A novel *ent*-kaurane diterpenoid from *Pteris semipinnata*

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The chemical investigation of the aerial parts of *Pteris semipinnata* furnished a novel ent-kaurane diterpenoid, and three known sesquiterpenoids with a 1-indanone skeleton, namely (2*R*)-pterosin B, (2*S*, 3*S*)-pterosin C and norpterosin C. The structure of 7β-hydroxy-11β, 16β-epoxy-*ent*-kauran-19-oic acid was established by spectroscopic methods, especially two dimensional NMR techniques.

Keywords: *Pteris semipinnata*, diterpenoid, sesquiterpenoid

The distinctive constituents of the genus of *Pteris* are *ent*-kaurane diterpenoids and C_{15} or C_{14} sesquiterpenoids (pterosins) with 1-indanone skeleta.[1–4] *Pteris semipinnata* L. (Pteridaceae) is widely distributed in China, and has been commonly used in folk medicine to treat toothache, diarrhea, jaundice and snake bites.[5] A previous phytochemical study of this plant resulted in the characterisation of a series of *ent*-kaurane diterpenoids and pterosins,[5–7] some of which inhibited the proliferation of several tumor cell lines.[8–10] In the present study, the systematic chemical investigation of this plant resulted in the isolation of a novel diterpenoid, 7β-hydroxy-11β, 16β-epoxy-*ent*-kauran-19-oic acid (**1**), and three known sesquiterpenoids. *i.e.*, (2*R*)-pterosin B (**2**), (2*S*, 3*S*)-pterosin C (**3**) and norpterosin C (**4**). The structure of **1** was established using spectroscopic methods, including 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY). We report here the isolation and structural determination of these compounds from *P. semipinnata*.

Compound **1** was obtained as colourless powder. Its molecular formula $C_{20}H_{30}O_4$ was determined by HR-ESIMS *m/z* 335.2228 (Calc. for $C_{20}H_{31}O_4$ [M + 1]$^+$ 335.2222), and indicated the existence of six degrees of unsaturation in **1**. In the IR spectrum, absorption at 3450 and 1723 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum (Table 1) of **1** displayed the presence of two oxygenated methine protons at δ 4.22 (1H, brs) and 3.30 (1H, brs), three methyls at δ 0.90 (3H, s), 1.06 (3H, s) and 1.23 (3H, s). Twenty carbon signals corresponding to 20 carbon atoms in the molecular formula were all resolved in the ^{13}C NMR spectrum. These comprised five quaternary carbons (one carbonyl at δ 179.1; one signal at δ 84.9 attributed to oxygenated carbon), five tertiary carbons (two oxygenated CH at δ 75.6 and 72.4), seven secondary carbons and three methyls. According to the ^{13}C NMR spectrum of **1**, there was no olefinic group. Since one carbonyl group accounted for only one degree of the unsaturation, the remaining five degrees of unsaturation indicated the presence of a penta-cyclic system in **1**.

Analysis of the ^{1}H NMR, ^{13}C NMR and HMQC spectra of **1** enabled us to assign all the protons to their bonding carbons.

Table 1 NMR data of **1** at 500 MHz in DMSO-d_6

No.	1	
	δ_H	δ_C
1	1.78 (1H, m) 1.02 (1H, m)	41.0
2α	1.67 (1H, m)	
2β	1.32 (1H, m)	18.8
3	2.05 (1H, d, 12.8) 1.00 (1H, m)	37.7
4	–	42.2
5	1.75 (1H, m)	46.5
6	1.82 (2H, m)	29.4
7	3.30 (1H, brs)	72.4
8	–	49.0
9	1.65 (1H, m)	52.8
10	–	36.4
11	4.22 (1H, brs)	75.6
12α	1.97 (1H, d, 11.1)	40.5
12β	1.76 (1H, m)	
13	2.15 (1H, t, 6.4)	45.0
14	1.74 (1H, m) 1.22 (1H, m)	42.3
15	1.77 (1H, m) 1.21 (1H, d, 11.0)	52.4
16	–	84.9
17	1.23 (3H, s)	23.3
18	1.06 (3H, s)	28.7
19	–	179.1
20	0.90 (3H, s)	17.3

The three partial structures **a** (C-1 to C-3), **b** (C-5 to C-7), and **c** (C-11 to C-14 and C-9), drawn in bold in Fig. 2, were established by ^{1}H–^{1}H COSY spectra. The planar structure of **1** was deduced from HMBC spectra. In the HMBC spectrum (Fig. 2), the linkage of C-3 and C-5 by C-4 was deduced from the cross-peaks of H_3-18/C-3, H_3-18/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 carboxylic acid group at C-4. The insertion of one oxygen between C-11 and C-16 was revealed by the HMBC correlation of H-11/C-16. This led to the planar structure of **1**.

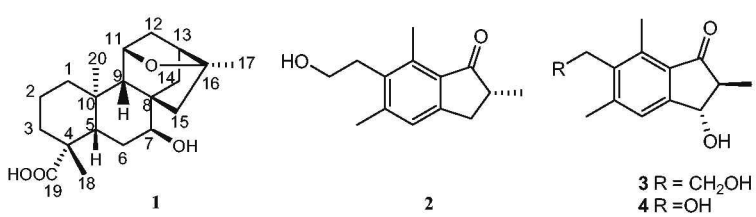

Fig. 1 Structure of compounds **1–4**.

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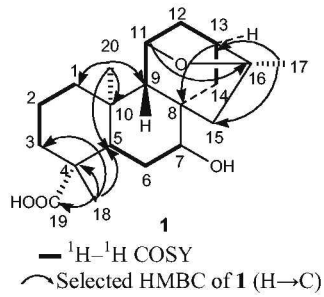

Fig. 2 Selected 2D NMR correlations of **1**.

The relative stereochemistry of **1** was deduced from the NOESY correlations. In the NOESY spectrum, the correlation of H-7/H-17, H-17/H-13, H-13/H-12α, H-12α/H-11, H-11/H-20, and H-20/H-2α indicated that the protons H-2α, H-7, H-11, H-12α, H-13, H-17, and H-20 were on the same face of the molecule and defined this as the α-orientation. The cross-peaks of H-2β/H-18 and H-18/H-5 observed in NOESY spectrum indicated that the protons H-18 and H-5 were on the β-face. As a consequence, the carboxylic acid group (COOH) possessed the α-configuration. The ^{1}H, ^{13}C NMR spectral data and 2D NMR experiments support the assignment of structure **1** to the new compound named as 7β-hydroxy-11β, 16β-epoxy-*ent*-kauran-19-oic acid.

Three known sesquiterpenoids were identified as (2*R*)-pterosin B (**2**), (2*S*,3*S*)-pterosin C (**3**)[11] and norpterosin C (**4**)[6] 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-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, People's Republic of China). Silica gel (200–300 mesh) was 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 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 08550P) was deposited at Zhejiang University of Technology, People's Republic of China.

Extraction and isolation

The dry aerial parts of *P. semipinnata* (6.8 kg) were coarsely powdered and percolated with 95% EtOH. After removal of the solvent, the crude extract (354 g) was suspended in H_2O (3 L) and extracted with EtOAc (each 5 × 500 mL) to afford an EtOAc soluble fraction. The EtOAc-soluble fraction (90 g) was subjected to silica gel column chromatography. Elution with petroleum ether/acetone (20:1–1:2) gave two major fractions 1–2. The first fraction (3.7 g) was separated by silica gel column chromatography and eluted with petroleum ether/acetone (10:1) to yield **2** (28 mg). Fraction 2 (12.0 g) was also separated by silica gel column chromatography eluted with $CHCl_3$/MeOH (100:1–50:1) to afford the diterpenoid **1** (18 mg), and sesquiterpenoids **3** (80 mg), and **4** (27 mg).

7β-Hydroxy-11β, 16β-epoxy-ent-*kauran-19-oic acid* (**1**): Colourless powder, $[\alpha]_D^{20}$ –78.1 ° (*c* 0.36, CH_3OH); IR (KBr): 3450, 2970, 1723, 1380, 1112, 960 cm^{-1}; ESIMS *m/z*: 335 $[M + 1]^+$; HR-ESIMS *m/z*: 335.2228 $[M + 1]^+$ (Calcd for $C_{20}H_{31}O_4$ 335.2222). ^{1}H NMR and ^{13}C NMR data: see Table 1.

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References

1 B. Qing and D.Y. Zhu, *Hua Xue Yan Jiu*, 2004, **15**, 72.
2 B. Qing and D.Y. Zhu, *Hua Xue Yan Jiu*, 2004, **15**, 66.
3 T. Murakami and Y. Saiki, *Biochem. Syst. Ecol.*, 1988, **17**, 131.
4 X. Ge, G. Ye, P. Li, W.J. Tang, J.L. Gao and W.M. Zhao, *J. Nat. Prod.*, 2008, **71**, 227.
5 J.H. Li, N.C. Liang, L.E. Mo, X. Zhang and C.W. He, *Acta Pharm. Sin.*, 1998, **33**, 641.
6 K. Aoyama, N. Tanaka, N. Suzuki, T. Murakami, Y. Saiki and C.M. Chen, *Chem. Pharm. Bull.*, 1977, **25**, 2461.
7 Q. Zhang and L.J. Xuan, *Chin. Chem. Lett.*, 2007, **18**, 1386.
8 J.H. Li, N.C. Liang, L.E. Mo, C.W. He and X. Zhang, *Chin. Pharm. Bull.*, 1999, **15**, 49.
9 C.W. He, N.C. Liang, L.E. Mo, J.H. Li and X. Zhang, *China J. Cancer Prev. Treat.*, 2002, **9**, 11.
10 Z.M. Liu, E.K. W. Ng, N.C. Liang, Y.F. Deng, B.C.S. Leung and G.G. Chen, *FEBS Lett.*, 2005, **579**, 1477.
11 T. Murakami, T Satake and C.M. Chen, *Yakugaku Zasshi*, 1985, **105**, 640.