Identification of New Dibenzofurans from *Distylium racemosum*

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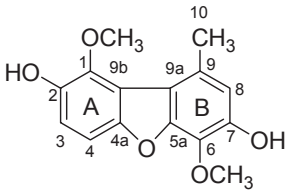
Distylium racemosum (Hamamelidaceae) is a large indeciduous tree distributed over Halla Mountain in Jeju Island, Korea. We are continuously searching for the bioactive compounds applicable as functional cosmetic ingredients from plants in Jeju.[1] From the ethanol extract of *D. racemosum* branches, we have found inhibition activities on elastase. Elastin degradation by elastase in dermis is associated with aged skin-wrinkle.[2] Since no chemical investigation has been reported from the branches of this plant, we have conducted a phytochemical study to identify the active constituents. In this study, we have isolated new dibenzofuran compounds, 2,7-dihydroxy-1,6-dimethoxy-9-methyldibenzofuran (**1**) and 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran (**2**).

From the dried *D. Recamosum* collected in Jeju Island, ethanol extract was prepared. The extract was successively partitioned into hexane, ethyl acetate and *n*-butanol. The ethyl acetate fraction was further purified by column chromatography over celite, silica gel and Sephadex LH-20 to afford the compounds **1** and **2**.



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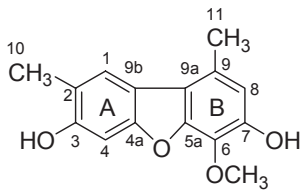

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The compound **1** was white amorphous powder, and its molecular formula was determined as $C_{15}H_{14}O_5$ (nine unsaturation number) based on the high resolution FAB-MS data. The UV spectrum of **1** showed absorption maxima at 290.4 nm, indicating the presence of conjugated or aromatic system(s). The ^{1}H NMR spectrum of **1** in pyridine-d_5 (Table 1) showed signals for one methyl group at δ 2.98 (3H, s), two methoxy groups at δ 4.11 (3H, s) and 4.12 (3H, s), three aromatic protons at δ 7.41 (1H, d), 7.32 (1H, d), 7.17 (1H, s). The ^{13}C NMR data indicated the presence of 12 sp^2 carbons corresponding to two aromatic rings, and additional one methyl and two *O*-methyl carbons. Since two aromatic rings accounted for eight unsaturation number, compound **1** was inferred to have another ring, an ether linkage between aromatic rings to construct a dibenzofuran nucleus.

^{1}H NMR analysis indicated that two protons at δ 7.32 (H-3) and 7.41 (H-4) are located in *ortho* position by their doublet coupling constants (J = 8.8 Hz). Since the proton at δ 7.17 is singlet, its position should be located in another aromatic ring in dibenzofuran skeleton. The connectivities of the substituents in dibenzofuran **1** were determined by HMBC and NOESY experiments. HMBC correlations in H-3/C-1, H-3/C-4a, H-4/C-2, H-4/C-9b showed the location of the carbons in ring A (Figure 1). The position of methoxy hydrogen (1-OCH$_3$) at δ 4.11 was assigned to be at C-1 by its $^3J_{CH}$ HMBC cross peak.

In ring B, singlet at δ 7.17 (H-8) showed HMBC correlation with signals at δ 132.2 (C-6) and 151.2 (C-7). Methyl (δ 2.98) and methoxy (δ 4.12) were verified to be attached in ring B by their HMBC correlation with δ 116.2 (C-8) and 129.4 (C-9), and δ 132.2 (C-6), respectively. NOESY experiment showed the crosspeak between methyl protons (δ_H 2.96) in ring B and methoxy protons (δ_H 4.12) in ring A. Since occurrence of NOESY correlation between substituents in different rings in **1** is possible at the positions of C-1 and C-9,[3] it is reasonable to assign the location of the methyl group at C-9 and the methoxy group at C-1. Methyl protons (H-10) also showed a NOESY correlation with a proton at δ_H 7.17, which supported the location of the proton signal (δ 7.17, H-8). The position of the carbon signal at δ_C 132.2, showing HMBC correlation with methoxy protons, was assigned to C-6 rather than C-7 by the consideration of calculated δ_C values in benzene ring.[4] Hyroxy protons at C-2 and C-7 were showed up in down field at δ 11.83 and 11.35 as singlets. From these spectroscopic data, the chemical structure of dibenzofuran **1** was completely determined.

The occurrence of dibenzofuran derivatives from natural sources is relatively few. It is interesting to note that a dibenzofuran, kehokorin B (**3**), has been recently isolated from myxomycete, and it shared the same structure in ring A compared to **1**.[3] When we compared the ^{13}C NMR data for the carbons in A ring between compounds **1** and **3**, it was found that their chemical shifts are well matching each other (Table 2).

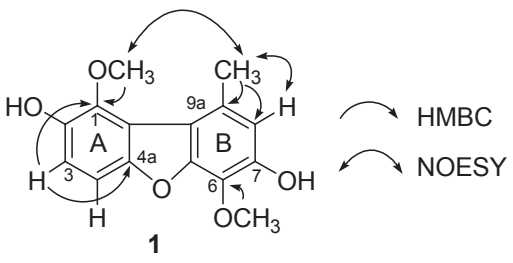

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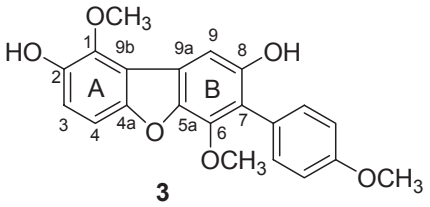
Figure 1. Key COSY and HMBC correlations in compound **1**

Table 1. 1D and 2D NMR data for **1** and **2** in 400 MHZ (for ^{1}H) NMR

Compound	**1** (pyridine-d_5)			**2** (CD_3OD)		
position	δ_C	δ_H (int, mult, *J* in Hz)	HMBC (H→C)	δ_C	δ_H (int, mult, *J* in Hz)	HMBC (H→C)
1	142.8			123.4	7.58 (1H, s)	C-3, C-4a, C-10
2	147.9			121.6		
3	116.0	7.32 (1H, d, 8.8)	C-1, C-4a	155.4		
4	107.5	7.41 (1H, d, 8.8)	C-2, C-9b	98.5	6.94 (1H, s)	C-9b
4a	151.6			157.3		
5a	150.8			149.6		
6	132.2			132.2		
7	151.2			148.4		
8	116.2	7.17 (1H, s)	C-6, C-7	114.0	6.60 (1H, s)	C-6, C-9a, C-11
9	129.4			127.6		
9a	117.2			118.4		
9b	120.2			118.3		
10	22.4	2.98 (3H, s)	C-8, C-9	16.7	2.30 (3H, s)	C-2, C-3
11				19.6	2.58 (3H, s)	C-8, C-9, C-9a
1-OCH_3	61.3	4.11 (3H, s)	C-1			
6-OCH_3	61.4	4.12 (3H, s)	C-6	61.5	4.03 (3H, s)	C-6
-OH		11.35 (1H, s)				
-OH		11.83 (1H, s)				



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The molecular formula of **2** was revealed as $C_{15}H_{14}O_4$ based on the high resolution FAB-MS and NMR data. The UV spectrum of **2** at λ_{max} 296.4 nm indicated the presence of aromatic systems. The ^{1}H NMR spectrum of **2** in CD_3OD (Table 1) showed signals for two methyl groups at δ_H 2.58 (3H, s) and 2.30 (3H, s), one methoxy group at δ_H 4.03 (3H, s), three aromatic hydrogens at δ_H 7.58 (1H, s), 6.94 (1H, s), 6.60 (1H, s). The ^{13}C NMR data indicated the presence of 12 sp^2 carbons, and two methyl and one methoxy carbons. It was reasonable to assume that **2** has also dibenzofuran nucleus based on comparative analysis of ^{1}H and ^{13}C NMR data with those of **1**.

The position of the substituents were established by 1D and 2D (HMBC and NOESY) NMR data. Three aromatic proton signals were all observed as singlets, which indicates that three protons are in separate spin system. The only possible orientation is that two protons are located in *para* positions in one ring and another proton is placed in the other ring. Closer examination of the NMR data indicated that **2** has the same chemical structure with **1** in ring B. For example, carbon signals and HMBC correlation (H-8/C-6, H-8/C-9a. H-8/C-11) were almost identical between **1** and **2** in ring B. HMBC correlations of proton signals at δ 7.58 with δ 155.4, 157.3, 16.7 as well as those of δ 6.94 with δ 118.3 established the carbon connectivities in ring A. Since proton signal at δ 7.58 showed NOESY crosspeak with signal at δ 2.58 (H-11), it is reasonable to assign a signal (δ_H 7.58) to H-1 based on stereochemical consideration. H-1 also showed another NOESY correlation with a signal at δ 2.30, which corroborated the attachment of the methyl group (C-10) to C-2. From these spectroscopic data, the compound **2** was identified as 3,7-dihydroxy-6-methoxy-2,7-dimethyldibenzofuran.

Table 2. Comparison of ^{13}C NMR data for the carbons in ring A of **1** and **3**

position	**1** (pyridine-d_5)	**3** (acetone-d_6)
1	142.8	142.8
2	147.9	146.3
3	116.0	117.6
4	107.5	108.0
4a	151.6	152.2
9b	120.2	119.3

The compounds **1** and **2** were examined for elastase inhibition activities.[5] The compound **2** (IC_{50} = 7.7 μg/ml) was more active than oleanolic acid (IC_{50} = 9.7 μg/ml), a compound commercially applied as the whitening ingredient in functional cosmetics. The compound **1** (IC_{50} = 97.4 μg/ml) showed slightly lower activity.

Experimental Section

Reagent and Equipment. Thin layer chromatography was performed on Merck prepared plates (silica gel 60 F-254 on aluminum). Column chromatography was performed using Merck silica gel 60 (230-400 mesh). Sephadex LH-20

(25-100 μm) for Gel filtration chromatography (GFC) was obtained from Fluka. The UV absorbance was performed with a Biochrom Libra S22 UV-visible spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JNM-LA 400 (JEOL) instrument, with chemical shift data reported in ppm relative to the solvent used. 2D NMR spectra were recorded on the same instrument using field gradient FG2 (inverse) probe. Melting point was recorded on Fisher Scientific 12-132T. High resolution MS data was obtained from the Korea Basic Science Institute in Seoul.

Isolation of the Compounds 1 and 2. The branches of *D. racemosum* were collected from Halla Botanical Garden in Jeju Island, and air-dried and cut into small pieces. The voucher specimen (J-242) was prepared and deposited in the laboratory of natural product, department of chemistry, Cheju National University.

The dried *D. racemosum* powder (2.5 kg) was suspended in 25 L of 70 % EtOH and mechanically stirred for 24 h at room temperature. The solution was filtered and the residue was extracted one more time. The filtrates were combined and concentrated under reduced pressure to give the oily extract (270 g). After the extract was suspended in 2 L of distilled water, successive solvent fractionation was performed using *n*-hexane, ethyl acetate and *n*-butanol. The EtOAc fraction (45 g) was chromatographed over celite with *n*-hexane, CH_2Cl_2, $CHCl_3$, Et_2O, EtOAc and MeOH successively. The obtained diethyl ether subfraction was chromatographed over reversed-phase silica gel with gradient solvent (H_2O-MeOH) system to provide 12 fractions (Fr-1 to Fr-12). The ninth fraction (Fr-9) was further purified by silica gel column chromatography (CC) with $CHCl_3$/MeOH (4/1) ,and the obtained subfraction was recrystallized to give the compound **1** (63.9 mg). The compound **2** (4.9 mg) was obtained from Fr-10 by Sephadex LH-20 CC with $CHCl_3$/MeOH (8/1).

2,7-Dihydroxy-1,6-dimethoxy-9-methyldibenzofuran (1). White amorphous powder; UV (CH_3OH) 290.4; Melting point 178-182^{o}C; HR-FABMS data [*m*/*z* 274.0832 $(M)^{+}$, calcd for $C_{15}H_{14}O_5$ 274.0841, △ -0.9 mmu]. For ^{1}H and ^{13}C NMR data, see Table 1; NOESY correlations (H↔H#) 6-OCH_3↔H_3-10, H_3-10↔H-8.

3,7-Dihydroxy-6-methoxy-2,7-dimethyldibenzofuran (2). White amorphous powder; UV (CH_3OH) 296.4; HR-FABMS data [*m*/*z* 258.0894 $(M)^{+}$, calcd for $C_{15}H_{14}O_4$ 258.0892, △ -0.2 mmu]. For ^{1}H and ^{13}C NMR data, see Table 1; NOESY correlations (H↔H#) H-1↔H_3-10, H-1↔H_3-11.

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