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A NEW FLAVONOID GLYCOSIDE FROM *Galium verum*

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*A new flavonoid glycoside, an apigenin 7-*O*-(3,4-di-*O*-acetyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**1**), was isolated from the 95% ethanol extract of* Galium verum *L. Its structure was elucidated by spectroscopic analysis.*

Keywords: Rubiaceae, *Galium verum* L., flavonoid glycoside.

Galium verum L. (Rubiaceae), widely distributed in China, is often used as natural dyestuff and food additive. As traditional Chinese medicine, it is often used for the treatment of phlebophlogosis and hepatitis [1]. Phytochemical investigations of *G. verum* L. have led to the isolation of several kinds of bioactive compounds such as iridoids, anthraquinones, chlorogenic acids, and flavonoids [2–5].

In our recent research, a new flavonoid glycoside, apigenin 7-*O*-(3,4-di-*O*-acetyl)-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**), was obtained. In this paper, we report the isolation and structure elucidation of this rarely acetylated flavonoid glycoside.

The molecular formula of **1** was determined to be $C_{31}H_{34}O_{16}$ on the basis of the HR-ESI-MS pseudo-ion peak at *m*/*z* 685.1742 $[M + Na]^+$ (calcd for $C_{31}H_{34}O_{16}Na$, 685.1745), and ESI-MS *m*/*z:* 685.3 $[M + Na]^+$, 661.2 $[M - H]^-$.

The IR spectrum (KBr) of **1** showed absorption bands for hydroxyl, conjugated carbonyl, and aromatic functional groups.

The ^{1}H NMR spectrum of **1** exhibited 1,4-disubstituted benzene signals at δ 7.87 (2H, d, J = 8.3, H-2′, 6′) and 6.92 (2H, d, J = 8.3, H-3′, 5′), *meta*-coupled aromatic proton signals at δ 6.48 (1H, d, J = 1.9, H-6) and 6.74 (1H, d, J = 1.9, H-8), and an aromatic proton signal at δ 6.64 (1H, s, H-3). The ^{13}C NMR spectrum disclosed 31 carbon signals, including 15 aromatic carbon signals for the flavone aglycone and 12 signals for the sugar moieties and two acetyl moieties at δ 173.1 (2 × $OCOCH_3$) and 21.0 (2 × $OCOCH_3$). Acid hydrolysis of **1** resulted in release of D-glucose and L-rhamnose, which was identified by HPTLC comparison of the hydrolysate with an authentic sample. The configurations of the glucose and rhamnose residues in **1** were assigned as *β*- and *α*-, based on the coupling constant of the anomeric protons at δ 5.07 (1H, d, J = 7.4, H-1″) and 4.70 (1H, d, J = 1.9, H-1‴).

On the basis of the above analyses of spectral data, the flavone aglycone was suggested as 5,7,4′-trihydroxyflavone. The ^{1}H NMR spectrum also showed two methyl proton signals at δ 1.99 (3H, s, 3‴-$OCOCH_3$) and 1.92 (3H, s, 4‴-$OCOCH_3$), and these methyl groups were assigned to the two acetyl moieties, which were connected to the C-3‴ and C-4‴ sites of the rhamnose unit through an oxygen atom by the HMBC correlations of H-3‴/3‴-O*C*OCH_3 and H-4‴/4‴-O*C*OCH_3, respectively. In the HMBC spectrum (Fig. 1), long-range correlations of H-1‴/C-6″ and H-6″/C-1‴ established a 1→6 linkage between the *α*-L-3,4-di-*O*-acetylrhamnopyranosyl unit and the *β*-D-glucopyranosyl moiety, and the correlation of H-1″/C-7 indicated that the sugar chain was connected to the C-7 position of the flavone aglycone through an oxygen atom. The ^{1}H NMR and ^{13}C NMR spectral data of **1**, supported by TOCSY, HMQC, and HMBC experiments, permitted assignment of all proton and carbon resonances. Consequently, compound **1** was determined to be apigenin 7-*O*-(3,4-di-*O*-acetyl)-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

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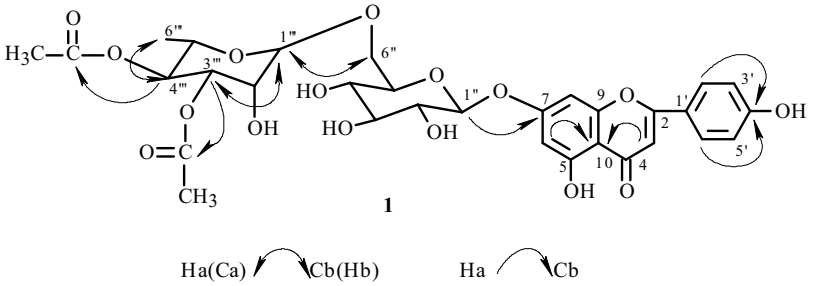

Fig. 1. Key HMBC correlations of compound **1**.

EXPERIMENTAL

The UV-Vis spectrum was performed on a Shimadzu UV-260 instrument. The IR spectrum was performed on a Bruker IR S-55 instrument. NMR spectra were recorded on a Bruker-ARX-600 spectrometer, using TMS as an internal standard. ESI-MS was performed on a Finnigan LCQ mass spectrometer. HR-ESI-MS was performed on a QSTARLCQ mass spectrometer. The optical rotation was measured on a Perkin–Elmer 241 polarimeter. Silica gel: 200–300 mesh, Qingdao Ocean Chemical Group Co. Ltd., P. R. China. TLC: HSGF254 precoated silica gel plates, 10–40 μm, Yantai Chemical Plant, Yantai, P. R. China. Sephadex LH-20 gel: Pharmacia.

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