Structure and Cytotoxicity of Diterpenoids from *Isodon eriocalyx*

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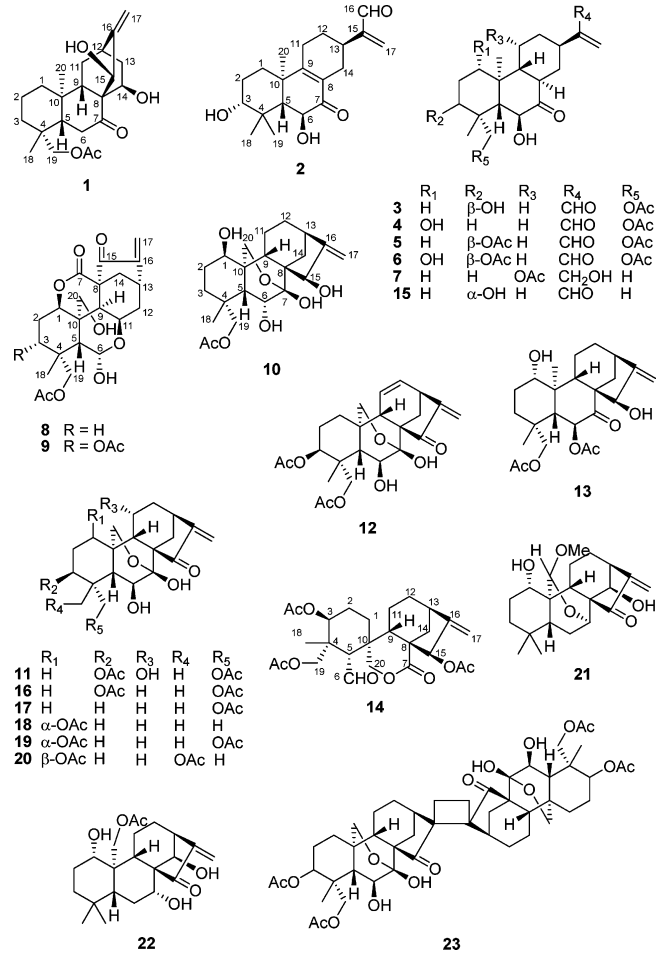
A new *ent*-atisanoid, eriocatisin A (**1**), six new *ent*-abietanoids, eriocasins B−E (**2**−**4**, **7**), 3-acetyleriocasin C (**5**), and 3β-acetoxyeriocasin D (**6**), and seven new *ent*-kauranoids, maoesins A−F (**8**, **10**−**14**) and 3α-acetoxy-maoesin A (**9**), together with 21 known compounds, were isolated from the aerial parts of *Isodon eriocalyx*. The structures of **1**−**14** were determined by spectroscopic data interpretation. All compounds isolated were evaluated for their in vitro growth inhibitory activity against the HT-29, BEL-7402, and SK-OV-3 human cancer cell lines. Compounds **17**, **18**, and **20** showed inhibitory effects for all three tumor cell lines used, with IC_{50} values in the range 2.1−7.3 μM.

Plants of the genus *Isodon* (Lamiaceae) are typified by the presence of bioactive *ent*-kaurane diterpenoids.[1] Since 1976, as part of a search for novel natural products as useful leads for the discovery of therapeutic agents to treat cancer, more than 60 species of this genus from mainland China have been investigated phytochemically. About 600 new diterpenoids with diverse skeletons have been isolated and characterized. Some of these *ent*-kauranoids possess potential antitumor activity and low toxicity,[1,2] such as eriocalyxin B,[3,4] oridonin,[5] and ponicidin.[5]

Isodon eriocalyx (Dunn) Hara, a perennial herb, is distributed mainly in the southwest of the People's Republic of China.[6] Previous phytochemical investigations on *I. eriocalyx* collected from Yunnan and Guizhou Provinces led to the discovery of a series of *ent*-kauranoids and several other compounds.[7−16] Since the secondary metabolites of the genus *Isodon* often differ when grown in different ecological environments,[17−21] *I. eriocalyx* collected in Luding County of Sichuan Province was chosen for detailed chemical investigation in order to search for structurally unique bioactive diterpenoids. As a result, a new *ent*-atisanoid (**1**), six new *ent*-abietanoids (**2**−**7**), and seven new *ent*-kauranoids (**8**−**14**), together with 21 known diterpenoids, were isolated. The structures of **1**−**14** were elucidated by spectroscopic methods and by comparison with reported data. Furthermore, the cytotoxic evaluation of all diterpenoids isolated was carried out against the HT-29, BEL-7402, and SK-OV-3 human tumor cell lines.

Results and Discussion

The ethyl acetate-soluble extract of the aerial parts of *I. eriocalyx* was subjected successively to silica gel, MCI gel CHP-20, Sephadex LH-20, and Lichroprep RP-18 gel column chromatography as well as to semipreparative HPLC, to afford 14 new diterpenoids (**1**−**14**). Also isolated were 21 known compounds identified as 3α,6β-dihydroxy-7,17-dioxo-*ent*-abieta-15(16)-ene (**15**),[22] maoecrystal J (**16**),[7] longikaurin C (**17**),[23] effusanin B (**18**),[24] effusanin D (**19**),[24] maoecrystal R (**20**),[9] kamebacetal A (**21**),[25] henryin (**22**),[25] maoecrystal M (**23**),[8] nodosin,[26] enmin,[27,28] xerophilusin H,[29] *ent*-kaurane-3β,16β-diol,[30] 3-acetyltrichorabdal C,[7] coetsin B,[31] rabdosichuanin A,[32] ludongnins F−I,[18] and sculponeatin C,[33] respectively, by comparing their spectroscopic data with those reported in the literature.



Eriocatisin A (**1**) was obtained as a white, amorphous powder. The positive HRESIMS (*m*/*z* 399.2138, calcd for $C_{22}H_{32}O_5Na$, 399.2147) showed the molecular formula to be $C_{22}H_{32}O_5$, representing an unsaturation value of seven. The IR spectrum showed absorptions at 3440, 1738, 1694, and 1658 cm^{-1}, which were attributed to OH, C=O, and C=C functional groups. In the ^1H NMR spectrum (Table 1), two methyl signals were observed as singlets at δ 0.79 and 1.06. A pair of oxygenated AB methylene signals

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Table 1. ^{1}H NMR Data of Compounds **1**−**7** (δ in ppm, J in Hz)

H	**1**[a,c]	**2**[a,d]	**3**[a,d]	**4**[a,d]	**5**[b,d]	**6**[a,c]	**7**[a,d]
1α	0.79 (overlap)	1.30 (m)	1.40 (m)		1.41 (overlap)		0.93 (dt, 13.0, 3.0)
1β	1.48 (overlap)	1.65 (brd, 12.9)	1.87 (overlap)	3.66 (t, 8.1)	1.57 (m)	4.12 (m)	1.81 (brd, 13.0)
2α	1.25 (m)	1.93 (2H, m)	1.90 (overlap)	1.99 (overlap)	1.92 (overlap)	2.38 (2H, m)	1.30 (overlap)
2β	1.49 (overlap)		2.07 (overlap)	1.89 (m)	1.87 (overlap)		1.49 (overlap)
3α	0.86 (dt, 13.6, 4.0)		4.11 (brs)	1.96 (overlap)	4.97 (brs)	5.63 (brs)	1.17 (overlap)
3β	1.62 (brd, 13.6)	3.50 (t, 7.8)		1.26 (overlap)			1.37 (overlap)
5β	1.56 (dd, 10.6, 6.4)	1.90 (d, 12.6)	2.15 (d, 12.3)	1.52 (d, 12.0)	1.70 (d, 12.9)	2.08 (d, 12.4)	1.21 (overlap)
6α	2.69 (d, 4.0)	4.71 (d, 12.6)	4.65 (d, 12.3)	4.71 (d, 12.0)	4.36 (d, 12.9)	4.80 (d, 12.4)	4.51 (d, 12.4)
6β	2.67 (s)						
8α			2.27 (t, 11.0)	2.38 (t, 15.2)	2.43 (overlap)	2.44 (t, 10.8)	2.81 (dt, 11.5, 3.0)
9β	2.53 (overlap)		1.02 (overlap)	1.27 (overlap)	1.23 (overlap)	1.44 (overlap)	1.19 (overlap)
11α	1.39 (m)	2.22 (overlap)	1.68 (overlap)	2.91 (d, 17.5)	1.96 (overlap)	2.91 (m)	
11β	1.70 (m)	2.10 (m)	1.02 (overlap)	1.51 (overlap)	1.36 (overlap)	1.62 (overlap)	5.62 (brs)
12α	2.51 (overlap)	1.72 (m)	1.71 (overlap)	1.41 (overlap)	2.18 (overlap)	1.39 (overlap)	1.40 (overlap)
12β		1.50 (overlap)	1.28 (dd, 24.5, 12.3)	1.06 (m)	1.33 (overlap)	1.09 (m)	2.17 (dd, 14.0, 2.5)
13α	2.26 (m)	2.73 (overlap)	2.48 (d, 12.3)	2.52 (t, 14.8)	2.48 (overlap)	2.54 (m)	2.63 (t, 12.5)
13β	1.81 (m)						
14α	4.75 (dd, 9.6, 3.6)	2.19 (overlap)	0.91 (m)	1.75 (dd, 17.0, 2.5)	1.12 (m)	1.80 (m)	2.56 (dd, 13.5, 2.5)
14β		2.94 (overlap)	2.18 (overlap)	2.23 (dd, 17.0, 3.5)	1.91 (overlap)	2.30 (m)	1.54 (t, 13.5)
15α	5.76 (brs)						
16a		9.61 (s)	9.58 (s)	9.57 (s)	9.52 (s)	9.56 (s)	4.42 (2H, s)
16b							
17a	5.50 (s)	6.12 (s)	6.16 (s)	6.15 (s)	6.28 (s)	6.18 (s)	5.42 (s)
17b	5.21 (s)	5.94 (s)	5.89 (s)	5.87 (s)	6.02 (s)	5.90 (s)	5.04 (s)
18	0.79 (3H, s)	1.85 (3H, s)	1.88 (3H, s)	1.51 (3H, s)	1.20 (3H, s)	1.62 (3H, s)	1.35 (3H, s)
19a	4.31 (dd, 11.1)	1.48 (3H, s)	4.84 (dd, 11.0)	4.92 (dd, 10.6)	4.48 (dd, 11.2)	4.94 (dd, 11.2)	1.22 (3H, s)
19b	3.97 (dd, 11.1)		4.70 (dd, 11.0)	4.64 (dd, 10.6)	4.28 (dd, 11.2)	4.75 (dd, 11.2)	
20	1.06 (3H, s)	1.20 (3H, s)	1.15 (3H, s)	1.45 (3H, s)	1.21 (3H, s)	1.50 (3H, s)	1.18 (3H, s)
OAc	1.93 (3H, s)		2.07 (3H, s)	2.05 (3H, s)	2.09 (3H, s)	2.08 (3H, s)	2.04 (3H, s)
					2.05 (3H, s)	2.02 (3H, s)	

[a] Recorded in C_5D_5N. [b] Recorded in $CDCl_3$. [c] Recorded at 400 MHz. [d] Recorded at 500 MHz.

Table 2. ^{13}C NMR Data of Compounds (**1**−**14**) (δ in ppm)

C	**1**[a,c]	**2**[a,d]	**3**[a,d]	**4**[a,c]	**5**[b,c]	**6**[a,d]	**7**[a,d]	**8**[a,d]	**9**[a,d]	**10**[a,c]	**11**[a,c]	**12**[b,c]	**13**[a,d]	**14**[b,c]
1	39.1 CH_2	35.6 CH_2	32.2 CH_2	76.8 CH	32.1 CH_2	72.2 CH	40.1 CH_2	77.7 CH	77.6 CH	64.9 CH	23.5 CH_2	22.6 CH_2	79.7 CH	21.0 CH_2
2	17.9 CH_2	28.1 CH_2	26.2 CH_2	30.0 CH_2	22.5 CH_2	34.6 CH_2	18.5 CH_2	23.8 CH_2	27.1 CH_2	27.4 CH_2	22.6 CH_2	22.2 CH_2	29.6 CH_2	22.5 CH_2
3	36.5 CH_2	77.6 CH	71.0 CH	33.8 CH_2	74.5 CH	74.1 CH	43.7 CH_2	28.7 CH_2	72.5 CH	28.5 CH_2	73.1 CH	72.0 CH	34.8 CH_2	71.8 CH
4	36.8 C	40.6 C	43.2 C	38.3 C	41.3 C	42.1 C	34.5 C	36.6 C	40.9 C	37.8 C	41.1 C	40.8 C	37.9 C	41.9 C
5	53.0 CH	56.2 CH	55.3 CH	58.8 CH	55.7 CH	55.8 CH	59.5 CH	46.1 CH	41.2 CH	54.3 CH	57.5 CH	57.2 CH	53.1 CH	56.6 CH
6	38.4 CH_2	73.9 CH	75.3 CH	74.9 CH	74.2 CH	74.4 CH	76.2 CH	91.4 CH	91.5 CH	73.7 CH	73.9 CH	72.8 CH	75.6 CH	201.9 CH
7	211.5 C	201.1 C	211.4 C	211.2 C	210.9 C	210.8 C	212.1 C	171.0 C	169.6 C	97.9 C	96.5 C	96.4 C	205.9 C	173.7 C
8	60.3 C	127.6 C	47.8 CH	47.8 CH	47.5 CH	47.7 CH	43.6 CH	55.3 C	54.9 C	52.0 C	60.0 C	59.5 C	59.9 C	50.0 C
9	44.6 CH	166.8 C	55.2 CH	56.6 CH	55.1 CH	56.2 CH	56.4 CH	38.5 CH	38.2 CH	36.6 CH	54.1 CH	51.2 CH	47.4 CH	37.9 CH
10	37.1 C	41.3 C	37.6 C	44.0 C	37.4 C	43.8 C	38.1 C	41.0 C	40.7 C	41.1 C	37.3 C	37.2 C	45.8 C	40.1 C
11	28.7 CH_2	24.4 CH_2	26.2 CH_2	29.3 CH_2	25.8 CH_2	29.3 CH_2	68.8 CH	61.1 CH	61.2 CH	15.1 CH_2	65.4 CH	122.5 CH	19.5 CH_2	16.6 CH_2
12	37.3 CH	26.8 CH_2	31.4 CH_2	31.8 CH_2	30.6 CH_2	31.7 CH_2	36.7 CH_2	37.8 CH_2	37.7 CH_2	27.4 CH_2	41.3 CH_2	137.3 CH	33.7 CH_2	31.8 CH_2
13	38.2 CH_2	31.0 CH	34.7 CH	34.8 CH	34.2 CH	34.7 CH	33.6 CH	35.2 CH	35.2 CH	37.2 CH	34.8 CH	37.1 CH	40.1 CH	37.0 CH
14	66.0 CH	28.2 CH_2	31.6 CH_2	31.9 CH_2	31.0 CH_2	31.8 CH_2	32.5 CH_2	31.5 CH_2	30.9 CH_2	32.6 CH_2	27.5 CH_2	31.8 CH_2	35.5 CH_2	32.7 CH_2
15	66.8 CH	153.7 C	154.8 C	154.9 C	153.9 C	154.9 C	154.6 C	202.2 C	202.2 C	75.4 CH	211.3 C	207.4 C	75.5 CH	81.5 CH
16	155.6 C	133.6 CH_2	133.0 CH_2	133.0 CH_2	133.3 CH_2	132.9 CH_2	107.9 CH_2	150.5 C	150.4 C	162.3 C	154.1 C	148.1 C	158.0 C	152.5 C
17	108.8 CH_2	194.6 CH	194.5 CH	194.6 CH	194.4 CH	194.5 CH	64.5 CH_2	118.8 CH_2	118.7 CH_2	107.2 CH_2	115.7 CH_2	117.0 CH_2	105.6 CH_2	110.2 CH_2
18	26.5 CH_3	31.5 CH_3	26.5 CH_3	30.0 CH_3	25.2 CH_3	24.5 CH_3	37.0 CH_3	26.9 CH_3	21.8 CH_3	27.2 CH_3	22.6 CH_3	20.5 CH_3	29.6 CH_3	22.8 CH_3
19	66.7 CH_2	16.3 CH_3	67.5 CH_2	67.0 CH_2	66.0 CH_2	66.4 CH_2	22.1 CH_3	66.9 CH_2	66.7 CH_2	67.1 CH_2	67.0 CH_2	65.6 CH_2	67.2 CH_2	67.6 CH_2
20	14.8 CH_3	20.3 CH_3	16.2 CH_3	11.4 CH_3	16.0 CH_3	11.3 CH_3	17.6 CH_3	61.6 CH_2	61.1 CH_2	66.3 CH_2	69.4 CH_2	67.6 CH_2	15.1 CH_3	68.7 CH_2
OAc	170.8 C		171.1 C	171.1 C	171.2 C	170.9 C	169.9 C	171.0 C	170.9 C	171.0 C	170.7 C	171.0 C	171.0 C	170.1 C
	20.7 CH_3		20.8 CH_3	20.9 CH_3	170.3 C	170.3 C	21.4 CH_3	20.7 CH_3	170.9 C	20.8 CH_3	170.5 C	170.3 C	170.7 C	170.1 C
					21.2 CH_3	21.0 CH_3			21.3 CH_3		21.0 CH_3	21.2 CH_3	21.2 CH_3	169.8 C
					21.0 CH_3	20.7 CH_3			20.6 CH_3		20.6 CH_3	20.9 CH_3	20.7 CH_3	21.0 CH_3
														21.0 CH_3
														20.7 CH_3

[a] Recorded in C_5D_5N. [b] Recorded in $CDCl_3$. [c] Recorded at 100 MHz. [d] Recorded at 125 MHz.

was observed at δ 3.97 (J = 11.1 Hz) and 4.31 (J = 11.1 Hz). The ^{13}C NMR and DEPT spectroscopic data (Table 2), displaying 22 carbon atoms, indicated the presence of an acetyl moiety on a diterpene structure consisting of three methyls, eight methylenes, five methines, and six quaternary carbon atoms. Since species of the genus *Isodon* have yielded numerous *ent*-kaurane diterpenoids,[1] it was considered that compound **1** may possess this same carbon skeleton. Two methyl carbon signals were observed at δ 14.8 and 26.5, corresponding to C-20 and C-18, respectively. In addition, two oxygenated methine carbons were observed at δ 66.0 (C-14) and 66.8 (C-15), and an oxygenated methylene carbon resonated at δ 66.7 (C-19). The ketone carbonyl group (δ 211.5) was placed at C-7. The above assignments were established by a HMBC experiment (Figure 1). The signals at δ 155.6 (C-16) and 106.3 (C-17), typical of an isolated double bond, supported the absence of an α,β-unsaturated ketone moiety in the D ring of **1**. The acetate carbonyl signal was observed at δ 170.8, while its methyl carbon resonated at δ 20.7.

Further analysis of the NMR spectroscopic data of **1** revealed that it differed from an *ent*-kauranoid in the D ring. ^{1}H−^{1}H COSY correlations of H-9 with H-11 and of H-11 with a methine proton (H-12), instead of H-11 with an oxymethine proton, suggested that

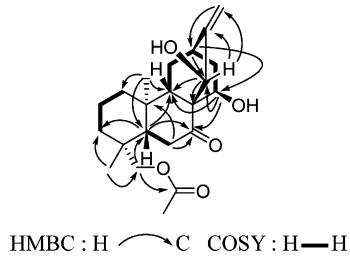

Figure 1. Key HMBC and COSY correlations of **1**.

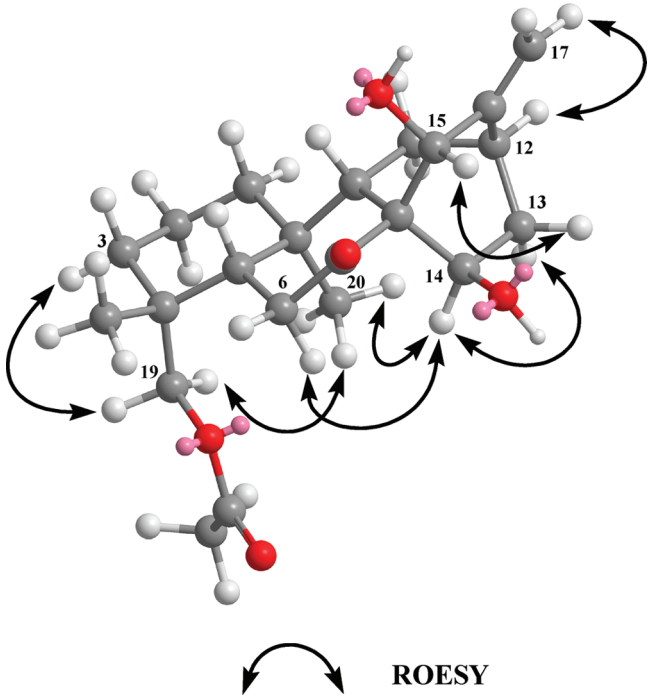

Figure 2. Key ROESY correlations of **1**.

1 possesses a migrated C-12−C-16 bond rather than a C-13−C-16 bond. This deduction explained all correlations in the HMBC spectrum (Figure 1). In addition, two hydroxy groups could be located at C-14 and C-15, respectively, on the basis of the HMBC correlations of H-14 with C-7, C-9, and C-15 and of H-15 with C-8, C-9, C-16, and C-17. Compound **1** was proposed as being based on a rearranged 16(13→12)-*abeo*-*ent*-kaurane skeleton (*ent*-atisane skeleton), a structural type found from only one other species of the genus *Isodon* so far.[34]

Considering that all the kauranoids isolated from the genus *Isodon* possess an *ent*-configuration, **1** was presumed to be an *ent*-atisane diterpenoid. A β-orientation for OH-14 was suggested from the strong ROESY correlations of H-14 with H_3-20, H-6α, and H-13α, as shown in a computer-generated 3D model (Figure 2). The relative configuration of OH-15 was confirmed by the correlation of H-15 with H-13β, as shown in Figure 2. In addition, the acetoxy group at C-19 was further supported by the correlation of H_2-19 with H_3-20. Thus, the structure of **1**, named eriocatisin A, was determined as 19-acetoxy-14β,15β-dihydroxy-7-oxo-*ent*-atis-16-ene.

Eriocasin B (**2**) was isolated as a white, amorphous powder. Its molecular formula was determined as $C_{20}H_{28}O_4$ from the positive HRESIMS quasi-molecular ion peak at *m*/*z* 355.1882 $[M + Na]^+$, indicating seven degrees of unsaturation. From the ^{13}C NMR spectrum (Table 1), a ketone carbon (δ_C 201.1), an aldehyde carbon (δ_C 194.6), three olefinic quaternary carbons (δ_C 166.8, 153.7, 127.6), an olefinic methylene (δ_C 133.6), two oxygenated methines (δ_C 77.6, 73.9), two methines (δ_C 56.2, 31.0), five methylenes (δ_C 35.6, 28.2, 28.1, 26.8, 24.4), two nonoxygenated quaternary carbons (δ_C 41.3, 40.6), and three methyls (δ_C 31.5, 20.3, 16.3) were observed. Compared with the *ent*-kaurane diterpenoids, a characteristic nonoxygenated quaternary carbon (C-8) was absent in the high-field region of the ^{13}C NMR spectrum of **2**. Thus, it was suspected that compound **2** is an *ent*-abietanoid.[22,34−39]

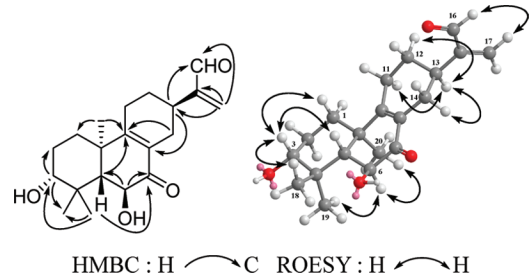

Figure 3. Key HMBC and ROESY correlations of **2**.

The NMR data of **2** were very similar to those of 3α,6β-dihydroxy-7,17-dioxo-*ent*-abieta-15(16)-ene (**15**)[22] (Table 1). The principal differences between these compounds were the absence of two methine signals (C-8 and C-9) for **15** and the appearance of a pair of tetrasubstituted double-bond signals (δ_C 127.6, s and δ_C 166.8, s) in the downfield region of **2**. Also, the carbonyl resonance at δ_C 212.1 (s, C-7) in **15** was shifted upfield to δ_C 201.1 in **2**. These changes suggested that an additional olefinic bond was present between C-8 and C-9 in **2**, which was conjugated with a ketone group. The HMBC correlation of H_3-20 to C-9 (δ_C 166.8, s) was also supporting evidence for the double bond between C-8 and C-9. Compound **2** was assigned the same relative configuration as that of **15** by comparison of their ROESY spectra (Figure 3). The ROESY correlations of H-3β with H-1β and H-5β, of H-6α with H_3-19 and H_3-20, and of H-13α with H-11α and H-14α confirmed that the hydroxy groups at C-3 and C-6 have 3α- and 6β-orientations and the same relative configuration of C-13, respectively. Therefore, the structure of **2** was elucidated as 3α,6β-dihydroxy-7,16-dioxo-*ent*-abieta-8(9),15(17)-diene.

Eriocasin C (**3**) gave a molecular formula of $C_{22}H_{32}O_6$ by HRESIMS. The NMR data indicated that **3** is very similar to **15**. However, an acetyl group in **3** was absent at C-19, as established by the HMBC spectrum. Moreover, on comparing with **15**, the upfield shift of C-1 (δ_C 32.2) and C-5 (δ_C 55.3) suggested a β-orientation of OH-3 in **3** due to the γ-gauche steric compression effect between OH-3 and H-1β and H-5β. This was further supported by the correlations of H-3α with H_3-18 and H_2-19 in the ROESY spectrum of **3**. Hence, compound **3** was assigned as 19-acetoxy-3β,6β-dihydroxy-7,16-dioxo-*ent*-abieta-15(17)-ene.

Eriocasin D (**4**) was assigned the same molecular formula as **3** ($C_{22}H_{32}O_6$). Since its spectroscopic data were very similar to those of **3**, it was presumed to be a functional group isomer in terms of the position of the hydroxy group. The signal at δ_C 16.2 (C-20) in **3** was shifted upfield to δ_C 11.4, suggesting the presence of a C-1α hydroxy group due to the γ-gauche steric compression effect between the OH-1 and CH_3-20 in **4**.[1] Accordingly, **4** was established as 19-acetoxy-1α,6β-dihydroxy-7,16-dioxo-*ent*-abieta-15(17)-ene.

The ^1H and ^{13}C NMR data of **5** were very similar to those of eriocasin C (**3**), and the only observed difference was that **5** possesses an additional acetyl group. This second acetyl group in **5** was assigned unambiguously to C-3β, because the signal at δ_H 4.11 (δ_C 71.0) due to H-3α in **3** was shifted downfield to δ_H 4.97 (δ_C 74.5) in **5**, as confirmed by the HMBC results. The relative configuration of the remaining substituents in **5** was determined to be the same as that in **3** from a ROESY experiment. Thus, 3-acetyleriocasin C (**5**) was established as 6β,19-diacetoxy-6β-hydroxy-7,16-dioxo-*ent*-abieta-15(17)-ene.

3β-Acetoxyeriocasin D (**6**) displayed a $[M + Na]^+$ peak at *m*/*z* 473.2140 in its HRESIMS, corresponding to the molecular formula

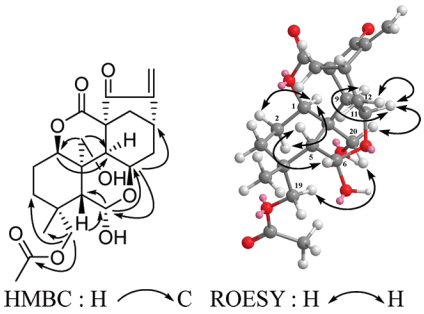

Figure 4. Key HMBC and ROESY correlations of **8**.

$C_{24}H_{34}O_7$, 16 amu more than that of compound **5**. Comparison of its NMR data with those of **5** revealed similarities except for signals of an α-hydroxy group at C-1 of **6**, causing a significant downfield chemical shift of C-2 (δ_C 34.6) and C-10 (δ_C 43.8) and an upfield chemical shift of C-20 (δ_C 11.3) due to the γ-gauche steric compression effect between OH-1 and CH_3-20. HMBC and ROESY experiments further supported the above assignments. Therefore, **6** was determined as 6*β*,19-diacetoxy-1α,6*β*-dihydroxy-7,16-dioxo-*ent*-abieta-15(17)-ene.

The molecular formula of eriocasin E (**7**) was determined as $C_{22}H_{34}O_5$ by HRESIMS in combination with its ^{1}H and ^{13}C NMR data. By comparison with the ^{13}C NMR data of compound **15**, the aldehyde carbon signal (δ_C 194.6) was replaced by an oxygenated methylene signal (δ_C 64.5) in **7**. Also, the olefinic methylene signal at δ_C 133.2 (C-17) in **15** was shifted upfield to δ_C 107.9 in **7**. It was concluded that the α,*β*-unsaturated aldehyde moiety in **15** was changed to an isolated double bond in **7**, as in rubescensins I−M and P.[40] Further evidence came from the HMBC spectrum of **7**, in which long-range ^{1}H−^{13}C correlations between H_2-17 and C-13, C-15, and C-16 were clearly displayed. Other differences were that **7** showed acetyl group signals (δ_C 169.9 and 21.4), and the oxygenated methine signal at δ_C 78.4 in **15** was shifted upfield to δ_C 68.8 in **7**. A HMBC correlation of H-11 (δ_H 5.62) with the acetyl carbonyl group (δ_C 169.9) revealed that the acetoxy group is attached to C-11 in **7**. ROESY correlations of H-11*β* with H-1*β*, H-9*β*, and H-12*β* confirmed the α-orientation of the acetoxy group. Accordingly, compound **7** was identified as 11α-acetoxy-6*β*,16-dihydroxy-7-oxo-*ent*-abieta-15(17)-ene.

The HRESIMS of compound **8** exhibited a molecular ion peak at *m/z* 443.1671 ($[M + Na]^+$), suggesting a molecular formula of $C_{22}H_{28}O_8$, with nine degrees of unsaturation. Its ^{1}H and ^{13}C NMR data indicated a skeleton of 6,7-*seco*-1,7-olide-*ent*-kaurane, similar to nodosin.[26] The most notable difference was that the C-19 methyl (δ_C 23.3) in nodosin was substituted by an acetoxy group in **8**, which was supported by the HMBC spectrum. Moreover, the signals at δ_C 102.1 (d, C-6), 66.1 (d, C-11), and 74.0 (t, C-20) in nodosin were shifted upfield to δ_C 91.4, 61.1, and 61.6, respectively, in **8**. In its HMBC spectrum, H-6 (δ_H 5.84) correlated to C-11 and vice versa, which suggested an oxygen bridge occurring between C-6 and C-11, instead of between C-6 and C-20. Until now, this type of linkage is rare among the natural *ent*-kaurane diterpenoids.[41] The signal of C-20 was in a relatively upfield region in the ^{13}C NMR spectrum, and H-20 (δ_H 5.13 and 4.55) only correlated to C-1, C-9, and C-10 rather than C-6 in the HMBC spectrum. From the molecular formula, C-20 was assumed to be substituted by a hydroxy group. Thus, the planar structure of compound **8** was formulated (Figure 4).

The relative configuration of **8** was determined from the ROESY spectrum (Figure 4). ROESY correlations of H-1/H-2α/H-2*β*, H-6/H-5*β*/H_3-18, and H-11/H-9α/H_2-20 indicated the presence of H-1α, H-6*β*, and H-11α functionalities. The key ROESY correlations and corresponding interatomic distances were depicted on a computer-modeled 3D structure of **8** (Figure 4). The relative configuration and preferred conformation from this procedure were consistent with those of **8** assigned by the ROESY spectrum. Consequently, compound **8** (maoesin A) was determined as 19-acetoxy-6α,20-dihydroxy-6,11*β*-epoxy-15-oxo-6,7-*seco*-*ent*-kaur-16-en-1*β*,7-olide.

The ^{1}H and ^{13}C NMR data of **9** were very similar to those of maoesin A (**8**), but it was evident that **9** possesses an acetoxy group. This group was assigned to C-3α because the methylene signals (δ_H 1.71 and δ_C 28.7) in **8** changed to oxygenated methine signals (δ_H 5.26 and δ_C 72.5) in **9**, which was confirmed by the HMBC spectrum. The acetoxy group was confirmed to have an α-orientation by the ROESY correlation of H-3/H_3-18. The relative configurations of the remaining substituents in **9** were the same as in **8** from the ROESY experiment. Thus, 3α-acetoxymaoesin A (**9**) was elucidated as 3α,19-diacetoxy-6α,20-dihydroxy-6,11*β*-epoxy-15-oxo-6,7-*seco*-*ent*-kaur-16-en-1*β*,7-olide.

Maoesin B (**10**), obtained as a white powder, showed a quasi-molecular ion peak $[M + H]^+$ at *m/z* 409.2215 in the HRFABMS, corresponding to the molecular formula $C_{22}H_{32}O_7$. This was corroborated by the ^{13}C and DEPT NMR spectra, which displayed 22 signals for the carbons of the diterpenoid skeleton substituted by an acetyl group (δ_C 171.0, 20.8). On the basis of the characteristic signals of three methines [δ_C 54.3 (C-5), 37.2 (C-13), and 36.6 (C-9)], three quaternary carbons [δ_C 52.0 (C-8), 41.1 (C-10), and 37.8 (C-4)], a hemiketal quarternary carbon [δ_C 97.9 (C-7)], and an oxygenated methylene [δ_C 66.3, δ_H 4.27 and 4.06 (each 1H, d, J = 9.7 Hz)], it was assumed that **10** is a 7*β*-hydroxy-7α,20-epoxy-*ent*-kauranoid, similar to xerophilusin H.[29] However, the signals at δ_C 162.3 (s) and 107.2 (t) in the ^{13}C NMR spectrum, which are typical of an isolated double bond, suggested the absence of an α,*β*-unsaturated ketone moiety in the D ring. Together with the presence of a proton signal at δ_H 5.23 (1H, brs) and a corresponding carbon signal at δ_C 75.4 (d) in the HSQC spectrum, it was evident that the C-15 ketone group of xerophilusin H was reduced to a hydroxy group in **10**. Comparison of the NMR data of these two compounds revealed them to be very similar except for the above-mentioned differences. The ROESY spectrum revealed the relative configuration of **10** to be the same as xerophilusin H except for the OH-6 orientation, which was found to be α by the correlations of H-6 with H-5 and H_3-18 in **10**. In addition, H-15 displayed an NOE correlation with H-13α, indicating that OH-15 is *β*-oriented. This was also supported by the relative upfield shift of the C-9 signal in **10**, which was caused by the γ-steric compression effect between H-9*β* and OH-15. Thus, compound **10** was established as 19-acetoxy-1*β*,6α,7*β*,15*β*-tetrahydoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Maoesin C (**11**) gave the molecular formula $C_{24}H_{32}O_9$, as determined by HRESIMS. Comparison of the spectroscopic data of **11** with that of maoecrystal J (**16**)[7] revealed similarities except for the occurrence of an additional hydroxy group at C-11, causing a significant downfield chemical shift of C-9 (δ_C 54.1) and C-12 (δ_C 41.3) for **11**. HMBC correlations of δ_H 4.48 (H-11) with δ_C 60.0 (C-8), 54.1 (C-9), and 34.8 (C-13) supported the above assignments. The α-orientation of the OH-11 group was apparent from the ROESY correlations of H-11 with H-1*β*, H-9*β*, and H-12*β*. The other substituents gave the same orientations as those in **16**. Therefore, **11** was elucidated as 3*β*,19-diacetoxy-6*β*,7*β*,11α-trihydoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

On comparing the NMR data of **12** with those of **16**, compound**12** showed evidence of having two more olefinic methines (δ_C 122.5 and 137.3) and two less methylenes (δ_C 16.7 and 29.4) than compound **16**. In its HMBC spectrum, the proton at δ 5.60 (dd, J = 9.4, 2.5 Hz, H-1) correlated to C-8 (δ 59.5, s), C-9 (δ 51.2, s), C-10 (δ 37.2, s), and C-13 (δ 37.1, t), and the proton at δ 6.44 (dd, J = 9.4, 6.6 Hz, H-1) correlated to C-9 and C-13, suggesting that a double bond occurred between C-11 and C-12. The configurations of all chiral centers in **12** were established as being consistent with those of **16** via a ROESY experiment.

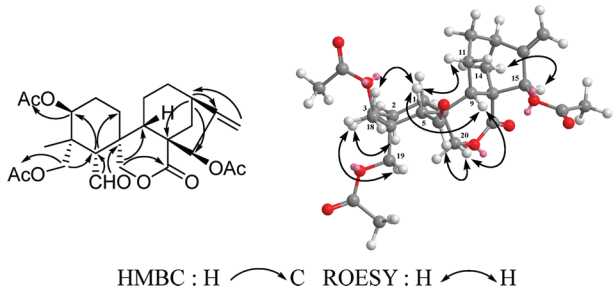

Figure 5. Key HMBC and ROESY correlations of **14**.

Consequently, compound **12** (maoesin D) was identified as 3β,19-diacetoxy-6β,7β,11α-trihydoxy-7α,20-epoxy-*ent*-kaur-11,16-dien-15-one.

Maoesin E (**13**), obtained as a white, amorphous powder, gave the molecular formula $C_{24}H_{34}O_7$ from its HRESIMS (*m*/*z* 457.2215 $[M + Na]^+$), indicating eight degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **13** displayed signals for two singlet methyls, six methylenes (one oxygenated), six methines (three oxygenated), four quaternary carbons (one ketonic carbon), an exocyclic double bond, and two acetyl groups. This was consistent with a skeleton of an *ent*-kaurane diterpenoid.[42]

The correlations in the HMBC spectrum of **13** from H-1 (δ_H 3.74) to C-3 (δ_C 34.8) and C-9 (δ_C 47.4), from H-6 (δ_H 6.01) to C-4 (δ_C 37.9), C-5 (δ_C 53.1), C-7 (δ_C 205.9), C-10 (δ_C 45.8), and the acetoxy group (δ_C 170.7), from H-15 (δ_H 5.51) to C-16 (δ_C 158.0), and from H-19 (δ_H 4.44) to C-3, C-4, C-5, C-18 (δ_C 29.6), and the acetoxy group (δ_C 171.0) helped assign the two hydroxy groups at C-1 and C-15 and the two acetoxy groups at C-6 and C-19, respectively. Similarly, the carbonyl group could be located at C-7 by the correlations of C-7 with H-5, H-6, and H-14. The relative configuration of **13** was established on the basis of ROESY correlations of H-1 with H-5β and H-9β, of H-6 with H_2-19 and H_3-20, and of H-15 with H-14β, which revealed that the substituent groups of C-1, C-6, and C-15 were α-, α-, and β-oriented, respectively. Therefore, compound **13** was assigned as 6β,19-diacetoxy-1α,15β-dihydoxy-*ent*-kaur-16-en-7-one.

Compound **14**, obtained as a white powder, gave a molecular formula of $C_{26}H_{34}O_9$, which was confirmed by HRESIMS (*m*/*z* 513.2100 $[M + Na]^+$). The analysis of ^1H and ^{13}C NMR (DEPT) data revealed that the compound closely resembled 3-acetyltrichorabdal C except for the D ring.[7] The quaternary carbon signal at δ_C 202.6 assigned to a ketone group in the latter compound was replaced by an oxygenated methine carbon signal at δ_C 81.5 in **14**, while signals for an acetoxy group (δ_H 2.22, 3H, s; δ_C 170.1, s and 21.0, q) were observed. The HMBC and ROESY NMR experiment further supported the above assignment (Figure 5). Thus, maoesin F was assigned as 3β,15β,19-triacetoxy-6-oxo-6,7-*seco*-*ent*-kaur-16-en-7,20-olide.

All compounds isolated were evaluated for their in vitro growth inhibitory effects against three human cancer cell lines (HT-29, BEL-7402, and SK-OV-3) using a previously described method (Table 4).[43] None of the compounds isolated showed high cytotoxic potency, but compounds **17**, **18**, and **20** exhibited activity (IC_{50} < 10 μM) for all three cell lines used.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 and a JASCO P-1020 polarimeter, respectively. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer. 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 with 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 (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The aerial parts of *I. eriocalyx* were collected in Luding County, Sichuan Province, People's Republic of China, in September 2007. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 200709126) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. eriocalyx* (10 kg) were extracted four times with 70% aqueous Me_2CO (4 × 35 L, each 3 days) at room temperature and filtered. The filtrate was dried and partitioned with EtOAc (4 × 20 L). The EtOAc partition (520 g) was applied to silica gel (200−300 mesh) column chromatography, eluting with $CHCl_3$−Me_2CO (1:0−0:1 gradient system), to give six fractions, which were decolorized on MCI gel, eluted with 90% MeOH−H_2O, to yield fractions A−F.

Fraction B (250 g), brown gum, was subjected to column chromatography over a silica gel (200−300 mesh) column, eluted with an ether−Me_2CO (1:0−0:1) gradient system, to obtain five fractions, B1−B5. Compound **20** (22.0 g) was precipitated from fraction B2, and coetsin B (93.0 g) was precipitated from fractions B4 and B5, respectively. After repeated column chromatography (silica gel, petroleum ether−Me_2CO, 9:1−2:1 gradient system), fraction B1 afforded *ent*-kaurane-3β,16β-diol (2 mg). Fraction B3 was separated by RP-18 (30%−100% MeOH−H_2O gradient system) and purified by semipreparative HPLC with 50% MeOH−H_2O, to afford compounds **1** (11 mg), **2** (3 mg), **3** (34 mg), **4** (4 mg), **5** (2 mg), **6** (4 mg), **7** (2 mg), **12** (52 mg), **14** (4 mg), **15** (6 mg), **16** (13 mg), **17** (50 mg), **18** (21 mg), **20** (96 mg), **22** (2 mg), 3-acetyltrichorabdal C (4 mg), and rabdosichuanin A (6 mg).

Fraction C (26 g) was separated by RP-18 chromatography (30%−100% MeOH−H_2O gradient system) into eight fractions, C1−C8. Compound **16** (5.2 g) was crystallized from fractions C5−C7. After repeated column chromatography (silica gel, petroleum ether−Me_2CO, 9:1−1:1 gradient system), fraction C1 afforded **19** (2 mg). Fraction C2 was separated by RP-18 (30%−100% MeOH−H_2O gradient system), then was purified by semipreparative HPLC with 45% MeOH−H_2O, to yield compounds **8** (1 mg), **9** (2 mg), nodosin (1 mg), enmin (2 mg), ludongnin F (3 mg), ludongnin G (2 mg), ludongnin H (4 mg), ludongnin I (5 mg), and sculponeatin C (2 mg). In the same way, **9** (2 mg) and xerophilusin H (1 mg) were isolated from fraction C3. Similarly, **13** (4 mg) and **17** (28 mg) were obtained from fractions C4 and C8, respectively.

Fraction D (38 g) was separated by RP-18 chromatography (30%−80% MeOH−H_2O gradient system) into five fractions, D1−D5. Compound **11** (10.0 g) was crystallized from fraction D3, and the mother liquor was passed through a silica gel column, eluted with a gradient system (petroleum ether−2-propanol, 9:1−1:1), to yield compound **20** (25 mg). Fraction E (25 g) was subjected to passage over RP-18 (55% MeOH−H_2O gradient system), then by semipreparative HPLC (45% MeOH−H_2O), to give **10** (100 mg) and **21** (3 mg). By similar means, **17** (17 mg) and **23** (6 mg) were obtained from fraction F (35 g).

Eriocatisin A (1): white, amorphous solid; $[\alpha]_D^{20}$ −41.1 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.55) nm; IR (KBr) ν_{max} 3440, 2932, 2870, 1738, 1694, 1658, 1460, 1445, 1394, 1374, 1305, 1241, 1181, 1080, 1035, 1009, 986, 956, 907, 868, 852, 819, 792, 760, 739, 707, 626, 568, 543 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive EIMS *m*/*z* 376 $[M]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 399.2138 (calcd for $C_{22}H_{34}O_5Na$ $[M + Na]^+$, 399.2147).

Eriocasin B (2): white, amorphous solid; $[\alpha]_D^{27}$ −16.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.40), 216 (3.57) nm; IR (KBr) ν_{max} 3450, 3426, 2934, 2873, 1721, 1690, 1656, 1625, 1461, 1379, 1272, 1254, 1175, 1123, 1101, 1079, 1061, 1036, 1013, 970, 927, 670 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 355 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m*/*z* 355.1882 (calcd for $C_{20}H_{28}O_4Na$ $[M + Na]^+$, 355.1885).

Eriocasin C (3): white, amorphous solid; $[\alpha]_D^{26}$ −8.9 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.54) nm; IR (KBr) ν_{max} 3540, 3466,

Table 3. ^{1}H NMR Data of Compounds **8**−**14** (δ in ppm, J in Hz)

H	**8**[a,c]	**9**[a,c]	**10**[a,d]	**11**[a,d]	**12**[b,c]	**13**[a,c]	**14**[b,c]
1α	5.35 (overlapped)	5.41 (brs)	3.76 (brs)	1.96 (overlap)	1.57 (dt, 16.0, 7.0)		1.58 (overlap)
1β				2.09 (overlap)	1.31 (brd, 16.0)	3.74 (m)	1.83 (overlap)
2α	2.30 (m)	2.66 (overlap)	1.85 (overlap)	1.83 (overlap)	1.76 (overlap)	1.99 (m)	1.91 (overlap)
2β	1.80 (m)	2.66 (overlap)	2.17 (overlap)	1.94 (overlap)	1.76 (overlap)	1.76 (overlap)	1.86 (overlap)
3α	1.71 (overlap)		1.74 (overlap)	5.42 (brs)	5.02 (brs)	1.17 (overlap)	5.02 (brs)
3β	1.71 (overlap)	5.26 (brs)	2.14 (overlap)			1.76 (overlap)	
5β	2.14 (brs)	2.64 (overlapped)	2.50 (d, 5.1)	2.25 (d, 7.1)	1.77 (overlap)	1.86 (d, 13.5)	2.95 (d, 3.2)
6α				4.53 (t, 7.1)	3.86 (overlap)	6.01 (d, 13.5)	9.92 (d, 3.2)
6β	5.84 (brs)	5.90 (brs)	4.45 (d, 5.4)				
9α	3.25 (d, 3.4)	3.35 (d, 3.2)					
9β			3.28 (m)	1.87 (overlap)	2.22 (d, 2.4)	2.74 (brd, 7.4)	2.49 (t, 6.0)
11α	4.87 (overlap)	4.90 (brs)	1.71 (overlap)		5.60 (dd, 9.4, 2.5)	2.34 (m)	1.55 (overlap)
11β			2.07 (m)	4.48 (d, 4.0)		3.34 (brd, 11.5)	1.55 (overlap)
12α	1.61 (dd, 7.2, 2.6)	1.60 (dd, 14.6, 5.8)	1.96 (overlap)	2.49 (dd, 15.0, 9.7)	6.44 (dd, 9.4, 6.6)	1.58 (overlap)	2.08 (overlap)
12β	2.21 (t, 7.2)	2.23 (dd, 14.6, 8.4)	1.84 (overlap)	1.61 (dd, 15.0, 4.7)		1.87 (overlap)	1.51 (overlap)
13α			2.66 (m)	3.11 (m)	3.44 (m)	2.69 (brs)	2.79 (m)
13β	3.01 (m)	3.02 (m)					
14α	2.24 (dd, 12.6, 2.1)	2.68 (overlap)	1.56 (m)	3.62 (d, 11.6)	1.77 (overlap)	2.13 (d, 11.6)	2.06 (overlap)
14β	3.23 (d, 12.6)	3.27 (d, 11.8)	2.17 (overlap)	2.60 (dd, 11.6, 4.0)	2.49 (dd, 14.5, 5.0)	1.60 (overlap)	1.85 (overlap)
15α			5.23 (brs)			5.51 (brs)	5.74 (brs)
17a	6.02 (brs)	6.00 (brs)	5.48 (brs)	5.92 (s)	5.94 (brs)	5.46 (brs)	5.13 (brs)
17b	5.34 (brs)	5.34 (brs)	5.17 (brs)	5.26 (s)	5.35 (brs)	5.08 (brs)	4.99 (brs)
18	1.05 (3H, s)	1.26 (3H, s)	1.34 (3H, s)	1.52 (3H, s)	1.15 (3H, s)	1.19 (3H, s)	1.21 (3H, s)
19a	4.86 (overlap)	5.28 (dd, 11.6)	4.83 (dd, 11.1)	4.72 (dd, 11.6)	4.38 (dd, 11.8)	4.46 (dd, 11.1)	4.16 (dd, 11.6)
19b	4.85 (overlap)	4.72 (dd, 11.6)	4.52 (dd, 11.1)	4.68 (dd, 11.6)	4.28 (dd, 11.8)	4.42 (dd, 11.1)	4.13 (dd, 11.6)
20a	5.13 (dd, 11.0)	5.16 (dd, 11.1)	4.27 (dd, 9.7)	5.38 (dd, 9.2)	3.84 (dd, 10.4)	1.75 (3H, s)	4.48 (dd, 10.8)
20b	4.55 (dd, 11.0)	4.13 (dd, 11.1)	4.06 (dd, 9.7)	4.23 (dd, 9.2)	3.74 (dd, 10.4)		4.42 (dd, 10.8)
OAc	1.94 (3H, s)	2.28 (3H, s)	2.05 (3H, s)	2.12 (3H, s)	2.10 (3H, s)	2.21 (3H, s)	2.22 (3H, s)
		1.94 (3H, s)		1.90 (3H, s)	2.09 (3H, s)	2.06 (3H, s)	2.11 (3H, s)
							2.06 (3H, s)

[a] Recorded in C_5D_5N. [b] Recorded in $CDCl_3$. [c] Recorded at 500 MHz. [d] Recorded at 400 MHz.

Table 4. IC_{50} Values (μM) of Diterpenoids from *I. eriocalyx* for Human Tumor Cell Lines[a]

compound	HT-29	BEL-7402	SK-OV-3
4	>10	>10	5.7
6	>10	>10	5.1
12	7.3	>10	>10
15	8.1	>10	7.3
16	4.2	>10	9.8
17	2.1	2.9	3.3
18	4.1	7.3	2.1
19	5.9	>10	9.1
20	2.4	4.0	3.0
21	9.8	>10	4.7
22	6.0	>10	5.9
23	>10	6.0	7.0
ADR[b]	0.092	0.067	0.17

[a] Compounds **1**−**3**, **5**, **7**−**11**, **13**, **14**, and other known compounds were inactive (IC_{50} > 10 μM) for all cell lines. [b] Adriamycin was used a positive control.

2926, 2856, 1721, 1707, 1685, 1675, 1644, 1629, 1455, 1441, 1395, 1377, 1307, 1258, 1226, 1178, 1120, 1080, 1062, 1041, 1028, 993, 959, 940, 909, 890, 861, 637, 448 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 415 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 415.2092 (calcd for $C_{24}H_{34}O_7Na$ [M + Na]$^+$, 415.2096).

Eriocasin D (4): white, amorphous solid; $[\alpha]_D^{18}$ −10.2 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 217 (3.56) nm; IR (KBr) ν_{max} 3446, 2932, 2867, 1735, 1692, 1657, 1628, 1459, 1446, 1393, 1375, 1248, 1176, 1129, 1115, 1093, 1064, 1037, 1005, 989, 962, 917, 888, 853, 831, 636, 606 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 415 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 415.2047 (calcd for $C_{22}H_{32}O_6Na$ [M + Na]$^+$, 415.2096).

3-Acetyleriocasin C (5): white, amorphous solid; $[\alpha]_D^{26}$ −1.7 (c 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 280 (3.02), 239 (2.98) nm; IR (KBr) ν_{max} 3448, 2938, 1738, 1643, 1461, 1378, 1243, 1184, 1114, 1065, 1035, 998, 925, 905, 846, 755, 682, 666, 639, 605, 581 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 457 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 457.2199 (calcd for $C_{24}H_{34}O_7Na$ [M + Na]$^+$, 457.2202).

3β-Acetoxyeriocasin D (6): white, amorphous solid; $[\alpha]_D^{26}$ 0 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.51) nm; IR (KBr) ν_{max} 3440, 2926, 2869, 2855, 1738, 1710, 1690, 1639, 1630, 1378, 1246, 1168, 1111, 1038, 995, 915, 603 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 473 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 473.2140 (calcd for $C_{24}H_{34}O_7Na$ [M + Na]$^+$, 473.2151).

Eriocasin E (7): white, amorphous solid; $[\alpha]_D^{23}$ −31.9 (c 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.28) nm; IR (KBr) ν_{max} 3442, 2927, 2867, 1735, 1703, 1692, 1639, 1630, 1461, 1441, 1390, 1372, 1258, 1240, 1174, 1149, 1128, 1103, 1084, 1058, 1036, 1018, 937, 902, 604, 578, 563 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 401 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 401.2308 (calcd for $C_{22}H_{34}O_5Na$ [M + Na]$^+$, 401.2303).

Maoesin A (8): white powder; $[\alpha]_D^{25}$ +17.5 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.55), 203 (3.53) nm; IR (KBr) ν_{max} 3433, 2929, 1738, 1711, 1640, 1460, 1453, 1433, 1372, 1246, 1198, 1149, 1123, 1037, 1007, 978, 935, 919, 874, 850, 670, 626, 607 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS m/z 443 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 443.1671 (calcd for $C_{22}H_{28}O_8Na$ [M + Na]$^+$, 443.1681).

3α-Acetoxymaoesin A (9): white powder; $[\alpha]_D^{26}$ +52.4 (c 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.62), 203 (3.54) nm; IR (KBr) ν_{max} 3435, 2927, 2854, 1738, 1713, 1639, 1550, 1460, 1437, 1378, 1245, 1148, 1124, 1059, 1007, 965, 934, 916, 850, 823, 794, 685, 670, 639, 606, 587, 559 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS m/z 501 [M + Na]$^+$; positive HRESIMS [M + Na]$^+$ m/z 501.1719 (calcd for $C_{22}H_{28}O_8Na$ [M + Na]$^+$, 501.1736).

Maoesin B (10): amorphous solid; $[\alpha]_D^{14}$ −14.0 (c 0.68, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.23) nm; IR (KBr) ν_{max} 3440, 2956, 2932, 2863, 1720, 1660, 1629, 1450, 1430, 1395, 1376, 1343, 1253, 1189, 1146, 1080, 1032, 970, 946, 907, 890, 877, 767, 661 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive FABMS m/z 409 [M + H]$^+$, 817 [2 M + H]$^+$, 391 [M − H_2O + H]$^+$; positive HRFABMS [M + Na]$^+$ m/z 409.2215 (calcd for $C_{22}H_{33}O_7$ [M + H]$^+$, 457.2226).

Maoesin C (11): colorless crystal; $[\alpha]_D^{27}$ −76.2 (c 0.16, acetone); UV (MeOH) λ_{max} (log ε) 238 (3.58) nm; IR (KBr) ν_{max} 3483, 3356, 3215, 2977, 2964, 2947, 2923, 2878, 1729, 1641, 1473, 1458, 1424, 1395, 1371, 1323, 1298, 1247, 1213, 1187, 1169, 1084, 1064, 1050, 1031, 985, 961, 938, 920, 908, 950, 686, 670, 646, 621, 609, 569, 534, 487, 468 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive

ESIMS *m/z* 487 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 487.1957 (calcd for $C_{24}H_{32}O_9Na$ $[M + Na]^+$, 487.1944).

Maoesin D (12): white powder; $[\alpha]_D^{26}$ −131.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 235 (3.38), 203 (3.51) nm; IR (KBr) ν_{max} 3387, 2942, 2866, 1739, 1640, 1450, 1379, 1248, 1191, 1079, 1064, 1027, 982, 944, 914, 884, 750, 688, 646, 604, 578, 535 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 487 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 487.1957 (calcd for $C_{24}H_{32}O_9Na$ $[M + Na]^+$, 487.1944).

Maoesin E (13): white, amorphous powder; $[\alpha]_D^{22}$ −60.4 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.58) nm; IR (KBr) ν_{max} 3441, 2934, 2874, 1739, 1719, 1336, 1458, 1444, 1396, 1376, 1242, 1114, 1084, 1034, 1000, 978, 902, 605 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; positive ESIMS *m/z* 457 $[M + Na]^+$, 891 $[2\ M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 457.2215 (calcd for $C_{24}H_{34}O_7Na$ $[M + Na]^+$, 457.2202).

Maoesin F (14): white, amorphous powder; $[\alpha]_D^{26}$ +22.9 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 241 (2.74) nm; IR (KBr) ν_{max} 3440, 2940, 1745, 1640, 1459, 1377, 1232, 1098, 1048, 997, 902, 754, 669, 604 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 3; positive ESIMS *m/z* 513 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 513.2100 (calcd for $C_{26}H_{34}O_9Na$ $[M + Na]^+$, 513.2100).

Cytotoxicity Bioassay. The cytotoxicity of all compounds isolated was tested against colon carcinoma (HT-29), hepatic carcinoma (BEL-7402), and ovarian (SK-OV-3) human cell lines using a method described in the literature,[43] with adriamycin as the positive control. The HT-29 and SK-OV-3 cell lines were obtained from American Type Culture Collection (ATCC) and were grown in McCoy's 5a medium (Gibco BRL, New York, NY) supplemented with 10% fetal bovine serum (FBS, HyClone). BEL-7402 cells, obtained from Shanghai Cell Bank, Chinese Academy of Sciences, were maintained in PRMI 1640 medium (Gibco BRL) with 10% FBS. Cells were plated in 96-well plates for 24 h, then treated in two replicates at five concentrations of tested compounds or positive control (ADR), and incubated for 72 h at 37 °C in a 5% CO_2 atmosphere. Next, cells were fixed with 10% precooled trichloroacetic acid for 1 h at 4 °C and stained for 15 min at room temperature with 100 μL of 4 mg/mL sulforhodamine B (SRB) solution (Sigma) in 1% AcOH. SRB was then removed and cells were quickly rinsed five times with 1% AcOH. After air-drying, protein-bound dye was dissolved in 150 μL of 10 mM Tris base for 5 min and was measured at 510 nm using an ELISA reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA). In these experiments, the negative reference agents were isochoric normal saline. The inhibition rate for cell proliferation was calculated as $(OD_{control} - OD_{treated})/OD_{control} - OD_{treated})/OD_{control} \times 100\%$. Results are expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) in μM.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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