ent-Kaurane Diterpenoids from *Isodon scoparius*

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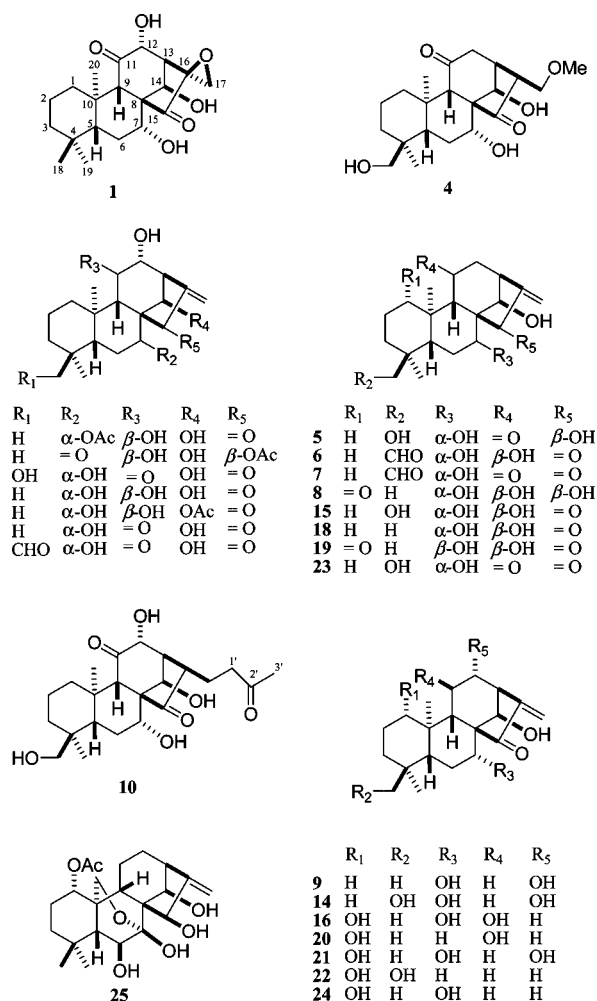
Nine new *ent*-kaurane diterpenoids, isoscoparins D−L (**1**−**9**), and an artificial product, the acetonide of rabdoloxin A (**10**), along with 16 known analogues (**11**−**26**), were isolated from the leaves of *Isodon scoparius.* The new structures were determined by 1D and 2D NMR spectroscopic analysis. Selected compounds were evaluated for their cytotoxicity against NB4, A549, PC-3, MCF-7, and SH-SY5Y cell lines.

ent-Kaurane diterpenoids, with diverse structures and promising cytotoxic activities for cancer cells in culture, have attracted considerable attention by natural product chemists.[1,2] More than 50 *Isodon* species have been investigated phytochemically, and a large number of new *ent*-kauranoids have been found by our group over the past 30 years.[2] Among these *ent*-kaurane diterpenoids, eriocalyxin B was found to induce apoptosis of murine t(8;21) leukemia cells effectively through modulation of the NF-κB, acute myeloid leukemia AML1-ETO, and mitogen-activated protein kinase (MAPK) pathways and may be a potential apoptosis inducer for treatment of murine t(8;21) leukemia.[3] *Isodon scoparius* (C. Y. Wu et H. W. Li) H. Hara (Lamiaceae), a dwarf shrub growing in the rocky mountains in the northwest district of Yunnan Province, People's Republic of China, has been used as an antipyretic agent by local inhabitants.[4] Previous phytochemical investigation of this herb resulted in three *ent*-clerodane diterpenoids, isoscoparins A−C, and an *ent*-labdane, (13*E*)-*ent*-labda-7,13-dien-15-oic acid.[5] In our continuing research program with the aim of discovering new diterpenoids with diverse structures and bioactivities, we have reinvestigated the aerial parts of *I. scoparius*, collected in Shangrila, Yunnan Province, and isolated 26 compounds, including nine new *ent*-kaurane diterpenoids, isoscoparins D−L (**1**−**9**), and a artificial product, the acetonide of rabdoloxin A (**10**), along with 16 known analogues (**11**−**26**). In this paper, we present the isolation and structure elucidation of these new compounds and the cytotoxicity evaluation of selected compounds isolated.

Results and Discussion

The 70% aqueous acetone extract of the air-dried and powdered aerial parts of *I. scoparius* was partitioned between EtOAc and H₂O to afford an EtOAc extract, which was subjected to silica gel column chromatography using a CHCl₃−Me₂CO mixture as eluent. Further purification by repeated normal column chromatography and semipreparative HPLC yielded nine new *ent*-kaurane diterpenoids, isoscoparins D−L (**1**−**9**), and a artificial product, the acetonide of rabdoloxin A (**10**). Also isolated were 16 known constituents, namely, rabdoloxin A (**11**),[6] rabdoloxin B (**12**),[6] rabdokunmin A (**13**),[7] rabdokunmin C (**14**),[7] rabdokunmin D (**15**),[7] rabdoinflexin B (**16**),[8] phyllostachysin D (**17**),[9] phyllostachysin F (**18**),[9] phyllostachysin G (**19**),[9] phyllostachysin H (**20**),[9] excisanin A (**21**),[10] excisanin K (**22**),[11] henryine A (**23**),[12] kamebanin (**24**),[13] lasiokau-

	R₁	R₂	R₃	R₄	R₅
2	H	α-OAc	β-OH	OH	=O
3	H	α-OH	β-OH	OH	β-OAc
11	OH	α-OH	=O	OH	=O
12	H	α-OH	β-OH	OH	=O
13	H	α-OH	β-OH	OAc	=O
17	H	α-OH	=O	OH	=O
26	CHO	α-OH	=O	OH	=O

	R₁	R₂	R₃	R₄	R₅
5	H	OH	α-OH	=O	β-OH
6	H	CHO	α-OH	β-OH	=O
7	H	CHO	α-OH	β-OH	=O
8	=O	H	α-OH	β-OH	β-OH
15	H	OH	α-OH	β-OH	=O
18	H	H	α-OH	β-OH	=O
19	=O	H	β-OH	β-OH	=O
23	H	OH	α-OH	=O	=O

	R₁	R₂	R₃	R₄	R₅
9	H	H	H	H	OH
14	H	OH	OH	H	OH
16	OH	H	OH	OH	H
20	OH	H	H	OH	H
21	OH	H	OH	H	OH
22	OH	OH	H	H	H
24	OH	H	OH	H	H

rinol (**25**),[14] and macrocalyxin E (**26**).[15] The structures of the known compounds were determined by spectroscopic data comparison with literature values.

Isoscoparin D (**1**), obtained as a white, amorphous powder, gave the molecular formula C₂₀H₂₈O₆ from its HRESIMS (*m/z* 387.1772 [M + Na]⁺, calcd 387.1783), indicating seven degrees of unsaturation. IR absorptions at 3426, and 1750 and 1709 cm⁻¹ implied the presence of hydroxyl and carbonyl groups, respectively. The ¹³C NMR and DEPT spectra of **1** displayed 20 carbon signals corresponding to three methyls, five methylenes (of which one was oxygenated), six methines (including three oxygenated methines),

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Table 1. ^{1}H NMR Data of Isoscoparins D−H (**1**−**5**) (C_5D_5N, 500 MHz, δ in ppm, J in Hz)

H	**1**[b]	**2**[a]	**3**[b]	**4**[a]	**5**[a]
1α	1.60 (1H, s)	1.93 (1H, overlap)	2.70 (1H, m)	1.80 (1H, overlap)	1.93 (1H, overlap)
1β	1.13 (1H, m)	0.99 (1H, overlap)	2.29 (1H, m)	1.20 (1H, m)	1.42 (1H, m)
2α	1.57 (1H, m)	1.57 (1H, overlap)	1.51 (1H, m)	1.41 (1H, m)	1.43 (1H, overlap)
2β	1.41 (1H, overlap)	1.25 (1H, overlap)	1.71 (1H, m)	1.56 (1H, m)	1.60 (1H, m)
3α	1.22 (1H, m)	0.99 (1H, overlap)	1.25 (1H, m)	1.28 (1H, d, 12.9)	1.25 (1H, br d, 12.9)
3β	1.41 (1H, overlap)	1.25 (1H, overlap)	1.45 (1H, m)	1.78 (1H, overlap)	1.79 (1H, td, 16.3, 5.0)
5β	1.08 (1H, dd, 12.3, 1.7)	1.08 (1H, br d, 12.2)	1.23 (1H, m)	1.85 (1H, d, 12.0)	1.93 (1H, d, 12.5)
6α	1.90 (1H, m)	1.82 (1H, m)	1.97 (1H, br d, 16.3)	2.08 (1H, m)	2.18 (1H, q, 15.3)
6β	2.03 (1H, m)	2.17 (1H, m)	1.18 (1H, m)	2.41 (1H, br d, 12.0)	2.48 (1H, dd, 15.3, 4.1)
7β	4.24 (1H, m)	6.00 (1H, dd, 11.9, 3.2)		4.79 (1H, br d, 11.6)	4.59 (1H, m)
9β	1.76 (1H, s)	2.04 (1H, br s)	2.25 (1H, br s)	2.09 (1H, s)	3.03 (1H, s)
11α					
11β		4.40 (1H, s)	3.90 (1H, d, 5.6)		
12α				2.91 (1H, br d, 20.0)	2.83 (1H, dd, 19.4, 3.7)
12β	3.90 (1H, br s)	4.79 (1H, br s)	4.00 (1H, t, 3.2)	2.78 (1H, dd, 20.0, 4.0)	2.67 (1H, dd, 19.4, 7.1)
13α	2.60 (1H, m)	3.79 (1H, br s)	2.75 (1H, d, 3.8)	3.08 (1H, br s)	3.19 (1H, s)
14α	5.68 (1H, s)	5.85 (1H, br s)	5.44 (1H, br d, 7.1)	5.75 (1H, s)	5.48 (1H, s)
15α			5.80 (1H, s)		6.00 (1H, d, 2.9)
16α				3.72 (1H, m)	
17a	3.14 (1H, d, 6.8)	6.34 (1H, s)	5.25 (1H, s)	3.80 (1H, dd, 9.7, 4.7)	5.66 (1H, s)
17b	3.08 (1H, d, 6.8)	5.49 (1H, s)	5.15 (1H, s)	3.55 (1H, t, 9.7)	5.33 (1H,s)
18a	0.93 (3H, s)	0.82 (3H, s)	0.85 (3H, s)	3.61 (1H, d, 10.6)	3.63 (1H, dd, 10.5, 5.4)
18b				3.25 (1H, d, 10.6)	3.27 (1H, dd, 10.5, 4.9)
19	0.90 (3H, s)	0.75 (3H, s)	0.87 (3H, s)	0.82 (3H, s)	0.86 (3H, s)
20	1.39 (3H, s)	1.58 (3H, s)	1.48 (3H, s)	1.18 (3H, s)	1.21 (3H, s)
OAc		1.93 (3H, s)	1.86 (3H, s)		
OMe				3.14 (3H, s)	3.14 (3H, s)

[a] Recorded in C_5D_5N. [b] Recorded in $(CD_3)_2CO$.

Table 2. ^{1}H NMR Data of Isoscoparins I−L (**6**−**9**) and Acetonide of Rabdoloxin A (**10**) (500 MHz, δ in ppm, J in Hz)

H	**6**[a]	**7**[a]	**8**[a]	**9**[b]	**10**[a]
1α	1.68 (1H, br d, 12.9)	1.65 (1H, overlap)		1.40 (1H, overlap)	1.91 (1H, overlap)
1β	0.94 (1H, td, 13.1, 3.4)	1.16 (1H, overlap)		1.15 (1H, m)	1.31 (1H, overlap)
2α	1.54 (1H, br d, 13.5)	1.40−1.49 (2H, m)	2.16 (1H, m)	1.39 (1H, overlap)	1.56 (1H, m)
2β	1.53 (1H, m)		2.89 (1H, m)	1.65 (1H, overlap)	1.40 (1H, m)
3α	1.06 (1H, overlap)	1.16 (1H, m)	1.63 (1H, m)	0.69 (1H, td, 13.5, 3.2)	1.31 (1H, overlap)
3β	1.24 (1H, td, 13.2, 4.3)	1.27 (1H, td, 16.3, 5.2)	1.46 (1H, td, 12.8, 5.0)	1.64 (1H, overlap)	1.80 (1H, m)
5β	1.61 (1H, d, 12.5)	1.64 (1H, td, 12.8)	1.36 (1H, overlap)	0.93 (1H, br d, 12.3)	1.92 (1H, overlap)
6α	2.09 (1H, q, 12.5)	2.18 (1H, q, 15.7)	2.10 (2H, overlap)	1.67 (1H, m)	2.17 (1H, m)
6β	1.68 (1H, br d, 12.5)	1.68 (1H, br d, 15.7)		1.94 (1H, dd, 12.7, 4.4)	2.46 (1H, m)
7β	4.92 (1H, br d, 12.0)	4.83 (1H, dd, 11.9, 3.9)	4.20 (1H, m)	4.27 (1H, m)	4.87 (1H, dd, 11.8, 3.3)
9β	2.00 (1H, s)	2.12 (1H, s)	2.94 (1H, s)	1.27 (1H, overlap)	2.32 (1H, br s)
11α	4.20 (1H, d, 3.5)		4.29 (1H, d, 4.8)	1.49 (1H, m)	
11β				1.66 (1H, overlap)	
12α	2.35 (1H, dd, 14.4, 3.2)	2.95 (1H, dd, 16.3, 3.5)	2.24 (1H, dd, 14.1, 3.3)	3.99 (1H, dd, 8.7, 4.6)	
12β	2.23 (1H, m)	2.69 (1H, m)	2.10 (1H,m)		4.44 (1H, br s)
13α	3.30 (1H, s)	3.42 (1H, s)	2.96 (1H, s)	3.00 (1H, d, 3.8)	3.25 (1H, br s)
14α	5.18 (1H, s)	5.61 (1H, s)	5.87 (1H, s)	5.16 (1H, d, 2.3)	6.35 (1H, s)
15α			4.87 (1H, s)		
16α					3.44 (1H, q, 7.3)
17a	6.29 (1H, s)	6.33 (1H, s)	5.66 (1H,s)	5.95 (1H, s)	2.21 (1H, m)
17b	5.41 (1H, s)	5.52 (1H, s)	5.37 (1H, s)	5.32 (1H, s)	1.71 (1H, overlap)
18a	9.21 (1H, s)	9.25 (1H, s)	0.72 (3H, s)	0.91 (3H, s)	3.65 (1H, br d, 10.6)
18b					3.28 (1H, br d, 10.6)
19	1.08 (3H, s)	1.10 (3H, s)	0.95 (3H, s)	0.88 (3H, s)	0.93 (3H, s)
20	1.02 (3H, s)	1.47 (3H, s)	1.34 (3H, s)	1.31 (3H, s)	1.07 (3H, s)
1′					2.54−2.69 (2H, m)
3′					1.92 (3H, s)
OMe					

[a] Recorded in C_5D_5N. [b] Recorded in $(CD_3)_2CO$.

and six quaternary carbons (including one oxygenated and two carbonyls) ·(Table 3). This was consistent with a skeleton of an *ent*-kaurane diterpenoid.[6]

Except for two carbonyls, the presence of five rings was necessary to meet the number of degrees of unsaturation required. Since four rings of an *ent*-kaurane diterpenoid accounted for four degrees of unsaturation, another ring was present. Two oxygenated methylene protons at δ_H 3.14 (1H, d, J = 6.8 Hz) and 3.08 (1H, d, J = 6.8 Hz), along with an oxygenated quaternary carbon at δ_C 61.6, suggested the presence of an epoxy ring between C-16 and C-17. This was supported by the HMBC correlations from H-13 (δ_H 2.60), H-14 (δ_H 5.68), and H$_2$-17 (δ_H 3.08 and 3.14) to C-16 (δ_C 61.6) and from H$_2$-17 (δ_H 3.08 and 3.14) to C-13 (δ_C 48.7), C-14 (δ_C 70.1), C-15 (δ_C 214.2), and C-16 (δ_C 61.6). In addition,

the correlations in the HMBC spectrum from H-7 (δ_H 4.24) to C-5 (δ_C 53.2), C-8 (δ_C 60.1), and C-14 (δ_C 70.1), from H-9 (δ_H 1.76), H-13 (δ_H 2.60), and H-14 (δ_H 5.68) to C-12 (δ_C 74.5), and from H-14 (δ_H 5.68) to C-7 (δ_C 74.0), C-9 (δ_C 69.2), and C-16 (δ_C 61.6) helped in assigning three oxygenated methines at C-7, C-12, and C-14, respectively. Similarly, two carbonyl groups could be located at C-11 and C-15 (Figure 1).

The relative configuration of **1** was established on the basis of ROESY correlations of H-7 with H-5β and H-9β, of H-14 with H-6α and H$_3$-20, and of H-17a with H-13α, which revealed that the substituent groups of C-7 and C-14 and the epoxy ring (C-16 and C-17) were α-, β-, and β-oriented, respectively. Since correlations were not observed between H-17a and H-12β in the ROESY experiment, this confirmed that the epoxy ring is β-configured. The

Table 3. ^{13}C NMR Data of Isoscoparins D−L (**1**−**9**) and Acetonide of Rabdoloxin A (**10**) (125 MHz, δ in ppm)

carbon	**1**[b]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[b]	**10**[a]
1	39.9 t	39.3 t	38.7 t	39.4 t	40.5 t	38.5 t	38.9 t	215.6 s	40.1 t	39.7 t
2	18.8 t	18.6 t	18.8 t	18.2 t	18.4 t	17.3 t	17.1 t	35.6 t	19.0 t	18.2 t
3	41.8 t	41.5 t	40.0 t	35.2 t	35.5 t	32.0 t	31.9 t	41.9 t	42.3 t	35.3 t
4	34.0 s	33.3 s	33.9 s	40.0 s	38.2 s	49.7 s	49.7 s	33.1 s	33.7 s	38.3 s
5	53.2 d	53.2 d	53.2 d	46.6 d	46.5 d	45.5 d	44.9 d	54.0 d	53.7 d	46.7 d
6	29.3 t	25.4 t	42.3 t	29.4 t	30.3 t	32.3 t	31.8 t	30.6 t	29.8 t	29.6 t
7	74.0 d	77.0 d	208.1 s	73.4 d	74.5 d	73.8 d	74.6 d	74.6 d	75.1 d	73.9 d
8	60.1 s	60.8 s	64.0 s	61.3 s	53.6 s	60.2 s	60.8 s	53.7 s	61.6 s	60.5 s
9	69.2 d	68.0 d	59.9 d	68.6 d	63.2 d	65.8 d	68.0 d	50.6 d	57.4 d	70.2 d
10	41.6 s	39.2 s	38.5 s	38.2 s	37.5 s	37.5 s	38.6 s	52.8 s	39.2 s	41.0 s
11	207.0 s	70.5 d	70.0 d	208.3 s	209.1 s	64.1 d	206.9 s	67.3 d	26.4 t	209.2 s
12	74.5 d	79.2 d	79.7 d	45.1 t	52.3 t	40.7 t	50.1 t	41.4 t	72.8 d	74.1 d
13	48.7 d	54.5 d	53.2 d	40.4 d	48.5 d	46.1 d	45.4 d	49.0 d	55.3 d	48.4 d
14	70.1 d	70.8 d	74.6 d	74.9 d	73.7 d	76.0 d	72.1 d	74.9 d	71.1 d	71.5 d
15	214.2 s	206.4 s	75.1 d	217.1 s	76.4 d	206.5 s	204.8 s	77.3 d	208.1 s	220.2 s
16	61.6 s	146.7 s	151.9 s	50.8 d	157.4 s	150.9 s	148.2 s	158.7 s	147.8 s	47.5 s
17	54.9 t	116.4 t	110.7 t	68.8 t	110.6 t	113.9 t	120.6 t	108.5 t	116.8 t	20.3 t
18	33.7 q	33.4 q	33.0 q	71.0 t	71.2 t	205.7 d	205.3 d	31.8 q	33.8 q	71.2 t
19	21.9 q	21.7 q	21.1 q	18.1 q	18.3 q	14.2 q	14.3 q	22.8 q	21.9 q	18.1 q
20	18.4 q	17.4 q	16.1 q	19.5 q	19.7 q	18.1 q	19.2 q	17.5 q	16.4 q	18.9 q
1′										41.6 t
2′										207.2 s
3′										29.6 q
OAc		169.4 s	171.0 s							
OMe		21.1 q	20.8 q	58.5 q						

^{a} Recorded in C_5D_5N. ^{b} Recorded in $(CD_3)_2CO$.
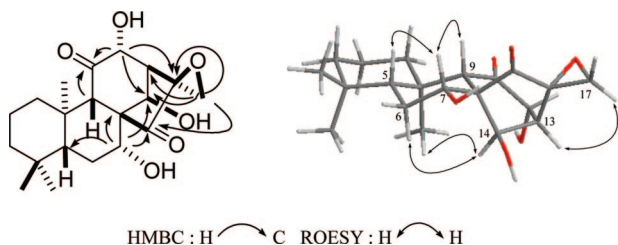
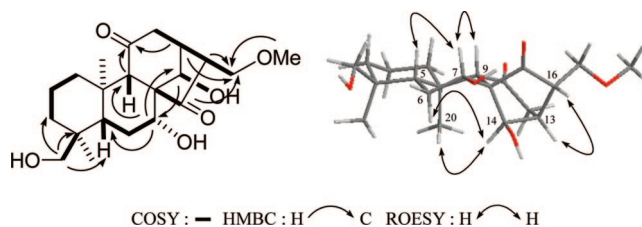**Figure 1.** Key HMBC and ROESY correlations of **1**.

Figure 2. Key COSY, HMBC, and ROESY correlations of **4**.

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. Consequently, compound **1** was assigned as 7α,12α,14β-trihydroxy-16β,17-epoxy-*ent*-kaur-11,15-dione.

The ^{1}H and ^{13}C NMR data of isoscoparin E (**2**) were similar to those of **12**, and the only difference was that an acetoxy group at C-7 in **2** replaced a hydroxyl group at the same position in the latter compound, which was proved by the correlations from H-7 (δ_H 6.00) to OAc (δ_C 169.4) in the HMBC experiment. The relative configurations of both **2** and **12** were identical according to the ROESY correlations observed. Consequently, **2** was characterized as 11β,12α,14β-trihydroxy-7α-acetoxy-*ent*-kaur-16-en-15-one.

Isoscoparin F (**3**) exhibited a peak at *m/z* 393.2266 ([M + H]$^+$, calcd 393.2277) in the HRESIMS, in agreement with the molecular formula, $C_{22}H_{32}O_6$, of **2**. Detailed comparison of the NMR data of **3** with those of **2** suggested that the acetoxyl and carbonyl groups at C-7 and C-15, respectively, in **2** were transposed in **3**. The signal at δ_C 68.0 (C-9) in **2** was shifted upfield to δ_C 59.9 in **3**, in combination with the singlet at δ_H 5.80 (H-15), and indicated the acetoxyl group was substituted at C-15β in **3**. Furthermore, the signal at δ_C 64.0 (C-8) showed that a carbonyl was placed at C-7. The HMBC correlations observed confirmed the deduction mentioned above. Accordingly, **3** was identified as 11β,12α,14β-trihydroxy-15β-acetoxy-*ent*-kaur-16-en-7-one.

The HRESIMS indicated the molecular formula of isoscoparin G (**4**) to be $C_{21}H_{32}O_6$. IR absorptions at 1741 and 1704 cm^{-1} implied the presence of two isolated ketone groups. The NMR data (Tables 1 and 3) of **4** resembled those of **23** except for the signals due to an oxygenated methylene at C-17 in **4** rather than an olefinic methylene at the same position in **23**, which was proved by the

HMBC correlations from H-17a (δ_H 3.80, dd, *J* = 9.7, 4.7 Hz) to C-13 (δ_C 40.4), C-15 (δ_C 217.1), C-16 (δ_C 50.8), and OMe (δ_C 58.5). A β-configuration of the oxygenated methylene (C-17) was concluded on the basis of the ^{1}H−^{1}H correlations of H-16 with H-13α, of H-13α with H-14α, and of H-14α with H$_3$-20, observed in the ROESY experiment of **4** (Figure 2). Thus, compound **4** was identified as 7α,14β,18-trihydroxy-16β-methoxymethyl-*ent*-kaur-11,15-dione.

The molecular formula of isoscoparin H (**5**) was established as $C_{20}H_{30}O_5$ by HRESIMS (*m/z* 373.1989 [M + Na]$^+$, calcd 373.1990). The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **23**, except that a hydroxyl with a β-orientation at C-15 in **5** replaced a ketone group at the same position in **23**. The unusual shift for C-9 (upfield by 4.9 ppm) caused by the γ-steric compression effect between OH-15β and H-9β confirmed this conclusion. Accordingly, **5** was elucidated as 7α,14β,15β,18-tetrahydroxy-*ent*-kaur-16-en-11-one.

Isoscoparin I (**6**) was shown to have the molecular formula $C_{20}H_{28}O_5$ by HRESIMS at *m/z* 371.1842 [M + Na]$^+$ (calcd 371.1834). The IR spectrum revealed absorption bands at 3381 (OH), 1716 (C=O), and 1650 (C=C) cm^{-1}. The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **18**, and further spectroscopic analysis revealed that the only difference was in the ring-A region. The signals of a CHO (δ_C 205.7, δ_H 9.21) in **6** rather than a methyl at C-18 in **18** were observed, which was supported by the HMBC spectrum, in which H-18 (δ_H 9.21) correlated to C-3 (δ_C 32.0), C-5 (δ_C 45.5), and C-19 (δ_C 14.2). Considering the fact that the ROESY correlations of **6** were consistent with those of **18**, compound **6** could be identified as 7α,11β,14β-trihydroxy-*ent*-kaur-16-en-15-oxo-18-al.

Isoscoparin J (**7**), obtained as a white powder, was assigned the molecular formula $C_{20}H_{26}O_5$. The absorptions at 1728, 1709, and

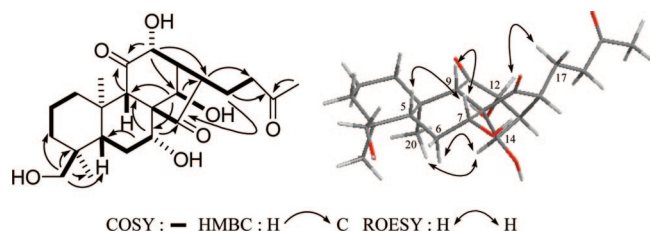COSY : ▬   HMBC : H ↷ C   ROESY : H ↷ H

Figure 3. Key COSY, HMBC, and ROESY correlations of **10**.

1645 cm^{-1} in the IR spectrum suggested the presence of an isolated, conjugated ketone. Comparison of the NMR data of **7** with those of **6** led to the deduction that the only difference was that a hydroxyl group at C-11 in **6** is replaced by a carbonyl in **7**, which was verified by the HMBC correlations of H$_2$-12 (δ_H 2.69 and 2.95) and H-9 (δ_H 2.12) with C-11 (δ_C 206.9). Thus, **7** was determined to be 7α,14β-dihydroxy-*ent*-kaur-16-en-11,15-dioxo-18-al.

Isoscoparin K (**8**) was found by HRESIMS to possess the molecular formula C$_{20}$H$_{30}$O$_5$, the same as **5**. Detailed analysis of the NMR spectra of **5** and **8** showed that a carbonyl (δ_C 215.6), a hydroxyl (δ_C 67.3), and a methyl (δ_C 31.8) occurred at C-1, C-11, and C-18 in **8**, respectively, in good agreement with the observed HMBC correlations. The β-orientation for OH-11 was determined from the ROESY correlations of H-11 (δ_H 4.29, d, J = 4.8 Hz) with H$_3$-20 (δ_H 1.34, s). Therefore, compound **8** was characterized as 7α,11β,14β,15β-tetrahydroxy-*ent*-kaur-16-en-1-one.

The molecular formula (C$_{20}$H$_{30}$O$_4$) of isoscoparin L (**9**) was inferred from its HRESIMS (*m/z* 357.2042). The ^{1}H and ^{13}C NMR data, as well as the HSQC and HMBC spectra, indicated that compound **9** resembled **21**, but with the presence of a methylene at δ_C 40.1 (C-1) instead of an oxygenated methine at δ_C 81.8 (C-1). The relative configuration of **9** was assigned by the ROESY correlations. The structure of isoscoparin M (**9**) was determined as 7α,12α,14β-trihydroxy-*ent*-kaur-16-en-15-one.

The molecular formula of **10** was determined as C$_{23}$H$_{34}$O$_7$ by HRESIMS (*m/z* 445.2219 [M + Na]$^+$, calcd 445.2202). Careful comparison of the ^{1}H and ^{13}C NMR data of **10** with those of rabdoloxin A (**11**) indicated that the two compounds are similar except for the ring-D region, and instead of an olefinic bond, a methylene (δ_C 20.3) and a methine (δ_C 47.5) in **10** were evident, in combination with the presence of a free ketone carbon at δ_C 220.2 in **10**; these revealed the exo-double bond of **10** was reduced. Further analysis the HMBC spectrum of **10** showed an acetonyl unit was located at C-17, which was proved by the HMBC correlations from H-14 (δ_H 6.35), H-16 (δ_H 3.44), and H-1' (δ_H 2.54–2.69) to C-17 (δ_C 20.3) and from H$_2$-17 (δ_H 1.71 and 2.21) to C-15 (δ_C 220.2) and C-2' (δ_C 207.2). The ^{1}H–^{1}H COSY experiment showed the presence of the fragment –CH(13)–CH(16)–CH$_2$(17)–CH$_2$(1')–, as shown in Figure 3. In the ROESY spectrum of **10**, the correlations between H-12β and H-17b indicated the relative configuration of H$_2$-17 to be β-oriented. Therefore, compound **10** was established as the acetonide of rabdoloxin A, which was verified by experiment to be an extraction artifact.

Due to the limited amount of material available, compounds **2**, **4**, **7**, **8**, **18**, **19**, and **26** were not tested for their 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,[16] with cisplatin, 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 most potently cytotoxic compounds for one or more cell lines were **3**, **6**, **9**, **12**, **13**, **17**, **23**, and **24**.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter. IR spectra were recorded

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

compd	NB4	A549	SH-SY5Y	PC-3	MCF-7
3	2.2	9.9	9.2	>10	3.9
6	4.4	>10	>10	>10	>10
9	0.7	6.4	6.8	>10	2.2
12	2.8	1.7	>10	>10	>10
13	2.7	2.8	>10	>10	>10
17	0.3	>10	>10	>10	>10
21	8.2	>10	>10	>10	>10
22	8.7	>10	>10	>10	>10
23	2.3	>10	5.1	7.0	>10
24	0.9	7.3	8.9	>10	8.4
cis-platin	1.0	27.0	24.6	15.0	60.9
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. [b] Compounds **1**, **5**, **10**, **11**, **14**–**16**, **20**, and **25** were inactive for all cell lines (IC$_{50}$ > 10 μM).

on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker 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. scoparius* were collected in Shangrila County, Yunnan Province, People's Republic of China, in August 2006. Voucher specimens (KIB 200608014) were stored in a dry and dark room and deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, before the plant material was extracted in September 2007, and were identified by Prof. Xi-Wen Li.

Extraction and Isolation. The milled aerial parts of *I. scoparius* (4.0 kg) were extracted with 70% aqueous acetone (3 × 8 L) at rt overnight. The extract was partitioned between EtOAc and H$_2$O. The EtOAc extract (380 g) was chromatographed on MCI gel CHP 20P (90% CH$_3$OH–H$_2$O, then 100% CH$_3$OH). The 90% CH$_3$OH fraction (285 g) was chromatographed over silica gel (200–300 mesh, 1.5 kg), eluted in a step gradient manner with CHCl$_3$–CH$_3$COCH$_3$ (1:0 to 0:1), to afford fractions A–F. Fraction B (12 g) was submitted to repeated chromatography over silica gel (petroleum ether–acetone, from 99:1 to 1:1) to give fractions B1–B4. Fraction B2 was purified by repeated chromatography over silica gel (petroleum ether–acetone, from 40:1 to 0:1) and a RP-18 column (30% → 60% MeOH–H$_2$O) to yield isolate **9** (12 mg). Compound **24** (4 mg) was purified from fraction B4 by semipreparative HPLC (35% MeOH–H$_2$O). Fraction C (37 g) was submitted to repeated chromatography over silica gel (petroleum ether–acetone, from 20:1 to 0:1) to obtain fractions C1–C6. Compounds **12** (840 mg) and **16** (90 mg) were crystallized from fractions C3 and C5, respectively. Fraction C1 was separated further by RP-18 column chromatography (40% MeOH–H$_2$O) to afford **3** (30 mg) and **6** (5 mg). Compound **18** (2 mg) was obtained by semipreparative HPLC (40% MeOH–H$_2$O) from C2. Fraction C4 afforded **13** (4 mg) and **23** (20 mg) by RP-18 column chromatography (30% → 60% MeOH–H$_2$O) and semipreparative HPLC (40% MeOH–H$_2$O). Separation of fraction D by silica gel column chromatography, eluted with petroleum ether–acetone (9:1 → 1:1), yielded **17** (120 mg) and mixtures D1–D6. Fraction D1 was subjected to RP-18 column chromatography (30% → 70% MeOH–H$_2$O) to afford **1** (5 mg), **10** (12 mg), and **20** (5 mg). Compounds **4** (2 mg) and **5** (4 mg) were obtained by RP-18 column chromatography (45% MeOH–H$_2$O) and then by semipreparative HPLC (42% MeOH–H$_2$O) from fraction D2. Isolates **2** (2 mg), **14** (18 mg), and **25** (15 mg) were obtained from fraction D4 by RP-18 column chromatography (30% → 60% MeOH–H$_2$O). Fraction D5 was further

chromatographed over a RP-18 column (40% MeOH−H$_2$O) followed by semipreparative HPLC (36% MeOH−H$_2$O) to give **7** (3 mg) and **8** (1 mg). Compound **19** (2 mg) was acquired by semipreparative HPLC (42% MeOH−H$_2$O) from fraction D6. Fraction E (35 g) was subjected to silica gel column chromatography, eluted with petroleum ether−acetone (4:1 → 1:1), to yield fractions E1−E5. Compound **26** (1 mg) was obtained from fraction E1 by RP-18 column chromatography (50% MeOH−H$_2$O). Fraction E3 was purified using RP-18 column chromatography (30% → 60% MeOH−H$_2$O) to afford **15** (12 mg) and **22** (8 mg). Semipreparative HPLC (35% MeOH−H$_2$O) was applied to furnish **11** (6 mg) from fraction E4. Compound **21** (20 mg) was obtained from fraction E5 by RP-18 column chromatography (32% MeOH−H$_2$O).

Isoscoparin D (1): white, amorphous powder; [α]$^{16.1}_D$ +25.1 (*c* 0.18, MeOH); UV (MeOH) λ$_{max}$ (log ε) 201.4 (3.21) nm; IR (KBr) ν$_{max}$ 3426, 2951, 2930, 1750, 1709, 1630, 1460, 1393, 1368, 1252, 1187, 1156, 1103, 1068, 1052, 1007, 989, 969 cm^{-1}; ^{1}H NMR [(CD$_3$)$_2$CO, 500 MHz], see Table 1; ^{13}C NMR [(CD$_3$)$_2$CO, 125 MHz], see Table 3; positive ESIMS *m/z* 387 [M + Na]$^+$; positive HRESIMS *m/z* 387.1772 [M + Na]$^+$ (calcd for C$_{20}$H$_{28}$O$_6$, 387.1783).

Isoscoparin E (2): colorless needle crystals; [α]$^{23.0}_D$ −48.7 (*c* 0.11, MeOH); UV (MeOH) λ$_{max}$ (log ε) 235.0 (3.40) nm; IR (KBr) ν$_{max}$ 3428, 2998, 2930, 2872, 1731, 1709, 1651, 1630, 1467, 1461, 1411, 1390, 1371, 1306, 1272, 1261, 1243, 1167, 1093, 1070, 1039, 1015, 985 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 1; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 415 [M + Na]$^+$; positive HRESIMS *m/z* 415.2091 [M + Na]$^+$ (calcd for C$_{22}$H$_{32}$O$_6$, 415.2096).

Isoscoparin F (3): colorless needle crystals; [α]$^{16.4}_D$ +22.5 (*c* 0.49, MeOH); UV (MeOH) λ$_{max}$ (log ε) 205.0 (3.62) nm; IR (KBr) ν$_{max}$ 3428, 2956, 2938, 2924, 2867, 2848, 1703, 1678, 1423, 1382, 1366, 1272, 1203, 1195, 1091, 1068, 1046, 1033, 1017 cm^{-1}; ^{1}H NMR [(CD$_3$)$_2$CO, 500 MHz], see Table 1; ^{13}C NMR [(CD$_3$)$_2$CO, 125 MHz], see Table 3; positive ESIMS *m/z* 393 [M + H]$^+$; positive HRESIMS *m/z* 393.2266 [M + H]$^+$ (calcd for C$_{22}$H$_{32}$O$_6$, 393.2277).

Isoscoparin G (4): white powder; [α]$^{23.1}_D$ −16.7 (*c* 0.18, MeOH); UV (MeOH) λ$_{max}$ (log ε) 203.6 (3.16) nm; IR (KBr) ν$_{max}$ 3422, 2931, 2874, 1741, 1704, 1451, 1389, 1230, 1194, 1143, 1096, 1040 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 1; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 403 [M + Na]$^+$; positive HRESIMS *m/z* 403.2109 [M + Na]$^+$ (calcd for C$_{21}$H$_{32}$O$_6$, 403.2096).

Isoscoparin H (5): white powder; [α]$^{16.3}_D$ +47.1 (*c* 0.34, MeOH); UV (MeOH) λ$_{max}$ (log ε) 202.0 (3.44) nm; IR (KBr) ν$_{max}$ 3298, 2954, 2935, 2914, 2867, 1691, 1448, 1416, 1392, 1351, 1329, 1293, 1260, 1237, 1201, 1134, 1111, 1096, 1054, 1037, 997 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 2; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 373 [M + Na]$^+$, 723 [2 M + Na]$^+$; positive HRESIMS *m/z* 373.1989 [M + Na]$^+$ (calcd for C$_{20}$H$_{30}$O$_5$, 373.1990).

Isoscoparin I (6): white powder; [α]$^{18.6}_D$ −101.5 (*c* 0.21, MeOH); UV (MeOH) λ$_{max}$ (log ε) 237.0 (3.49) nm; IR (KBr) ν$_{max}$ 3381, 2934, 2893, 1716, 1650, 1450, 1412, 1319, 1261, 1250, 1162, 1101, 1066, 1002, 977 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 2; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 371 [M + Na]$^+$, 719 [2 M + Na]$^+$; positive HRESIMS *m/z* 371.1842 [M + Na]$^+$ (calcd for C$_{20}$H$_{28}$O$_5$, 371.1834).

Isoscoparin J (7): white powder; [α]$^{23.3}_D$ +1.5 (*c* 0.34, MeOH); UV (MeOH) λ$_{max}$ (log ε) 229.0 (3.54) nm; IR (KBr) ν$_{max}$ 3363, 2938, 2875, 1728, 1709, 1645, 1452, 1417, 1391, 1242, 1200, 1136, 1107, 1093, 1068, 965, 948 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 2; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 369 [M + Na]$^+$, 715 [2 M + Na]$^+$; positive HRESIMS *m/z* 369.1685 [M + Na]$^+$ (calcd for C$_{20}$H$_{26}$O$_5$, 369.1677).

Isoscoparin K (8): amorphous powder; [α]$^{18.7}_D$ −25.0 (*c* 0.06, MeOH); UV (MeOH) λ$_{max}$ (log ε) 203.6 (3.60) nm; IR (KBr) ν$_{max}$ 3420, 2932, 2871, 1700, 1632, 1461, 1447, 1385, 1259, 1120, 1099, 1074, 1055, 1035, 994 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 2; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 373 [M + Na]$^+$, 723 [2 M + Na]$^+$; positive HRESIMS *m/z* 373.1996 [M + Na]$^+$ (calcd for C$_{20}$H$_{30}$O$_5$, 373.1990).

Isoscoparin L (9): colorless needle crystals; [α]$^{16.3}_D$ −58.5 (*c* 0.19, MeOH); UV (MeOH) λ$_{max}$ (log ε) 228.6 (3.42) nm; IR (KBr) ν$_{max}$ 3374, 2926, 2870, 2853, 1717, 1647, 1459, 1394, 1372, 1364, 1258, 1231, 1192, 1162, 1093, 1076, 1048, 994 cm^{-1}; ^{1}H NMR [(CD$_3$)$_2$CO, 500

MHz], see Table 2; ^{13}C NMR [(CD$_3$)$_2$CO, 125 MHz], see Table 3; positive ESIMS *m/z* 357 [M + Na]$^+$, 691 [2 M + Na]$^+$; positive HRESIMS *m/z* 357.2042 [M + Na]$^+$ (calcd for C$_{20}$H$_{30}$O$_4$, 357.2041).

Acetonide of rabdoloxin A (10): white powder; [α]$^{18.6}_D$ +19.0 (*c* 0.16, MeOH); UV (MeOH) λ$_{max}$ (log ε) 201.4 (3.39) nm; IR (KBr) ν$_{max}$ 3398, 2933, 2875, 1738, 1708, 1551, 1447, 1364, 1306, 1144, 1098, 1055, 1000, 970 cm^{-1}; ^{1}H NMR (C$_5$D$_5$N, 500 MHz), see Table 1; ^{13}C NMR (C$_5$D$_5$N, 125 MHz), see Table 3; positive ESIMS *m/z* 445 [M + Na]$^+$; positive HRESIMS *m/z* 445.2219 [M + Na]$^+$ (calcd for C$_{23}$H$_{34}$O$_7$, 445.2202).

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). Cisplatin, paclitaxel, and etoposide were used as positive controls.

Supporting Information Available: 1D, 2D NMR and MS spectra of **1** and 1D NMR and MS spectra of **2**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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