Natural Product Sciences
19(3) : 215-220 (2013)

Phenolic Compounds from *Desmodium caudatum*

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Abstract – Three *C*-glucosyl flavones (**1 - 3**), one xanthone (**4**), and four flavanonols (**5 - 8**) were isolated by various chromatographic methods from the leaves and stems of *Desmodium caudatum* (Thunb.) DC. Chemical structures of these compounds were elucidated by 1D and 2D NMR and mass spectroscopy. The compounds were identified as swertisin (**1**), spinosin (**2**), 7-methyl-apigenin-6-*C*-*β*-glucopyranosyl-2"-*O*-*β*-D-xylopyranoside (**3**), 1,3,5,6-tetrahydroxyxanthone (**4**), yokovanol (**5**), aromadendrin (**6**), 2'-hydroxy yokovanol (**7**), and 2'-hydroxy neophellamuretin (**8**). Compounds **2 - 4** were first isolated from *D. caudatum*, as well as the spectroscopic data for compound **3**.

Keywords – *Desmodium caudatum*, Leguminosae, *C*-glucosyl flavone, Xanthone, Flavanonol

Introduction

Desmodium caudatum (Thunb.) DC is a small tree of the Leguminosae distributed in China, India, and South Korea. Its leaves and stems have been used as an insecticide (Aritomi and Kawasaki, 1968). According to ancient Chinese literature, the whole plant is used for treatment of febrile diseases, rheumatic arthritis, and bacillary dysentery in folk medicine (Ma *et al*., 2011). Earlier studies on *D. caudatum* revealed alkaloids and flavonoids has been isolated from the leaves, whereas alkaloids and flavonoids have been isolated from roots and stems (Aritomi and Kawasaki, 1968; Ueno *et al*., 1978; Sasaki *et al*., 2012). However, the phytochemical study of *C*-glucosyl flavone from *D. caudatum* has not been determined in depth. In this study, Three *C*-glucosyl flavones (**1 - 3**), one xanthone (**4**), and four flavanonols (**5 - 8**) were isolated by various chromatographic methods from the leaves and stems of *D. caudatum*.

Experimental

General experimental procedures – Optical rotations were determined using a Jasco DIP-370 automatic polarimeter. The FTIR spectra were obtained using a Jasco Report-100 infrared spectrometer. The NMR spectra were recorded using a JEOL ECA 600 spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz), and the ESI-MS using an Aglient 1200 LC-MSD Trap spectrometer. Column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230, and 230 - 400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins, and thin layer chromatography (TLC) was performed using pre-coated silica-gel 60 F_{254} and RP-18 F_{254}S plates (both 0.25 mm, Merck, Darmstadt, Germany).

Plant material – The leaves and stems of *D. caudatum* were collected in Jeju, Korea, in August 2010 and identified by Professor Young Ho Kim. A voucher specimen (CNU 10107) was deposited at the Herbarium of College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation – Dried leaves and stems of plant (1.0 kg) were extracted with MeOH under reflux for 9 h (5 L × 3 times). The MeOH extract (83.0 g) was concentrated under vacuum to give a gummy residue, which was then suspended in H_2O (800 mL). This solution was extracted with *n*-hexane and *n*-BuOH to give 26.0 g of *n*-hexane soluble fraction, 10.5 g of *n*-BuOH soluble fraction and 46.0 g of H_2O soluble fraction. The *n*-hexane extract 26.0 g was subjected to silica gel column chromatography with a stepwise gradient of CH_2Cl_2 and MeOH to give 5 fractions (Fr. 1A–1E). Fraction 1C was separated using a RP C_{18} column with a MeOH–H_2O (2 : 1, 1.5 L) elution solvent to give compound **7** (48.0 mg). Fraction 1D was separated using a RP C_{18} column with a MeOH–H_2O (2.2 : 1, 1 L) elution solvent to give compound **8** (21.0 mg). Fraction 1E was separated using a reversed-phase (RP) C_{18} column with MeOH–H_2O (1.5 : 1 → 2 : 1 → 2.3 : 1) elution solvent to give compound **4**

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Table 1. The NMR spectroscopic data of compounds **1 - 3**

Pos.	**1**		**2**		**3**	
	δ_C[a,c]	δ_H[a,d] (*J* in Hz)	δ_C[a,c]	δ_H[a,d] (*J* in Hz)	δ_C[b,c]	δ_H[b,d] (*J* in Hz)
2	164.2 164.3	–	164.2 164.3	–	164.5 165.1	–
3	103.5	6.84, 6.86 (1H, s)	103.5 103.6	6.72, 6.74 (1H, s)	102.4 102.5	6.48, 6.51 (1H, s)
4	182.4 182.8	–	182.5 182.8	–	182.4 182.7	–
5	160.1 160.8	–	160.2 161.0	–	160.7 161.1	–
6	110.1 110.2	–	109.0 109.1	–	108.5 108.7	–
7	164.4 165.4	–	164.4 165.6	–	165.2 165.7	–
8	90.7 91.5	6.82 6.83 (1H, s)	90.8 91.3	6.85 6.89 (1H, s)	90.0 90.2	6.57 (1H, s)
9	157.2 157.3	–	157.5 157.6	–	157.8 157.9	–
10	104.6 105.1	–	104.7 105.0	–	104.4 104.5	–
1'	121.3	–	121.5	–	120.9	–
2'	129.0	7.97 (1H, d, 8.5)	129.0	7.86 (1H, d, 8.5)	128.1 128.2	7.72, 7.74 (1H, d, 8.5)
3'	116.5	6.92 (1H, d, 8.5)	116.5	6.82 (1H, d, 8.5)	116.1 116.2	6.78, 6.80 (1H, d, 8.5)
4'	161.9	–	161.8	–	162.6 162.9	–
5'	116.5	6.92 (1H, d, 8.5)	116.5	6.82 (1H, d, 8.5)	116.1 116.2	6.78, 6.80 (1H, d, 8.5)
6'	129.0	7.97 (1H, d, 8.5)	129.0	7.86 (1H, d, 8.5)	128.1 128.2	7.72, 7.74 (1H, d, 8.5)
1"	73.1 73.3	4.55, 4.57 (1H, d, 10)	71.2 71.5	4.55, 4.56 (1H, d, 10)	71.4 71.9	4.80, 4.85 (1H, d, 9.7)
2"	71.3 71.4	3.03 (1H, m)	80.3 80.5	4.16, 4.30 (1H, m)	80.3 80.5	4.34, 4.52 (1H, t, 9.0)
3"	79.6	3.13 (1H, m)	78.8 79.2	3.29 (1H, m)	78.6 78.9	3.62 (1H, m)
4"	70.1 70.7	3.95, 4.13(1H, m)	70.9	3.01 (1H, m)	70.3 70.6	3.37, 3.46 (1H, m)
5"	82.2	3.10 (1H, m)	82.1	3.03 (1H, m)	81.2	3.35 (1H, m)
6"	62.2	3.31, 3.66 (2H, m)	61.9	3.22, 3.56 (2H, m)	61.7 61.9	3.64, 3.85 (2H, m)
1"'	–	–	105.8 106.0	4.04, 4.05 (1H, d, 8.5)	105.3 105.7	4.19, 4.23 (1H, d, 6.9)
2"'	–	–	75.0 75.2	2.72 (1H, m)	74.1 74.3	3.02 (1H, m)
3"'	–	–	77.0 77.2	2.91 (1H, m)	76.2 76.3	3.15 (1H, m)
4"'	–	–	69.6 69.9	2.90 (1H, m)	69.5	3.17 (1H, m)
5"'	–	–	76.8 76.9	2.91 (1H, m)	65.5	3.24, 3.38 (2H, m)
6"'	–	–	61.1	3.06 (2H, m)	–	–
7-OMe	56.8 57.0	3.85, 3.88 (3H, s)	56.5 57.0	3.77, 3.78 (3H, s)	55.3 55.6	3.79, 3.82 (3H, s)

Assignments were done by HMQC, HMBC, and ^{1}H-^{1}H COSY experiments; [a]Measured in DMSO-d_6; [b]Measured in methanol-d_4; [c]150 MHz; [d]600 MHz

(16.0 mg), **5** (25.0 mg) and **6** (38.0 mg). The *n*-BuOH extract was subjected to silica gel column chromatography with a $CHCl_3$–MeOH–H_2O (10 : 1 : 0.1 → 7 : 1 : 0.1 → 4 : 1 : 0.1 → 2 : 1 : 0.1 → 1 : 1 : 0.2) elution solvent to give 5 fractions (Fr. 2A–2E). Fraction 2B was separated using a RP C_{18} column with acetone-H_2O (0.4 : 1) elution solvent to give compound **1** (55.0 mg). Fraction 2C was separated using a RP C_{18} column with a MeOH–H_2O (0.45 : 1) elution solvent to give compound **3** (38.0 mg). Fraction 2E was separated using a silica gel column chromatography with a $CHCl_3$–MeOH–H_2O (3 : 1 : 0.12) elution solvent to give compound **2** (46.0 mg).

Swertisin (1): White amorphous powder; UV (MeOH): λ_{max} 274, 329 nm; ESI-MS: *m/z* 445 $[M-H]^-$; ^1H and ^{13}C NMR (see Table 1).

Spinosin (2): Yellow amorphous powder; UV (MeOH): λ_{max} 270, 334 nm; ESI-MS: *m/z* 607 $[M-H]^-$; ^1H and ^{13}C NMR (see Table 1).

7-Methyl-apigenin-6-*C*-*β*-glucopyranosyl-2''-*O*-*β*-D-xylopyranoside (3): White amorphous powder; mp 221 - 223 °C; α_D^{28} = –27.6 (*c* 0.25, MeOH); UV (MeOH): λ_{max} 270, 334 nm; IR (KBr): ν_{max} 3372, 1650, 1605 cm^{-1}; ESI-MS: *m/z* 577.3 $[M-H]^-$; ^1H and ^{13}C NMR (see Table 1).

1,3,5,6-Tetrahydroxyxanthone (4): Yellow amorphous powder; mp 235 - 238 °C; UV (MeOH): λ_{max} (log ε) 248 (4.25), 286 (3.65), 317 (3.61) nm; EI-MS, *m/z* 260 $[M]^+$. ^1H NMR (600 MHz, methanol-d_4) δ: 6.39 (1H, d , *J* = 2.0 Hz, H-2), 6.15 (1H, d , *J* = 2.0 Hz, H-4), 6.84 (1H, d , *J* = 9.2 Hz, H-7), 7.53 (1H, d , *J* = 9.2 Hz, H-8); ^{13}C NMR (150 MHz, methanol-d_4): 159.3 (C-1), 95.1 (C-2), 166.7 (C-3), 98.9 (C-4), 164.7 (C-4a), 147.6 (C-4b), 133.6 (C-5). 152.9 (C-6), 113.7 (C-7), 117.5 (C-8), 114.9 (C-8a), 103.1 (C-8b), 181.7 (C = O).

Yokovanol (5): White amorphous powder; ESI-MS: *m/z* 361 $[M-H]^-$; ^1H NMR (600 MHz, methanol-d_4) δ: 4.91 (1H, d , *J* = 8.2 Hz, H-2), 4.62 (1H, d , *J* = 8.2 Hz, H-3), 5.81 (1H, s, H-6), 7.31 (1H, d , *J* = 8.5 Hz, H-2', 6'), 6.74 (1H, d , *J* = 8.5 Hz, H-3'), 6.73 (1H, d , *J* = 8.5 Hz, H-5'), 6.32 (1H, d , *J* = 10.5 Hz, H-1"), 5.41 (1H, d , *J* = 10.5 Hz, H-2"), 1.29 (1H, s, H-4"), 1.31 (1H, s, H-5"); ^{13}C NMR (150 MHz, methanol-d_4): 83.7 (C-2), 72.2 (C-3), 197.7 (C-4), 163.3 (C-5), 96.9 (C-6), 162.3 (C-7), 101.9 (C-8), 156.8 (C-9), 101.1 (C-10), 127.7 (C-1'), 129.0 (C-2', 6'), 114.9 (C-3', 5'), 157.9 (C-4'), 114.8 (C-1"), 126.5 (C-2"), 78.0 (C-3"), 27.1 (C-4"), 27.3 (C-5").

Aromadendrin (6): White amorphous powder; ESI-MS: *m/z* 287 $[M-H]^-$; ^1H NMR (600 MHz, methanol-d_4) δ: 4.88 (1H, d , *J* = 8.5 Hz, H-2), 4.43 (1H, d , *J* = 8.5 Hz, H-3), 5.82 (1H, d , *J* = 1.8 Hz H-6), 5.83 (1H, d , *J* = 1.8 Hz H-8), 7.25 (1H, d , *J* = 8.5 Hz, H-2', 6'), 6.73 (1H, d, *J* = 8.5 Hz, H-3', 5'); ^{13}C NMR (150 MHz, methanol-d_4): 83.6 (C-2), 72.3 (C-3), 197.1 (C-4), 163.9 (C-5), 96.0 (C-6), 167.5 (C-7), 95.0 (C-8), 163.1 (C-9), 100.4 (C-10), 127.9 (C-1'), 129.0 (C-2', 6'), 114.7 (C-3', 5'), 157.9 (C-4').

2'-Hydroxy yokovanol (7): Yellow granules; mp 185 - 192 °C; α_D^{28} = +54.0 (MeOH, c 0.25); HR-ESI-MS: *m/z* 369.0996 $[M-H]^-$ (calcd for $C_{20}H_{17}O_7$: 369.0980); ^1H NMR (600 MHz, methanol-d_4) δ: 5.34 (1H, d, *J* = 11.2 Hz, H-2), 4.72 (1H, d, *J* = 11.2 Hz, H-3), 5.82 (1H, s, H-6), 6.26 (1H, d, *J* = 1.8 Hz, H-3'), 6.27 (1H, dd, *J* = 8.1, 1.8 Hz, H-5'), 7.14(1H, d, *J* = 8.1 Hz, H-6'), 6.36 (1H, d, *J* =8.0 Hz, H-1"), 5.44 (1H, d, *J* = 8.0 Hz, H-2"), 1.32 (3H, s, H-4"), 1.32 (3H, s, H-5"); ^{13}C NMR (150 MHz, methanol-d_4): 78.8 (C-2), 71.1 (C-3), 198.0 (C-4), 163.3 (C-5), 96.4 (C-6), 162.2 (C-7), 101.8 (C-8), 157.3 (C-9), 101.1 (C-10), 114.0 (C-1'), 157.3 (C-2'), 102.3 (C-3'), 158.8 (C-4'), 106.5 (C-5'), 129.4 (C-6'), 115.0 (C-1"), 126.2 (C-2"), 77.9 (C-3"), 27.3 (C-4"), 27.4 (C-5").

2'-Hydroxy neophellamuretin (8): Yellow granules; mp 217 - 225 °C; α_D^{28} = +15.0 (MeOH, c 0.25); HR-ESI-MS: *m/z* 371.1127 $[M-H]^-$ (calcd for $C_{20}H_{19}O_7$: 371.1131); ^1H NMR (600 MHz, methanol-d_4) δ: 5.40 (1H, d, *J* = 11.2 Hz, H-2), 4.76 (1H, d, *J* =11.2 Hz, H-3), 5.96 (1H, s, H-6), 6.37 (1H, d, *J* = 2.1 Hz, H-3'), 6.36 (1H, dd, *J* = 8.1, 2.1 Hz, H-5'), 7.24 (1H, d, *J* = 8.1 Hz, H-6'), 3.13 (2H, d, *J* =6.8 Hz, H-1"), 5.14 (1H, t, *J* = 6.8 Hz, H-2"), 1.61 (3H, s, H-4"), 1.54 (3H, s, H-5"); ^{13}C NMR (150 MHz, methanol-d_4): 79.8 (C-2), 72.4 (C-3), 199.3 (C-4), 163.0 (C-5), 96.4 (C-6), 166.2 (C-7), 109.2 (C-8), 161.8 (C-9), 102.0 (C-10), 115.9 (C-1'), 158.7 (C-2'), 103.7 (C-3'), 160.0 (C-4'), 107.8 (C-5'), 130.6 (C-6'), 22.4 (C-1"), 123.7 (C-2"), 131.8 (C-3"), 26.1 (C-4"), 17.8 (C-5").

Results and Discussion

The methanol extract of *D. caudatum* was subjected to various separation procedures and 8 phenolic compounds were isolated (Fig. 1). Their structures were elucidated by comparing spectroscopic data to published values. Compounds **1** - **8** were identified as swertisin (**1**) (Joseph *et al*., 1988), spinosin (**2**; Wu *et al*., 2011), 7-methyl-apigenin-6-*C*-*β*-glucopyranosyl-2"-*O*-*β*-D-xylopyranoside (**3**), 1,3,5,6-tetrahydroxyxanthone (**4**; Nielsen and Arends, 1979), yokovanol (**5**; Ito *et al*., 1989), aromadendrin (**6**; Manez *et al*., 1988),), 2'-hydroxy yokovanol (**7**; Sasaki *et al*., 2012), and 2'-hydroxy neophellamuretin (**8**; Sasaki *et al*., 2012).

Compound **3** was obtained as a white amorphous powder, α_D^{28} = –27.6 (*c* 0.25, MeOH), and its basic ion peak at *m/z* 577.3 $[M-H]^-$, observed by negative ion

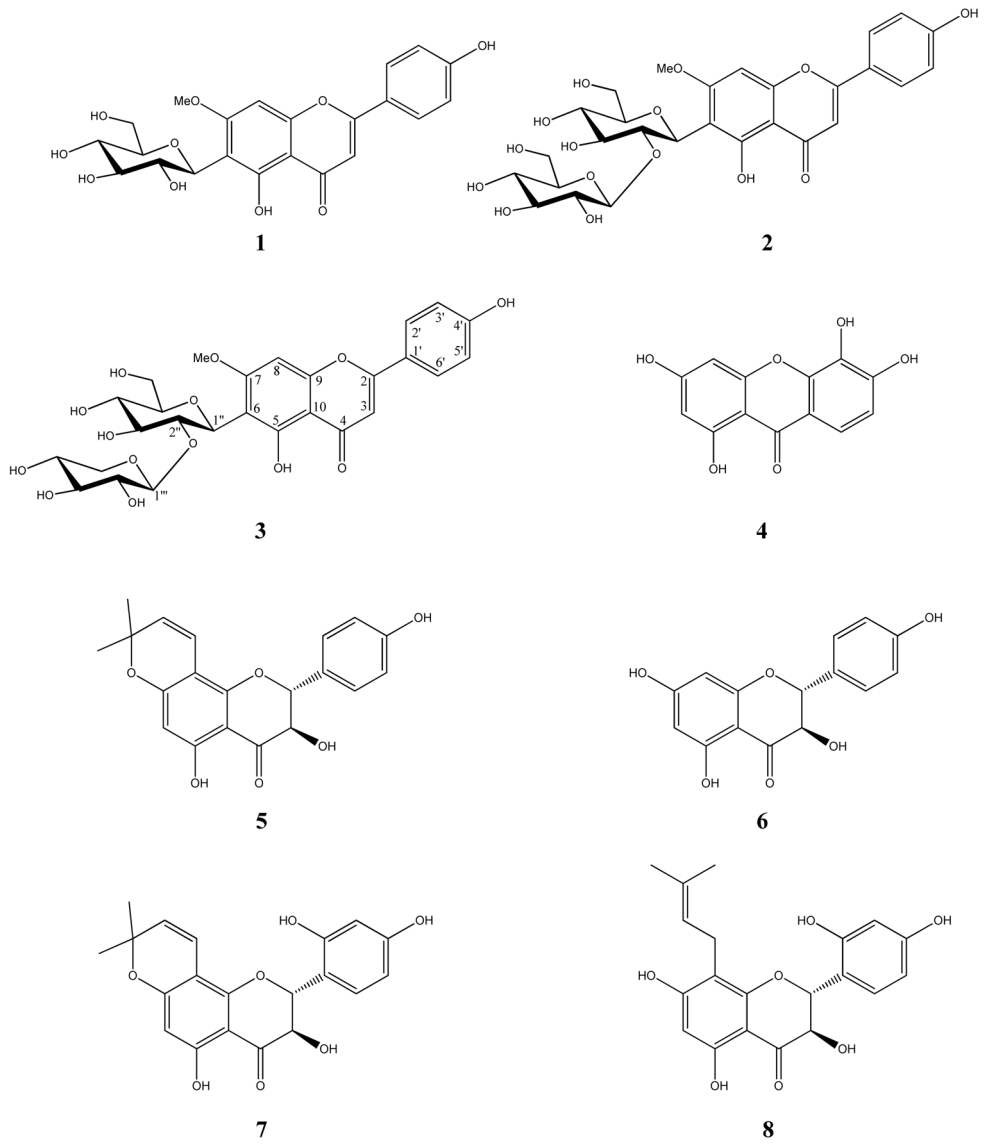

Fig. 1 Structures of compounds **1 - 8** from leaves and stems of *D. caudatum.*

electrospray ionization mass spectrometry (ESI-MS), revealed its molecular formula, $C_{27}H_{30}O_{14}$. Its infrared (IR) spectrum contained absorption bands of a hydroxyl group at 3372 cm^{-1}, a carbonyl group at 1650 cm^{-1} and a phenyl group at 1605 cm^{-1}. The ultraviolet (UV) absorption maxima at λ_{max} 270 and 334 nm suggested that compound **3** is a flavone derivative (Wallace and Mabry, 2010). ^1H and ^{13}C NMR, and distortionless enhancement by polarization transfer (DEPT) spectra of compound **3**, suggested the presence of an apigenin derivative and two glycosyl moieties. The ^1H NMR spectrum of compound **3** (in methanol-d_4) showed signals for six aromatic protons at δ_H 6.48/6.51 (s), 6.57 (s), 6.78/6.80 (d, J = 8.5 Hz, 2H), and 7.72/7.74 (d, J = 8.5 Hz, 2H), along with a methoxyl group at δ_H 3.79, 3.82 (s). Among them, the appearance of four aromatic proton signals which were observed at δ_H 6.78/6.80 (d, J = 8.5Hz, H-3', 5') and 7.72/7.74 (d, J = 8.5Hz, H-2', 6') with a characteristic pattern of an A_2B_2 system confirmed the disubstitution of a B-ring at the 1' and 4'- positions. Proton signals at δ_H 6.48/6.51 (1H, s) and 6.57 (1H, s) were assigned to H-3 and H-8 by HMQC and HMBC spectra. ^{13}C NMR and DEPT spectra revealed 27 carbon signals including 11 sugar carbons (Table 1). Characteristic signals at δ_C 102.4/102.5 and 90.0/90.2 were assigned to C-3 and C-8, while δ_C 116.1/116.2 and 128.1/128.2 were assigned to C-3', 5' and C-2', 6' of a B-ring by HMQC and HMBC spectra, and by comparison with reported data. The residual methoxyl group (δ_H 3.79/

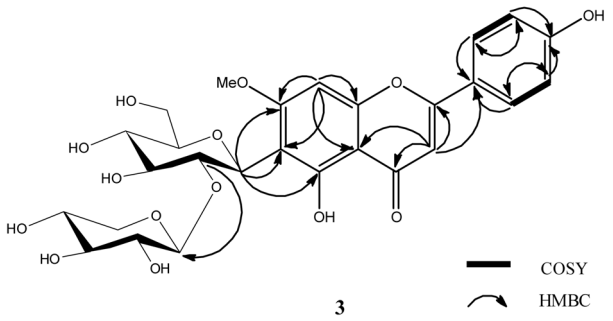

Fig. 2. ^{1}H-^{1}H COSY and HMBC correlations of compound **3**.

3.82) was located at the 7-position of apigenin, which was based on the appearance of HMBC correlations for methoxyl group (δ_H 3.79/3.82) and C-7 (δ_C 165.2/165.7). Therefore, the flavone moiety of compound **3** was determined as 7-methoxyl-apigenin (Fig. 1). In the ^{1}H NMR spectrum, two signals of anomeric protons were observed at δ_H 4.80/4.85 (1H, d, J = 9.7 Hz) and δ_H 4.19/4.23 (1H, d, J = 6.9 Hz), indicating the presence of two sugar moieties. A combination of ^{13}C NMR, COSY, HMQC, and HMBC data allowed the identification of the sugar moieties as glucopyranose and xylopyranose (Fig. 2). In addition, the ^{13}C NMR spectrum revealed that anomeric carbons of the glucopyranose and xylopyranose moieties at δ_C 71.4/71.9 and 105.3/105.7, corresponded to a *C*-linked glucopyranose and an *O*-linked xylopyranose. The configurations of the anomeric positions, glucopyranose and xylopyranose, were assigned as β and β, respectively, based on their large coupling constants between H-1 and H-2 of the sugar ring protons ($J_{1,2}$ values: glc, 9.7 Hz; xyl, 6.9 Hz). Connectivities of the sugar moiety were provided by the HMBC spectrum. HMBC correlations were observed from H-1" to C-5, C-6, and C-7, indicating that the flavone *C*-glycopyranoside moiety of compound **3** was 7-methyl-apigenin-6-*C*-β-glucopyranoside. The HMBC correlation was also observed for C-2"/H-1"', indicating that the xylopyranose moiety was attached to the C-2" hydroxyl of the glucopyranose. The D-configuration of the xylopyranose was determined using silica gel thin-layer chromatography (TLC) following acid hydrolysis, and comparison with those of authentic standards. Consequently, we concluded the structure of compound **3** to be 7-methyl-apigenin-6-*C*-β-glucopyranosyl-2"-*O*-β-D-xylopyranoside. We are the first to report the isolation of compound **3** from *D. caudatum*, as well as its spectroscopic data. Some signals in the ^{1}H- and ^{13}C-NMR spectra of compounds **1** - **3** were observed as pairs (Table 1). Duplication of these signals could be attributable to the presence of rotational conformers. Previously, the presence of rotational conformers was reported for flavone 6-*C* glycoside, which contained additional substituents such as a methoxyl or glucosyl group in C-5 and C-7. This phenomenon may have been observed due to the presence of rotational hindrance at the C (sp3)-C (sp2) glucosyl-flavone linkage in flavone 6-C-glucoside with substituents at C-7. Therefore, two conformers in compounds **1** - **3** may be produced by rotation hindrance by a methoxy group at C-7 and a hydroxyl group at C-5 (Ohtsuki *et al*., 2010).

In this study, the systematic investigation of the leaves and stems of *D. caudatum* led to eight isolated phenolic compounds. All compounds were uncommonly found in the genus *Desmodium*. Compounds **2** - **4** were isolated from this species for the first time. Additionally, compounds **3** and **4** have not been reported in any species of *Desmodium*, as well as the family Leguminosae. These results may be useful to complete the constituents of *D. caudatum*.

Acknowledgments

This study was supported by the Priority Research Center Program (2009-0093815) and Basic Science Research Program (2010-0023645) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, and the Ministry of Knowledge Economy (MKE), Korea Institute for Advancement of Technology (KIAT) through the Inter-ER Cooperation Projects (R0002019), Republic of Korea.

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Received March 17, 2013
Accepted May 8, 2013