New Dibenzofurans from the Branches of *Distylium racemosum* Sieb. et Zucc

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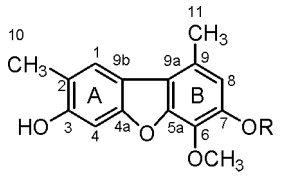
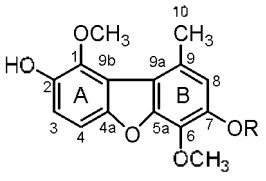
Distylium genus which belongs to the family Hamamelidaceae comprises of about 18 species of evergreen shrubs and trees, native to eastern and southeastern Asia. As a part of our study to find biologically active compounds from Jeju Island plants,[1] we previously reported the isolation of two new dibenzofurans, **3** and **4** from the branches of *Distylium racemosum* Sieb. et Zucc.[2] *D. racemosum* is an evergreen tree distributed over Halla Mountain in Jeju Island, Korea.[3] Continuous studies on the organic extract of this species has led to the isolation of two additional new dibenzofurans, 7-[(β-D-glucopyranosyl)oxy]-3-hydroxy-6-methoxy-2,9-dimethyldibenzofuran (**1**) and 2-hydroxy-1,6,7-trimethoxy-9-methyldibenzofuran (**2**) along with two known compounds, abscisic acid (**5**) and 6''-*O*-galloylsalidroside (**6**). Herein, we report the structure determination and tyrosinase inhibitory activities of the isolated compounds.

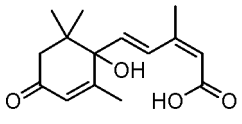
The 70% EtOH extract of the dried branches of *D. racemosum* was partitioned successively into hexane, ethyl acetate and *n*-butanol. The ethyl acetate soluble fraction was subjected to column chromatography over celite, followed by purification over reversed-phase silica gel and Sephadex LH-20 afforded two new compounds, **1** and **2**, along with the known compounds, **5** and **6**.



1 R = β–D-Glc
3 R = H



2 R = CH_3
4 R = H



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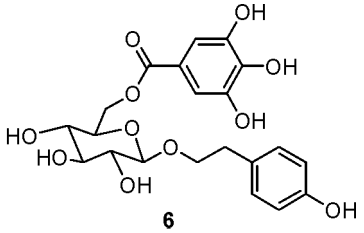

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Compound **1** was obtained as a light yellow amorphous powder. Its molecular formula was determined as $C_{21}H_{24}O_9$ (ten unsaturations) based on HR-FAB-MS, which showed a $[M+Na]^+$ peak at *m/z* 443.1317 (calcd *m/z* 443.1318). The UV absorption maximum at 289 nm suggested the presence of conjugated double bonds or aromatic system(s). The ^{1}H NMR spectrum (Table 1) of **1** showed signals corresponding to two methyls (δ 2.65 and 2.31), one methoxy (δ 4.07) and three aromatic protons (δ 7.65, 7.03 and 6.91), as well as signals assignable to a sugar moiety [δ 5.01 (1H, d, J = 7.3 Hz) and 3.40-4.00 (6H)]. The ^{13}C NMR spectrum showed signals for all the 21 carbons including 12 aromatic, two methyl, one methoxy and six oxygen-bearing sugar carbons. Since two aromatic rings and a sugar ring accounted for unsaturation number of nine, compound **1** is inferred to have additional ring. Therefore, it was assumed that **1** has same dibenzofuran skeleton as of **3**, identified in our previous study.[2]

The position of the substituents in the dibenzofuran nucleus was established by 1D and 2D (HMBC and NOESY) NMR data. As shown in Table 1, three aromatic protons were all appeared as singlets. Considering the ^{1}H-^{1}H coupling criteria, it is not possible to arrange three singlet aromatic protons in the same ring in a dibenzofuran compound. Therefore, singlet protons at δ 7.65 (H-1) and 6.91 (H-4) should be placed in ring A in *para* position to each other, because both singlets showed HMBC correlations with C-4a in ring A (Figure 1). A methyl group (C-10) was placed at C-2, because its proton (H-10) showed HMBC cross peaks with C-1, C-2 and C-3. The NOESY cross peaks of H-1 was observed with H_3-10 (δ 2.31) and H_3-11 (δ 2.65), which suggested that H-1 is placed between two methyl groups. Therefore, we can assign the connection of the methyl (C-11) to C-9 in the ring B. A sharp singlet at δ 4.07 (3H) was ascribed to a methoxy group and was placed at C-6 as it showed ^3J correlation with this carbon (134.5 ppm) in its HMBC spectrum. The methyl proton (H-11) in the ring B showed a NOESY peak with H-8 aromatic proton, which in turn showed NOESY correlation with anomeric proton (H-1') of a sugar moiety, thereby suggesting the sugar residue is placed at C-7 position. This was further confirmed by HMBC correlation observed between H-1' and C-7. The ^{1}H and ^{13}C NMR signal showed that the sugar unit is a hexose. The observed chemical shifts and ^{1}H-^{1}H coupling constants indicated that the hexose is glucose. The coupling constant (J = 7.5 Hz) of the anomeric proton revealed that the glucose is in β-configuration. Based on the above spectral studies, compound **1** was characterized as 7-[(β-D-glucopyranosyl)oxy]-3-hydroxy-6-methoxy-2,9-dimethyldibenzofuran, a glycosylated derivative of **3**.

Table 1. 1D and 2D NMR data for compounds **1** and **2** with CD_3OD

Compound	**1**[a]			**2**[b]		
no	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	123.8	7.65 (1H, s)	C-3, C-4a	142.8		
2	122.1			147.4		
3	156.4			116.3	6.96 (1H, d, 8.8)	C-1, C-4
4	98.5	6.91 (1H, s)	C-4a	107.7	7.15 (1H, d, 8.8)	C-3, C-4a, C-9b
4a	157.7			152.5		
5a	149.4			151.1		
6	134.5			134.4		
7	148.7			152.4		
8	115.8	7.03 (1H, s)	C-6, C-7	111.8	6.82 (1H, s)	C-6, C-7, C-10
9	127.6			129.8		
9a	121.5			119.7		
9b	117.7			119.2		
10	16.1	2.31 (3H, s)	C-1, C-2, C-3	22.2	2.85 (3H, s)	C-8, C-9, C-9a
11	19.7	2.65 (3H, s)	C-8, C-9, C-9a			
1-OCH_3	-			61.6	3.91 (3H, s)	C-1
6-OCH_3	62.1	4.07 (3H, s)	C-6	61.7	3.98 (3H, s)	C-6
7-OCH_3	-			57.4	3.92 (3H, s)	C-7
1'	104.2	5.01 (1H, d, 7.5)	C-7			
2'	75.3	3.53 (dd, 7.5, 7.5)				
3'	78.4	3.40 - 3.42 (m)				
4'	71.6	3.40 - 3.42 (m)				
5'	78.2	3.48 (ddd, 8.8, 3.2, 2.9)				
6'	62.7	4.00 (dd, 12.4, 2.9) 3.82 (dd, 12.4, 3.2)				

[a]Recorded on 400 MHz (for ^1H) NMR. [b]Recorded on 500 MHz (for ^1H) NMR

Compound **2**, isolated as white amorphous powder, showed a peak at *m/z* 288.1092 $(M)^+$ in its HR-FAB mass spectrum corresponding to the molecular formula $C_{16}H_{16}O_5$. Inspection of ^1H and ^{13}C NMR spectra of **2** revealed that it has also dibenzofuran nucleus and its chemical shifts in aromatic rings are very similar to those of the compound **4**, isolated in our previous study.[2] However, some difference was found in the substituents of dinezofuran between **2** and **4**. The compound **2** showed one methyl and three methoxy groups, whereas **4** showed one methyl, two methoxy and two hydroxy groups. This strongly implied that one hydroxy group in **4** is methylated to produce trimethoxy dibenzofuran analogue **2**. The location of methoxy groups was determined by HMBC study. Among the three methoxy group, the one at δ 3.91 was placed at C-1, based on its HMBC correlation with this carbon (142.8 ppm), further confirmed by NOESY correlation with methyl proton (δ 2.85, H_3-10) of ring B. Similarly, the other two methoxy groups at δ 3.98 and 3.92 were placed at C-6 and C-7 because they showed HMBC cross peaks with the corresponding carbons, respectively. Taken these data together, compound **2** was identified as 2-hydroxy-1,6,7-trimethoxy-9-methyldibenzofuran, where -OH at C-7 of **4** is methylated to -OCH_3. The comparison of the spectroscopic data for **1**-**4** also corroborated the proposed structures.

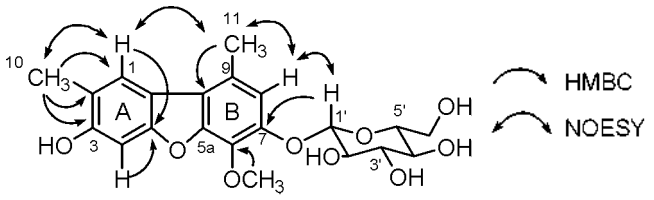

Figure 1. Key HMBC and NOESY correlations in compound **1**.

The known compounds isolated in this study were identified as abscisic acid (**5**)[4] and 6''-*O*-galloylsalidroside (**6**) by comparison of their spectral data with published values. Abscisic aicd is an abscission-accelerating plant hormone isolated from young fruit of cotton.[5] Phenol glucoside **6** has been isolated for the first time from *Quercus stenophylla*.[6] Interestingly, these two compounds were isolated for the first time from *D. racemosum*.

The isolated compounds, **1**-**6** were examined for their tyrosinase inhibition activities.[7] For the synthesis of melanin in human melanocytes, tyrosinase is the key enzyme mediating oxidation of L-tyrosine to DOPA and further to DOPA chrome. Therefore, tyrosinase inhibitors have attracted attention as cosmeceutical ingredients to control overproduction of melanin in the skin epidermis. Among the compounds, dibenzofurans **1** (IC_{50} 122.1 μg/mL) and **3** (IC_{50} 76.4 μg/mL) showed moderate activities compared to arbutin (IC_{50} 48.7 μg/mL), a commercial whitening ingredient used in cosmetic formulation. The other isolated compounds (**2**, **4**-**6**) exhibited very weak activities (IC_{50} > 300 μg/mL).

Experimental Section

Reagent and equipment. Solvents for extraction and open column chromatography were of reagent grade and used

without further purification. Optical rotation was recorded using a Jasco P-1030 polarimeter. UV absorptions were measured in MeOH on a Biochrom Libra S22 UV-visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-LA 400 (JEOL) or Ultrashield Plus 500 (Bruker) instrument, with chemical shift values (δ) reported in ppm relative to the solvent used. Flash column chromatography (CC) was carried out using Merck silica gel 60 (230-400 mesh). Sephadex LH-20 (25-100 μm) for Gel filtration chromatography (GFC) was obtained from Fluka. Thin layer chromatography was performed on Merck prepared plates (silica gel 60 F-254 on aluminium). High resolution Mass data were obtained from Korea Basic Science Institute (Seoul).

Plant material. The branches of *D. racemosum* were collected from Halla Botanical Garden, Jeju Island. A voucher specimen (J-242) is deposited in the Laboratory of Natural Products, Department of chemistry, Cheju National University, Jeju, Korea.

Extraction and isolation. Shade-dried and powdered branches of *D. racemosum* (2.5 kg) were extracted with 70% EtOH at room temperature for 24 hr. The oily extract (270 g) was suspended in H_2O and then successively partitioned with hexane, ethyl acetate and *n*-butanol. The EtOAc soluble fraction (45 g) was chromatographed over celite with successive elution of hexane, CH_2Cl_2, $CHCl_3$, Et_2O, EtOAc and MeOH. Reversed-phase C_{18} silica gel column chromatography of the Et_2O subfraction (DE fr) eluted with H_2O/MeOH system gave 12 fractions (DE-I to DE-XII). Fractions DE-V and DE-VI were combined and purified with Sephadex LH-20 CC using $CHCl_3$/MeOH (3/1 and 4/1) to afford compounds **6** (1.0 mg) and **5** (63.9 mg). Fraction DE-X was also purified by Sephadex LH-20 CC with $CHCl_3$/MeOH (8/1) to yield compound **2** (9.6 mg). The EtOAc fraction of the celite column (EA fr) was subjected to silica gel CC eluted with H_2O/MeOH gradient to give 15 fractions (EA-I to EA-XV). The fraction EA-V was chromatographed over Sephadex LH-20 using gradient $CHCl_3$/H_2O/MeOH to yield seven subfractions (EA-V-1 to EA-V-7). Fraction EA-V-1 was subjected to Sephadex LH 20 CC followed by silica gel CC with Hex/EtOAc/MeOH (10/5/8) afforded **1** (4.4 mg).

7-[(β-D-Glucopyranosyl)oxy]-3-hydroxy-6-methoxy-2,9-dimethyldibenzofuran (1). Light yellow amorphous powder; UV (CH_3OH) 289 nm; $[\alpha]_D^{20}$ = -16.0 (*c* 0.0021, MeOH); 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, H_3-11↔H-8, H-8↔H-1'; HR-FABMS: *m/z* 443.1317 $[M+Na]^-$ (calcd for $C_{21}H_{24}O_9Na$ 443.1318, △ 0.1 mmu).

2-Hydroxy-1,6,7-trimethoxy-9-methyldibenzofuran (2). White amorphous powder; UV (CH_3OH) 289 nm; For ^{1}H and ^{13}C NMR data, see Table 1; NOESY correlations (H#↔H#) 1-OCH_3↔H_3-10; HR-FABMS: *m/z* 288.1029 $(M)^+$ (calcd for $C_{16}H_{16}O_5$ 288.0998, △ -3.1 mmu).

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