Cytotoxic Diterpenoids and Sesquiterpenoids from *Pteris multifida*

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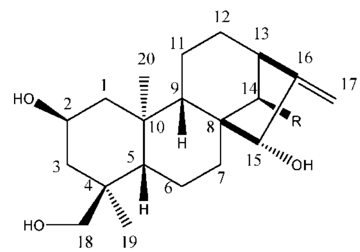
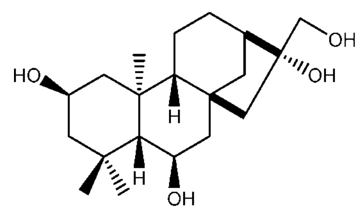
Three new *ent*-kaurane diterpenoids, named pterokaurane M_1-M_3 (**1–3**) and three new C_{14} pterosin-sesquiterpenoids, named multifidoside A−C (**4–6**), along with 18 known compounds, were isolated from the whole plants of *Pteris multifida*. The structures of **1**−**6** were established using spectroscopic methods, including extensive 2D NMR and CD analyses. Compounds **4** and **5** showed cytotoxicity against the HepG2 tumor cell line with IC_{50} values of less than 10 μM.

Pteris multifida Poir. (Pteridaceae) is a plant widely distributed in the southeast of China, and the whole plant has been used in traditional Chinese medicine as an antitumor and anti-inflammatory agent.[1–4] Previous phytochemical investigation of the genus *Pteris*[5–9] revealed C-20-nonoxygenated *ent*-kaurane diterpenoids and C_{14} sesquiterpenoids (pterosins) with 1-indanone skeletons. *ent*-Kauranes, found in several plant families, mainly in the *Isodon* species (Labiatae), have attracted interest due to their structural diversity and antitumor activity.[10] E. Fujita and Han-Dong Sun have reviewed systematically on the *Isodon* diterpenoids, concerning the isolation, structural elucidation and biological evaluation.[11,12] Pterosin sesquiterpenoids were first isolated from the bracken fern, *Pteridium aquilinum* var. *latiusculum* (Pteridaceae).[13] About 50 pterosin sesquiterpenoids, 2,5,7-trimethyl-indan-1-one derivatives, have been isolated from ferns of the same family.[14] Among them, pterosin Z and acetyl-Δ^2-dehydropterosin B were found to be cytotoxic.[15] In the course of our efforts to find new antitumor compounds, a systematic chemical investigation of the title plant was undertaken. As a result, three new *ent*-kaurane diterpenoids, pterokauranes M_1-M_3 and three new C_{14} sesquiterpenoids, multifidosides A−C, 10 known diterpenoids and eight known C_{14} sesquiterpenoids were identified. The new compounds (**1**−**6**) were evaluated for their cytotoxic activity against HepG2, K562, KB, and LoVo cell lines *in vitro*. We herein report the structure elucidation of these new compounds and their cytotoxicity.



1 R = H
2 R = OH



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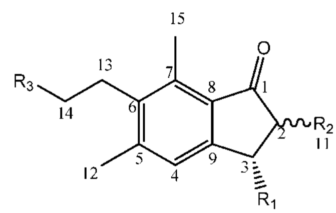
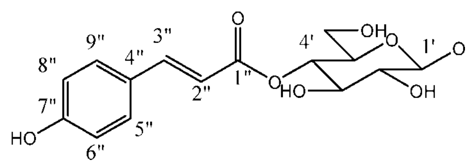


	R_1	R_2	R_3
4	M	β-CH_3	OH
5	M	α-CH_3	OH
6	H	α-CH_3	M

M =



Results and Discussion

The 95% EtOH extract of the whole plant of *Pteris multifida* was partitioned between ethyl acetate and water, and the ethyl acetate-soluble fraction was chromatographed over silica gel, Sephadex LH-20, and RP-18 gel columns to yield compounds (**1**–**6**).

Compound **1**, isolated as a white amorphous solid, had the molecular formula $C_{20}H_{32}O_3$ by HREIMS (*m/z* 320.2342; calcd 320.2351), indicating five degrees of unsaturation in its structure. The IR spectrum of **1** showed absorption bands due to OH (3311 cm^{-1}) and C═C double bond (1660 cm^{-1}) groups. Two tertiary methyl groups [δ_H 0.64 (3H, s), 0.96 (3H, s); δ_C 18.5 (q), 19.1 (q)], one olefinic group [δ_H 5.04 (1H, s), 4.94 (1H, s); δ_C 159.6 (s), 107.8 (t)], and three oxygenated carbons [δ_C 81.4 (d), 70.0 (t), 62.8 (d)] were deduced from its ^{1}H and ^{13}C NMR spectra. The upfield region (below δ_C 60.0) of its ^{13}C NMR spectrum exhibited signals of seven methylene, three methine, and three quaternary carbons. The ^{1}H−^{1}H-COSY and HMQC spectra of **1** indicated the presence of the structural fragments $-CH_2CHCH_2-$ (C-1−C-2−C-3), $-CHCH_2CH_2-$ (C-5−C-6−C-7), and $-CHCH_2CH_2CH-$ (C-9−C-11−C-12−C-13). Analysis of the HMBC spectrum demonstrated that the structural fragments are consistent with an *ent*-kaurene skeleton based on cross peaks of H-5 (δ_H 1.02, 1H,

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overlapped) with C-1, C-4, C-6, C-7, C-9, C-10, C-18, and Me-19, 20, of H-9 (δ_H 0.93, 1H, overlapped) with C-1, C-8, C-10, C-11, C-14, C-15, and Me-20. The oxygenated methylene signal was assigned to C-18 because the equatorial C-18 CH_2OH signal (δ_C 70.0) should be more downfield than the axial C-19 CH_2OH signal (about δ_C 65.0).[16] In the ROESY spectrum of **1**, NOE correlation signals were clearly observed between H-2 and H-1α, H-3α, Me-19, and Me-20 and between H-15 and H-17a, H-9, and H-14β, which revealed the OH groups at C-2 and C-15 to be β- and α-oriented, respectively. Therefore, compound **1** was elucidated as 2β, 15α,18-trihydroxy-*ent*-kaur-16-ene.

Comparison of the ^{13}C NMR data of **2** with those of **1** revealed that they were quite similar, except that a methylene group at C-14 in **1** was replaced by a hydroxymethine function in **2**. The correlation of H-14 (δ_H 4.46, s) with C-7 (δ_C 32.9), C-15 (δ_C 83.5), and C-16 (δ_C 160.0) in its HMBC spectrum confirmed the above deduction. In the ROESY spectrum of **2**, NOE correlations were observed between H-14 and H-7α, H-7β, H-12α, H-13, and Me-20, which revealed the OH group at C-14 to be β-oriented. Cross-peaks in the ROESY spectrum of **2** indicated that the corresponding substituents (except for C-14) in compound **2** had the same orientations as those of compound **1**. Comparison of the NMR data of compound **2** with its C-4 epimer, the known compound pterokauran P_4,[7,16] which had C-19 oxygenation instead of C-18, confirmed the assignments above. Therefore, compound **2** was deduced as 2β, 14β, 15α, 18-tetrahydroxy-*ent*-kaur-16-ene.

The molecular formula of compound **3** was deduced as $C_{20}H_{34}O_4$ from its HREIMS and NMR data, requiring one less degree of unsaturation than **1** and **2**. Compound **3** had no olefinic group at C-16 and C-17 according to the ^{13}C NMR spectrum. An OH group at C-17 was evident in **3** on the basis of a signal (δ_C 66.4, t) in the ^{13}C NMR spectrum. Comparison of IR and 1D and 2D NMR data of **3** with 2β,6β,16α-trihydroxy-*ent*-kaurane and 2β,15α,16α,17-tetrahydroxy-*ent*-kaurane[17] indicated that C-2, C-6, and C-16 should be oxygenated. ROESY correlations of H-2 with H-1α, H-3α, Me-19, and Me-20 and of H-6 with H-7α, Me-19, and Me-20, **3** were consistent with an *ent*-kaurane diterpenoid having OH groups at C-2, C-6, and C-16 in β-, β-, and α-orientations, respectively. Thus, compound **3** was elucidated as 2β,6β,16α,17-tetrahydroxy-*ent*-kaurane.

Compound **4** had the molecular formula $C_{29}H_{34}O_{10}$, as determined by HRESIMS. Acid hydrolysis **4** yielded *p*-coumaric acid, glucose, and its aglycone. The latter had the same retention time as (2*S*,3*S*)-pterosin C in the HPLC experiment.[6] The ^1H NMR spectrum of **4** showed an anomeric proton at δ 5.27 (1H, d, J = 7.2 Hz), demonstrating the β-conformation of the sugar. The ^1H and ^{13}C NMR data showed the presence of an (*E*)-*p*-coumaroyl group [δ_H 6.61 (d, J = 15.6 Hz, H-2″), 7.96 (d, J = 15.6 Hz, H-3″), 7.16 (2H, d, J = 8.1 Hz, H-6″, H-8″), 7.55 (2H, d, J = 8.1 Hz, H-5″, H-9″); δ_C 167.1 (C-1″)], a β-glucopyranose moiety [δ_H 5.27 (d, J = 7.2 Hz, H-1′); δ_C 62.0 (C-6′), 72.4 (C-4′), 75.4 (C-2′), 75.8 (C-5′), 76.4 (C-3′), 105.6 (C-1′)], and a 1-indanone skeleton identical to that of (2*S*,3*S*)-pterosin C.[6] The ^{13}C NMR spectrum of **4** showed 14 carbon signals for the 1-indanone skeleton, which were resolved into one carbonyl, one penta-substituted aromatic ring, two methine, two methylene, and three methyl carbons through DEPT experiments. The β-glucopyranose unit was connected to C-3, as indicated by a HMBC correlation of H-3 to the anomeric carbon of the glucose unit and of the anomeric proton to C-3. From the significant downfield shift (δ_H 5.84) of H-4′ in comparison with (2*S*,3*S*)-pterosin C 3-*O*-β-glucoside, the coumaroyl group was suggested to be linked to the C-4′ hydroxyl group via an ester bond, a conclusion supported by an HMBC correlation of H-4′ to the ester carbonyl at δ_C 167.1 of the coumaroyl group. The *trans*-configuration of the methyl at C-2 and the OH at C-3 in **4** was proved by $J_{2,3}$ (3.6 Hz).[6,18] The absolute configuration of compound **4** was determined from its CD spectrum, which showed a positive Cotton effect at 322 nm in MeOH, indicating that the OH group at C-3 exists in pseudoaxial conformation irrespective of the configuration at C-2.[19,20] Accordingly, compound **4** was assigned as (2*S*,3*S*)-pterosin C 3-*O*-β-(4′-*p*-coumaroyl)-glucopyranoside.

Compound **5** had molecular formula $C_{29}H_{34}O_{10}$, identical to that of **4**. The NMR data of **5** were similar to those of **4**, except for the chemical shifts and J values due to H-2, H-3, and Me-11 of the 1-indanone skeleton. The $J_{2,3}$ (6.6 Hz) value observed indicated **5** to be a *cis*-2,3 isomer.[18] The CD spectrum exhibited a positive Cotton effect at 327 nm in MeOH, indicating that **5** has the same absolute configuration as (2*R*,3*S*)-pterosin C.[19,20] Thus, compound **5** was determined to be (2*R*,3*S*)-pterosin C 3-*O*-β-(4′-*p*-coumaroyl)-glucopyranoside.

Compound **6** had the molecular formula $C_{29}H_{34}O_9$, as determined by the HRESIMS. Acid hydrolysis and analysis of the NMR data of **6** revealed the presence of an (*E*)-*p*-coumaroyl moiety, a β-glucopyranose unit, and an aglycone with a 1-indanone skeleton identical to that of (2*R*)-pterosin B.[6] The ^1H and ^{13}C NMR data of **6** were closely related to those of (2*R*)-pterosin B 14-*O*-β-glucopyranoside,[21] except for the presence of an additional (*E*)-*p*-coumaroyl group. The significant downfield shift of H-4′ of the glucose moiety (δ_H 5.79) in comparison with (2*R*)-pterosin B 14-*O*-β-glucopyranoside suggested the linkage site of the coumaroyl group via an ester bond, which was further supported by the HMBC correlation of H-4′ to the ester carbonyl at δ_C 167.2. The absolute configuration of **6** was also determined from the CD spectrum, which showed a positive Cotton effect at 302 nm in MeOH, indicating the (2*R*)-configuration.[19,20] Accordingly, compound **6** was identified to be (2*R*)-pterosin B 14-*O*-β-(4′-*p*-coumaroyl)-glucopyranoside.

The structures of 18 known diterpenoids and sesquiterpenoids isolated from the title plant were identified as pterokaurane P_1,[7] pterokaurane P_1 2-*O*-β-glucopyranoside,[7] 2β,6β,15α-trihydroxy-*ent*-kaur-16-ene,[17] pterokaurane P_3,[6] 2β,15α-dihydroxy-*ent*-kaur-16-ene,[6] creticoside A,[6] 2β,6β,16α-trihydroxy-*ent*-kaurane,[17] 2β,15α,16α,17-tetrahydroxy-*ent*-kaurane,[17] 2β,14β,15α,16α,17-pentahydroxy-*ent*-kaurane,[17] siegesbeckiol,[22] (2*S*,3*S*)-pterosin C,[6] (2*R*,3*S*)-pterosin C,[21] (2*S*,3*S*)-pterosin C 3-*O*-β-glucopyranoside,[6] (2*S*,3*S*)-pterosin Q,[6] (2*R*)-pterosin B,[6] (2*S*,3*S*)-pterosin S,[6] (2*S*,3*S*)-pterosin S 14-*O*-β-glucopyranoside,[6] (2*R*)-pterosin B 14-*O*-β-glucopyranoside,[21] by comparison of their spectroscopic data with literature values.

The six new compounds (**1**−**6**) were evaluated for their cytotoxicity against HepG2, K562, KB, and LoVo cells, using SRB or WST-1 methods, with adriamycin and taxotere as positive controls (Table 3). Compounds **4** and **5** demonstrated significant inhibitory activity against HepG2 cells, with IC_{50} values of 8.69 and 9.26 μM, respectively, and also displayed inhibitory effect on K562 cells with IC_{50} values of 10.63 and 9.57 μM, respectively. No significant activity was observed among the three new *ent*-kaurane diterpenoids, which confirmed that the α,β-unsaturated ketone function acting as a Michael acceptor is essential to their cytotoxicity.[10] Our study provides insights into the cytotoxicity of the pterosin-sesquiterpenoids **4**−**6**, and these results support the pharmacological basis of this plant being used as a traditional herbal medicine for the treatment of cancer.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were measured on a Shimadzu UV-2550 spectrometer. CD spectra were obtained on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Perkin-Elmer 577 spectrometer using KBr disks. NMR spectra were measured on either a Bruker AM-400 or Bruker AM-300 spectrometer with TMS as internal standard. EIMS and HREIMS (70 eV) were carried out on a Finnigan-MAT 95 mass spectrometer, and ESIMS was carried out on a Finnigan LCQ-DECA mass spectrometer. HRESIMS were measured on a Thermo Electron Corporation FT-mass spectrometer. All solvents used were analytical grade (Sinopharm Chemical

Table 1. ^{13}C and ^{1}H NMR Data of Compounds **1**–**3** (δ ppm)

position	**1** (d_6-DMSO)		**2** (C_5D_5N)		**3** (C_5D_5N)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	49.3 t	α 1.95, dd (11.1, 2.4)	50.3 t	α 2.48, dd (12.0, 3.6)	50.5 t	α 2.48, m
		β 0.46, t (11.7)		β 1.07, t (12.0)		β 1.05, t (11.4)
2	62.8 d	α 3.70, m	64.3 d	α 4.33, m	63.8 d	α 4.30, m
3	44.8 t	α 1.34, m	46.1 t	α 2.13, m	54.8 t	α 2.10, m
		β 1.23, m		β 2.10, m		β 1.62, m
4	38.6 s		39.5 s		36.2 s	
5	47.8 d	β 1.02, m	48.8 d	β 1.70, m	60.9 d	β 1.21, m
6	18.5 t	α 1.10, m	19.2 t	α 1.30, t (7.0)	68.3 d	α 4.23, m
		β 1.42, m		β 1.80, m		
7	34.8 t	α 1.45, m	32.9 t	α 1.54, m	38.7 t	α 2.00, m
		β 1.34, m		β 1.75, m		β 2.15, m
8	47.1 s		52.8 s		45.1 s	
9	54.0 d	β 0.93, m	56.7 d	β 1.43, br s	56.7 d	β 1.18, m
10	40.5 s		41.4 s		43.1 s	
11	18.0 t	α 1.51, m	18.3 t	α 1.58, m	18.8 t	α 1.70, m
		β 1.32, m		β 1.39, m		β 1.66, m
12	32.6 t	α 1.51, m	28.2 t	α 2.90, br d (14.1)	26.8 t	α 1.87, m
		β 1.38, m		β 1.65, br d (14.1)		β 1.52, m
13	41.8 d	α 2.59, br s	51.3 d	α 3.05, br s	46.2 d	α 2.40, br s
14	36.0 t	α 1.72, d (11.1)	76.6 d	α 4.46, s	54.0 t	α 2.26, dd (12.3, 3.6)
		β 1.24, br d (11.1)				β 2.03, m
15	81.4 d	β 3.59, s	83.5 d	β 4.05, s	54.3 t	α 1.94, m
						β 1.75, m
16	159.6 s		160.0 s		81.3 s	
17	107.8 t	a 5.04, s	110.6 t	a 5.60, s	66.4 t	a 4.12, d (11.1)
		b 4.94, s		b 5.25, s		b 4.04, d (11.1)
18	70.0 t	a 3.13, d (10.5)	71.4 t	a 3.70, d (10.5)	37.8 q	1.64, s
		b 2.82, d (10.5)		b 3.39, d (10.5)		
19	18.5 q	0.64, s	18.9 q	0.90, s	23.2 q	1.31, s
20	19.1 q	0.96, s	19.7 q	1.10, s	20.1 q	1.17, s

Table 2. ^{13}C and ^{1}H NMR Data of Compounds **4**–**6** (C_5D_5N, δ ppm)

position	**4**		**5**		**6**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	205.7 s		207.4 s		209.5 s	
2	52.3 d	3.11, m	49.1 d	2.98, m	42.6 d	2.56, m
3	84.2 d	5.08, d (3.6)	76.3 d	5.60, d (6.6)	33.7 t	α 2.42, dd (16.5, 3.6)
						β 3.05, dd (16.5, 7.8)
4	126.2 d	7.72, s	126.7 d	7.75, s	126.0 d	6.93, s
5	144.6 s		144.4 s		144.4 s	
6	138.7 s		138.9 s		135.5 s	
7	136.7 s		137.1 s		137.5 s	
8	132.0 s		131.7 s		132.3 s	
9	150.8 s		151.2 s		152.6 s	
11	13.8 q	1.59, d (7.5)	11.9 q	1.44, d (7.5)	16.6 q	1.22, d (7.5)
12	21.1 q	2.27, s	21.2 q	2.32, s	21.0 q	2.28, s
13	33.0 t	3.10, t (6.9)	33.1 t	3.08, t (7.2)	29.5 t	3.11, t (7.5)
14	60.8 t	3.93, t (6.9)	60.8 t	3.91, t (7.2)	68.3 t	3.81, m
15	14.0 q	2.76, s	13.9 q	2.78, s	13.5 q	2.72, s
1′	105.6 d	5.27, d (7.2)	104.2 d	5.29, d (7.8)	104.6 d	4.99, d (7.5)
2′	75.4 d	4.15, m	75.3 d	4.30, m	75.3 d	4.20, m
3′	76.4 d	4.50, m	76.7 d	4.45, t (9.0)	76.5 d	4.42, t (9.6)
4′	72.4 d	5.84, t (9.6)	72.7 d	5.79, t (9.2)	72.6 d	5.79, t (9.6)
5′	75.8 d	4.28, m	75.8 d	4.24, m	76.0 d	4.08, m
6′	62.0 t	4.12–4.30	62.4 t	4.14–4.28	62.3 t	4.09–4.23
1″	167.1 s		167.2 s		167.2 s	
2″	115.0 d	6.61, d (15.6)	115.0 d	6.59, d (15.6)	114.9 d	6.58, d (15.9)
3″	145.7 d	7.96, d (15.6)	145.7 d	7.96, d (15.6)	145.7 d	7.94, d (15.9)
4″	126.0 s		126.1 s		125.9 s	
5″, 9″	130.7 d	7.55, d (8.1)	130.7 d	7.55, d (8.1)	130.7 d	7.53, d (8.7)
6″, 8″	116.7 d	7.16, d (8.1)	116.7 d	7.18, d (8.1)	116.7 d	7.14, d (8.7)
7″	161.3 s		161.4 s		161.5 s	

Reagent Co., Ltd., Shanghai, People's Republic of China). Column chromatography was performed either on silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany), and Sephadex LH-20 (25–100 μm; Pharmacia). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The whole plant of *Pteris multifida* Poir. was collected in Pan'an County, Zhejiang Province, People's Republic of China, in June 2006. The sample was identified by Prof. Jin-Gui Shen of the Shanghai Institute of Materia Medica, and a voucher specimen (SIMM 20060616) was deposited in the Herbarium of

Table 3. Cytotoxic Activities of Compounds **1**−**6** against Four Tumor Cell Lines

	IC_{50} (μM)			
compound	K562	HepG2	KB	LoVo
1	NE[a]	NE	26.35	20.34
2	NE	NE	69.60	76.34
3	81.86	82.43	53.66	91.66
4	10.63	8.69	14.16	11.28
5	9.57	9.26	23.82	NA[b]
6	64.56	NE	45.34	NA
taxotere[c]	NA	NA	1.14×10^{-3}	1.97×10^{-3}
adriamycin[c]	0.09	0.06	NA	NA

[a] NE = $IC_{50} > 100$ μM. [b] NA = no cytotoxic activity ($IC_{50} > 400$ μM). [c] Positive control substances.

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Extraction and Isolation. Air-dried and powdered whole plants of *Pteris multifida* Poir. (5 kg) were extracted with 95% EtOH (10 L × 3, each 2 days) at room temperature. After evaporation of the solvent *in vacuo* at 55 °C, the residue was dissolved in H_2O (1 L) and then extracted successively with petroleum ether (60–90 °C, 1 L × 3) and EtOAc (1 L × 3). The EtOAc extract (41.6 g) was subjected to column chromotography (CC) over silica gel (100–200 mesh) and eluted with a mixture of $CHCl_3$−MeOH (100% $CHCl_3$, 150:1, 100:1, 80:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1) to give fractions A−J. Fraction C (7.3 g) was subjected to silica gel CC using petroleum ether−Me_2CO (6:1) as the eluent to give 2*β*,15α-dihydroxy-*ent*-kaur-16-ene[6] (360 mg) and (2*R*)-pterosin B[6] (20 mg). Fraction D (2.7 g) was subjected to silica gel CC and eluted in a step gradient manner with petroleum ether−Me_2CO (from 6:1 to 1:1) to give **1** (60 mg), pterokaurane P_1[7] (105 mg), and 2*β*,6*β*,15α-trihydroxy-*ent*-kaur-16-ene[17] (21 mg). Fraction E (2.9 g) was chromatographed on RP-18 eluted with a MeOH−H_2O (50−100%) gradient to afford three subfractions E1−E3. 2*β*,6*β*,16α-Trihydroxy-*ent*-kaurane[17] (5 mg) and siegesbeckiol[22] (4 mg) were obtained from E1 (0.3 g) and E2 (204 mg), respectively, by recrystallization from Me_2CO. Fraction E3 (0.8 g) was 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 (2*S*,3*S*)-pterosin C[6] (10 mg) and (2*R*,3*S*)-pterosin C[21] (5 mg). Fraction F (1.6 g) was divided into five subfractions F1−F5 by passing through a RP-18 column eluted with MeOH−H_2O (from 30 to 100%). 2*β*,15α,16α,17-Tetrahydroxy-*ent*-kaurane[17] (8 mg) and 2*β*,14*β*,15α,16α,17-pentahydroxy-*ent*-kaurane[17] (17 mg) were obtained from F2 (0.9 g) by repeated silica gel CC eluted with $CHCl_3$−Me_2CO (from 8:1 to 2:1). Compound **2** (8 mg) was obtained from F3 (50 mg) by silica gel CC eluted with petroleum ether−Me_2CO (1:1). Pterokaurane P_3[6] (5 mg) was obtained from F4 (47 mg) by recrystallization from EtOH. Fraction G (1.8 g) was chromatographed on RP-18 eluted with a MeOH−H_2O (30−100%) gradient to afford four subfractions, G1−G4. G1 (0.2 g) was subjected to a Sephadex LH-20 column, eluted with MeOH, to give **3** (5 mg). (2*S*,3*S*)-Pterosin Q[6] (180 mg) was obtained from G2 (0.5 g) by recrystallization from Me_2CO. G4 (0.7 g) was subjected to silica gel CC, eluted with petroleum ether−Me_2CO (1.5:1), to give (2*S*,3*S*)-pterosin S[6] (25 mg). Fraction H (2.6 g) was divided into subfractions H1−H6 by chromatography over a RP-18 column, eluted with MeOH−H_2O (from 15 to 100%). Fraction H1 (0.1 g) was subjected to Sephadex LH-20 column, eluted with MeOH, to give pterokaurane P_1 2-*O*-*β*-glucopyranoside[7] (6 mg). H2 (0.4 g) was chromatographed on silica gel using $CHCl_3$−Me_2CO (1.5:1) as solvent and finally purified by semipreparative HPLC (MeOH−H_2O, 55:45) to yield **4** (28 mg) and **5** (6 mg). (2*S*,3*S*)-Pterosin S 14-*O*-*β*-glucopyranoside[6] (40 mg) was obtained from H3 (70 mg) by recrystallization from MeOH. (2*S*,3*S*)-Pterosin C 3-*O*-*β*-glucopyranoside[6] (5 mg) and (2*R*)-pterosin B 14-*O*-*β*-glucopyranoside[21] (15 mg) were purified from H4 (0.2 g) by semipreparative HPLC (MeOH−H_2O, 60:40). Fraction H5 (0.7 g) was subjected to a Sephadex LH-20 column, eluted with MeOH, to give creticoside A[6] (120 mg). Fraction H6 (0.1 g) was subjected to RP-18 CC with MeOH−H_2O (35:65) as eluent to afford **6** (6 mg).

Pterokaurane M_1 (1): white amorphous powder; $[\alpha]^{20}_D$ −99.0 (*c* 0.08, MeOH); IR (KBr) υ_{max} 3311, 3222, 3002, 2924, 2852, 1660, 1456, 1387, 1041, 897 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 320 $[M]^+$ (1), 302 (26), 287 (24), 272 (34), 271 (100), 253 (33), 244 (7), 215 (5), 213 (8), 203 (16), 185 (8), 173 (7), 145 (14), 121 (22); HREIMS *m/z* 320.2342 (calcd for $C_{20}H_{32}O_3$, 320.2351).

Pterokaurane M_2 (2): white amorphous powder; $[\alpha]^{22}_D$ −112.0 (*c* 0.10, MeOH); IR (KBr) υ_{max} 3410, 2929, 2869, 1639, 1458, 1387, 1257, 1101, 1036, 903, 825, 606 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 336 $[M]^+$ (20), 318 (39), 300 (44), 287 (34), 270 (44), 269 (100), 251 (18), 201 (25), 183 (19), 175 (15), 167 (28), 145 (29), 133 (37), 121 (71), 119 (64), 91 (57), 81 (37); HREIMS *m/z* 336.2301 (calcd for $C_{20}H_{32}O_4$, 336.2300).

Pterokaurane M_3 (3): white amorphous powder; $[\alpha]^{22}_D$ −43.0 (*c* 0.15, MeOH); IR (KBr) υ_{max} 3415, 2999, 2929, 2870, 1720, 1639, 1452, 1416, 1259, 1230, 1065, 1034, 1003, 881 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 338 $[M]^+$ (1), 320 (10), 305 (16), 302 (19), 289 (100), 271 (97), 269 (51), 259 (38), 229 (95), 215 (29), 173 (27), 161 (27), 147 (57); HREIMS *m/z* 338.2447 (calcd for $C_{20}H_{34}O_4$, 338.2457).

Multifidoside A (4): white amorphous powder; $[\alpha]^{22}_D$ −17.6 (*c* 0.45, MeOH); CD (MeOH) $[\theta]_{322.5}$ $+14.0 \times 10^3$; UV (MeOH) λ_{max} (log ϵ) 218 (4.62), 260 (4.19), 314 (4.35) nm; IR (KBr) υ_{max} 3408, 2962, 1697, 1630, 1603, 1514, 1331, 1261, 1159, 1038, 833, 519 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; negative ESIMS *m/z* 541 $[M - H]^-$; positive HRESIMS *m/z* 565.2054 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_{10}Na$, 565.2050).

Multifidoside B (5): white amorphous powder; $[\alpha]^{22}_D$ −80.0 (*c* 0.25, MeOH); CD (MeOH) $[\theta]_{327.0}$ $+2.76 \times 10^3$; UV (MeOH) λ_{max} (log ϵ) 218 (4.62), 260 (4.20), 310 (4.33) nm; IR (KBr) υ_{max} 3419, 3250, 2931, 2889, 1695, 1643, 1605, 1583, 1514, 1340, 1277, 1167, 1038, 827, 611 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; negative ESIMS *m/z* 541 $[M - H]^-$; positive HRESIMS *m/z* 565.2047 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_{10}Na$, 565.2050).

Multifidoside C (6): white amorphous powder; $[\alpha]^{22}_D$ −17.7 (*c* 0.30, MeOH); CD (MeOH) $[\theta]_{302.5}$ $+1.08 \times 10^3$; UV (MeOH) λ_{max} (log ϵ) 218 (4.53), 260 (4.14), 314 (4.28) nm; IR (film) υ_{max} 3408, 3016, 2926, 1689, 1630, 1603, 1514, 1443, 1377, 1327, 1157, 1030, 833, 754 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; negative ESIMS *m/z* 525 $[M - H]^-$; positive HRESIMS *m/z* 549.2096 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_9Na$, 549.2101).

Acid Hydrolysis of Compounds 4–6. In separate reactions, a mixture of 2 mg of compounds **4**, **5**, or **6** and 1 mL of 10% H_2SO_4 was heated in a boiling water bath for 30 min, respectively. After being cooled, the reaction mixtures were diluted with H_2O and allowed to stand overnight. The aglycone was then centrifuged, washed with H_2O, and chromatographed using HPLC (MeOH−H_2O, 55:45). The t_R values of the peaks in HPLC were indistinguishable from those of (2*S*,3*S*)-pterosin C, (2*R*,3*S*)-pterosin C, and (2*R*)-pterosin B, respectively. (Inertsil ODS-3 column (5 μm, 250 × 4.6 mm), column temperature 25 °C, flow rate, 1.0 mL/min; t_R, 14.09, 13.38, and 47.92 min, respectively). The supernatant was extracted several times with Et_2O, and the combined Et_2O extracts were subjected to TLC, in which, *p*-coumaric acid was identified by TLC comparison with an authentic sample. The mother liquor was neutralized with Ag_2CO_3, then concentrated to a small volume, and checked by co-TLC with authentic sugar samples, with *n*-BuOH−pyridine−H_2O (9:5:4) as developing solvent.

Cell Cultures. Human HepG2 (hepatocellular carcinoma), K562 (human leukemia), KB (cervix carcinoma), and LoVo (colon adenocarcinoma) cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI1640 medium with 10% FBS (fetal bovine serum). In each case, 100 U/mL of penicillin and 100 U/mL of streptomycin were added.

Cytotoxicity Assay. Cells were cultured in 96-well microtiter plates for the assay. After incubation for 24 h and treatment with 10^{-2} to 10^2 μM of the test compounds for 72 h, growth inhibition of the cancer cells was evaluated by the SRB method (adherent cells: HepG2, KB, and LoVo) or WST-1 method (suspended cell: K562), as described in the literature.[23,24] The activity is shown as IC_{50} value. Results are expressed as the mean value of triplicate data points. Adriamycin and taxotere were used as positive controls.

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