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Two new acetylated flavan glycosides from rhizomes of the fern *Abacopteris penangiana*

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Two new acetylated flavan glycosides (2*S*,4*R*)-4,5,7-trihydroxy-4′-methoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetylglucopyranoside-7-*O*-β-D-glucopyranoside (**1**) and (2*S*,4*R*)-5,7-dihydroxy-4,4′-dimethoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetylglucopyranoside-7-*O*-β-D-glucopyranoside (**2**) were isolated from the rhizomes of *Abacopteris penangiana*. Their structures were elucidated on the basis of spectroscopic methods including IR, HR-ESI-MS, 1D and 2D NMR, and chemical evidences.

Keywords: *Abacopteris penangiana*; flavan-4-ol glycosides; flavonoids

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1. Introduction

Abacopteris penangiana (Hook.) Ching (Thelypteridaceae family), commonly named as *Pizhenxinyuejue*, is widely distributed in the south of China, and used as traditional Chinese medicine for the treatment of upper respiratory tract infections and dysentery [1,2]. Phytochemical investigations on thelypteridaceous ferns have demonstrated the occurrence of some rare flavan-4-ol glycosides [3–6]. Previous research on *A. penangiana* has resulted in the isolation of several flavan-4-ol and flavonol glycosides [7–9], and some flavan-4-ol glycosides showed significant cytotoxic activities *in vitro* [7]. As part of the systematic chemical investigation on thelypteridaceous species, we initiated a chemical investigation of *A. penangiana*. In this article, we report on the isolation and structural elucidation of two new acetylated flavan-4-ol glycosides (Figure 1) from *Abacopteris penangiana* and its related bioactivity assay.

2. Results and discussion

Compound **1** was isolated as colorless needles (MeOH) with mp 160–162°C and $[\alpha]_D^{20} - 3$ ($c = 0.53$, MeOH). The molecular formula was established as $C_{32}H_{42}O_{16}$ on the basis of its HR-ESI-MS at *m/z* 705.2388 $[M + Na]^+$. The IR spectrum showed the presence of hydroxyl (3439 cm^{-1}), carbonyl (1742 cm^{-1}), and phenyl (1610, 1516 cm^{-1}) absorptions. The ^1H NMR spectrum showed characteristic signals of three methyls at δ_H 1.99 (3H, s), 2.22 (3H, s), and 2.06 (3H, s), one methoxyl at δ_H 3.75 (3H, s), two anomeric protons at δ_H 4.74 (d, $J = 7.2$ Hz) and 4.43 (d, $J = 7.6$ Hz), and four aromatic protons as two doublets at δ_H 7.38 (2H, d, $J = 8.8$ Hz) and 6.97 (2H, d, $J = 8.8$ Hz), suggesting a *para*-substituted phenyl group (Table 1).

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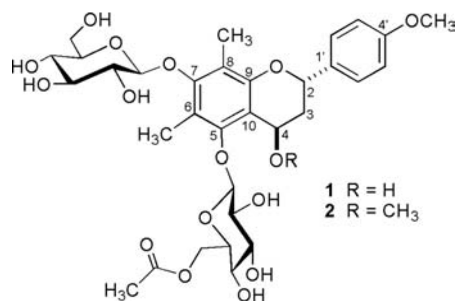

Figure 1. Structures of compounds **1** and **2**.

The ^{13}C NMR spectrum of **1** showed the existence of 9 sp^2 quaternary, 4 sp^2 methine, 12 sp^3 methine, 3 sp^3 methylene, and 4 methyl carbons (Table 1). The ^{1}H–^{1}H COSY correlations (Figure 2) between H-2 (δ_H 5.09) and H_2-3 (δ_H 2.01 and 1.87), H_2-3 and H-4 (δ_H 4.96), and the HMBC correlations (Figure 2) from H-4 to C-5 (δ_C 150.8), C-9 (δ_C 151.4), and C-10 (δ_C 115.4), Me-6 (δ_H 2.22) to C-5 (δ_C 150.8), C-6 (δ_C 117.5), and C-7 (δ_C 154.1), and Me-8 (δ_H 2.06) to C-7 (δ_C 154.1), C-8

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (DMSO-d_6).

	1		**2**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2	5.09 brd (12.0)	72.1	5.10 brd (12.0)	72.0
3	2.01 brd (14.0)	36.8	2.01 m	32.9
	1.87 brt (14.0, 12.0)	–	1.83 brt (14.0, 12.0)	–
4	4.96 brs	57.2	4.99 brs	66.0
5	–	150.8	–	152.7
6	–	117.5	–	117.1
7	–	154.1	–	154.0
8	–	116.0	–	115.8
9	–	151.4	–	151.1
10	–	115.4	–	111.8
Me-6	2.22 s	11.2	2.18 s	11.2
Me-8	2.06 s	9.9	2.02 s	10.1
OMe-4			3.34 s	55.1
1′	–	133.5		133.7
2′,6′	7.38 d (8.8)	127.7	7.38 d (8.8)	127.6
3′,5′	6.97 d (8.8)	114.0	6.96 d (8.8)	113.9
4′	–	159.0	–	159.0
OMe-4′	3.75 s	55.2	3.75 s	55.2
O-glc-*5*				
1″	4.74 d (7.2)	103.8	4.55 d (7.8)	105.3
2″	3.36 m	74.0	3.28 m	74.1
3″	3.32 m	75.8	3.24 m	76.3
4″	3.23 m	69.9	3.17 m	69.9
5″	3.42 m	73.5	3.36 m	73.6
6″	4.17 brd (12.0)	63.5	4.26 brd (11.0)	63.5
	4.04 dd (5.9, 12.0)		4.02 dd (11.0, 6.8)	
O-glc-*7*				
1‴	4.43 d (7.6)	103.8	4.46 d (7.6)	104.3
2‴	3.30 m	74.1	3.21 m	74.4
3‴	3.18 m	76.5	3.15 m	76.5
4‴	3.07 brt (9.1)	70.2	3.07 brt (9.1)	70.0
5‴	2.99 m	77.0	2.97 m	76.9
6‴	3.62 brd (10.2)	61.4	3.60 brd (11.0)	61.4
	3.40 m		3.30 m	
CH_3*CO*	–	170.4	–	170.3
*CH_3*CO	1.99 s	20.9	2.00 s	20.9

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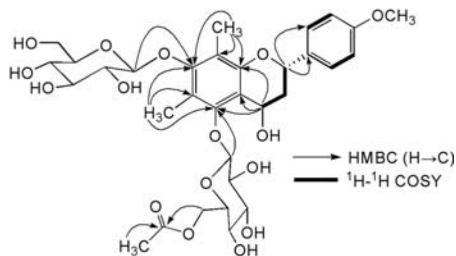

Figure 2. Selected 2D NMR correlations for compound **1**.

(δ_C 116.0), and C-9 (δ_C 151.4) revealed the occurrence of a 4,5,7-trisubstituted 6,8-dimethylchromane ring. The HMBC correlations (Figure 2) from H-2 (δ_H 5.09) to C-1′ (δ_C 133.5) and C-2′ (δ_C 127.7), and H-2′, 6′ (δ_H 7.38) to C-2 (δ_C 72.1) indicated that the *para*-substituted phenyl group is attached to C-2 of the chromane ring. Analysis of the above structural characteristics indicated that **1** is a flavan-4-ol type glycoside with two sugar units. The NMR spectra of the sugar units indicated the presence of two β-D-glucoses, which were confirmed by acid hydrolysis and GC analysis of the thiazolidine derivative. As observed, the HMBC correlations from H-1″ (δ_H 4.74) to C-5 (δ_C 150.8) and H-1‴ (δ_H 4.43) to C-7 (δ_C 154.1) suggested that two D-glucoses were attached to C-5 and C-7, respectively. The presence of an acetyl group was verified from the correlation of Me (δ_H 1.99) to C=O (δ_C 170.4), and the attachment of the acetyl group at C-6″ of the sugar located at C-5 was evidenced from the HMBC correlation of H-6″ (δ_H 4.17, 4.04) to the carbonyl carbon (δ_C 170.4).

The coupling constants of H-2 (δ_H 5.09, brd, $J = 12.0$ Hz) and H_2-3 (δ_H 2.01, brd, $J = 14.0$ Hz; 1.87, brt, $J = 14.0$ and 12.0 Hz) suggested that the stereochemistry relationship between H-2 and H-4 was *trans* [10], and this was supported by a positive Cotton effect at 283 nm ($\Delta\epsilon + 1.74$) observed in the CD spectrum of **1**. The configurations at C-2 and C-4 were finally determined as 2*S* and 4*R* by comparing CD spectrum and the coupling constants of H-2 and H-4 of **1** with those of abacopterin D [8]. On the basis of these evidences, compound **1** was determined to be (2*S*,4*R*)-4,5,7-trihydroxy-4′-methoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetylglucopyranoside-7-*O*-β-D-glucopyranoside.

Compound **2** was isolated as a white amorphous powder with $[\alpha]_D^{20} -12$ ($c = 0.26$, MeOH). The molecular formula was established as $C_{33}H_{44}O_{16}$ on the basis of its HR-ESI-MS at *m/z* 719.2518 $[M + Na]^+$. The IR spectrum showed the presence of hydroxyl (3422 cm^{-1}), carbonyl (1724 cm^{-1}), and phenyl (1612, 1517 cm^{-1}) absorptions. The ^{1}H and ^{13}C NMR spectral data of **2** were similar to those of **1**, except for the appearance of a methoxyl group. An HMBC correlation of OMe-4 (δ_H 3.34, s) to C-4 (δ_C 66.0) established the location of the methoxyl at C-4. Thus, compound **2** was assigned as (2*S*,4*R*)-5,7-dihydroxy-4,4′-dimethoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetylglucopyranoside-7-*O*-β-D-glucopyranoside (Figure 1).

Compounds **1** and **2** were tested for *in vitro* cytotoxicity against a human hepatoblastoma cell line (HepG2) using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) method [11]. Both of them showed no cytotoxicity against HepG2 cells and their IC_{50} values were higher than 100 μM. The positive control was 5-fluorouracil with the IC_{50} value of 9.7 μM.

3. Experimental

3.1 General experimental procedures

Melting points were determined using an X-4 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter. CD spectra were obtained on an ASCO J-810 spectropolarimeter. UV spectra were measured on a UV-756 MC UV–vis spectrophotometer and IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer. NMR spectra

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were recorded at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR on a Bruker AM-400 spectrometer. The HR-ESI-MS spectrum was recorded on a Mariner spectrometer. The GC was performed on a GC-14C gas chromatograph (Shimadzu, Tokyo, Japan) with a DB-17 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) (J&W Scientific, Rancho Cordova, CA, USA). Column chromatography was performed with silica gel (Qingdao Marine Chemical Company, Qingdao, China), octadecyl-silica (ODS) (230–400 mesh, Fluka BioChemika, Buchs, Switzerland), and Sephadex LH_{20} (25–100 μm, Fluka BioChemika, Buchs, Switzerland).

3.2 Plant material

The rhizomes of *A. penangiana* (Hook.) Ching were collected in October 2004 from Wufeng County of Hubei Province, China, and they were identified by Prof. Jinlan Ruan. A voucher specimen (PZX0310) has been deposited in the Tongji College of Pharmacy, Huazhong University of Science and Technology.

3.3 Extraction and isolation

The dried rhizomes (5.0 kg) were pulverized and extracted with MeOH (10 liters × 5). The MeOH extract was concentrated under vacuum to leave a residue, which was suspended in H_2O (3 liters) and extracted with $CHCl_3$ (3 liters × 3), EtOAc (3 liters × 3), and *n*-BuOH (3 liters × 3), sequentially. The *n*-BuOH extract (30 g) was chromatographed on a silica gel (1.0 kg) column, eluting with a $CHCl_3$–MeOH–H_2O gradient system (5:1:0.1, 4:1:0.15, 2:1:0.2, 1:1:0.5) to yield 10 fractions (B_1–B_{10}). Fraction B_1 (1.4 g) was passed through a silica gel column ($CHCl_3$–MeOH, 4:1 → 3:2), and then subjected to RP C_{18} column chromatography (MeOH–H_2O, 2:3 → 1:1) to give **2** (16 mg). Fraction B_2 (1.7 g) was chromatographed on a Sephadex LH_{20} column, using $CHCl_3$–MeOH (1:2) as eluent, then further purified on silica gel columns, eluting with EtOAc–MeOH (7:1 → 6:1) to yield **1** (10 mg).

*3.3.1 (2S,4R)-4,5,7-Trihydroxy-4′-methoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetyl glucopyranoside-7-*O*-β-D-glucopyranoside (**1**)*

Colorless needles (MeOH); mp 160–162°C; $[\alpha]_D^{20}$ −3 ($c = 0.53$, MeOH); CD (MeOH) $\Delta\epsilon_{224\,nm}$ −5.73, $\Delta\epsilon_{283\,nm}$ + 1.74; UV (MeOH) (log ϵ) λ_{max}: 228 (4.15), 276 (3.51) nm; IR (KBr) ν_{max}: 3439, 2931, 1742, 1610, 1516, 1452, 1250, 1152, 1076, 836 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 705.2388 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_{16}Na$, 705.2371).

*3.3.2 (2S,4R)-5,7-Dihydroxy-4,4′-dimethoxy-6,8-dimethylflavan-5-*O*-β-D-6-acetylglucopyranoside-7-*O*-β-D-glucopyranoside (**2**)*

White amorphous powder; $[\alpha]_D^{20}$ − 12 ($c = 0.26$, MeOH); CD (MeOH) $\Delta\epsilon_{214\,nm}$−6.49, $\Delta\epsilon_{237\,nm}$ + 2.50, $\Delta\epsilon_{277\,nm}$ + 0.78; UV (MeOH) (log ϵ) λ_{max}: 231 (4.02), 282 (3.48) nm; IR (KBr) ν_{max}: 3422, 2929, 1724, 1612, 1517, 1453, 1253, 1149, 1073, 835 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 719.2518 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{16}Na$, 719.2527).

3.4 Acidic hydrolysis

Each compound (4 mg) was hydrolyzed with 9% HCl (1.5 ml) at 90°C for 5 h. After being cooled, the reaction mixture was filtered, and then the filtrate was freeze-dried. The residues were prepared as thiazolidine derivatives for GC analysis

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according to the methods described in the literature [8].

3.5 Cytotoxicity bioassay

Cytotoxic activity was determined against a human hepatoblastoma cell line (HepG 2) using the previously described MTT method [11].

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