Cytotoxic *ent*-Kauranoids from the Medicinal Plant *Isodon xerophilus*

Li-Mei Li,[†] Zhi-Ying Weng,[†,‡] Sheng-Xiong Huang,[†] Jian-Xin Pu,[†] Sheng-Hong Li,[†] Hao Huang,[⊥] Bing-Bing Yang,[‡] Yu Han,[§] Wei-Lie Xiao,[†] Ma-Lin Li,[‡] Quan-Bin Han,*[,†] and Han-Dong Sun*[,†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Kunming Medical College, Yunnan Province Key Laboratory of the Natural Medicine Pharmacology, Kunming 650000, People's Republic of China, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, People's Republic of China, and Guiyang Medical College, Guiyang 550004, People's Republic of China

Received May 3, 2007

Bioassay-directed fractionation of the leaves of the medicinal plant *Isodon xerophilus* led to the isolation of a series of potential antitumor molecules. Thirteen new (**1**−**13**) and 23 (**14**−**36**) known diterpenoids were isolated and identified, representing *ent*-kauranoids of several structural types. The structures of **1**−**13** were determined by means of spectroscopic studies. The absolute configurations of the new compounds were clarified by CD spectroscopic studies or were postulated on biogenetic grounds. All compounds obtained were evaluated for their cytotoxic activity against the K562, MKN45, and HepG2 cell lines. Compounds **1**, **2**, **11**, **16**−**19**, **23**, **26**−**28**, **30**, and **32** demonstrated significant cytotoxic activity for one or more cell lines.

In the past 30 years, a large number of *ent*-kauranoids, exhibiting a wide range of bioactivities with low toxicity, have been isolated from the genus *Isodon* (Labiatae) by our group.[1] Among these *ent*-kauranoids, eriocalyxin B, isolated from *Isodon eriocalyx*, was found to inhibit nuclear factor-κB (NF-κB) activation by interfering with the binding of both p65 and p50 to the response element in a noncompetitive manner.[2] Recently, eriocalyxin B has been proved 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. Eriocalyxin B may thus be a potential apoptosis inducer and therapeutic reagent for treatment of murine t(8;21) leukemia.[3]

Isodon xerophilus (C. Y. Wu et H. W. Li) H. Hara, a perennial shrub native to Yunnan Province, People's Republic of China, has been used as an antitumor, anti-inflammatory, and antibacterial agent by local people.[4] Previous investigations have led to the characterization of several bioactive *ent*-kaurane diterpenoids from this plant.[5−9] An AcOEt extract prepared from the leaves of *I. xerophilus* exhibited cytotoxicity against K562 (human chronic myelogenous leukemia) cells, with an IC_{50} value of 2.3 μg/mL, during an initial bioassay. Therefore, with the intent of discovering new *ent*-kauranoids with potential antitumor properties, we performed a bioguided fractionation of the AcOEt extract. As a result, 36 *ent*-kauranoids, including 13 new compounds (**1**−**13**), were obtained. The structures of **1**−**13** were elucidated mainly by means of MS and NMR spectroscopic experiments, and their absolute configurations were determined by CD spectroscopic studies and on biogenetic grounds. The biological evaluation of all the diterpenoids isolated was carried out using human cancer cell lines. The current paper describes the isolation, identification, and biological evaluation of the *ent*-kauranoid constituents of *I. xerophilus*.

Results and Discussion

The leaves of *I. xerophilus* were extracted exhaustively with petroleum ether and ethyl acetate, sequentially. Bioassay-directed fractionation of the ethyl acetate extract (423 g) by repeated column chromatography on silica gel and RP-18 silica gel, followed by preparative and semipreparative high-performance liquid chromatography (HPLC), led to the isolation of 13 new *ent*-kauranoids (**1**−**13**), for which the NMR spectroscopic data are expressed in Tables 1−3. Also isolated were 23 known analogues, namely, xerophilusin G (**14**),[7] xerophilusin H (**15**),[6] longikaurin B (**16**),[7] longikaurin D (**17**),[10] maoecrystal I (**18**),[11] rosthorin A (**19**),[12] rabdoternin D (**20**),[13] rabdoternin C (**21**),[13] xerophilusin I (**22**),[7] xerophilusin K (**23**),[7] a mixture of hebeirubescensins G and H (**24** and **25**),[14] rabdoternin E (**26**),[13] xerophilusin B (**27**),[5] ponicidin (**28**),[15] macrocalin B (**29**),[16] xerophilusin A (**30**),[5] xerophilusin D (**31**),[9] xerophilusin N (**32**),[8] trichorabdal B (**33**),[17] phyllostachysin A (**34**),[18] xerophilusin F (**35**),[9] and enanderinanin J (**36**).[19] The known diterpenoids **14**−**36** were identified by comparing their spectroscopic data with those reported in the literature.

The molecular formula of compound **1**, obtained as colorless needles, was inferred by HRESIMS as $C_{20}H_{26}O_6$. The 1D NMR spectra of **1** (Tables 1 and 3) showed two angular methyls, seven methines (four of which were oxygenated), five methylenes (including an exomethylene), and six quaternary carbons (including two carbonyl carbons). In addition, the partial structure of an exomethylene conjugated with a carbonyl group on a five-membered ring was revealed by its NMR [δ_C at 208.3 (s), 149.9 (s), 115.1 (t); δ_H at 6.25 (br s), 5.40 (br s)], IR (ν_{max} 1709 (br), 1647 cm^{-1}), and UV [λ_{max} (log ϵ) 226 (3.84) nm] spectra. Analysis of these data, along with knowledge of the structural types of diterpenoids isolated previously from the genus *Isodon*,[1] indicated that compound **1** is an *ent*-kaur-16-en-15-one diterpenoid. Moreover, the angular methyl of C-20 was oxidized to an aldehyde group, which was confirmed by the HMBC spectrum, in which H-20 correlated to C-1 and C-10. The other carbonyl carbon was placed at C-6 by HMBC correlations from H-5 and H-7 to C-6. In a similar manner, the three hydroxyl groups were linked to C-7, C-11, and C-14, respectively (Figure 1). In the ROESY spectrum, H-7 showed correlation to H-14, H-11 correlated to H-1α, and H-20 showed correlations to H-12α and H-14, revealing the β-orientation of the hydroxyl groups attached to C-7, C-11, and C-14 (Figure 2). Thus, the structure of compound **1** was elucidated as 7β,11β,14β-trihydroxy-*ent*-kaur-20-al-6,15-dioxo-16-ene.

Compound **2** exhibited the same molecular formula ($C_{20}H_{26}O_6$) and similar NMR data to **1**. These two compounds were determined as being isomers, differing from each other only in the oxygenation patterns of C-6 and C-7. The substituents at C-6 and C-7 were found to be a hydroxyl and a carbonyl group in **2**, respectively, instead

* To whom correspondence should be addressed. Tel: 86-871-5223251. Fax: 86-871-5216343. E-mail: hdsun@mail.kib.ac.cn.
[†] Kunming Institute of Botany.
[‡] Kunming Medical College.
[⊥] Shanghai Institute of Organic Chemistry.
[§] Guiyang Medical College.

10.1021/np070205m CCC: $37.00
Published on Web 08/01/2007

Table 1. ^{1}H NMR Data of Compounds **1**−**7** in C_5D_5N (δ in ppm)

position	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[b]	**7**[a]
1	2.86 (br d, 9.5)	3.78 (d, 13.6)	3.66 (br s)	1.19−1.24 (overlap)	3.73 (br s)	4.01 (br s)	3.64 (br s)
	1.17−1.27 (overlap)	1.25−1.28 (overlap)		0.89 (dt, 13.6, 4.5)			
2	1.29−1.35 (2H, m)	1.36−1.43 (2H, m)	1.88−1.94 (overlap)	1.46−1.52 (m)	1.95−2.00 (m)	1.86 (2H, d, 7.8)	2.24 (d, 11.7)
			1.80−1.84 (m)	1.33−1.36 (m)	1.86 (dd, 14.0, 3.0)		2.06 (d, 11.7)
3	1.09−1.17 (2H, m)	1.25−1.28	2.11−2.18	2.30−2.36 (overlap)	2.12−2.27 (m)	2.07−2.13 (m)	4.27 (br s)
		(2H, overlap)	(2H, overlap)	1.02 (t, 13.4)	2.14−2.18 (m)	1.69 (br d, 13.4)	
5	3.97 (s)	1.97 (d, 12.4)	2.39 (d, 6.6)	1.64 (d, 6.5)	2.57 (d, 5.2)	2.49 (d, 4.3)	2.61−2.70 (overlap)
6		6.12 (d, 12.4)	4.64 (dd, 9.8, 6.6)	4.54 (dd, 10.2, 6.5)	4.62 (t, 5.0)	4.47−4.50 (overlap)	4.54−4.58 (m)
7	5.47 (s)						
9	3.46 (br s)	2.44 (d, 10.2)	2.70 (dd, 12.0, 5.6)	1.62−1.69 (overlap)	3.57−3.61 (m)	2.64 (d, 9.1)	2.61−2.70 (overlap)
11	4.57 (br s)	4.33−4.38 (m)	2.13−2.18 (overlap)	1.62−1.65 (overlap)	2.06−2.10 (m)	4.65−4.71 (m)	1.99−2.03 (m)
			1.88−1.94 (overlap)	1.19−1.24 (overlap)	1.68−1.73 (overlap)		1.80−1.89 (m)
12	2.22 (br d, 9.5)	2.38−2.42 (m)	2.34−2.38 (m)	2.30−2.36 (overlap)	2.32−2.35 (m)	3.00−3.09 (m)	2.32−2.40 (m)
	2.04 (br d, 13.5)	2.13 (t, 11.5)	1.52−1.59 (m)	1.52−1.56 (m)	1.68−1.73 (overlap)	1.91 (dd, 14.0, 8.6)	1.52−1.60 (m)
13	3.28 (br s)	3.17 (br s)	3.18 (d, 8.8)	3.16 (d, 9.4)	2.86 (d, 9.0)	3.26 (d, 9.4)	3.18 (d, 7.4)
14	4.34 (s)	4.72 (s)	5.31 (s)	5.12 (s)	5.77 (s)	5.21 (s)	5.24 (s)
15					5.04 (s)		
17	6.25 (br s)	6.07 (br s)	6.22 (br s)	6.27 (br s)	5.68 (br s)	6.24 (br s)	6.25 (br s)
	5.40 (br s)	5.33 (br s)	5.43 (br s)	5.50 (br s)	5.30 (br s)	5.51 (br s)	5.46 (br s)
18	1.06 (s)	1.47 (s)	1.65 (s)	1.60 (s)	1.59 (s)	1.43 (s)	1.71 (s)
19	1.19 (s)	1.17 (s)	4.36 (d, 10.6)	4.25 (d, 10.6)	4.44 (d, 10.8)	4.81 (d, 11.2)	4.65−4.70 (2H, m)
			4.13 (d, 10.6)	4.05 (d, 10.6)	4.20 (d, 10.8)	4.47−4.50 (overlap)	
20	10.35 (s)	10.74 (s)	4.35 (d, 9.6)	4.27 (d, 9.8)	4.28 (d, 9.7)	4.34 (d, 10.5)	4.24 (d, 8.4)
			4.19 (d, 9.6)	3.97 (d, 9.8)	4.25 (d, 9.7)	4.21 (d, 10.5)	4.07 (d, 8.4)
OAc						1.99 (s)	1.95 (s)
OH-6			7.05 (d, 9.8)	6.89 (d, 10.2)	8.53 (d, 4.7)		

[a] 400 MHz. [b]500 MHz.

of a carbonyl and a hydroxyl group in **1**. Compound **2** was therefore characterized as 6β,11β,14β-trihydroxy-*ent*-kaur-20-al-7,15-dioxo-16-ene.

For each of diterpenoids **3**−**11**, a 7α,20-epoxy-*ent*-kaurane diterpenoid skeleton was evident from the NMR spectrum, and all showed the presence of three methines (C-5, C-9, and C-13), three quaternary carbons (C-4, C-8, and C-10), a hemiketal quaternary carbon (C-7), and a noticeable oxygenated methylene (C-20).[1] Moreover, for compounds **3**−**8**, the methyls due to C-19 were replaced by hydroxyl or acetoxyl groups. In comparison with xerophilusin G (**14**),[7] the ^{13}C NMR signal of C-19 in **3** was shifted upfield by $\Delta\delta$ 2.1 ppm and was attributed to the absence of an acetyl group at C-19. Accordingly, compound **3** was elucidated as 1β,6β,7β,14β,19-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. Compound **4** differs structurally from **3** only at C-1. The hydroxyl group at C-1 in **3** was absent in **4**, which resulted in upfield shifts of the ^{13}C NMR signals of C-1, C-2, and C-10 and downfield shifts of the ^{13}C NMR signals of C-3, C-5, and C-9 in **4** arising from the absence of γ-gauche steric compression effects of OH-1β with H-3β, H-5β, and H-9β, apparent for **3**. Diterpenoid **4** was thus identified as 6β,7β,14β,19-tetrahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. Compound **5** is a reductive product of **3**. The conjugated carbonyl carbon (C-15) was reduced to a hydroxyl group. The OH-15 was assigned with a β-orientation due to the significant upfield signal of C-9 (δ_C 38.8), which was caused by the γ-gauche steric compression effect between OH-15β and H-9β. Therefore, compound **5** was concluded to be 1β,6β,7β,14β,15β,-19-hexahydroxy-7α,20-epoxy-*ent*-kaur-16-ene. Compound **6** has one more hydroxyl group than compound **14**, which was confirmed by HRESIMS, giving a molecular formula of $C_{22}H_{30}O_9$. Careful analysis of its 2D NMR spectra helped to locate the hydroxyl group at C-11 with β-orientation, which resulted in significant downfield shifts of the ^{13}C NMR signals of C-9, C-11, and C-12 in **6**, in comparison with those of **14**. Consequently, the structure 1β,6β,7β,-11β,14β-pentahydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one was assigned to compound **6**. Diterpenoid **7** was found to be an isomer of **6**. The compounds differ in the shift in position of a hydroxyl group at C-11 in **6** to C-3 in **7**, which was demonstrated by the observation of HMBC correlations of H-1, H_2-2, Me-18, and H_2-19 to C-3. The α-orientation of OH-3 was proved by a ROESY NMR correlation between H-3β and Me-18. Compound **7** was thus established as 1β,3α,6β,7β,14β-pentahydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. Like **7**, compound **8** is also an isomer of compound **6**. A hydroxyl with β-orientation at C-14 in **7** was transposed to C-11 with α-orientation in **8** by interpretation of the HMBC and ROESY spectra. Accordingly, the structure of compound **8** was elucidated as 1β,3α,6β,7β,11α-pentahydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Compounds **9**−**11** were also assigned as 7α,20-epoxy-*ent*-kaurane diterpenoids and shared some other common characteristics, such as having two angular methyls and a β-oriented hydroxyl at C-14, with the absence of a hydroxyl at C-1. Compound **9** exhibited a quasi-molecular ion peak at *m*/*z* 403.1726 $[M + Na]^+$ in its positive HRESIMS, corresponding to a molecular formula of $C_{20}H_{28}O_7$. The above information suggested that this compound possesses an additional hydroxyl in comparison with rosthorin A (**19**).[12] Comparison of the NMR data of **9** with those of **19** disclosed that the main structural difference between these compounds is in

Table 2. ^{1}H NMR Data of Compounds **8**−**13** in C_5D_5N (δ in ppm)

position	**8**[a]	**9**[b]	**10**[c]	**11**[b]	**12**[a]	**13**[a]
1	4.63−4.66 (overlap)	2.23−2.38 (m)	1.36−1.45 (overlap)	1.22−1.34 (2H, overlap)	2.43 (d, 14.4)	1.49−1.54 (2H, overlap)
		1.77−1.90 (overlap)	1.13−1.17 (overlap)		1.90−1.99 (m)	
2	2.31 (br d, 10.8)	1.77−1.90 (2H, overlap)	1.36−1.47 (2H, overlap)	1.26−1.31 (2H, overlap)	1.40−1.51 (2H, overlap)	1.72−1.78 (m)
	2.15 (br d, 12.0)					1.23−1.28 (m)
3	4.31 (br s)	3.59 (br d, 11.1)	1.43−1.49 (m)	1.28−1.34 (overlap)	1.39−1.47 (overlap)	1.39−1.42 (overlap)
			1.13−1.17 (overlap)	1.05−1.13 (m)	1.15−1.18 (m)	1.12−1.18 (m)
5	2.64−2.69 (overlap)	1.76 (d, 4.9)	1.39−1.45 (overlap)	1.46 (d, 5.0)	1.56 (d, 5.5)	1.59 (d, 9.0)
6	4.63−4.66 (overlap)	4.43−5.00 (overlap)	5.29 (s)	4.14−4.18 (overlap)	4.19 (dd, 9.5, 5.5)	4.28 (dd, 11.6, 9.0)
9	2.56 (d, 3.5)	2.20 (d, 9.8)	2.70 (dd, 9.6, 1.6)	2.21 (d, 8.8)	2.22 (d, 7.5)	2.17 (d, 11.0)
11	5.19 (d, 4.2)	4.43−5.00 (overlap)	4.94−4.99 (m)	5.36−5.41 (m)	5.10 (overlap)	5.61−5.68 (m)
12	2.47−2.52 (m)	3.01−3.08 (m)	2.90−2.97 (m)	3.08−3.20 (m)	2.93−3.01 (m)	2.92−3.06 (m)
	1.65−1.72 (m)	1.90−1.96 (m)	1.40−1.45 (overlap)	1.56 (dd, 14.5, 7.0)	1.89 (dd, 14.4, 7.6)	1.47−1.53 (overlap)
13	3.13−3.16 (m)	3.26 (d, 9.2)	2.58 (d, 8.9)	2.72 (d, 8.3)	3.25 (d, 8.6)	3.17 (d, 9.0)
14	3.70 (d, 11.5)	5.20 (s)	4.46 (s)	5.03 (s)	5.57 (s)	5.18 (s)
	2.64−2.69 (overlap)					
15			4.98 (s)			
16				2.98−3.00 (m)		
17	5.92 (br s)	6.24 (br s)	5.39 (br s)	3.89−3.94 (2H, m)	6.25 (br s)	6.32 (br s)
	5.22 (br s)	5.49 (br s)	5.30 (br s)		5.50 (br s)	5.59 (br s)
18	1.76 (s)	1.57 (s)	0.86 (s)	1.18 (s)	1.20 (s)	1.40 (s)
19	4.66−4.75 (2H, m)	1.38 (s)	1.16 (s)	1.01 (s)	1.03 (s)	1.21 (s)
20	5.61 (d, 8.5)	4.40 (d, 9.9)	4.15 (d, 10.2)	4.14−4.18 (overlap)	5.40 (s)	5.00 (s)
	4.09 (d, 8.5)	4.19 (d, 9.9)	3.87 (d, 10.2)	4.00 (d, 10.2)		
OMe				3.23 (s)	3.48 (s)	3.33 (s)
OAc	1.90 (s)		2.18 (s)	2.08 (s)		2.07 (s)
			1.98 (s)			
OH-6					7.10 (d, 9.5)	6.03 (d, 11.6)

[a] 400 MHz. [b]500 MHz. [c]Measured in $CDCl_3$, 500 MHz.

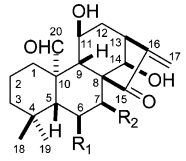
Table 3. ^{13}C NMR Data of Compounds **1**−**13** in C_5D_5N (125 or 100 MHz, δ in ppm)[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**[b]	**11**	**12**	**13**
1	34.3	36.3	65.1	31.0	65.5	65.0	66.8	68.8	29.1	29.2	29.2	30.2	31.9
2	19.2	19.6	27.7	19.0	27.6	26.2	31.3	31.4	28.3	18.4	18.9	18.7	20.2
3	41.7	43.1	28.2	35.9	28.1	28.4	71.4	71.7	77.7	40.7	41.3	41.6	42.1
4	32.4	35.8	39.1	36.6	39.2	38.3	43.1	43.4	40.6	33.9	34.1	34.3	33.2
5	54.9	59.2	56.6	61.5	53.6	56.3	51.8	53.0	61.9	54.9	60.4	61.5	58.5
6	210.3	75.3	73.4	73.1	72.4	73.1	73.4	74.2	73.6	73.7	73.7	74.6	73.9
7	76.3	211.3	99.0	98.6	100.5	98.8	98.9	96.9	98.7	97.3	98.0	99.7	99.8
8	60.4	70.2	62.4	62.7	53.1	62.0	62.3	59.8	62.9	53.6	62.4	62.8	62.7
9	59.8	62.2	48.4	52.8	38.8	54.2	48.1	50.1	59.2	46.5	54.1	60.7	56.6
10	58.7	57.5	41.3	38.9	41.0	43.2	41.9	43.2	37.8	37.0	37.8	40.6	41.5
11	63.8	65.8	16.5	16.9	14.6	61.1	16.3	66.1	61.0	64.2	65.0	63.5	66.4
12	38.3	41.9	30.4	30.2	32.6	41.1	30.2	41.5	42.0	38.8	38.1	42.4	37.7
13	46.6	44.7	44.0	43.8	46.4	44.0	44.0	35.1	44.4	44.8	39.3	44.7	43.2
14	75.4	79.0	73.9	73.5	73.2	73.7	74.0	28.0	73.8	76.3	76.2	74.3	72.7
15	208.3	201.7	209.3	208.7	76.6	209.0	209.5	212.2	209.3	71.7	220.5	210.0	207.2
16	149.9	148.6	153.1	153.0	161.2	152.0	152.8	154.6	152.5	156.1	56.7	152.6	151.6
17	115.1	118.6	119.5	119.6	109.3	119.9	119.7	115.0	119.4	111.9	74.4	119.5	121.1
18	30.9	35.4	28.4	28.2	27.7	27.2	22.2	22.9	29.3	32.1	33.3	33.9	35.9
19	21.0	21.6	65.1	64.5	64.7	67.0	67.4	67.8	16.4	22.2	22.4	22.7	23.2
20	204.9	206.1	67.0	67.2	66.6	67.2	66.5	68.7	67.4	66.6	66.7	103.7	104.8
OMe											58.3	55.9	55.0
OAc						170.9	170.8	170.8		170.4	169.9		169.9
						20.7	20.7	20.7		168.9	21.3		21.4
										21.4			
										21.4			

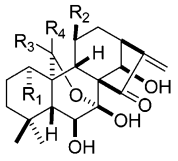
[a] Assignments are based on 1D and 2D NMR experiments. [b] Measured in $CDCl_3$.

ring A. Detailed analysis of the 2D NMR spectra led to the conclusion that the hydroxyl at C-3 is in the α-orientation. Accordingly, compound **9** was assigned as 3α,6*β*,7*β*,11*β*,14*β*-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. The NMR data of **10** were very similar to those of rabdoternin D (**20**),[13] and the only observed difference was that **10** has an acetyl group. The acetyl group was assigned to C-11 because the signal at δ_H 4.30 (δ_C 60.6) due to C-11 in **20** was shifted downfield to δ_H 4.94−4.99 (δ_C 64.2) in **10**, which was confirmed from the HMBC correlation of H-11 with the acetyl carbonyl. The relative configuration of the substituents in **10** was found to be the same as that in **20** as determined from a ROESY experiment. Thus, **10** was elucidated as 7*β*,14*β*,15*β*-trihydroxy-6*β*,11*β*-diacetoxy-7α,20-epoxy-*ent*-kaur-16-ene. Compound **11** was assigned the molecular formula $C_{23}H_{34}O_8$ from the positive HRESIMS. It showed no α,*β*-unsaturated ketone group absorptions in its UV and IR spectra. Its NMR spectra were closely

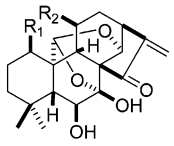
Chart 1



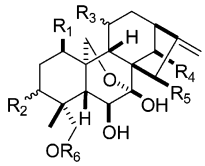
	R_1	R_2
1	=O	OH
2	OH	=O



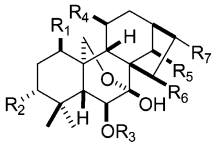
	R_1	R_2	R_3	R_4
12	H	OH	H	OMe
13	H	OAc	H	OMe
24	OH	H	H	OH
25	OH	H	OH	H
26	OH	H	OMe	H



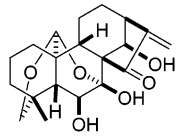
	R_1	R_2
27	H	H
28	OH	H
29	H	OH
30	H	OAc



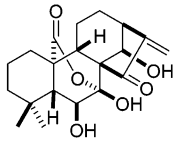
	R_1	R_2	R_3	R_4	R_5	R_6
3	OH	H	H	OH	=O	H
4	H	H	H	OH	=O	H
5	OH	H	H	OH	OH	H
6	OH	H	β–OH	OH	=O	Ac
7	OH	α–OH	H	OH	=O	Ac
8	OH	α–OH	α–OH	H	=O	Ac
14	OH	H	H	OH	=O	Ac
15	OH	H	H	H	=O	Ac
16	H	H	H	OH	=O	Ac
17	H	H	α-OH	H	=O	Ac
18	OH	β-OH	H	H	=O	Ac



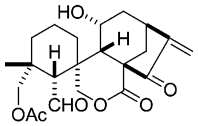
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
9	H	OH	H	OH	OH	=O	$=CH_2$
10	H	H	Ac	OAc	OH	OH	$=CH_2$
11	H	H	H	OAc	OH	=O	α–CH_2OCH_3
19	H	H	H	OH	OH	=O	$=CH_2$
20	H	H	Ac	OH	OH	OH	$=CH_2$
21	H	H	Ac	H	OH	OAc	$=CH_2$
22	OH	H	H	H	H	=O	$=CH_2$
23	H	H	H	OAc	OH	=O	$=CH_2$



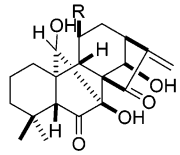
31



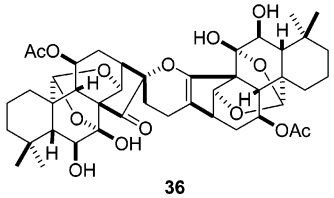
32



33



34 R=OH
35 R=OAc



36

similar to those of xerophilusin K (**23**)[7] except for the signal for ring D. The exomethylene in **23** was replaced by a methine [δ_H 2.99 (H-16); δ_C 56.7 (C-16)] and a methoxymethyl group [δ_H 3.23 (OMe), 3.89−3.94 (H_2-17); δ_C 58.3 (OMe) and 74.4 (C-17)] in **11**. The α-configuration of the methoxymethyl group in **11** was deduced from the ROESY NMR correlations between H-16β and H-12β. At the same time, the ^{13}C NMR signal of C-12 (δ_C 38.1) in **11** shifted downfield slightly, in comparison with C-12 in **23** (δ_C 37.4), which confirmed the above conclusion.[10] Therefore, the structure of **11** was established as 6β,7β,14β-trihydroxy-11β-acetoxy-16α-methoxymethyl-7α,20-epoxy-*ent*-kaur-15-one.

When comparing the ^{13}C NMR spectroscopic data of **12** and **13** with those of compounds **3**−**11**, differences were observed at C-20. This position was determined to be a hemiacetal carbon in

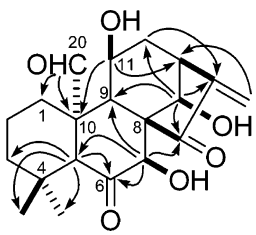


Figure 1. Selected HMBC correlations for **1**.

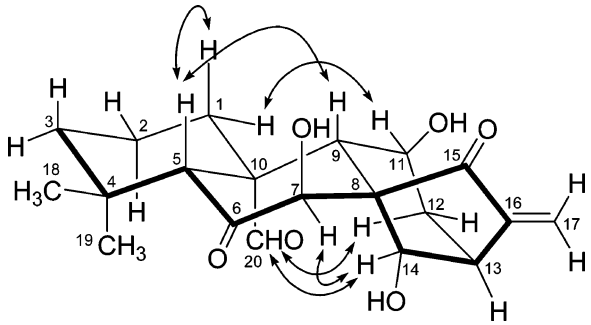


Figure 2. Selected ROESY correlations for **1**.

Table 4. Cytotoxic Activity of Compounds **1**−**36**[a]

compound	K562	MKN45	HepG2
1	0.04	2.8	0.19
2	1.0	>10	4.5
11	1.5	3.9	1.9
13	3.6	2.1	4.9
16	1.6	0.49	1.2
17	0.56	0.34	0.56
18	>10	1.3	>10
19	0.33	5.4	6.4
22	7.6	6.2	4.0
23	1.4	5.6	2.2
24/25	5.3	>10	>10
26	2.4	>10	>10
27	4.1	0.40	3.9
28	1.3	>10	0.96
30	1.6	>10	>10
31	9.0	3.9	4.2
32	1.2	4.8	>10
34	6.6	3.0	ND
35	4.3	>10	4.9
cisplatin[d]	2.7	3.5	3.3

[a] Results are expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) in μM, and data were obtained from triplicate experiments. [b] Compounds **4**−**6**, **10**, **12**, **14**, **15**, **20**, **21**, **29**, **33**, and **36** showed IC_{50} values > 10 μM for all cell lines tested. [c] Compounds **3** and **7**−**9** were not evaluated. [d] Positive control substance.

compounds **12** and **13** instead of the oxygenated methylenes found in compounds **3**−**11**. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) of **12** were similar to those of rabdoternin F.[13] They differ from each other only in the locations of the hydroxyls present. The hydroxyls were assigned at C-6, C-7, C-11, and C-14 in **12** by the following key HMBC NMR spectroscopic correlations: from H-6 to C-4, C-5, and C-7; from H-11 to C-9 and C-12; and from H-14 to C-9, C-12, C-15, and C-16. Similarly, a methoxyl could be placed at C-20 due to the HMBC correlation between OMe and C-20. The 20*S* configuration was verified from ROESY correlation between H-20 and Me-19. Therefore, compound **12** was characterized as (20*S*)-6β,7β,11β,14β-tetrahydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. For compound **13**, an acetyl group occurred at C-11 when compared with **12**, which resulted in downfield shifts of C-11 and upfield shifts of C-9 and C-12 in its ^{13}C NMR spectrum (Table 3). Therefore, compound **13** was elucidated as (20*S*)-6β,7β,14β-trihydroxy-11β-acetoxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

The absolute configurations of the new compounds were proposed from their CD spectra or on biogenetic grounds. Compounds **3**, **4**, **6**−**8**, and **12** showed a first negative Cotton effect at 335−340 nm in their CD spectra, corresponding to an enone system.[20] Compound **11** displayed a negative Cotton effect at 303 nm in its CD spectrum, consistent with a saturated ketone.[21,22] The other six new compounds (**1**, **2**, **5**, **9**, **10**, and **13**) were elucidated as *ent*-kaurane diterpenoids on biogenetic grounds.[1]

All of the isolates **1**−**36** were evaluated for their cytotoxic activity against the K562 (chronic myelogenous leukemia), MKN45 (stomach adenocarcinoma), and HepG2 (hepatocellular carcinoma) human cell lines. Among these diterpenoids, **1**, **2**, **11**, **16**−**19**, **23**, **26**−**28**, **30**, and **32** were found to be the most cytotoxic for one or more cell lines. Compound **1** exhibited an IC_{50} value of 0.04 μM against the K562 cell line. In addition, several of these compounds showed some selective cytotoxic activity (Table 4).

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Electrospray-ionization (ESIMS), high-resolution electrospray-ionization (HRESIMS), and fast atom bombardment (FABMS) mass spectra were acquired on an API QSTAR time-of-flight mass spectrometer and a VG Autospec-3000 mass spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a diode-array detector and a Zorbax SB-C_{18} (Agilent, 9.4 mm × 25 cm) column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatographic apparatus with a Shimadzu PRC-ODS (K) column (34 mm × 15 cm). Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), silica gel H (60 μm, Qingdao Marine Chemical Factory), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), and MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). All solvents including petroleum ether (60−90 °C) were distilled prior to use.

Plant Material. The leaves of *Isodon xerophilus* were collected in Yuanyang County, Yunnan Province, People's Republic of China, in August 2001. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 01082815) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Cell Cultures. Chronic myelogenous leukemia (K562), stomach adenocarcinoma (MKN45), and hepatocellular carcinoma (HepG2) human cell lines were obtained from Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI1640 medium with hormone-free 15% heat-inactivated FBS (fetal bovine serum). In each case, 2 mM glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin were added.

Cytotoxicity Assay. K562 cells were seeded into 96-well plates at an initial density of 5×10^4 cells/mL, and MKN45 and HepG2 cells were seeded at 4×10^4 cells/mL. After incubation with the indicated concentrations of compounds (10^2−10^{-2} μM) for 48 h, cell viability was assayed at 450 and 650 nm on Cell Counting Kit-8 (CCK-8; Dojindo, Gaithersburg, MD).[23] Pure compounds were tested against MKN45 and HepG2 cell lines using established protocols.[24] Testing against the K562 cell line was carried out according to established protocols.[25]

Extraction and Isolation. The air-dried, milled plant material (7.5 kg) was soaked with acetone (3 × 12 L, each 3 days) at room temperature and filtered. The filtrate was evaporated in vacuo to afford a residue, which was dissolved in H_2O (4 L) and then extracted with petroleum ether (3 × 3 L) and ethyl acetate (3 × 4 L), sequentially. The EtOAc extract (504 g) was decolorized using MCI gel, eluted with 90% MeOH−H_2O, to yield a yellowish gum (423 g). The gum was separated on a silica gel column, eluted with $CHCl_3$−Me_2CO (1:0 → 0:1, gradient system), to obtain seven fractions, A−G. Separation of fraction B (33.4 g) by silica gel column chromatography, eluted with petroleum ether−acetone (6:1 → 2:1, gradient system), yielded **27** (103.5 mg) and **32** (305.2 mg) and mixtures B1 (7.8 g) and B2 (19.2 g). Fraction B1 was subjected to RP-18 column chromatography (40% → 80% MeOH−H_2O, gradient system) to afford fractions B11 (2.3 g) and B12 (4.0 g) and **22** (17.1 mg). Compound **36** (820.5 mg) was obtained from B11 by silica gel column chromatography (petroleum ether−2-propanol, 12:1). Part of B12 (1.0 g) was purified by silica gel column chromatography (petroleum ether−2-propanol, 15:1 → 5:1, gradient system) and then by preparative HPLC (55% MeOH−H_2O) to give **23** (37.5 mg) and **35** (40.3 mg). Fraction B2 was further chromatographed over a RP-18 column (30% → 80% MeOH−H_2O, gradient system) to afford fractions B21 (11.0 g), B22 (970.0 mg), and B23 (48.4 mg). Subsequently, compounds **30** (2.5 g), **10** (52.8 mg), and **21** (15.6 mg) were purified by silica gel column chromatography from fraction B21 (petroleum ether−2-propanol, from 20:1 to 10:1), B22 (petroleum ether−acetone, from 5:1 to 4:1), and B23 ($CHCl_3$−Me_2CO, from 30:1 to 20:1), respectively. Fraction C (112 g) was applied to a silica gel column, eluted with a gradient system (petroleum ether−acetone, 4:1 → 1:1), to afford four main subfractions, C1−C4. Compounds **1** (12.7 mg), **13** (2.1 mg), and **30** (5.2 g) were obtained from C1 (28.5 g) by RP-18 column chromatography eluted with a gradient system (30% → 70% MeOH−H_2O). Part of C2 (558.0 mg) was separated using a silica gel column ($CHCl_3$−MeOH, 50:1 → 20:1, gradient system), then by preparative HPLC (40% MeOH−H_2O), to give **20** (18.6 mg). A solid residue (MA, 1.3 g) was precipitated

from C3. Part of MA (120.8 mg) was chromatographed by preparative HPLC (55% MeOH−H_2O) to yield **15** (36.3 mg), **16** (22.1 mg), **17** (6.4 mg), and **31** (7.1 mg). Compound **33** (232.7 mg) was acquired from the remainder of MA (15.8 g) by repeated column chromatography. Fraction C4 (33.0 g) was subjected to a silica gel column chromatography ($CHCl_3$−MeOH, 70:1 → 30:1, gradient system). Compound **11** (10.0 mg) was obtained by elution of $CHCl_3$−MeOH (50:1) and was finally purified by semipreparative HPLC (35% MeCN−H_2O). Compounds **14** (11.8 g) and **19** (13.6 g) were crystallized from fractions D (24.6 g) and E (95.2 g), respectively. The mother liquid of **19** was chromatographed on a silica gel column, using a petroleum ether−acetone (3:1 → 1:1) gradient system as eluants, to afford E1 (7.4 g), E2 (34.5 g), and E3 (25.0 g). Fraction E1 was chromatographed over a RP-18 column (30% → 50% MeOH−H_2O) to give E11 (525.2 mg) and E12 (330.0 mg). Compounds **24** and **25** (42.2 mg), **26** (9.0 mg), **28** (64.5 mg), and **29** (59.2 mg) were purified from E11 by preparative HPLC (45% MeOH−H_2O). E12 was further separated by silica gel column chromatography and reversed-phase semipreparative HPLC to afford **2** (15.0 mg) and **12** (27.3 mg). Compound **4** (28.5 mg) was obtained from E3 by RP-18 column chromatography (30% → 50% MeOH−H_2O) and preparative HPLC (20% MeCN−H_2O). Fraction F (28.7 g) was subjected to column chromatography over a RP-18 column (30% → 60% MeOH−H_2O, gradient system) to provide F1 (650.0 mg) and F2 (1.3 g). Compound **18** (3.2 mg) was purified from F1 by silica gel column chromatography, eluted with petroleum ether−acetone (from 3:1 to 1:1) and $CHCl_3$−MeOH (25:1), successively, and F2 was applied to a silica gel column and developed by gradient elution ($CHCl_3$−MeOH, 30:1 → 10:1) to afford F21 (158.2 mg), F22 (117.8 mg), and F23 (53.5 mg). Preparative HPLC (20% MeOH−H_2O for F21, 30% MeOH−H_2O for F22, and 15% MeCN−H_2O for F23) was used to purify **3** (40.0 mg), **5** (3.2 mg), **6** (10.1 mg), **7** (15.6 mg), **8** (7.2 mg), **9** (3.3 mg), and **34** (12.5 mg) from F21−F23.

Xerophilusin I (1): colorless needles; mp 219−220 °C; $[\alpha]_D^{25}$ −40.0 (*c* 1.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 241 (3.77) nm; IR (KBr) ν_{max} 3434 (br), 2965, 2948, 2927, 2907, 2869, 2847, 1709 (br), 1647, 1438, 1408, 1390, 1367, 1299, 1260, 1199, 1133, 1095, 1065, 988, 957, 757, 730, 532 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 385 $[M + Na]^+$, 747 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 385.1635 (calcd for $C_{20}H_{26}O_6Na$ $[M + Na]^+$, 385.1627).

Xerophilusin II (2): white needles; mp 195−197 °C; $[\alpha]_D^{26}$ +50.0 (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (3.84) nm; IR (KBr) ν_{max} 3440 (br), 2929−2871 (br), 1726−1697 (br), 1644, 1393, 1370, 1287, 1256, 1161, 1097, 1042, 1003, 946, 537 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 385 $[M + Na]^+$, 747 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 385.1631 (calcd for $C_{20}H_{26}O_6Na$ $[M + Na]^+$, 385.1627).

Xerophilusin III (3): white, amorphous powder; $[\alpha]_D^{26}$ −97.1 (*c* 1.46, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (3.88) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 340 (−1.9), 236 (−7.2), 206 (+5.7); IR (KBr) ν_{max} 3528−3312 (br), 2970, 2953, 2896, 2875, 1700, 1636, 1458, 1439, 1326, 1269, 1175, 1083, 1069, 1026, 1014, 992, 962, 933, 916, 764, 632 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 403 $[M + Na]^+$, 783 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 403.1725 (calcd for $C_{20}H_{28}O_7Na$ $[M + Na]^+$, 403.1732).

Xerophilusin IV (4): white, amorphous powder; $[\alpha]_D^{25}$ −80.4 (*c* 0.84, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.91) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 335 (−1.7), 239 (−6.6), 208 (+5.3); IR (KBr) ν_{max} 3375 (br), 2933, 2873, 1709, 1643, 1448, 1213, 1180, 1085, 1060, 1021, 959, 911, 683, 635, 580 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 387 $[M + Na]^+$, 751 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 387.1792 (calcd for $C_{20}H_{28}O_6Na$ $[M + Na]^+$, 387.1783).

Xerophilusin V (5): white, amorphous powder; $[\alpha]_D^{26}$ −86.4 (*c* 0.71, C_5H_5N); UV (MeOH) λ_{max} (log ϵ) 204 (3.80) nm; IR (KBr) ν_{max} 3450−3300 (br), 2959, 2931, 2899, 2865, 1631, 1451, 1346, 1260, 1211, 1190, 1082, 1022, 979, 893, 781, 744, 661, 640, 571, 522 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 405 $[M + Na]^+$, 787 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 405.1882 (calcd for $C_{20}H_{30}O_7Na$ $[M + Na]^+$, 405.1889).

Xerophilusin VI (6): white, amorphous powder; $[\alpha]_D^{26}$ −116.1 (*c* 0.98, C_5H_5N); UV (MeOH) λ_{max} (log ϵ) 238 (3.71) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 340 (−1.0), 243 (−9.5), 209 (+4.1); IR (KBr) ν_{max} 3375 (br), 2945 (br), 1735, 1714, 1644, 1452, 1376, 1237, 1190, 1087, 1035, 987, 969, 927, 639 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 461 $[M + Na]^+$, 899 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 461.1781 (calcd for $C_{22}H_{30}O_9Na$ $[M + Na]^+$, 461.1787).

Xerophilusin VII (7): white, amorphous powder; $[\alpha]_D^{25}$ −84.9 (*c* 1.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.73) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 340 (−1.8), 244 (−7.8), 202 (+4.8); IR (KBr) ν_{max} 3383 (br), 2939, 1715 (br), 1643, 1450, 1395, 1374, 1243, 1195, 1082, 1032, 988, 600 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 461 $[M + Na]^+$, 899 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 461.1783 (calcd for $C_{22}H_{30}O_9Na$ $[M + Na]^+$, 461.1787).

Xerophilusin VIII (8): white, amorphous powder; $[\alpha]_D^{26}$ −92.4 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (3.74) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 340 (−2.0), 245 (+3.2), 202 (+7.8); IR (KBr) ν_{max} 3418 (br), 2940, 1718, 1706, 1641, 1474, 1398, 1367, 1300, 1269, 1253, 1083, 1066, 1033, 619, 478 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 461 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 461.1789 (calcd for $C_{22}H_{30}O_9Na$ $[M + Na]^+$, 461.1787).

Xerophilusin IX (9): white, amorphous powder; $[\alpha]_D^{26}$ −69.8 (*c* 0.63, C_5H_5N); UV (MeOH) λ_{max} (log ϵ) 238 (3.69) nm; IR (KBr) ν_{max} 3406 (br), 2953, 2936, 2876, 1709, 1643, 1446, 1190, 1083, 1046, 1022, 951, 645, 581 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 403 $[M + Na]^+$, 783 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 403.1726 (calcd for $C_{20}H_{28}O_7Na$ $[M + Na]^+$, 403.1732).

Xerophilusin X (10): white, amorphous powder; $[\alpha]_D^{18}$ +21.0 (*c* 0.83, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (2.82) nm; IR (KBr) ν_{max} 3433 (br), 2938, 2874, 1736 (br), 1637, 1451, 1369, 1263, 1244, 1229, 1052, 1028, 969, 918, 573, 537 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive FABMS *m/z* 451 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 473.2154 (calcd for $C_{24}H_{34}O_8Na$ $[M + Na]^+$, 473.2151).

Xerophilusin XI (11): white, amorphous powder; $[\alpha]_D^{25}$ −27.5 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.29) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 303 (−1.8), 257 (+1.2), 214 (−4.5); IR (KBr) ν_{max} 3410−3261 (br), 2947, 2869, 1728 (br), 1450, 1365, 1242, 1176, 1088, 1061, 1028, 971, 958, 574 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive FABMS *m/z* 439 $[M + H]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 461.2156 (calcd for $C_{23}H_{34}O_8Na$ $[M + Na]^+$, 461.2151).

Xerophilusin XII (12): colorless crystals; mp 210−211 °C; $[\alpha]_D^{26}$ −43.0 (*c* 1.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (3.81) nm; CD (MeOH) λ_{max} nm ($\Delta\epsilon$) 339 (−1.7), 239 (−11.1), 203 (+10.3); IR (KBr) ν_{max} 3333 (br), 2935−2873 (br), 1714, 1643, 1449, 1201, 1173, 1105, 1092, 1053, 1028, 985, 953, 941, 715, 597, 579 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 417 $[M + Na]^+$, 811 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 417.1887 (calcd for $C_{21}H_{30}O_7Na$ $[M + Na]^+$, 417.1889).

Xerophilusin XIII (13): white, amorphous powder; $[\alpha]_D^{26}$ −36.8 (*c* 1.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 241 (3.80) nm; IR (KBr) ν_{max} 3343 (br), 2890 (br), 1735, 1712, 1644, 1440, 1211, 1175, 1092, 1030, 975, 950, 715, 597, 579 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 3; positive ESIMS *m/z* 459 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 459.1990 (calcd for $C_{23}H_{32}O_8Na$ $[M + Na]^+$, 459.1995).

Acknowledgment. Financial support of the Natural Science Foundation of Yunnan Province (No. 2004C0008Z) and the National Natural Science Foundation of China (No. 20502026 to Q.-B.H.) is gratefully acknowledged.

References and Notes

(1) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673−698.
(2) Leung, C. H.; Grill, S. P.; Lam, W.; Gao, W. L.; Sun, H. D.; Cheng, Y. C. *Mol. Pharmacol.* **2006**, *70*, 1946−1955.
(3) Wang, L.; Zhao, W. L.; Yan, J. S.; Liu, P.; Sun, H. P.; Zhou, G. B.; Weng, Z. Y.; Wu, W. L.; Weng, X. Q.; Sun, X. J.; Chen, Z.; Sun, H. D.; Chen, S. J. *Cell Death Differ.* **2007**, *14*, 306−317.
(4) Wu, C. Y.; Li, H. W. In *Flora Republicae Popularis Sinicae*; Beijing Academic Press: Beijing, 1987; Vol. 66, p 494.
(5) 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.
(6) Hou, A. J.; Jiang, B.; Yang, H.; Zhao, Q. S.; Lin, Z. W.; Sun, H. D. *Acta Bot. Yunnan.* **2000**, *22*, 197−200.
(7) 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.

(8) Li, S. H.; Niu, X. M.; Peng, L. Y.; Zhang, H. J.; Yao, P.; Sun, H. D. *Planta Med.* **2002**, *68*, 944−946.
(9) Hou, A. J.; Yang, H.; Liu, Y. Z.; Zhao, Q. S.; Lin, Z. W.; Sun, H. D. *Chin. J. Chem.* **2001**, *19*, 365−370.
(10) Zhang, R. P.; Zhang, H. J.; Lin, Z. W.; Zhen, Y. L.; Sun, H. D. *Phytochemistry* **1992**, *31*, 4237−4240.
(11) Shen, X. Y.; Isogai, A.; Furihata, K.; Kaniwa, H.; Sun, H. D.; Suzuki, A. *Phytochemistry* **1989**, *28*, 855−858.
(12) Li, G. Y.; Wang, Y. L. *Acta Pharm. Sin.* **1984**, *19*, 590−592.
(13) Takeda, Y.; Takeda, K.; Fujita, T.; Sun, H. D.; Minami, Y. *Phytochemistry* **1994**, *35*, 1513−1516.
(14) Huang, S. X.; Zhou, Y.; Pu, J. X.; Li, R. T.; Li, X.; Xiao, W. L.; Lou, L. G.; Han, Q. B.; Ding, L. S.; Peng, S. L.; Sun, H. D. *Tetrahedron* **2006**, *62*, 4941−4947.
(15) Fujita, E.; Taoka, M.; Shibuya, M., Fujita, T.; Shingu, T. *J. Chem. Soc., Perkin Trans.* **1973**, *1*, 2277−2281.
(16) Cheng, P. Y.; Lin, Y. L.; Xu, G. Y. *Acta Pharm. Sin.* **1984**, *19*, 593−598.
(17) Fujita, K.; Node, M.; Sai, M.; Fujita, E.; Shingu, T.; Watson, W. H.; Grossie, D. A.; Zabel, V. *Chem. Pharm. Bull.* **1989**, *37*, 1465−1469.
(18) Fujita, T.; Sun, H. D.; Yoshio, T.; Yoshinori, M.; Teruyoshi, M.; Lin, Z. W.; Xu, Y. L. *J. Chem. Soc., Chem. Commun.* **1985**, 1738−1739.
(19) Na, Z.; Li, S. H.; Xiang, W.; Zhao, A. H.; Li, C. M.; Sun, H. D. *Chin. J. Chem.* **2002**, *20*, 884−886.
(20) Hong, S. S.; Lee, S. A.; Han, X. H.; Jin, H. Z.; Lee, J. H.; Lee, D.; Lee, J. J.; Hong, J. T.; Kim, Y.; Ro, J. S.; Hwang, B. Y. *J. Nat. Prod.* **2007**, *70*, 632−636.
(21) Takeda, Y.; Ichihara, T.; Yamasaki, K.; Otsuka, H.; Utsumi, H. *Phytochemistry* **1993**, *32*, 145−150.
(22) Isogai, A.; Shen, X. Y.; Furihata, K; Kaniwa, H.; Sun, H. D.; Suzuki, A. *Phytochemistry* **1989**, *28*, 2427−2432.
(23) Wang, Y. Y.; Zhou, G. B.; Yin, T.; Chen, B.; Shi, J. Y.; Liang, W. X.; Jin, X. L.; You, J. H.; Yang, G.; Shen, Z. X.; Chen, J.; Xiong, S. M.; Chen, G. Q.; Xu, F.; Liu, Y. W.; Chen, Z.; Chen, S. J. *Proc. Natl. Acad. Sci.* **2005**, *102*, 1104−1109.
(24) Seo, E.-K.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Mukherjee, R.; Farnsworth, N. R.; Kinghorn, A. D. *Phytochemistry* **2000**, *55*, 35−42.
(25) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M. *J. Nat. Prod.* **1993**, *56*, 30−38.

NP070205M