A new labdane diterpenoid from *Pteris semipinnata*

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A new labdane diterpenoid, 15-*O*-β-D-glucopyranosyl-labda-8(17), 13*E*-diene-3β, 7β-diol **(1)**, was isolated from the aerial parts of *Pteris semipinnata*, along with two known compounds, namely paniculoside III **(2)**, and pterosin C 3-*O*-β-D-glucoside **(3)**. The structure of **1** was established on the basis of spectroscopic data, including IR, HR-ESI-MS, 1D and 2D NMR spectroscopy.

Keywords: *Pteris semipinnata*, diterpenoid, sesquiterpenoid

Pteris semipinnata L. (Pteridaceae), a traditional Chinese medicinal plant which is widely distributed in Southern China, has been used to treat hepatitis, enteritis and snake bite.[1] Studies on the chemical constituents of *P. semipinnata* L. date back to the 1970s, when Aoyama and co-authors reported the isolation of a new sesquiterpenoid, norptersin C.[2] A series of *ent*-kaurane diterpenoids and illudalane-type sesquiterpenoids, two characteristic chemotaxonomical constituents of the genus of *Pteris*,[3–7] were isolated from this plant and show anti-tumour bioactivity.[8–11] During our continuing search for bioactive components from Chinese medicinal plants, a systematic investigation of the chemical constituents of *Pteris semipinnata* L. was carried out. This led to the isolation of a new labdane diterpenoid **(1)**, together with two known metabolites (**2–3**). The structure of **1** was established on the basis of spectroscopic data, including IR, HR-ESI-MS, 1D and 2D NMR spectra. We report here the isolation and structural determination of these compounds from *P. semipinnata*.

Compound 1 was obtained as a colourless amorphous powder. It was assigned the molecular formula $C_{26}H_{44}O_8$ from HR-ESIMS measurement which showed a pseudomolecular ion peak at *m/z* 507.2925 $[M + Na]^+$ (Calcd for $C_{26}H_{44}NaO_8$, 507.2934). The IR spectrum exhibited bands at 3405 and 1650 cm^{-1} due to hydroxyl and double bond groups, respectively. The glycosidic nature of 1 was indicated by anomeric resonances [δ_H 4.29 (1H, d, J = 7.8 Hz); δ_C 102.4] (Table 1). The ^{1}H NMR spectrum of 1 revealed the presence of four tertiary methyl groups (δ 1.68, 0.79, 1.03 and 0.72), a pair of exocyclic double bond protons (δ 5.26 and 4.72), and a trisubstituted double bond signal at δ 5.31 (1H, t, J = 6.7 Hz). It also showed signals due to two oxygenated methines at δ 3.90 (1H, dd, J = 11.1, 5.2 Hz) and 3.24 (1H, m), and an oxygen-bearing methylene at δ 4.28 (2H, m), together with signals arising from the glucosyl moiety. The ^{13}C NMR spectrum of 1 exhibited the presence of 26 C-atoms comprising four quaternary, ten tertiary, eight secondary C-atoms, as well as four methyl groups. Among them, except for the six carbon signals assigned for glucosyl moiety, two downfield carbon signals (δ 79.6 and 74.8) were ascribed to methines linked with oxygen. Two out of the five degrees of unsaturation were due two double bond groups, and the remaining three degrees of unsaturation were accounted for by three rings. The data mentioned above showed that 1 was a labdane-type diterpene with glucosyl moiety.

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-12), and **d** (C-14 to C-15) drawn with bold bond were established by using a combination of 2D NMR spectra measured in CD_3OD (Fig. 2). The linkage of four structural fragments **a**-**d** was finally made by the HMBC experiment (Fig. 2). In the HMBC, the proton signal of C-18 (δ 0.79) was correlated with the C-3, C-4, and C-5 to connect fragments **a** and **b**; the connections of the C-7 and C-9 to the C-8 were deduced by the HMBC correlations of H-17/C-7, H-17/C-8, and H-17/C-9; C-20, C-1 and C-9 were shown to be attached to the C-10 by the strong HMBC correlations of H_3-20/C-1, H_3-20/C-9, and H_3-20/C-10, respectively; the methyl signal at δ 1.68 showed correlations with C-12, C-13, and C-14, indicating the linkage of fragments **c** and **d** by C-13. The glycosyl moiety was allocated to C-15 by the strong correlations between C-15 and H-1′ (δ 4.29 d, 7.8 Hz) The planar structure of **1** was thus outlined.

Table 1 ^{1}H NMR and ^{13}C Data of **1** in CD_3OD at 500 MHz (δ ppm)

Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1	1.76 (1H, m) 1.14 (1H, m)	38.4	14	5.31 (1H, t, 6.7)	122.1
2	1.66 (2H, m)	28.9	15	4.28 (2H, m)	66.4
3	3.24 (1H, m)	79.6	16	1.68 (3H, s)	16.4
4	–	40.4	17	5.26 (1H, s) 4.72 (1H, s)	104.4
5	1.18 (1H, m)	53.9	18	0.79 (3H, s)	16.5
6	2.03 (1H, ddd, 11.9, 5.3, 2.2) 1.31 (1H, m)	34.6	19	1.03 (3H, s)	29.1
7	3.90 (1H, dd, 11.1, 5.2)	74.8	20	0.72 (3H, s)	15.4
8	-	151.7	1p	4.29 (1H, d, 7.8)	102.4
9	1.53 (1H, m)	55.1	2p	3.18 (1H, m)	75.3
10	–	40.2	3p	3.34 (1H, m)	78.5
11	1.63 (1H, m) 1.55 (1H, m)	22.8	4p	3.29 (1H, m)	72.0
12	2.15 (1H, m) 1.90 (1H, m)	39.2	5p	3.26 (1H, m)	78.4
13	–	142.7	6p	3.87 (1H, dd, 11.9, 2.1) 3.67 (1H, dd, 11.9, 5.6)	63.1

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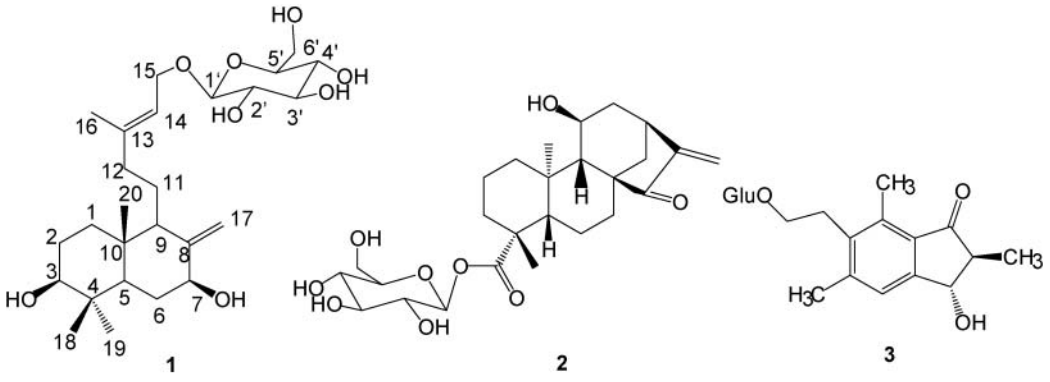

Fig. 1 Structure of compounds **1**–**3.**

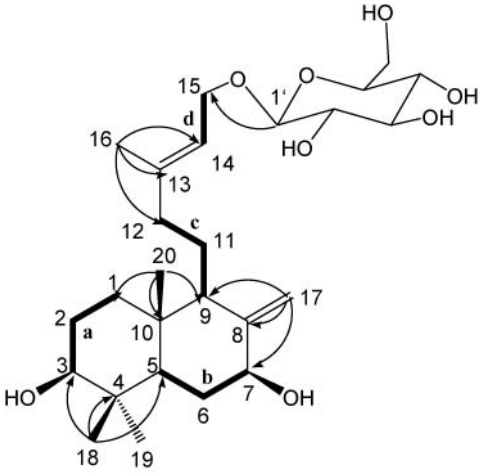

Fig. 2 ^{1}H–^{1}H COSY (—) and Key HMBC (↷) correlations of **1.**

The relative stereochemistry of **1** was completely established by the coupling constants of ^{1}H NMR and the correlations in the NOESY spectrum. The β-configuration of the hydroxyl group at C-3 was revealed by the correlation between H-3 and H-19. The strong correlation of H-7/H-5 showed that the hydroxyl at C-7 was located on the β-face. Comparison with the data of gomojosides L–O [12] indicated the *E*-geometry of Δ^{13}, which was confirmed by NOE correlation of H-15/H-16. The large coupling constant of H-1′ at δ 4.29 (1H, d, J = 7.8 Hz) showed the β-configuration of the sugar. The structure of **1** was thereby established as 15-*O*-β-D-glucopyranosyl-labda-8(17), 13*E*-diene-3β, 7β-dio1. To the best of our knowledge, though there were a series of diterpenoids isolated from this genus[13], the isolation of labdane-type diterpenoid was reported for the first.

The structures of two known compounds also isolated from the title plant were identified as paniculoside III **(2)**[14] and pterosin C 3-*O*-β-D-glucoside **(3)**[15]by comparison of their spectroscopic data with literature values.

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-500 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, P.R. China). Silica gel (200−300 mesh), Lichroprep RP-18 (40−63 μm; Merck), 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, P. R. China) was used for TLC.

Plant material. P. semipinnata was collected from Guiling area in Guangxi Province of P. R. China and identified by Prof. Hai-Bo Bai of the College of City, Zhejiang University. A voucher specimen (ZJUT 081005P) was deposited at Zhejiang University of Technology, P. R. China.

Extraction and isolation. Air-dried and powdered aerial parts of *P. semipinnata* (6.0 kg) were extracted with 95% EtOH at room temperature. After evaporation of the solvent *in vacuo*, the residue was dissolved in H_2O (3 L) and then extracted successively with EtOAc (0.5 L × 5) and BuOH (0.5 L × 5). The BuOH extract (88.0 g) was subjected to column chromatography (CC) containing MCI gel CHP 20P and eluted with MeOH–H_2O (0:10−5:5) to give fractions A-C. Fraction A (13.5 g) was applied to a reversed phase C_{18} silica gel column eluted with MeOH-H_2O (1:9) to afford **3** (12 mg). Fraction B (8.1 g) was subjected to silica gel CC using $CHCl_3$–MeOH (3:1) as the eluent to give **2** (25 mg). Fraction C (2.7 g) was purified on a C_{18} column eluting with MeOH-H_2O (4:6−5:5) to furnish **1** (1.05 g).

15-O-β-D-Glucopyranosyl-labda-8(17), 13E-diene-3β, 7β-dio1 **(1)**, White amorphous powder; $[\alpha]^{20}_{D}$ −56.2° (*c* 0.40, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3405, 2930, 2854, 1650, 1448, 1385, 1077, 1036, 897, 617, 578; ESIMS *m/z* 507 [M + Na]$^{+}$; HRESIMS *m/z* 507.2925 [M + Na]$^{+}$ (Calcd for $C_{26}H_{44}NaO_8$, 507.2934); ^{1}H and ^{13}C NMR: see Table 1.

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