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FLAVONOID GLUCOSIDES FROM LICORICE

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Key Word Index—*Glycyrrhiza uralensis*; Leguminosae; licorice; flavonoid glycosides; liquiritigenin 4′-*O*-*β*-apiofuranosyl(1 → 2)-*β*-glucopyranoside; liquiritigenin 7,4′-diglucoside; apigenin 6,8-di-*C*-glucoside.

Abstract—Two new flavanone glycosides, liquiritigenin 4′-apiosyl(1 → 2)-glucoside and liquiritigenin 7,4′-diglucoside together with a known flavone, apigenin 6,8-di-*C*-glucoside, have been isolated from licorice.

Licorice, the dried root of *Glycyrrhiza uralensis* (Leguminosae), is prescribed in many Chinese traditional medicines as a flavouring, a diluting agent and an anti-inflammatory agent. It has been reported to contain triterpenoids (glycyrrhizin, etc.) and a variety of flavonoids [1–4] (isoflavonoids, chalcones and flavones). As part of our chemical examination of phenolic constituents in Chinese crude drugs, we have undertaken a further analysis of licorice. This has resulted in the isolation and structural characterization of two new flavanone glycosides (**1** and **2**), together with apigenin 6,8-di-*C*-glucoside (**3**).

Compound **1** showed UV absorptions at 274 and 312 nm, characteristic of flavanones. The ^{1}H NMR spectrum of **1** exhibited two anomeric proton signals at δ 5.38 (*br s*) and 4.95 (*d*, J = 7 Hz). Enzymatic hydrolysis of **1** with crude hesperidinase [5] yielded liquiritigenin (7,4′-dihydroxy flavanone) (**1a**), glucose and apiose. The sugar sequence and the configuration in **1** were determined by the analogy of the chemical shifts (Table 1) with those of the corresponding chalcone glycoside licurazid [6], which contains a *β*-apiofuranosyl(1 → 2)-*β*-glucopyranosyl moiety. The location of the sugar moiety in **1** was confirmed by comparison of the ^{13}C NMR spectrum of **1** with that of **1a**. On going from **1a** to **1**, the carbon resonances of C-1′, C-3′ and C-5′ in the flavanoid B-ring were displaced downfield by 3.0, 0.9 and 0.9 ppm, respectively, while the carbon resonances arising from the A- and C-rings remained unchanged. From these observations, the structure of **1** was assigned as liquiritigenin 4′-*O*-*β*-apiofuranosyl(1 → 2)-*β*-glucopyranoside.

Compound **2** showed UV absorption similar to **1** (270 and 313 nm). Enzymatic hydrolysis of **2** yielded **1a** and glucose. The occurrence of two *β*-glucosyl moieties in **2** was deduced from the fact that its ^{1}H NMR spectrum showed two anomeric proton signals at δ4.99 and 4.90 (each *d*, J = 7 Hz). Furthermore, these two glucose residues were shown to be attached to the A- and B-rings in the flavanoid skeleton by comparison of the ^{13}C NMR resonances in **2** with those of **1a**; the carbon resonances of C-6, C-8, C-4a, C-1′, C-3′ and C-5′ in **2** were shifted downfield by 0.4, 0 9, 1.7, 2.7, 1.0 and 1.0 ppm, respectively. Accordingly, the structure of **2** was determined as liquiritigenin 7,4′-di-*O*-*β*-glucopyranoside.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR and ^{13}C NMR spectra were recorded at 100 and 25 05 MHz, respectively, and chemical shifts are given in the δ (ppm) scale with TMS as internal standard. TLC was performed on silica gel and compounds were detected by

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Table 1 ^{13}C NMR spectral data of **1a**, **1**, **2** and **4***

	1a	1	2		4	
2	78.7	78.4	78.6		142.9	C-α
3	43.0	42.9	42.9		118.9	β
4	189.6	189.4	189.8		190.7	7′
5	128.1	128.0	127.7		132.5	1′
6	110.2	110.2	110.6		108.4	2′
7	164.2	164.1	163.0		165.6	3′
8	102.3	102.2	103 2		102.4	4′
8a	162.7	162.6	162 3		165.6	5′
4a	113 3	113.2	115.0		112 4	6′
1′	129.0	132.0	131.7		128.1	1
2′	127.9	127 7	127.7		130.4	2
3′	114.8	115.7	115.8		116.1	3
4′	157.8	156.9	157.0		158.8	4
5′	114.8	115.7	115.8		116.1	5
6′	127 8	127 7	127.7		130.4	6
glc						
1		98 3	99.4	100.0	98.1	
2		76.6	72.9	72.9	76.7	
3		75.5	76.3	76.3	75.6	
4		69.7	69.3	69.5	69.9	
5		75.8	76.8	76.1	75.9	
6		60.4	60.4	60.4	60.4	
api[7]						
1		108.3			108.4	
2		76.6			76.7	
3		79.0			79.0	
4		73.7			73.8	
5		64.0			64.1	

*Measured in DMSO-d_6 at 25.05 MHz with TMS as internal standard. δ values in ppm.

spraying with 10% H_2SO_4 and heating. Sugars were detected on PC by aniline hydrogen phthalate reagent.

Isolation. The aq. suspension of MeOH extracts of licorice (commercial name: Tohoku-kanzo in Japanese; *Glycyrrhiza uralensis* Fisch. et DC.) [8] was successively extracted with EtOAc and *n*-BuOH. The *n*-BuOH layer was evapd under red. pres. to give a residue (60 g), which was chromatographed over Sephadex LH-20. Elution with H_2O containing increasing amounts of MeOH yielded five fractions. Fraction 2 was separated by MCI gel CHP20P (high-porosity polystyrene gel) using EtOH–H_2O (1:4) and then by Avicel cellulose CC eluted with H_2O to give **2** (44 mg) and **3** (162 mg). MCI gel CHP20P chromatography of fraction 3 using MeOH–H_2O (1:1) afforded **1** (280 mg). Fraction 1 contained triterpenoid glycosides, while fractions 4 and 5 contained known flavonoid glycosides.

Liquiritigenin 4′-apiosyl(1 → 2)glucoside (**1**). An amorphous, white powder; $[\alpha]_D^{19}$ − 79.6° (MeOH; *c* 1.37). Found. C, 55.76: H, 5.62. $C_{25}H_{30}O_{13}$ requires: C, 56.25; H, 5.48%. CD (MeOH; *c* 1.8 × 10^{-3}) $[\theta]^{20}$ (nm): + 5.69 × 10^3 (326), 0 (315), − 8.67 × 10^3(297). UV λ_{max}^{MeOH} nm (log ε): 274 (4.25), 312 (4.04). FDMS *m/z*: 573 $[M+Na]^+$, 551 $[M+1]^+$, 133 [pentose]$^+$ ^{1}H NMR (DMSO-d_6): δ 7.64 (1H, *d*, *J* = 8 Hz. H-5), 7.43 (2H, *d*, *J* = 8 Hz, H-2′, H-6′), 7.02 (2H, *d*, *J* = 8 Hz, H-3′, H-5′), 6.52 (1H, *dd*, *J* = 2, 8 Hz, H-6), 6.37 (1H, *d*, *J* = 2 Hz, H-8), 5.52 (1H, *dd*, *J* = 3, 12 Hz, H-2), 5.38 (1H, *br s*, api H-1). 4.95 (1H, *d*, *J* = 7 Hz, glc H-1), 3.15 (1H, *dd*, *J* = 12, 17 Hz, H-3), 2 67 (1H, *dd*, *J* = 3, 17 Hz, H-3).

Enzymatic hydrolysis of **1**. **1** (30 mg) in aq. soln was incubated with crude hesperidinase (30 mg) at 40° for 16 hr. The reaction mixture was extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over dry Na_2SO_4 and evapd to dryness to yield colourless needles (EtOH, 6.3 mg, mp 208°, $[\alpha]_D^{18}$ − 35.0° (MeOH; *c* 0.60), which were shown to be identical to **1a**. The aq. layer was concd and run on PC giving apiose (R_f 0.42; *n*-BuOH–pyridine–H_2O, 6:4:3) and glucose (R_f 0.23).

Liquiritigenin 7,4′-diglucoside (**2**). An amorphous powder, $[\alpha]_D^{19}$ − 94.9° (MeOH; *c* 1.1). Found: C, 54.30; H, 5.56 $C_{27}H_{32}O_{14}\cdot H_2O$ requires: C, 54 16; H, 5.73%. CD (MeOH; *c* 2.08 × 10^{-3}) $[\theta]^{20}$ (nm). + 7 25 × 10^2 (334), 0 (325), − 2.08 × 10^3 (307). UV λ_{max}^{MeOH} nm (log ε): 270 (4.36), 313 (3.97). FDMS *m/z*: 603 $[M+Na]^+$, 581 $[M+1]^+$, 163 [hexose]$^+$ ^{1}H NMR (DMSO-d_6): δ 7.72 (1H, *d*, *J* = 8 Hz, H-5), 7.45 (2H, *d*, *J* = 8 Hz, H-2′, H-6′), 7 06 (2H, *d*, *J* = 8 Hz, H-3′, H-5′), 6.75 (1H, *dd*, *J* = 2, 8 Hz, H-6), 6.68 (1H, *br s*, H-8), 5.59 (1H, *dd*, *J* = 3, 12 Hz, H-2), 4.99 and 4.90 (each 1H, *d*, *J* = 7 Hz, glc H-1), 2.73 (1H, *dd*, *J* = 3, 17 Hz, H-3).

Enzymatic hydrolysis of **2**. **2** (10 mg) in aq. soln was incubated with crude hesperidinase (5 mg) as for **1** and **1a** and glucose were detected

Apigenin 6,8-di-C-glucoside (**3**) Pale yellow needles (H_2O), mp 245–247°, $[\alpha]_D^{19}$ + 37.1° (pyridine; *c* 0 43) **3** was identified by UV, ^{1}H NMR and ^{13}C NMR spectral analyses.

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