New Isoflavone Glycoside from the Woods of *Sophora japonica*

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Sophora japonica L. (Leguminosae), commonly called Scholar Tree, is a well-known traditional medicine used for the treatment of bleeding and antihemorrhagic agent.[1] Many isoflavonoids and their glycosides have been reported from the root, bark and fruit of *S. japonica*.[2-5] The structures of isoflaflavones in the woods of *S. japonica* have less studied. In the study, the EtOH extract of the woods of *S. japonica* was separated by repeated column chromatography to give eight isoflavonoids, namely, 5,6',7-trihydroxy-3',4'-methylenedioxyisoflavone 6'-O-β-D-glycoside (**1**) and seven known compounds. These compounds were identified as, irisolidone, biochanin A, formononetin, dihydroformononetin, puerol A, biochanin A-7-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside and (-)-maackiain. Compound **1** is new isoflavone glycoside. Formononetin and dihydroformononetin were isolated from this plant for the first time. The known compounds were elucidated by comparison with authentic samples (MS, NMR) or with reported spectral data. The isolation and structural elucidation of the new compound **1** are described in the present study.

Compound **1** obtained from the CH_2Cl_2-soluble part of ethanol extract of the woods of *S. japonica* was isolated as yellow amorphous powder and showed the molecular ion peak at *m/z* 477 $[M+H]^+$ and another fragment at *m/z* 315 $[M-162+H]^+$ (loss of glucose) in the positive ion FAB mass spectrum (corresponding to a formula of $C_{22}H_{20}O_{12}$). The IR spectrum of compound **1** showed absorption bands at 3413 cm^{-1} (OH) and 1653 cm^{-1} (α,β-unsaturated carbonyl group, C=O).[6] The structure of compound **1** was identified by using ^1H-^1H COSY, DEFT, HMBC and HMQC experiments. Carbons having proton and their protons were precisely matched by the HMQC experiment. The proton signal at δ 8.10 ppm assignable to H-2 and carbon signal at δ 157.9 ppm (C-2) in the ^1H and ^{13}C NMR spectra (Table 1), respectively, were suggestive of an isoflavone type skeleton.[7] The HMBC correlations of proton signal at δ 8.10 ppm (H-2) to the carbon signals at δ 121.2 ppm (C-3), δ 182.3 ppm (C-4), δ 159.8 ppm (C-9), δ 114.9 ppm (C-1') showed that compound **1** is an isoflavone derivative (Fig. 1). The ^1H NMR spectrum of **1** showed signals for four aromatic protons. Two meta-coupled doublets at δ 6.21 ppm (J = 2.2 Hz) and δ 6.35 ppm (J = 2.2 Hz) could be assigned to H-6 and H-8, respectively and two singlets at δ 6.78 ppm and δ 6.94 ppm are attributed to H-2' and H-5', respectively. It was supported by ^{13}C NMR and DEPT spectra, which showed a total of 22 carbons consisting of two methylenes, ten methines and ten quaternary carbons. The ^1H and ^{13}C NMR spectra revealed one anomeric signal ($\delta_{H-1''}$ 4.79 ppm and $\delta_{C-1''}$ 103.9 ppm) together with five carbon signals in the region of sugar indicated compound **1** to be an isoflavone-O-glycoside.[8] The ^1H NMR signal at δ 5.96 ppm (2H, *s*), ^{13}C NMR signal at δ 103.0 ppm and IR absorption band at 932 cm^{-1}, revealed the presence of a methylenedioxy group in compound **1**.[9-11] The HMBC spectrum showed cross peaks between OCH_2O δ_H 5.96 ppm (H-7') and C-3' (δ 144.3 ppm) and C-4' (δ 149.9 ppm), respectively, determined the position

Table 1. NMR assignments for compound **1**

Atom	^1H	^{13}C	HMBC
2	8.10[a] (1H, *s*)	157.9	C-1', C-3, C-4, C-9
3	-	121.2	
4	-	182.3	
5	-	163.7	
6	6.21 (1H, *d*, 2.2[b])	100.2	C-5, C-8, C-10
7	-	166.0	
8	6.35 (1H, *d*, 2.2)	94.9	C-6, C-7, C-9, C-10
9	-	159.8	
10	-	106.4	
1'	-	114.9	
2'	6.78 (1H, *s*)	111.7	C-3, C-3', C-4', C-6'
3'	-	144.3	
4'	-	149.9	
5'	6.94 (1H, *s*)	100.8	C-1', C-3', C-4', C-6'
6'	-	151.8	
7'	5.96 (2H, *s*)	103.0	C-3', C-4'
1''	4.79 (1H, *d*, 8.0)	103.9	C-6'
2''	3.27 (1H, *m*)	74.8	
3''	3.37 (1H, *m*)	78.2	
4''	3.35 (1H, *m*)	78.0	
5''	3.30 (1H, *m*)	71.3	
6''	3.64 (1H, *dd*, 5.5, 12.0) 3.84 (1H, *dd*, 2.0, 12.0)	62.6	

[a]δ in ppm from TMS. [b]coupling constants (J) in Hz are given in parentheses.

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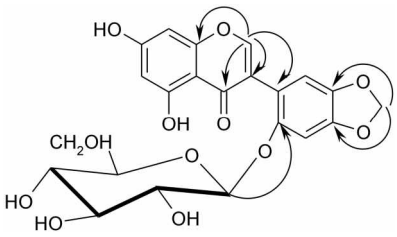

Figure 1. Selected HMBC correlations of compound **1**.

of methylenedioxy group to be between C-3' and C-4' (Fig. 1). The anomeric proton of the glucose residue at δ_H 4.79 ppm with a large coupling constant of 8.0 Hz implied that the glucose moiety must have a β-glucopyranose form. A cross peak in the HMBC spectrum between H-1'' (δ_H 4.79) of the glucose unit with C-6' (δ_C 151.8) of the isoflavone moiety indicated that the glucose residue was attached to the 6'-hydroxyl of the isoflavone moiety. Acid hydrolysis of a small amount of compound **1** afforded a substance identified by spectroscopic methods as 5,6',7-trihydroxy-3',4'-methylenedioxyisoflavone. From the above evidence, the structure of compound **1** was concluded to be 5,6',7-trihydroxy-3',4'-methylenedioxyisoflavone 6'-O-β-D-glycoside and this is the first report of this compound in the literature.

Experimental Section

General methods. MPs: uncorr. IR: KBr disc. ^{1}H and ^{13}C NMR in MeOH-*d4* with TMS as int. standard. NMR spectra were obtained using a Varian UI 500 spectrometer at the operating frequency of 500 MHz (^{1}H) and 125 MHz (^{13}C) at Korea Basic Science Institute in Seoul. FAB-MS (thioglycerol + NaI matrix); EI-MS: JEOL JMS-600W, direct inlet at 70 eV.

Plant material. The woods of *S. japonica* were collected at Jiri mountain, Kyungnam, the southern part of South Korea during June, 1996, and were identified by Dr. Y. H. Kwon (National Arboretum, Korea). The voucher specimens were deposited at the Korea Forest Research Institute, Seoul, Korea.

Extraction and isolation. Air-dried and powdered woods of *S. japonica* were extracted with EtOH at room temperature for 72 h. The EtOH extract was partitioned between water and *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 soluble fraction (51.55 g) was subjected to column chromatography on Sehpadex LH-20 eluted with MeOH-EtOH (1 : 1, v/v) to yield 9 sets of fraction (SJD1-SJD9). Fraction SJD5 (29.17 g) was rechromatographed on silica gel column with CH_2Cl_2-MeOH (150 : 1-50 : 1, v/v) to give 17 subfractions (SJD5-1-SJD5-17). Fraction SJD5-17 (4.65 g) was subjected to column chromatography on silica gel with benzene-MeOH (5 : 1, v/v) to give 8 fractions (SJD5-17-1-SJD5-17-8). Fraction SJD5-17-6 (256 mg) was rechromatographed on silica gel eluted with EtOAc-MeOH-H_2O (20 : 2 : 1, v/v/v) to give compound **1** (67.1 mg).

Acid hydrolysis. A solution of compound **1** in 10 mL of 5% HCl was heated for 2 h. A reaction mixture was extracted with EtOAc. The EtOAc soluble fraction (aglycone) and insoluble fraction (sugar) were concentrated and identified by spectral evidence.

5,6',7-trihydroxy-3',4'-methylenedioxyisoflavone 6'-O-β-D-glycoside. Yellow amorphous powder, mp 169 °C. IR (KBr) ν_{max} 3413, 1653, 1485, 1036, 932 cm^{-1}, EI-MS 70 eV *m/z* (rel. int.): 314 [M-glucose]$^-$ (100), 300 [M-glucose-OCH_2O]$^+$ (32), 162, FAB-MS: *m/z* 499 [M+Na]$^+$, 477 [M+H]$^-$, 315 [M-162+H]$^-$, ^{1}H, ^{13}C NMR and HMBC data are listed in Table 1.

5,6',7-trihydroxy-3',4'-methylenedioxyisoflavone. ^{1}H-NMR (500 MHz, MeOH-*d4*): 5.91 (2H, s, OCH_2O, H-7'), 6.25 (1H, *d*, *J* = 2.0 Hz, H-6), 6.38 (1H, *d*, *J* = 2.0 Hz, H-8), 6.50 (1H, *s*, H-2'), 6.74 (1H, *s*, H-5'), 8.05 (1H, *s*, H-2). ^{13}C-NMR (125 MHz, MeOH-*d4*): 93.7 (C-8), 98.2 (C-6), 99.1 (C-5'), 101.4 (C-OCH_2O, C-7'), 105.1 (C-10), 109.7 (C-2'), 110.2 (C-1'), 121.0 (C-3), 141.1 (C-3'), 148.8 (C-4'), 150.7 (C-6'), 156.1 (C-2), 158.6 (C-9), 162.5 (C-5), 164.9 (C-7), 181.2 (C-4).

References

1. Ishida, H.; Umino, T.; Tsuji, K.; Kosuge, T. *Chem. Pharm. Bull.* **1987**, *35*, 857-860.
2. Kimura, M.; Hiromi, Y. *Yakugaku Zasshi* **1984**, *104*, 340-346.
3. Komatsu, K.; Yokae, I.; Shirakaki, Y. *Yakugaku Zasshi* **1976**, *96*, 254-257.
4. Shirataki, Y.; Takaya, Y.; Yokoe, I.; Komatsu, M. *Chem. Pharm. Bull.* **1987**, *35*, 1637-1640.
5. Takeda, T.; Ishiguro, I.; Masegi, M.; Ogihara, Y. *Phytochemistry* **1977**, *16*, 619-620.
6. Kim, E. M.; Jung, H. R.; Min, T. J. *Bull. Korean Chem. Soc.* **2001**, *22*, 59-62.
7. Yankep, E.; Mbafor, J. T.; Fomum, Z. T.; Steinbeck, C.; Messanga, B. B.; Nyasse, B.; Budzikiewicz, H.; Lenz, C.; Schmickler, H. *Phytochemistry* **2001**, *56*, 363-368.
8. Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier: New York, U. S. A., 1989; pp 192-196.
9. Eliane, O.; Ferreira, D. A. D. *Phytochemistry* **2000**, *53*, 145-147.
10. Jeon, Y. W.; Jung, J. W.; Kang, M.; Chung, I. K.; Lee, W. *Bull. Korean Chem. Soc.* **2002**, *23*, 391-394.
11. Wei, W. X.; Gong, X. G.; Ishrud, O.; Pan, Y. J. *Bull. Korean Chem. Soc.* **2002**, *23*, 896-898.