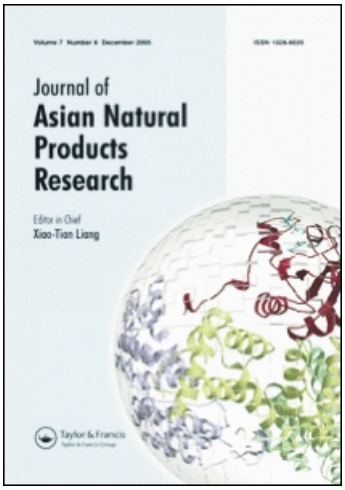

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Two new flavan-4-ol glycosides from *Abacopteris penangiana*

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ORIGINAL ARTICLE

Two new flavan-4-ol glycosides from *Abacopteris penangiana*

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Two new flavan-4-ol glycosides, (2*R*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-6″-*O*-acetyl-glucopyranoside (**1**) and (2*R*,4*S*)-5,7-*O*-β-D-di-glucopyranosyloxy-4′-methoxy-6,8-dimethyl-4,2″-oxidoflavane (**2**), were isolated from the rhizomes of *Abacopteris penangiana*. Their structures were elucidated by spectroscopic methods. Compounds **1** and **2** showed significant anticancer activities against HeLa and L929 cell lines *in vitro*.

Keywords: Amaranthaceae; *Abacopteris penangiana*; flavan-4-ol glycoside; antitumor activity

1. Introduction

Abacopteris penangiana (Hook.) Ching is a fern growing at an altitude of 1900–3600 m in the south of China. As a folk medicine, it has been used to treat infections in the pharynx and larynx [1]. Several infrequent flavan-4-ol glycosides have been isolated from submarginal ferns [2–5].

This paper deals with the isolation and structural elucidation of two new flavan-4-ol glycosides, named (2*R*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-6″-*O*-acetyl-glucopyranoside (**1**) and (2*R*,4*S*)-5,7-*O*-β-D-di-glucopyranosyl-oxy-4′-methoxy-6,8-dimethyl-4,2″-oxido-flavane (**2**) (Figure 1), as well as their cytotoxic activities. Their structures were established by spectroscopic methods, especially by a series of 2D NMR experiments (^{1}H–^{1}H COSY, NOESY, HSQC, and HMBC) and HRMS.

2. Results and discussion

Compound **1** was obtained as an amorphous white powder. Its HR-ESI-MS showed an $[M + Na]^+$ ion at *m/z* 525.1721, corresponding to the molecular formula $C_{26}H_{30}O_{10}$. Its UV spectrum showed the absorption maxima at 281 (log ε 4.01), 274 (log ε 4.03), 222 (log ε 4.75), and 207 (log ε 4.97) nm, and the IR spectrum showed the presence of a hydroxyl group (3452 cm^{-1}), carbonyl (1660 cm^{-1}), and aromatic rings (1616 and 1516 cm^{-1}).

The ^{1}H NMR spectrum (Table 1) of **1** revealed the presence of two methyls at δ_H 2.05 and 2.08 (each 3H, s), an acetyl methyl group (δ_H 2.06, 3H, s), one methoxyl (δ_H 3.81, 3H, s), an AA′XX′

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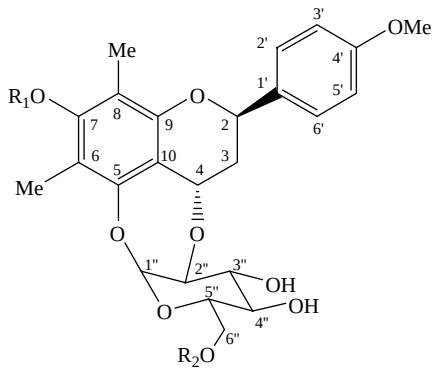

1 $R_1 = H$ $R_2 = CH_3CO$

2 $R_1 = \beta$-D-Glu $R_2 = H$

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

coupling system proton at δ_H 7.43 (2H, dd, $J = 2.0$, 8.7 Hz, H-2′, 6′) and 6.93 (2H, dd, $J = 2.0$, 8.7 Hz, H-3′, 5′), two oxygenated methine protons at δ_H 4.86 (1H, br d, $J = 11.6$ Hz) and 5.09 (1H, dd, $J = 1.7$, 4.7 Hz), one methylene at δ_H 1.95 (1H, ddd, $J = 3.0$, 11.6, 16.5 Hz), 2.30 (1H, dd, $J = 2.0$, 16.5 Hz), and an anomeric proton signal at δ_H 5.11 (1H, d, $J = 8.4$ Hz).

Its ^{13}C NMR spectrum showed 26 carbons, including one acetyl, two benzene rings, one glucose, one methoxy, two oxygenated methines, one methylene, and two methyl carbon signals. From the above observations, compound **1** was deduced to be a flavan-4-ol glycoside, and its ^{13}C NMR spectral data were similar to those of 5,7-dihydroxy-4′-methoxy-6,8-dimethyl-2″,4(*S*)-oxido-2(*R*)-flavan-5-β-D-glucopyranoside (eruberin A) [6], except for an additional acetyl group.

From the ^1H–^1H COSY spectrum, a substructure, —O—CH—CH_2—CH—O—, was concluded from the correlations of H_2-3 (δ_H 1.95, 2.30) with H-2 (δ_H 4.86) and H-4 (δ_H 5.09). The coupling constants of H-2 (δ_H 4.86, br d, $J = 11.6$ Hz), H-3*ax* (δ_H 1.95, ddd, $J = 3.0$, 11.6, 16.5 Hz), H-3*eq* (δ_H 2.30, dd, $J = 2.0$, 16.5 Hz), H-4 (δ_H 5.09, dd, $J = 1.7$, 4.7 Hz), and the values of $[\alpha]_D$ were consistent with those of eruberin A [6], which suggested that the configurations at C-2 and C-4 were 2*R* and 4*S*.

In the HMBC spectrum, the anomeric proton H-1″ (δ_H 5.11, glucose) correlated with the signal at δ_C 151.2 (C-5), H-2″ (δ 3.61) with C-4 at δ_C 67.2, and H-4 at δ_H 5.09 with C-2″ at δ_C 76.5. The above observations indicated that the anomeric carbon (C-1″) was connected to C-5 and C-2″ to C-4 through *O*-linkages. On the other hand, the signal at δ_H 3.81 (4′-OMe) showed correlations with the signal at δ_C 160.9 (C-4′). The signals at δ_H 4.45, 4.19 (H_2-6″) were correlated with the acetyl carbonyl carbon at δ_C 172.8, which indicated that the acetyl group was assigned at position C-6″ (Figure 2). Therefore, the structure of compound **1** was deduced to be (2*R*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-6″-*O*-acetyl-glucopyranoside (Figure 1).

Compound **2**, an amorphous white powder, had the molecular formula $C_{30}H_{38}O_{14}$ from HR-ESI-MS. The ^1H and ^{13}C NMR spectral data (Table 1) were similar to those of compound **1**, except for the presence of an additional glucose and the absence of an acetyl group at C-6″. The HMBC correlation between the anomeric proton (δ_H 4.65, Glu H-1‴) and C-7 at δ_C 156.2 indicated that another glucose moiety was located at C-7 of the flavan-4-ol. The ^1H and ^{13}C NMR signal assignments were achieved by the combination of ^1H–^1H COSY, HSQC, and HMBC spectral elucidation. Thus, the structure of compound **2** was elucidated to be (2*R*,4*S*)-5,7-*O*-β-D-di-glucopyranosyloxy-4′-methoxy-6,8-dimethyl-4,2″-oxidoflavane.

The antineoplastic activity of compounds **1** and **2** was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay with two tumor cell lines: human neuroblastoma cell line (HeLa) and human leukemia cell line (L929). Compounds **1** and **2** showed significant

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of **1** and **2** (CD_3OD, TMS, δ in ppm).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2	4.86 (1H, br d, 11.6 Hz)	74.8 (d)	4.86 (1H, br d, $J = 11.6$ Hz)	74.9 (d)
3	1.95 (1H, ddd, $J = 3.0$, 11.6, 16.5 Hz, H-3*ax*), 2.30 (1H, dd, $J = 2.0$, 16.5 Hz, H-3*eq*)	38.5 (t)	1.95 (1H, ddd, $J = 3.0$, 11.6, 16.5 Hz, H-3*ax*), 2.32 (1H, dd, $J = 2.0$, 16.5 Hz, H-3*eq*)	38.2 (t)
4	5.09 (1H, dd, $J = 1.7$, 4.7 Hz)	67.2 (d)	5.11 (1H, dd, $J = 1.7$, 4.7 Hz)	67.0 (d)
5		151.2 (s)		151.6 (s)
6		111.1 (s)		117.2 (s)
6-Me	2.05 (3H, s)	9.4 (s)	2.08 (3H, s)	10.8 (s)
7		156.2 (s)		156.2 (s)
8		109.6 (s)		118.1 (s)
8-Me	2.08 (3H, s)	8.7 (q)	2.29 (3H, s)	10.4 (q)
9		152.9 (s)		152.9 (s)
10		105.4 (s)		109.7 (s)
1′		134.9 (s)		134.6 (s)
2′/6′	7.43 (2H, dd, $J = 2.0$, 8.7 Hz)	128.7 (d)	7.42 (2H, dd, $J = 2.0$, 8.7 Hz)	128.7 (d)
3′/5′	6.93 (2H, dd, $J = 2.0$, 8.7 Hz)	114.9 (d)	6.94 (2H, dd, $J = 2.0$, 8.7 Hz)	114.9 (d)
4′		160.9 (s)		161.0 (s)
4′-OMe	3.81 (3H, s)	55.8 (q)	3.81 (3H, s)	55.8 (q)
5-Glu				
1″	5.11 (1H, d, $J = 8.4$ Hz)	102.4 (d)	5.17 (1H, d, $J = 8.0$ Hz)	102.7 (d)
2″	3.61 (1H, m)	76.5 (d)	3.41 (1H, m)	79.1 (d)
3″	3.29 (1H, m)	75.8 (d)	3.32 (1H, m)	76.1 (d)
4″	3.19 (1H, m)	71.7 (d)	3.37 (1H, m)	71.8 (d)
5″	3.54 (1H, ddd, $J = 2.0$, 2.2, 3.7 Hz)	76.1 (d)	3.60 (1H, m)	76.7 (d)
6″	4.45 (1H, dd, $J = 2.1$, 11.8 Hz), 4.19 (1H, dd, $J = 5.9$, 11.8 Hz)	64.6 (t)	3.63 (1H, dd, $J = 1.5$, 6.0 Hz), 3.67 (1H, dd, $J = 1.5$, 6.0 Hz)	63.0 (t)
6″-$OCOCH_3$	2.06 (3H, s)	172.8 (s), 20.8 (q)		
7-Glu				
1‴			4.65 (1H, d, $J = 7.5$ Hz)	105.6 (d)
2‴			3.41 (1H, m)	78.0 (d)
3‴			3.17 (1H, m)	75.8 (d)
4‴			3.52 (1H, m)	71.7 (d)
5‴			3.42 (1H, m)	78.0 (d)
6‴			3.77 (1H, dd, $J = 2.2$, 11.9 Hz), 3.90 (1H, dd, $J = 2.2$, 11.9 Hz)	62.9 (t)

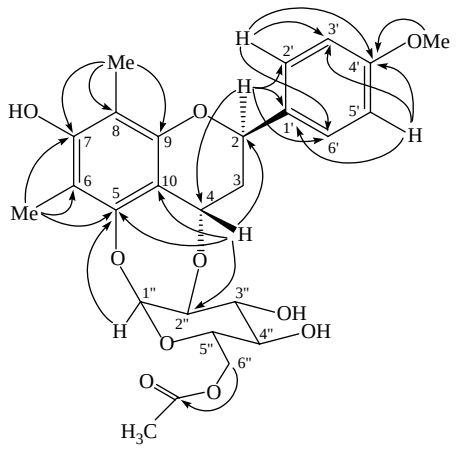

Figure 2. Key HMBC correlations of compound **1**.

inhibitory effect against HeLa (IC_{50} 22.52, 37.68 μg/ml) and L929 (IC_{50} 36.75, 64.39 μg/ml) cell lines, respectively.

3. Experimental

3.1 General experimental procedures

IR spectra were recorded as KBr pellets by a NICOLET 380 spectrometer (Thermo Electron Corporation, Waltham, MA, USA). UV spectra were obtained on a $UVIKON_{XS}$ recording spectrometer (BIO-TEK, Winooski, VT, USA). Optical rotation was measured with an MC 241 digital polarimeter (Perkin-Elmer, Waltham, MA, USA). The NMR samples were run on a Bruker AVANCE 300 instrument (^1H NMR, 300 MHz; ^{13}C NMR, 75 MHz) using tetramethylsilane as the internal standard for both ^1H and ^{13}C NMR analyses. MS data were obtained on an IonSpec 4.7-Tesla Fourier-transform mass spectrometer. Silica gel (300–400 mesh; Qingdao Marine Chemical Co. Ltd, Qingdao, China) was used for column chromatography. TLC plates were performed on silica gel F_{254}. All compounds were detected by spraying with Ce_2SO_4, followed by heating. Flash chromatography was carried out on a column (C18 HS 40M 1621-1; Biotage, Inc., Suite C Charlotte, NC, USA). HPLC separation was performed on a JASCO Gulliver Series with PU-2089 (pump), and RI-2031 and UV-2075 (detectors). The GPC column (gel permeation chromatography, Jordi, K-806M, 10.8 mm × 500 mm × 2) was used as the preparative HPLC with a mobile phase $CHCl_3$.

3.2 Plant material

The rhizomes of *A. penangiana* (Hook.) Ching were collected in Jianshi County, Hubei Province of China in November 2004 and identified by Prof. Ding-Rong Wan (School of Life Sciences, South-Central University for Nationalities). A voucher specimen (D20050103) has been deposited at the School of Pharmaceutical Sciences, Tianjin Medical University, China.

3.3 Extraction and isolation

The dried aerial parts (5.4 kg) of *A. penangiana* were crushed and extracted three times with EtOH (95%, 20 liters each) under reflux for 6 h. The EtOH extract was concentrated *in vacuo* to give a residue (750 g), which was suspended in H_2O, and then partitioned with petroleum ether (PE), EtOAc, and *n*-BuOH, successively. The PE extract (32.5 g) was chromatographed on a silica gel (100–200 mesh) column eluting with solvents of increasing polarity [650 g silica gel; PE–EtOAc (8:1, 5:1, 3:1, 1:1, 1:2, 1:4), EtOAc, EtOAc–MeOH (19:1, 9:1, 4:1), MeOH)] to yield 17 fractions (1–17). Fraction 12 (1.6 g) was chromatographed on a silica gel flash column ($CHCl_3$–MeOH) and further purified by HPLC (GPC, $CHCl_3$) to yield compounds **1** (12.2 mg) and **2** (7.2 mg).

3.3.1 (2R,4S)-6,8-Dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-O-β-D-6″-O-acetyl-glucopyranoside (1)

An amorphous white powder; $[\alpha]_D^{25} + 81$ ($c = 1.00$, MeOH); IR (KBr) ν_{max}: 3452, 2891, 1724, 1616, 1516, 1471, 1373, 1339, 1248, 1143, 1068, 942, 835 cm^{-1}; UV

(MeOH) λ_{max} (log ε): 206 (4.63), 227 (4.39), 274 (3.87), 280 (3.89) nm; ^{1}H and ^{13}C NMR spectral data see Table 1; positive HR-ESI-TOF-MS *m/z*: 525.1721 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_{10}Na$, 525.1737).

3.3.2 (2R,4S)-5,7-O-β-D-Di-glucopyranosyloxy-4′-methoxy-6,8-dimethyl-4,2″-oxidoflavane

An amorphous white powder; $[\alpha]_D^{25} + 63$ ($c = 0.45$, MeOH); IR (KBr) ν_{max}: 3417, 2889, 1613, 1515, 1461, 1343, 1250, 1069, 947, 894, 832 cm^{-1}; UV (MeOH) λ_{max} (log ε): 206 (4.63), 224 (4.40), 281(3.89) nm; ^{1}H and ^{13}C NMR spectral data see Table 1; positive HR-ESI-TOF-MS *m/z*: 645.2169 $[M + Na]^+$ (calcd for $C_{30}H_{38}O_{14}Na$, 645.2159).

3.4 Cytotoxicity assay

Procedure of the bioassay was reported in the previous paper [7].

Acknowledgement

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