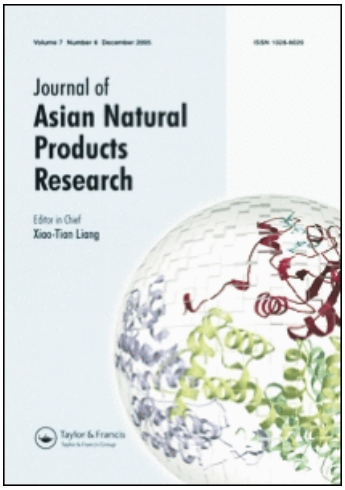

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ORIGINAL ARTICLE

Acylated flavone *C*-glycosides from *Hemistepta lyrata*

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Two new acylated flavone *C*-glycosides, 6″-*O*-(2‴-methylbutyryl)isoswertisin (**1**) and 6″-*O*-(2‴-methylbutyryl)isoswertiajaponin (**2**), together with four known acylated flavone *C*-glycosides, were isolated for the first time from the whole plants of *Hemistepta lyrata* (Compositae). Their structures were elucidated on the basis of chemical and spectroscopic methods including HR-ESI-MS, ESI-MS, UV, IR, and 1D and 2D NMR spectral techniques.

Keywords: *Hemistepta lyrata*; Compositae; acylated flavone *C*-glycosides; 6″-*O*-(2‴-methylbutyryl)isoswertisin; 6″-*O*-(2‴-methylbutyryl)isoswertiajaponin

1. Introduction

Hemistepta lyrata Bunge (Compositae) is an herbaceous plant growing on mountain slopes, wasteland, and along roadsides in the eastern and southern areas of Asia and Australia [1]. The whole dried plant of *H. lyrata*, commonly known as 'NiHuCai' in China, is used as a folklore medicine for reducing fever and detoxification, eliminating stagnated blood, and dispersing swelling [2]. In extensive investigations aimed at the discovery of new bioactive flavonoids from *H. lyrata* growing in the Changbai Mountain, Jilin Province of China, six acylated flavone *C*-glycosides were isolated and characterized from the ethyl acetate soluble part of the ethanol extract, including two new acylated flavone *C*-glycosides, 6″-*O*-(2‴-methylbutyryl)isoswertisin (**1**) and 6″-*O*-(2‴-methylbutyryl)isoswertiajaponin (**2**), as well as four known acylated flavone *C*-glycosides (**3**–**6**). We report herein the isolation and structural characterizations of the six acylated flavone *C*-glycosides.

2. Results and discussion

Compound **1** was obtained as a yellow powder and exhibited a positive magnesium hydrochloric acid test. The molecular formula was determined as $C_{27}H_{30}O_{11}$ by HR-ESI-MS at *m/z* 529.1712 $[M-H]^-$. The absorption bands at 3396, 1655, and 1498 cm^{-1} in the IR spectrum and the absorption maxima at 334, 314, and 262 nm in the UV spectrum are characteristic of a flavone derivative [3,4]. The ^1H NMR spectrum of **1** indicated signals of two sets of doublets at δ 7.98 (2H, d, $J = 8.8$ Hz) and 6.92 (2H, d, $J = 8.8$ Hz), due to the protons H-2′, 6′ and H-3′, 5′ of a 4′-hydroxyphenyl moiety, and two singlets at δ 6.84 and 6.53, due to the protons at C-3 and C-6 in rings C and A of a flavone, respectively. In addition to the 15 aglycone carbon signals, the ^{13}C NMR spectrum

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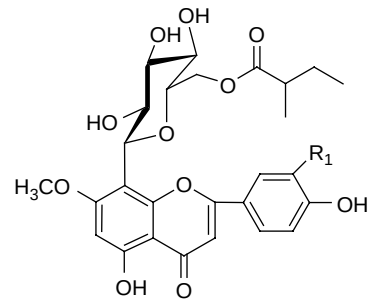
revealed six sugar carbon signals, in which a carbon signal at δ 56.5 was ascribable to a methoxyl, and another five signals (δ 175.8, 40.0, 26.1, 11.0, 16.2) were apparently due to an acyl group. The signal of C-6 at δ 95.0 exhibited the presence of a methoxyl group attached to the C-7 position. The sugar moiety was determined to be glucose from ^{1}H and ^{13}C NMR spectral data. The resonances of the glucosyl residue were assigned from ^{1}H–^{1}H COSY, HSQC, and HMBC spectral data using the anomeric proton H-1″ at δ 4.75 (1H, d, $J = 10.0$ Hz) as a starting point. The carbon signals of the glucosyl at δ 73.3, 70.7, 78.3, 70.5, 78.5, and 63.6 suggested that **1** is a flavone *C*-glycoside [5]. The site of the sugar linkage to the aglycone in **1** was considered to be at the C-8 position since the C-8 signal appeared at δ 105.3 in the ^{13}C NMR spectrum. The result was further confirmed by the appearance of cross-peaks of the anomeric proton of the sugar at δ 4.75 with the carbons at δ 105.3 (C-8), 163.4 (C-7), and 155.2 (C-9) in the HMBC spectrum. The ^{1}H and ^{13}C NMR spectral data of the aglycone and sugar moieties of **1** were similar to those of isoswertisin [6], except for an upfield shift of 3.1 ppm for C-5″ (δ 78.5) and a downfield shift of 2.3 ppm for C-6″ (δ 63.6), indicating the acylation of C-6″. The ^{13}C NMR signals ascribable to an acyl group, namely, the carbonyl signal at δ 175.8 (C-1‴) and four aliphatic carbon signals at δ 40.0 (C-2‴), 26.1 (C-3‴), 11.0 (C-4‴), and 16.2 (C-5‴), together with the aliphatic ^{1}H NMR signals of a doublet at δ 0.85 (3H, d, $J = 7.0$ Hz), a triplet at δ 0.70 (3H, t, $J = 7.4$ Hz), and three multiplets at δ 2.16 (1H, m), 1.44 (1H, m), and 1.27 (1H, m), indicated the presence of a 2-methylbutyryl group [7]. The position of the acyl group was confirmed at C-6″ of the sugar moiety by the long-range correlation between the proton at δ 4.10 (H-6″) and the carbonyl signal at δ 175.8 (C-1‴) from the HMBC spectrum. Based on the above observations, the structure



1 R_1 = H
2 R_1 = OH

Figure 1. Chemical structures of compounds **1** and **2**.

of **1** was determined as 6″-*O*-(2‴-methylbutyryl)isoswertisin, a new acylated flavone *C*-glycoside (Figure 1).

Compound **2** was obtained as a yellow powder and assigned the molecular formula of $C_{27}H_{30}O_{12}$, as established from the quasi-molecular ion peak at *m/z* 545.1663 $[M - H]^-$ in the HR-ESI-MS, 16 mass units greater than that of **1**. It was similar to **1** in the magnesium hydrochloric acid test, UV, and IR spectra, suggesting that **2** also has a flavone skeleton [3,4]. Comparison of the ^{1}H NMR spectral data of **2** with those of **1** indicated the presence of an ABX system, instead of the AA′BB′ system in **1**, due to the protons H-5′ (δ 6.84, d, $J = 8.4$ Hz), H-2′ (δ 7.40, d, $J = 2.1$ Hz), and H-6′ (δ 7.46, dd, $J = 8.4$, 2.1 Hz) of a 3′,4′-dihydroxyphenyl moiety. The ^{13}C NMR spectrum revealed 27 carbon signals, which suggested that the structure is a flavonoid containing a sugar moiety and a methylbutyryl group. The site of the sugar linkage to the aglycone in **2** was unambiguously determined at the C-8 position by the appearance of cross-peaks of the anomeric proton H-1″ (δ 4.72, d, $J = 9.9$ Hz) with the carbon signals at δ 105.2 (C-8), 163.3 (C-7), and 155.2 (C-9) in the HMBC spectrum. From these data, the sugar substituent at C-8 of the aglycone moiety gave a pattern of ^{13}C NMR signals similar to those in isoswertiajaponin [8]. The signals of C-5″

(δ 78.6) and C-6″ (δ 63.9) of the sugar moiety showed an upfield shift of 3.3 ppm and a downfield shift of 2.5 ppm, respectively, compared with the corresponding data (δ 81.8, 61.4) of isoswertiajaponin. The position of the methylbutyryl group was confirmed at C-6″ of the sugar moiety by the long-range correlation between the proton at δ 4.40 (H-6″) and the carbonyl signal at δ 175.8 (C-1‴) from the HMBC spectrum. Thus, the structure of **2** was determined as 6″-*O*-(2‴-methylbutyryl)isoswertiajaponin, a new acylated flavone *C*-glycoside (Figure 1).

In addition, four known acylated flavone *C*-glycosides, 2″-*O*-(2‴-methylbutyryl)isoswertiajaponin (**3**) [9], 2″-*O*-(2‴-methylbutyryl) isoswertisin (**4**) [10], 2″-*O*-(2‴-methylbutyryl)orientin (**5**) [10], and 2″-*O*-(3‴,4‴-dimethoxybenzoyl) orientin (**6**) [10], were also isolated and identified by spectroscopic methods including UV, ESI-MS, and 1D and 2D NMR spectral techniques.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X4 micro-melting apparatus and are uncorrected. UV spectra were measured with a Shimadzu UV-2100 spectrometer in methanol with absorption given in nm and IR spectra with a Perkin-Elmer FT-IR spectrometer as KBr pellets with absorption given in cm^{-1}. HR-ESI-MS and ESI-MS were obtained with a Bruck micro-TOFQ mass spectrometer. NMR spectra were measured on a Bruck AV-500 FT-NMR in DMSO-d_6, using visual DMSO-d_6 resonances (^{1}H δ 2.49, ^{13}C δ 39.5) for internal reference. All chemical shifts (δ) are given in ppm. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China) and RP-18 reversed-phase silica gel (S-50 μm; YMC, Kyoto, Japan). TLC analysis was carried out on precoated TLC plates with silica gel 60 F_{254} and silica gel RP-18 60 F_{254} (Merck, Darmstadt, Germany, 0.25 mm). Detection was achieved by spraying with 10% H_2SO_4 in MeOH, followed by heating. Preparative HPLC was performed on a Shimadzu LC-6AD pump connected with a Shimadzu SPD-20A UV–vis detector (at 254 nm), using Shim Pak ODS column (250 mm × 21.2 mm, i.d., 5 μm; Shimadzu, Kyoto, Japan). All solvents used for the chromatographic separations were distilled before use.

3.2 Plant material

The whole plants of *H. lyrata* were collected from Changbai Mountain, Jilin Province of China, in September 2008, and authenticated by Prof. Zhongkai Yan, Jilin Academy of Chinese Medicine Sciences, China. A voucher specimen (HLNHC20080902) is deposited at the Institute of Phytochemistry, Jilin Academy of Chinese Medicine Sciences, China.

3.3 Extraction and isolation

The air-dried and ground whole plant material (40 kg) was extracted twice with 60% ethanol under reflux for 2 h and the solvent was evaporated under reduced pressure to give a brown residue (8.4 kg). The residue (6.3 kg) was suspended in water and successively partitioned with petroleum ether, chloroform, and ethyl acetate.

The EtOAc-soluble fraction (50 g) was chromatographed over a silica gel column (10 × 90 cm, 2.0 kg), eluted with a $CHCl_3$–MeOH (19:1, 9:1, 1:1, and 0:10) and separated into 16 fractions (Fr. 1–16) on the basis of TLC analyses. Fr. 8 was purified by RP-18 reversed-phase silica gel column (3 × 25, 100 g) chromatography, eluted with MeOH–H_2O (50:50) and preparative HPLC (RP-18 column: 250 mm × 21.2 mm, i.d., 5 μm; flow rate: 4 ml/min) using MeOH–H_2O (55:45) as the mobile phase to yield compound **4**

(68.85 mg, $t_R = 98$ min). Fr. 9 was separated by RP-18 reversed-phase silica gel column (3 × 25, 100 g) chromatography, eluted with a gradient of increasing MeOH (50–100%) in water and preparative HPLC (RP-18 column: 250 mm × 21.2 mm, i.d., 5 μm; flow rate: 4 ml/min) employing MeOH–H_2O (60:40) as the mobile phase to give compound **1** (8.33 mg, $t_R = 116$ min). Fr. 13 was subjected to RP-18 reversed-phase silica gel column chromatography with a MeOH–H_2O (50:50) solvent system to afford 11 subfractions (Sub. 13-I–13-XI). Sub. 13-VIII and 13-X were isolated by preparative HPLC (RP-18 column: 250 mm × 21.2 mm, i.d., 5 μm; flow rate: 4 ml/min) using MeOH–H_2O (45:55) and (55:45) as the mobile phase to obtain compound **3** (17.17 mg, $t_R = 190$ min) and compound **2** (5.64 mg, $t_R = 85$ min), respectively. Fr. 14 was loaded on a column of RP-18 reversed-phase silica gel and eluted with a gradient of increasing MeOH (30–50%) in water to yield 17 subfractions (Sub. 14-I–14-XVII). Compound **6** (6.91 mg, $t_R = 120$ min) and compound **5** (5.39 mg, $t_R = 152$ min) were obtained from Sub. 14-XIV and 14-XV by preparative HPLC (RP-18 column: 250 mm × 21.2 mm, i.d., 5 μm; flow rate: 3 ml/min) employing MeOH–H_2O (45:55) as the mobile phase, respectively.

Table 1. ^{13}C NMR spectral data for compounds **1** and **2** (125 MHz, DMSO-d_6, δ in ppm).

C	**1**	**2**
2	164.3	164.6
3	102.4	102.1
4	182.2	182.0
5	161.4	161.4
6	95.0	95.0
7	163.4	163.3
8	105.3	105.2
9	155.2	155.2
10	104.4	104.4
1′	121.1	120.9
2′	128.7	113.6
3′	115.9	146.2
4′	161.4	149.8
5′	115.9	115.5
6′	128.7	119.2
1″	73.3	73.2
2″	70.7	70.6
3″	78.3	78.5
4″	70.5	70.5
5″	78.5	78.6
6″	63.6	63.9
1‴	175.8	175.8
2‴	40.0	40.0
3‴	26.1	26.1
4‴	11.0	11.0
5‴	16.2	16.3
7-OCH_3	56.5	56.5

*3.3.1 6″-O-(2‴-Methylbutyryl) isoswertisin (**1**)*

Yellow powder; UV λ_{max} (CH_3OH): 334, 314, 262 nm; IR ν_{max} (KBr): 3396, 1655, 1498 cm^{-1}; ^{1}H NMR spectral data (500 MHz, DMSO-d_6) δ: 7.98 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.92 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 6.84 (1H, s, H-3), 6.53 (1H, s, H-6), 4.75 (1H, d, $J = 10.0$ Hz, H-1″), 4.36 (1H, d, $J = 11.8$ Hz, H_a-6″), 4.10 (1H, dd, $J = 11.8$, 5.2 Hz, H_b-6″), 3.88 (3H, s, H-OCH_3), 2.16 (1H, m, H-2‴), 1.44 (1H, m, H_a-3‴), 1.27 (1H, m, H_b-3‴), 0.85 (3H, d, $J = 7.0$ Hz, H-5‴), 0.70 (3H, t, $J = 7.4$ Hz, H-4‴); ^{13}C NMR spectral data (125 MHz, DMSO-d_6): see Table 1; HR-ESI-MS: *m/z* 529.1712 $[M - H]^-$ (calcd for $C_{27}H_{29}O_{11}$, 529.1710).

*3.3.2 6″-O-(2‴-Methylbutyryl) isoswertiajaponin (**2**)*

Yellow powder; UV λ_{max} (CH_3OH): 340, 274 nm; IR ν_{max} (KBr): 3407, 1622, 1504 cm^{-1}; ^{1}H NMR spectral data (500 MHz, DMSO-d_6) δ: 7.46 (1H, dd, $J = 8.4$, 2.1 Hz, H-6′), 7.40 (1H, d, $J = 2.1$ Hz, H-2′), 6.84 (1H, d, $J = 8.4$ Hz, H-5′), 6.66 (1H, s, H-3), 6.50 (1H, s, H-6), 4.72 (1H, d, $J = 9.9$ Hz, H-1″), 4.40 (1H, d, $J = 11.9$ Hz, H_a-6″), 4.08 (1H, dd, $J = 11.9$, 5.6 Hz, H_b-6″), 3.86

(3H, s, H-OCH_3), 2.20 (1H, m, H-2‴), 1.43 (1H, m, H_a-3‴), 1.26 (1H, m, H_b-3‴), 0.87 (3H, d, $J = 6.9$ Hz, H-5‴), 0.69 (3H, t, $J = 7.4$ Hz, H-4‴); ^{13}C NMR spectral data (125 MHz, DMSO-d_6): see Table 1; HR-ESI-MS: *m/z* 545.1663 $[M - H]^-$ (calcd for $C_{27}H_{29}O_{12}$, 545.1659).

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