl)-cyclohexanone (1): amorphous white powder; $[\alpha]_D^{23}$ +7.27° (*c* 0.11, MeOH); ORD (*c* 2.0 × 10^{-2}, MeOH) [*θ*] (nm) −2500 (222), 0 (224), +15 000 (228); UV λ_{max} (MeOH) (log ϵ) 236 (s, 4.09), 260 (s, 3.34), 287 (s, 3.30), 296 (3.41) nm; IR (KBr) ν_{max} 3486, 2932, 1702, 1664, 1459, 1392, 1328, 1258, 1247, 1179, 1157, 1127, 903, 862, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 1.57 (1H, td, J = 12.1, 3.3 Hz, H-4), 1.61 (1H, td, J = 12.6, 3.1 Hz, H-3), 1.70 (1H, td, J = 13.2, 3.6 Hz, H-5), 1.85 (1H, d, J = 13.2 Hz, H-4), 1.89 (1H, m, H-3), 2.08 (1H, septet, J = 2.9 Hz, H-5), 2.39 (1H, td, J = 9.4, 5.6 Hz, H-6), 2.48 (1H, d, J = 9.4 Hz, H-6), 2.80 (1H, m, H-2), 3.19 (1H, dd, J = 14.5, 10.4 Hz, H-7), 3.50 (1H, dd, J = 14.5, 3.8 Hz, H-7); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.3 (C-4), 27.8 (C-5), 32.7 (C-3), 36.9 (C-7), 42.0 (C-6), 50.1 (C-2), 112.1 (C-2'), 112.9 (C-3'), 117.5 (C-6'), 132.5 (C-1'), 140.4 (C-4'), 140.6 (C-5'), 211.7 (C-1); HRFABMS, see text.

2,3,6-Tribromo-4,5-dihydroxybenzyl alcohol (2): amorphous white powder; ^1H NMR (CD_3OD, 300 MHz) δ 4.99 (2H, s, H-7); ^{13}C NMR (CD_3OD, 75 MHz) δ 74.5 (C-7), 114.2 (C-2), 115.2 (C-3), 119.7 (C-6), 129.4 (C-1), 144.6 (C-4), 146.6 (C-5).

DPPH Radical Scavenging Effect. The DPPH radical scavenging effect was evaluated according to the method first employed by Blois.[9] A methanol solution (4 mL) of varying sample concentrations (1.5−45 μM) was added to 1.0 mL DPPH methanol solution (1.5 × 10^{-1} M). After mixing gently and leaving for 30 min at room temperature, the optical density was measured at 520 nm using a spectrophotometer. The antioxidant activity of each sample was expressed in terms of IC_{50} (μg/ mL or μM required to inhibit DPPH radical formation by 50%) and calculated from the log-dose inhibition curve.

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