Cytotoxic Triterpene Dilactones from the Stems of *Kadsura ananosma*

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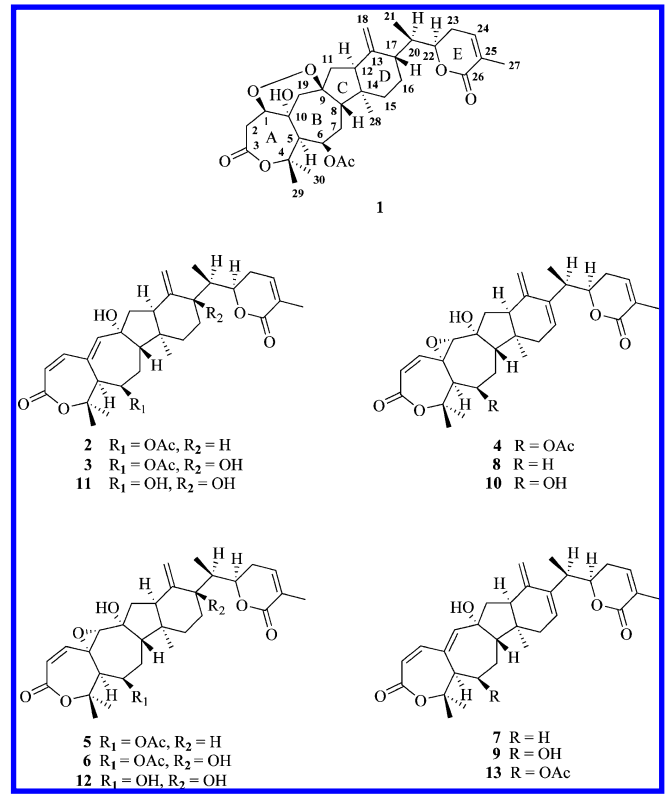
Six new triterpene dilactones with a rare rearranged pentacyclic skeleton, longipedlactones K−P (**1**−**6**), and seven known analogues (**7**−**13**) were isolated from the stems of *Kadsura ananosma*. Compound **1** was found to possess a unique peroxide bridge between C-1 and C-9 in rings A and B. The structures of these new compounds were established on the basis of spectroscopic data analysis, especially of their 2D NMR spectra. In the evaluation of the in vitro cytotoxicity of these compounds against a small panel of human cancer cell lines, compounds **3**, **7**, **9**, and **13** were found to be the most potent against HL-60 acute leukemia cell.

Phytochemically, plants of the genus *Kadsura* (Magnoliaceae) have been reported to contain dibenzocyclooctadiene lignans[1−4] and lanostane[5,6] and cycloartane triterpenoids,[7,8] and such compounds have exhibited antitumor,[9] anti-HIV,[10,11] and cytotoxic activities.[12−14] In the course of research on the bioactive constituents of members of this genus, several triterpenoids and lignans with unprecedented new skeletons, such as kadsuphilactone A,[15] taiwankadsurins A−C,[16] kadlongilactones A and B,[12] and longipedlacones A−I,[13] have been discovered in recent years.

Kadsura ananosma Kerr is a liana indigenous to Yunnan Province, People's Republic of China.[17] Previous work has led to the isolation of lanostane triterpenoids, sesquiterpenoids, and lignans from this plant.[14,18−21] We have reinvestigated the stems of *K. ananosma*. As a result, six new triterpene dilactones, named longipedlactones K−P (**1**−**6**), and seven known triterpenoids, longipedlactones A (**7**), D (**8**), and F−J (**9**−**13**),[13,22] were isolated. In this paper, the isolation, structure elucidation, and cytotoxicity of these compounds are reported herein.



Results and Discussion

A 70% aqueous acetone extract of the stems of *K. ananosma* was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography and HPLC to afford six new triterpenoids, longipedlactones K−P (**1**−**6**), together with seven known compounds. The structures of the known compounds were identified as longipedlactones A (**7**), D (**8**), and F−J (**9**−**13**) by comparing their spectroscopic data (MS, ^{1}H NMR, ^{13}C NMR) with reported values.[13,22]

Longipedlactone K (**1**) was isolated as an amorphous powder and gave a molecular formula of $C_{32}H_{44}O_9$, as determined by HRESIMS ($[M + Na]^+$ *m/z* 595.2898, calcd 595.2883), requiring 11 degrees of unsaturation. The IR absorptions of **1** indicated the presence of hydroxy (3433 cm^{-1}) and lactone (1721 cm^{-1}) functional groups. The ^{1}H NMR spectrum showed signals for an olefinic proton signal (δ_H 6.70), an exocyclic methylene (δ_H 4.86, d, $J = 1.2$ Hz and δ_H 4.80, d, $J = 1.2$ Hz), three oxygenated methine protons (δ_H 4.12, 5.78, 4.65), a secondary methyl group, and five tertiary methyl groups (Table 1). The ^{13}C NMR and DEPT spectra exhibited 32 carbon signals, consisting of five methyls, eight methylenes, nine methine carbons (including three oxidized carbons), eight quaternary carbons (including three oxidized, two olefinic, and two ester groups), and an acetyl group (170.6, C, and 21.3, CH_3). These data were consistent with the elemental formula obtained from the HRESIMS and suggested that **1** is a pentacyclic triterpene, possessing a longipedlactone skeleton.[13] Analysis of its ^{1}H and ^{13}C NMR spectra indicated that **1** shows a close resemblance with longipedlactone I (**12**). Differences between **1** and **12** were the absence of a double bond (δ_C 146.7, CH, C-1 and δ_C 126.8, CH, C-2) and a trisubstituted epoxide (δ_C 61.0, C, C-10 and δ_C 66.7, CH, C-19) in **12** and the presence of an oxygenated methine (δ_C 83.3, CH, C-1), an oxygenated quaternary carbon (δ_C 73.4, C, C-10), and two methylenes (δ_C 36.5, CH_2, C-2 and δ_C 50.1, CH_2, C-19) (Table 2) in **1**. This was further confirmed by HMBC correlations from H-1 to C-3, C-5, and C-19 and from H-19 to C-8 and C-9 (Figure 1). Apart from two double bonds, three carbonyl groups, and the pentacyclic skeleton, there was one remaining element of unsaturation for **1**. From the HRESIMS, it was deduced

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Table 1. ^{1}H NMR Data of Compounds **1**−**3** in C_5D_5N (400 MHz, δ in ppm, J in Hz)

H	**1**[a]	**2**	**3**
1	4.12 (t, 4.2)	6.85 (d, 12.4)	6.83 (d, 12.3)
2α	2.85 (overlap)	6.04 (d, 12.4)	6.04 (d, 12.3)
2β	3.40 (dd, 14.8, 3.7)		
5	2.16 (d, 3.5)	4.48 (s)	4.50 (s)
6	5.78 (dd, 6.8, 4.2)	6.11 (br s)	6.09 (br s)
7α	1.72 (overlap)	2.53−2.45 (m)	2.47 (overlap)
7β	2.10 (overlap)	2.17 (overlap)	2.17 (overlap)
8	2.26 (overlap)	2.18 (overlap)	2.46 (overlap)
11α	2.13 (overlap)	2.23 (overlap)	2.20 (overlap)
11β	1.77 (overlap)	1.92 (overlap)	2.03 (overlap)
12	2.33 (overlap)	2.91 (dd, 13.2, 6.8)	3.12 (dd, 13.2, 6.8)
15α	1.20−1.15 (m)	0.94−0.86 (m)	1.39−1.37 (m)
15β	1.90−1.86 (m)	1.25−1.22 (m)	1.39−1.37 (m)
16α	1.71 (overlap)	1.44−1.41 (m)	2.06 (overlap)
16β	1.64 (overlap)	1.44−1.41 (m)	1.74−1.69 (m)
17	2.08 (overlap)	2.05 (overlap)	
18a	4.86 (d, 1.2)	5.01 (d, 1.7)	5.57 (s)
18b	4.80 (d, 1.2)	4.75 (d, 1.7)	5.27 (s)
19α	1.96 (d, 14.0)	6.62 (s)	6.57 (s)
19β	2.58 (d, 14.0)		
20	2.24 (overlap)	2.05 (overlap)	2.24 (overlap)
21	0.86 (d, 6.9)	0.98 (d, 5.3)	1.25 (d, 6.7)
22	4.65 (br d, 12.2)	4.44 (br d, 13.1)	4.89 (br d, 12.7)
23α	2.26 (overlap)	1.95 (overlap)	2.75−2.70 (m)
23β	2.32 (overlap)	2.21 (overlap)	2.45 (overlap)
24	6.70 (d, 6.0)	6.50 (d, 5.7)	6.50 (d, 4.2)
27	1.81 (s)	1.97 (s)	1.99 (s)
28	0.82 (s)	1.09 (s)	1.07 (s)
29	1.81 (s)	1.47 (s)	1.46 (s)
30	1.43 (s)	1.50 (s)	1.49 (s)
HO-9		6.80 (s)	
HO-10	4.41 (s)		
HO-17			5.94 (s)
AcO-6	1.94 (s)	2.13 (s)	2.10 (s)

[a] Measured in $(CD_3)_2CO$.

that a peroxy bridge is present in **1**. The HMBC correlations observed from the proton signal of OH to C-1, C-5, and C-10 indicated that a hydroxy group is located at C-10, so C-9 and C-1 are connected through a peroxy bridge. ^{1}H NMR signals indicated that an AB spin system doublet (δ_H 2.58, d, J = 14.0 Hz and δ_H 1.96, d, J = 14.0 Hz, H_2-19) appeared in the low-field region, due to the induction effect of the neighboring peroxy bridge.[23] In addition, a hydroxy group at C-6 (δ_C 66.1, CH) in **12** was replaced by an acetyl group (δ_C 69.9, CH) in **1**, and the presence of the methine carbon signal at δ_C 44.6 in **1** indicated that the OH group at C-17 in **12** was absent. HMBC correlations were observed from H-6 (δ_H 5.78) to the acetyl carbonyl (δ_C 170.6) and from H-17 (δ_H 2.08) to C-12, 15, and 18. The ROESY (Figure 1) correlations of HO-10 with H-5α and H_2-19 indicated that HO-10 is α-oriented. On the basis of a computer-generated 3D structure obtained by CHEM 3D ULTRA V 10.0 with MM2 force-field calculations for energy minimization, if the relative configuration of the peroxy bridge was α-oriented (Figure 1a), the interatomic distance between H-1 and HO-10α and between H-8β and H-11β would both be approximately 3.60 Å, so the ROESY correlations of H-1 with HO-10α and that of H-8β with H-11β could not be observed. However, correlations between H-1 and HO-10α and between H-8β and H-11β were clearly observed in the ROESY spectrum (Figure 1b), which indicated that the peroxy bridge in **1** is β-oriented. This was further assigned by the shortest interatomic distances being approximately 2.28 Å between H-1 and HO-10α and 2.68 Å between H-8β and H-11β. The other details of the relative configuration of **1** were determined to be the same as **12** (Figure 1). Therefore, the structure of **1** was determined as shown, and this has been named longipedlactone K.

Longipedlactone L (**2**) was isolated as an amorphous powder. The HRESIMS ($[M + Na]^+$ m/z 561.2843, calcd 561.2828) indicated its molecular formula to be $C_{32}H_{42}O_7$, requiring 12 degrees of unsaturation. The UV spectrum of **2** showed absorption maxima at 228, 235, and 276 nm, indicating the occurrence of one or more conjugated systems. The IR spectrum showed the presence of a hydroxy group (3435 cm^{-1}) and lactone groups (1736 and 1717 cm^{-1}). Analysis of the ^{1}H NMR, ^{13}C NMR, and DEPT data revealed the occurrence of six methyls (one secondary methyl and five tertiary methyls), six methylenes (one olefinic), 11 methines (two oxygenated and four olefinic), and nine quaternary carbons (three carbonyls, two oxygenated and three olefinic). Analysis of the NMR spectroscopic data of **2** showed this compound to have close structural resemblance to **13**, with these compounds differing only in the absence of a double bond between C-16 and C-17 (δ_C 125.8, CH and 140.2, C) in **2**, which were replaced by a methylene carbon (δ_C 24.8, CH_2, C-16) and a methine carbon (δ_C 43.4, CH, C-17), respectively. HMBC correlations from H-17 to C-12, C-18, and C-21 were observed (Figure 2). In the ROESY spectrum, the correlation between H-17 and H-18b indicated that H-17 is β-oriented (Figure 2). The rest of the relative configuration of **2** was determined to be the same as that of **13** from further analysis of the ROESY spectrum. Consequently, the structure of **2** (longipedlactone L) was established as shown.

Longipedlactone M (**3**) was isolated as an amorphous powder with the molecular formula $C_{32}H_{42}O_8$, as determined by HRESIMS ($[M + Na]^+$ m/z 577.2777, calcd 577.2777). Comparison of the NMR data of **3** and **2** indicated these to be almost identical. The presence of a quaternary carbon signal (δ_C 75.1, C, C-17) in **3** suggested that C-17 was substituted by a hydroxy group. This was confirmed by HMBC correlations from H-12, H-15, H-16, H-18, H-21, and HO-17 to C-17. The ROESY correlations of HO-17 with H-18b and H-16β indicated that HO-17 adopts a β-orientation. The relative configuration of **3** was assigned as being the same as that of **2**. Hence, compound **3** (longipedlactone M) was elucidated as shown.

Longipedlactone N (**4**) was isolated as an amorphous powder. The molecular formula, $C_{32}H_{40}O_8$, was deduced from the HRESIMS ($[M + Na]^+$ m/z 575.2629, calcd 575.2620). Detailed comparison of the NMR data of **4** with those of **13** indicated that these two compounds are closely comparable. The only significant difference was that a double bond (δ_C 140.4, C-10; 148.2, C-19) in **13** was replaced by a trisubstituted epoxide (δ_C 58.7, C, C-10; 66.7, CH, C-19) in **4** (Table 3). This assignment was in accord with the observed changes of chemical shifts for C-1 and C-2, from δ_C 145.7 and 119.2 in **13** to δ_C 145.5 and 124.9 in **4**, and confirmed by the HMBC correlations from H-2 and H-5 to C-1 and C-10 and from H-19 to C-1, C-9, C-10, and C-11. The ROESY correlations, from H-19 to H-8β and H-11β, indicated an α-orientation of the epoxy ring. Accordingly, longipedlactone N was assigned as **4**.

Longipedlactone O (**5**) was obtained as an amorphous powder. Its molecular formula, $C_{32}H_{42}O_8$, was determined by the $[M + Na]^+$ ion peak at m/z 577.2792 (calcd 577.2777). The ^{1}H and ^{13}C NMR data of **5** were similar to those of **4**, except for the absence of signals for a double bond (δ_C 124.3, CH, C-16 and δ_C 138.2, C, C-17) in **4** and evidence for a methylene (δ_C 24.7, CH_2) and a methine (δ_C 43.5, CH) in **5**. The HMBC and ^{1}H−^{1}H COSY spectra were used to support the above deductions. The ROESY correlations of HO-17 with H-18b and H-21β indicated that HO-17 is in a β-orientation. The relative configuration of **5** was determined otherwise to be the same as that of **4**. Thus, the structure of longipedlactone O was proposed as **5**.

Longipedlactone P (**6**), isolated as an amorphous powder, gave the molecular formula $C_{32}H_{42}O_9$ by HRESIMS at m/z 593.2709 ($[M + Na]^+$, calcd 593.2726). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **6** showed similarities with the analogous values for compound **3**. However, signals for a trisubstituted epoxide (δ_C 59.9, C, C-10 and δ_C 66.9, CH, C-19) were evident in **6**, instead of a double bond (δ_C 140.4, C, C-10 and δ_C 147.6, CH, C-19) as in **3**. The HMBC correlations from H-2 and H-5 to C-10 and from H-19

Table 2. ^{13}C NMR Data of Compounds **1**−**6** in C_5D_5N (δ in ppm)

carbon	**1**[a]	**2**[b]	**3**[b]	**4**[c]	**5**[b]	**6**[b]
1	83.3 (CH)	145.6 (CH)	145.7 (CH)	145.5 (CH)	146.5 (CH)	146.5 (CH)
2	36.5 (CH_2)	119.4 (CH)	119.3 (CH)	124.9 (CH)	126.1 (CH)	126.1 (CH)
3	169.3 (C)	166.5 (C)	166.6 (C)	164.8 (C)	166.2 (C)	166.5 (C)
4	82.7 (C)	79.8 (C)	79.8 (C)	79.9 (C)	81.0 (C)	81.0 (C)
5	57.5 (CH)	50.5 (CH)	50.4 (CH)	50.7 (CH)	52.0 (CH)	51.9 (CH)
6	69.9 (CH)	69.0 (CH)	69.0 (CH)	68.7 (CH)	69.9 (CH)	69.8 (CH)
7	30.7 (CH_2)	32.5 (CH_2)	32.5 (CH_2)	29.0 (CH_2)	30.9 (CH_2)	31.0 (CH_2)
8	49.0 (CH)	47.4 (CH)	48.0 (CH)	48.1 (CH)	47.6 (CH)	48.6 (CH)
9	92.2 (C)	79.3 (C)	79.3 (C)	76.1 (C)	77.6 (C)	77.7 (C)
10	73.4 (C)	140.4 (C)	140.4 (C)	58.7 (C)	59.9 (C)	59.9 (C)
11	44.1 (CH_2)	47.9 (CH_2)	48.0 (CH_2)	49.6 (CH_2)	47.7 (CH_2)	47.7 (CH_2)
12	52.3 (CH)	53.7 (CH)	53.9 (CH)	51.7 (CH)	54.3 (CH)	54.7 (CH)
13	147.6 (C)	148.0 (C)	153.1 (C)	146.4 (C)	148.0 (C)	153.0 (C)
14	47.1 (C)	44.2 (C)	44.1 (C)	41.4 (C)	43.6 (C)	43.6 (C)
15	32.8 (CH_2)	35.1 (CH_2)	34.1 (CH_2)	36.1 (CH_2)	35.2 (CH_2)	35.1 (CH_2)
16	25.5 (CH_2)	24.8 (CH_2)	34.8 (CH_2)	124.3 (CH)	24.7 (CH_2)	34.7 (CH_2)
17	44.6 (CH)	43.4 (CH)	75.1 (C)	138.2 (C)	43.5 (CH)	75.3 (C)
18	113.5 (CH_2)	115.5 (CH_2)	116.0 (CH_2)	108.1 (CH_2)	115.3 (CH_2)	115.6 (CH_2)
19	50.1 (CH_2)	147.7 (CH)	147.6 (CH)	66.7 (CH)	66.8 (CH)	66.9 (CH)
20	38.0 (CH)	41.0 (CH)	44.7 (CH)	38.1 (CH)	40.1 (CH)	43.2 (CH)
21	13.4 (CH_3)	13.4 (CH_3)	9.3 (CH_3)	14.5 (CH_3)	13.4 (CH_3)	9.6 (CH3)
22	80.1 (CH)	79.1 (CH)	79.5 (CH)	79.8 (CH)	79.2 (CH)	79.6 (CH)
23	24.5 (CH_2)	23.4 (CH_2)	27.0 (CH_2)	25.6 (CH_2)	23.5 (CH_2)	27.2 (CH_2)
24	140.4 (CH)	140.1 (CH)	141.4 (CH)	138.5 (CH)	140.1 (CH)	141.4 (CH)
25	128.4 (C)	128.1 (C)	127.7 (C)	127.0 (C)	128.0 (C)	127.6 (C)
26	166.1 (C)	166.3 (C)	166.6 (C)	165.0 (C)	166.1 (C)	166.1 (C)
27	17.0 (CH_3)	17.2 (CH_3)	17.1 (CH_3)	16.0 (CH_3)	17.2 (CH_3)	17.1 (CH_3)
28	22.3 (CH_3)	26.1 (CH_3)	25.7 (CH_3)	25.0 (CH_3)	25.6 (CH_3)	24.9 (CH_3)
29	31.4 (CH_3)	26.0 (CH_3)	26.0 (CH_3)	24.4 (CH_3)	25.4 (CH_3)	25.5 (CH_3)
30	32.6 (CH_3)	29.8 (CH_3)	29.8 (CH_3)	28.5 (CH_3)	29.6 (CH_3)	29.6 (CH_3)
OAc	170.6 (C)	170.5 (C)	170.6 (C)	168.8 (C)	170.0 (C)	170.1 (C)
	21.3 (CH_3)	21.2 (CH_3)	21.3 (CH_3)	20.0 (CH_3)	21.2 (CH_3)	21.3 (CH_3)

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

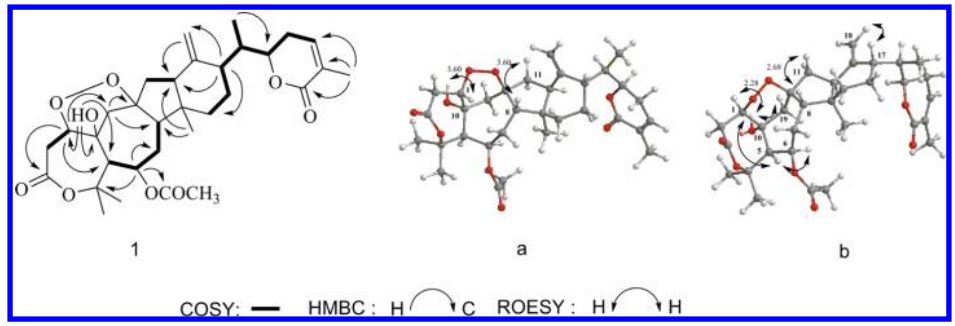

Figure 1. Key COSY, HMBC, and ROESY correlations of **1**.

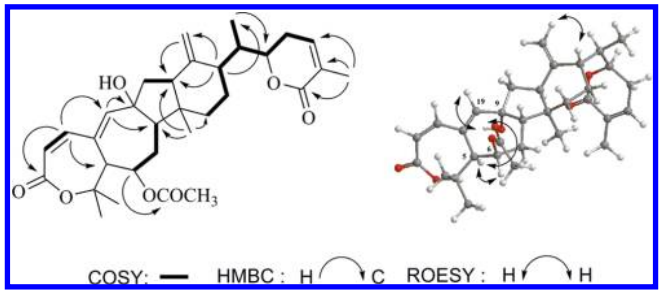

Figure 2. Key COSY, HMBC, and ROESY correlations of **2**.

to C-9, C-10, and C-11 verified that the epoxide group could be positioned between C-10 and C-19. The ROESY correlations from H-19 to H-8β and H-11β suggested that the epoxy ring is α-oriented, and the remaining relative configuration was confirmed to be the same as that of **3**. Thus, the structure of longipedlactone P was elucidated as **6**.

Compounds **1**−**4**, **6**−**10**, **12**, and **13** were assayed for their cytotoxicity against the HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3 human tumor cell lines by the MTT method,[24] and the results are shown in Table 4. The most broadly cytotoxic compound found was longipedlactone J (**13**), while compounds **2**, **3**, **7**, and **9** also showed some evidence of cytotoxicity against the HL-60 cell line. Due to limitations in the amounts available, compounds **5** and **11** were not tested in the bioassay used.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tensor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm × 25 cm) column. Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μM, Merck, Darmstadt, Germany), and MCI gel (75−150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were

Table 3. ^{1}H NMR Data of Compounds **4**−**6** in C_5D_5N (δ in ppm, J in Hz)

H	**4**[a]	**5**[b]	**6**[b]
1	6.01 (d, 12.0)	5.98 (d, 12.4)	5.94 (d, 12.4)
2	6.34 (d, 12.0)	6.39 (d, 12.4)	6.40 (d, 12.4)
5	3.22 (s)	3.22 (s)	3.25 (s)
6	5.92 (s)	5.97 (s)	5.98 (d, 6.2)
7α	2.17 (overlap)	2.15 (overlap)	2.28 (overlap)
7β	2.01 (overlap)	2.15 (overlap)	2.10 (overlap)
8	2.18 (overlap)	2.23 (overlap)	2.28 (overlap)
11α	2.59 (dd, 16.5, 9.5)	2.16 (overlap)	2.18 (overlap)
11β	2.15 (overlap)	2.02 (overlap)	2.12−2.06 (m)
12	3.07 (t, 10.5)	2.92 (dd, 13.0, 6.9)	3.18 (dd, 13.3, 6.5)
15α	1.78 (dd, 21.0, 3.5)	0.99 (overlap)	1.47 (overlap)
15β	2.03 (overlap)	1.36−1.31 (m)	1.60−1.53 (m)
16α	5.74 (s)	1.53−1.49 (m)	2.13 (overlap)
16β		1.53−1.49 (m)	1.82−1.76 (m)
17		2.05 (overlap)	
18a	5.14 (s)	5.04 (s)	5.61 (d, 2.5)
18b	4.93 (s)	4.77 (s)	5.29 (d, 2.5)
19	3.54 (s)	3.51 (s)	3.45 (s)
20	2.99 (t, 8.5)	2.14 (overlap)	2.71 (br s)
21	1.09 (d, 7.0)	0.94 (d, 6.4)	1.21 (d, 6.9)
22	4.48−4.44 (m)	4.47 (br d, 13.0)	4.98−4.94 (m)
23α	2.14 (overlap)	1.98 (overlap)	2.81−2.74 (m)
23β	2.15 (overlap)	2.20 (overlap)	2.52−2.44 (m)
24	6.45 (d, 5.8)	6.49 (d, 6.2)	6.48 (d, 6.2)
27	1.85 (s)	1.95 (s)	1.95 (s)
28	1.12 (s)	1.03 (s)	1.01 (s)
29	1.66 (s)	1.69 (s)	1.70 (s)
30	1.41 (s)	1.42 (s)	1.43 (s)
HO-9		6.84 (s)	6.88 (s)
AcO-6	2.07 (s)	2.20 (s)	2.13 (s)

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

Table 4. Cytotoxic Activities of Compounds **1**−**4**, **6**−**10**, **12**, and **13** against Tumor Cell Lines[a]

	HL-60	SMMC-7721	A-549	PANC-1	SK-BR-3
2	7.3	>10	>10	>10	>10
3	4.1	>10	>10	>10	>10
7	3.5	>10	>10	>10	>10
9	2.3	>10	>10	8.8	7.4
13	2.0	>10	>10	5.3	2.8
cisplatin	1.7	>10	>10	>10	>10

[a] Results are expressed as IC_{50} values in μM. Cell lines: HL-60 acute leukemia; SMMC-7721 liver cancer; A-549 lung cancer; PANC-1 pancreatic cancer; SK-BR-3 colon cancer. Compounds **1**, **4**, **6**, **8**, **10**, and **12** were inactive for all cell lines (IC_{50} > 10 μM).

monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The stems of *K. ananosma* were collected in Simao Country of Yunnan Province, People's Republic of China, in August 2006, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 08102009) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered stems of *K. ananosma* (10 kg) were extracted with 70% aqueous Me_2CO (40 L × 3) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (300 g) was chromatographed on MCI gel CHP 20P (90% CH_3OH-H_2O). The 90% CH_3OH fraction (245 g) was subjected to silica gel (200−300 mesh, 3.0 kg) column chromatography, eluting with a $CHCl_3-Me_2CO$ gradient system (9:1, 8:2, 2:1, 1:1, 0:1), to give fractions 1−5. Fraction 2 (38 g) was chromatographed on silica gel (petroleum ether−Me_2CO, 25:1−2:1) to give eight subfractions. Fraction 2.2 (350 mg) was subjected to RP-18 column chromatography (40%−80% gradient CH_3OH-H_2O) to afford subfractions 2.2.1−2.2.5. Subfraction 2.2.4 (40 mg) was chromatographed by semipreparative HPLC (70% CH_3OH-H_2O) to give **7** (15 mg) and **8** (4 mg). Fraction 2.3 (300 mg) was subjected to chromatography over RP-18 (40%−80% gradient CH_3OH-H_2O) to give subfractions 2.3.1−2.3.5. Subfraction 2.3.3 (32 mg) was separated by semipreparative HPLC (70% CH_3OH-H_2O) to yield **2** (5 mg) and **13** (4 mg). Fraction 2.4 (598 mg) was applied to a silica gel column (eluted with petroleum ether−Me_2CO, 18:1), RP-18 chromatography (30%−80% gradient CH_3OH-H_2O), and then semipreparative HPLC, with 65% CH_3OH-H_2O, to yield **4** (34 mg) and **5** (2 mg). Compound **1** (5 mg) was crystallized from fraction 2.6 directly. Fraction 3 (20 g) was subjected to column chromatography on silica gel with a $CHCl_3-CH_3OH$ gradient system (100:1, 50:1, 25:1, 10:1, 5:1), with final purification by semipreparative HPLC (60% CH_3OH-H_2O), to yield **3** (19 mg), **6** (9 mg), **9** (6 mg), and **10** (5 mg). Compounds **11** (2 mg) and **12** (12 mg) were obtained from fraction 4 (9 g) after repeated silica gel column chromatography (petroleum ether−Me_2CO, 15:1), followed by semipreparative HPLC (60% CH_3OH-H_2O).

Longipedlactone K (1): white powder; $[\alpha]^{22}_D$ −112.5 (*c* 0.08, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 237 (3.45), 200 (3.30), 191 (3.31) nm; IR (KBr) ν_{max} 3433, 2928, 1721, 1636, 1363, 1245, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 595 (100) $[M + Na]^+$; positive HRESIMS *m/z* 595.2898 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_9Na$, 595.2883).

Longipedlactone L (2): white powder; $[\alpha]^{22}_D$ −182.9 (*c* 0.08, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 276 (3.73), 235 (3.55), 228 (3.54) nm; IR (KBr) ν_{max} 3435, 2928, 1736, 1717, 1375, 1238, 1130, 1034, cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 561 (100) $[M + Na]^+$; positive HRESIMS *m/z* 561.2843 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_7Na$, 561.2828).

Longipedlactone M (3): white powder; $[\alpha]^{22}_D$ −229.0 (*c* 0.11, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 276 (3.96), 218 (3.74), 203 (3.79) nm; IR (KBr) ν_{max} 3441, 2930, 1698, 1375, 1239, 1131, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 577 (100) $[M + Na]^+$; positive HRESIMS *m/z* 577.2777 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_8Na$, 577.2777).

Longipedlactone N (4): white powder; $[\alpha]^{21}_D$ −69.2 (*c* 0.09, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 237 (3.57), 217 (3.27), 203 (3.24) nm; IR (KBr) ν_{max} 3443, 2928, 1702, 1639, 1382, 1240, 1132, 1038 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 575 (100) $[M + Na]^+$; positive HRESIMS *m/z* 575.2629 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_8Na$, 575.2620).

Longipedlactone O (5): white powder; $[\alpha]^{21}_D$ −125.0 (*c* 0.22, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 238 (3.69), 224 (3.22), 218 (3.23) nm; IR (KBr) ν_{max} 3440, 2929, 1741, 1706, 1639, 1377, 1238, 1130, 1037 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 577 (100) $[M + Na]^+$; positive HRESIMS *m/z* 577.2792 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_8Na$, 577.2777).

Longipedlactone P (6): white powder; $[\alpha]^{21}_D$ −4.0 (*c* 0.08, $CHCl_3$−MeOH, 1:1); UV ($CHCl_3$−MeOH, 1:1) λ_{max} (log ε) 237 (4.01), 221 (3.68), 217 (3.68) nm; IR (KBr) ν_{max} 3439, 2926, 1718, 1618, 1270, 1111 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 593 (100) $[M + Na]^+$; positive HRESIMS *m/z* 593.2709 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_9Na$, 593.2726).

Cytotoxicity Assay. The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3. 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).[24] Briefly, 100 μL adherent cells were 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 initial density of 1 × 10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with 10-hydroxycamptothecin (Sigma) as positive control. 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.[24]

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

References and Notes

(1) Gao, X. M.; Pu, J. X.; Huang, S. X.; Yang, L. M.; Huang, H.; Xiao, W. L.; Zheng, Y. T.; Sun, H. D. *J. Nat. Prod.* **2008**, *71*, 558–563.
(2) Shen, Y. C.; Lin, Y. C.; Cheng, Y. B.; Chiang, M. Y.; Liou, S. S.; Khalil, A. T. *Phytochemistry* **2009**, *70*, 114–120.
(3) Ookawa, N.; Ikeya, Y.; Sugama, K.; Taguchi, H.; Maruno, M. *Phytochemistry* **1995**, *39*, 1187–1191.
(4) Liu, J. S.; Li, L. *Phytochemistry* **1995**, *38*, 241–245.
(5) Wang, N.; Li, Z. L.; Song, D. D.; Li, W.; Fu, H. W.; Koike, K.; Pei, Y. H.; Jing, Y. K.; Hua, H. M. *J. Nat. Prod.* **2008**, *71*, 990–994.
(6) Li, H. R.; Wang, L. Y.; Miyata, S.; Kitanaka, S. *J. Nat. Prod.* **2008**, *71*, 739–741.
(7) Wang, W.; Liu, J. Z.; Han, J.; Xu, Z. G.; Liu, R. X.; Liu, P.; Wang, W. X.; Ma, X. C.; Guan, S. H.; Guo, D. *Planta Med.* **2006**, *72*, 450–457.
(8) Sy, L. K.; Brown, G. D. *Tetrahedron* **1999**, *55*, 119–132.
(9) Chen, D. F.; Zhang, S. X.; Kozuka, M.; Sun, Q. Z.; Feng, J.; Wang, Q.; Mukainaka, T.; Nobukuni, Y.; Tokuda, H.; Nishino, H.; Wang, H. K.; Morris-Natschke, S. L.; Lee, K. H. *J. Nat. Prod.* **2002**, *65*, 1242–1245.
(10) Chen, D. F.; Zhang, S. X.; Chen, K.; Zhou, B. N.; Wang, P.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **1996**, *59*, 1066–1068.
(11) Chen, D. F.; Zhang, S. X.; Xie, L.; Xie, J. X.; Chen, K.; Kashiwada, Y.; Zhou, B. N.; Wang, P.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem.* **1997**, *5*, 1715–1723.
(12) Pu, J. X.; Xiao, W. L.; Lu, Y.; Li, R. T.; Li, H. M.; Zhang, L.; Huang, S. X.; Li, X.; Zhao, Q. S.; Zheng, Q. T.; Sun, H. D. *Org. Lett.* **2005**, *7*, 5079–5082.
(13) Pu, J. X.; Li, R. T.; Xiao, W. L.; Gong, N. B.; Huang, S. X.; Lu, Y.; Zheng, Q. T.; Lou, L. G.; Sun, H. D. *Tetrahedron* **2006**, *62*, 6073–6081.
(14) Chen, Y. G.; Hai, L. N.; Liao, X. R.; Qin, G. W.; Xie, Y. Y.; Halaweish, F. *J. Nat. Prod.* **2004**, *67*, 875–877.
(15) Shen, Y. C.; Lin, Y. C.; Chiang, M. Y.; Yeh, S. F.; Cheng, Y. B.; Liao, C. C. *Org. Lett.* **2005**, *7*, 3307–3310.
(16) Shen, Y. C.; Lin, Y. C.; Cheng, Y. B.; Kuo, Y. H.; Liaw, C. C. *Org. Lett.* **2005**, *7*, 5297–5300.
(17) Liu, Y. H. *Flora of China*; Science Press: Shanghai, 1996; Vol. 30, issue 1, p 234.
(18) Chen, Y. G.; Xie, Y. Y.; Cheng, K. F.; Cheung, K. K.; Qin, G. W. *Phytochemistry* **2001**, *58*, 1277–1280.
(19) Chen, Y. G.; Song, X. P.; Hai, L. N. A. F.; Bi, Y. M.; Liao, X. R. *Pol. J. Chem.* **2006**, *80*, 1677–1681.
(20) Chen, Y. G.; Song, X. P.; Hai, L. N.; Lv, Y. P. A. F.; Halaweish, F.; Liao, X. R. *Pharmazie* **2006**, *61*, 891–892.
(21) Zou, C.; Pu, X. Y.; Zhou, J. *Acta Bot. Yunnan.* **1993**, *15*, 196–200.
(22) Pu, J. X.; Yang, L. M.; Xiao, W. L.; Li, R. T.; Lei, C.; Gao, X. M.; Huang, S. X.; Li, S. H.; Zheng, Y. T.; Huang, H.; Sun, H. D. *Phytochemistry* **2008**, *69*, 1266–1272.
(23) Chen, G. F.; Li, Z. L.; Tang, C. M.; He, X.; Chen, K.; Pan, D. J.; Hu, C. Q.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. *Heterocycles* **1990**, *31*, 1903–1906.
(24) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493–497.

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