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CHEMICAL CONSTITUENTS OF *Pteris multifida*

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Phytochemical study of the whole plant of Pteris multifida *Poir. afforded eight compounds **1–8**. Their structures were established on the basis of spectroscopic methods. Compound **1** was a new natural pterosin, (2*R*)-acetyl pterosin B, and was evaluated for cytotoxic activity against human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480.*

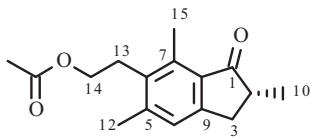
Keywords: *Pteris multifida* Poir., Pteridaceae, (*2R*)-acetyl pterosin B.

Pteris multifida Poir. (Pteridaceae), widely distributed in China, has been used as a traditional chinese medicine for the treatment of enteritis, hepatitis, bacterial dysentery, hematemesis, hematuria, tonsillitis, parotitis, and eczema [1]. A series of *ent*-kaurane diterpenoids and pterosins with antitumor bioactivity from this plant has been reported [2–5]. In the continuing search for biologically active compounds from Chinese medicinal plants, a systematic chemical investigation of the title plant was undertaken. The present study results in the isolation and structural determination of eight compounds, including a new natural pterosin, (2*R*)-acetyl pterosin B (**1**), three known C_{14} illudane sesquiterpenoids **2**, **3,** and **8**, and four *ent*-kaurane diterpenoids **4–7** from an EtOH extract of *Pteris multifida* Poir. The new natural pterosin (2*R*)-acetyl pterosin B (**1**) was evaluated for cytotoxic activity against human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 but did not show cytotoxic activity. We herein report the isolation, structure elucidation, and the cytotoxicity of compound **1**.

The powdered whole plants of *Pteris multifida* Poir. (7 kg) were repeatedly extracted with 90% EtOH at room temperature. The extract was then concentrated under reduced pressure to give a brown syrup, which was diluted with water and extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The petroleum ether and ethyl acetate-soluble fraction was chromatographed over silica gel, Sephadex LH-20, and RP-18 columns to yield compounds **1–8**.

The structures of compounds **1–8** were elucidated on the basis of their MS, ^{1}H NMR, and ^{13}C NMR spectra. All experimental data were in agreement with the respective data in the literature.

Compound **1** was isolated as a colorless powder. Its formula, $C_{16}H_{20}O_3$, was established by analysis of the ^{13}C NMR and DEPT spectra and confirmed by HR-ESI-MS ($[M + Na]^+$ *m/z* 283.1299, calcd 283.1310), which indicated seven degrees of unsaturation.



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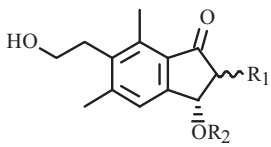

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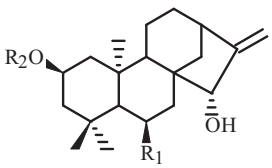

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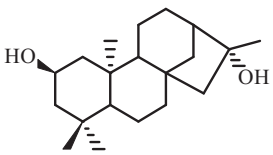

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2: $R_1 = \alpha CH_3$, R_2 = H; **3:** $R_1 = \beta CH_3$, R_2 = H
4: R_1 = OH, R_2 = H; **5:** $R_1 = R_2$ = H; **7:** R_1 = H, R_2 = Glc
8: $R_1 = \beta CH_3$, R_2 = Glc

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TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data, ^{1}H–^{1}H COSY, and HMBC Correlations for **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	^{1}H–^{1}H COSY	HMBC
1		210.6 (s)*		
2	2.60 (m)	42.9 (d)	H-3, H-10	C-1, C-3, C-10
3	3.25 (dd, J = 16.7, 7.8)	34.3 (t)	H-2	C-1, C-2, C-4, C-8, C-9, C-10
	2.58 (dd, overlap)			C-4, C-9
4	7.10 (s)	126.2 (d)	H-12	C-3, C-6, C-8, C-12
5		144.7 (s)		
6		134.4 (s)		
7		138.4 (s)		
8		132.6 (s)		
9		153.2 (s)		
10	1.27 (d, J = 7.3)	16.9 (q)	H-2	C-1, C-2, C-3
12	2.39 (s)	21.3 (q)	H-4	C-4, C-5, C-6
13	3.05 (t, J = 7.7)	28.3 (t)	H-14	C-5, C-6, C-7, C-14
14	4.14 (t, J = 7.6)	63.2 (t)	H-13	C-6, C-13, OAc
15	2.67 (s)	14.0 (q)		C-6, C-7, C-8
OAc	2.05 (s)	171.4 (s)		
		21.5 (q)		171.4

*Multiplicities deduced by DEPT.

The ^{1}H NMR spectra displayed the presence of two aromatic methyl groups at δ 2.67 and 2.39 (each s), one methyl doublet at δ 1.27 (d, J = 7.2 Hz), one ethyl group at δ 3.05 (t, J = 7.7 Hz) and 4.14 (t, J = 7.6 Hz), one methylene group at δ 3.25 (dd, J = 16.7, 7.8 Hz) and 2.58 (dd, overlap), one methine group at 2.60 (m), and an acetyl proton at δ 2.05 (s). A typical signal at δ 7.10 (1H, s) was also observed in the ^{1}H NMR spectrum, which was assignable to the H-atom of a penta-substituted phenyl ring. The above-mentioned evidence and the seven degrees of unsaturation suggested that compound **1** is a sesquiterpenoid with an indan-1-one skeleton [3, 6]. The UV absorption at 215, 260, and 298 nm and IR absorption at 1697 and 1602 also displayed characteristics of pterosin-type compounds [6]. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of **1** with those of pterosin B ((2*R*)-2,3-dihydro-6-(2-hydroxyethyl)-2,5,7-trimethyl-1*H*-inden-1-one) showed that the only structural difference was the presence of an acetyl group in **1** not found in pterosin B [7], and this was supported by its molecular composition. The presence of HMBC correlations between the ethyl group signal at δ 4.14 (t, J = 7.6 Hz, H-14) and acetyl carbonyl at δ_C 171.4 (s) suggested that the acetyl group was located at C-14. The absolute configuration of **1** was also determined from the CD spectrum, which showed a positive Cotton effect at 302 nm in MeOH, indicating the (2*R*)-configuration [8, 9]. Thus, **1** was an acetyl derivative of pterosin B and was established as (*2R*)-(2,4,6-trimethyl-3-oxo-2,3-dihydro-1*H*-inden-5-yl)ethyl acetate. (2*R*)-Acetyl pterosin B (**1**) was obtained synthetically from pterosin B with acetic anhydride in dried pyridine, but as a natural product, (2*R*)-acetyl pterosin B (**1**) was isolated here for the first time from nature [10].

The structures of known compounds **2–8** were identified as (2*R*,3*S*)-pterosin C (**2**) [6], (2*S*,3*S*)-pterosin C (**3**) [11], 2*β*,6*β*,15*α*-trihydroxy-*ent*-kaur-16-ene (**4**), 2*β*,15*α*-dihydroxy-*ent*-kaur-16-ene (**5**) [6], 2*β*,16*α*-dihydroxy-*ent*-kaurane (**6**) [8], creticoside A (**7**) [6], and (2*S*,3*S*)-pterosin C 3-*O*-*β*-D-glucopyranoside (**8**) [6]. All of these known compounds were identified by comparison of physical data with literature values and from spectroscopic evidence.

Compound **1** was evaluated for cytotoxic activities against five human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480, where they proved to be inactive (IC_{50} > 40 μM).

EXPERIMENTAL

Air-dried whole plants of *Pteris multifida* (7 kg) were extracted three times with 90% EtOH at room temperature, yielding a residue (600 g) after evaporation under reduced pressure. The residue was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH successively, yielding petroleum-ether solubles (50 g), EtOAc solubles (42 g), and *n*-BuOH solubles (90 g). The petroleum ether-soluble fraction was subjected to silica gel column chromatography eluted with petroleum ether–EtOAc (9:1–1:1) to afford eight fractions (I–VIII). Fraction V (10 g) was further separated by silica gel column chromatography and then by Sephadex LH-20 column chromatography ($CHCl_3$–MeOH elution) to give **1** (5 mg) and

5 (7 mg). The EtOAc extract (42 g) was subjected to column chromotography (CC) over silica gel and eluted with a mixture of petroleum ether–EtOAc (9:1–1:1), and EtOAc to give fractions A–I. Fraction C (3.3 g) was subjected to silica gel CC using petroleum ether–EtOAc (5:1) as the eluent to give **6** (3 mg). Fraction D (5.7 g) was subjected to silica gel CC and eluted in a step gradient manner with petroleum ether–EtOAc (from 6:1 to 1:1) to give **4** (21 mg). Fraction E (3.9 g) was chromatographed on RP-18 eluted with a MeOH–H_2O (50–100%) gradient, then subjected to silica gel CC, eluted with $CHCl_3$–Me_2CO (3:1), and finally purified by semipreparative HPLC (MeOH–H_2O, 45:55) to give **3** (10 mg) and **2** (15 mg). Fraction H3 (1.6 g) was chromatography over an RP-18 column, eluted with MeOH–H_2O (from 15 to 100%), and then subjected to Sephadex LH-20 column chromatography eluting with MeOH to give **7** (5 mg). Fraction H5 (1.2 g) was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to give **8** (9 mg).

(2*R*)-Acetyl Pterosin B. $C_{16}H_{20}O_3$, colorless gum, $[\alpha]_D^{20}$ –20.1° (*c* 0.84, $CHCl_3$). Mass spectrum *m*/*z* 283.1299 $[M + Na]^+$ (HR-ESI-MS; calcd 283.1310). UV ($CHCl_3$, λ_{max}, nm): 215, 260, 298. IR (KBr, ν, cm^{-1}): 2921, 1745, 1697, 1602, 1451, 1232, 1012.

Table 1 gives the ^1H NMR and ^{13}C NMR spectroscopic data.

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