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α-Glucosidase Inhibitors from the Roots of *Sophora flavescens*

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Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, or action, or both. Postprandial hyperglycemia has been an early indicator of abnormality in glucose metabolism leading to type 2-diabetes.[1] Therefore, reduction of postprandial hyperglycemia is critical in the early stage of diabetes therapy.[2,3] Since α-glucosidase plays significant roles in the adjustment of insulin, inhibition of this enzyme has become one of the therapeutic targets to retard glucose absorption, finally resulting in suppression of postprandial hyperglycemia.[4] Therefore, suppressing carbohydrate digestion by the application of α-glucosidase inhibitors (*e.g.* acarbose, miglitol, and voglibose) is considered an alternative treatment for diabetes.[5]

The dried roots of *S. flavescens* (Leguminosae) are commonly used as the traditional Chinese medicine and possess various physiological properties such as anti-bacterial, anti-inflammatory, antipyretic, anti-asthmatic, anti-ulcerative, and anti-neoplastic effects.[6] A series of isoprenylated or lavandulylated flavonoids have been isolated from this plant.[7-12] Some of these compounds exhibited significant antibacterial activity against Gram-positive bacteria,[7] antiviral activity against herpes simplex virus types I and II,[8] as well as cytotoxic activity against human myeloid leukemia HL-60 cells,[10,12] and potent inhibitory activity against cGMP phosphodiesterase 5.[11] The present study describes the isolation and structural elucidation of a new compound (**1**) and eight known compounds (**2-9**) from the methanol extract of the roots of *S. flavescens*. The inhibitory effect of the isolated compounds on the α-glucosidase activity was evaluated.

The dried, chipped roots of *S. flavescens* were extracted with MeOH. The MeOH extract was suspended in water, and then partitioned with CH_2Cl_2 and EtOAc succesively to give CH_2Cl_2 (A), EtOAc (B), and aqueous fractions (C), respectively. Fr. B was subjected to multiple column chromatograph using silica gel and YMC C_{18} to yield one new compound (**1**) and eight known compounds (**2-9**). The known compounds were identified to be isoxanthohumol (**2**),[13] norkurarinone (**3**),[14] kurarinone (**4**),[15] (2*S*)-2'-methoxykurarinone (**5**),[10] kushenol T (**6**),[7] norkurarinol (**7**),[15] kuraridin (**8**),[15] and formononetin (**9**)[16] by comparison of the NMR and MS data with those reported in the literature.

Compound **1** was isolated as a yellow amorphous powder, and its molecular formula was established to be $C_{21}H_{22}O_6$ by HRESITOFMS at *m/z* 369.1320 $[M-H]^-$ (calcd for $C_{21}H_{21}O_6$, 369.1338). The IR spectrum of **1** revealed hydroxyl and carbonyl groups based on absorption bands at 3279 and 1605 cm^{-1}, respectively. The ^1H NMR spectrum of **1** showed an aromatic proton singlet at δ_H 6.10 (H-6), and a typical A_2B_2 system at δ_H 6.73 (2H, d, J = 8.4 Hz, H-3', 5') and 7.28 (2H, d, J = 8.4 Hz, H-2', 6'). The ^1H NMR of **1** further showed the signals of a 3,3-dimethylallyl group at δ_H 1.58 (3H, s, H_3-5''), 1.61 (3H, s, H_3-4''), 3.22 (2H, m, H-1''), and 5.12 (1H, t, J = 7.2 Hz, H-2''). The position of 3,3-dimethyl-

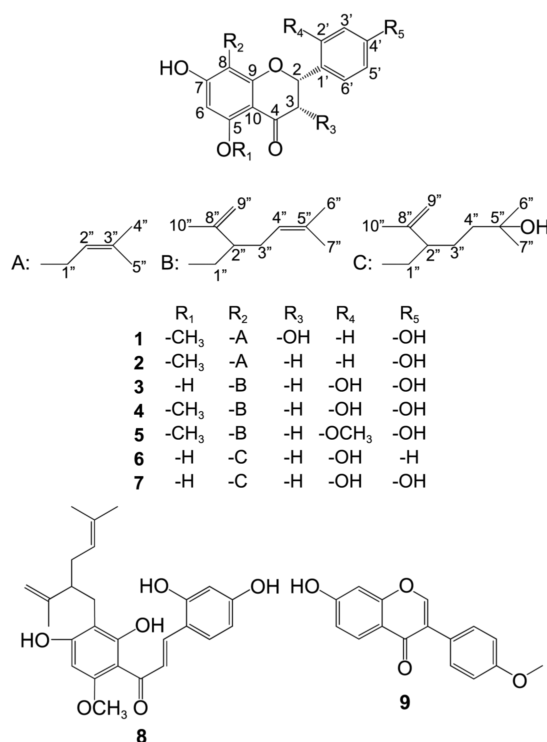

Figure 1. Structures of compounds **1-9**.

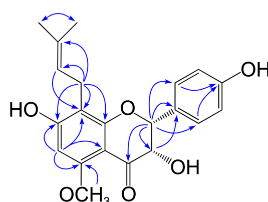

Figure 2. Key HMBC correlations of compound **1**.

allyl group was assigned to C-8 by the HMBC correlations from H_2-1" to C-7 (δ_C 164.4), C-8 (δ_C 109.8), and C-9 (δ_C 162.7). The signals of a methoxyl group at 3.75 (3H, s) and two oxymethine groups at 5.37 (1H, d, J = 3.6 Hz, H-2) and 4.24 (1H, d, J = 3.6 Hz, H-2) were also observed in the ^{1}H NMR spectrum of **1**. The methoxyl group was determined to locate at C-5 based on the HMBC correlation of signal at δ_H 3.75 with δ_C 161.9 (C-5). The small coupling constant (J = 3.6 Hz) of H-2 and H-3 revealed that these protons are located on the same side. The absolute configurations of H-2 and H-3 were determined by CD spectrum. The CD spectrum of **1** showed positive and negative Cotton effects at 341.4 and 274.2 nm, respectively. These signs and positions are in good agreement with those of the reported compound, (2*R*,3*S*)-*cis*-dihydrokaemferol,[17,18] indicating the absolute configurations of C-2 and C-3 to be *R* and *S*, respectively. Based on the above evidence, together with comparison of the NMR data of **1** with those of similar compound,[19] the structure of **1** was established as (2*R*)-3α,7,4'-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone.

α-Glucosidases are enzymes that digest dietary carbohydrates in the small intestine and hence the inhibitors of this enzyme can delay the absorption of carbohydrates in food to suppress postprandial hyperglycemia. The effects of the isolated compounds on the inhibition of α-glucosidases activity were evaluated. The result showed that compound **8** was most effective with IC_{50} value of 3.6 μM, whereas compounds **3-7** displayed similar inhibitory effects with IC_{50} values in a range of 14.0-18.0 μM. Based on the comparison of the structures and activities of the tested compounds, we found that all the 8-lavandulyl flavanone (**3-5**), 8-(5-hydroxy-2-isopropenyl-5-methylhexyl) flavanone (**6** and **7**), and 8-lavandulyl chalcone (**8**) significantly inhibited α-glucosidase activity, while two 8-dimethylallyl flavanones (**1** and **2**) and an isoflavone (**9**) were inactive. Thus, the lavandulyl or 5-hydroxy-2-isopropenyl-5-methylhexyl group at C-8 is essential for the α-glucosidase inhibitory effect of the flavanone compounds.

In conclusion, these results provide a scientific support for the use of the roots of *S. flavescens* and warrant further studies to develop new agents for the prevention and treatment of the diabetes.

Experimental

General Procedures. Optical rotation was determined using a Jasco DIP-370 digital polarimeter. The FT-IR spectra were measured using a Jasco Report-100 infrared spectrometer. Electrospray ionization (ESI) mass spectra were obtained using an Agilent 1200 LC-MSD Trap spectrometer. HR-ESI-TOF mass spectra were obtained using a JEOL JMS-T100LC spectrometer. The NMR spectra were recorded on a Jeol ECA 600 spectrometer using TMS as an internal standard. CD spectra were recorded with a Jasco J-720 spectropolarimeter. TLC was performed on Kieselgel 60 F254 (1.05715; Merck, Darmstadt, Germany) or RP-18 F254s (Merck) plates. Spots were visualized by spraying with 10% aqueous H_2SO_4 solution, followed by heating. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and YMC RP-18 resins.

Plant Materials. The roots of *S. flavescens* was purchased from herbal market at Kumsan, Chungnam, Korea, in August 2010. The plant material was identified by one of us (Y. H. Kim). A voucher specimen (CNU10106) was deposited at herbarium, College of Pharmacy, Chungnam National University.

Extraction and Isolation. The dried stem bark of *S. flavescens* (3 kg) was extracted with hot MeOH. After concentration, the MeOH extract (250 g) was suspended in water and then partitioned successively with CH_2Cl_2 and EtOAc to give CH_2Cl_2 (A), EtOAc (B) and water (C) fractions, respectively. Fraction B was subjected to silica gel column chromatography, eluted with acetone in CH_2Cl_2 (0-100%, stepwise) to yield five fractions (B1-5). Fraction B2 was then separated by column chromatography over YMC reverse-phase, using MeOH-H_2O (2:1) to afford **8** (30 mg) and **9** (15 mg). Fraction B3 was separated by column chromatography over silica gel, using CH_2Cl_2-EtOAc (4:1) as eluent to give three subfractions (B3A-C). Subfractiob B3A was chromatographed over silica gel column, eluting with CH_2Cl_2-acetone (5:1) to provide **3** (50 mg). Subfraction B3B was then chromatographed over YMC reverse-phase, using MeOH-H_2O (3:1), and further purified by column chromatography over silica gel, eluting with CH_2Cl_2-MeOH (15:1) to obtain **1** (12 mg) and **6** (7 mg). Subfraction B3C was separated by YMC reverse-phase chromatography, using MeOH-H_2O (2:1) as eluent, and further purified by column chromatography over silica gel, eluting with CH_2Cl_2-MeOH (12:1) to obtain **2** (10 mg) and **7** (45 mg). Fraction B4 was separated by column chromatography over silica gel, eluted with CH_2Cl_2-Acetone (4:1) to provide three subfractions (B4A-C). Subfraction B4B was then purified by column chromatography over YMC reverse-phase, using MeOH-H_2O (2:1) to afford **4** (45 mg) and **5** (10 mg).

(2*R*)-3α,7,4'-Trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (1): pale yellow, amorphous powder; $[\alpha]_D^{25}$ −71.5 (c 0.25, MeOH); FT-IR (CH_3CN) ν_{max} 3279, 2968, 1605, 1599, 1516, 1256, 1087, 816 cm^{-1}. HRESITOFMS m/z 369.1320 $[M-H]^-$ (calcd for $C_{21}H_{21}O_6$, 369.1338). CD (MeOH) nm (Δε): 341.4 (+39.93), 274.2 (−26.60), 242.2 nm (−25.45). ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR data (methanol-d_4, 150 MHz), see Table 1.

Isoxanthohumol (2): pale yellow, amorphous powder, $[\alpha]_D^{25}$ −25 (c = 0.5, MeOH).

Table 1. NMR data for compound **1**

Position	δ_C[a,b]	DEPT	δ_H[a,c]	HMBC
2	82.0	CH	5.37 d (3.6)	C-3, 4, 1′, 2′, 6′
3	74.1	CH	4.24 d (3.6)	C-2, 4, 1′
4	192.2	C		
5	161.9	C		
6	93.4	CH	6.10 br s	C-5, 7, 8, 10
7	164.4	C		
8	109.8	C		
9	162.7	C		
10	104.0	C		
1′	128.3	C		
2′	129.7	CH	7.28 d (8.4)	C-2, 4′, 6′
3′	115.8	CH	6.73 d (8.4)	C-2′, 4′, 5′
4′	158.2	C		
5′	115.8	CH	6.73 d (8.4)	C-3′, 4′, 6′
6′	129.7	CH	7.28 d (8.4)	C-2, 2′, 4′
1″	22.6	CH_2	3.22 m	C-7, 8, 9, 2″, 3″
2″	123.9	CH	5.12 t (7.2)	C-8, 1″, 4″, 5″
3″	131.7	C		
4″	25.9	CH_3	1.58 s	C-2″, 3″, 5″
5″	17.9	CH_3	1.61 s	C-2″, 3″, 4″
5-OCH_3	56.0	CH_3	3.75 s	C-5

[a]Recorded in CD_3OD. [b]150 MHz. [c]600 MHz. *J* values (Hz) are in parentheses.

Norkurarinone (3): yellow, amorphous powder, $[\alpha]_D^{25}$ +10.3 (c = 0.7, MeOH).

Kurarinone (4): yellow, amorphous powder, $[\alpha]_D^{25}$ +13.5 (c = 0.2, MeOH).

(2*S*)-2'-Methoxykurarinone (5): pale yellow, amorphous powder, $[\alpha]_D^{25}$ −35.2 (c = 0.1, MeOH).

Kushenol T (6): yellow, amorphous powder, $[\alpha]_D^{25}$ −118.5 (c = 0.2, MeOH).

Norkurarinol (7): yellow, amorphous powder, $[\alpha]_D^{25}$ −25.5 (c = 0.1, MeOH).

Kuraridin (8): yellow, amorphous powder, $[\alpha]_D^{25}$ −25.5 (c = 0.1, MeOH).

Formononetin (9): pale yellow, amorphous powder.

Table 2. α-Glucosidase inhibitory effects of compounds **1-9**

Compound	IC_{50} (μM)
1	107.8 ± 5.7
2	86.9 ± 6.1
3	17.7 ± 2.6
4	14.5 ± 1.3
5	14.0 ± 1.2
6	16.7 ± 2.1
7	18.0 ± 1.5
8	3.6 ± 0.8
9	119.6 ± 9.4
Acarbose	2.9 ± 0.2

The values are mean ± SD (n = 3).

Inhibition Assay for α-Glucosidase Activity. A mixture of samples (50 μL), 0.1 M phosphate buffer (pH 7.0, 50 μL) containing α-glucosidase solution (0.3 U/mL), and distilled water (50 μL) was incubated in 96 well plates at 37 °C for 15 min. Then a solution of 3 mM *p*-NPG in 0.1 M phosphate buffer (pH 7.0, 100 μL) was added to each well. The reaction mixtures were incubated at 37 °C for 10 min and stopped by adding 0.1 M Na_2CO_3. The absorbance was recorded at 405 nm by FLUOstar Optima (BMG Labtech, Offenburg, Germany). The results were expressed as a percent of α-glucosidase inhibition and calculated according to the following equation:

$$\% \text{ inhibition} = [(\text{Abs}^{\text{control}} - \text{Abs}^{\text{extract}})/\text{Abs}^{\text{control}}] \times 100$$

Acarbose was used as a positive control.

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