ent-Kaurane Diterpenoids from *Isodon pharicus*

Yong Zhao,[†,‡] Jian-Xin Pu,*[,†] Sheng-Xiong Huang,[†] Li-Sheng Ding,[⊥] Ying-Li Wu,[§] Xian Li,[†] Li-Bin Yang,[†] Wei-Lie Xiao,[†] Guo-Qiang Chen,[§] and Han-Dong Sun*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, College of Chemistry and Engineering, Yunnan Normal University, Kunming 650092, People's Republic of China, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China, and Department of Pathophysiology and Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, Shanghai Jiao-Tong University School of Medicine (SJTU-SM) College, Shanghai 200025, People's Republic of China

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Phytochemical investigation of the aerial parts of *Isodon pharicus* led to the isolation of 13 new *ent*-kaurane diterpenoids, compounds **1**−**13**, together with 12 known analogues (**14**−**25**). The structures of the new compounds were determined by means of extensive spectroscopic techniques including interpretation of 1D and 2D NMR spectra. Selected compounds were evaluated for their cytotoxicity against NB4, A549, PC-3, MCF-7, and SH-SY5Y cell lines.

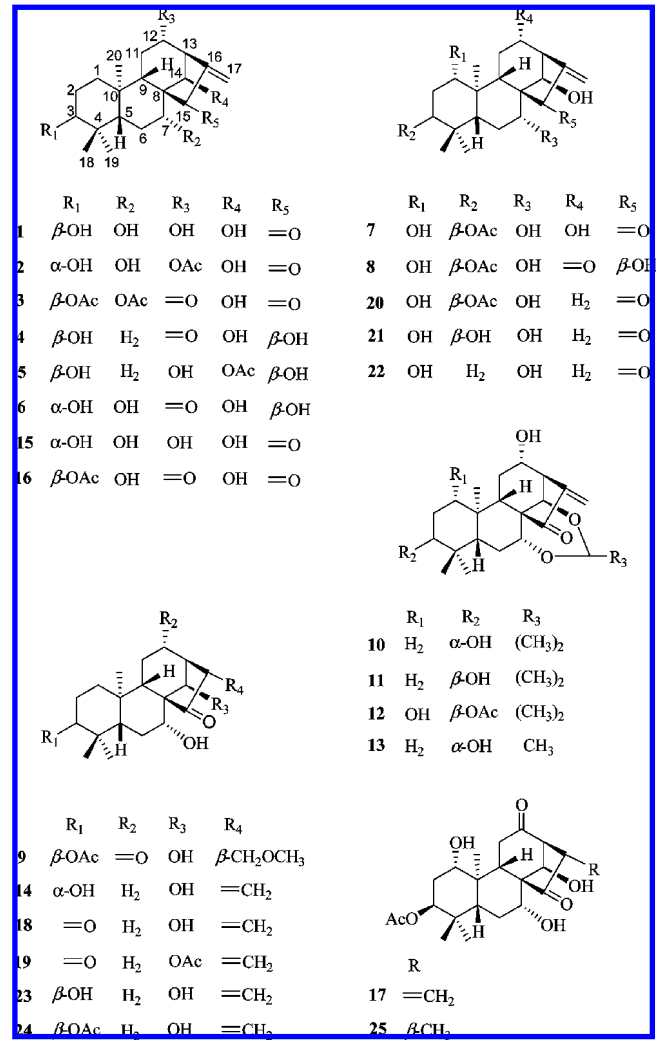
Over the past 30 years, a large number of *ent*-kauranoids, with a wide range of bioactivities and low cytotoxicities, have been isolated from the genus *Isodon* (Lamiaceae) by our group.[1,2] Moreover, the secondary metabolites of this genus have been proven to exhibit characteristics of biodiversity attributed to their different ecological environments.[3−8] *Isodon pharicus* (Prain) Hara, mainly distributed in the northwest of Sichuan Province and the southern district of the Tibetan Region, People's Republic of China, has been used for deinsectization and treatment of inflammation of the eyes.[9] Previous studies on this plant resulted in the isolation of nine *ent*-kuaranoids, including a dimeric *ent*-kauranoid.[10−13] In the course of searching for more biologically active *ent*-kauranoids, we have investigated the aerial parts of *I. pharicus*, collected in Lhasa, Tibet Autonomous Region. As a result, 13 new *ent*-kaurane diterpenoids, compounds **1**−**13**, together with 12 known analogues (**14**−**25**), were obtained. We describe herein the isolation and structure elucidation of these new compounds and the cytotoxicity evaluation of selected compounds.

Results and Discussion

The 70% aqueous acetone extract of the air-dried and powdered aerial parts of *I. pharicus* was partitioned between EtOAc and H_2O to afford an EtOAc extract (273 g), which was subjected to silica gel column chromatography using a $CHCl_3$−Me_2CO mixture as eluent. Further purification by repeated normal-phase column chromatography and semipreparative HPLC yielded 13 new *ent*-kaurane diterpenoids, compounds **1**−**13**, along with 12 known constituents, namely, pseuratas A−C (**14**−**16**),[14] pseurata F (**17**),[15] glaucocalyxins A and B (**18** and **19**),[16] isodomedin (**20**),[17] minherygin G (**21**),[18] kamebanin (**22**),[19] wangzaozin A (**23**),[20] leukamenin E (**24**),[21] and dihydropseurata F (**25**).[15] The structures of the known compounds were determined by comparing spectroscopic data with literature values.

3-Epipseurata B (**1**), a white amorphous powder, showed a pseudomolecular ion at *m/z* 373 $[M + Na]^+$ in the positive ESIMS. IR absorptions at 3421, 1716, and 1643 cm^{-1} implied the presence of hydroxy, carbonyl, and α,β-unsaturated ketone functions. The ^{13}C NMR spectrum showed 20 resonances (Table 3), in agreement with HRESIMS data (*m/z* 373.1993, calcd 373.1990), suggesting a molecular formula of $C_{20}H_{30}O_5$ for **1**. The HSQC spectrum resolved the 20 carbon signals as three methyl, five methylene (including one sp^2 methylene), seven methine (of which four were oxygenated), and five quaternary carbons (including one sp^2 carbon and one carbonyl), which was consistent with a skeleton of an *ent*-kaur-16-en-15-one.[21] Four oxymethine protons at δ_H 3.38 (1H, dd, J = 3.0, 6.1 Hz), 4.33 (1H, m), 4.03 (1H, dd, J = 1.4, 3.3 Hz), and 5.19 (1H, s), observed in the ^1H NMR spectrum (Table 1), were

Chart 1



* Corresponding author. Tel: (86) 871-5223251. Fax: (86) 871-5216343. E-mail: pujianxin@mail.kib.ac.cn or hdsun@mail.kib.ac.cn.
† Kunming Institute of Botany, Chinese Academy of Sciences.
‡ Yunnan Normal University.
⊥ Chengdu Institute of Biology, Chinese Academy of Sciences.
§ Shanghai Jiao-Tong University.

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Table 1. ^{1}H NMR Data of Compounds **1**−**5** (δ in ppm, J in Hz)

H	**1**[a,c]	**2**[b,d]	**3**[b,d]	**4**[a,c]	**5**[a,c]
1α	1.34 (1H, overlap)	1.52 (1H, overlap)	1.04 (1H, m)	1.40 (2H, m)	1.41 (2H, m)
1β	1.29 (1H, m)	0.72 (1H, dt, 12.8, 4.4)	1.28 (1H, br d, 13.7)		
2α	1.98 (1H, m)	1.72 (2H, overlap)	1.58 (1H, m)	1.52 (1H, m)	1.53 (1H, overlap)
2β	1.53 (1H, overlap)		1.75 (1H, overlap)	1.91 (1H, m)	1.76 (1H, m)
3α	3.38 (1H, dd, 6.1, 3.0)		4.76 (1H, s)	3.33 (1H, br s)	3.31 (1H, dd, 7.2, 2.7)
3β		3.36 (1H, dd, 10.8, 5.1)			
5β	1.52 (1H, overlap)	0.97 (1H, br d, 11.9)	1.76 (1H, overlap)	1.47 (1H, br s)	1.33 (1H, overlap)
6α	1.72 (1H, m)	2.04 (1H, m)	1.73 (1H, overlap)	1.41 (1H, m)	1.45 (1H, overlap)
6β	1.89 (1H, m)	2.27 (1H, m)	2.17 (1H, m)	1.49 (1H, m)	1.33 (1H, overlap)
7α				2.27 (1H, br d, 12.4)	1.62 (1H, m)
7β	4.33 (1H, m)	4.86 (1H, dd, 11.9, 3.7)	5.97 (1H, dd, 10.5, 3.5)	1.50 (1H, m)	1.49 (1H, overlap)
9β	1.38 (1H, d, 9.9)	1.49 (1H, overlap)	1.75 (1H, overlap)	2.06 (1H, br s)	1.90 (1H, d, 10.0)
11α	1.68 (1H, d, 16.7)	1.66 (2H, overlap)	2.47−2.55 (2H, m)	2.03 (1H, m)	1.49 (1H, overlap)
11β	1.53 (1H, overlap)			2.63 (1H, dd, 16.5, 10.4)	1.96 (1H, m)
12β	4.03 (1H, dd, 3.3, 1.4)	5.18 (1H, br s)			3.91 (1H, m)
13α	2.60 (1H, m)	3.52 (1H, d, 2.8)	4.14 (1H, s)	3.17 (1H, br s)	2.61 (1H, d, 4.4)
14α	5.19 (1H, s)	5.46 (1H, br s)	5.41 (1H, s)	4.41 (1H, s)	5.83 (1H, s)
15α				4.40 (1H, s)	4.09 (1H, m)
17a	5.99 (1H, s)	6.34 (1H, s)	6.25 (1H, s)	5.19 (1H, d, 2.5)	5.13 (1H, t, 1.4)
17b	5.36 (1H, s)	5.49 (1H, s)	5.51 (1H, s)	5.01 (1H, s)	4.96 (1H, s)
18	0.98 (3H, s)	1.17 (3H, s)	0.87 (3H, s)	0.94 (3H, s)	0.91 (3H, s)
19	0.90 (3H, s)	1.06 (3H, s)	0.70 (3H, s)	0.79 (3H, s)	0.82 (3H, s)
20	1.35 (3H, s)	1.23 (3H, s)	0.84 (3H, s)	0.79 (3H, s)	1.35 (3H, s)
OAc		2.12 (3H, s)	2.03 (3H, s)		1.97 (3H, s)
			1.93 (3H, s)		

[a] Recorded in $(CD_3)_2CO$. [b] Recorded in C_5D_5N. [c] Recorded at 400 MHz. [d] Recorded at 500 MHz.

Table 2. ^{1}H NMR Data of Compounds **6**−**9** (δ in ppm, J in Hz)

H	**6**[a,d]	**7**[a,c]	**8**[a,d]	**9**[b,c]
1α	1.67 (1H, m)			1.25 (1H, br d, 12.9)
1β	0.92 (1H, m)	3.51 (1H, dd, 11.9, 4.2)	3.63 (1H, dd, 11.2, 4.4)	1.00 (1H, m)
2α	1.59 (2H, m)	1.61 (1H, m)	1.73 (1H, m)	1.56 (1H, m)
2β		1.96 (1H, m)	1.97 (1H, m)	1.72 (1H, m)
3α		4.63 (1H, t, 2.7)	4.65 (1H, d, 2.6)	4.77 (1H, br s)
3β	3.16 (1H, m)			
5β	0.93 (1H, dd, 12.4, 1.6)	1.34 (1H, d, 11.4)	1.40 (1H, d, 12.1)	1.62 (1H, d, 12.1)
6α	1.72 (1H, q, 17.6)	1.80 (2H, m)	1.79 (1H, q, 12.1)	1.89 (1H, q, 12.1)
6β	2.03 (1H, overlap)		1.88 (1H, m)	2.11 (1H, m)
7α				
7β	3.93 (1H, m)	4.18 (1H, dd, 11.4, 4.4)	3.91 (1H, dd, 11.7, 3.9)	4.82 (1H, br d, 11.8)
9β	1.86 (1H, br d, 10.2)	1.62 (1H, d, 9.4)	2.23 (1H, d, 9.9)	1.72 (1H, d, 10.8)
11α	2.00 (1H, overlap)	3.11 (1H, d, 16.7)	3.69 (1H, d, 16.7)	2.46 (1H, d, 17.9)
11β	2.67 (1H, dd, 16.6, 10.2)	1.44 (1H, m)	2.66 (1H, dd, 16.7, 9.9)	2.76 (1H, dd, 17.9, 10.8)
12β		3.91 (1H, t, 4.4)		
13α	3.11 (1H, s)	3.02 (1H, d, 2.9)	3.10 (1H, s)	3.65 (1H, d, 6.4)
14α	4.75 (1H, s)	5.17 (1H, s)	4.78 (1H, s)	5.47 (1H, s)
15α	5.31 (1H, d, 5.5)		5.35 (1H, s)	
16α				3.77 (1H, m)
17a	5.28 (1H, d, 2.6)	5.94 (1H, s)	5.26 (1H, d, 1.7)	3.90 (1H, m)
17b	5.08 (1H, s)	5.33 (1H, s)	5.06 (1H, s)	3.74 (1H, dd, 9.8, 4.7)
18	1.00 (3H, s)	0.85 (3H, s)	0.87 (1H, s)	0.85 (3H, s)
19	0.78 (3H, s)	0.93 (3H, s)	0.94 (3H, s)	0.76 (3H, s)
20	0.83 (3H, s)	1.39 (3H, s)	0.96 (3H, s)	0.86 (3H, s)
OAc		2.00 (3H, s)	2.02 (3H, s)	2.05 (3H, s)
OMe				3.14 (3H, s)

[a] Recorded in $(CD_3)_2CO$. [b] Recorded in C_5D_5N. [c] Recorded at 400 MHz. [d] Recorded at 500 MHz.

located at C-3, C-7, C-12, and C-14, respectively, which was proven by the HMBC correlations from H-3 to C-5, C-18, and C-19, from H-7 to C-5, C-8, C-14, and C-15, from H-12 to C-9 and C-14, and from H-14 to C-7, C-8, C-15, and C-16. The ^{1}H−^{1}H COSY correlations of H-1/H-2/H-3, H-5/H-6/H-7, and H-9/H-11/H-12/H-13/H-14 established the spin systems of −CH_2(C-1)−CH_2(C-2)−CH(C-3)−, −CH(C-5)−CH_2(C-6)−CH(C-7)−, and −CH(C-9)−CH_2(C-11)−CH(C-12)−CH(C-13)−CH(C-14)−, respectively, as shown in Figure 1. These features indicated the gross structure of **1** as 3,7,12,14-tetrahydroxy-*ent*-kaur-16-en-15-one.

The relative configuration of **1** was judged from the ROESY correlations of H-3 with Me-19, of H-7 with H-9β, of H-12 with H-17b, and of H-14 with H-6α and Me-20, which suggested the substituents at C-3, C-7, C-12, and C-14 were β-, α-, α-, and β-oriented, respectively. Compared with **15**, the upfield signals at δ_C 33.1 (C-1) and δ_C 46.3 (C-5), caused by the γ-steric compression effect between HO-3β and H-1β and H-5β, along with the small coupling constant of H-3, confirmed that H-3 in **1** was α-oriented. The 3D structure of **1** obtained using a molecular modeling program with MM2 force-field calculations for energy minimization was in good agreement with the observed ROESY correlations, as shown in Figure 1. Therefore, compound **1** was elucidated as 3β,7α,12α,14β-tetrahydroxy-*ent*-kaur-16-en-15-one.

The ^{1}H and ^{13}C NMR data of 12-*O*-acetylpseurata B (**2**) were similar to those of **15**, and the only difference was that an acetoxy group at C-12 in **2** replaced a hydroxy group at the same position in the latter compound, which was proven by the correlations of H-12 (δ_H 5.18) with C-9, C-13, C-14, and OAc in the HMBC

Table 3. ^{13}C NMR Data of Compounds **1**−**9** (δ in ppm)

carbon	**1**[a,c]	**2**[b,d]	**3**[b,d]	**4**[a,c]	**5**[a,c]	**6**[a,d]	**7**[a,c]	**8**[a,d]	**9**[b,c]
1	33.1 t	38.2 t	32.9 t	33.6 t	33.7 t	39.0 t	76.4 d	76.7 d	32.7 t
2	26.1 t	28.0 t	22.9 t	26.1 t	25.8 t	27.9 t	34.0 t	37.6 t	22.8 t
3	75.2 d	77.7 d	76.9 d	75.3 d	75.6 d	78.1 d	78.8 d	78.6 d	77.2 d
4	39.0 s	38.7 s	36.9 s	39.4 s	38.1 s	39.4 s	37.1 s	37.2 s	36.8 s
5	46.3 d	55.1 d	47.9 d	48.6 d	48.5 d	53.0 d	46.7 d	46.8 d	47.7 d
6	30.4 t	30.0 t	24.9 t	19.4 t	20.1 t	29.8 t	28.9 t	29.7 t	29.0 t
7	75.3 d	74.4 d	75.2 d	29.5 t	31.1 t	75.4 d	75.0 d	75.2 d	74.3 d
8	61.5 s	60.9 s	62.3 s	52.1 s	51.0 s	55.0 s	61.9 s	55.9 s	61.3 s
9	57.2 d	55.1 d	50.0 d	46.5 d	50.4 d	46.6 d	58.5 d	47.8 d	51.3 d
10	38.0 s	39.3 s	40.1 s	38.1 s	38.2 s	39.5 s	44.3 s	45.0 s	39.4 s
11	26.3 t	23.5 t	36.0 t	36.7 t	26.3 t	36.4 t	28.9 t	34.2 t	36.8 t
12	72.7 d	74.6 d	206.5 s	209.8 s	75.1 d	209.2 s	73.3 d	210.3 s	209.4 s
13	55.1 d	51.2 d	64.3 d	67.2 d	54.8 d	67.5 d	55.3 d	67.7 d	60.0 d
14	71.1 d	71.2 d	72.4 d	73.3 d	75.1 d	75.4 d	71.5 d	75.6 d	73.1 d
15	208.4 s	207.7 s	203.4 s	79.3 d	80.0 d	73.5 d	209.2 s	73.3 d	215.6 s
16	147.6 s	146.1 s	144.3 s	154.2 s	154.6 s	154.3 s	147.9 s	154.7 s	52.3 d
17	117.1 t	119.0 t	119.8 t	109.3 t	107.2 t	110.4 t	116.9 t	109.9 t	68.8 t
18	29.1 q	28.8 q	27.8 q	28.9 q	29.4 q	28.6 q	28.1 q	28.0 q	27.8 q
19	22.4 q	16.5 q	21.6 q	22.3 q	22.5 q	16.2 q	21.9 q	22.0 q	21.6 q
20	16.3 q	16.1 q	16.1 q	16.7 q	16.1 q	17.2 q	13.0 q	13.8 q	16.3 q
OAc		170.0 s	170.3 s 169.7 s		170.5 s		170.9 s	170.5 s	170.3 s
		21.3 q	21.1 q 21.0 q		20.9 q		21.0 q	21.1 q	21.0 q
OMe									59.0 q

[a] Recorded in $(CD_3)_2CO$. [b] Recorded in C_5D_5N. [c] Recorded at 100 MHz. [d] Recorded at 125 MHz.

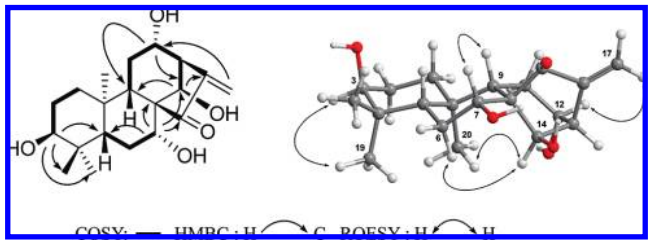

Figure 1. Key HMBC and ROESY correlations of **1**.

experiment. The presence of the fragment for $-CH_2$(C-1)$-CH_2$(C-2)$-$CH(C-3)$-$, $-$CH(C-5)$-CH_2$(C-6)$-$CH(C-7)$-$, and $-$CH(C-9)$-CH_2$(C-11)$-$CH(C-12)$-$CH(C-13)$-$CH(C-14)$-$ were confirmed by the correlations of H-1/H-2/H-3, H-5/H-6/H-7, and H-9/H-11/H-12/H-13/H-14 observed in the $^1H-^1H$ COSY spectrum. The relative configuration of **2** was assigned on the basis of the ROESY correlations of H-3 with H-1β and H-5β, H-7 with H-5β and H-9β, H-12 with H-17b, and H-14 with H-6α and H_3-20. Thus, **2** was elucidated as 3α,7α,14β-trihydroxy-12α-acetoxy-*ent*-kaur-16-en-15-one.

7-*O*-Acetylpseurata C (**3**) exhibited a molecular formula of $C_{24}H_{32}O_7$ as determined by the positive HRESIMS (*m*/*z* 455.2067 ([M + Na]$^+$, calcd 455.2045). Comparison of the NMR data of **3** with those of **16** revealed that the only difference was that a hydroxy group at C-7 in **16** is acetylated in **3**. The HMBC correlations of H-7 (δ_H 5.97) with OAc (δ_C 169.7) confirmed this conclusion. Moreover, the correlations observed in the ROESY spectrum of **3** indicated that the orientations of the substituents in **3** are the same as those of **16**. Thus, compound **3** was characterized as 14β-hydroxy-3β,7α-diacetoxy-*ent*-kaur-16-en-12,15-dione.

The molecular formula of compound **4** was analyzed as $C_{20}H_{30}O_4$ from its HRESIMS and NMR data. The absorptions of an α,β-unsaturated ketone moiety were not observed in its UV and IR spectra. Analysis of its 2D NMR spectra and comparison with **3** showed the absence of a substituent at C-7 and the disappearance of two *O*-acetyl groups in **4**, which was supported by H_2-7 (δ_H 2.27, br d, J = 12.4 Hz, δ_H 1.50, m) correlations with C-5 (δ_C 48.6), C-9 (δ_C 46.5), and C-14 (δ_C 73.3), and H-7 (δ_H 2.27), H-9 (δ_H 2.06), H-14 (δ_H 4.41), and H_2-17 (δ_H 5.19, 5.01) all correlated with C-15 (δ_C 79.3) in the HMBC experiment. In addition, in the ^{13}C NMR spectrum, the upfield shift for C-9 from δ_C 50.0 in **3** to δ_C 46.5 in **4**, caused by the γ-steric compression effect between HO-15β and H-9β, along with the upfield shift for C-8 (δ_C 52.1) in **4**, assigned the H-15 in **4** to be α-oriented. Observation of the correlations of H-3 with H_3-19, H-14 with H-6α and H_3-20, and H-15 with H-7α and H-13α in its ROESY experiment allowed H-3, H-14, and H-15 to be assigned as α-oriented. Subsquently, compound **4** was elucidated as 3β,14β,15β-trihydroxy-*ent*-kaur-16-en-12-one.

Compound **5**, a white amorphous powder, was found to possess the molecular formula $C_{22}H_{34}O_5$ from the HRESIMS pseudomolecular ion [M + Na]$^+$ at *m*/*z* 401.2287 (calcd for 401.2303). Comparison of the NMR data of **5** with those of **4** indicated that a carbonyl group at C-12 in **4** is reduced to a hydroxy group in **5** and the hydroxy group at C-14 in **4** is replaced by an *O*-acetyl group in **5**, which was verified by the HMBC correlations of H-12 (δ_H 3.91) with C-9 (δ_C 50.4) and C-14 (δ_C 75.1) and H-14 (δ_H 5.83) with C-12 (δ_C 75.1) and OAc (δ_C 170.5), as well as the significant upfield shift for C-13 (Δ 12.4 ppm) and C-11 (Δ 10.4 ppm), compared with those of **4**. In addition, the HO-12α, AcO-14β, and HO-15β were assigned by the correlations of H-12 with H-17b, H-14 with H-13α and H_3-20, and H-15 with H-7α observed in the ROESY experiment. Meanwhile, the upfield shifts at C-8 (δ_C 51.0) and C-9 (δ_C 50.4) confirmed the α-orientation of H-15. Thus, **5** was determined as 3β,12α,15β-trihydroxy-14β-acetoxy-*ent*-kaur-16-ene.

Compound **6** was assigned the molecular formula $C_{20}H_{30}O_5$ by the positive HRESIMS. Its NMR data were similar to those of **4** except for the ring-A and -B regions. A hydroxy group at C-7 (δ_H 3.93, m, δ_C 75.4) in **6** substituted the C-7 methylene group in **4**. The H-3β was deduced from the abnormal upfield shift of C-19 (Δ 6.1 ppm) and the relative downfield shifts of C-1 (Δ 5.4 ppm) and C-5 (Δ 4.4 ppm) because of the absence of γ-steric compression effect between HO-3β and H-1β and H-5β in **6**. This was confirmed by the ROESY correlations of H-3 with H-1β and H-5β, and of H-7 with H-5β and H-9β. Consequently, the structure of **6** was elucidated as 3α,7α,14β,15β-tetrahydroxy-*ent*-kaur-16-en-12-one.

12-Deoxyisodomedin (**7**) was isolated as a white powder, whose molecular formula was established as $C_{22}H_{32}O_7$ by HRESIMS and NMR data. The ^1H and ^{13}C NMR spectra of **7** indicated the similarity to those of **17**, and the only difference observed was the

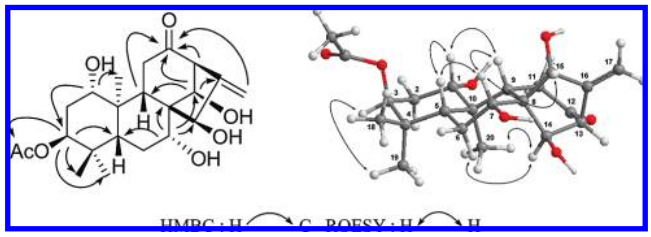

Figure 2. Key HMBC and ROESY correlations of **8**.

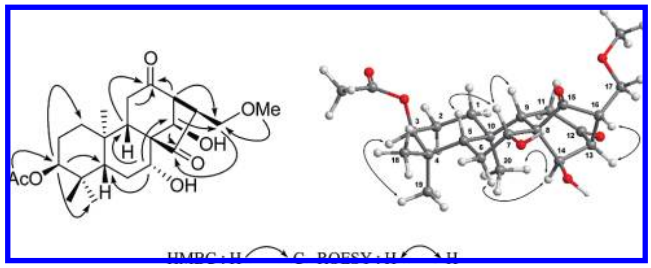

Figure 3. Key HMBC and ROESY correlations of **9**.

presence of a hydroxy with α-orientation at C-12 in **7** instead of a keto group at the same position in **17**. The correlations of H-12 (δ_H 3.91) with C-9 (δ_C 58.5) and C-14 (δ_C 71.5) observed in the HMBC and H-12/H-17b observed in the ROESY spectrum of **7** confirmed this conclusion. Accordingly, **7** was characterized as 1α,7α,12α,14β-tetrahydroxy-3β-acetoxy-*ent*-kaur-16-en-15-one.

Dihydropseurata F (**8**), obtained as a white powder, has a molecular formula of $C_{22}H_{32}O_7$ from its HRESIMS. The IR spectrum revealed absorption bands at 3418 (OH) and 1717 (CO). Comparison of the spectroscopic data of **8** with those of **17** suggested that a carbonyl group at C-15 in **17** was reduced to a hydroxy group in **8**, which was proven by the HMBC correlations from H-15 (δ_H 5.35) to C-7 (δ_C 75.2), C-9 (δ_C 47.8), and C-17 (δ_C 109.9). The upfield signals at δ_C 55.9 (C-8) and δ_C 47.8 (C-9) indicated the presence of H-15α. This was proven by the ROESY spectrum of **8**, as shown in Figure 2. Consequently, compound **8** was assigned as 1α,7α,14β,15β-tetrahydroxy-3β-acetoxy-*ent*-kaur-16-en-12-one.

17-Methoxydihydropseurata C (**9**) was assigned the molecular formula $C_{23}H_{34}O_7$ by the positive HRESIMS. The NMR data of **9** were similar to those of **16** except for the ring-D region. The exomethylene group in **16** was replaced by a methine proton [δ_H 3.77 (m, H-16); δ_C 52.3 (C-16)] and a methoxymethyl group [δ_H 3.14 (s, OMe), 3.90 (m, H-17a), and 3.74 (dd, J = 9.8, 4.7 Hz, H-17b); δ_C 59.0 (OMe) and 68.8 (C-17)] in **9**, which was confirmed by the COSY correlations of H-13/H-16/H_2-17. The β-orientation of the methoxymethyl group was established by the ROESY correlations of H-16/H-13α (Figure 3). Therefore, the structure of **9** was represented as 16(*R*)-7α,14β-dihydroxy-17-methoxy-3β-acetoxy-*ent*-kaur-12,15-dione.

Pseurata B acetonide (**10**) exhibited a quasimolecular ion peak at *m/z* 413.2321 $[M + Na]^+$ in its HRESIMS, corresponding to $C_{23}H_{34}O_5$, with seven degrees of unsaturation. Comparison of the NMR data of **10** with those of pseurata B (**15**) revealed that the two compounds resembled each other, and the only difference was that **10** has one more degree of unsaturation when taken in conjunction with the three more carbon signals including a quaternary carbon [δ_C 97.5 (C-1′)] and two methyls at [δ_C 31.3 (C-2′); δ_C 25.4 (C-3′)]; this indicated that **10** could be an acetonide of **15**. The above conclusion was proven by HMBC correlations of H-7/C-1′, H-14/C-1′, and H-3′ and H-2′/C-1′. Thus, compound **10** was identified as 3α,7α,12α,14β-tetrahydroxy-*ent*-kaur-16-en-15-one 7,14-acetonide.

The molecular formulas of compounds **11**−**13** were determined as $C_{23}H_{34}O_5$, $C_{25}H_{36}O_7$, and $C_{22}H_{32}O_5$, respectively, according to their HRESIMS. Examination of their NMR data as well as a detailed comparison of their 1D, 2D NMR spectra with those of compounds **1**, **7**, and **15**, respectively, suggested that **11** and **12** were the acetonides of **1** and **7**, respectively, and **13** was an acetal of **15**. Therefore, compounds **11**−**13** were elucidated as 3β,7α,12α,14β-tetrahydroxy-16-*ent*-kaur-15-one 7,14-acetonide, 1α,7α,12α,14β-tetrahydroxy-3β-acetoxy-*ent*-kaur-16-en-15-one 7,14-acetonide, and 3α,7α,12α,14β-tetrahydroxy-*ent*-kaur-16-en-15-one 7,14-acetal, respectively. Compounds **10**−**12** could be artifacts of **15**, **1**, and **7**, respectively, since acetone was used in the course of extraction and isolation. Similarly, compound **13** was most likely generated from condensation of **15** with acetaldehyde, which is produced by the oxidation of ethanol in chloroform.

Table 4. Cytotoxicity Data for Selected Isolates from *I. pharicus* in Selected Human Cell Lines[a]

compd	NB4	A549	SH-SY5Y	PC-3	MCF-7
2	8.32	>10	8.12	>10	>10
9	8.74	>10	>10	7.42	>10
10	3.56	6.02	>10	>10	>10
14	7.69	2.92	>10	>10	9.05
16	2.08	7.62	>10	>10	>10
18	2.90	5.39	>10	>10	8.73
19	7.86	6.22	>10	>10	>10
20	7.29	>10	>10	>10	>10
23	9.02	9.29	>10	>10	5.80
24	4.00	>10	>10	>10	>10
paclitaxel	0.1	0.1	0.2	0.2	0.1
etoposide	1.3	1.7	1.7	13.6	7.6

[a] Results are expressed as IC_{50} values in μM. Cell lines: NB4 acute promyelocytic leukemia; A549 lung cancer; PC-3 prostate cancer; MCF-7 breast cancer; SH-SY5Y human neuroblastoma. Compounds **1**, **3**, **5**, **7**, **8**, **15**, **17**, and **21** were inactive for all cell lines (IC_{50} > 10 μM).

Due to the limited amount of material available, compounds **4**, **6**, **11**, **12**, **13**, **22**, and **25** were not tested for cytotoxicity. The other diterpenoids were evaluated for cytotoxic activities against the NB4 (acute promyelocytic leukemia), A549 (lung cancer), PC-3 (prostate cancer), MCF-7 (breast cancer), and SH-SY5Y (neuroblastoma) human cell lines, using the sulforhodamine B (SRB) method, as reported previously,[21] with paclitaxel and etoposide as the positive controls. As may be seen from Table 4, none of these compounds was broadly cytotoxic for all cell lines represented. The moderate cytotoxic compounds for one or more cell lines were **10**, **14**, **16**, **18**, and **24**.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker DRX-400 and a DRX-500 instrument with TMS as internal standard. Mass spectra were obtained on a VG Auto Spec-3000 spectrometer or on a Finnigan MAT 90 instrument. 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 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 MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} on glass plates (Qingdao Marine Chemical Inc.) using various solvent systems.

Plant Material. The aerial parts of *I. pharicus* were collected in the Lhasa area, Tibet Autonomous Region, People's Republic of China, in October 2005. Voucher specimens (KIB 20051006) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and were identified by Prof. Xi-Wen Li.

Extraction and Isolation. The milled aerial parts of *I. pharicus* (7.0 kg) were extracted with 70% aqueous acetone (3 × 40 L) at room temperature overnight. The extract was partitioned between EtOAc and

H_2O. The EtOAc extract (380 g) was chromatographed on MCI gel CHP 20P (90% CH_3OH-H_2O, then 100% CH_3OH). The 90% CH_3OH fraction (273 g) was chromatographed over silica gel (200−300 mesh, 1.5 kg), eluted in a step gradient manner with $CHCl_3$−acetone (1:0 to 0:1), to afford fractions A−F. Fraction A (9 g) was submitted to repeated chromatography over silica gel (petroleum ether−acetone, from 99:1 to 1:1) to give fractions A1−A4. Fraction A2 was purified by repeated chromatography over silica gel (petroleum ether−acetone, from 99:1 to 2:1) and RP-18 column (30% → 60% MeOH−H_2O) to yield compounds **3** (7 mg, 0.00010%) and **19** (6 mg, 0.00009%). Compounds **9** (12 mg, 0.00017%), **10** (5 mg, 0.00007%), and **24** (4 mg, 0.00006%) were purified from fraction A3 by RP-18 column (30% → 60% MeOH−H_2O) and semipreparative HPLC (42% MeOH−H_2O). Fraction A4 gave compounds **11** (2 mg, 0.00003%), **12** (2 mg, 0.00003%), and **13** (3 mg, 0.00004%) by RP-18 column (30% → 60% MeOH−H_2O) followed by semipreparative HPLC (40% MeOH−H_2O). Fraction B (24 g) was submitted to repeated chromatography over silica gel (petroleum ether−acetone, from 40:1 to 0:1) to obtain fractions B1−B4. Compound **16** (350 mg, 0.005%) was crystallized from fraction B2. Compound **2** (227 mg, 0.00324%) was crystallized from fraction B4. Separation of fraction C by silica gel column chromatography, eluted with petroleum ether−acetone (9:1 → 1:1), yielded mixture fractions C1−C5. Compounds **5** (4 mg, 0.00006%) and **20** (125 mg, 0.00179%) were obtained by semipreparative HPLC (45% MeOH−H_2O) from C2. Fraction C3 afforded compounds **14** (12 mg, 0.00017%) and **18** (3 mg, 0.00004%) by RP-18 column chromatography (30% → 60% MeOH−H_2O) and semipreparative HPLC (40% MeOH−H_2O). Compound **4** (1 mg, 0.00001%) was purified by fraction C4. Compound **22** (2 mg, 0.00003%) was obtained by RP-18 column chromatography (37% MeOH−H_2O) from fraction C5. Fraction D (20 g) was subjected to silica gel column chromatography, eluted with petroleum ether−acetone (9:1 → 1:1), to yield fractions D1−D5. Compound **25** (1 mg, 0.00001%) was purified by semipreparative HPLC (32% MeOH−H_2O) from fraction D2. Separation of D3 by RP-18 column chromatography (30% MeOH−H_2O) led to the isolation of compound **23** (6 mg, 0.00009%). Fraction D5 was further chromatographed over a RP-18 column (37% MeOH−H_2O) followed by semipreparative HPLC (32% MeOH−H_2O) to give compounds **1** (4 mg, 0.00006%) and **6** (2 mg, 0.00003%). Fraction E (37 g) was subjected to silica gel column chromatography, eluted with petroleum ether−acetone (4:1 → 1:1), to yield fractions E1−E5. Compound **21** (5 mg, 0.00007%) was separated from fraction E1 by recrystallization from MeOH. Fraction E2 was purified using RP-18 column chromatography (30% → 60% MeOH−H_2O) to afford compounds **7** (12 mg, 0.00017%) and **15** (38 mg, 0.00054%). Semipreparative HPLC (35% MeOH−H_2O) was applied to give compound **8** (9 mg, 0.00013%) from fraction E3. Compound **17** (2.4 g, 0.03429%) was crystallized from fraction E4.

3-Epipseurata B (1): white, amorphous powder; $[\alpha]^{19.9}_D$ −37.9 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (3.44) nm; IR (KBr) ν_{max} 3421, 2963, 2915, 1750, 1716, 1699, 1643, 1455, 1391, 1372, 1257, 1227, 1108, 1079, 1069, 1032, 1012, 989, 958, 671 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz], see Table 1; ^{13}C NMR [$(CD_3)_2CO$, 100 MHz], see Table 3; positive ESIMS *m/z* 373 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 373.1993 (calcd for $C_{20}H_{30}O_5$, 373.1990).

12-*O*-Acetylpseurata B (2): white powder; $[\alpha]^{20.2}_D$ −15.3 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 229.4 (3.52) nm; IR (KBr) ν_{max} 3354, 2934, 1739, 1650, 1441, 1372, 1235, 1098, 1034, 993 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 415 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 415.2104 (calcd for $C_{22}H_{32}O_6$, 415.2096).

7-*O*-Acetylpseurata C (3): amorphous powder; $[\alpha]^{18.4}_D$ +64.9 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 205.4 (3.28) nm; IR (KBr) ν_{max} 3442, 2956, 1726, 1641, 1375, 1248, 1089, 1057, 1034, 981 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 3; positive ESIMS *m/z* 455 $[M + Na]^+$; positive HRESIMS *m/z* 455.2067 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_7$, 455.2045).

3*β*,14*β*,15*β*-Trihydroxy-*ent*-kaur-16-en-12-one (4): white powder; $[\alpha]^{19.8}_D$ +73.3 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 205.4 (3.45) nm; IR (KBr) ν_{max} 3429, 2939, 2872, 1698, 1447, 1416, 1389, 1233, 1054, 986, 969, 916, 764 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz], see Table 1; ^{13}C NMR [$(CD_3)_2CO$, 100 MHz], see Table 3; positive ESIMS *m/z* 357 $[M + Na]^+$; positive HRESIMS *m/z* 357.2043 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4$, 357.2041).

3*β*,12α,15*β*-Trihydroxy-14*β*-acetoxy-*ent*-kaur-16-ene (5): white powder; $[\alpha]^{19.8}_D$ +27.3 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 204.6 (3.52) nm; IR (KBr) ν_{max} 3433, 2941, 2876, 1717, 1450, 1432, 1376, 1264, 1127, 1090, 1029, 991, 919, 887 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz], see Table 2; ^{13}C NMR [$(CD_3)_2CO$, 100 MHz], see Table 3; positive ESIMS *m/z* 401 $[M + Na]^+$; positive HRESIMS *m/z* 401.2287 $[M + Na]^+$ (calcd for, $C_{22}H_{34}O_5$, 401.2303).

3α,7α,14*β*,15*β*-Tetrahydroxy-*ent*-kaur-16-en-12-one (6): white, amorphous powder; $[\alpha]^{19.2}_D$ +56.6 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 204.6(3.44) nm; IR (KBr) ν_{max} 3396, 2932, 1713, 1449, 1392, 1369, 1096, 1029, 963, 916, 593, 522 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 500 MHz], see Table 1; ^{13}C NMR [$(CD_3)_2CO$, 125 MHz], see Table 3; positive ESIMS *m/z* 373 $[M + Na]^+$; positive HRESIMS *m/z* 373.1977 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_5$, 373.1990).

12-Deoxyisodomedin (7): white powder; $[\alpha]^{18.9}_D$ +28.9 (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ε) 202.0 (3.99) nm; IR (KBr) ν_{max} 3398, 2959, 2921, 1732, 1717, 1645, 1467, 1430, 1375, 1256, 1082, 1024, 1001 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz], see Table 2; ^{13}C NMR [$(CD_3)_2CO$, 100 MHz], see Table 3; positive ESIMS *m/z* 431 $[M + Na]^+$; positive HRESIMS *m/z* 431.2058 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_7$, 431.2045).

Dihydropseurata F (8): white powder; $[\alpha]^{19.5}_D$ +74.2 (*c* 0.46, MeOH); UV (MeOH) λ_{max} (log ε) 206.0 (3.46) nm; IR (KBr) ν_{max} 3418, 2952, 1717, 1466, 1450, 1376, 1260, 1182, 1116, 1088, 1036, 988, 904 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 500 MHz], see Table 2; ^{13}C NMR ($(CD_3)_2CO$, 125 MHz), see Table 3; positive ESIMS *m/z* 431 $[M + Na]^+$; positive HRESIMS *m/z* 431.2073 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_7$, 431.2045).

17-Methoxydihydropseurata C (9): amorphous powder; $[\alpha]^{18.6}_D$ +63.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 202.4 (3.30) nm; IR (KBr) ν_{max} 3431, 2919, 2851, 1687, 1605, 1502, 1407, 1290, 1262, 1205, 1162, 1019 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz), see Table 2; ^{13}C NMR (C_5D_5N, 100 MHz), see Table 3; positive ESIMS *m/z* 445 $[M + Na]^+$; positive HRESIMS *m/z* 445.2200 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_7$, 445.2202).

Pseurata B acetonide (10): white powder; $[\alpha]^{19.9}_D$ −49.7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (3.76) nm; IR (KBr) ν_{max} 3439, 2989, 2933, 1732, 1650, 1465, 1374, 1261, 1196, 1157, 1113, 1086, 1018, 975, 869 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] δ 5.35 (1H, s, H-17a), 4.93 (1H, s, H-17b), 4.59 (1H, d, J = 3.0 Hz, H-14α), 4.12 (1H, m, H-7*β*), 4.09 (1H, s, H-12*β*), 3.15 (1H, m, H-3*β*), 3.04 (1H, d, J = 3.0 Hz, H-13α), 2.05−1.98 (2H, m, H_2-6), 1.97 (1H, overlap, H-2*β*), 1.67 (1H, overlap, H-1α), 1.61 (2H, overlap, H_2-11), 1.60 (1H, overlap, H-2α), 1.55 (3H, s, Me-3′), 1.26 (3H, s, Me-20), 1.23 (1H, overlap, H-9*β*), 1.10 (3H, s, Me-2′), 1.01 (3H, s, Me-18), 0.97 (1H, overlap, H-1*β*), 0.82 (3H, s, Me-19), 0.78 (1H, dd, J = 9.8, 2.3 Hz, H-5*β*); positive ESIMS *m/z* 413 $[M + Na]^+$; positive HRESIMS *m/z* 413.2321 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_5$, 413.2303); ^{13}C NMR [$(CD_3)_2CO$, 100 MHz] δ 206.9 (s, C-15), 146.6 (s, C-16), 116.6 (t, C-17), 97.5 (s, C-1′), 78.2 (d, C-3), 72.2 (d, C-12), 71.6 (d, C-7), 67.2 (d, C-14), 54.8 (s, C-8), 54.7 (d, C-9), 51.8 (d, C-13), 51.8 (d, C-5), 39.3 (s, C-4), 38.3 (s, C-10), 38.2 (t, C-1), 31.3 (q, C-2′), 28.5 (q, C-18), 28.2 (t, C-6), 27.8 (t, C-2), 26.5 (t, C-11), 25.4 (q, C-3′), 16.4 (q, C-20), 15.8 (q, C-19); positive ESIMS *m/z* 413 $[M + Na]^+$; positive HRESIMS *m/z* 413.2321 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_5$, 413.2303).

3-Epipseurata B acetonide (11): white powder; $[\alpha]^{19.9}_D$ −49.7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 231.6 (3.49) nm; IR (KBr) ν_{max} 3442, 2989, 2940, 2874, 1733, 1650, 1463, 1376, 1260, 1197, 1157, 1114, 1092, 1039, 1022, 993, 869 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.16 (1H, s, H-17a), 5.56 (1H, s, H-17b), 4.93 (1H, s, H-14α), 4.29 (1H, t, J = 8.9 Hz, H-7*β*), 4.18 (1H, br s, H-12*β*), 3.44 (1H, s, H-3α), 3.07 (1H, d, J = 2.3 Hz, H-13α), 1.97 (2H, m, H_2-6), 1.89 (1H, m, H-11*β*), 1.67 (2H, m, H_2-2), 1.61 (1H, m, H-11α), 1.58 (3H, s, Me-3′), 1.47 (1H, d, J = 9.6 Hz, H-9*β*), 1.37 (1H, overlap, H-1α), 1.37 (1H, overlap, H-5*β*), 1.24 (3H, s, Me-2′), 1.23 (3H, s, Me-20), 1.22 (1H, overlap, H-1*β*), 0.98 (3H, s, Me-18), 0.90 (3H, s, Me-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.4 (s, C-15), 144.2 (s, C-16), 118.1 (t, C-17), 97.3 (s, C-1′), 75.5 (d, C-3), 72.1 (d, C-12), 70.7 (d, C-7), 66.3 (d, C-14), 54.4 (s, C-8), 53.1 (d, C-9), 51.0 (d, C-13), 44.5 (d, C-5), 37.4 (s, C-4), 37.3 (s, C-10), 32.0 (t, C-1), 30.7 (q, C-2′), 28.0 (q, C-18), 27.1 (t, C-6), 25.6 (t, C-2), 25.2 (q, C-3′), 24.8 (t, C-11), 21.9 (q, C-19), 15.8 (q, C-20); positive ESIMS *m/z* 413 $[M + Na]^+$; positive HRESIMS *m/z* 413.2307 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_5$, 413.2303).

12-Deoxyisodomedin acetonide (12): white powder; $[\alpha]^{19.9}_D$ +51.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 231.2 (3.48) nm; IR (KBr) ν_{max} 3440, 2988, 1734, 1650, 1376, 1258, 1197, 1180, 1159, 1115,

1039, 987, 954 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 500 MHz] δ 5.95 (1H, s, H-17a), 5.33 (1H, s, H-17b), 4.95 (1H, s, H-14α), 4.65 (1H, br s, H-3α), 4.45 (1H, d, J = 2.9 Hz, HO-12α), 4.11 (1H, dd, J = 12.7, 5.9 Hz, H-7β), 4.03 (1H, d, J = 3.1 Hz, H-12β), 3.55 (1H, m, H-1β), 3.50 (1H, d, J = 6.1 Hz, HO-1α), 3.15 (1H, br d, J = 14.4 Hz, H-11β), 3.04 (1H, d, J = 2.7 Hz, H-13α), 2.09 (1H, m, H-6α), 2.02 (3H, s, OAc), 1.96 (1H, m, H-2β), 1.84 (1H, m, H-6β), 1.70 (1H, m, H-2α), 1.62 (1H, overlap, H-11α), 1.62 (1H, overlap, H-9β), 1.56 (3H, s, Me-3′), 1.37 (3H, s, Me-20), 1.24 (1H, d, J = 12.3 Hz, H-5β), 1.11 (3H, s, Me-2′), 0.97 (3H, s, Me-19), 0.89 (3H, s, Me-18); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz] δ 207.5 (s, C-15), 170.5 (s, OAc), 147.4 (s, C-16), 116.1 (t, C-17), 97.5 (s, C-1′), 79.0 (d, C-3), 76.0 (d, C-1), 72.8 (d, C-12), 71.5 (d, C-7), 67.5 (d, C-14), 55.6 (s, C-8), 55.6 (d, C-9), 52.2 (d, C-13), 46.1 (d, C-5), 43.8 (s, C-10), 37.2 (s, C-4), 34.0 (t, C-2), 31.2 (q, C-2′), 28.3 (t, C-11), 27.9 (q, C-18), 27.7 (t, C-6), 25.5 (q, C-3′), 21.9 (q, C-19), 21.0 (q, OAc), 12.3 (q, C-20); positive ESIMS m/z 471 $[M + Na]^+$; positive HRESIMS m/z 471.2349 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_7$, 471.2358).

Pseurata B acetal (13): white powder; $[\alpha]^{20.1}_D$ −65.9 (c 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 231.4 (3.50) nm; IR (KBr) ν_{max} 3439, 2960, 2932, 2872, 1732, 1649, 1406, 1254, 1126, 1096, 1026, 994 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 500 MHz] δ 5.95 (1H, s, H-17a), 5.36 (1H, s, H-17b), 5.11 (1H, q, J = 5.0 Hz, H-1′), 4.85 (1H, s, H-14α), 4.59 (1H, d, J = 3.0 Hz, HO-12α), 4.13 (1H, dd, J = 12.6, 5.6 Hz, H-7β), 4.06 (1H, m, H-12β), 3.52 (1H, d, J = 5.3 Hz, HO-3α), 3.16 (1H, m, H-3β), 3.07 (1H, d, J = 3.7 Hz, H-13α), 2.14 (1H, q, J = 12.6 Hz, H-6α), 1.75 (1H, m, H-6β), 1.65 (1H, m, H-1α), 1.59 (2H, m, H_2-11), 1.57 (2H, m, H_2-2), 1.28 (3H, s, Me-20), 1.25 (1H, d, J = 9.3 Hz, H-9β), 1.05 (3H, d, J = 5.0 Hz, Me-2′), 1.03 (3H, s, Me-18), 0.86 (1H, overlap, H-5β), 0.84 (1H, overlap, H-1β), 0.83 (3H, s, Me-19); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz] δ 207.1 (s, C-15), 146.7 (s, C-16), 116.8 (t, C-17), 91.7 (d, C-1′), 78.2 (d, C-3), 73.2 (d, C-12), 73.3 (d, C-14), 72.6 (d, C-7), 55.4 (s, C-8), 55.3 (d, C-9), 52.1 (d, C-13), 51.5 (d, C-5), 39.5 (s, C-4), 38.4 (t, C-1), 38.2 (s, C-10), 28.6 (q, C-18), 27.8 (t, C-2), 26.6 (t, C-11), 23.0 (t, C-6), 21.4 (q, C-2′), 16.2 (q, C-20), 15.9 (q, C-19); positive ESIMS m/z 399 $[M + Na]^+$, 775 $[2\,M + Na]^+$; positive HRESIMS m/z 399.2152 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_5$, 399.2147).

Cellular Proliferation Assay. Colorimetric assays were performed to evaluate compound activity. The NB4 acute promyelocytic leukemia cell line, the A549 lung cancer cell line, the PC-3 prostate cancer cell line, the MCF-7 breast cancer cell line, and the SH-SY5Y neuroblastoma cell line were treated with various concentrations of compounds (0, 0.01, 0.1, 1, 10, 50 μM) in 96-well culture plates for 48 h in 200 μL of media and pulsed with 10 μL of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (WST-8; Cell Counting Kit-8; Dojindo, Kumamoto, Japan) to each well for 4 h. WST-8 is converted to WST-8-formazan upon bioreduction in the presence of an electron carrier, 1-methoxy-5-methylphenazinium methyl sulfate, which is abundant in viable cells. Absorbance readings at a wavelength of 450 nm were taken on a spectrophotometer (Multiscan MK3, Thermo Labsystems). The concentration resulting in 50% of cell-growth inhibition (IC_{50}) was calculated using the Probit program in SPSS 7.5 for windows 98 (SPSS Inc., Chicago). Paclitaxel and etoposide were used as positive controls.[22]

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Supporting Information Available: 1D, 2D NMR and MS spectra of **1**−**13**, 1D NMR spectral data of **15**−**17**, and key correlations of **1**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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