Pterisolic Acids A—F, New *ent*-Kaurane Diterpenoids from the Fern *Pteris semipinnata*

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Six new *ent*-15-oxokauran-19-oic acid derivatives, named pterisolic acids A—F (1—6), were isolated from the ethanol extract of the fern *Pteris semipinnata* (Pteridaceae), and the structures of these new *ent*-kauranoids were elucidated on the basis of extensive spectroscopic studies and single crystal X-ray diffraction analysis.

Key words *Pteris semipinnata*; *ent*-kauranoid; pterisolic acid

The genus *Pteris* (Pteridaceae) is a rich source of bioactive *ent*-kaurane diterpenoids and pterosins.[1—7] As part of Bio-BioPha to assemble a large-scale natural product library which is very valuable in the discovery of new drug leads from nature,[8—11] the further phytochemical investigation on the whole plants of *Pteris semipinnata* afforded six new *ent*-15-oxokauran-19-oic acid derivatives, named pterisolic acids A—F (**1**—**6**), along with known *ent*-kauranoids, *ent*-9α-hydroxy-15-oxo-16β(*H*)-kauran-19-oic acid (**7**),[2] *ent*-9α-hydroxy-15-oxo-16-kauren-19-oic acid (**8**),[2] *ent*-6α,9α-dihydroxy-15-oxo-16-kauren-19-oic acid (**9**),[3] *ent*-9α-hydroxy-15-oxo-16-kauren-19-oic acid β-D-glucopyranosyl ester (**10**),[3] *ent*-6α,9α-dihydroxy-15-oxo-16-kauren-19-oic acid β-D-glucopyranosyl ester (**11**),[3] and *ent*-6α,11α-dihydroxy-15-oxo-16-kauren-19-oic acid β-D-glucopyranosyl ester (**12**).[3] Herein, we report the isolation and structure elucidation of these new constituents.

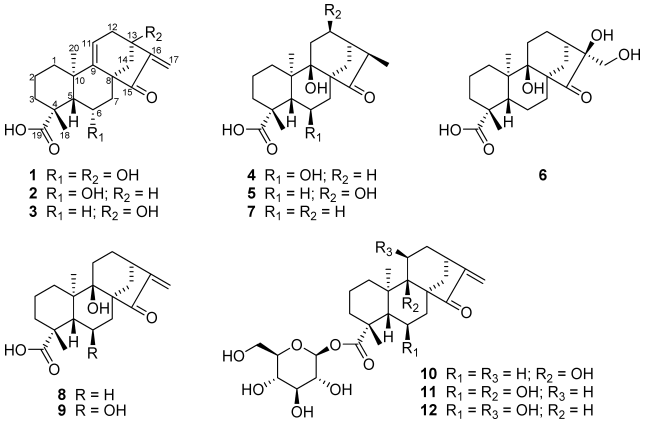

Fig. 1. *ent*-Kaurane Diterpenoids Isolated from *Pteris semipinnata*

Results and Discussion

Compound **1**, obtained as amorphous powder, had a molecular formula of $C_{20}H_{26}O_5$ based on the positive high resolution-electrospray ionization-mass spectrum (HR-ESI-MS), showing a quasi-molecular ion peak at *m*/*z* 347.1856 (Calcd for $C_{20}H_{27}O_5$, 347.1858). The ^{1}H-NMR spectrum (Table 1) showed the following legible signals: two olefinic protons at δ_H 6.10, 5.74 (each br s) due to an exocyclic methylene group, a trisubstituted olefinic proton at δ_H 5.77 (dd, J=4.0, 3.0 Hz), an oxygenated methine proton at δ_H 5.12 (br dd, J=7.7, 6.8 Hz), and two methyl singlets at δ_H 1.39 and 1.04. The ^{13}C-NMR spectrum (Table 3) revealed 20 carbon resonances, including an α,β-unsaturated ketone group at δ_C 204.9 (s), 154.3 (s), and 119.0 (t), a carboxylic carbon at δ_C 181.5 (s), two olefinic carbons at δ_C 149.6 (s) and 123.6 (d), as well as two oxygen-bearing carbons at δ_C 75.1 (s) and

Table 1. ^{1}H-NMR Data of Pterisolic Acids A—C (**1**—**3**) in CD_3OD (δ_H 3.30 ppm)

No.	1	2	3
1	1.12 (H_β, ddd, 13.4, 13.4, 4.0) 1.98 (H_α, br d, 13.4)	1.13 (H_β, ddd, 13.2, 13.2, 4.0) 1.98 (H_α, br d, 13.2)	1.10 (H_β, ddd, 13.4, 13.4, 5.0) 1.85 (H_α, br d, 13.4)
2	1.48 (H_β, m), 1.85 (H_α, m)	1.48 (H_β, m), 1.85 (H_α, m)	1.42 (H_β, m), 1.88 (H_α, m)
3	0.99 (H_β, ddd, 13.7, 13.4, 4.0) 2.16 (H_α, br d, 13.7)	0.99 (H_β, ddd, 13.6, 13.2, 4.0) 2.16 (H_α, br d, 13.6)	0.97 (H_β, ddd, 13.4, 13.1, 4.0) 2.09 (H_α, br d, 13.4)
5	1.38 (d, 7.7)	1.41 (d, 7.7)	1.71 (dd, 10.4, 7.0)
6	5.12 (br dd, 7.7, 6.8)	5.11 (br dd, 7.7, 6.6)	2.13—2.20 (m)
7	1.78 (H_β, br d, 15.4) 2.43 (H_α, dd, 15.4, 6.8)	1.75 (H_β, br d, 15.4) 2.39 (H_α, dd, 15.4, 6.6)	1.77, 1.97 (each m)
11	5.77 (dd, 4.0, 3.0)	5.66 (dd, 3.3, 3.3)	5.67 (dd, 4.5, 2.7)
12	2.37 (H_β, ddd, 17.1, 4.0, 1.0) 2.63 (H_α, dd, 17.1, 3.0)	2.21 (br d, 18.0) 2.68 (ddd, 18.0, 4.4, 3.3)	2.28 (H_β, ddd, 16.7, 4.5, 1.5) 2.61 (H_α, dd, 16.7, 2.7)
13	—	3.10 (br dd, 5.1, 4.4)	—
14	1.82 (H_β, dd, 10.4, 1.0) 1.85 (H_α, d, 10.4)	1.68 (H_α, br d, 11.0) 1.85 (H_β, dd, 11.0, 5.1)	1.68 (H_β, dd, 10.7, 1.5) 1.85 (H_α, d, 10.7)
17	5.74, 6.10 (each br s)	5.61, 5.98 (each br s)	5.61, 5.97 (each br s)
18	1.39 (s)	1.39 (s)	1.23 (s)
20	1.04 (s)	1.05 (s)	1.07 (s)

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66.1 (d). The above NMR data and its biological source suggested that this compound should be an *ent*-15-oxo-16-kauren-19-oic acid derivative.[1—4,12] The heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2) from the proton at δ_H 5.12 (1H, br dd, J=7.7, 6.8 Hz) to the carbons at δ_C 45.5 (s, C-4) and 54.7 (s, C-8), and from the protons at δ_H 6.10, 5.74 (each 1H, br s, H-17) to the carbon at δ_C 75.1 (s), were observed, revealing the presence of hydroxy groups at C-6 and C-13. While the observable HMBC correlations (Fig. 2) from the olefinic proton at δ_H 5.77 (dd, J=4.0, 3.0 Hz) to the carbons at δ_C 54.7 (s, C-8), 40.6 (s, C-10), and 75.1 (s, C-13) indicated that a trisubstituted double bond was located at C-9. No rotating frame Overhauser enhancement spectroscopy (ROESY) correlation between δ_H 1.04 (3H, s, Me-20) and δ_H 1.39 (3H, s) allowed to the assignment of C19-oic acid. The ROESY correlation (Fig. 2) of H-6↔Me-18 indicated that these protons were cofacial and β-oriented, and the more detailed ROESY information has been summarized in Fig. 2. Accordingly, the structure of **1** was elucidated as *ent*-6β,13-dihydroxy-15-oxo-9(11),16-kauradien-19-oic acid, named pterisolic acid A.

Compound **2**, obtained as amorphous powder, had the molecular formula $C_{20}H_{26}O_4$ according to its positive HR-ESI-MS at *m/z* 353.1726 (Calcd for $C_{20}H_{26}O_4Na$, 353.1728). The NMR data (Tables 1, 3) were very similar to those of pterisolic acid A (**1**), and the major difference was that a non-oxygenated methine signal (δ_H 3.10, δ_C 38.1) replaced the oxygenated quaternary carbon (δ_C 75.1) in **1**, meanwhile, the HMBC correlations from the proton at δ_H 3.10 (1H, br dd, J=5.1, 4.4 Hz) to the carbons at δ_C 121.5 (d, C-11), 206.7 (s, C-15) and 118.5 (t, C-17) were observed, thus it was safe to draw the conclusion that the structure of **2** was *ent*-6β-hydroxy-15-oxo-9(11),16-kauradien-19-oic acid. The configuration at C-6 was deduced to be the same as that of **1** based on their accordant NMR data including coupling constants. As a result, the structure of **2** was established and named pterisolic acid B.

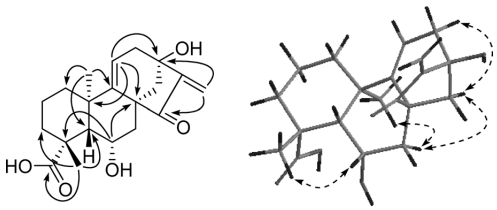

Fig. 2. Significant HMBC and ROESY Correlations of **1**

Compounds **3** and **2** shared the same molecular formula $C_{20}H_{26}O_4$ according to its positive HR-ESI-MS at *m/z* 331.1909 (Calcd for $C_{20}H_{27}O_4$, 331.1909). The NMR data (Tables 1, 3) were also similar to those of pterisolic acid A (**1**), but there were two significant differences: a) the oxygenated methine signal (δ_H 5.12, δ_C 66.1) in **1** disappeared and was replaced by an upfield methylene carbon (δ_C 19.8); b) the ^{13}C-NMR signals of C-5 and C-7 were shifted upfield by 9.2 and 8.9 ppm, respectively, which suggested that **3** was 6-deoxypterisolic acid A. This deduction was further validated by the following HMBC correlations: from the protons at δ_H 1.23 (3H, s, Me-18) and 1.07 (3H, s, Me-20) to the carbon at δ_C 48.4 (d, C-5), and from the protons at δ_H 5.67 (1H, dd, J=4.5, 2.7 Hz, H-11) and 5.61, 5.97 (each 1H, br s, H-17) to the carbon at δ_C 74.7 (s, C-13). Thus the structure of **3** was established as *ent*-13-hydroxy-15-oxo-9(11),16-kauradien-19-oic acid, named pterisolic acid C.

Compound **4** had a molecular formula of $C_{20}H_{30}O_5$ based on the positive HR-ESI-MS, showing a quasi-molecular ion peak at *m/z* 373.1991 (Calcd for $C_{20}H_{30}O_5Na$, 373.1990). The ^{1}H-NMR spectrum (Table 2) showed an oxygenated methine proton at δ_H 4.44 (ddd, J=10.8, 9.9, 3.9 Hz) and three methyl signals at δ_H 1.10 (d, J=7.1 Hz), 1.44 and 1.13 (each s). The ^{13}C-NMR spectrum (Table 3) revealed 20 carbon resonances, including a saturated ketone group at δ_C 224.7 (s), a carboxylic carbon at δ_C 182.0 (s), as well as two oxygen-bearing carbons at δ_C 77.0 (s) and 69.0 (d). These NMR character suggested that **4** should be a dioxygenated derivative of *ent*-15-oxokauran-19-oic acid. The HMBC correla-

Table 2. ^{1}H-NMR Data of Pterisolic Acids D—F (**4**—**6**)

No.	**4**[a)]	**4**[b)]	**5**[a)]	**6**[a)]
1	1.40 (H_α, m), 1.72 (H_β, m)	1.30 (H_α, m), 1.58—1.72 (H_β, m)	1.44 (H_α, m), 1.81 (H_β, m)	1.45 (H_α, m), 1.80 (H_β, m)
2	1.41 (H_β, m), 1.84 (H_α, m)	1.27 (H_β, m), 1.58—1.72 (H_α, m)	1.44 (H_β, m), 1.91 (H_α, m)	1.43 (H_β, m), 1.93 (H_α, m)
3	1.06 (H_β, ddd, 13.4, 13.4, 4.3)	0.89 (H_β, m)	1.01 (H_β, ddd, 13.3, 13.0, 4.1)	1.02 (H_β, ddd, 13.3, 13.3, 4.1)
	2.06 (H_α, br d, 13.4)	1.88 (H_α, br d, 13.6)	2.08 (H_α, br d, 13.3)	2.07 (H_α, m)
5	1.90 (d, 9.9)	1.69 (d, 10.2)	1.81 (m)	1.79 (m)
6	4.44 (ddd, 10.8, 9.9, 3.9)	4.20 (ddd, 11.6, 10.2, 4.1)	1.80—1.89 (m)	1.78—1.89 (m)
7	1.40 (H_α, dd, 13.1, 3.9)	1.14 (H_α, dd, 12.8, 4.1)	1.18 (H_α, m)	1.10 (H_α, ddd, 13.3, 3.3, 2.7)
	2.19 (H_β, dd, 13.1, 10.8)	2.09 (H_β, dd, 12.8, 11.6)	2.09 (H_β, m)	2.09 (H_β, m)
11	1.04 (H_β, m)	0.93 (H_β, m)	1.08 (H_β, dd, 13.1, 11.6)	1.40 (H_β, m)
	2.15 (H_α, ddd, 15.6, 6.7, 2.7)	1.98 (H_α, ddd, 15.4, 6.3, 1.6)	2.40 (H_α, dd, 13.1, 6.4)	2.12 (H_α, m)
12	1.75 (H_α, m), 1.83 (H_β, m)	1.58—1.72 (m)	4.16 (ddd, 11.6, 6.4, 3.4)	1.73 (H_α, m), 2.02 (H_β, m)
13	2.42 (m)	2.30 (m)	2.58 (br ddd, 7.0, 4.7, 3.4)	2.48 (m)
14	1.61 (H_β, ddd, 12.7, 4.0, 2.1)	1.48 (H_β, ddd, 12.4, 3.7, 2.1)	1.54 (H_β, dd, 13.1, 4.7)	1.65 (H_β, ddd, 13.3, 3.8, 2.6)
	2.61 (H_α, br d, 12.7)	2.45 (H_α, br d, 12.4)	2.53 (H_α, br d, 13.1)	2.64 (H_α, br d, 13.3)
16	2.30 (qd, 7.1, 6.9)	2.22 (qd, 7.2, 6.9)	2.38 (qd, 7.4, 7.0)	—
17	1.10 (d, 7.1)	0.98 (d, 7.2)	1.27 (d, 7.4)	3.43, 3.55 (each d, 11.7)
18	1.44 (s)	1.36 (s)	1.19 (s)	1.19 (s)
20	1.13 (s)	1.00 (s)	1.09 (s)	1.17 (s)
6-OH		4.06 (br s)		
9-OH		3.58 (s)		

a, *b*) Measured in CD_3OD (δ_H 3.30 ppm) and DMSO-d_6 (δ_H 2.49 ppm), respectively.

Table 3. ^{13}C-NMR Data of Pterisolic Acids A—F (**1**—**6**) in CD_3OD (δ_C 49.0 ppm)

No.	1	2	3	4	5	6
1	41.7 (t)	41.8 (t)	42.8 (t)	33.2 (t)	32.4 (t)	32.6 (t)
2	21.0 (t)	21.1 (t)	21.3 (t)	19.9 (t)	19.9 (t)	20.0 (t)
3	39.9 (t)	40.0 (t)	39.1 (t)	40.4 (t)	38.9 (t)	38.9 (t)
4	45.5 (s)	45.6 (s)	45.1 (s)	45.5 (s)	44.7 (s)	44.7 (s)
5	57.6 (d)	57.7 (d)	48.4 (d)	55.1 (d)	49.9 (d)	50.1 (d)
6	66.1 (d)	66.5 (d)	19.8 (t)	69.0 (d)	21.4 (t)	21.2 (t)
7	35.5 (t)	35.5 (t)	26.6 (t)	39.5 (t)	30.3 (t)	30.5 (t)
8	54.7 (s)	52.0 (s)	53.9 (s)	57.8 (s)	57.3 (s)	57.8 (s)
9	149.6 (s)	150.0 (s)	150.5 (s)	77.0 (s)	77.1 (s)	78.2 (s)
10	40.6 (s)	40.7 (s)	40.7 (s)	47.4 (s)	45.6 (s)	46.1 (s)
11	123.6 (d)	121.5 (d)	124.1 (d)	30.8 (t)	40.8 (t)	31.9 (t)
12	44.5 (t)	37.5 (t)	44.2 (t)	26.5 (t)	73.4 (d)	29.0 (t)
13	75.1 (s)	38.1 (d)	74.7 (s)	35.6 (d)	42.4 (d)	38.0 (d)
14	48.4 (t)	40.2 (t)	49.5 (t)	39.6 (t)	37.6 (t)	35.2 (t)
15	204.9 (s)	206.7 (s)	203.1 (s)	224.7 (s)	224.8 (s)	222.6 (s)
16	154.3 (s)	152.6 (s)	154.8 (s)	48.5 (d)	49.2 (d)	81.7 (s)
17	119.0 (t)	118.5 (t)	117.1 (t)	10.4 (q)	11.9 (q)	66.2 (t)
18	30.8 (q)	30.8 (q)	29.1 (q)	32.8 (q)	29.6 (q)	29.6 (q)
19	181.5 (s)	181.6 (s)	181.5 (s)	182.0 (s)	181.7 (s)	181.8 (s)
20	26.0 (q)	26.0 (q)	23.3 (q)	19.0 (q)	18.0 (q)	18.0 (q)

tions (Fig. 3) from the oxygenated methine proton at δ_H 4.44 (ddd, J=10.8, 9.9, 3.9 Hz) to the carbons at δ_C 45.5 (s, C-4) and 57.8 (s, C-8), and from the protons at δ_H 1.13 (3H, s, Me-20) to the carbons at δ_C 33.2 (t, C-1), 55.1 (d, C-5) and 77.0 (s), were observed, therefore two hydroxy groups were located at C-6 and C-9. The detectable ROESY correlations (DMSO-d_6, Fig. 3) of H-6↔Me-20, H-6↔H-14α, Me-20↔H-14α, H-16↔H-14β, and Me-17↔H-12β were indicative of α-orientation of H-6 and H-16, and the correlations of 9-OH↔H-1β, H-5, H-7β, and H-11β revealed the β-orientation of C-9 hydroxy group. This *ent*-kauranoid was finally confirmed by single crystal X-ray diffraction, and a perspective ORTEP diagram of the molecule was shown in Fig. 4. As a result, the structure of **4** was determined as *ent*-6α,9α-dihydroxy-15-oxo-16β(*H*)-kauran-19-oic acid, named pterisolic acid D.

Pterisolic acid E (**5**) shared the same molecular formula $C_{20}H_{30}O_5$ with **4** on the basis of its positive HR-ESI-MS at *m*/*z* 373.1996 (Calcd for $C_{20}H_{30}O_5Na$, 373.1990). The NMR data (Tables 2, 3) were generally similar to those of **4**, which indicated that the *ent*-kauranoid held a different stereochemistry or hydroxy substitution pattern. The HMBC correlations from the protons at δ_H 1.09 (3H, s, Me-20) to the carbons at δ_C 32.4 (t, C-1), 49.9 (d, C-5) and 77.1 (s), and from the oxygenated methine proton at δ_H 4.16 (ddd, J=11.6, 6.4, 3.4 Hz) to the carbons at δ_C 37.6 (t, C-14) and 49.2 (d, C-16), were observed, which allowed to position two hydroxy groups at C-9 and C-12. The α-orientation of H-12 and H-16 was based on the ROESY correlations of H-12↔Me-20, H-12↔H-14α, Me-20↔H-14α, and H-16↔H-14β. Consequently, the structure of **5** was elucidated as *ent*-9α,12α-dihydroxy-15-oxo-16β(*H*)-kauran-19-oic acid.

Pterisolic acid F (**6**), amorphous powder, possessed the molecular formula $C_{20}H_{30}O_6$, determined by the positive HR-ESI-MS at *m*/*z* 389.1943 (Calcd for $C_{20}H_{30}O_6Na$, 389.1940). The ^{1}H-NMR spectrum (Table 2) displayed two AB system protons at δ_H 3.43, 3.55 (each 1H, d, J=11.7 Hz) due to a hydroxylmethyl group and two methyl singlets at δ_H 1.19 and

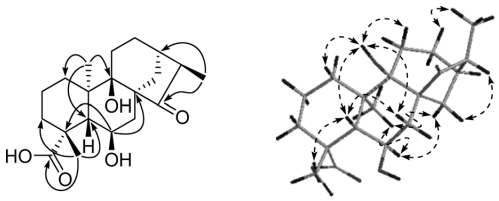

Fig. 3. Significant HMBC and ROESY Correlations of **4**

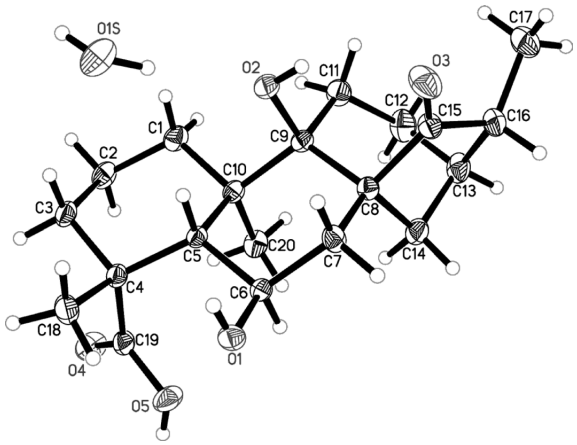

Fig. 4. X-Ray Crystal Structure of **4**

1.17, and the ^{13}C-NMR spectrum (Table 3) also exhibited 20 carbon resonances, including a saturated ketone signal at δ_C 222.6 (s), a carboxylic carbon at δ_C 181.8 (s), and three oxygen-bearing carbons at δ_C 81.7 (s), 78.2 (s) and 66.2 (t), which suggested that **6** was a trioxygenated derivative of *ent*-15-oxokauran-19-oic acid. According to the HMBC correlations from the protons at δ_H 1.17 (3H, s, Me-20) to the carbons at δ_C 32.6 (t, C-1), 50.1 (d, C-5) and 78.2 (s), and from the protons at δ_H 3.43, 3.55 (each 1H, d, J=11.7 Hz) to the carbons at δ_C 38.0 (d, C-13), 222.6 (s, C-15) and 81.7 (s), the position of three hydroxy groups was established at C-9, C-16 and C-17. The hydroxy group at C-16 was assigned as β-oriented by the clear ROESY correlations of Me-20↔H-14α and H_2-17↔H-14β. As a result, the structure of pterisolic acid F was determined as *ent*-9α,16α,17-trihydroxy-15-oxokauran-19-oic acid.

To the best of our knowledge, this type of *ent*-15-oxokauran-19-oic acids was found only in a few genera, such as *Pteris* and *Adenostemma* (Compositae), which suggested their chemotaxonomic significance. We found that it was necessary for those early reported *ent*-9α-hydroxy-15-oxokauran-19-oic acids to interchange the ^{13}C-NMR assignments of C-1 and C-14.[2,3,12]

Experimental

General Experimental Procedures Optical rotations were measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. NMR spectra were carried out on either Bruker DRX 500 MHz or Bruker Avance III 600 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with the deuterated solvent as an internal standard. ESI-MS (including HR-ESI-MS) were measured on an API QSTAR Pulsar i (MDS Sciex, Concord, Ontario, Canada) mass spectrometer. Single crystal X-ray crystallographic data were collected on a Bruker APEX II DUO diffractometer. Silica gel 200—300 mesh (Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and MCI gel CHP 20P (75—150 μm, Mitsubishi Chemical Corp.,

Tokyo, Japan) were used for normal pressure column chromatography. Fractions were monitored and analyzed by TLC (Qingdao Marine Chemical Inc., China), in combination with Agilent 1200 series HPLC system equipped by Eclipse XDB-C18 column (5 μm, 4.6×150 mm).

Plant Material The whole plants of *P. semipinnata* were collected in Hekou County of Yunnan Province, China, in August 2008, and identified by Mr. Yu Chen of Kunming Institute of Botany, CAS. A voucher specimen (No. BBP2010011PS) was deposited at BioBioPha.

Extraction and Isolation Dried and powdered whole plants (8.5 kg) of *P. semipinnata* were extracted with 95% ethanol at room temperature. The alcohol extract was concentrated to give a residue (*ca.* 1.0 kg), which was fractionalized by silica gel column chromatography eluted with a solvent system of petroleum ether/acetone (20 : 1, 10 : 1, 7 : 1, 5 : 1, 3 : 1, 1 : 1, 0 : 1) and then pure methanol to yield fractions A—H, respectively. Fraction B was repeatedly isolated and purified by silica gel ($CHCl_3$/MeOH, 100 : 0→100 : 1) and Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) to afford **2** (10 mg). Fraction C was subjected to silica gel using $CHCl_3$/MeOH (100 : 0→50 : 1), and the fraction eluted by $CHCl_3$/MeOH 50 : 1 was further isolated by silica gel using petroleum ether/acetone (20 : 1, 10 : 1, 8 : 1) to get subfractions I, II and III, respectively. Subfraction II was further isolated and purified by silica gel ($CHCl_3$/MeOH, 120 : 1→80 : 1), MCI (pure MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1 or 0 : 1) to afford **3** (53 mg) and **5** (9.0 mg), and compound **4** (250 mg) was obtained from subfraction III in the same way. Fraction D was isolated and purified by silica gel ($CHCl_3$/MeOH, 100 : 1→50 : 1), MCI (pure MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) to afford **1** (255 mg), and compound **6** (62 mg) was acquired from fraction E in the similar way. The retention times (t_R) of **1**—**3** from analysis-type HPLC (50%→100% MeOH in H_2O over 6.0 min followed by 100% MeOH to 10 min, 1.0 ml/min, 20 °C) were 5.6, 6.8 and 5.4 min, respectively.

Pterisolic acid A (**1**): Colorless powder, $[\alpha]_D^{27}$ +53.4° (c=0.12, MeOH). TLC: Rf=0.21 ($CHCl_3$: MeOH=20 : 1). UV λ_{max} (MeOH): 231, 280 (sh), 345 nm. IR (KBr): 3463, 1701, 1687, 1648, 1452, 1218, 1029, 1017, 951 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 1 and 3, respectively. ESI-MS (pos.): 347 $[M+H]^+$, 369 $[M+Na]^+$. HR-ESI-MS (pos.): 347.1856 (Calcd for $C_{20}H_{27}O_5$, 347.1858).

Pterisolic acid B (**2**): Colorless powder, $[\alpha]_D^{27}$ +21.6° (c=0.10, MeOH). TLC: Rf=0.44 ($CHCl_3$: MeOH=20 : 1). UV λ_{max} (MeOH): 236, 276 (sh), 344 nm. IR (KBr): 3386, 1710, 1640, 1445, 1215, 1048, 1019, 953 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 1 and 3, respectively. ESI-MS (pos.): 353 $[M+Na]^+$. HR-ESI-MS (pos.): 353.1726 (Calcd for $C_{20}H_{26}O_4Na$, 353.1728).

Pterisolic acid C (**3**): Colorless powder, $[\alpha]_D^{27}$ +133.6° (c=0.11, MeOH). TLC: Rf=0.48 ($CHCl_3$: MeOH=20 : 1). UV λ_{max} (MeOH): 238 (sh), 276 (sh), 349 nm. IR (KBr): 3453, 1726, 1707, 1648, 1466, 1222, 1150, 1106, 1029, 953 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 1 and 3, respectively. ESI-MS (pos.): 331 $[M+H]^+$, 353 $[M+Na]^+$. HR-ESI-MS (pos.): 331.1909 (Calcd for $C_{20}H_{27}O_4$, 331.1909).

Pterisolic acid D (**4**): Colorless columnar crystals (acetone), $[\alpha]_D^{27}$ −72.2° (c=0.11, MeOH). TLC: Rf=0.29 ($CHCl_3$:MeOH=20:1). IR (KBr): 3423, 1715, 1691, 1447, 1231, 1213, 1035 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 2 and 3, respectively. ESI-MS (pos.): 373 $[M+Na]^+$. HR-ESI-MS (pos.): 373.1991 (Calcd for $C_{20}H_{30}O_5Na$, 373.1990).

Pterisolic acid E (**5**): Colorless powder, $[\alpha]_D^{27}$ −49.8° (c=0.11, MeOH). TLC: Rf=0.26 ($CHCl_3$: MeOH=20 : 1). IR (KBr): 3439, 1717, 1467, 1449, 1213 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 2 and 3, respectively. ESI-MS (pos.): 373 $[M+Na]^+$. HR-ESI-MS (pos.): 373.1996 (Calcd for $C_{20}H_{30}O_5Na$, 373.1990).

Pterisolic acid F (**6**): Colorless powder, $[\alpha]_D^{27}$ −39.8° (c=0.10, MeOH). TLC: Rf=0.16 ($CHCl_3$: MeOH=20 : 1). IR (KBr): 3440, 1715, 1689, 1469, 1450, 1281, 1048, 1018 cm^{-1}. ^{1}H- and ^{13}C-NMR data: see Tables 2 and 3, respectively. ESI-MS (pos.): 389 $[M+Na]^+$. HR-ESI-MS (pos.): 389.1943 (Calcd for $C_{20}H_{30}O_6Na$, 389.1940).

X-Ray Crystallographic Analysis of 4 $C_{20}H_{30}O_5 \cdot H_2O$, molecular weight (MW)=368.46, orthorhombic, space group $P2_12_12_1$, a=7.2995(8) Å, b=12.8722(14) Å, c=19.6270(2) Å, V=1844.1(4) Å^3, Z=4, ρ_{calcd}=1.327 g/cm^3, λ=0.71073 Å, μ(Mo$K\alpha$)=0.097 mm^{-1}, F(000)=800, and T=296(2) K. A colorless columnar crystal of dimensions 0.52×0.32×0.23 mm was selected for X-ray analysis. A total of 19722 reflections, collected in the range $1.89° \leq \theta \leq 30.03°$, yielded 5239 unique reflections. The crystal structure was solved by direct methods using SHELXS-97, and refined by full-matrix least-squares methods on F^2. Hydrogen atoms were fixed at calculated positions. The final R indices [$I>2\sigma(I)$] were R_1=0.0338, wR_2=0.0895, and the goodness-of-fit on F^2 was 1.026. Crystallographic data for **4** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication (CCDC No. 806366). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk).

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