ent-Kaurane Diterpenoids from *Isodon albopilosus*

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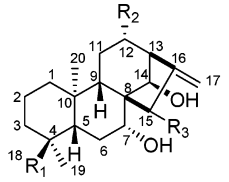
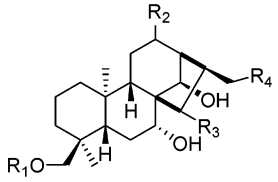
Nine new diterpenoids, albopilosins B−J (**1**−**9**), together with six known analogues, albopilosin A (**10**), macrocalyxin C (**11**), rabdokunmin C (**12**), excisanin (**13**), amethystonoic acid (**14**), and coetsanoic acid (**15**), were isolated from the aerial parts of *Isodon albopilosus.* The structures of **1**−**9** were established using spectroscopic methods including extensive 1D and 2D NMR analysis. The diterpenoids isolated were evaluated for their inhibitory activities against HepG2 (hepatoma) cells. Compounds **7** and **13** were the most active, with both having IC_{50} values of <15 μM.

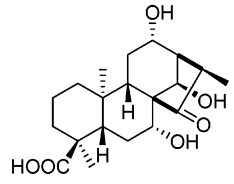
Isodon species (Labiatae) are a prolific source of new diterpenoids with diverse functionalities, of which some have antitumor and anti-inflammatory activities.[1,2] Our group has investigated phytochemically more than 50 *Isodon* species distributed in mainland China. About 500 new diterpenoids with highly oxygenated structures have been isolated and characterized.[3] In the course of our continuing investigations into the chemistry of this genus, a series of new *ent*-kaurane diterpenoids, albopilosins B−J (**1**−**9**), and six known compounds, albopilosin A (**10**),[4] macrocalyxin C (**11**),[5] rabdokunmin C (**12**),[6] excisanin C (**13**),[7] amethystonoic acid (**14**),[5] and coetsanoic acid (**15**),[8] were isolated from an acetone extract of the aerial parts of *Isodon albopilosus* (C. Y. Wu & H. W. Li) H. Hara, collected in Maoxian, Sichuan Province, People's Republic of China. All of these compounds were evaluated for their cytotoxic activity against HepG2 (hepatoma) cells. This paper reports the structure elucidation of these new compounds and the results of bioactivity testing toward this tumor cell line.



	R_1	R_2	R_3
1	CH_2OH	OH	β-OAc
2	CHO	OH	β-OAc
3	CH_2OH	H	β-OAc
6	CH_2OH	OH	H
7	CHO	H	=O
8	COOH	H	=O
10	CH_2OAc	OH	=O
11	CHO	OH	=O
12	CH_2OH	OH	=O
13	CH_2OH	H	=O
14	COOH	OH	=O



	R_1	R_2	R_3	R_4
4	H	=O	β-OH	H
5	Ac	α-OH	=O	OH
9	Glc	α-OH	=O	H



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Results and Discussion

The acetone extract of the aerial parts of *I. albopilosus* was partitioned between petroleum ether (60−90 °C) and water and then between ethyl acetate and water. The ethyl acetate extract was chromatographed over MCI gel, silica gel, and RP-18 gel columns and further purified by high-performance liquid chromatography (HPLC) using reversed-phase columns to yield 15 *ent*-kaurane diterpenoids (**1**−**15**).

Albopilosin B (**1**) was isolated as colorless needles. A positive HRESIMS measurement established the molecular formula as $C_{22}H_{34}O_6$, requiring six degrees of unsaturation. The IR spectrum showed absorption bands due to hydroxyl groups (3442 cm^{-1}), an ester group (1724 cm^{-1}), and a C=C double bond (1638 cm^{-1}). Observed in the ^1H and ^{13}C NMR (including DEPT) spectra (see Table 1) were two tertiary methyl groups [δ_H 0.89 (3H, s), 1.73 (3H, s); δ_C 18.2 (q), 17.4 (q)], an olefinic group [δ_H 5.37 (1H, br s), 5.19 (1H, br s), δ_C 152.3 (s), 109.6 (t)], an acetoxyl group [δ_H 1.99 (3H, s); δ_C 21.0 (q), 171.2 (s)], and five oxygenated carbons [δ_C 75.7 (d), 73.9 (d), 72.8 (d), 76.2 (d), 71.4 (t)]. In addition, the upfield region of the ^{13}C NMR and DEPT spectra exhibited five methylenes, three methines, and three quaternary carbons. Considering the NMR data and the

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Table 1. ^{13}C NMR Data of Compounds **1**−**9** (C_5D_5N, 100 MHz, δ in ppm)[a]

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**[b]	**8**	**9**		
1	40.5 t	39.5 t	40.5 t	39.9 t	38.9 t	40.4 t	38.5 t	39.2 t	38.3 t	Glc	
2	18.6 t	17.2 t	18.7 t	18.3 t	17.9 t	18.5 t	17.0 t	18.4 t	18.0 t	1′	105.5 d
3	35.8 t	32.2 t	35.7 t	35.6 t	35.7 t	35.8 t	31.9 t	37.3 t	35.9 t	2′	74.8 d
4	38.0 s	49.7 s	37.9 s	38.0 s	36.6 s	38.7 s	49.4 s	47.6 s	38.7 s	3′	78.5 d
5	46.6 d	45.3 d	46.8 d	46.7 d	47.6 d	46.8 d	45.5 d	48.1 d	45.9 d	4′	71.5 d
6	30.6 t	33.2 t	30.4 t	30.6 t	30.0 t	30.8 t	30.4 t	32.8 t	29.9 t	5′	78.6 d
7	75.7 d	74.9 d	75.4 d	76.1 d	74.8 d	78.4 d	73.9 d	74.2 d	74.9 d	6′	62.7 t
8	54.4 s	54.5 s	54.6 s	56.2 s	61.6 s	52.6 s	61.8 s	62.2 s	60.7 s		
9	51.8 d	51.4 d	50.2 d	47.3 d	57.1 d	60.2 d	53.9 d	55.2 d	56.4 d		
10	38.5 s	37.3 s	39.4 s	39.1 s	38.6 s	38.1 s	38.5 s	39.5 s	37.6 s		
11	26.4 t	26.1 t	17.8 t	38.7 t	27.0 t	26.2 t	17.1 t	17.6 t	26.5 t		
12	73.9 d	73.6 d	33.0 t	214.0 s	66.7 d	73.6 d	30.8 t	31.4 t	66.6 d		
13	59.3 d	59.3 d	50.6 d	66.2 d	52.7 d	62.0 d	45.9 d	47.0 d	51.0 d		
14	72.8 d	72.7 d	76.9 d	75.6 d	71.6 d	74.2 d	74.9 d	75.6 d	71.5 d		
15	76.2 d	76.1 d	75.3 d	71.7 d	220.4 s	41.5 t	207.4 s	207.7 s	222.1 s		
16	152.3 s	151.9 s	154.7 s	37.1 d	49.5 d	153.2 s	147.3 s	149.8 s	43.4 d		
17	109.6 t	109.8 t	108.9 t	10.6 q	58.7 t	106.6 t	118.3 t	116.4 t	9.8 q		
18	71.4 t	206.2 d	71.2 t	71.0 t	72.7 t	71.4 t	205.7 d	181.3 s	78.6 t		
19	18.2 q	14.4 q	18.3 q	18.0 q	17.5 q	18.2 q	14.1 q	17.2 q	18.0 q		
20	17.4 q	17.0 q	18.9 q	17.7 q	17.0 q	17.3 q	18.2 q	18.4 q	17.1 q		
OAc	171.2 s	171.3 s	171.3 s		170.8 s						
	21.0 q	21.1 q	21.1 q		20.6 q						

[a] The assignments were based on DEPT, $^1H-^1H$ COSY, HMQC, and HMBC experiments. [b] Recorded in $CDCl_3$.

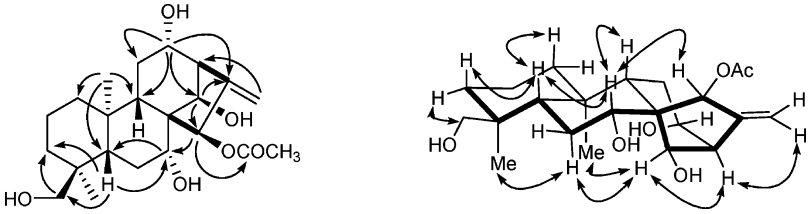

Figure 1. Key HMBC and ROESY correlations for compound **1**.

structural types of diterpenoids isolated from *I. albopilosus* previously,[4] compound **1** was assigned tentatively as an *ent*-kauranoid, which was confirmed by the measurement of 2D NMR data. In the $^1H-^1H$ COSY and HMQC spectra of **1**, the presence of the partial structures $-CH_2CH_2CH_2-$ (C-1 to C-3) and $-CHCH_2CH-$ (C-5 to C-7) was inferred. Through careful analysis of the HMBC spectrum (Figure 1), the partial structures were correlated to constitute an *ent*-kauranoid on the basis of the observation of the cross-peaks of H-5 (δ_H 1.79, 1H, br d, J = 12.4 Hz) with C-1, C-3, C-6, C-9, C-18, and Me-19, 20, of H-9 (δ_H 2.05, 1H, br d, J = 9.5 Hz) with C-1, C-8, C-10, C-11, C-14, C-15, and Me-20, and of H-13 (δ_H 3.28, 1H, br d, J = 4.0 Hz) with C-8, C-11, C-14, C-15, and C-17. According to the correlations in the HMBC (Figure 1) and $^1H-^1H$ COSY spectra of **1**, the acetoxyl group was placed at C-15 and four hydroxyl groups at C-7, C-12, C-14, and C-18, respectively. The relative configuration of **1** was established by a ROESY NMR experiment, in which correlations of H-7 with H-5, H-9, and H-15 and of H-14 with H-6α and Me-20 were observed clearly (Figure 1). This suggested that the substituents at C-7, C-12, C-14, and C-15 possess α-, α-, β-, and β-orientations, respectively. Therefore, compound **1** was elucidated as 7α,12α,14β,18-tetrahydroxy-15β-acetoxy-*ent*-kaur-16-ene.

Albopilosin C (**2**) was assigned the molecular formula $C_{22}H_{32}O_6$, as deduced from the positive HRESIMS (*m/z* 415.2105 $[M + Na]^+$). Comparison of the spectroscopic data of **2** with those of **1** revealed that they were quite similar except for the moiety at C-18. Observation of the presence of an aldehyde group (δ_C 206.2, d) and the absence of a oxymethylene carbon in the ^{13}C NMR spectrum of **2** showed that an aldehyde group at the C-18 position was evident for **2** instead of a hydroxyl group at the same position in **1**. Moreover, the cross-peaks in the ROESY spectrum of **2** indicated that the corresponding substituents in **2** had the same orientations as those in **1**. Thus, compound **2** was determined as 7α,12α,14β-trihydroxy-15β-acetoxy-*ent*-kaur-16-en-18-al.

Albopilosin D (**3**) was assigned the molecular formula $C_{22}H_{34}O_5$ from its HRESIMS and NMR data. Comparison of the spectroscopic data of **3** with those of **1** (Table 1) showed similarities except that a hydroxyl group at C-12 in **1** was replaced by a methylene group (δ_C 17.8) in **3**. Examination of its 2D NMR data allowed **3** to be deduced as 7α,14β,18-trihydroxy-15β-acetoxy-*ent*-kaur-16-ene.

Albopilosin E (**4**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{20}H_{32}O_5$ by HRESIMS ($[M + Na]^+$ at *m/z* 375.2145, calcd 375.2147). Its IR, MS, and NMR spectroscopic data suggested **4** to be an *ent*-kaurane diterpenoid, with a ketone group and four oxygenated carbons. A careful analysis of the 2D NMR spectral data and comparison with rabdokunmin C (**12**) led to the conclusion that the C-7, C-14, C-15, and C-18 positions were each substituted by a hydroxyl group, and a ketone was at C-12, on the basis of the HMBC correlations of H_2-11 (δ_H 2.56 and 2.97, each 1H) with C-12 (δ_C 214.0, s) and H-16 (δ_H 3.37, 1H, m) with C-12. Moreover, because of the ROESY correlations of H-7 with H-15, H-5, and H-9 and of H-14 with H-6α and Me-20, **4** was deduced as being an *ent*-kaurane diterpenoid having the substituents at C-7, C-14, C-15, and C-16 in the α-, β-, β-, and β-orientations, respectively. Therefore, **4** was determined as (16*R*)-methyl-7α,14β,15β,18-tetrahydroxy-*ent*-kaur-12-one.

The molecular formula of compound **5** was analyzed as $C_{22}H_{34}O_7$ from its HRESIMS and NMR data. The ^1H and ^{13}C NMR spectra of **5** were similar to those of the known compound **10**, except for the absence of an olefinic group at C-16 and C-17. A hydroxyl group at the C-17 position

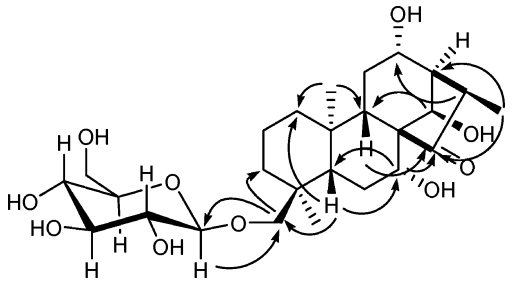

Figure 2. Key HMBC correlations for compound **9**.

was evident for **5** on the basis of the observation of a signal (δ_C 58.7, t) in the ^{13}C NMR spectrum. Further 2D NMR experiments allowed a determination of **5** as (16*R*)-7α,12α,-14*β*,17-tetrahydroxy-18-acetoxy-*ent*-kaur-15-one.

Albopilosin G (**6**), an amorphous powder, was found to possess the molecular formula $C_{20}H_{32}O_4$ from the HRESIMS pseudomolecular ion $[M + Na]^+$ at *m/z* 359.2194 (calcd 359.2198). The IR peaks at 3444 and 1638 cm^{-1} and the ^{13}C NMR signals at δ_C 153.2 (s), 106.6 (t), 78.4 (d), 73.6 (d), 74.2 (d), and 71.4 (t) (Table 1) revealed the presence of an olefinic group and four oxygenated carbons. Comparison of the ^{13}C NMR spectroscopic data of **6** and **12** showed that the signal for C-15 was at δ_C 41.5 (t) in **6** instead of at δ_C 206.5 (s) as observed in **12**, indicating that C-15 in **6** is a methylene carbon. This was confirmed by the observation of an AB system (δ_H 3.81 and 2.22, 1H each, d, J = 16.9 Hz) in the ^1H NMR spectrum. Thus, **6** was elucidated as 7α,12α,14*β*,18-tetrahydroxy-*ent*-kaur-16-ene.

Compound **7** was isolated as colorless needles. Its molecular formula, $C_{20}H_{28}O_4$, was established from the quasimolecular ion peak at *m/z* 355.1891 $[M + Na]^+$ in the HRESIMS. The IR peaks at 1709 and 1642 cm^{-1} suggested the presence of an α,*β*-unsaturated carbonyl group, which was confirmed by the strong absorption at 234 (3.91) nm in the UV spectrum. Comparison of the spectroscopic data of **7** with those of **11** showed they were closely similar except that a hydroxyl group at C-12 in **11** was replaced by a methylene group (δ_C 17.8) in **7**. Albopilosin H (**7**) was therefore concluded to be 7α,14*β*-dihydroxy-*ent*-kaur-16-en-15-oxo-18-al.

Inspection of UV, IR, and NMR data of albopilosin I (**8**) indicated considerable similarity to compound **7**. The only difference between **7** and **8** was that a carboxyl group rather than an aldehyde group was located at the C-18 in **8**. Thus, compound **8** was determined to be 7α,14*β*-dihydroxy-*ent*-kaur-16-en-15-oxo-18-oic acid.

Compound **9** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{42}O_{10}$ on the basis of the ion peak at *m/z* 537.2673 $[M + Na]^+$ in the HRESIMS. The IR spectrum suggested the presence of hydroxyl groups (3417 cm^{-1}) and a ketone group (1727 cm^{-1}). Acid hydrolysis of **9** afforded D-glucose, which was detected by TLC and identified by its optical rotation value. The anomeric proton ^1H NMR signal at δ_H 4.67 (1H, d, J = 7.7 Hz) suggested the presence of a *β*-D-glucopyranoside moiety. Besides the signals for the *β*-D-glucopyranosyl group, the close similarity of the ^1H and ^{13}C NMR data to those of dihydrorabdokunmin C suggested that **9** is a glucoside derivative.[6,8] The ^{13}C NMR signal for C-18 shifted downfield from δ_C 71.3 in dihydrorabdokunmin C to δ_C 78.6 in **9**, which indicated that the *β*-D-glucopyranosyl moiety is located at C-18. This was confirmed by the HMBC correlations between H_2-18 (δ_H 3.63 and 3.41, 1H each, d, J = 9.4 Hz) and C-1′ (δ_C 105.5, d) and between H-1′ (δ_H 4.67, 1H, d, J = 7.4 Hz) and C-18 (δ_C 78.6, t) (Figure 2). Thus, **9** was elucidated as (16*R*)-methyl-7α,12α,14*β*-trihydroxy-*ent*-kaur-15-oxo-18-*O*-*β*-D-glucopyranoside.

The structures of the known compounds isolated were identified as albopilosin A (**10**),[4] macrocalyxin C (**11**),[5] rabdokunmin C (**12**),[6] excisanin C (**13**),[7] amethystonoic acid (**14**),[5] and coetsanoic acid (**15**),[8] by comparison of their spectroscopic data with literature values.

All diterpenoids were evaluated for their cytotoxicity against HepG2 (hepatoma) cells. As determined by an Alamar Blue assay,[9,10] **7** and **13** demonstrated significant inhibitory activity against HepG2 cells with IC_{50} values of 13.31 and 13.17 μM, respectively. Compounds **10**−**12** were less active, with IC_{50} values of 24.91, 30.82, and 42.08 μM, respectively, while compounds **1**−**6**, **8**, **9**, **14**, and **15** were noncytotoxic. Interestingly, compounds **8** and **14**, with an active center (cyclopentanone conjugated with an exomethylene group), were completely inactive, which suggests that the carboxyl group present in each of these molecules resulted in the loss of cytotoxicity.

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were carried out on a Perkin-Elmer model 241 polarimeter. UV spectra were obtained in a UV 210A spectrometer. IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a VG Auto Spec-3000 spectrometer or on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were measured on either a Bruker AM-400 or a Bruker DRX-500 instrument with TMS as internal standard. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Column chromatography was performed either on silica gel (200−300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), silica gel H (10−40 μm; Qingdao Marine Chemical Inc.), or Lichroprep RP-18 gel (40−63 μm; Merck, Darmstadt, Germany). 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 aerial parts of *I. albopilosus* were collected in Maoxian, Sichuan Province, People's Republic of China, in July 2004. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 04092101) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

Extraction and Isolation. Air-dried and powdered aerial parts of *I. albopilosus* (1.1 kg) were extracted with acetone (5 L × 4, each 2 days) at room temperature. After evaporating the solvents in vacuo at 45 °C, a residue (85 g) was obtained. This residue was dissolved in H_2O (0.5 L) and then extracted successively with petroleum ether (60−90 °C, 0.3 L × 3) and EtOAc (0.5 L × 5). The EtOAc extract (30 g) was subjected to column chromatography over silica gel (200−300 mesh) and eluted with $CHCl_3$−Me_2CO (from 1:0 to 0:1) to give fractions A−E. Fraction B (2.3 g) was subjected to silica gel column chromatography and eluted in a step gradient manner with $CHCl_3$−Me_2CO (from 60:1 to 3:1) to give compound **7** (25 mg). Fraction C (11.2 g) was chromatographed on RP-18 eluted with a MeOH−H_2O (30%−100%) gradient system to afford four main fractions, C1−C4. Compounds **10** (2.1 g) and **11** (1.5 g) were obtained from C3 (2.9 g) and C2 (3.3 g), respectively, by recrystallization from Me_2CO. The remainder of C2 (0.4 g) was separated by semipreparative HPLC (MeOH−H_2O, 55:45) to afford compound **2** (12 mg). C4 (1.2 g) was subjected to silica gel column chromatography, eluted with cyclohexane−isopropyl alcohol (30:1), to give compounds **3** (17 mg) and **13** (35 mg). Fraction D (10.5 g) was divided into subfractions D1−D5 by passage over a RP-18 column, eluted with MeOH−H_2O (from 30% to 100%). Compounds **1** (5 mg) and **12** (300 mg) were

obtained from D2 (1.2 g) by repeated silica gel column chromatography eluted with $CHCl_3$−Me_2CO (8:1). Subfraction D3 (0.6 g) was chromatographed on silica gel using cyclohexane−isopropyl alcohol (20:1) as solvent and finally purified by semipreparative HPLC (MeOH−CH_3CN−H_2O, 50:5:45) to yield compounds **4** (4 mg), **5** (5 mg), and **6** (4 mg). Compound **8** (22 mg) was obtained from D4 (3.3 g) by recrystallization from MeOH. Separation of fraction E (5.8 g) over RP-18 eluted with a MeOH−H_2O (30%−100%) gradient system afforded compounds **14** (50 mg), **15** (10 mg), and **9** (30 mg).

Albopilosin B (1): colorless needles; mp 128−130 °C; $[\alpha]^{19}_D$ −80.1 (*c* 0.27, MeOH); UV (MeOH) λ_{max} log (ϵ) 204 (4.68) nm; IR (KBr) ν_{max} 3442, 2930, 1724, 1638, 1435, 1374, 1250, 1043, 873 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 7.23 (1H, s, H-15α), 5.65 (1H, br s, H-14α), 5.37 (1H, br s, H-17a), 5.19 (1H, br s, H-17b), 4.35 (1H, br s, H-12*β*), 4.24 (1H, m, H-7*β*), 3.67 (1H, d, *J* = 10.5 Hz, H-18a), 3.32 (1H, d, *J* = 10.5 Hz, H-18b), 3.28 (1H, br d, *J* = 4.0 Hz, H-13α), 2.44 (1H, m, H-6*β*), 2.05 (1H, br d, *J* = 9.5 Hz, H-9*β*), 2.03 (1H, overlapped, H-11*β*), 2.00 (1H, overlapped, H-6α), 1.99 (3H, s, OAc), 1.95 (1H, overlapped, H-11α), 1.86 (1H, overlapped, H-3*β*), 1.85 (1H, overlapped, H-1α), 1.79 (1H, br d, *J* = 12.4 Hz, H-5*β*), 1.73 (3H, s, Me-20), 1.70 (1H, m, H-2*β*), 1.42 (1H, m, H-2α), 1.38 (1H, br d, *J* = 12.3 Hz, H-3α), 0.90 (1H, m, H-1*β*), 0.89 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 417.2264 (calcd for $C_{22}H_{34}O_6Na$ $[M + Na]^+$, 417.2253).

Albopilosin C (2): amorphous solid; mp 126−128 °C; $[\alpha]^{19}_D$ −58.5 (*c* 0.23, MeOH); UV (MeOH) λ_{max} log (ϵ) 204 (4.52) nm; IR (KBr) ν_{max} 3431, 2932, 1724, 2871, 1723, 1632, 1443, 1373, 1242, 1044, 989 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 9.21 (1H, s, H-18), 7.20 (1H, s, H-15α), 5.55 (1H, br s, H-14α), 5.39 (1H, br s, H-17a), 5.21 (1H, br s, H-17b), 4.34 (1H, br s, H-12*β*), 4.16 (1H, br d, *J* = 11.9 Hz, H-7*β*), 3.25 (1H, br d, *J* = 3.6 Hz, H-13α), 2.18 (3H, s, OAc), 2.16 (1H, m, H-6α), 2.02 (1H, br d, *J* = 9.2 Hz, H-9*β*), 1.98 (1H, m, H-11α), 1.91 (1H, m, H-11*β*), 1.81 (1H, m, H-1α), 1.78 (1H, m, H-6*β*), 1.64 (1H, overlapped, H-2α), 1.64 (3H, s, Me-20), 1.62 (1H, br d, *J* = 12.3 Hz, H-5*β*), 1.42 (1H, m, H-2*β*), 1.28 (1H, m, H-3*β*), 1.15 (1H, overlapped, H-3α), 1.12 (3H, s, Me-19), 0.84 (1H, m, H-1*β*); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 415.2105 (calcd for $C_{22}H_{34}O_6Na$ $[M+Na]^+$, 415.2096).

Albopilosin D (3): colorless needles; mp 218−220 °C; $[\alpha]^{19}_D$ −91.5 (*c* 0.30, MeOH); UV (MeOH) λ_{max} log (ϵ) 204 (4.03) nm; IR (KBr) ν_{max} 3433, 2927, 2870, 1721, 1633, 1458, 1372, 1257, 1046, 890 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 7.09 (1H, s, H-15α), 5.37 (1H, br s, H-17a), 5.19 (1H, br s, H-17b), 4.94 (1H, br s, H-14α), 4.14 (1H, br d, *J* = 11.2 Hz, H-7*β*), 3.64 (1H, d, *J* = 10.5 Hz, H-18a), 3.29 (1H, d, *J* = 10.5 Hz, H-18b*)*, 2.96 (1H, br d, *J* = 2.4 Hz, H-13α), 2.38 (1H, dd, *J* = 11.2, 2.8 Hz, H-6*β*), 2.02 (3H, s, OAc), 1.96 (1H, br d, *J* = 12.0 Hz, H-6α), 1.95 (1H, overlapped, H-11α), 1.81 (1H, overlapped, H-12α), 1.80 (1H, overlapped, H-3*β*), 1.79 (1H, overlapped, H-9*β*), 1.76 (1H, m, H-1α), 1.73 (1H, m, H-2*β*), 1.72 (1H, br d, *J* = 12.1 Hz, H-5*β*), 1.66 (1H, m, H-11*β*), 1.57 (1H, br d, *J* = 11.2 Hz, H-12*β*), 1.47 (1H, m, H-2α), 1.36 (1H, br d, *J* = 13.1 Hz, H-3α), 1.11 (3H, s, Me-20), 0.86 (3H, s, Me-19), 0.85 (1H, overlapped, H-1*β*); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 401.2307 (calcd for $C_{22}H_{34}O_5Na$ $[M + Na]^+$, 401.2303).

Albopilosin E (4): white amorphous powder; $[\alpha]^{19}_D$ +6.5 (*c* 0.44, MeOH); UV (MeOH) λ_{max} end absorption; IR (KBr) ν_{max} 3424, 2926, 2870, 2854, 1696, 1460, 1052, 960 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 5.65 (1H, br d, *J* = 9.8 Hz, H-15α), 5.33 (1H, br s, H-14α), 4.57 (1H, br d, *J* = 8.0 Hz, H-7*β*), 3.65 (1H, d, *J* = 10.6 Hz, H-18a), 3.27 (1H, d, *J* = 10.6 Hz, H-18b*)*, 3.37 (1H, q like, *J* = 8.2 Hz, H-16α), 3.12 (1H, d, *J* = 6.0 Hz, H-13α), 2.97 (1H, dd, *J* = 17.4, 10.3 Hz, H-11*β*), 2.56 (1H, br d, *J* = 17.4 Hz, H-11α), 2.47 (1H, br d, *J* = 10.2 Hz, H-6*β*), 2.00 (1H, m, H-6α), 2.41 (1H, br d, *J* = 10.3 Hz, H-9*β*), 1.86 (1H, br d, *J* = 12.1 Hz, H-5*β*), 1.78 (1H, br d, *J* = 13.4 Hz, H-3*β*), 1.30 (1H, m, H-3α), 1.61 (1H, m, H-1α), 0.80 (1H, m, H-1*β*), 1.58 (1H, m, H-2*β*), 1.42 (1H, m, H-2α), 1.25 (3H, d, *J* = 8.0 Hz, Me-17), 0.98 (3H, s, Me-20), 0.77 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 375.2145 (calcd for $C_{20}H_{32}O_5Na$ $[M + Na]^+$, 375.2147).

Albopilosin F (5): colorless needles; mp 228−230 °C $[\alpha]^{19}_D$ −61.1 (*c* 0.28, MeOH); UV (MeOH) λ_{max} end absorption; IR (KBr) ν_{max} 3425, 2929, 1724, 1252, 1250, 1037, 918 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 5.98 (1H, br s, H-14α), 4.85 (1H, br s, H-12*β*), 4.71 (1H, dd, *J* = 11.8, 3.8 Hz, H-7*β*), 4.60 (1H, dd, *J* = 10.8, 5.0 Hz, H-17a), 4.17 (1H, t like, *J* = 10.6 Hz, H-17b), 4.01 (1H, d, *J* = 11.1 Hz, H-18a), 3.71 (1H, d, *J* = 11.1 Hz, H-18b), 3.90 (1H, dd, *J* = 12.1, 8.0 Hz, H-13α), 3.44 (1H, br d, *J* = 4.8 Hz, H-16α), 2.13 (1H, m, H-6*β*), 1.98 (1H, m, H-6α), 1.97 (1H, overlapped, H-11α), 1.34 (1H, overlapped, H-11*β*), 1.89 (3H, s, OAc), 1.80 (1H, overlapped, H-3*β*), 1.27 (1H, m, H-3α), 1.75 (1H, m, H-2*β*), 1.32 (1H, overlapped, H-2α), 1.63 (1H, overlapped, H-1α), 0.60 (1H, m, H-1*β*), 1.60 (1H, overlapped, H-9*β*), 1.40 (1H, br d, *J* = 11.9 Hz, H-5*β*), 1.62 (3H, s, Me-20), 0.78 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 433.2203 (calcd for $C_{22}H_{34}O_7$ Na $[M + Na]^+$, 433.2202).

Albopilosin G (6): amorphous powder; $[\alpha]^{19}_D$ +28.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} log (ϵ) 207 (3.96) nm; IR (KBr) ν_{max} 3444, 2923, 2852, 1638, 1465, 1385, 1098, 1040, 996, 879 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 5.51 (1H, br s, H-14α), 5.09 (1H, br s, H-17a), 5.08 (1H, br s, H-17b), 4.30 (1H, br s, H-12*β*), 4.12 (1H, br d, *J* = 10.7 Hz, H-7*β*), 3.81 (1H, d, *J* = 16.9 Hz, H-15α), 2.22 (1H, d, *J* = 16.9 Hz, H-15*β*), 3.71 (1H, d, *J* = 10.5 Hz, H-18a), 3.34 (1H, d, *J* = 10.5 Hz, H-18b), 3.23 (1H, br d, *J* = 3.4 Hz, H-13α), 2.39 (1H, m, H-6*β*), 2.07 (1H, m, H-6α), 1.99 (1H, m, H-11*β*), 1.87 (1H, overlapped, H-11α), 1.87 (1H, overlapped, H-3*β*), 1.41(1H, m, H-3α), 1.83 (1H, overlapped, H-1α), 0.81 (1H, m, H-1*β*), 1.71 (1H, overlapped, H-2*β*), 1.46 (1H, m, H-2α), 1.75 (1H, br d, *J* = 12.0 Hz, H-5*β*), 1.52 (1H, br d, *J* = 9.6 Hz, H-9*β*), 1.70 (3H, s, Me-20), 0.90 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; ESIMS (positive mode) *m/z* 359 $[M + Na]^+$ (100), 695 $[2M + Na]^+$ (98); HRESIMS (positive ion) *m/z* 359.2194 (calcd for $C_{20}H_{32}O_4Na$ $[M + Na]^+$, 359.2198).

Albopilosin H (7): colorless crystals; mp 170−172 °C; $[\alpha]^{19}_D$ −115.7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} log (ϵ) 234 (3.83) nm; IR (KBr) ν_{max} 3412, 2995, 2935, 2868, 1728, 1642, 1442, 1364, 1259, 1160, 1075, 990, 877 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 9.20 (1H, s, H-18), 6.14 (1H, br s, H-17a), 5.39 (1H, br s, H-17b), 4.83 (1H, br s, H-14α), 4.39 (1H, dd, *J* = 12.0, 4.4 Hz, H-7*β*), 3.03 (1H, br s, H-13α), 1.81 (1H, m, H-6*β*), 1.39 (1H, overlapped, H-6α), 1.94 (1H, m, H-12*β*) 1.75 (1H, overlapped, H-12α), 1.80 (1H, overlapped, H-1α), 0.78 (1H, m, H-1*β*), 1.67 (1H, m, H-2*β*), 1.42 (1H, m, H-2α), 1.62 and 1.35 (each 1H, overlapped, H_2-11), 1.37 and 1.30 (each 1H, overlapped, H_2-3), 1.49 (1H, br d, *J* = 12.5 Hz, H-5*β*), 1.35 (1H, overlapped, H-9*β*), 1.09 (3H, s, Me-20), 1.07 (3H, s, Me-19); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; ESIMS (positive mode) *m/z* 355 $[M + Na]^+$(100), 333 $[M + H]^+$(20) (98); HRESIMS (positive ion) *m/z* 355.1891 (calcd for $C_{20}H_{28}O_4Na$ $[M + Na]^+$, 355.1885).

Albopilosin I (8): colorless crystals; mp 169−171 °C; $[\alpha]^{19}_D$ −108.2 (*c* 0.52, MeOH); UV (MeOH) λ_{max} log (ϵ) 234 (3.91) nm; IR (KBr) ν_{max} 3377, 2978, 2932, 2869, 1729, 1715, 1648, 1478, 1391, 1259, 1223, 1092, 999, 934 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 6.30 (1H, br s, H-17a), 5.37 (1H, br s, H-17b), 5.13 (1H, br s, H-14α), 4.98 (1H, br d, *J* = 11.0 Hz, H-7*β*), 3.23 (1H, br s, H-13α), 2.19 (1H, m, H-6α), 2.01 (1H, overlapped, H-6*β*), 2.00 (1H, overlapped, H-3α), 1.71 (1H, m, H-3*β*), 1.95 (1H, overlapped, H-12*β*) 1.57 (1H, m, H-12α), 1.64 (1H, m, H-2*β*), 1.49 (1H, overlapped, H-2α), 1.64 (1H, overlapped, H-1α), 0.68 (1H, m, H-1*β*), 1.53 and 1.37 (each 1H, overlapped, H_2-11), 2.20 (1H, overlapped, H-5*β*), 1.48 (1H, overlapped, H-9*β*), 1.41 (3H, s, Me-19), 1.07 (3H, s, Me-20); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (negative ion) *m/z* 347.1864 (calcd for $C_{20}H_{27}O_5$ $[M - H]^-$, 347.1858).

Albopilosin J (9): amorphous powder; $[\alpha]^{19}_D$ −43.0 (*c* 0.41, MeOH); UV (MeOH) λ_{max} end absorption; IR (KBr) ν_{max} 3417, 2930, 2876, 1727, 1457, 1076, 918 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ_H 5.90 (1H, br s, H-14α), 4.98 (1H, br d, *J* = 11.2 Hz, H-7*β*), 4.35 (1H, br s, H-12*β*), 3.63 (1H, d, *J* = 9.4 Hz, H-18a), 3.41 (1H, d, *J* = 9.4 Hz, H-18b), 3.33 (1H, m, H-16α), 2.94 (1H, br d, *J* = 3.0 Hz, H-13α), 2.32 (1H, br d, *J* = 12.5 Hz, H-6*β*), 1.97 (1H, m, H-6α), 1.81 (1H, m, H-11*β*), 1.38 (1H, overlapped,

H-11α), 1.72 (1H, m, H-3β), 1.28 (1H, overlapped, H-3α), 1.52 (1H, br d, J = 12.9 Hz, H-1α), 0.31 (1H, m, H-1β), 1.31 and 1.27 (2H, overlapped, H_2-2), 1.75 (1H, br d, J = 12.5 Hz, H-5β), 1.40 (1H, br d, J = 9.6 Hz, H-9β), 1.17 (3H, d, J = 7.2 Hz, Me-17), 1.59 (3H, s, Me-20), 0.75 (3H, s, Me-19), 4.67 (1H, d, J = 7.4 Hz, H-1′), 4.04 (1H, t like, J = 8.1 Hz, H-2′), 4.12 (1H, t like, J = 8.7 Hz, H-3′), 4.25 (1H, t like, J = 8.7 Hz, H-4′), 3.87 (1H, m, H-5′), 4.56 (1H, br d, J = 11.5 Hz, H-6′a), 4.41 (1H, dd, J = 11.5, 4.4 Hz, H-6′b); ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1; HRESIMS (positive ion) *m/z* 537.2673 (calcd for $C_{26}H_{42}O_{10}$ Na $[M + Na]^+$, 537.2676).

Acid Hydrolysis of 9. Albopilosin J (**9**, 10 mg) was heated with 2 mL of 2.5 M HCl at 65 °C for 4 h. After 4 mL of H_2O was added and the mixture was extracted with EtOAc, the aqueous phase was evaporated in vacuo to yield 3 mg of D-glucose, which was detected by TLC and optical rotation, $[\alpha]^{19}_D$ +70.5 (*c* 0.10, H_2O).

Cytotoxicity Assay. Compounds were tested for cytotoxicity against HepG2 (hepatoma) cells using an Alamar Blue assay.[9,10] Drug stock solutions were prepared in DMSO and stored at −80 °C. Upon dilution with phosphate-buffered saline (PBS) into culture medium, the final DMSO concentration was <1% DMSO (v/v). HepG2 cells were maintained in RPMI-1640 medium containing 10% FCS and 1% P/S at 37 °C in a humidified atmosphere with 5% CO_2. The day before the experiments were carried out, HepG2 cells were harvested with trypsin/EDTA, centrifuged (200*g*, 4 min), resuspended in medium, and seeded in 96-well plates (3×10^4 cells/well and 100 μL/well). After 12 h incubation, the medium was exchanged and cells were incubated with different concentrations of the drugs (100−0.412 μM) for 24 h at 37 °C in 5% CO_2 atmosphere. Half an hour before the end of incubation, 33% EtOH was added as the positive control. After incubation, 10 μL of Alamar Blue was added to each well, which was incubated for another 2 h. Then, the fluorescence was monitored at 560 nm excitation wavelength and 580 nm emission wavelength on a Victor fluorimeter plate reader (Wallac Instruments). The IC_{50} values were determined using the software GraphPad Prism 4.0.

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