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A New Isoflavone Glycoside from the Stem Bark of *Sophora japonica*

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A new isoflavone glycoside, 6-methoxy-7-hydroxy-4'-*O*-β-D-glucosyl isoflavone, glycitein-4'-*O*-β-D-glucoside (**10**), along with nine known flavonoids, were isolated from the stem bark of *Sophora japonica*. The structures of these compounds were determined by analysis of spectroscopic data (1D -, 2D - NMR and HRMS). The inhibitory effects of all the isolated compounds on aldose reductase were evaluated *in vitro*. Among these compounds, daidzein (**1**), puerol A (**4**), and paratensein-7-*O*-glucoside (**9**) exhibited potent inhibitory effects, with IC_{50} values of 3.2, 6.4, and 1.9 μM, respectively.

Key words: *Sophora japonica*, Stem bark, Isoflavone glycoside, Glycitein-4'-*O*-β-D-glucoside, Aldose reductase

INTRODUCTION

The stem bark of *Sophora japonica* (Leguminosae) has been used as an anti-inflammatory and analgesic agents in traditional Chinese medicine (Zhong Hua Ben Cao Editorial Commitee, 1999). The chemical constituents cyclosin, puerol A, trifolirhizin, and genistein have been isolated from the stem bark of this plant (Park et al., 2002).

Aldose reductase (EC 1.1.1.21; AR) is a key enzyme in the polyol pathway, in which catalyzes glucose to sorbitol, and then sorbitol dehydrogenase converts sorbitol to fructose (Yabe-Nishimura, 1998). The significance of this enzyme has been indicated in the pathogenesis of diabetic complications such as neuropathy, retinopathy, and cataract (Santiago, 1993; Feldman et al., 1997). Thus, the inhibition of aldose reductase may be of therapeutic use in diabetic complications. During a search for aldose reductase inhibitors from plant sources, an *n*-BuOH-soluble fraction of the stem barks of *Sophora japonica* showed the best inhibition activity (data not shown). This paper deals with the isolation, structure elucidation, and aldose reductase inhibitory activity of compounds from the barks of *Sophora japonica*.

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MATERIALS AND METHODS

General procedures

Melting point were determined on a Fisher Johns melting point apparatus. UV/Vis spectra were obtained using a V-530 spectrophotometer (JASCO). Optical rotation was measured on a DIP 1000 Digital Polarimeter (JASCO). IR spectra were obtained using a FTIR-4200 (JASCO). MS spectra were obtained using a Autospec M363 (Micromass). NMR spectra were recorded on a DPX 400 and AVANCE 600 (Bruker). Chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using Kieselgel 60, 400-230 mesh (Merck) and YMC gel ODS-A, 150 μm (YMC). TLC was performed on glass-backed Kieselgel 60 F_{254} and RP F_{254s} plates. HPLC analysis was performed using a Spectra system (Thermo Separation Products).

Plant material

The stem bark of *S. japonica* was collected from the Herbal garden of Kangwon National University (June, 2007) in Korea and identified by Prof. Y. S. Kwon, College of Pharmacy, Kangwon National University. A voucher specimen (KNUH-B-0701) was deposited in the herbarium of the College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation

The dried stem bark of *S. japonica* (1.7 kg) was extracted with MeOH at room temperature for a week. The MeOH extract (288 g) was suspended in water and then partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, successively, leaving a residual water-soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of *n*-hexane fraction (40 g), $CHCl_3$ fraction (7 g), and *n*-BuOH fraction (70 g). Among the solvent fractions, a *n*-BuOH soluble fraction exhibited 65% inhibition on aldose reductase at 100 μg/mL. Column chromatography was performed to isolate the active compounds from the *n*-BuOH soluble fraction,. The *n*-BuOH soluble fraction (60 g) was column chromatographed on a silica gel (500 g, 9 × 60 cm) using an isocratic elution with $CHCl_3$: MeOH (4 : 1), in order to divide the fraction into six fractions (Fr. 01 - Fr. 06). Fr. 01 (1 g) was re-chromatographed on silica gel by elution with benzene : EtOAc (2 : 1) and divided into six sub-fractions (Fr. 011-Fr. 016). Fr. 016 was further purified by silica gel column chromatography [3.8 × 50 cm, CHCl : MeOH (13 : 1)] to give compounds **1** (51 mg) and **2** (9 mg), respectively. Fr. 2 (7 g) was re-chromatographed on a silica gel column (500 g, 6.5 × 50 cm) by elution with EtOAc : MeOH (9 : 1) and divided into five sub-fractions (Fr. 021-Fr. 025). Fr. 023 was recrystallized from MeOH to give compound **3** (355 mg). Fr. 022 was re-chromatographed on a silica gel column (80 g, 2.5 × 45 cm) by elution with benzene : EtOAc (2:1) to give compound **4** (42.8 mg). Fr. 024 was re-chromatographed on a silica gel column (80 g, 2.5 × 50 cm) and ODS column (70 g, 2.5 × 50 cm) by elution with water saturated-EtOAc : MeOH (19 : 1) and MeOH:H_2O (60 : 40) to give compounds **5** (100 mg) and **6** (12 mg), respectively. F03 (6.5 g) was subjected to MPLC on a Combiflash Retrieve using Redisep C-18 column (130 g), eluting with MeOH : H_2O (40 : 60) to yield five sub-fractions (Fr.031 - Fr.035). Fr. 032 was re-chromatographed on a silica gel column (80 g, 2.5 × 50 cm) by elution with $CHCl_3$: MeOH (6 : 1) to give compounds **7** (2.7 mg) and **8** (54 mg). Fr. 034 was re-chromatographed on a silica gel column (80 g, 2.5 × 50 cm) and Sephadex LH20 (50 g, 2.5 × 50 cm) by elution with EtOAc : MeOH (15 : 1) and MeOH : H_2O (60 : 40), to give compounds **9** (50 mg) and **10** (66.7 mg), respectively. The purity of isolated compounds (> 95%) were determined by HPLC using a Intersil column (ODS-2, 150 × 4.5 mm) with acetonitrile : water (20 : 70).

Acid hydrolysis of 10

Compound **10** (10 mg) was refluxed with 5% HCl (2 mL) in water for 1 h. The reaction mixture was diluted with water and fractionated by EtOAc. The EtOAc-soluble fraction was concentrated and column-chromtographed on silica gel ($CHCl_3$: MeOH = 19 : 1) gave compound **10a** (2.3 mg). The remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by TLC with authentic sugar.

Compound 10

Mp : 147~150°C; UV (MeOH) λ_{max} 257, 324 nm; UV (MeOH + NaOAc) λ_{max} 261, 345 nm; UV (MeOH + NaOH) λ_{max} 258, 348 nm; IR (ATR)$_{max}$ 3371 (OH), 1620 (C=O), 1597, 1509, 1473 (C=C), 1163, 1069, 1034 (C-O) cm^{-1}; ^{1}H-NMR (400 MHz, MeOH-d_4) δ : See Table I; ^{13}C-NMR (100 MHz, MeOH-d_4) δ : See Table I; FAB-

Table I. ^{1}H- and ^{13}C-NMR data for **10** (in MeOH-d_4)[a]

Pposition	**10**	
	H (*J* = Hz)	C
2	8.08 (s)	154.45
3		124.81
4		177.50
5	7.47 (s)	105.06
6		149.26
7		156.76
8	6.83 (s)	103.97
9		154.74
10		117.06
1'		127.66
2'	7.45 (d, 8.8)	131.38
3'	7.15 (d, 8.8)	117.64
4'		158.92
5'	7.15 (d, 8.8)	117.64
6'	7.45 (d, 8.8)	131.38
1"	4.95 (d, 7.5)	102.27
2"	3.42 ~ 3.51 (m)	74.94
3"		77.98
4"		71.39
5"		78.16
6"	3.73 (dd, 5.1, 12.0) 3.50 (m)	62.54
OCH_3	3.92 (s)	56.53

[a]The assignments were based on DEPT, COSY, HSQC, and HMBC experiments.

MS m/z : 447 $[M+H]^+$; HR-FAB-MS m/z : 447.1178 $[M+H]^+$ (calcd for 447.1291).

Compound 10a

^{1}H-NMR (600 MHz, MeOH-d_4) δ : 3.97 (3H, s, OCH_3), 6.87 (2H, d, J = 8.5 Hz, H-3', H-5'), 6.89 (1H, s, H-8), 7.39 (2H, d, J = 8.5 Hz, H-2', H-6'), 7.56 (1H, s, H-5), 8.14 (1H, s, H-2); EI-MS m/z : 284 $[M]^+$, 166, 118.

Assay for rat lens aldose reductase inhibitory activity

According to method modified from Hayman and Kinoshita (Hayman and Kinoshita, 1965), rat lens homogenate was prepared. In summary, the lenses were removed from the eyes of Sprague-Dawley rats (Daehan Biolink Co. Ltd) weighing 320-350 g. The lenses were homogenized in 100 mM sodium phosphate buffer (pH 6.2). The supernatant was obtained by centrifugation of the homogenate at 10,000 rpm at 4°C for 20 min and was divided into 1 mL, and stored at −40°C.

Enzyme activities were assayed spectrophotometically on a JASCO V-530 spectrophotometer. The activities of rat lens aldose reductase (RLAR) were measured according to the procedure of Nishimura et al. (1991). The reaction mixture contained 0.15 mM NADPH, 10 mM DL-glyceraldehyde, 100 μL of RLAR and 10 μL of the test sample or dimethylsulfoxide (DMSO) in a total volume of 1 mL of 100 mM sodium phosphate buffer (pH 6.2). After the reaction mixtures were incubated in advance at 25°C for 3 min, the reaction was started by addition of the enzyme, and then the decrease in absorbance was measured at 340 nm for 6 min using a JASCO V-530 spectrophotometer.

The inhibition activity (%) was estimated as follows : [1 − (ΔA sample/min − ΔA blank/min)/(ΔA control/min − ΔA blank/min)] × 100.

ΔA sample/min showed a decrease in absorbance for 6 min with a sample, ΔA blank/min with DMSO and water instead of a sample and a substrate, respectively, and ΔA control/min with DMSO instead of a sample. The 50% inhibition concentrations of the RLAR inhibitory activity were calculated from the log-dose inhibition curve against test concentrations.

RESULTS AND DISCUSSION

Repeated silica gel, ODS, and Sephadex LH 20 column chromatography led to the isolation of compounds **1**-**10**. Compounds **1**-**9** were identified by comparison with spectral data from the literature of daidzein (**1**) (Lee et al., 2008), calycosin (**2**) (Park et al., 2002), trifolirhizin (**3**) (Park et al., 2002), puerol A (**4**) (Park et al., 2002), ononin (**5**) (Hwang et al., 1997), 5-hydroxypseudobaptigenin-7-*O*-glucoside (**6**) (Laman and Volynets, 1975), glycitin (**7**) (Park et al., 1999), calycosin-7-*O*-glucoside (**8**) (Lee et al., 2008), and paratensein-7-*O*-glucoside (**9**) (Ahut et al., 1984), respectively.

Compound **10** was obtained as a brownish powder and produced a *quassi* molecular ion $[M + H]^+$ at m/z 447.1178 by HR-FAB-MS, consistent with an elemental formula of $C_{22}H_{22}O_{10}$. The IR spectrum of **10** showed absorption bands at 3371 (OH), 1620 (α, β-unstaturated C=O), 1597, 1509, 1473 (aromatic C=C), 1163, 1069, 1034 (C-O) cm^{-1}. The presence of a singlet at δ 8.08 in the ^{1}H-NMR spectrum and UV absorption band at 254 and 324 nm suggested that it was an isoflavonoid. The ^{1}H-NMR spectrum of **10** exhibited signals from the H-5 and H-8 position of the isoflavone ring at δ 7.47 (1H, s) and 6.83 (1H, s), respectively. Two AB - type doublets at δ 7.15 (2H, d, J = 8.8 Hz) and 7.45 (2H, d, J = 8.8 Hz) indicated the presence of H-3', H-5' and H-2', H-6', respectively. A singlet at δ 3.92 (3H) indicated the presence of a methoxyl group. In addition, an anomeric proton at δ 4.95 (1H, d, J = 7.5 Hz) was assigned. The signals of methoxyl and anomeric proton showed an HMBC correlation with the C-6' (δ 149.26) and C-4' (δ 158.92) signals, respectively, in the HMBC spectrum (Fig. 1), respectively. Upon acid hydrolysis of **10**, glycitein (**10a**) (Park et al., 1999) and glucose were identified by ^{1}H-NMR and TLC, respectively. The anomeric configuration of glucose in **10** was concluded to be of β-form because of the large J value (7.5 Hz) of its anomeric proton. Consequently, the structure of **10** was established as glycitein-4'-*O*-β-D-glucoside. All of the isolated compounds were examined for rat lens aldose reducatase inhibitory activity. Daidzein

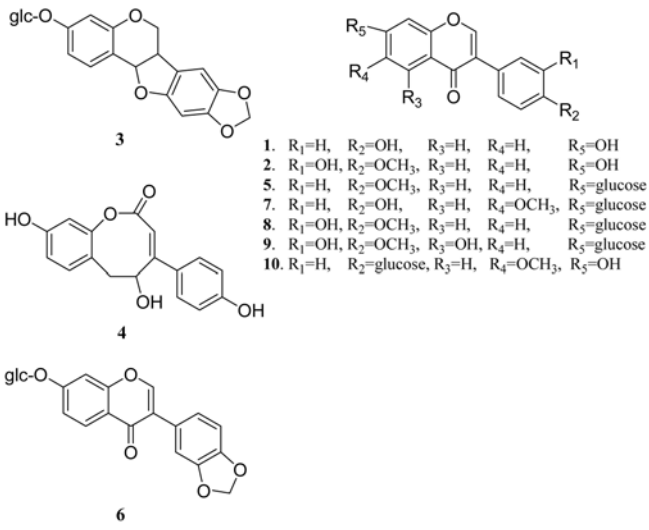

Fig. 1. The structures of **1-10** isolated from *S. japonica*

Table II. The inhibitory effects **1-10** of *S. japonica* on aldose reductase

Compound	Inhibitory effect (IC_{50} values)[a]	
	μg/mL	μM
1	0.8	3.2
2	8.3	29.2
3	6.9	15.4
4	1.9	6.4
5	33.7	78.5
6	44	95.7
7	15.1	34.5
8	34.6	77.6
9	0.9	1.9
10	8.6	19.3
Quercetin[b]	5.3	1.9

[a]The concentration required for a compound for a 50% inhibition (IC_{50}) of human recombinant aldose reductase. IC_{50} values were calculated from the dose inhibition curve. Inhibitory effect was expressed as mean of triplicate experiments.
[b]Quercetin was used as a positive control.

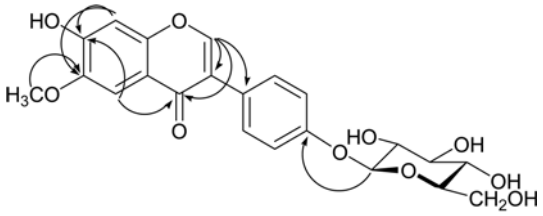

Fig. 2. Important HMBC (H→C) correlations of **10**

(**1**), puerol A (**4**), and paratensein-7-*O*-glucoside (**9**) showed potent inhibitory activity with IC_{50} values of 3.2, 6.4, and 1.9 μM, respectively, as shown Table II. Calycosin (**2**), trifolirhizin (**3**), glycitin (**7**), and glycitein-4'-*O*-*β*-D-glucoside (**10**) showed mild inhibitory activity with IC_{50} values of 29.2, 15.4, 34.5, and 19.3 μM, respectively. Ononin (**5**), 5-hydroxypseudobaptigenin-7-*O*-glucoside (**6**), and calycosin-7-*O*-glucoside (**8**) showed very weak inhibitory activity in the present study. In conclusion, daidzein (**1**), puerol A (**4**), and paratensein-7-*O*-glucoside (**9**) were isolated from the bark of *S. japonica* as a potent inhibitor. Though glycitein-4'-*O*-β-D-glucoside (**10**) was newly isolated from plant sources, it was showed mild inhibitory activity with an IC_{50} value of 19.4 μM.

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