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CYTOTOXIC PHENOLIC GLYCOSIDES FROM *Boschniakia himalaica*

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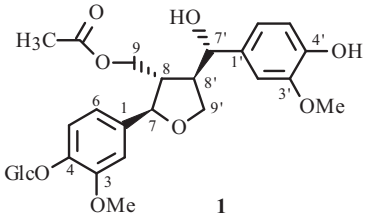
*A new lignan glycoside, 9-acetyl lanicepside B (**1**), was isolated from the whole plant of* Boschniakia himalaica*, along with five known compounds, lanicepside A (**2**), plantainoside D (**3**), plantamajoside (**4**), (–)-woonenoside XI (**5**), and syringin (**6**). The new compound's structure was established on the basis of detailed 1D and 2D NMR spectroscopic analysis and chemical evidence. 9-Acetyl lanicepside B (**1**) showed weak* in vitro *cytotoxic activities against A549 and P388 with IC_{50} values of 64.7 μM and 72.5 μM, respectively. Syringin (**6**) showed moderate* in vitro *cytotoxic activities against A549 and HL-60 with IC_{50} values of 32.5 μM and 41.8 μM, respectively.*

Keywords: *Boschniakia himalaica*, lignan glycosides, phenolic glycosides, cytotoxicity.

Boschniakia himalaica Hook. f. et. Thomson (Orobanchaceae) is a parasitic herb growing on the roots of the genus *Rhododendron* (Betulaceae) and distributed widely in Yunnan, Sichuan, and Tibet, China [1]. This plant, served traditionally as a decoction, has been one of the commonly-used folk medicines in the minority nationality areas of Yunnan Province. Pharmacological studies show that this plant exhibits a variety of activities, including antitussive, anti-inflammatory, and antioxidative activities, and is also used to relieve abdominal pain and syndromes such as gastralgia [2, 3]. Previous chemical studies of *B. himalaica* have led to the isolation of lignans, lignan glucosides, triterpenoids, and sterols [4, 5]. To depict the chemical constituents related to its bioactivities, a further investigation on the title plant was carried out. On the basis of the traditional usage-decoction, the polar fraction was selected for detailed isolation. Herein, we reported the isolation and structural elucidation of a new lignan glycoside, 9-acetyl lanicepside B (**1**), along with five known compounds, lanicepside A (**2**) [6], plantainoside D (**3**) [7], plantamajoside (**4**) [8], (–)-woonenoside XI (**5**) [9], and syringin (**6**) [10]. All compounds were evaluated for their cytotoxic activities against three tumor cell lines, A549, HL-60, and P388.



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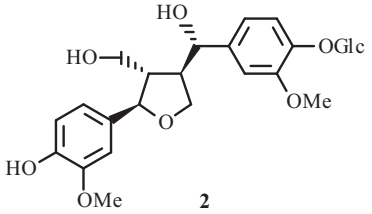

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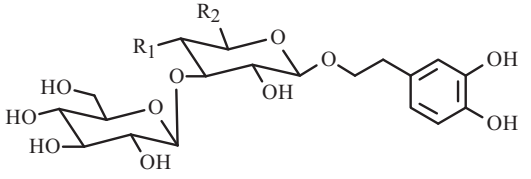

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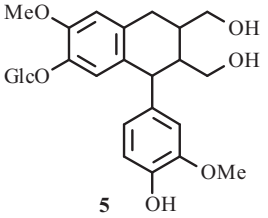
3: R_1 = OH, R_2 = OCOCH=CH-$C_6H_3(OH)_2$
4: R_1 = OCOCH=CH-$C_6H_3(OH)_2$, R_2 = OH



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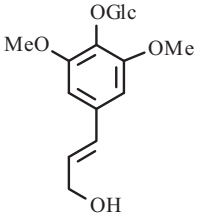

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TABLE 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for Compound **1** (methanol-d_4, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1		134.1	7′	4.50 (d, J = 7.7)	76.6
2	6.92 (d, J = 1.6)	111.7	8′	2.54 m	50.8
3		149.6	9′α	3.93 (dd, J = 9.3, 7.6)	71.6
4		148.0	β	4.27 (dd, J = 9.3, 4.3)	
5	6.77 (d, J = 8.1)	116.6	3-OMe	3.85 s	57.2
6	6.79 (dd, J = 8.1, 1.6)	120.9	3′-OMe	3.84 s	50.7
7	4.55 (d, J = 8.4)	85.9	$CO\underline{C}H_3$	2.05 s	21.2
8	2.12 m	51.5	$\underline{C}OCH_3$		172.2
9	3.20 m	64.3	Glc		
	3.30 m		1″	4.85 (d, J = 7.7)	103.2
1′		140.0	2″	3.48 m	75.4
2′	6.96 (d, J = 1.8)	112.7	3″	3.46 m	78.3
3′		151.4	4″	3.39 m	71.8
4′		147.9	5″	3.39 m	78.6
5′	7.11 (d, J = 8.5)	118.5	6″	3.66 (dd, J = 12.0, 4.7)	63.0
6′	6.85 (dd, J = 8.5, 1.8)	121.1		3.85 m	

TABLE 2. Cytotoxic Activity of Compounds **1–6** (IC_{50}, μM; mean ± SD, n = 3)

Compound	Cell lines			Compound	Cell lines		
	A549	HL-60	P388		A549	HL-60	P388
1	64.7 ± 4.3	> 100	72.5 ± 3.9	**5**	89.7 ± 3.7	> 100	85.1 ± 5.6
2	90.5 ± 5.7	> 100	N.d.[b]	**6**	32.5 ± 5.1	41.8 ± 4.7	92.6 ± 7.2
3	> 100	> 100	> 100	Colchicine[a]	6.8 ± 1.7	13.6 ± 2.1	0.8 ± 0.03
4	N.d.[b]	> 100	> 100				

[a]Colchicine as positive control; [b]N.d.: not determined.

Dried powdered whole plant of *B. himalaica* (500 g) were extracted with 95% EtOH. The crude EtOH extract was subjected to repeated column chromatography over polyamide resin and silica gel, and preparative HPLC as well as preparative TLC, to afford compounds **1–6**.

Compound **1**, obtained as light yellow powder, $[\alpha]_D^{20}$ –19.0° (*c* 0.1, MeOH), has the molecular formula $C_{28}H_{36}O_{13}$, determined on the basis of a quasi-molecular ion $[M + Na]^+$ at *m/z* 603.2059 generated by HR-ESI-MS, ^{13}C NMR, and DEPT spectra. The UV spectrum showed absorption maxima at 220 and 276 nm, and the IR spectrum showed the presence of hydroxyl groups (3366 cm^{-1}), ester carbonyl groups (1745 cm^{-1}), and aromatic moieties (1605, 1517 cm^{-1}), indicating a phenolic moiety.

The ^{1}H NMR spectrum (Table 1) displayed signals of three aromatic protons at δ 6.92 (d, J = 1.6 Hz), 6.79 (dd, J = 8.1, 1.6 Hz), and 6.77 (d, J = 8.1 Hz), assignable to one set of ABX system, as well as three other aromatic protons at δ 7.11 (d, J = 8.5 Hz), 6.96 (d, J = 1.8 Hz), and 6.85 (dd, J = 8.5, 1.8 Hz), ascrible to another set of ABX system. The ^{1}H NMR spectrum also displayed the presence of two oxygenated methylenes, one at δ 3.20 (m) and 3.30 (m), the other at δ 4.27 (dd, J = 9.3, 4.3 Hz) and 3.93 (dd, J = 9.3, 7.6 Hz), two oxygenated methines at δ 4.55 (d, J = 8.4) and 4.50 (d, J = 7.7), two methines at δ 2.54 (m) and 2.12 (m), an acetyl methyl group at δ 2.05 (s), correlated with an ester C=O at δ_C 172.2 in the HMBC spectrum, as well as an anomeric proton at δ 4.85 (d, J = 7.7 Hz), and oxygenated methine and methylene protons ascribed to a sugar moiety. All these resonances were almost identical with those of lanicepside B [6], except for an additional acetyl group. Complete acid hydrolysis with HCl yielded D-glucose, which was determined by comparison with the derivatives of authentic samples by GC analysis. On the basis of the above partial structures, the molecular framework was constructed by the HMBC experiment. The HMBC correlations between the anomeric proton at δ_H 4.85 and C-4 at δ_C148.0 confirmed that the glucose unit was located at C-4. Another HMBC long-range correlation between H-9 at δ_H 3.20 and the C=O moiety at δ_C 172.2 confirmed that the acetyl unit was located at C-9.

The relative stereochemistry of **1** was established on the basis of ^{1}H NMR data and ROESY experiments. The strong cross peaks of H-7 to H_2-9 and H-9′α, H_2-9 to H-8′ and H-7, and H-8′ to H-9′α and H_2-9 indicated that H-7, H_2-9, and H-8′ had α orientations, similar to those of lanicepsides A and B [6], tinosposide B [11], and tanegosides A, B, and C [12]. The relative configuration of H-7′ was determined as having a β orientation by comparison of the chemical shift and coupling constant of H-7′ with those of lanicepsides A and B [6], tinosposide B [11], and tanegool [13], further supported by the ROESY correlation between H-7′ and H-9β. Thus, the structure of compound **1** was identified as shown and named 9-acetyl lanicepside B.

Compounds **2–6** were identified as lanicepside A (**2**) [6], plantainoside D (**3**) [7], plantamajoside (**4**) [8], (–)-woonenoside XI (**5**) [9], and syringin (**6**) [10] by comparing their physical and spectral data with the data reported in the literature.

All compounds isolated form this herb were evaluated against three tumor cell lines, A549, HL-60, and P388 (Table 2). 9-Acetyl lanicepside B (**1**) showed weak *in vitro* cytotoxic activities against A549 and P388 with IC_{50} values of 64.7 μM and 72.5 μM, respectively. Syringin (**6**) showed moderate *in vitro* cytotoxic activities against A549 and HL-60 with IC_{50} values of 32.5 μM and 41.8 μM, respectively.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a Perkin–Elmer polarimeter 341. IR spectra were determined on a Nicolet Magna FT-IR 750 spectrometer (ν_{max}, cm^{-1}). The NMR spectra were recorded on Bruker DRX-400 NMR and Varian UNITYINOVA 600 spectrometers for ^{1}H and ^{13}C NMR. The chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard, and coupling constants (J) are in Hz. The ESI-MS and HR-ESI-MS data were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. Preparative and semipreparative HPLC system: two PrepStar SD-1 solvent delivery modules, a ProStar UV-Vis 320 detector and a ProStar 701 fraction collector (Varian, Walnut Creek, CA, USA); a LinChrospher 100 RP-18 (Merck, Darmstadt, Germany) column (220 × 25 mm i.d.; particle size 5 μm) for preparative isolation; a XTerra^{TM} C_{18} column (3.5 μm; 250 × 10 mm, waters) for semi-preparative isolation. Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200–300 and 400–600 mesh) was used for column chromatography. Precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Plant Material. The whole plant of *B*. *himalaica* were collected in Dali County, Yunnan Province, P. R. China in September, 2006 and identified by Prof. Shukun Chen of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China. A voucher specimen (20060913B) is deposited in the Herbarium of South-Central University for Nationalities, Wuhan, P. R. China.

Extraction and Isolation. The air-dried whole plant of *B*. *himalaica* (200 g) were percolated with 95% aq. EtOH (1 L × 3) at room temperature. The EtOH extract was filtered and concentrated under reduced pressure. Then the concentrated extract was suspended in H_2O and partitioned with *n*-BuOH (1 L × 3). The *n*-BuOH fraction (8 g) was subjected to CC over polyamide resin (300 g) and eluted with 0%, 20, 40, 60, and 80% aq. EtOH in a step manner. The fraction eluted with 20% aq. EtOH was applied to a silica gel column ($CHCl_3$–MeOH–H_2O 9:3:0.2) to afford subfractions 1.1–1.6. Subfraction 1.2 was submitted to prep. TLC ($CHCl_3$–MeOH–H_2O 9:3:0.2) to yield **1** (2 mg) and **2** (5 mg). Subfraction 1.3 was submitted to semipreparative HPLC (H_2O–MeOH 95% : 5%→75%:25%, 35 min) to give **3** (7 mg, 9.6 min) and **4** (8 mg, 10.9 min). Subfraction 1.4 was subjected to CC over silica gel and then preparative TLC ($CHCl_3$–MeOH–H_2O 8:3:0.2) to give **5** (11 mg) and **6** (13 mg).

9-Acetyl Lanicepside B (1). Light yellow powder; $[\alpha]_D^{25}$ –19.0° (*c* 0.1, MeOH). UV (MeOH, λ_{max}, nm): 220, 276. IR (ν_{max}, film, cm^{-1}): 3366, 2926, 1745, 1605, 1517, 1465, 1264, 1227, 1161, 1068, 1025, 817. ^{1}H and ^{13}C NMR, see Table 1; HR-ESI-MS *m/z* 603.2059 $[M + Na]^+$ ($C_{28}H_{36}O_{13}Na$, calcd 603.2054).

Cytotoxicity Assay. Cell lines A549 (human lung carcinoma), HL-60 (human promyelocytic leukemia), and P388 (murine leukemia) were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cytotoxic assay was performed as previously described [14]. Briefly, three cell lines suspended in RPMI 1640 containing 10% FBS were seeded at 1×10^4 cells (100 μL) per well in a flat 96-well plate and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. After 24 h, the medium containing different concentrations of compounds **1–6** was added, and 0.1% DMSO was used as solvent control. After that, the cells were fixed with EtOH–H_2O (95:5, v/v), stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH. The absorbance in control and drug-treated wells was measured in an automated microplate reader (Bio-Rad 550) at 550 nm.

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