Flavone Glycosides from *Lonicera gracilipes* var. *glandulosa*[1]

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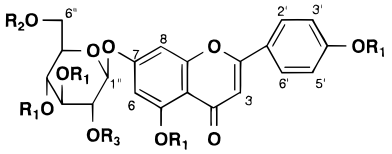
A new flavone glycoside, apigenin 7-*O*-(2^G-rhamnosyl)-gentiobioside (**1**), and 7 known flavone glycosides—cosmosiin, apigenin 7-*O*-neohesperidoside, apigenin 7-*O*-gentiobioside, apigenin 7-*O*-sophoroside, luteolin 7-*O*-glucoside, luteolin 7-*O*-neohesperidoside, and luteolin 7-*O*-gentiobioside—were isolated from the leaves of *Lonicera gracilipes* var. *glandulosa*. Their structures were elucidated on chemical and physicochemical properties.

Our previous studies on the MeOH extract of *Lonicera gracilipes* var. *glandulosa* Maxim. fresh leaves have led to the isolation of three new polyhydric alcohol glycosides[2] and phenolic compounds such as chlorogenic acid derivatives and a new coumarin glycoside.[3,4] This paper reports the isolation and identification of seven known apigenin and luteolin 7-glycosides and a new apigenin 7-*O*-(2^G-rhamnosyl)gentiobioside (**1**).

On the basis of a comparison with reported spectroscopic data, seven known compounds were identified as cosmosiin (**2**), apigenin 7-*O*-neohesperidoside (**3**), apigenin 7-*O*-gentiobioside (**4**), apigenin 7-*O*-sophoroside (**5**), luteolin 7-*O*-glucoside, luteolin 7-*O*-neohesperidoside, and luteolin 7-*O*-gentiobioside.[5−8]



1 R_1=H, R_2=glc, R_3=rha

2 R_1=R_2=R_3=H

3 R_1=R_2=H, R_3=rha

4 R_1=R_3=H, R_2=glc

4a R_1=R_3=Ac, R_2=glc(Ac)$_4$

5 R_1=R_2=H, R_3=glc

Compound **1** was isolated as colorless needles, mp 309−310 °C. The FABMS of **1** showed an ion at *m*/*z* 741[M + H]$^+$ in the high mass region. The UV spectrum showed characteristic flavone absorption at 266 and 334 nm. Hydrolysis of **1** with 5% HCl yielded apigenin, glucose, and rhamnose. The ^{1}H-NMR spectrum showed the presence of a flavone skeleton [δ 6.57 (1H, d, J = 2.2 Hz, H-6), 6.70 (1H, s, H-3), 6.79 (1H, d, J = 2.2 Hz, H-8)], two glucosyl anomeric protons [δ 4.39 (1H, d, J = 7.7 Hz) and 5.26 (1H, d, J = 7.3 Hz)], and a rhamnosyl anomeric proton [δ 5.22 (1H, d, J = 1.5 Hz)]. The two AB-type coupling proton signals, δ 6.99 and 7.90, showed that a hydroxyl group was at C-4 of the B-ring. In the NOESY spectrum of **1**, cross-peaks were observed between the inner glucosyl anomeric proton and H-6 and H-8. Chemical shifts in the ^{13}C-NMR spectrum (in MeOH−d_4) of **1** were compared with those of cosmosiin (**2**), apigenin 7-*O*-neohesperidoside (**3**), and the gentiobiosyl moiety of kaempferol 3-*O*-gentiobioside, and the ^{13}C-NMR assignments were confirmed with the help of DEPT spectrum and ^{13}C−^{1}H COSY. In the HMBC spectrum, cross-peaks were observed between a terminal glucosyl anomeric proton and an inner glucosyl C-6″ at δ 70.1 and between a rhamnosyl anomeric proton and an inner glucosyl C-2″ at δ 79.7. Thus, the structure of **1** was determined to be apigenin 7-*O*-α-L-rhamnopyranosyl(1→2)-*O*-[β-D-glucopyranosyl(1→6)-*O*-β-D-glucopyranoside].

The glucosyl linkage of apigenin 7-diglucoside (**4** and **5**), which is unclear from literature reports,[9,10] has been determined to be 7-*O*-β-D-glucopyranosyl(1→6)-*O*-β-D-glucopyranoside and 7-*O*-β-D-glucopyranosyl(1→2)-*O*-β-D-glucopyranoside, respectively, on the basis of spectroscopic evidence (^{1}H- and ^{13}C-NMR, NOESY, and HMBC). As the crude powder of **4** containing a little impurity did not separate by HPLC, it was then acetylated, and the acetate (**4a**) was purified by chromatography on a Si gel column. The structure of **4a** was confirmed on the basis of spectroscopic evidence.

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively. Chemical shifts are given on the δ (ppm) scale with TMS as internal standard. Preparative HPLC was carried out using an ODS-120A (7.8 mm i.d. × 30 cm) column with UV detector. GC (by column) was performed on a G-column (Chemicals Inspection and Testing, Japan), column length 40 m, column inside diameter 1.2 mm, liquid phase G-205, film thickness 5 μm, carrier gas N_2, flow rate 20 mL/min (0.6 kg/cm^2), column temperature 250 °C, detector FID.

Plant Material. As reported previously.[2]

Isolation and Purification. The general extraction procedure was previously reported. The *n*-BuOH-soluble fraction was concentrated under reduced pressure to afford a residue (15.2 g) that was chromatographed on a Si gel column ($CHCl_3$−MeOH−H_2O, 30:10:1) and a Sephadex LH-20 column (MeOH−H_2O, 1:1) and then subjected to preparative HPLC (MeOH−H_2O, 3:7) to give **1** (10 mg), **2** (8 mg), **3** (15 mg), **4** (13 mg), **5** (7 mg), luteolin 7-*O*-glucoside (21 mg), luteolin 7-*O*-neohesperidoside (16 mg), and luteolin 7-*O*-gentiobioside (12 mg).

Apigenin 7-*O*-(2^G-rhamnosyl)gentiobioside (1): colorless needles; mp 309−310 °C (MeOH); $[\alpha]^{25}_D$ −102° [*c* 0.1, Me_2CO−H_2O (4:1)]; FABMS *m*/*z* 741 [M + H]$^+$;

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UV λ_{max} (MeOH) (log ϵ) 266 (4.17), 334 (4.28) nm; IR ν_{max} (KBr) 3355, 1655, 1623 sh, 1608, 1513, 1495 cm^{-1}; ^1H NMR (CD_3OD) δ 1.31 (3H, d, J = 6.2 Hz, H-6‴), 3.99 (1H, dd, J = 3.3, 1.8 Hz, H-2‴), 4.18 (1H, d, J = 9.9 Hz, H_A-6″), 4.39 (1H, d, J = 7.7 Hz, H-1‴), 5.22 (1H, d, J = 1.8 Hz, H-1⁗), 5.26 (1H, d, J = 7.3 Hz, H-1″), 6.57 (1H, d, J = 2.2 Hz, H-6), 6.70 (1H, s, H-3), 6.79 (1H, d, J = 2.2 Hz, H-8), 6.99 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.90 (2H, d, J = 8.8 Hz, H-2′ and H-6′); ^{13}C NMR (CD_3OD) δ 18.0 (C6⁗), 62.5 (C-6‴), 70.1 (C-6″ and C-5⁗), 71.0 (C-4‴), 71.4(C-4″), 71.8 (C-2⁗ or C-3⁗), 71.9 (C-2⁗ or C-3⁗), 73.6 (C-4⁗), 74.8 (C-2‴), 76.9 (C-5‴), 77.6 (C-3″ or C-5″), 77.7 (C-3″ or C-5″), 78.2 (C-3‴), 79.7 (C-2″), 96.3 (C-8), 99.6 (C-1″), 101.0 (C-6 and C-1‴), 102.5 (C-1⁗), 104.6 (C-3), 107.1 (C-10), 117.2 (C-3′ and C-5′), 123.0 (C-1′), 129.7 (C-2′ and 6′), 158.8 (C-9), 162.9 (C-4′), 163.0 (C-5), 164.0 (C-7), 167.0 (C-2), 184.1 (C-4). Acid hydrolysis of **1** with 5% HCl yielded apigenin, D-glucose, and L-rhamnose. Apigenin was identified by HPLC, and the sugars were determined as their TMSi ether derivatives by gas chromatography. L-Rhamnose had a t_R of 3.9, 5.2 min (standard, for L-rhamnose, t_R 3.9, 5.2 min); D-glucose had a t_R of 10.0, 12.6 min (standard for D-glucose, t_R 10.0, 12.6 min).

Apigenin 7-*O*-gentiobioside (4). Compound **4** was a white powder. The ^1H-NMR spectrum of the crude powder of **4** showed the absence of acetyl groups. The crude powder of **4** was acetylated with Ac_2O in pyridine, and the nonaacetate (**4a**) was purified by chromatography on a Si gel column with C_6H_6−EtOAc (7:3). Compound **4a**: colorless needles; mp 258−260 °C (MeOH); $[\alpha]^{20}_D$ −31.8° (*c* 1.0, $CHCl_3$); FABMS *m*/*z* 973 $[M + H]^+$; UV λ max (MeOH) (log ϵ) 255 (4.24), 301 (4.33) nm; IR ν_{max} ($CHCl_3$) 1757, 1642, 1616, 1507 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.64, 1.80, 1.94, 2.01, 2.04, 2.06, 2.07, 2.35, 2.46 (each 3H, s, CH_3CO × 9), 3.70 (2H, m, H-6″ and H-5⁗), 3.95 (2H, m, H-6″ and H-5″), 4.14 (1H, dd, J = 12.5, 2.2 Hz, H-6⁗), 4.25 (1H, dd, J = 12.5, 4.5 Hz, H-6⁗), 4.53 (1H, d, J = 8.1 Hz, H-1⁗), 5.20 (1H, d, J = 7.0 Hz, H-1″), 6.59 (1H, s, H-3), 6.69 (1H, d, J = 2.2 Hz, H-6), 7.00 (1H, d, J = 2.2 Hz, H-8), 7.29 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 7.93 (2H, d, J = 8.8 Hz, H-2′ and H-6′); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5 (×2), 20.6 (×3), 20.7, 21.1, 21.2 (each *C*H_3CO), 61.8 (C-6⁗), 68.3 (C-4⁗), 68.4 (C-6″), 68.7 (C-4″), 70.9 (C-2″ and C-2⁗), 72.1 (C-5⁗), 72.5 (C-3″ or C-3⁗), 72.6 (C-3⁗ or C-3″), 73.4 (C-5″), 98.1 (C-1″), 100.9 (C-1⁗), 102.3 (C-8), 108.6 (C-6), 109.4 (C-3), 112.9 (C-10), 122.4 (C-3′ and C-5′), 127.7 (C-2′ and C-6′), 128.9 (C-1′), 150.8 (C-5), 153.3 (C-4′), 158.5 (C-9), 160.1 (C-7), 161.8 (C-6), 168.9, 169.2, 169.4, 169.5, 169.7, 170.1 (× 2), 170.6 (× 2) (each CH_3CO), 176.3 (C-4).

Apigenin 7-*O*-sophoroside (5): white powder; mp 267−268 °C; $[\alpha]^{23}_D$ −56.6° [*c* 01., Me_2CO−H_2O (4:1)]; FABMS *m*/*z* 595 $[M + H]^+$; acid hydrolysis, apigenin and D-glucose; UV λ_{max} (MeOH) (log ϵ) 266 (4.41), 330 (4.35) nm; IR ν_{max} (KBr) 3375, 1657, 1608, 1600 sh, 1569, 1500 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.49 (1H, d, J = 7.3 Hz, H-1‴), 5.22 (1H, d, J = 7.3 Hz, H-1″), 6.43 (1H, d, J = 2.0 Hz, H-6), 6.83 (1H, d, J = 2.0 Hz, H-8), 6.85 (1H, s, H-3), 6.96 (2H, d, J = 8.9 Hz, H-3′ and H-5′), 7.97 (2H, d, J = 8.9 Hz, H-2′ and H-6′); ^{13}C NMR (DMSO-d_6) δ 60.0 (C-6″ and C-6⁗), 69.3 (C-4″ and C-4⁗), 73.9 (C-2⁗), 75.5 (C-3⁗), 75.6 (C-3″), 76.7 (C-5⁗), 78.9 (C-5″), 81.8 (C-2″), 94.8 (C-8), 98.2 (C-1″), 99.4 (C-6), 103.0 (C-1⁗), 104.7 (C-3), 105.3 (C-10), 115.9 (C-3′ and C-5′), 120.9 (C-1′), 128.6 (C-2′ and C-6′), 156.8 (C-9), 161.0 (C-4′), 162.6 (C-5), 164.3 (C-7), 164.4 (C-2), 181.9 (C-4).

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References and Notes

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