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TWO FLAVONE 2′-GLUCOSIDES FROM *SCUTELLARIA BAICALENSIS*

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Key Word Index—*Scutellaria baicalensis*. Labiatae; flavones; 5,2′,6′-trihydroxy-6,7,8- trimethoxyflavone 2′-glucoside; 5,2′,6′-trihydroxy -6,7-dimethoxyflavone 2′-glucoside.

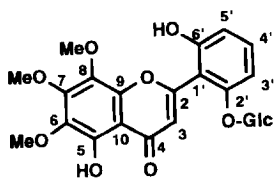
Abstract—Two new flavone glucosides, 5,2′,6′-trihydroxy-6,7,8-trimethoxyflavone 2′-*O*-glucoside and 5,2′,6′-trihydroxy-6,7-dimethoxyflavone 2′-*O*-glucoside were isolated from the aqueous methanol extract of the roots of *Scutellaria baicalensis*. From the extract, seven phenolics, 5,7,2′,6′-terahydroxyflavone, 5,7,2′,5′-tetrahydroxy-8,6′-dimethoxyflavone, skullcapflavone II, baicalin, baicalin methyl ester, wogonin 7-glucuronide and 3,5,7,2′,6′-pentahydroxyflavanone were also isolated.

INTRODUCTION

Scutellaria baicalensis Georgi (Labiatae) is one of the important medicinal herbs widely used for the treatment of various inflammatory diseases, hepatitis, tumors and diarrhea in East Asian countries such as China, Korea and Japan [1]. This plant has been reported to contain a large number (over 40) of flavonoids, frequently found as the glucuronides, and other constituents such as phenethyl alcohols, sterols, essential oils and amino acids [2]. In the present flavonoid study of *S. baicalensis* two new flavone glucosides (**1** and **2**) were isolated from the roots and their structures determined by UV, ^{1}H and ^{13}C NMR spectral evidence. Seven known phenolics (**3**–**9**) were also isolated.

RESULTS AND DISCUSSION

By the combination of Sephadex LH-20, MCI GEL CHP 20P, silica gel and Bondapak C18 Porasil B column chromatography nine phenolics (**1**–**9**) were isolated from the aqueous methanol extract of the roots of *S. baicalensis*. Among these, seven were identified as the known compounds: 5,7,2′,6′-tetrahydroxyflavone (**3**) [3], 5,7,2′,5′ tetrahydroxy-8,6′-dimethoxyflavone (**4**) [4], skullcapflavone II (**5**) [5], baicalin (6) [6], baicalin methyl ester (**7**) [3], wogonin 7-*O*-glucronide (**8**) [6] and 3,5,7,2′,6′-pentahydroxyflavanone (**9**) [7] by the analyses of their ^{1}H and ^{13}C NMR spectral data.



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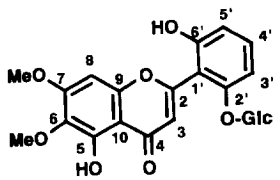

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Compound **1**, obtained as pale yellow amorphous powder, exhibited $[M-H]^-$ peak at *m/z* 521.1292 ($C_{24}H_{25}O_{13}$) in the high resolution negative SIMS. The ^{1}H NMR spectrum (Table 1) of **1** showed three methoxyl (δ3.81, 3.82 and 4.00), a non-coupled olefinic (δ6.35), ABC-type aromatic (δ6.69, 6.79 and 7.25) and chelated hydroxyl (δ12.70) proton signals. This signal pattern resembled that of **5** which has 5(OH), 6,7,8,2′,6′-substituted flavone structure. In the ^{1}H NMR spectrum of **1**, one anomeric proton (δ5.00) signal was also observed, indicating the presence of one sugar moiety. The ^{13}C NMR spectrum of **1** (Table 2) exhibited the signals arising from one flavone (15 carbons, C-2 ~ 10 and 1′ ~ 6′) and a hexose (δ63.0, 71.7, 74.9, 78.3, 78.4 and 107.9) whose chemical shifts were in good agreement with those of β-D-glucose.

The ^{1}H–^{1}H NOESY spectrum of **1** revealed a NOE correlation cross-peak between the glucose C-1 and C-3′ (B-ring) protons suggesting that the glucose moiety was linked to the C-2′ position. In the NOESY spectrum, there was no cross peak between three methoxyl and

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Table 1. ^{1}H NMR spectral data of **1** and **2** at 270 MHz (δ values) (in acetone-d_6 + D_2O)

H	1	2
3	6.35 1H, *s*	6.35 1H, *s*
8	—	6.41 1H, *s*
3′	6.79 1H, *d* (8)	6.74 1H, *d* (8)
4′	7.25 1H, *t* (8)	7.22 1H, *t* (8)
5′	6.69 1H, *d* (8)	6.67 1H, *d* (8)
Glucose		
1	5.00 1H, *d* (7)	4.98 1H, *d* (7)
2	3.20–3.50 *m*	3.19–3.50 *m*
3	3.20–3.50 *m*	3.19–3.50 *m*
4	3.20–3.50 *m*	3.19–3.50 *m*
5	3.20–3.50 *m*	3.19–3.50 *m*
6	3.62 1H, *dd* (12, 6)	3.58 1H, *dd* (12, 6)
	3.75 1H, *dd* (12, 2)	3.78 1H, *dd* (12, 2)
OMe	3.81 3H, *s*	3.85 6H, *s*
	3.82 3H, *s*	
	4.00 3H, *s*	
OH	12.70 1H, *s*	12.70 1H, *s*

Coupling constants (J in Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of **1** and **2** at 67.5 MHz (δ values) (in acetone-d_6 + D_2O)

C	1	2
2	163.2	163.4
3	113.9	113.6
4	184.5	184.1
5	150.7	151.6
6	136.5	130.1
7	154.3	159.9
8	133.6	96.8
9	148.3	157.6
10	108.4	105.7
1′	112.2	111.8
2′	157.8	158.0
3′	111.3	111.1
4′	133.7	133.7
5′	102.4	101.8
6′	158.1	158.2
Glucose		
1	107.9	107.2
2	74.9	74.5
3	78.3	77.7
4	71.7	71.0
5	78.4	77.9
6	63.0	62.5
OMe	61.5	57.3
	62.5	62.3
	63.1	

aromatic (B-ring) protons suggesting that the methoxyl groups were attached to the A-ring. The presence of a characteristic signal for a chelated hydroxyl (δ12.70, at C-5) in the ^{1}H NMR spectrum of **1** confirmed the positions of the three methoxyl groups at C-6, 7 and 8. The position of the hydroxyl group at C-5 was supported also by the bathochromic shift observed in the UV spectrum of **1** on the addition of $AlCl_3$ and $AlCl_3$ + HCl. From the combined spectral data above, **1** was concluded to be 5, 2′,6′-trihydroxy-6,7,8-trimethoxyflavone 2′-*O*-β-D-glucopyranoside .

Compound **2**, a pale yellow amorphous powder, exhibited $[M-H]^-$ peak at m/z 491.1188 ($C_{23}H_{23}O_{12}$) in the high resolution negative SIMS. The ^{1}H NMR spectrum (Table 1) of **2** showed two methoxyl (δ3.85, ×2), an anomeric (δ4.98), one olefinic (δ6.35, *s*), one non-coupled (δ6.41) and ABC-type (δ6.67, 6.74 and 7.22) aromatic proton signals. The ^{13}C NMR spectrum (Table 2) of **2** also indicated the presence of two methoxyls, one D-glucose moiety and a 5,6,7,2′,6′-substituted flavone.

The ^{1}H–^{1}H NOESY spectrum of **2** showed two cross-peaks between glucose C-1 and C-3′ protons and between the methoxyl and C-8 protons. This indicated the positions of the glucose and one of the methoxyl groups at C-2′ and C-7, respectively. The configuration of the glucose C-1 was concluded to β from the J value (7 Hz) of the anomeric signal in the ^{1}H NMR spectrum of **2**. The characteristic signal of a chelated hydroxyl (δ12.70, at C-5) in the ^{1}H NMR spectrum and bathochromic shift observed in the UV spectrum (on addition of $AlCl_3$ and $AlCl_3$ + HCl) suggested the presence of a free hydroxyl group at C-5 position. Therefore, **2** was characterized as 5,2′,6′-trihydroxy-6,7-dimethoxyflavone 2′-*O*-β-D-glucopyransoide.

Although several flavone glucosides have been characterised previously from *S. baicalensis*, **1** and **2** are the first in which the glucose moieties are connected to the B-ring.

EXPERIMENTAL

^{1}H and ^{13}C NMR were measured at 270 and 67.5 MHz, respectively, locked to the major deuterium resonance of the solvent [$(CD_3)_2CO$, C_5D_5N and $(CD_3)_2SO$].

Plant material. The roots of *Scutellaria baicalensis* were collected from the plants grown in the field at Tsukuba Medicinal Plant Research Station. Voucher specimens are deposited at Faculty of Agriculture, Saga University.

Extraction and isolation. Lyophilized roots (219 g) were mashed and extracted at room temp. with 60% aq. MeOH (750, 500, 450 and 400 ml). The extract, after concn under red. pres., was subjected to Sephadex LH-20 (4.5 × 23 cm) CC and eluted first with H_2O with increasing amounts of MeOH and then with EtOH to afford six fractions (Frs 1–6). Fr. 1 was separated by Bondapak C18 Porasil B (H_2O–MeOH) and MCI GEL CHP 20P (H_2O–MeOH) CC to give **6** (185 mg), **7** (6 mg). and **8** (26 mg). Fr. 2 was separated by silica gel ($CHCl_3$–MeOH) and Bondapak C18 Porasil B (H_2O–MeOH) CC to afford **1** (23 mg) and **2** (24 mg). Frs 3–6 were purified by silica gel ($CHCl_3$–MeOH and/or $CHCl_3$–EtOH) CC to

give **5** (189 mg) (from Fr. 3), **4** (78 mg) (from Fr. 4), **9** (161 mg) (from Fr. 5) and **3** (28 mg) (from Fr. 6).

5,2',6'-Trihydroxy-6,7,8-trimethoxyflavone 2'-O-β-D-glucopyranoside (**1**). A pale yellow amorphous powder, $[\alpha]_D^{25}$ -14.6° (MeOH; *c* 0.1) ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; High resolution negative SI MS *m/z* (rel. int): 521.1292 $[M-H]^-$ (65), UV λ_{max}^{MeOH} nm (log ε): 268.0 (4.04); λ + $AlCl_3$ 397.0 (3.33), 279.5 (4.01); λ + $AlCl_3$/HCl 399.5 (3.33), 280.0 (4.02); λ + NaOAc 268.5 (4.08); + H_3BO_3 268.0 (4.09) nm.

5,2',6'-Trihydroxy-6,7-dimethoxyflavone 2'-O-β-D-glucopyranoside (**2**). A pale yellow amorphous powder, $[\alpha]_D^{25}$ − 5.6° (MeOH; *c* 0.2) ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; High resolution negative SI MS *m/z* (rel. int): 491.1188 [M-H]$^-$ (82), UV λ_{max}^{MeOH} (log ε): 264.0 (4.44); + $AlCl_3$: 395.0 (3.83), 274.5 (4.47); + $AlCl_3$/HCl 396.0 (3.83), 274.5 (4.48): + NaOAc 264.0 (4.41): + H_3BO_3 264.0 (4.42) nm.

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