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IDENTIFICATION OF A NEW FLAVONE GLYCOSIDE FROM *Codonopsis nervosa*

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*A new flavone glycoside, luteolin 7-*O*-[(6‴-caffeoyl)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**1**), was isolated from* Codonopsis nervosa*, along with three other known compounds, luteolin 7-*O*-β-D-glucopyranoside (**2**), luteolin 7-*O*-gentiobioside (**3**), and tangshenoside VI (**4**). Their structures were determined on the basis of 1D and 2D NMR, IR, and HR-ESI-MS.*

Keywords: *Codonopsis nervosa*, flavone glycoside, luteolin.

Codonopsis nervosa Chipp Nannf is a plant growing mainly in eastern Tibet, southeastern Qinghai, western Sichuan, northwestern Yunnan, and southeastern Gansu provinces in China at 3500–4500 m elevation range [1]. To the best of our knowledge, no phytochemical investigation of this plant has previously been undertaken. This paper reports the isolation and structural elucidation of a new flavone glycoside, luteolin 7-*O*-[(6‴-caffeoyl)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (**1**), along with three known compounds obtained from the aerial parts of this plant.

The dried aerial parts (4.0 kg) of *Codonopsis nervosa* were extracted with 95% EtOH to give the crude extract (0.4 kg). The crude extract was then suspended in H_2O (50°C) and partitioned successively with petroleum ether, $CHCl_3$, ethyl acetate, and *n*-BuOH. Compounds **1**, **2**, **3**, and **4** were obtained from the *n*-BuOH-soluble fraction using column chromatography. The structures of compounds **2**, **3**, and **4** were confirmed as luteolin 7-*O*-*β*-D-glucopyranoside (**2**), luteolin 7-*O*-gentiobioside (**3**), and tangshenoside VI (**4**) by comparison of their physical properties and spectral data with the reported data of the known compounds [2–4].

Compound **1** was obtained as a yellow amorphous powder. The HR-ESI-MS showed $[M + Na]^+$ at *m/z* 795.1777, corresponding to the pseudomolecular formula $C_{36}H_{36}O_{19}Na$ $[M + Na]^+$, which requires *m/z* 795.1777. The ^{13}C NMR spectrum showed 36 carbon resonances. Further DEPT experiments revealed that these 36 carbons were composed of two methylene groups, 21 methine groups, and 13 quarternary carbon groups. The ^1H NMR spectrum indicated the presence of two doublets (1H, δ 6.53, J = 1.6 Hz and 1H, δ 6.77, J = 1.6 Hz), which were typical of the two *meta*-related H-6 and H-8 protons of ring A of the flavonoid unit. The ABX system of ring B (δ 7.43, dd, J =7.6, J = 2.0 Hz; δ 7.39, d, J = 2.0 Hz; δ 6.88, d, J = 7.6 Hz), with the H-3 singlet at δ 6.67, led to the identification of the aglycon as luteolin. A second aromatic ABX system (δ 6.91, dd, J = 8.4, 1.6 Hz; δ 6.70, d, J = 8.4 Hz; δ 6.98, br.s), together with two coupled doublets (J = 15.8 Hz) at δ 6.21 and 7.39 and the signal of an ester C=O at δ_C 166.9 from the ^{13}C NMR, revealed the presence of a *trans*-caffeoyl moiety. The remaining 12 resonances of the ^{13}C NMR spectrum of **1** were two *β*-D-glucopyranoses 1→6 linked to form the gentiobiose determined by the downfield shift of the inner sugar methylene (C-6″). The caffeoyl group was located at C (6‴) of the Glc moiety due to the downfield shift observed for this proton in the ^1H NMR spectrum. This was also corroborated by the HMBC cross-peaks CH_2 (6‴)/C (*α*) (Fig. 1). The attachment of the gentiobiose moiety at C (7) was established by the HMBC cross-peak H-C (1″)/C (7). Therefore, **1** is luteolin 7-*O*-[(6‴-caffeoyl)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside.

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TABLE 1. NMR Data of Compound **1** (^{1}H: 400 MHz; ^{13}C:100 MHz, DMSO-d_6)

C atom	δ_H (J/Hz)	δ_C	C atom	δ_H (J/Hz)	δ_C
2		165.0	4″	3.31 (br.s)	70.0
3	6.67 (s)	102.7	5″	3.75 (q)	75.6
4		182.0	6″	3.68 (q)	69.7
5		157.1	Glc-1‴	4.28 (d, J = 7.6)	104.2
6	6.53 (d, J = 1.6)	99.8	2‴	3.09 (q)	73.6
7		163.0	3‴	3.17–3.27 (m)	76.6
8	6.77 (d, J = 1.6)	95.0	4‴	3.17–3.27 (m)	70.1
9		161.4	5‴	3.40 (q)	74.2
10		105.5	6‴	4.13–4.18 (m)	63.7
1′		121.5	Caffeoyl-1⁗		125.5
2′	7.39 (d, J = 2.0)	113.6	2⁗	6.98 (br.s)	115.2
3′		145.8	3⁗		146.0
4′		152.0	4⁗		149.0
5′	6.88 (d, J = 7.6)	116.2	5⁗	6.70 (d, J = 8.4)	115.9
6′	7.43 (dd, J = 7.6, J = 2.0)	119.4	6⁗	6.91 (dd, J = 8.4, J = 1.6)	121.5
Glc-1″	5.02 (d, J = 6.8)	100.2	C-α	6.21 (d, J = 15.8)	113.6
2″	3.31 (br.s)	73.4	C-β	7.39 (d, J = 15.8)	145.8
3″	3.17–3.27 (m)	76.8	C=O		166.9

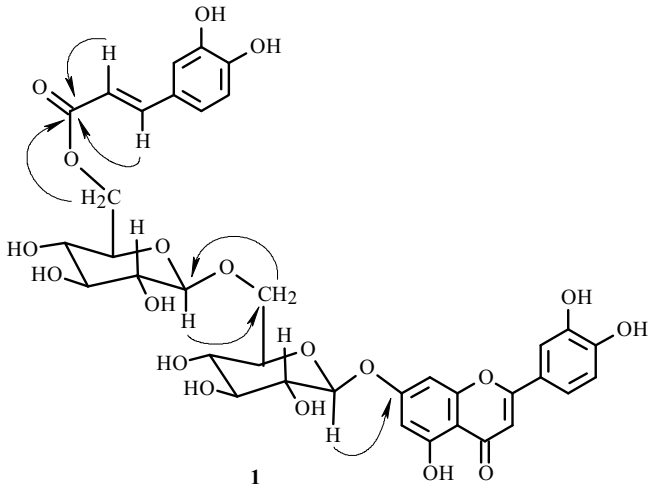

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Fig. 1. Key HMBC correlations of compound **1**.

EXPERIMENTAL

Instruments and Materials. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. IR was measured with a ThermoFisher Nicolet 6700 spectrometer using KBr pellets in cm^{-1}. ^{1}H and ^{13}C NMR spectra were taken on a Bruker AC-E 200 and Varian Unity INOVA 400/54 NMR spectrometers, in DMSO-d_6 with TMS as internal standard. HR-ESI-MS were recorded on a VG Auto Spec 3000 or Finnegan MAT 90 instrument. Column chromatography was performed on silica gel G and H (Qingdao Ocean Chemical Factory, China), RP-18 silica gel (Merck), Sephadex LH-20 (Merck), and D-101 macroporous resin (Rohm & Haas).

The plant *Codonopsis nervosa* Chipp Nannf was collected in Tibet, China, in September 2008. A voucher specimen was identified by Associate Senior Pharmacist GeSangSuoLang of the Tibetan Institute of Food and Drug Control.

Extraction and Isolation. Aerial parts of *Codonopsis nervosa* Chipp Nannf (4 kg) were air dried and extracted with 95% EtOH three times at room temperature with each process lasting a week. After removal of the EtOH by evaporation, the resulting residue (0.4 kg) was suspended in H_2O (50°C) and then partitioned successively with petroleum ether (7 × 5 L), $CHCl_3$ (5 × 5 L), EtOAc (5 × 5 L), and *n*-BuOH (6 × 5 L) to yield the crude material 126, 3, 12, and 65 g, respectively. The *n*-BuOH fraction (65 g) was then dissolved in H_2O and filtered. The filtrate was purified on a D-101 macroporous resin column (10 × 40 cm) and eluted successively with H_2O and 25%, 50%, 75%, and 95% (*v/v*) EtOH to afford five fractions (A–E).

Fraction C was applied to an RP-18 silica gel column with MeOH–H_2O 1:9–1:0 to provide fractions C-1–C-7. Fraction C-3 was then applied to another RP-18 silica gel column with MeOH–H_2O 1:1, then to a Sephadex LH-20 column with H_2O as the eluent to furnish **1** (25 mg), **2** (21 mg), and **3** (26 mg). In a similar manner, fraction C-4 was applied to an RP-18 silica gel column with MeOH–H_2O 1:1, then to a Sephadex LH-20 column with MeOH–H_2O 1:1 to produce **4** (60 mg).

Luteolin 7-*O*-[(6‴-caffeoyl)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (1). Yellow amorphous powder. $[\alpha]_D^{20}$ –20° (*c* 0.16, MeOH). IR (KBr, ν, cm^{-1}): 3367, 2925, 2855, 1695, 1656, 1606, 1496, 1446, 1383, 1344, 1302, 1260, 1176, 1115, 1069, 846, 820, 770, 712, 690, 630, 595, 521. ^1H NMR and ^{13}C NMR, see Table 1. HR-ESI-MS $[M + Na]^+$ *m/z* 795.1777, calcd for $C_{36}H_{36}O_{19}Na$ $[M + H]^+$, 795.1777.

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