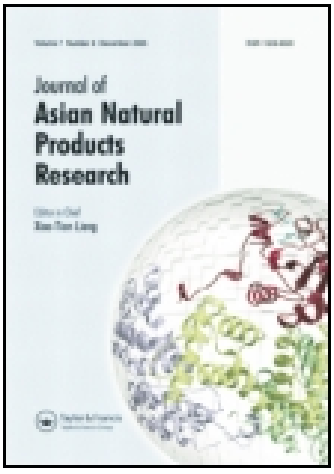

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Two new acetylated flavonoid glycosides from *Phyllanthus urinaria*

Chun Wu[a], Chun-Shan Wei[b], Shao-Fu Yu[a], Bai-Lian Liu[a], Yao-Lan Li[a], Wen-Cai Ye[a], Guang-Dong Tong[b]* and Guang-Xiong Zhou[a]*

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Two new acetylated flavonoid glycosides, quercetin 3-*O*-α-L-(2,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**1**) and quercetin 3-*O*-α-L-(3,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**2**), together with two known compounds, quercetin (**3**) and quercetin 3-*O*-α-L-rhamnopyranoside (**4**), were isolated from the ethanol extract of *Phyllanthus urinaria*. The structures of the new compounds were determined on the basis of extensive spectroscopic data including IR, HR-ESI-MS, 1D NMR, and 2D NMR.

Keywords: Euphorbiaceae; *Phyllanthus urinaria*; acetylated flavonoid glycoside

1. Introduction

Phyllanthus urinaria Linnea (Euphorbiaceae) is widely distributed in tropical and subtropical regions in Asian countries. It is traditionally believed that this plant can reduce heat, relieve inflammation, remove food stagnancy, protect the liver, detoxify body from poison, and increase the flow of urine [1]. It has long been used in folk medicine for liver protection, hepatitis B, diabetes, nephrolithiasis, jaundice, and dropsy [2–6]. Earlier chemical studies on *P. urinaria* indicated that the major constituents of the plant are flavonoids, lignans, tannins, coumarins, and benzenoids [7–11]. Recently, we carried out a systematic chemical study on the 95% ethanol extract of the aerial part of *P. urinaria*, which resulted in the isolation of two new acetylated flavonoid glycosides, quercetin 3-*O*-α-L-(2,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**1**) and quercetin 3-*O*-α-L-(3,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**2**), together with two known compounds, quercetin (**3**) [12] and quercetin 3-*O*-α-L-rhamnopyranoside (**4**) [13] (Figure 1). In this study, we report the isolation and structural elucidation of the new compounds.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{31}H_{34}O_{17}$ based on its HR-ESI-MS data. The UV spectrum of **1** exhibited absorption maxima at 265, 346 (sh), and 352 nm, suggesting the presence of a flavone skeleton. Examination of the ^{1}H and ^{13}C NMR spectral data of **1** (Table 1) indicated that the molecule consisted of a flavone, two sugars, and two acetyl moieties. In ^{1}H NMR spectrum, two doublets were observed at δ_H 6.39 (d, $J = 1.5$ Hz) and 6.63 (d, $J = 1.5$ Hz), assignable to H-6 and H-8 of the ring A of a flavonoid unit. Three *ortho*- and *meta*-coupled aromatic proton signals at

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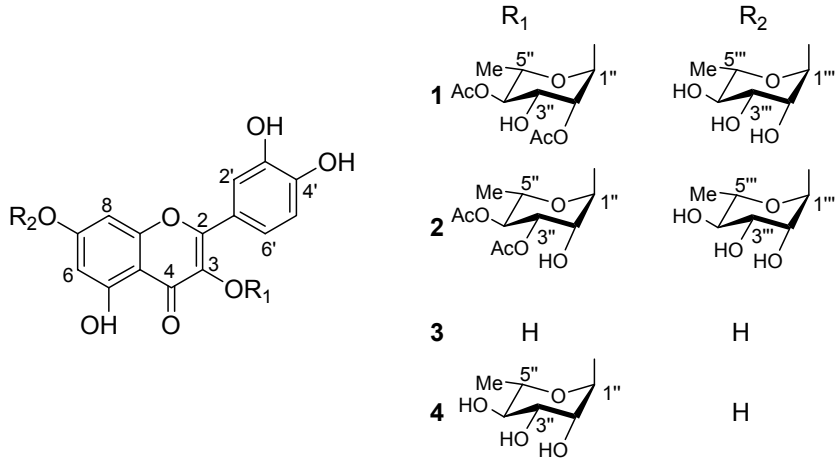

Figure 1. Structures of compounds **1**–**4**.

δ_H 6.92 (H-5′, d, $J = 8.2$ Hz), 7.28 (H-6′, dd, $J = 8.2$, 1.9 Hz), and 7.34 (H-2′, d, $J = 1.9$ Hz) indicated the presence of a 3′,4′-substituted phenyl group in ring B. In addition, two anomeric protons [δ_H 5.40 (H-1″) and 5.55 (H-1‴)] and two methyl protons [δ_H 0.75 (H-6″, d, $J = 6.2$ Hz) and 1.13 (H-6‴, d, $J = 6.0$ Hz)] indicated the presence of two rhamnose residues. Acid hydrolysis of **1** afforded quercetin (**3**) and rhamnose, which were identified by comparison of the ^{1}H and ^{13}C NMR spectra and high performance liquid chromatography (HPLC) R_t value with those of authentic markers. In ^{13}C NMR spectrum (Table 1), the carbon signal assignable to C-2 (δ_C 158.2) in the aglycone moiety was shifted to a lower field by about 11.4 ppm, compared with that of quercetin (**3**, δ_C 146.8), indicating that one of the two rhamnose residues was located at C-3 (δ_C 133.6). The remaining rhamnose residue was deduced to be attached at C-7 (δ_C 161.9), on the basis of the chemical shifts of H-6 (δ_H 6.39) and H-8 (δ_H 6.63), which were deshielded by 0.20 and 0.22 ppm, respectively, compared with the corresponding signals of quercetin (**3**, δ_H 6.19 and 6.41, respectively). These elucidation were further confirmed by the HMBC correlations of H-1″/C-3 and H-1‴/C-7 (Figure 2). Based on ^{1}H–^{1}H

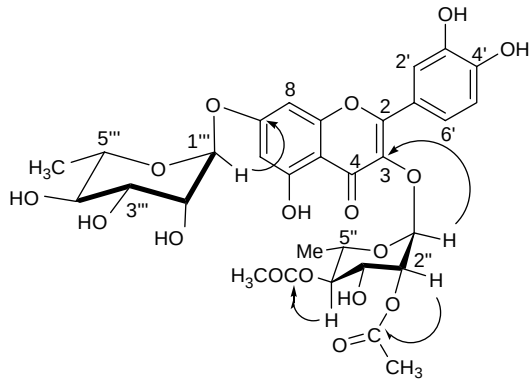

Figure 2. Key HMBC (H → C) correlations of compound **1**.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data for compounds **1** and **2** (DMSO-d_6, δ in ppm, J in Hz).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
2	–	158.2	–	158.1
3	–	133.6	–	134.5
4	–	177.6	–	177.8
5	–	160.9	–	161.0
6	6.39 (d, 1.5)	99.7	6.45 (d. 1.9)	98.5
7	–	161.9	–	162.1
8	6.63 (d, 1.5)	99.8	6.76 (d. 1.9)	99.6
9	–	156.2	–	156.2
10	–	105.8	–	105.8
1′	–	120.2		120.4
2′	7.34 (d, 1.9)	115.8	7.31 (d, 1.8)	115.7
3′	–	145.5	–	145.5
4′	–	149.0	–	148.9
5′	6.92 (d, 8.2)	115.6	6.91 (d, 8.2)	115.5
6′	7.28 (dd, 8.2, 1.9)	120.3	7.27 (dd, 8.2, 1.8)	121.2
1″	5.40 (d, 1.8)	98.2	5.68 (d, 1.7)	101.5
2″	5. 34 (dd, 3.4, 1.8)	71.8	4.26 (dd, 2.8, 1.7)	67.4
3″	3.92 (dd, 9.7, 3.4)	66.0	5.15 (dd, 9.9, 2.8)	70.3
4″	4.63 (t, 9.7)	73.0	4.63 (t, 9.9)	69.9
5″	3.37 (m)	68.0	3.29 (m)	68.0
6″	0.75 (d, 6.2)	17.1	0.78 (d, 6.2)	16.9
1‴	5.55 (br s)	98.5	5.56 (br s)	98.5
2‴	3.85 (dd, 3.0, 1.9)	69.8	3.85 (dd, 3.0, 1.9)	69.8
3‴	3.64 (dd, 9.2, 3.0)	70.3	3.65 (dd, 9.7, 3.0)	70.1
4‴	3.32 (t, 9.2)	71.2	3.31 (t, 9.7)	71.6
5‴	3.43 (m)	69.9	3.42 (m)	69.7
6‴	1.13 (d, 6.0)	18.2	1.13 (d, 6.1)	17.9
2″-$COCH_3$	2.01 (s)	170.0	–	–
2″-$\overline{C}OCH_3$	–	20.8	–	–
3″-$CO\overline{C}H_3$	–	–	1.99 (s)	169.9
3″$\overline{C}OCH_3$	–	–	–	20.5
4″$CO\overline{C}H_3$	2.05 (s)	169.7	2.02 (s)	169.7
4″-$\overline{C}O\underline{C}H_3$	–	20.9	–	20.8

correlation spectroscopy (COSY) spectrum, and compared with the corresponding ^{1}H NMR signals of quercetin 3-*O*-α-L-rhamnopyranoside (**4**) which was unacetylated (Table 1), the connectivities of the acetyl groups of **1** were established. Two protons H-2″ (δ_H 5.34) and H-4″ (δ_H 4.63) of the one rhamnose residue were shifted to a lower field by 1.11 and 1.22 ppm from **4** [H-2″ (δ_H 4.23) and H-4″ (δ_H 3.41)], convinced that two hydroxyl groups at C-2″ and C-4″ of rhamnose residue were acetylated. This was further supported by HMBC experiment (Figure 2), in which long-range correlations of H-2″/acetoxyl carbon at δ_C 170.0 and H-4″/acetoxyl carbon at δ_C 169.7 were observed. Thus, the structure of **1** was elucidated as quercetin 3-*O*-α-L-(2,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside.

Compound **2**, a yellow amorphous powder, had a molecular formula of $C_{31}H_{34}O_{17}$ on the basis of the HR-ESI-MS data. The UV spectrum of **2** exhibited absorption maxima at 268, 339 (sh), and 356 nm. The ^{1}H and ^{13}C NMR data of **2** (Table 1) were similar to those of **1**,

suggesting that the molecule of **2** also consisted of quercetin as an aglycone, two rhamnosyl and two acetyl groups. Comparison of the ^{1}H NMR spectral (Table 1) data of **2** with those of quercetin 3-*O*-α-L-rhamnopyranoside (**4**, H-3″ at δ_H 3.75 and H-4″ at δ_H 3.41, respectively) revealed that two protons at H-3″ (δ_H 5.15) and H-4″ (δ_H 4.63) of the one rhamnose residue were shifted to a lower field by 1.40 and 1.22 ppm (more than 1 ppm), respectively. This indicated that two hydroxyl groups at C-3″ and C-4″ of rhamnose residue were acetylated. This conclusion was further confirmed by HMBC correlations of H-3″/acetoxyl carbon at δ_C 169.9 and H-4″/acetoxyl carbon at δ_C 169.7. Finally, the structure of **2** was determined to be quercetin 3-*O*-α-L-(3,4-di-*O*-acetyl) rhamnopyranoside-7-*O*-α-L-rhamnopyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a JASCO P-1030 automatic digital polarimeter (JASCO, Tokyo, Japan). UV spectra were measured using a JASCO V-550 UV-Vis spectrophotometer. IR spectra were measured using a JASCO FT/IR-480 plus infrared spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded by Bruker AV-400 spectrometer with tetramethylsilane as the internal standard, and chemical shifts were expressed in δ values (ppm). HR-ESI-MS data were obtained from an Agilent 6210 liquid chromatography mass spectrometer detector time of flight (LC/MSD TOF) (Agilent, Pala Alto, CA, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), octadecylsilyl (ODS; 50 μm, YMC, Tokyo, Japan), and Sephadex LH-20 (25–100 μm, Fluka, Buchs, Switzerland) were used for column chromatography (CC). Preparative HPLC was carried out by a Varian instrument equipped with UV detectors (Varian, Salt Lake City, UT, USA) and a reversed-phase (RP) C18 column (5 μm, 20 mm × 250 mm; Cosmosil). Thin-layer chromatography (TLC) was carried out using precoated silica gel plates (GF_{254}, Yantai Chemical Industry Research Institute, Yantai, China). All the reagents were purchased from Tianjin Damao Chemical Company (Tianjin, China).

3.2 Plant material

The plant material was collected from Shenzhen, Guangdong, China, and was authenticated by G.X. Zhou at the Department of Pharmacognosy, College of Pharmacy, Jinan University. A voucher specimen (No. 2012YXZ0213) was also deposited in the Department.

3.3 Extraction and isolation

The dried aerial parts of *P. urinaria* (10 kg) were soaked in 95% ethanol and extracted by percolation. The extract (900 g) was obtained from the soaked solution after evaporation of the solvent under reduced pressure. This extract was suspended in distilled water, and then partitioned with petroleum ether, chloroform, and ethyl acetate (EtOAc), respectively.

The EtOAc extract (130 g) was subjected to silica gel CC (200–300 mesh, 1.3 kg) eluting with $CHCl_3$–CH_3OH (100:0 to 0:100) in gradient to yield eight fractions (fractions A–H) based on TLC patterns. Fraction C (3.8 g) was chromatographed on ODS column eluting with CH_3OH–H_2O (1:9 to 1:0) to afford three subfractions (C1–C3). Subfraction C2 (900 mg) was subjected to Sephadex LH-20 CC with the eluent of 70% CH_3OH–H_2O to yield compounds **3** (20.1 mg) and **4** (19.8 mg). Fraction D (4.5 g) was subjected to an ODS column eluting with CH_3OH–H_2O (1:9 to 1:0) in gradient to afford four subfractions (D1–D4). Subfraction D3 (1.0 g) was applied to

Sephadex LH-20 CC by eluting with CH_3OH to afford three fractions (D3A–D3C). Fraction D3A (200 mg) was purified by semi-preparative RP-C_{18} HPLC with the eluent 25% CH_3CN–H_2O (254 nm, 4 ml/min) to give compound **1** (14.9 mg, $t_R = 25.6$ min) and compound **2** (15.5 mg, $t_R = 33.4$ min)

*3.3.1 Quercetin 3-O-α-L-(2,4-di-O-acetyl) rhamnopyranoside-7-O-α-L-rhamnopyranoside (**1**)*

Yellow amorphous powder; $C_{31}H_{34}O_{17}$; $[\alpha]_D^{20}$ −127.2 ($c = 0.11$, CH_3OH); UV (MeOH) λ_{max} (log ε): 265 (4.4), 346 (sh), and 352 (4.5) nm; IR (KBr) ν_{max}: 3440, 1745, 1650, and 1610 cm^{-1}; ^1H and ^{13}C NMR spectral data (DMSO-d_6): see Table 1. HR-ESI-MS: *m/z* 679.1863 $[M + H]^+$ (calcd for $C_{31}H_{35}O_{17}$, 679.1874).

*3.3.2 Quercetin 3-O-α-L-(3,4-di-O-acetyl) rhamnopyranoside-7-O-α-L-rhamnopyranoside (**2**)*

Yellow amorphous powder; $C_{31}H_{34}O_{17}$; $[\alpha]_D^{20}$ −129.5 ($c = 0.12$, CH_3OH); UV (MeOH) λ_{max} (log ε): 266 (4.3), 339 (sh), and 356 (4.6) nm; IR (KBr) ν_{max}: 3415, 1720, 1650, and 1602 cm^{-1}; ^1H and ^{13}C NMR spectral data (DMSO-d_6): see Table 1. HR-ESI-MS: *m/z* 679.1865 $[M + H]^+$ (calcd for $C_{31}H_{35}O_{17}$, 679.1874).

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