JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Structure and Cytotoxicity of Diterpenoids from *Isodon adenolomus*

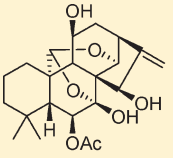
Wei Zhao,[†,‡] Jian-Xin Pu,[*,†] Xue Du,[†] Jia Su,[†] Xiao-Nian Li,[†] Jian-Hong Yang,[†] Yong-Bo Xue,[†,‡] Yan Li,[†] Wei-Lie Xiao,[†] and Han-Dong Sun[*,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China

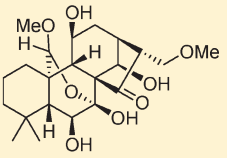
[‡]Graduate School of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China

Supporting Information

ABSTRACT: Twelve new diterpenoids, isoadenolins A–L (**1**–**12**), and 24 known ones were isolated from the aerial parts of *Isodon adenolomus*. Their structures were identified using spectroscopic data, and the absolute configurations of **1** and **14** were determined by single-crystal X-ray diffraction. Selected compounds were evaluated for their in vitro cytotoxicity against human tumor HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines. Compounds **9**, **13**–**16**, and **21** showed significant inhibitory effects on all five cells, with IC_{50} values in the range 0.7–9.7 μM.



1



4

The genus *Isodon* includes about 150 species and is a cosmopolitan and important genus of the family Lamiaceae. It has been reported to contain many diterpenoids with diverse structures, and interesting antibacterial, anti-inflammatory, and antitumor activities have been reported.[1] Over the past 30 years more than 60 *Isodon* species from China have been phytochemically investigated by our group, and the structures of more than 600 new diterpenoids (mainly *ent*-kauranoids) isolated and elucidated.[2]

Isodon adenolomus (Hand-Mazz.) H. Hara (Lamiaceae), a perennial herb, is distributed mainly in the southeast of China.[3] Our previous investigations of this species led to the discovery of seven *ent*-kauranoids.[4,5] Since the secondary metabolites of the genus *Isodon* often differ when grown in different ecological environments,[6−11] the aerial parts of this plant were collected in the Shangrila region of Yunnan Province, P. R. China, which had not been studied before. Investigation of this material has led to the isolation of 12 new *ent*-kaurane diterpenoids, isoadenolins A–L (**1**–**12**), and 24 known analogues. This paper reports the isolation, structure elucidation, and cytotoxic evaluation of selected compounds.

RESULTS AND DISCUSSION

The 70% aqueous acetone extract of air-dried and powdered aerial parts of *I. adenolomus* was partitioned between EtOAc and H_2O, and the EtOAc solubles were subjected to silica gel, MCI CHP-20 gel, Sephadex LH-20, and Lichroprep RP-18 gel column chromatography (CC) and semipreparative HPLC. The procedure yielded 12 new *ent*-kaurane diterpenoids, which we have named isoadenolins A–L (**1**–**12**), and 24 known compounds. The known compounds were identified as lasiokaurin (**13**),[12] xerophilusin N (**14**),[13] effusanin B (**15**),[14] longikaurin E (**16**),[15] xerophilusin J (**17**),[16] lasiokaurinin (**18**),[17] macrocalin B (**19**),[18] ponicidin (**20**),[19] xerophilusin B (**21**),[20] adenolin B,[21] adenolin D,[21] adenolin C,[21] adenolin A,[21] longikaurin A,[22] lasiodonin acetonide,[23] rabdoternin A,[24] rabdoternin E,[25] phyllostacin I,[26] nervosanin A,[27] lasiokaurinol,[17] xerophilusin XII,[13] longikaurin F,[15] longikaurin D,[15] and adenolin E.[4] The structures of the known compounds were determined by comparing spectroscopic data with literature values, and the absolute configurations of **1** and **14** were determined by X-ray analysis (CCDC 808511 and 808512, respectively).

Compound **1** was isolated as colorless crystals. Its molecular formula was determined as $C_{22}H_{30}O_7$ from the positive HRESIMS quasi-molecular ion peak at m/z 429.1885 $[M + Na]^+$, requiring eight degrees of unsaturation. The IR spectrum showed absorptions at 3415 and 1639 cm^{-1}, which were attributed to OH and C=C functional groups, respectively. The ^{13}C NMR and DEPT spectroscopic data (Table 1) exhibited 22 carbon signals, consisting of two methyls, five methylenes (including an olefinic one), eight methines (five oxygenated), five quaternary carbons (one oxygenated), and an acetyl group. Signals characteristic of a hemiketal quaternary carbon (δ_C 100.5, C-7) and two significant oxygenated methines (δ_C 97.7, C-20, and δ_C 71.3, C-14) indicated that **1** was a 7α,20:14α,20-diepoxy-*ent*-kaurane diterpenoid, which was confirmed by the HMBC correlations from H-20 to C-1, C-7, C-10, and C-14 (Figure 1). HMBC correlations from H-11 (δ_H 4.33) to C-8, C-9, and C-13 and from H-15 (δ_H 5.29) to C-7, C-9, and C-17 permitted the assignment of OH groups at C-11 and C-15, respectively, while HMBC correlations from H-6 (δ_H 5.55) to the acetyl carbonyl (δ_C 170.4) led to the location of an acetyl group at C-6.

In the ROESY spectrum, H-6 showed correlation to H_3-19α, H-11 correlated to H-12α, and H-15 correlated to H-13α and

Received: February 14, 2011
Published: May 02, 2011

dx.doi.org/10.1021/np200140j | *J. Nat. Prod.* 2011, 74, 1213–1220

Table 1. ^{13}C NMR Spectroscopic Data for Isoadenolins A–L (1–12) (δ in ppm)

position	1[a,b]	2[a,d]	3[a,b]	4[a,c]	5[a,b]	6[a,b]	7[a,c]	8[a,c]	9[a,b]	10[a,c]	11[a,b]	12[a,b]
1	28.4 CH_2	74.9 CH	72.0 CH	29.6 CH_2	31.1 CH_2	31.1 CH_2	29.5 CH_2	76.4 CH	74.2 CH	74.2 CH	74.3 CH	75.2 CH
2	18.9 CH_2	26.6 CH_2	30.2 CH_2	18.7 CH_2	18.9 CH_2	18.6 CH_2	18.7 CH_2	26.1 CH_2	24.8 CH_2	24.8 CH_2	25.0 CH_2	26.0 CH_2
3	41.0 CH_2	39.4 CH_2	39.9 CH_2	41.3 CH_2	41.6 CH_2	41.2 CH_2	41.1 CH_2	39.4 CH_2	38.9 CH_2	38.8 CH_2	38.6 CH_2	38.6 CH_2
4	33.7 C	33.9 C	33.5 C	34.2 C	34.2 C	34.1 C	34.2 C	32.9 C	33.5 C	33.5 C	33.7 C	34.3 C
5	61.7 CH	63.6 CH	63.4 CH	61.8 CH	58.6 CH	55.9 CH	58.2 CH	59.4 CH	52.5 CH	52.3 CH	51.5 CH	55.4 CH
6	72.6 CH	73.0 CH	72.9 CH	74.4 CH	73.8 CH	74.4 CH	73.5 CH	74.3 CH	75.2 CH	75.0 CH	73.6 CH	72.1 CH
7	100.5 C	102.2 C	101.9 C	99.7 C	101.4 C	100.0 C	101.2 C	99.9 C	96.7 C	99.4 C	100.4 C	107.4 C
8	50.8 C	56.2 C	57.7 C	63.2 C	53.9 C	53.9 C	53.5 C	62.6 C	61.3 C	64.7 C	56.1 C	53.6 C
9	48.4 CH	45.1 CH	44.6 C	54.8 CH	52.0 CH	51.8 CH	47.6 CH	54.7 CH	143.1 C	139.3 C	143.5 C	43.9 CH
10	42.6 C	47.7 C	48.4 C	40.4 C	40.3 C	40.5 C	40.1 C	43.6 C	43.2 C	43.0 C	42.5 C	48.1 C
11	63.9 CH	20.0 CH_2	19.9 CH_2	63.1 CH	63.6 CH	63.5 CH	68.4 CH	19.9 CH_2	117.4 CH	118.7 CH	116.3 CH	19.7 CH_2
12	36.7 CH_2	26.1 CH_2	19.3 CH_2	41.9 CH_2	44.9 CH_2	43.8 CH_2	41.4 CH_2	30.7 CH_2	35.8 CH_2	35.5 CH_2	79.4 CH	32.5 CH_2
13	41.7 CH	41.1 CH	35.7 CH	40.5 CH	47.2 CH	46.8 CH	46.4 CH	43.3 CH	36.3 CH	44.3 CH	47.8 CH	45.6 CH
14	71.3 CH	70.1 CH	70.9 CH	77.5 CH	73.2 CH	76.3 CH	76.5 CH	72.8 CH	32.1 CH_2	76.4 CH	39.2 CH_2	72.5 CH
15	71.3 CH	200.1 C	211.5 C	221.3 C	76.4 CH	73.2 CH	73.5 CH	208.2 C	203.7 C	203.0 C	79.1 CH	74.8 CH
16	157.0 C	149.7 C	53.6 CH	60.7 CH	160.6 C	160.6 C	159.1 C	152.8 C	152.2 C	151.4 C	159.1 C	159.3 C
17	110.0 CH_2	118.9 CH_2	68.5 CH_2	74.4 CH_2	109.2 CH_2	109.7 CH_2	110.1 CH_2	120.2 CH_2	118.1 CH_2	120.8 CH_2	109.6 CH_2	110.3 CH_2
18	31.4 CH_3	31.1 CH_3	31.0 CH_3	33.5 CH_3	33.9 CH_3	32.6 CH_3	34.5 CH_3	35.5 CH_3	34.0 CH_3	33.9 CH_3	33.8 CH_3	31.0 CH_3
19	23.1 CH_3	23.4 CH_3	23.0 CH_3	22.4 CH_3	22.9 CH_3	22.7 CH_3	22.8 CH_3	22.8 CH_3	23.4 CH_3	23.3 CH_3	22.8 CH_3	20.9 CH_3
20	97.7 CH	97.1 CH	97.5 CH	103.4 CH	103.7 CH	103.6 CH_2	103.2 CH	100.3 CH_2	67.9 CH_2	68.0 CH_2	69.2 CH_2	171.6 CH_2
OAc	21.6 CH_3	21.9 CH_3				21.4 CH_3	21.5 CH_3	21.4 CH_3	21.2 CH_3	21.2 CH_3	21.2 CH_3	21.8 CH_3
	170.4 C	170.8 C				169.1 C	170.3 C	170.1 C	170.2 C	170.2 C	170.3 C	170.7 C
OMe			58.6 CH_3	55.5 CH_3	55.8 CH_3	55.8 CH_3	55.7 CH_3	55.0 CH_3				
OMe				58.3 CH_3								

[a] Recorded in C_5D_5N. [b] Recorded at 400 MHz. [c] Recorded at 500 MHz. [d] Recorded at 600 MHz.

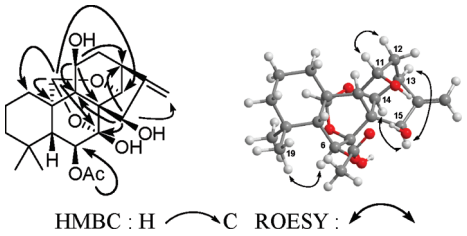


Figure 1. Key HMBC and ROESY correlations of **1**.

H-14β, revealing H-6, H-11, and H-15 to be α-oriented (Figure 1). To confirm the structure and determine its absolute configuration, **1** was crystallized from MeOH to afford a crystal of the monoclinic space group $P2_12_12_1$, which was analyzed by X-ray crystallography. On the basis of seven oxygen atoms in the molecule, the final refinement on the Cu Kα data resulted in a Flack parameter of 0.00 (15), allowing unambiguous assignment of the absolute configuration (Figure 2). The nine chiral centers, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-13, and C-14, were thus determined as *S*, *S*, *R*, *S*, *R*, *R*, *S*, *S*, and *R*, respectively. Thus, the structure of **1** was determined to be 6β-acetoxy-7β,11β,15β-trihydroxy-7α,20:14α,20-diepoxy-*ent*-kaur-16-ene, and it was given the trivial name isoadenolin A.

Compound **2** was isolated as a white powder with the molecular formula $C_{22}H_{28}O_7$, as determined by HRESIMS ($[M + Na]^+$ *m*/*z* 427.1741, calcd 427.1732), with nine degrees of unsaturation. The NMR data (Tables 1 and 2), C-7 (δ_C 102.2), C-20 (δ_C 97.1), and C-14 (δ_C 70.1), indicated that **2** was a 7α,20:14α,20-diepoxy-*ent*-kaurane diterpenoid similar to **20**,

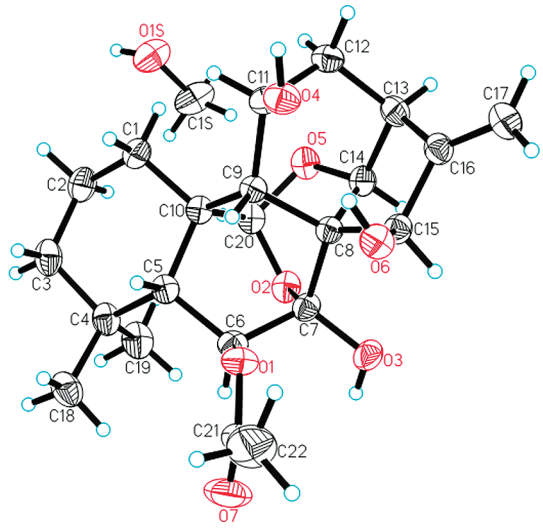


Figure 2. X-ray crystal structure of **1**.

except for an acetyl group at C-1. HMBC correlations from H-1 (δ_H 5.07) to the acetyl carbonyl (δ_C 170.8), from H-20 (δ_H 5.92) to C-7, and from H-14 (δ_H 4.95) to C-20 confirmed the above conclusion. The AcO-1 was α-oriented, as indicated by the ROESY correlations of H-1/H-5β. Consequently, compound **2** was identified as 1α-acetoxy-6β,7β-dihydroxy-7α,20:14α,20-diepoxy-*ent*-kaur-16-en-15-one, and it was named isoadenolin B.

Compound **3** was assigned as $C_{21}H_{30}O_7$, deduced from the HRESIMS and its NMR data. The ^{1}H and ^{13}C NMR data

Table 2. ^{1}H NMR Spectroscopic Data for Isoadenolins A−F (1−6) (δ in ppm, J in Hz)

position	1[a,b]	2[a,c]	3[a,c]	4[a,c]	5[a,b]	6[a,b]
1α	1.69 (d, 13.0)			2.32	2.60 (d, 14.1)	2.58 (m)
1β	1.19 (m)	5.07	3.85 (m)	1.81 (m)	2.11 (m)	2.09 (m)
2α	1.39−1.44 (m)	1.92 (m)	2.00 (m)	1.44	1.47 (m)	1.46 (m)
2β		1.72				
3α	1.30 (d, 13.0)	1.28 (m)	1.42 (m)	1.38	1.39	1.33, (m)
3β	1.00 (m)		1.31 (m)	1.14	1.18	1.06
5β	1.42	1.72	1.65 (br s)	1.55 (d, 5.0)	1.64 (d, 4.8)	1.62 (d, 7.1)
6α	5.55	4.95	4.22 (br s)	4.16 (d, 5.0)	4.17 (d, 4.8)	5.72 (d, 7.1)
8α						
9β	2.83 (s)	2.97 (d, 6.0)	2.86	3.39 (d, 5.0)	3.05 (m)	3.00 (d, 10.6)
11α	4.33 (d, 5.0)	1.63 (m)	1.71	4.92 (br s)	5.10	5.08
11β			2.45 (m)			
12α	2.58 (m)	2.51 (m)	2.84	2.80	1.14	2.90
12β	2.03 (d, 13.0)	1.43 (m)	1.72	2.09 (m)	2.01 (m)	1.98 (m)
13α	2.90 (m)	3.22 (m)	2.99	2.78	2.90	2.88
14α				5.35 (br s)	5.83 (br s)	5.34
14β	4.63 (d, 5.8)	4.95	5.12			
15α	5.29				5.36 (s)	5.58 (d, 2.9)
16α			2.99			
16β				2.35		
17a	5.55	6.16 (s)	3.98 (m)	3.95 (m)	5.65 (s)	5.63 (s)
17b	5.29	5.29	3.75 (t, 8.8)		5.32 (s)	5.34 (s)
18	0.86 (s)	1.01 (s)	1.06 (s)	1.16 (s)	1.13 (s)	0.81 (s)
19	0.83 (s)	0.93 (s)	0.94 (s)	0.99 (s)	1.06 (s)	1.09 (s)
20a	5.42 (s)	5.92 (s)	5.95 (s)	5.30 (s)	5.44 (s)	5.44 (s)
20b						
OAc	2.24 (s)	2.13 (s)				2.20 (s)
OMe			3.24 (s)	3.21 (s)	3.48 (s)	3.46 (s)
OMe				3.42 (s)		

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

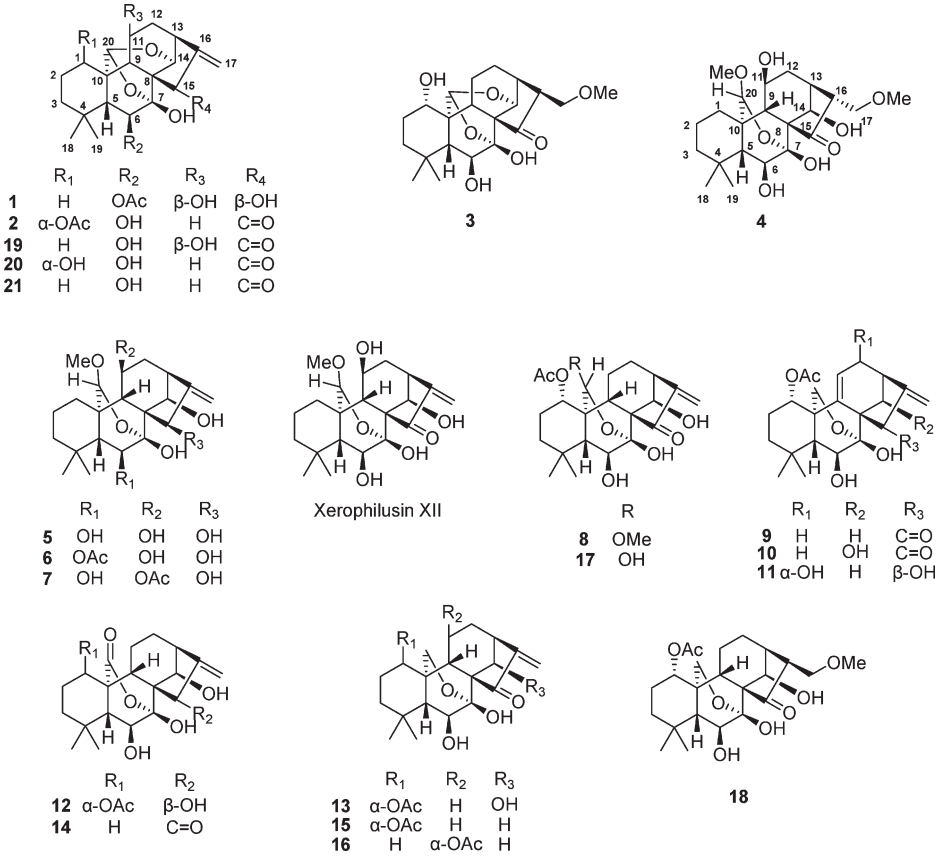
(Tables 1 and 2) revealed that the compound closely resembled **20** except for the D ring. The double bond between C-16 and C-17 in **20** was replaced by an OCH_3 group at C-17 (δ_C 68.5) and a methine at C-16 (δ_C 53.6) in **3**, which was supported by the HMBC correlations from H-16 (δ_H 2.99) to C-17, from H_2-17 (δ_H 3.98, 3.75) to C-13, and from MeO-17 (δ_H 3.24) to C-17. ROESY correlations of H-16/H-14β, H-1/H-9/H-11β, and H-6/H_3-19α indicated that the orientations of H-16, H-1, and H-6 were α, β, and α, respectively. Thus, compound **3** was assigned as 16(*S*)-1α,6β,7β-trihydroxy-17-methoxy-7α,20:14α,20-diepoxy-*ent*-kaur-15-one, and it was named isoadenolin C.

Compound **4** was isolated as a white, amorphous powder. Its molecular formula ($C_{22}H_{34}O_8$) indicated six degrees of unsaturation. The IR spectrum showed absorptions at 3425 and 1724 cm^{-1}, which were attributed to OH and carbonyl groups. The ^{13}C NMR and DEPT spectra (Table 1) displayed signals of four methyls (including two OCH_3), five methylenes (one oxygenated), eight methines (four oxygenated), and five quaternary carbons (one oxygenated and one ketone), which were very similar to those of xerophilusin XII. The principal differences between them were a characteristic double bond (C-16 and C-17) in xerophilusin XII changing into a methine (δ_C 60.7, C-16) and an oxygenated methylene (δ_C 74.4, C-17) in **4** on the basis of HMBC correlations from H-16 (δ_H 2.35) to C-12 and C-14 and from H-17 (δ_H 3.95) to the methoxy carbon (Figure 3), which was supported by the shift of C-13 ($\Delta\delta_C$ −4.2) in **4** relative to xerophilusin XII due to the γ-gauche steric compression effect between MeO-17 and H-13α, and the absence of the double bond (C-16 and C-17). The relative configuration of **4** was the same as that of xerophilusin XII except that H-16 was β-oriented in **4**, which was confirmed by the ROESY correlation of H_2-17 with H-13α and no correlation of H-16 with H-13α. The configuration at C-20 was assigned as *S* from the ROESY correlation between H-20 and H_3-19α (Figure 3). Therefore, the structure of **4** was elucidated as 16(*R*),20(*S*)-6β,7β,11β,14β-tetrahydroxy-17,20-dimethoxy-7α,20-epoxy-*ent*-kaur-15-one, and it was named isoadenolin D.

Compound **5** gave a molecular formula of $C_{21}H_{32}O_7$ by HRESIMS. The NMR data (Tables 1 and 2) demonstrated that **5** was very similar to xerophilusin XII, and the only differences were that the ^{13}C NMR signals of C-8, C-15, C-16, and C-17 had changed to δ_C 53.9, 76.4, 160.6, and 109.2 in **5** instead of δ_C 62.8, 210.0, 152.6, and 119.5) in xerophilusin XII. This indicated that a ketone carbon was absent at C-15 in **5**, as established by the HMBC spectrum from H-15 (δ_H 5.36) to C-13, C-14, and C-16, and was further supported by the shift of C-9 ($\Delta\delta_C$ −8.7) in **5** relative to xerophilusin XII, due to the γ-gauche steric compression effect between OH-15β and H-9β. The other three OH groups were connected to C-6, C-11, and C-14 by the HMBC

dx.doi.org/10.1021/np200140j |J. Nat. Prod. 2011, 74, 1213–1220

Chart 1



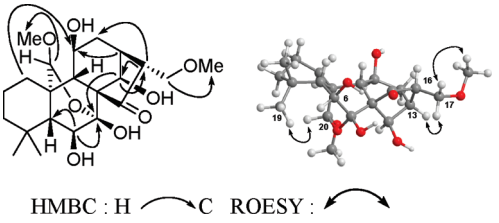


Figure 3. Key HMBC and ROESY correlations of **4**.

correlations from H-6 (δ_H 4.17) to C-14, C-5, and C-9, from H-11 (δ_H 5.10) to C-9 and C-10, and from H-14 (δ_H 5.83) to C-14 and C-16. The relative configurations of OH-6, OH-11, OH-14, and OH-15 were assigned on the basis of ROESY correlations of H-6/H_3-19α, H-11/H-14/H-13α, and H-15/H-13α. Hence, compound **5** was 20(*S*)-6*β*,7*β*,11*β*,14*β*,15*β*-pentahydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene, and it was named isoadenolin E.

Compounds **6** and **7** both had the molecular formula $C_{23}H_{34}O_8$, based on HRMS and NMR data, requiring seven degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **6** and **7** (Tables 1, 2, and 3) were similar to those of compound **5**, except that signals of an acetoxy group were evident in **6** and **7**. HMBC correlations in **6** from H-6 (δ_H 5.72) to the acetyl carbonyl (δ_C 169.1), from H-11 (δ_H 5.08) to C-10 and C-11, from H-14 (δ_H 5.34) to C-9, and from H-15 (δ_H 5.58) to C-16 verified that the acetyl carbonyl and the OH groups were at C-6, C-11, C-14, and C-15 in **6**. The acetyl group was at C-11 in **7**, based on the HMBC correlation from H-11 (δ_H 6.08) to the acetyl carbonyl (δ_C 170.3), which was supported by the downfield shift of C-11 from δ_C 63.6 in **5** to δ_C 68.4 in **7**. HMBC correlations from H-6 (δ_H 4.13) to C-5 and C-4, from H-14 (δ_H 5.28) to C-9, and from H-15 (δ_H 5.78) to C-16 and C-17 placed the OH groups at C-6, C-14, and C-15 in **7**. The relative configurations of **6** and **7** were determined to be the same as those of **5**, based on detailed analysis of the ROESY spectrum.

Compound **8** had the molecular formula $C_{23}H_{32}O_8$. Comparison of its NMR data (Tables 1 and 3) with those of **17** revealed similarities except for the signal of an OCH_3 group in **8** instead of a hydroxy group in **17**, which was supported by the HMBC correlation from MeO (δ_H 3.47) to C-20. The ROESY spectrum of H-20/H-11α and H-20/MeO-20 showed that C-20 in **8** had an *R*-configuration. Therefore, **8** was 20(*R*)-1α-acetoxy-6*β*,7*β*,14*β*-trihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one and was named isoadenolin H.

The HRESIMS of compound **9** suggested a molecular formula of $C_{22}H_{28}O_6$, with nine degrees of unsaturation. Its ^{1}H and ^{13}C NMR data (Tables 1 and 3) indicated a 7α,20-epoxy-*ent*-kaurane, similar to **15**. The most notable difference was that a methine (C-9) and a methylene (C-11) in **15** were changed into a double bond in **9**. This was supported by HMBC correlations from H-11 (δ_H 5.37) to C-10 and C-8, from H-13 and H_2-14 to C-11 (δ_C 117.4), and from H-1, H_2-20, H-12, and H_2-14 to C-9 (δ_C 143.1). The ROESY experiment indicated that they had the same relative configuration. Consequently, compound **9** was determined to be

Table 3. ^{1}H NMR Spectroscopic Data for Isoadenolins G−L (7−12) (δ in ppm, J in Hz)

position	7[a,c]	8[a,c]	9[a,c]	10[a,c]	11[a,c]	12[a,b]
1α	2.06 (m)					
1β	1.35	4.86 (dd, 11.5, 5.1)	5.16 (dd, 11.6, 4.9)	5.14 (dd, 11.3, 4.7)	5.25 (dd, 14.5 6.4)	5.05
2α	1.47−1.35	1.97	1.82	1.82 (t, 4.3)	1.86 (d, 14.0)	2.23 (m)
2β		1.76 (d, 3.9)	1.45	1.41 (t, 9.0)	1.51 (d, 14.0)	2.00 (m)
3α	1.47−1.35	1.37	1.45	1.31	1.33 (m)	1.35 (m)
3β			1.32		1.24	
5β	1.59 (d, 4.8)	1.64 (d, 9.4)	1.79	1.76 (d, 9.0)	2.26 (d, 8.8)	1.85 (d, 5.5)
6α	4.13 (d, 4.8)	4.35 (t, 10.7)	4.44	4.37	4.51 (d, 8.8)	4.27 (t, 4.8)
8α						
9β	3.18	2.02				3.21 (t, 6.2)
11α	6.08 (m)	1.80 (m)	5.37 (d, 2.9)	5.41 (s)	5.37 (d, 4.0)	1.61 (m)
11β		1.49 (m)				1.45
12α	3.15	2.27 (m)	2.57 (m)	2.71 (dd, 9.0, 4.0)		2.21
12β	1.62 (m)	1.49	2.01		5.85 (d, 2.6)	1.49
13α	2.85 (d, 7.9)	3.12 (d, 9.4)	2.99 (br. s)	3.19 (s)	3.03 (d, 4.8)	2.80 (d, 9.1)
14α	5.28 (s)	5.17 (s)	2.71 (dd, 11.1, 4.7)	4.84 (s)	2.69 (m)	5.68 (s)
14β			2.07		2.34 (m)	
15α	5.78 (s)				4.90 (s)	4.96 (s)
16α						
17a	5.69 (s)	6.25 (s)	6.03 (s)	6.29 (s)	5.66 (s)	5.66 (s)
17b	5.37 (s)	5.47 (s)	5.45 (s)	5.62 (s)	5.32 (s)	5.30 (s)
18	1.02 (s)	1.35 (s)	1.30 (s)	1.20 (s)	1.22 (s)	1.09 (q)
19	1.14 (s)	1.21 (s)	1.12 (s)	0.97 (s)	1.12 (s)	1.02 (q)
20a	5.31 (s)	5.29 (s)	4.41	4.40 (d, 9.0)	4.51 (d, 10.7)	
20b			4.18 (d, 8.7)	4.16 (d, 9.0)	4.18 (d, 10.7)	
OAc	2.01 (s)	2.03 (s)	2.04 (s)	2.03 (s)	2.03 (s)	2.07 (s)
OH					6.72 (d, 4.8)	
OMe	3.41 (s)	3.47 (s)				

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

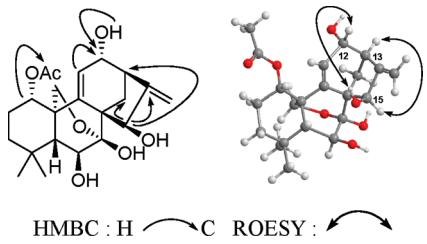


Figure 4. Key HMBC and ROESY correlations of **11**.

1α-acetoxy-6β,7β-dihydroxy-7α,20-epoxy-*ent*-kaur-9(11),16-dien-15-one, and it was named isoadenolin I.

The ^{1}H and ^{13}C NMR data (Tables 1 and 3) of compound **10** were very similar to those of **9**, but it was evident that **10** had an additional OH group. The OH group was assigned to C-14 since the methylene signal (δ_C 32.1, C-14) in **9** changed to an oxygenated methine signal (δ_C 76.4, C-14) in **10**, which was confirmed by the HMBC spectrum from H-14 to C-8, C-12, C-15, and C-17. H-14 was β-oriented, based on ROESY correlations of H-12α/H-14/H-13α. The other substituents had the same orientations as those in **9**. Therefore, **10** was 1α-acetoxy-6β,7β,14β-trihydroxy-7α,20-epoxy-*ent*-kaur-9(11),16-dien-15-one, and it was given the trivial name isoadenolin J.

Compound **11**, obtained as a white powder, gave a molecular formula of $C_{22}H_{30}O_7$ (HRESIMS). The ^{1}H and ^{13}C NMR data (Tables 1 and 3) revealed that it closely resembled **9**, except for an additional OH group at C-12 and the reduction of a carbonyl group at C-15. This was confirmed by HMBC correlations from H-11 to C-12 (δ_C 79.9) and from H-15 (δ_H 4.90) to C-13, C-14, and C-16 (Figure 4). The OH group (δ_H 6.72, d, J = 4.8 Hz) was assigned at C-15 by the HMBC from OH-15 (δ_H 6.72) to C-15. H-12 was β-oriented on the basis of ROESY correlations of OH-15β/H-12, H-12/H-13α, and H-15/H-13α (Figure 4). Thus, **11** was elucidated as 1α-acetoxy-6β,7β,12α,15β-tetrahydroxy-7α,20-epoxy-*ent*-kaur-9(11),16-diene and was named isoadenolin K.

Compound **12** had the molecular formula $C_{22}H_{30}O_8$. The ^{13}C and DEPT NMR spectra (Table 1) displayed 22 signals for the carbons of the diterpenoid skeleton, substituted by an acetoxy group (δ_C 170.7, 21.8). Comparison of the spectroscopic data of **12** with that of **14** revealed similarities, except for the occurrence of an additional acetyl group at C-1 and an OH at C-15 in **12**, which was confirmed by HMBC correlations from H-1 (δ_H 5.05) to the acetyl carbonyl (δ_C 170.3) and from H-15 (δ_H 4.96) to C-8, C-14, and C-17. The α-orientation of the AcO-1 group and β-orientation of the OH-15 group were apparent from the ROESY correlations of H-1 with H-5β and H-9β and of H-15 with H-13α and H-12α. Therefore, **12** was elucidated as 1α-acetoxy-6β,7β,14β,15β-tetrahydroxy-*ent*-kaur-16-en-7α,20-olide, and it was given the name isoadenolin L.

The absolute configuration of **14** was determined by X-ray analysis (Figure 5).

dx.doi.org/10.1021/np200140j |J. Nat. Prod. 2011, 74, 1213–1220

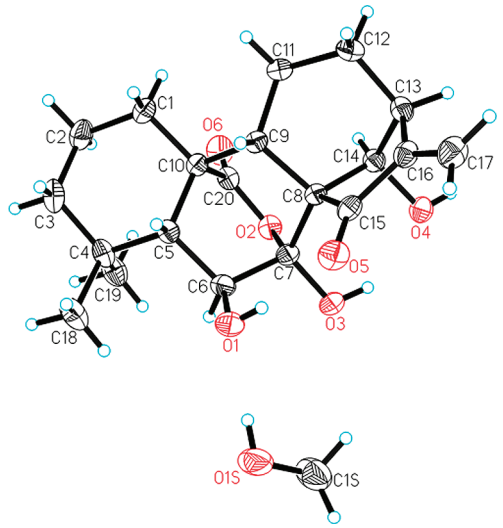


Figure 5. X-ray crystal structure of **14**.

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

compound[a]	HL-60	SMMC-7721	A-549	MCF-7	SW480
9	2.6	3.0	5.0	2.6	3.1
11	3.5	5.7	14.1	3.0	3.1
13	3.2	2.0	7.6	5.3	2.3
14	3.8	3.6	9.7	7.9	3.5
15	0.7	1.0	3.5	0.7	0.8
16	1.8	1.9	3.9	1.9	2.4
17	7.6	4.6	21.7	9.7	2.7
18	6.8	4.8	20.4	6.0	4.9
19	12.4	9.1	31.9	26.9	10.9
20	7.4	5.0	16.5	16.3	2.8
21	0.8	1.3	2.0	1.1	0.9
DDP[b]	1.5	14.5	14.1	15.0	16.9
paclitaxel[b]	0.01	<0.008	<0.008	<0.008	<0.008

[a] Other compounds than selected ones were inactive (IC_{50} > 10 μM) for all or one of cell lines. [b] DDP (cisplatin) and paclitaxel were used as positive controls.

Selected compounds were evaluated for their in vitro growth inhibitory effects against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using a previously described method.[28] The results are summarized in Table 4. Compounds **9**, **13**, **14**, **15**, **16**, and **21** exhibited significant activity (IC_{50} < 10 μM) for all five cell lines, suggesting that the carbonyl conjugated with an exomethylene group is the active center.[2]

EXPERIMENTAL SECTION

General Experimental Procedures. X-ray data were collected using a Bruker APEX DUO instrument. Optical rotations were measured with Horiba SEPA-300 and Jasco P-1020 polarimeters, respectively. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for IR spectra, using 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) were 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 (CC) was performed using silica gel (200−300 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. 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. adenolomus* were collected in the region of Shangrila, Yunnan Province, People's Republic of China, in August 2008. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 200809127) 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. adenolomus* (10 kg) were extracted four times with 70% aqueous Me_2CO (4 × 100 L, each 3 days) at room temperature and filtered. The filtrate was evaporated under reduced pressure and then partitioned with EtOAc (4 × 60 L). The EtOAc partition (900 g) was applied to silica gel (200−300 mesh) CC, eluting with $CHCl_3$−Me_2CO (1:0−0:1 gradient), to give six fractions. The fractions 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 CC on silica gel (200−300 mesh), eluted with ether−Me_2CO (1:0−0:1 gradient), to obtain fractions B1−B5. Compound **19** (1.0 g) and adenolin C (5 mg) were precipitated from fractions B2, B4, and B5, respectively. After repeated CC (silica gel, petroleum ether−Me_2CO, 9:1−2:1 gradient), fraction B1 afforded adenolin B (2 mg). Fraction B3 was separated by RP-18 (30%−100% MeOH−H_2O), then by HPLC with 50% MeOH−H_2O, to obtain compounds **1** (10 mg), **2** (2 mg), **3** (4 mg), **4** (2 mg), **5** (2 mg), **6** (6 mg), **11** (4 mg), **13** (8 mg), **14** (40 mg), **15** (100 mg), **16** (42 mg), **17** (90 mg), **19** (5 mg), **21** (8 mg), adenolin D (4 mg), and adenolin C (6 mg).

Fraction C (150 g) was separated by RP-18 (30−100% MeOH−H_2O) into fractions C1−C8. After repeated CC (silica gel, petroleum ether−Me_2CO, 9:1−1:1), fraction C1 afforded **18** (8 mg). Fraction C2 was separated by RP-18 (30−100% MeOH−H_2O), then by HPLC with 45% MeOH−H_2O, to yield compounds **7** (2 mg), **8** (5 mg), adenolin A (4 mg), longikaurin A (5 mg), lasiodonin acetonide (6 mg), rabdoternin A (4 mg), rabdoternin E (8 mg), phyllostacin I (10 mg), and nervosanin A (5 mg). In the same way, lasiokaurinol (3 mg) were isolated from fraction C3. Also, **12** (8 mg) was obtained from fractions C4 and C8, respectively.

Fraction D (180 g) was separated by RP-18 CC (30−80% MeOH−H_2O) into fractions D1−D5. Compound **10** (2 mg) crystallized from fraction D3, and the mother liquid was passed through a silica gel column, eluted with petroleum ether−2-propanol, 9:1−1:1, to yield xerophilusin XII (50 mg). Fraction E was subjected to RP-18 (55% MeOH−H_2O), then by HPLC (45% MeOH−H_2O), to give **9** (150 mg) and **20** (6 mg). Similarly, longikaurin F (30 mg), longikaurin D (7 mg), and adenolin E (12 mg) were obtained from fraction F.

*Isoadenolin A (**1**):* colorless crystals; mp 183.0−183.7 °C; $[\alpha]^{26}_{D}$ −59.4 (*c* 2.40, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.54) nm; IR (KBr) ν_{max} 3415, 2985, 2931, 2870, 1738, 1639, 1440, 1397, 1372, 1311, 1246, 1140, 1119, 1085, 1048, 1016, 997, 975, 943, 909, 849, 774, 722, 673, 650, 568, 533 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 3; positive ESIMS *m/z* 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 429.1885 (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

*Isoadenolin B (**2**):* white powder; mp 185−187 °C; $[\alpha]^{14}_{D}$ −76.8 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 227 (2.93) nm; IR (KBr) ν_{max} 3427, 2929, 2875, 1776, 1689, 1656, 1639, 1459, 1048, 1033 cm^{-1};

^{1}H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS m/z 427 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 427.1741 (calcd for $C_{22}H_{28}O_7Na$, 427.1732).

Isoadenolin C (**3**): white powder; mp 202−205 °C; $[\alpha]^{27}_D$ −97.9 (c 1.53, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.11) nm; IR (KBr) ν_{max} 3431, 2930, 2871, 1732, 1637, 1392, 1085, 967, 775, 597 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS m/z 417 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 417.1885 (calcd for $C_{21}H_{30}O_7Na$, 417.1889).

Isoadenolin D (**4**): white solid; mp 150−152 °C; $[\alpha]^{23}_D$ −27.8 (c 0.81, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.17) nm; IR (KBr) ν_{max} 3425, 2958, 2927, 1724, 1629, 1639, 1461, 1377, 1287, 1175, 1176, 1107, 1032, 1054, 983, 943, 580 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS m/z 449 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 449.2143 (calcd for $C_{22}H_{34}O_8Na$, 449.2151).

Isoadenolin E (**5**): white solid; mp 157−159 °C; $[\alpha]^{26}_D$ +8.3 (c 1.63, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.52) nm; IR (KBr) ν_{max} 3419, 2930, 2869, 1630, 1447, 1367, 1251, 1198, 1176, 1102, 1072, 1029, 984, 942 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; positive ESIMS m/z 419 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 419.2052 (calcd for $C_{21}H_{32}O_7Na$, 419.2045).

Isoadenolin F (**6**): white solid; mp 214−216 °C; $[\alpha]^{27}_D$ +29.2 (c 3.17, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.53) nm; IR (KBr) ν_{max} 3446, 3345, 2981, 2934, 2868, 1763, 1722, 1640, 1629, 1459, 1461, 1449, 1370, 1357, 1207, 1104, 1055, 981, 941, 910, 860, 848, 720, 660, 572 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; negative ESIMS m/z 437 $[M - H]^-$; negative HRESIMS $[M - H]^-$ m/z 437.2167 (calcd for $C_{23}H_{33}O_8$, 437.2175).

Isoadenolin G (**7**): white solid; mp 160−162 °C; $[\alpha]^{15}_D$ +6.8 (c 9.14, MeOH); UV (MeOH) λ_{max} (log ε) 217 (2.72) nm; IR (KBr) ν_{max} 3429, 2924, 1710, 1629 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 2 and 3; positive ESIMS m/z 461 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 461.2154 (calcd for $C_{23}H_{34}O_8Na$, 461.2151).

Isoadenolin H (**8**): white solid; mp 142−144 °C; $[\alpha]^{15}_D$ −37.3 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.14) nm; IR (KBr) ν_{max} 3427, 2948, 2872, 1737, 1712, 1641, 1441, 1241, 1058, 959 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 2 and 3; positive ESIMS m/z 437 $[M + H]^+$; positive HRESIMS $[M + H]^+$ m/z 437.2180 (calcd for $C_{23}H_{33}O_8$, 437.2175).

Isoadenolin I (**9**): white solid; mp 172−174 °C; $[\alpha]^{27}_D$ +18.6 (c 1.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.52), 237 (3.51) nm; IR (KBr) ν_{max} 3423, 2950, 2908, 1739, 1711, 1641, 1631, 1460, 1238, 1042, 973, 951, 917, 607 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 2 and 3; positive ESIMS m/z 411 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 411.1775 (calcd for $C_{22}H_{28}O_6Na$ $[M + Na]^+$, 411.1783).

Isoadenolin J (**10**): white powder; mp 133−135 °C; $[\alpha]^{15}_D$ +36.8 (c 0.72, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.06) nm; IR (KBr) ν_{max} 3430, 2922, 2853, 1743, 1706, 1638, 1629, 1476, 1450 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 3; positive ESIMS m/z 427 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 427.1734 (calcd for $C_{22}H_{28}O_7Na$, 427.1732).

Isoadenolin K (**11**): white powder; mp 153−156 °C; $[\alpha]^{26}_D$ +6.4 (c 1.11, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.65) nm; IR (KBr) ν_{max} 3416, 2950, 2910, 1738, 1722, 1640, 1527, 1460, 1433, 1372, 1242, 1191, 1129, 1088, 1043, 968, 951, 924, 863, 847, 775, 664, 629, 607, 559, 551 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 3; positive ESIMS m/z 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 429.1886 (calcd for $C_{22}H_{30}O_7Na$ $[M + Na]^+$, 429.1889).

Isoadenolin L (**12**): amorphous solid; mp 210−212 °C; $[\alpha]^{26}_D$ −27.6 (c 1.21, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.57) nm; IR (KBr) ν_{max} 3423, 2957, 2934, 1738, 1639, 1630, 1460, 1452, 1396, 1375, 1363, 1253, 1219, 1178, 1067, 1033, 989, 957, 905, 873, 809, 777, 677 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS m/z 445 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 445.1844 (calcd for $C_{22}H_{30}O_8Na$, 445.1838).

X-ray Crystal Structure Analysis. Colorless crystals of **1** and **14** were obtained from CH_3OH. Intensity data were collected at room temperature on an Bruker APEX DUO diffractometer equipped with an APEX II CCD, using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97.[29] Refinements were performed with SHELXL-97[29] using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H-atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. Crystallographic data (excluding structure factor tables) for the structures reported have been deposited with the Cambridge Crystallographic Data Center as supplementary publications no. CCDC 808511 for **1** and CCDC 808512 for **14**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44(0) (1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk].

Isoadenolin A (**1**): $C_{23}H_{34}O_8$, M_w = 438.5, orthorhombic, space group, $P2_12_12_1$, Z = 4, a = 6.7560(1) Å, b = 11.1010(2) Å, c = 29.6561(4) Å; α = β = γ = 90°, V = 2224.2(6) Å^3, μ(Cu Kα) = 0.81 mm^{-1}, ρ_{calc} = 1.31 g cm^{-3}; S = 1.09, final R indices: R_1 = 0.036 and wR_2 = 0.0927 for 3822 observed from 16 291 independent and 3843 measured reflections (θ_{max} = 66.5, $I > 2\sigma(I)$ criterion and 289 parameters); maximum and minimum residues are 0.18 and −0.23 e Å^{-3}, respectively. The Flack[30] parameter value was x = −0.00(15), indicating that the absolute structure has been determined correctly.

Xerophilusin N (**14**): $C_{21}H_{30}O_7$, M_w = 394.5, orthorhombic, space group, $P2_12_12_1$, Z = 4, a = 6.4226(1) Å, b = 8.7598(1) Å, c = 35.3066(4) Å; α = β = γ = 90°, V = 1986.37(5) Å^3, μ(Cu Kα) = 0.81 mm^{-1}, ρ_{calc} = 1.32 g cm^{-3}; S = 1.06, final R indices: R_1 = 0.038 and wR_2 = 0.101 for 3366 observed from 7652 independent and 3378 measured reflections (θ_{max} = 65.1, $I > 2\sigma(I)$ criterion and 261 parameters); maximum and minimum residues are 0.22 and −0.19 e Å^{-3}, respectively. The Flack[30] parameter value was x = −0.08(16), indicating that the absolute structure has been determined correctly.

Cytotoxicity Assay. The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, MCF-7, and SW-480. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO).[21] Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1 × 10^5 cells/mL in 100 μL of medium. Each cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS−50% DMF after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[31]

■ ASSOCIATED CONTENT

Supporting Information. This material (^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOESY, HRESIMS, IR, and UV spectra of compounds **1**, **4**, **9**, and **12**; ^{1}H, ^{13}C NMR, DEPT, and ESIMS spectra of compounds **2**−**3**, **5**−**8**, **10**, and **11**; X-ray data

of compounds **1** and **14**) is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: (86) 871-5223251. Fax: (86) 871-5216343. E-mail: pujianxin@mail.kib.ac.cn; hdsun@mail.kib.ac.cn.

ACKNOWLEDGMENT

This work was supported financially by the NSFC-Joint Foundation of Yunnan Province (no. U0832602 to H.-D.S.), the major direction projection foundation of CAS intellectual innovation project (no. 2010KIBA05 to J.-X. P.), the Major State Basic Research Development Program of China (no. 2009CB522300 and 2009CB940900), and the Science and Technology Program of Yunnan Province (no. 2008IF010 and 2008CD162).

REFERENCES

(1) Sun, H. D.; Xu, Y. L.; Jiang, B. *Diterpeniods from Isodon Species*; Science Press: Beijing, 2001; Vol. 1, p 93.

(2) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673–698.

(3) Zhang, R. P.; Zhang, H. J.; Lin, Z. W.; Zhen, Y. L.; Sun, H. D. *Phytochemistry* **1992**, *31*, 4237–4240.

(4) Sun, H. D.; Lin, Z. W.; Niu, F. D.; Lin, L. Z.; Chai, H. B.; Pezzuto, J. M.; Cordell, G. A. *Phytochemistry* **1995**, *38*, 437–442.

(5) Zhang, R. P.; Zhang, H. J.; Lin, Z. W.; Zheng, Y. L.; Sun, H. D. *Phytochemistry* **1992**, *31*, 4237–4240.

(6) Han, Q. B.; Zhang, J. X.; Zhao, A. H.; Sun, H. D.; Lu, Yu.; Wu, Y. S.; Zheng, Q. T. *Tetrahedron* **2004**, *60*, 2373–2377.

(7) Han, Q. B.; Lu, Yu.; Zhang, L. L.; Zheng, Q. T.; Sun, H. D. *Tetrahedron Lett.* **2004**, *45*, 2833–2837.

(8) Han, Q. B.; Jang, B.; Zhang, J. X.; Niu, X. M.; Sun, H. D. *Helv. Chim. Acta* **2003**, *86*, 773–777.

(9) Han, Q. B.; Li, R. T.; Zhang, J. X.; Sun, H. D. *Helv. Chim. Acta* **2004**, *87*, 1119–1124.

(10) Han, Q. B.; Zhao, A. H.; Zhang, J. X.; Lu, Yu.; Zhang, L. L.; Zheng, Q. T.; Sun, H. D. *J. Nat. Prod.* **2003**, *66*, 1391–1394.

(11) Huang, S. X.; Xiao, W. L.; Li, L. M.; Li, S. H.; Zhou, Y.; Ding, L. S.; Luo, L. G.; Sun, H. D. *Org. Lett.* **2006**, *8*, 1157–1160.

(12) Fujita, E.; Taoka, M. *Chem. Pharm. Bull.* **1972**, *20*, 1752–1754.

(13) Li, L. M.; Weng, Z. Y.; Huang, S. X.; Pu, J. X.; Li, S. H.; Huang, H.; Yang, B. B.; Han, Y.; Xiao, W. L.; Li, M. L.; Han, Q. B.; Sun, H. D. *J. Nat. Prod.* **2007**, *70*, 1295–1301.

(14) Fujita, T.; Takeda, Y.; Shingu, T.; Ueno, A. *Chem. Lett.* **1980**, 1635–1638.

(15) Fujita, T.; Takeda, Y.; Shingu, T. *Heterocycles* **1981**, *16*, 227–230.

(16) Hou, A. J.; Zhao, Q. S.; Li, M. L.; Jiang, B.; Lin, Z. W.; Sun, H. D.; Zhou, Y. P.; Lu, Y.; Zheng, Q. T. *Phytochemistry* **2001**, *58*, 179–183.

(17) Fujita, E.; Taoka, M.; Fujita, T. *Chem. Pharm. Bull.* **1974**, *22*, 280–285.

(18) Li, X.; Weng, Z. Y.; Li, Y.; Pu, J. X.; Huang, S. X.; Xiao, W. L.; Sun, H. D. *Helv. Chim. Acta* **2008**, *91*, 1130–1136.

(19) Fujita, E.; Fujita, T.; Shibuya, M. *Tetrahedron Lett.* **1966**, *7*, 3153–3162.

(20) Hou, A. J.; Li, M. L.; Jiang, B.; Lin, Z. W.; Ji, S. Y.; Zhou, Y. P.; Sun, H. D. *J. Nat. Prod.* **2000**, *63*, 599–601.

(21) Zhang, R. P.; Zhang, H. J.; Lin, Z. W.; Zhen, Y. L.; Sun, H. D. *Phytochemistry* **1992**, *31*, 4237–4240.

(22) Fujita, T.; Takeda, Y.; Shingu, T. *Chem. Commun.* **1980**, 205–207.

(23) Li, L. M.; Li, G. Y.; Huang, S. X.; Li, S. H.; Zhou, Y.; Xiao, W. L.; Lou, L. G.; Ding, L. S.; Sun, H. D. *J. Nat. Prod.* **2006**, *69*, 645–649.

(24) Takeda, Y.; Takeda, K.; Fujita, T.; Sun, H. D.; Minami, Y. *Chem. Pharm. Bull.* **1990**, *38*, 439–442.

(25) Takeda, Y.; Takeda, K.; Fujita, T.; Sun, H. D.; Minami, Y. *Phytochemistry* **1994**, *35*, 1513–1516.

(26) Li, X.; Weng, Z. Y.; Li, Y.; Pu, J. X.; Huang, S. X.; Xiao, W. L.; Sun, H. D. *Helv. Chim. Acta* **2008**, *91*, 1130–1136.

(27) Wang, X. R.; Hu, H. P.; Wang, H. P.; Wang, S. Q.; Shinichi, U.; Fujita, T. *Phytochemistry* **1994**, *37*, 1367–1370.

(28) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, P.; Vaigro-Wolff, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.

(29) Sheldrick, G. M. *SHELXL97*; University of Göttingen: Germany, 1997.

(30) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881.

(31) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493–497.