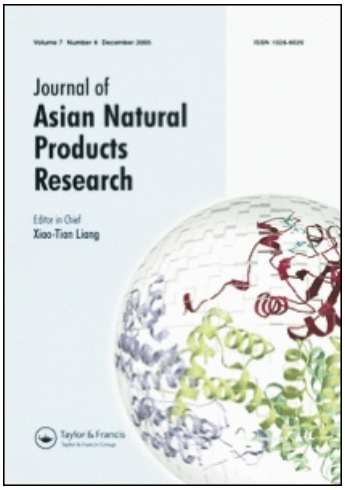

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ORIGINAL ARTICLE

Two new glycosides from *Leonurus japonicus*

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Two new glycosides, 1,6-di-*O*-syringoyl-β-D-glucopyranose (**1**) and quercetin 3-*O*-[(3-*O*-syringoyl-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranoside] (**2**), along with seven known compounds were isolated from the MeOH extract of *Leonurus japonicus*. The structures of these compounds were elucidated by spectral analysis.

Keywords: *Leonurus japonicus*; Labiatae; glycoside

1. Introduction

The whole plant of *Leonurus japonicus* Houtt (Labiatae) is a tonic and an antipyretic, and has been used in the treatment of cardiovascular, puerperal, and menstrual diseases in Chinese medicine [1]. Several alkaloids, diterpenes, and flavonoids have been isolated from related species in the same family [2–8]. In the course of our continuing study of the bioactive natural products, we investigated the aerial parts of *L. japonicus*. From this source, two new phenolic glycosides, 1,6-di-*O*-syringoyl-β-D-glucopyranose (**1**) and quercetin 3-*O*-[(3-*O*-syringoyl-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranoside] (**2**) (Figure 1), were isolated together with 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one [9], 6β-hydroxy-15,16-epoxylabda-8,13(16),14-trien-7-one [9,10], genkwanin [11], apigenin [12], quercetin [13], tiliroside [14], and uracil [15]. The present paper describes the isolation and structural elucidation of these two new glycosides. The pharmacological study revealed that compound **2** exhibited antioxidative activity.

2. Results and discussion

Compound **1** was obtained as a colorless powder and gave a molecular formula of $C_{24}H_{28}O_{14}$ according to HR-FAB-MS. The ^{1}H NMR spectrum of **1** displayed one set of sugar signals at δ 3.49, 3.57 (2H), 3.81, 4.34, 4.70, and 5.71 (1H, d, $J = 8.0$ Hz, H-1), and their corresponding carbon signals appeared at δ 63.9, 70.7, 72.8, 75.3, 76.8, and 95.1 in the ^{13}C NMR spectrum of **1**, which suggested the presence of a β-linked glucopyranosyl unit in the structure of **1**. In addition, its ^{1}H NMR spectrum showed one singlet for four methoxy groups at δ 3.88 (3′,5′,3″,5″-OCH_3) and two sets of symmetric singlets

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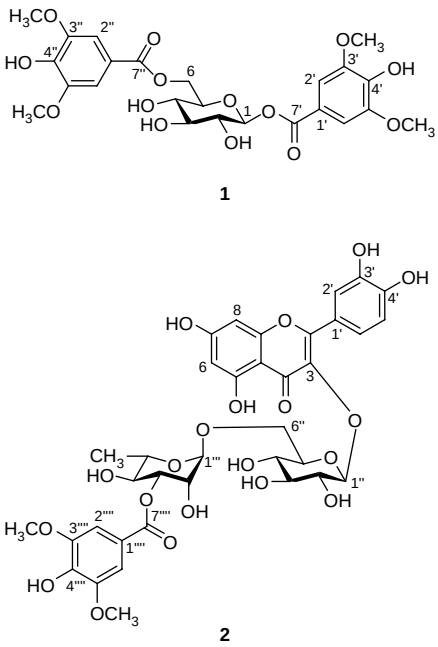

Figure 1. Structures of compounds **1** and **2**.

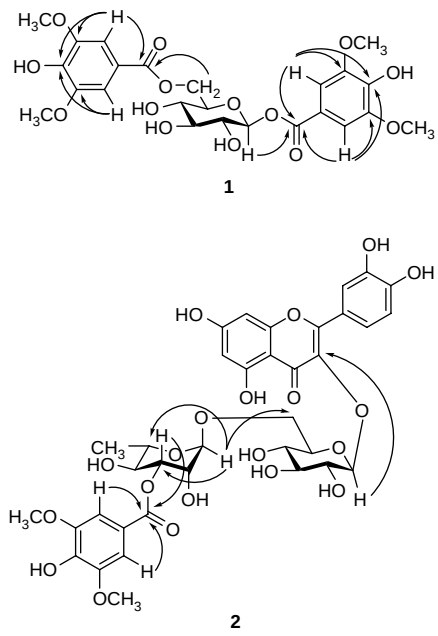

Figure 2. Key HMBC correlations of **1** and **2**.

at δ 7.33 (2H, s, H-2′, 6′) and 7.37 (2H, s, H-2″, 6″) attributed to a pair of 1,3,4,5-tetrasubstituted aromatic rings. The ^{13}C NMR spectrum exhibited methoxy signals at δ 55.7, eight aromatic carbon signals at δ 107.1, 107.4, 119.2, 120.0, 140.9, 141.5, 147.7, and 147.8, and two carbonyl carbon signals at δ 165.5 and 166.8 except for the carbon signals from a glucopyranosyl unit. The HMBC correlations (Figure 2) from H-2′ (H-2″) and H-6′ (H-6″) to C-3′–C-5′ (C-3″–C-5″) and carbonyl carbon C-7′ (C-7″) as well as from the methoxy protons 3′,5′-OCH_3 (3″,5″-OCH_3) to C-3′ (C-3″) and C-5′ (C-5″) indicated that there were two syringoyl groups in the structure of **1**. Since HMBC correlations were observed between H-1 and C-7′ as well as between H_2-6 and C-7″, these two syringoyl groups were assigned to be at C-1 and C-6 positions of the glucopyranosyl portion. Based on the above data, compound **1** was elucidated to be 1,6-di-*O*-syringoyl-β-D-glucopyranose.

Compound **2** was obtained as a yellow powder and gave a molecular formula of $C_{36}H_{38}O_{20}$, according to HR-FAB-MS. Its spectral characteristics showed the existence of a quercetin core, a syringoyl moiety, and two six-membered sugar units. In the ^{1}H NMR spectrum, one broad singlet at δ 12.56 (5-OH) for a chelated hydroxyl group, a pair of *m*-coupled doublets at δ 6.16 (J = 2.0 Hz, H-6) and 6.38 (J = 2.0 Hz, H-8), and one set of ABX-type signals at δ 6.81 (1H, d, J = 8.4 Hz, H-5′), 7.52 (1H, d, J = 2.0 Hz, H-2′), and 7.65 (1H, dd, J = 2.0, 8.4 Hz, H-6′) were attributed to the protons of the quercetin moiety. The presence of a syringoyl moiety was deduced by the observation of the proton signals at δ 3.79 (6H, s, 3‴, 5‴-OCH_3), 7.25 (2H, s, H-2‴, 6‴) and the carbon signals at δ 107.4 (C-2‴, 6‴), 120.0 (C-1‴), 140.6 (C-4‴), 147.5 (C-3‴, 5‴), 165.5 (C-7‴). The remaining 12 carbons and their corresponding proton signals suggested the presence of

a rutinose moiety [α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] [16–19], which contained the anomeric protons of rhamnopyranosyl and glucopyranosyl portions with an α-configuration (δ 5.33, $J = 7.6$ Hz, H-1″) and a β-configuration (δ 4.50, $J = 1.2$ Hz, H-1‴), respectively. The linkage of these two sugars was supported by the HMBC correlation (Figure 2) between H-1‴ (δ 4.50) and C-6″ (δ 65.1). Moreover, the HMBC correlations from H-2⁗, H-6⁗, and H-3‴ to the carbonyl carbon at δ 165.5 (C-7⁗) revealed that the syringoyl moiety was attached to C-3‴ through an ester linkage. Meanwhile, the correlation between C-3 (δ 133.6) and the anomeric proton of the glucopyranosyl portion (δ 5.33, H-1″) showed that the glucopyranosyl group was located at the C-3 position of quercetin through an ether linkage. Based on the above data, compound **2** was elucidated to be quercetin 3-*O*-[(3-*O*-syringoyl-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranoside].

Compound **2** was tested for antioxidative activity and showed DPPH free radical scavenging capacity with an IC_{50} value of around 38 μM.

3. Experimental

3.1 General experimental procedures

The ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. Optical rotations were taken with a JASCO DIP-370 digital polarimeter. UV spectra were measured on a Hitachi U-3200 spectrophotometer. HR-FAB-MS spectra were obtained using Finnigan/Thermo Quest MAT 95XL spectrometer. Reversed-phase HPLC was performed using a Hewlett-Packard series 1100 pump system equipped with Hewlett-Packard UV/vis detector. Cosmosil 5C18-MS-II columns were used with dimensions of 4.6 mm × 250 mm for analytical work and 10.0 mm × 250 mm for semi-preparative isolation.

3.2 Plant material

The aerial parts of *L. japonicus* were collected from a herb store in Taipei, Taiwan. A voucher specimen has been deposited at the Herbarium of the National Research Institute of Chinese Medicine, Taiwan.

3.3 Extraction and isolation

The aerial parts of *L. japonicus* (19.5 kg) were extracted at 50°C with MeOH (140 liters × 3). The combined extracts were evaporated *in vacuo* to give a black residue, which was suspended in water (12 liters) and centrifuged (9000 rpm, 35 min) to give water-soluble and water-insoluble portions. The water-soluble portion was partitioned between EtOAc and H_2O to give EtOAc and H_2O layers. Then, the EtOAc layer was applied to a silica gel column eluted with gradient solvent systems of *n*-hexane–EtOAc and EtOAc–MeOH to yield nine fractions (Fr-1–Fr-9). Fraction Fr-2, the eluate of *n*-hexane–EtOAc (3:1), was further purified by semi-preparative HPLC with a gradient system of H_2O–MeOH to give 6-hydroxy-15,16-epoxylabda-5,8,13(16),14-tetraen-7-one. Fraction Fr-5, the eluate of *n*-hexane–EtOAc (1:5), was repeatedly chromatographed on silica gel (*n*-hexane–EtOAc = 1:6) and Sephadex LH-20 (MeOH) columns to give genkwanin and apigenin. Fraction Fr-6, the eluate of EtOAc, was reseparated on Sephadex LH-20 (MeOH) and silica gel (*n*-hexane–EtOAc = 1:5–0:1) columns to yield quercetin and tiliroside. Separation of fraction Fr-7, the eluate of EtOAc–MeOH (10:1), by a silica gel column (EtOAc–MeOH = 20:1–10:1) and semi-preparative HPLC (a gradient system of H_2O–MeOH) afforded uracil and **1** (8.6 mg). Fraction Fr-9, the eluate of EtOAc–MeOH (4:1), was repeatedly chromatographed on Sephadex LH-20 (MeOH) and semi-preparative HPLC (a gradient system of H_2O–MeOH) to give

6β-hydroxy-15,16-epoxylabda-8,13(16), 14-trien-7-one. The H_2O layer was directly subjected to a Diaion HP-20 column, successively eluting with H_2O and MeOH. The MeOH eluate was evaporated to dryness, and then repeatedly chromatographed on silica gel (EtOAc–MeOH = 1:0–1:1, $CHCl_3$–MeOH = 8:1) and Sephadex LH-20 (MeOH) columns to give **2** (88.0 mg).

*3.3.1 1,6-Di-O-syringoyl-β-D-glucopyranose (**1**)*

Colorless powder; mp 130–132°C; $[\alpha]_D$ +33 ($c = 0.01$, MeOH); UV λ_{max}^{MeOH} (log ε): 217 (4.05), 278 (4.37) nm; IR (KBr) ν_{max}: 3442, 2925, 1706, 1608, 1513, 1462, 1339, 1219, 1113 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ: 3.49 (1H, m, H-4), 3.57 (2H, m, H-2, 3), 3.81 (1H, m, H-5), 3.88 (12H, s, 3′,5′,3″,5″-OCH_3), 4.34 (1H, dd, $J = 6.5$, 11.0 Hz, H-6a), 4.70 (1H, dd, $J = 1.5$, 11.0 Hz, H-6b), 5.71 (1H, d, $J = 8.0$ Hz, H-1), 7.33 (2H, s, H-2′, 6′), 7.37 (2H, s, H-2″, 6″); ^{13}C NMR (CD_3OD, 100 MHz) δ: 55.7 (3′,5′,3″,5″-OCH_3), 63.9 (C-6), 70.7 (C-4), 72.8 (C-2), 75.3 (C-5), 76.8 (C-3), 95.1 (C-1), 107.1 (C-2″, 6″), 107.4 (C-2′, 6′), 119.2 (C-1′), 120.0 (C-1″), 140.9 (C-4″), 141.5 (C-4′), 147.7 (C-3″, 5″), 147.8 (C-3′, 5′), 165.5 (C-7′), 166.8 (C-7″); HR-FAB-MS: *m/z* 540.1494 $[M]^+$ (calcd for $C_{24}H_{28}O_{14}$, 540.1480).

*3.3.2 Quercetin 3-O-[(3-O-syringoyl-α-L-rhamnopyranosyl)-(1 → 6)-β-D-glucopyranoside] (**2**)*

Yellow powder; mp 200–201°C; $[\alpha]_D$ +25 ($c = 0.20$, MeOH); UV λ_{max}^{MeOH} (log ε): 265 (4.05), 380 (4.32) nm; IR (KBr) ν_{max}: 3401, 1712, 1653, 1606, 1503, 1336, 1212, 1110 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.13 (3H, d, $J = 6.0$ Hz, H-6‴), 3.26–3.73 (7H, sugar protons), δ 3.67 (1H, m, H-5‴), 3.79 (6H, s, 3⁗,5⁗-OCH_3), 4.50 (1H, d, $J = 1.2$ Hz, H-1‴), 4.83 (1H, dd, $J = 3.0$, 9.6 Hz, H-3‴), 5.33 (1H, d, $J = 7.6$ Hz, H-1″), 6.16 (1H, d, $J = 2.0$ Hz, H-6), 6.38 (1H, d, $J = 2.0$ Hz, H-8), 6.81 (1H, d, $J = 8.4$ Hz, H-5′), 7.25 (2H, s, H-2⁗, 6⁗), 7.52 (1H, d, $J = 2.0$ Hz, H-2′), 7.65 (1H, dd, $J = 2.0$, 8.4 Hz, H-6′), 12.56 (1H, br s, 5-OH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 18.0 (C-6‴), 56.2 (3⁗,5⁗-OCH_3), 65.1 (C-6″), 68.0, 68.1, 68.5, 71.2, 73.1, 74.3 (6C, sugar carbons), 69.1 (C-5‴), 74.9 (C-3‴), 93.7 (C-8), 98.8 (C-6), 100.0 (C-1‴), 102.2 (C-1″), 104.0 (C-10), 107.4 (C-2⁗, 6⁗), 115.3 (C-5′), 116.1 (C-2′), 120.0 (C-1⁗), 121.1 (C-1′), 122.0 (C-6′), 133.6 (C-3), 140.6 (C-4⁗), 144.9 (C-3′), 147.5 (C-3⁗, 5⁗), 148.6 (C-4′), 156.4 (C-9), 156.5 (C-2), 161.3 (C-5), 164.3 (C-7), 165.5 (C-7⁗), 177.4 (C-4); HR-FAB-MS: *m/z* 791.2047 $[M + H]^-$ (calcd for $C_{36}H_{39}O_{20}$, 791.2035).

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