# A new diterpenoid glucoside from *Pteris semipinnata*

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The chemical investigation of the aerial parts of *Pteris semipinnata* afforded a new ent-kaurane diterpenoid glucoside, pteriside (**1**), and three known sesquiterpenoids, namely (2*R*)-pterosin B (**2**), (2*S*, 3*S*)-pterosin C (**3**) and pterosin C 3-*O*-β-D-glucoside (**4**). The structure of **1** was established by spectral methods, especially two dimensional NMR techniques.

**Keywords:** *Pteris semipinnata*, diterpenoid, sesquiterpenoid

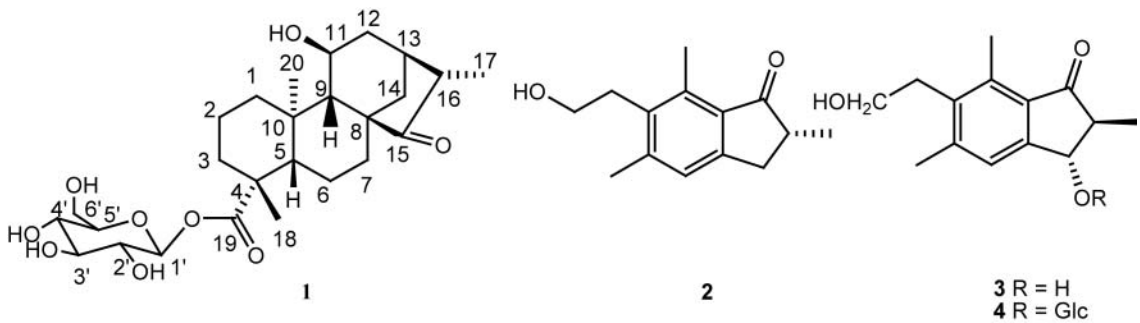
*Pteris semipinnata* L. (Pteridaceae) is a plant which is widely distributed in China. The whole plant has been used in traditional Chinese medicine (TCM) to treat toothache, diarrhoea, jaundice and snake bites.[1] Previous studies on the genus showed that the major secondary metabolites were sesquiterpenoids (known as pterosins) with a 1-indanone skeleton and *ent*-kaurane diterpenoids were their major secondary metabolites.[2–4] The isolation of a series of *ent*-kaurane diterpenoids and illudalane-type sesquiterpenoids have been reported from this plant.[5–8] Some of these inhibited the proliferation of several tumor cell lines.[9–11] In the present study, the systematic chemical investigation of *Pteris semipinnata* resulted in the isolation of a new diterpenoid glucoside, pteriside (**1**), and three known sesquiterpenoids. *i.e.*, (2*R*)-pterosin B (**2**), (2*S*, 3*S*)-pterosin C (**3**) and pterosin C 3-*O*-β-D-glucoside (**4**). The structure of **1** was established using spectroscopic methods, including 2D NMR ($^1$H–$^1$H COSY, HMQC, HMBC, and NOESY).

Pteriside **1** was obtained as a colourless powder. Its molecular formula $C_{26}H_{40}O_9$ was determined by HR-ESIMS at *m/z* 497.2746 (Calcd for $C_{26}H_{41}O_9$ $[M + 1]^+$ 497.2751), revealing the existence of seven degrees of unsaturation in **1**. In the IR spectrum, absorption at 1750 and 1710 cm$^{-1}$ indicated the presence of ketone and ester carbonyls, respectively. This was confirmed by signals at δ 228.5 and 178.4 in the $^{13}$C NMR spectrum. The glycosidic nature of **1** was indicated by anomeric resonance [$\delta_H$ 5.40 (1H, d, $J$ = 7.9 Hz); $\delta_C$ 95.9] (Table 1). The interpretation of NMR spectra of **1** showed that the sugar was glucose. Apart from the sugar moiety, the $^1$H NMR spectrum (Table 1) of **1** displayed the presence of an oxygenated methine proton at δ 3.99 (1H, d, $J$ = 3.8 Hz), three methyls at δ 0.95 (3H, s), 1.00 (3H, d, $J$ = 7.8 Hz) and 1.24 (3H, s). Twenty-six carbon signals corresponding to 26 carbon atoms in the molecular formula were all resolved in the $^{13}$C NMR spectrum, comprising five quaternary carbons (two carbonyls), 10 tertiary carbons, eight secondary carbons and three methyls. According to the $^{13}$C NMR spectrum of **1**, there was no olefinic group. The two carbonyl groups and a sugar accounted for three degree of the unsaturation, the remaining four degrees of unsaturation were accounted for by the presence of four rings in **1**. The data mentioned above showed **1** was an *ent*-kaurane diterpene with glucosyl moiety.[12]

**Table 1** NMR data of **1** at 400 MHz in $CD_3OD$

| NO | **1** | | NO | **1** | |
|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | | $\delta_H$ | $\delta_C$ |
| 1 | 1.90 (1H, m)<br>1.15 (1H, m) | 40.9 | 14 | 1.95 (1H, m)<br>1.90 (1H, m) | 39.9 |
| 2 | 1.92 (1H, m)<br>1.45 (1H, m) | 20.1 | 15 | – | 228.5 |
| 3 | 2.18 (1H, d, 12.0)<br>1.10 (1H, m) | 39.1 | 16 | 2.68 (1H, dq, 7.8, 2.0) | 48.4 |
| 4 | – | 45.3 | 17 | 1.00 (3H, d, 7.8 ) | 16.8 |
| 5 | 1.18 (1H, m) | 58.2 | 18 | 1.24 (3H, s) | 29.3 |
| 6 | 1.90 (2H, m) | 21.6 | 19 | – | 178.4 |
| 7 | 1.70 (1H, m)<br>1.40 (1H, m) | 36.1 | 20 | 0.95 (3H, s) | 16.6 |
| 8 | – | 53.1 | 1′ | 5.40 (1H, d, 7.9) | 95.9 |
| 9 | 1.32 (1H, m) | 63.9 | 2′ | 3.34 (1H, m) | 74.3 |
| 10 | – | 40.3 | 3′ | 3.40 (1H, m) | 79.0 |
| 11 | 3.99 (1H, d, 3.8) | 67.0 | 4′ | 3.36 (1H, m) | 71.4 |
| 12 | 2.30 (1H, m)<br>1.30 (1H, m) | 36.1 | 5′ | 3.41 (1H, m) | 78.9 |
| 13 | 2.10 (1H, m) | 38.6 | 6′ | 3.82 (1H, d, 12.4)<br>3.68 (1H, dd, 12.4, 2.0) | 62.7 |

The proton and corresponding carbon signals in the NMR spectrum of **1** were unequivocally assigned by HMQC experiment. The four partial structures **a** (C-1 to C-3), **b** (C-5 to C-7), **c** (C-9 and C-11 to C-17), and sugar moiety drawn with bold bond were established using a combination of 2D NMR spectra measured in $CD_3OD$ (Fig. 2). The linkage of four structural fragments was finally made by the HMBC experiment (Fig. 2). In the HMBC spectrum, the linkage of C-3 and C-5 via C-4 was deduced from the cross-peaks between $H_3$-18/C-3, $H_3$-18/




**1** **2** **3** R = H, **4** R = Glc

**Fig. 1** Structures of **1**–**4**.

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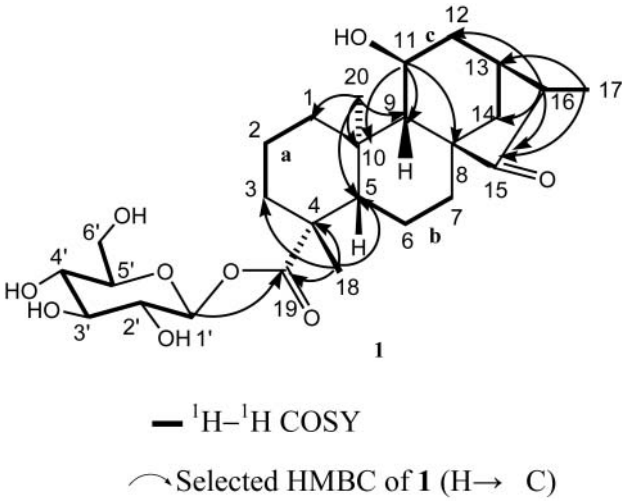


**Fig. 2** Selected 2D NMR correlations of **1**.

C-4, and $H_3$-18/C-5. The linkage of C-1 and C-9 by C-10 was solved by the same method. The HMBC correlations of $H_3$-18/C-4 and $H_3$-18/C-19 showed the presence of a carbonyl group at C-4. The cross-peaks of H-11/C-9 and H-11/C-8 indicated the linkage of C-8 and C-9. A carbonyl at C-15 was revealed by the HMBC correlations of H-17/C-15 and H-16/C-15. The signal at δ 5.40 showed correlation with C-19, suggesting the sugar was located at C-19 to form an ester. The planar structure of **1** was thus established. The aglycone of **1** was very similar to (16*R*)-ent-11α-hydroxy-15-oxokauran-19-oic acid (**2a**),[13] but the $^{13}$C NMR data of **1** showed apparent differences with those of **2a**, especially those of C-13–C-17, which indicated the aglycone of **1** had different configuration at C-16.

The relative stereochemistry of **1** was mainly deduced from the NOESY correlations. The large coupling constant of H-1′ at δ 5.40 indicated the β-configuration of the sugar. The cross-peak between H-18/H-5 observed in the NOESY spectrum indicated that the protons H-18 and H-5 were on the same face and assumed as β-orientation. As a consequence, the ester group (C-19) was in the α-face. In the NOESY spectrum, the correlations between H-20/H-11, H-11/H-13, H-13/H-17, and H-16/H-17 indicated the protons of H-11, H-13, H-17, and H-20 were in the α-orientation. The $^1$H, $^{13}$C NMR spectral data and 2D NMR experiments support the assignment of structure **1** to the new compound which was named pteriside.

Three known sesquiterpenoids were identified as (2*R*)-pterosin B (**2**)[14], (2*S*,3*S*)-pterosin C (**3**)[15] and pterosin C 3-*O*-β-D-glucoside (**4**)[14] by comparison of their spectroscopic data with those reported in the literature.

## Experimental

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as internal standard. ESIMS was recorded on an Agilent 6210 Lc/Tof mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh) and MCI CHP20P gel (75–150 μm; Mitsubishi Chemical Industries Ltd.) were used for column chromatography, and a precoated silica gel $GF_{254}$ plate (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) was used for TLC.

*Plant material: P. semipinnata* was collected from Lijiang area in Yunnan Province of P. R. China and authenticated by Prof. Yong-Hong Zhang of the Fujian Medical University, P. R. China. A voucher specimen (No.20090816P) was deposited with the Lishui Techonology College.

*Extraction and isolation:* The aerial parts of *P. semipinnata* (5.0 kg) were percolated with aq. 95% EtOH. After removal of the solvent, the crude extract (272 g) was suspended in $H_2O$ (5 L) and extracted with EtOAc (5 × 500 mL) to afford the EtOAc soluble fraction. The EtOAc-soluble fraction (38 g) was subjected to a silica gel column eluted with $CHCl_3$/MeOH (50:1–3:1) to give three major fractions 1–3. The fraction 1 (2.7 g) was separated by silica gel column chromatography eluted with petroleum ether/acetone (8:1) to yield **2** (48 mg) and **3** (80 mg). Fraction 2 (3.0 g) was also separated by silica gel column chromatography and eluted with $CHCl_3$/MeOH (8:1–6:1) to afford **1** (18 mg). The fraction 3 (3.8 g) was subjected to column chromatography containing MCI CHP20P gel (MeOH/$H_2O$ 0:10 → 3:7) to give **4** (104 mg).

*Pteriside* (**1**): Colourless powder, $[\alpha]^{20}_D$ −34.0° (*c* 0.1, $CH_3OH$); IR (KBr): 3430, 2934, 1750, 1710, 1382, 1114, 956 cm$^{-1}$; ESIMS *m/z*: 497 [M + 1]$^+$; HR-ESIMS *m/z*: 497.2746 [M + 1]$^+$ (Calcd for $C_{26}H_{41}O_9$ 497.2751). $^1$H NMR and $^{13}$C NMR data: see Table 1.

*(2R)-pterosin B* (**2**): Colourless powder, $[\alpha]^{20}_D$ −30.0° (*c* 0.1, $CHCl_3$); m.p. 108–109 °C; $^1$H NMR and $^{13}$C NMR data were identical with literature data[14].

(2*S*,3*S*)-pterosin C (**3**): Colourless powder, $[\alpha]^{20}_D$ +88.2° (*c* 0.1, MeOH); m.p. 152–155 °C; $^1$H NMR and $^{13}$C NMR data were identical with literature data.[15]

*Pterosin C 3-O-β-D-glucoside* (**4**)**:** Colourless powder, $[\alpha]^{20}_D$ +22.0° (*c* 0.2, MeOH); m.p. 216–217 °C; $^1$H NMR and $^{13}$C NMR data were identical with literature data.[14]

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