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Anti-oxidative Phenolic Compounds from Sophorae Fructus

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Abstract – Four isoflavonoids and three flavonoids, and a gallotannin were isolated from the fruits of *Sophora japonica* (Leguminosae). Their structures were identified as genistein (**1**), sophoricoside (**2**), genistein-4'-*O*--L-rhamnopyranoside (**3**), genistein-4'-*O*-α-L-rhamnopyranosly-(1→2)-β-D-glucopyranoside (**4**), kaempferol-3-*O*-α-D-sophoroside (**5**), kaempferol-3-*O*-β-D-glucopyranosyl-(1→2) -α-L-rhamnopyranosly-(1→6)-β-D-glu -copyranoside (**6**), rutin (**7**) and gallic acid 4-*O*-β-D-(6'-*O*-galloyl)-glucopyranoside (**8**) by chemical and spectroscopic analysis and comparisons with previously reported spectral data. Compounds **3** and **8** were isolated for the first time from this plant. Anti-oxidative activity was evaluated for the isolated compounds. **8** exhibited potent anti-oxidative activity against the radical scavenging ability of DPPH with the IC_{50} value of 17.1 μg/ml.
Keywords – Sophorae Fructus, isoflavonoids, flavonoids, gallotannin, antioxidative activity, DPPH

Introduction

Sophora japonica L.(Leguminosae) has common name as Chinese scholar tree or Japanese pagoda tree (Kim, 1995) and is distributed throughout Korea, China and Japan. It is a wide-branching, highly ornamental tree with a rounded crown (Michael, 1986). This tree is tolerant of pollution, heat, and drought and casts a light shade, allowing turf grasses to grow up the trunk. The leaves of this tree used in the traditional medicine for the treatment of hemorrhoids, stanching and decreasing blood pressure, anti-inflammation and the flower is used for the prevention against paralysis to the patients who has high blood pressure (Nature and Medicin study association of Korea, 1985). Genistein, sophoricoside, sophorabioside, kaempferol glucoside-C, sophoraflavonoloside and rutin were isolated from Sophorae Fructus (Park *et al.*, 2002). The flavonoids of the woods and roots (Park *et al.*, 2002; Takeda *et al.*, 1977; Shirataki *et al.*, 1987) had been studied. Various pharmacological activities including antihypertensive, antiarrhythmic, antiinflammatory, antiallergic, hypochl-olesterolemic, antihepatotoxic and antitumor were reported on the fruits of this plant (Harbone and Wiliams, 2000; Wenzel *et al.*, 2000). As part of our continuing search for anti-oxidative phenolic compounds from natural sources, activity-guided isolation of 80% acetone extract of Sophorae Fructus yielded four isoflavonoids (**1**-**4**), three flavonoids (**5**-**7**) and one phenolic acid (**8**). The structural elucidation of compounds **3** and **8** which were isolated for the first time from this plant and DPPH radical scavenging activity for the all isolated compounds were described here-in.

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Materials and Methods

General experimental procedures – ^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR spectra were obtained on a Varian Unity INOVA 500 spectrometer (Varian, Inc., U.S.A.). Chemical shifts were expressed in parts per million (ppm) relative to TMS as the internal standard, and coupling constants (*J*) were given in Hz. MS were obtained on a Varian Saturn 4D mass spectrometer (Varian, Inc., USA) and JEOL JMS HX-110/110A tandem mass spectrometer (JEOL Ltd., Japan). HPLC was performed on a Waters LC 600E pump using a Waters PorasilTM column (5 μm, 300×7.8 i.d.) and Supelco Silica column (3 μm, 150×4.6 i.d.). TLC was carried out on Merck silica gel F254-precoated glass plates and RP-18 F254s plates.

Plant material – The fruits of *Sophora japonica* were purchased from a commercial supplier at Kyeng-dong Chinese herb medicine market in August of 2003. A voucher specimen has been deposited at the herbarium, College of Pharmacy, Chung-Ang University.

Extraction and isolation – The fruits of *Sophora japonica* (4 kg) were extracted with 80% aqueous acetone

(3 × 10 L) for 3 days. After removal of Me_2CO in vacuo, the aqueous solution was filtered. The filtrate was concentrated and then applied to a column of Sephadex LH-20 (2 kg, 10 × 70 cm). Elution with H_2O containing increasing propertion of MeOH afforded 5 fractions, I(52.5 g), II(8 g), III(21.5 g), IV(9 g), and V(10 g). Repeated column chromatography of fraction I on MCI gel with a H_2O : MeOH gradient, followed by Sephadex LH-20 column chromatography with 60% MeOH yielded **3** (112 mg), **4** (42 mg), and **8** (22 mg). Column chromat-ography of fraction III over MIC gel with H_2O : MeOH gradient furnished **5** (129 mg), and **6** (30 mg). Column chromatography of fraction with MCI gel column chromatography (solvent, $H_2O \rightarrow$100% MeOH) yielded **7** (229 mg). Column chromatography of fraction with silica gel using $CHCl_3$ - MeOH - H_2O (70 : 30 : 4) as the eluent to yield **1** (95 mg) and **2** (264 mg).

Genistein (1) – yellow amorpous powder, Negative-FAB MS *m/z* : 269 ($[M-H]^-$), ^{1}H-NMR(300 MHz, DMSO-d_6) : δ 12.98(1H, s, 5-OH), 8.31(1H, s, H-2), 7.39(2H, d, *J*=8.4 Hz, H-2',6'), 6.84(2H, d, *J*=8.4 Hz, H-3',5'), 6.39(1H, d, *J*=1.8 Hz, H-8), 6.24(1H, d, *J*=1.8 Hz, H-6). ^{13}C-NMR(75 MHz, DMSO-d_6) : (Table 1).

Sophoricoside (2; Genistein-4'-*O*-β-D-glucopyran oside) – yellow amorpous powder, Negative-FAB MS *m/z* : 431 ($[M-H]^-$), ^{1}H-NMR(500 MHz, DMSO-d_6) : 12.90(1H,

Table 1. ^{13}C-NMR data of compounds **1-7** from Sophorae Fructus. (*75 MHz and **125 MHz, DMSO-d_6)

C	1*	2**	3*	4**	5**	6**	7*
2	154.1	154.4	154.8	154.6	155.6	156.2	156.9
3	122.4	124.2	124.4	124.5	132.8	132.7	133.5
4	180.4	180.0	180.3	180.3	177.5	177.3	177.7
5	162.2	162.0	162.2	162.2	161.2	161.1	161.5
6	99.0	99.1	99.1	99.3	98.6	98.2	98.8
7	164.5	164.5	164.7	164.6	164.0	164.3	164.3
8	93.7	93.8	94.0	94.1	93.5	93.7	93.7
9	157.7	157.6	157.8	157.9	156.3	156.4	156.7
10	104.5	104.4	104.5	104.7	103.9	103.7	104.1
1'	121.3	121.9	122.1	122.2	120.9	120.9	121.8
2'	130.3	130.2	130.2	130.5	130.9	130.9	115.4
3'	115.2	116.0	115.8	115.9	115.2	115.2	145.0
4'	157.6	157.3	157.2	157.2	159.9	159.8	148.6
5'	115.2	115.1	115.8	115.2	115.2	115.2	116.4
6'	130.3	130.0	130.5	130.5	130.9	130.9	121.3
g-1		100.3		98.3	97.9	98.7	101.3
g-2		73.2		72.2	82.4	69.7	74.2
g-3		76.7		76.8	77.4	76.4	76.5
g-4		69.7		70.8	69.5	69.7	70.6
g-5		77.0		77.6	77.0	69.5	76.0
g-6		60.7		60.8	60.5	68.1	67.1
r-1			100.7	100.7		100.3	100.9
r-2			70.4	70.7		82.2	70.4
r-3			70.7	70.1		70.2	70.1
r-4			76.3	77.0		71.8	71.9
r-5			68.5	68.6		66.0	68.3
r-6			17.9	18.2		17.6	17.7
g-1'					103.9	103.9	
g-2'					74.3	75.5	
g-3'					76.6	77.0	
g-4'					69.7	70.5	
g-5'					76.5	76.5	
g-6'					60.8	60.8	

s, 5-OH), 8.37(1H, s, H-2), 7.49(2H, d, *J*=8.8 Hz, H-2',6'), 7.09(2H, d, *J*=8.8 Hz, H-3',5'), 6.39(1H, d, *J*=2.0 Hz, H-8), 6.23(1H, d, *J*=2.0, H-6), 4.91(1H, d, *J*=7.3 Hz, glc-1). ^{13}C-NMR(125 MHz, DMSO-d_6) : (Table 1).

Genistein-4'-*O*-α-L-rhamnopyranoside (3) – yellow amorpous powder, Negative-FAB MS *m/z* : 415 ([M-H]$^-$), ^{1}H-NMR(300 MHz, DMSO-d_6) : δ 12.91(1H, s, 5-OH), 8.39(1H, s, H-2), 7.50(2H, d, *J*=9.0 Hz, H-2',6'), 7.06(2H, d, *J*=9.0 Hz, H-3',5'), 6.42(1H, d, *J*=2.4 Hz, H-8), 6.21(1H, d, *J*=2.4 Hz, H-6), 5.15 (1H, s, rha-1), 1.21 (3H, d, *J*= 6.3 Hz, rha-6). ^{13}C-NMR(75 MHz, DMSO-d_6) : (Table 1).

Genistein-4'-*O*-α-L-rhamnopyranosly-(12)-β-D-glucopyranoside (4) – yellow amorpous powder, Negative-FAB MS *m/z* : 577 ([M-H]$^-$), ^{1}H-NMR(500 MHz, DMSO-d_6) : δ 12.89(1H, s, 5-OH), 8.35(1H, s, H-2), 7.50(2H, d, *J*=8.7 Hz, H-2',6'), 7.07(2H, d, *J*=8.7 Hz, H-3',5'), 6.41(1H, d, *J*=2.0 Hz, H-8), 6.25(1H, d, *J*=2.0 Hz, H-6), 5.15 (1H, s, rha-1), 5.07(1H, d, *J*=7.4 Hz, glc-1), 1.23(3H, d, *J*=6.2 Hz, rha-6). ^{13}C-NMR(125 MHz, DMSO-d_6) : (Table 1).

Kaempferol-3-*O*-β-D-sophoroside (5) yellow amorpous powder, Negative-FAB MS *m/z* : 609 ([M-H]$^-$), ^{1}H-NMR(500 MHz, DMSO-d_6) : δ 12.66(1H, s, 5-OH), 8.05(2H, d, *J*=8.8 Hz, H-2',6'), 6.91(2H, d, *J*=8.8 Hz, H-3',5'), 6.44(1H, d, *J*=1.9 Hz, H-8), 6.20(1H, d, *J*=1.9 Hz, H-6), 5.70(1H, d, *J*=7.0 Hz, glc-1), 4.62(1H, d, *J*=7.8 Hz, glc-1'). ^{13}C-NMR(125 MHz, DMSO-d_6) : (Table 1).

Kaempferol-3-*O*-β-D-glucopyranosyl-(1→2)-α-Lrhamnopyranosly-(1→6)-β-D-glucopyranoside (6) –yellow amorpous powder, Negative-FAB MS *m/z* : 756 ([M-H]$^-$), ^{1}H-NMR(500 MHz, DMSO-d_6) : δ 12.62(1H, s, 5-OH), 7.99(2H, d, *J*=8.8 Hz, H-2',6'), 6.90(2H, d, *J*=8.8 Hz, H-3',5'), 6.44(1H, d, *J*=1.9 Hz, H-8), 6.20(1H, d, *J*=1.9 Hz, H-6), 5.54(1H, d, *J*=7.0 Hz, glc-1), 4.59(1H, d, *J*=7.8 Hz, glc-1'), 0.94(3H, d, *J*=6.1 Hz, rha-6). ^{13}C-NMR(125 MHz, DMSO-d_6) : (Table 1).

Rutin (7) – yellow amorpous powder, Negative-FAB MS *m/z* : 609 ([M-H]$^-$), ^{1}H-NMR(300 MHz, DMSO-d_6) : δ 12.61(1H, s, 5-OH), 7.57(1H, d, *J*=2.1 Hz, H-6'), 7.54(1H, s, H-2'), 6.85(1H, d, *J*=8.7 Hz, H-5'), 6.36(1H, d, *J*=1.8 Hz, H-8), 6.21(1H, d, *J*=1.8 Hz, H-6), 5.36(1H, d, *J*=7.5 Hz, glc-1), 4.39(1H, s, rha-1), 0.99(3H, d, *J*=6.3 Hz, rha-6). ^{13}C-NMR(75 MHz, DMSO-d_6) : (Table 1).

Gallic acid 4-*O*-β-D-(6'-*O*-galloyl)-glucopyranoside (8) – white amorpous powder, Negative-FAB MS *m/z* : 483 ([M-H]$^-$), ^{1}H-NMR(500 MHz, DMSO-d_6) : δ 6.99(2H in total, s, H-2,6), 6.93(2H in total, s, galloyl H), 4.75(1H, d, *J*=7.7 Hz, anomeric H), 4.38(1H, d, *J*=10.6 Hz, H-6'). ^{13}C-NMR(125 MHz, DMSO-d_6) : δ 105.1 (glc-1), 73.7 (glc-2), 75.6 (glc-3), 69.3 (glc-4), 74.3 (glc-5), 63.1 (glc-6), 127.6 (C-1), 108.7 (C-2, 6), 150.1 (C-3, 5), 136.6 (C-4), 165.8 (-COO-), 119.3 (galloyl-1), 108.8 (galloyl-2, 6), 145.5 (galloyl-3, 5), 138.6 (galloyl-4), 167.1 (-COO-)

DPPH radical scavenging activity – The antioxidant activity was determined on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrzyl (DPPH) free radical by a previously described method with a slight modification. 50 L of the compound in EtOH was added to 0.95 of a DPPH solution (7.887 mg DPPH in 200 ml EtOH). After mixing gently and standing at for 30 min, the optical density was measured at 520 nm using a microplate reader spectrophotometer VERSAmax (Molecular Devices, CA, U.S.A.).

Results And Discussions

Chromatographic isolation of 80% aqueous acetone extract of Sophorae Fructus (Leguimnosae) afforded eight phenolic compounds **1** (Harborne and Mabry, 1982; Park *et al.*, 2002), **2** (Philip *et al.*, 1998; Djerassi *et al.*, 1994; Lewis and Wahala, 1998), **4** (Jeffrey *et al.*, 1999), **5** (Lee *et al.*, 2002;Djerassi *et al.*, 1994), **6** (Agrawal, 1989) and **7** (Kim, 2000; Takeda *et al.*, 1997) which were isolated previously from same plant except for **3** (Saxena and Madhuri, 1998) and **8** (Nonaka and Nishioka, 1983).

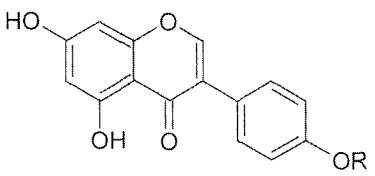
Compound **3** was obtained as brown powder. The ^{1}H-NMR spectrum of **3** showed two pairs of doublet signals which exhibited A_2B_2 type of B-ring at δ 7.50 (d, 2H) and δ 7.06 (d, 2H) assigned to be H-2', 6' and H-3', 5', respectively, and another two pairs of doublet signals at δ 6.42 (d, 1H) and δ 6.21 (d, 1H) assigned to be H-8 and H-6 of A-ring, respectively, and a singlet signal at δ 8.39 (1H, H-2). The ^{1}H-NMR spectrum of **3** also showed anomeric proton at δ 5.15 (1H, s) and rhamnose methyl signal at δ 1.21 (3H, d, *J*=6.3 Hz). These result suggested that **3** was genistein rhamnopyranoside. The ^{13}C-NMR spectrum of **3** was similar to those of sophoricoside (**2;** genistein-4'-*O*-β-D-glucopyranoside), except for the additional signals of an rhamnose (δ 100.7, 70.4, 70.7, 76.3, 68.5 and 17.9) instead of glucose. The negative FAB-mass spectrum of **3** exhibited a molecular ion due to [M-H]$^-$ at *m/z* 415. From these results, **3** could be established as genistein-4'-*O*-α-L-rhamnopyranoside (Saxena and Madhuri, 1998).

Compound **8** was obtained as colorless prisms and characterized as a gallotannin by its blue colouration with ferric chloride. The ^{1}H-NMR spectrum of **8** showed an anomeric proton at 4.75 (1H, d, *J*=7.7 Hz) and two pairs of singlet signals at δ 6.99 (s, 2H) and 6.93 (s, 2H)

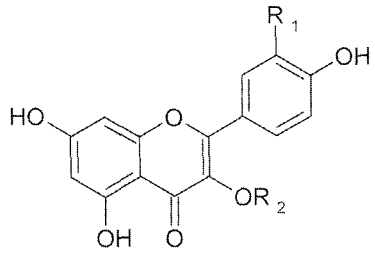
Table 2. Antioxidative activities of compounds **1** - **8** isolated from Sophorae Fructus.

Compounds	IC_{50} (μg/ml)
1	377.8
2	>1000
3	616.7
4	680.6
5	>1000
6	>1000
7	38.3
8	17.1
*L-Ascorbic acid	16.4

(*) Used as a positive control.
Each data represents three independent tests assayed in triplicate.



	R
1	H
2	-Glc
3	-Rha
4	-Glc $^{2}_{1}$ Rha



	R_1	R_2
5	H	-Glc $^{2}_{1}$ Glc
6	H	-Glc $^{6}_{1}$ Rha $^{2}_{1}$ Glc
7	OH	-Glc $^{6}_{1}$ Rha

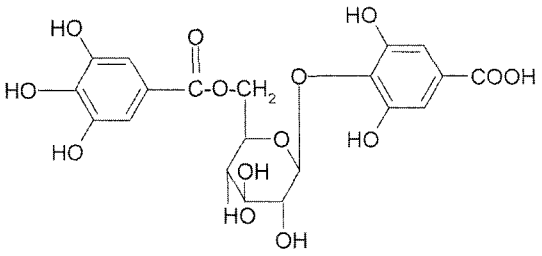


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Fig. 1. Structures of compounds 1-8.

assigned to be H-2, 6 and galloyl H, respectively. The ^{13}C-NMR spectrum of **8** also showed 1 mol of glucose signals at δ 105.1 (glc-1), 73.7 (glc-2), 75.6 (glc-3), 69.3 (glc-4), 74.3 (glc-5), 63.1 (glc-6) and two pairs of galloyl moieties at δ 127.6 (C-1), 108.7 (C-2 and 6), 150.1 (C-3 and 5), 136.6 (C-4) and 165.8 (ester-COO-) and 119.3 (galloyl 1), 108.8 (galloyl 2 and 6), 145.5 (galloyl 3 and 5), 138.6 (galloyl 4) and 167.1 (-COO-). Especially, the existences of singlet signal of H-2 and 6 at δ 6.99 in ^{1}H-NMR spectrum of **8** and free carboxyl carbon signal at δ 165.8 with another ester carbonyl signal at 167.1 and lowfield shifted C-6' of glucose signal at δ 63.1 in ^{13}C-NMR spectrum of **8** indicated that **8** is gallic acid 4-*O*-glucoside having a another galloyl group at the C-6' position of glucose. The negative FAB-mass spectrum of **8** exhibited a molecular ion due to $[M-H]^-$ at *m/z* 483. From these results, the structure of **8** was established as gallic acid 4-*O*-β-D-(6'-*O*-galloyl)-glucopyranoside (**8**, Nonaka and Nishioka, 1983).

The antioxidant activities were tested using all the isolated compounds (**1**-**8**) by DPPH free radical scavenging method. Among them, gallic acid 4-*O*-β-D-(6'-*O*-galloyl)-glucopyranoside (**8**) exhibited a potent, free radical scavenging effect with an IC_{50} value of 17.1 μg/ml compared with positive control, L-ascorbic acid (IC_{50} = 16.4 μg/ml) and rutin (**7**) also showed moderate DPPH radical scavenging effect with an IC_{50} value of 38.3 μg/ml.

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