

Kadlongilactones A and B, Two Novel Triterpene Dilactones from *Kadsura longipedunculata* Possessing a Unique Skeleton

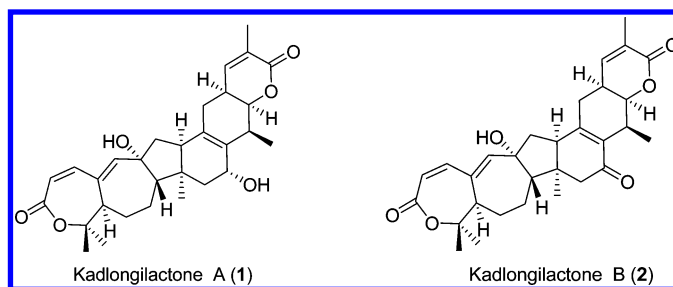
Jian-Xin Pu,^{†‡} Wei-Lie Xiao,[†] Yang Lu,[§] Rong-Tao Li,^{†||} Hong-Mei Li,^{||} Li Zhang,[§] Sheng-Xiong Huang,[†] Xian Li,[†] Qin-Shi Zhao,[†] Qi-Tai Zheng,[§] and Han-Dong Sun^{*,†}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, P. R. China, and Graduate School of the Chinese Academy of Sciences, Beijing 10039, P. R. China

hdsun@mail.kib.ac.cn

Received September 7, 2005

ABSTRACT



Two novel triterpene dilactones with an unprecedented rearranged hexacyclic skeleton, kadlongilactones A (1) and B (2), have been isolated from the leaves and stems of *Kadsura longipedunculata* Finet et Gagnep (Schisandraceae). Their structures were established by comprehensive 1D and 2D NMR spectroscopic analysis, coupled with single-crystal X-ray crystallographic diffraction. Compounds 1 and 2 exerted significant inhibitory effects against human tumor K562 cells with $IC_{50} = 1.40$ and $1.71 \mu\text{g/mL}$, respectively.

The family Schisandraceae consists of the genera *Schisandra* and *Kadsura*. Several species of both genera have been reported to contain dibenzocyclooctadienlignans, lanostane triterpenoid acids, and lactones, which have been found to possess some beneficial pharmacological effects, including antihepatitis, antitumor, and anti-HIV activities.^{1–6}

In our recent phytochemical research on some species of the genus *Schisandra*, we reported the isolation and structure elucidation of several highly oxygenated nortriterpenoids with new skeletons, including micrandilactones A–G, lancifodilactones A–G, and henridilactones A–D. Some of the nortriterpenes exhibited significant anti-HIV activities.^{7–14}

* To whom correspondence should be addressed. Ph: (86) 871-5223251. Fax: (86) 871-5216343.

[†] Kunming Institute of Botany.

[‡] Graduate School of the Chinese Academy of Sciences.

[§] Institute of Materia Medica.

^{||} Kunming University of Science and Technology.

(1) Chen, D. F.; Zhang, S. X.; Wang, H. K.; Zhang, S. Y.; Sun, Q. Z.; Cosebtino, L. M.; Lee, K. H. *J. Nat. Prod.* **1999**, 62, 94–97.

(2) Chen, D. F.; Zhang, S. X.; Wang, H. K.; Zhou, B. N.; Wang, P.; Cosebtino, L. M.; Lee, K. H. *J. Nat. Prod.* **1996**, 59, 1066–1068.

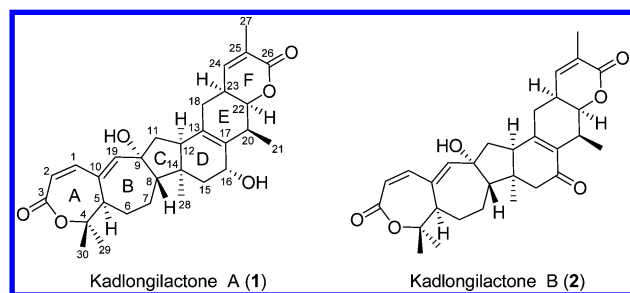
(3) Kuo, Y. H.; Kuo, L. Y.; Chen, C. F. *J. Org. Chem.* **1997**, 62, 3242–3245.

(4) Kuo, Y. H.; Li, S. Y.; Wu, M. D.; Huang, R. L.; Kuo, L. M.; Chen, C. F. *Chem. Pharm. Bull.* **1999**, 47, 1047–1048.

(5) Liu, G. T. *Acta Pharm. Sin.* **1983**, 18, 714–720.

(6) Liu, J. S.; Li, L. *Phytochemistry* **1995**, 38, 241–246.

Aimed at finding potentially bioactive and novel secondary metabolites from another genus (*Kadsura*), we investigated the leaves and stems of *Kadsura longipedunculata* Finet et Gagnep. From this plant two novel triterpene derivatives, kadlongilactones A (**1**) and B (**2**), were isolated and identified to have an unprecedented rearranged hexacyclic backbone derived from cycloartane. The two compounds were also tested for their cytotoxicities against K562 cells. In this paper, we present the isolation, structural elucidation, and biological evaluation of the two novel compounds.



The leaves and stems of *K. longipedunculata* were collected in the Erlang mountain region of Sichuan Province, China, in August 2004 and identified by Prof. Xi-Wen Li. The air-dried and powdered stems and leaves (11 kg) were extracted with 70% aqueous Me₂CO (4 × 30 L) at room temperature to yield an extract, which was successively extracted with petroleum ether and EtOAc. The EtOAc extract (300 g) was chromatographed on a silica gel column (1.5 kg) eluting with gradient CHCl₃ in Me₂CO to yield six fractions (I–VI). Fraction II was subjected to further separation on a silica gel column chromatography and semipreparative HPLC (Agilent 1100 HPLC system, Zorbax SB-C-18, Agilent, 9.4 mm × 25 cm, MeOH–H₂O 70:30) to yield **1** (20.3 mg) and **2** (10.6 mg).

(7) Xiao, W. L.; Li, R. T.; Li, S. H.; Li, X. L.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. *Org. Lett.* **2005**, *7*, 1263–1266.

(8) Xiao, W. L.; Zhu, H. J.; Shen, Y. H.; Li, R. T.; Li, S. H.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. *Org. Lett.* **2005**, *7*, 2145–2148.

(9) Li, R. T.; Zhao, Q. S.; Li, S. H.; Han, Q. B.; Sun, H. D.; Lu, Y.; Zhang, L. L.; Zheng, Q. T. *Org. Lett.* **2003**, *5*, 1023–1026.

(10) Li, R. T.; Han, Q. B.; Zheng, Y. T.; Zhao, Q. S.; Sun, H. D. *Chem. Commun.* **2005**, 2936–2938.

(11) Li, R. T.; Xiao, W. L.; Shen, Y. H.; Zhao, Q. S.; Sun, H. D. *Chem. Eur. J.* **2005**, *11*, 2989–2996.

(12) Li, R. T.; Li, S. H.; Zhao, Q. S.; Lin, Z. W.; Sun, H. D.; Lu, Y.; Wang, C.; Zheng, Q. T. *Tetrahedron Lett.* **2003**, *44*, 3531–3534.

(13) Li, R. T.; Xiang, W.; Li, S. H.; Lin, Z. W.; Sun, H. D. *J. Nat. Prod.* **2004**, *67*, 94–97.

(14) Li, R. T.; Shen, Y. H.; Xiang, W.; Sun, H. D. *Eur. J. Org. Chem.* **2004**, 807–811.

(15) Kadlongilactones A (**1**): colorless crystals, mp 212–214 °C; [α]_D²⁶ –155.3 (c 0.39, C₅H₅N); positive ESIMS *m/z* 517 [M + Na]⁺; positive HR-ESIMS found 517.2569, calcd for C₃₀H₃₈O₆Na 517.2566. UV (MeOH) λ_{max} (log ϵ): 365 (2.94), 279 (4.65), 212 (4.48) nm. IR (KBr) ν_{max} 3446, 2940, 2907, 1694, 1615, 1143, 1132 cm^{–1}. NMR data can be found in Table 1.

The molecular composition of kadlongilactone A (**1**),¹⁵ C₃₀H₃₈O₆, was established from HR-ESIMS ([M + Na]⁺, *m/z* 517.2569) and ¹³C NMR spectroscopic data, indicating 12 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl groups (3446 cm^{–1}) and carbonyl groups (1615 and 1694 cm^{–1}). Analysis of the ¹H and ¹³C NMR (Table 1) and HSQC spectra revealed that **1** contains

Table 1. ¹H and ¹³C NMR Assignments of Kadlongilactones A (**1**) and B (**2**)^a

position	1 δ_{H} (mult, <i>J</i> , Hz)	1 δ_{C} (mult)	2 δ_{H} (mult, <i>J</i> , Hz)	2 δ_{C} (mult)
1	6.53 (d, 12.2)	144.2 (d)	6.63 (d, 12.2)	143.8 (d)
2	5.96 (d, 12.2)	119.2 (d)	6.01 (d, 12.2)	119.6 (d)
3		166.5 (s)		166.4 (s)
4		80.3 (s)		80.2 (s)
5	4.09 (d, 9.3)	48.5 (d)	4.06 (d, 9.8)	48.5 (d)
6 α	2.16 (m)	28.4 (t)	2.21 (overlap)	28.3 (t)
6 β	1.25 (m)		1.21 (overlap)	
7 α	2.31 (overlap)	27.8 (t)	2.16 (overlap)	27.8 (t)
7 β	1.78 (m)		1.64 (m)	
8	1.63 (dd, 2.0, 12.7)	56.0 (d)	1.59 (m)	57.8 (d)
9		79.1 (s)		79.5 (s)
10		145.6 (s)		146.1 (s)
11 α	2.47 (dd, 7.4, 13.2)	49.2 (t)	2.28 (overlap)	45.2 (t)
11 β	1.37 (overlap)		1.54 (m)	
12	2.70 (m)	51.0 (d)	2.87 (m)	50.9 (d)
13		133.2 (s)		151.1 (s)
14		40.9 (s)		43.7 (s)
15 α	1.93 (overlap)	45.7 (t)	2.46 (d, 13.4)	52.0 (t)
15 β	1.93 (overlap)		2.60 (d, 13.4)	
16	4.64 (brs)	64.1 (d)		200.5 (s)
17		131.3 (s)		132.2 (s)
18 α	1.98 (overlap)	32.1 (t)	2.09 (overlap)	31.6 (t)
18 β	2.06 (m)		2.09 (overlap)	
19	6.22 (s)	148.2 (d)	6.31 (s)	146.5 (d)
20	3.23 (m)	34.4 (d)	3.17 (brs)	32.9 (d)
21	1.57 (d, 7.3)	14.7 (q)	1.47 (d, 7.4)	14.0 (q)
22	4.48 (dd, 2.0, 4.4)	80.1 (d)	4.51 (dd, 1.8, 4.9)	78.8 (d)
23	2.26 (overlap)	33.3 (d)	2.25 (overlap)	32.8 (d)
24	6.71 (dd, 1.5, 6.4)	146.1 (d)	6.71 (dd, 1.2, 6.1)	145.0 (d)
25		127.9 (s)		128.5 (s)
26		166.7 (s)		166.3 (s)
27	1.95 (s)	17.2 (q)	1.96 (s)	17.1 (q)
28	1.54 (s)	27.4 (q)	1.18 (s)	24.8 (q)
29	1.41 (s)	29.4 (q)	1.42 (s)	29.4 (q)
30	1.43 (s)	25.8 (q)	1.43 (s)	25.8 (q)
9-OH	6.53 (s)		6.74 (s)	

^a Data were recorded in C₅D₅N on Bruker DRX (¹H 500 MHz) and DRX (¹³C 125 MHz); chemical shifts (δ) are expressed in ppm with reference to the most downfield signal of C₅D₅N (δ 8.71 ppm) for ¹H and to the center peak of the most downfield signal of C₅D₅N (δ 149.9 ppm) for ¹³C, respectively.

two α,β -unsaturated carbonyl carbons, seven quaternary carbons (including four olefinic carbons and two oxygenated carbons), eleven methines (four olefinic and two oxygenated ones), five methylenes, and five methyls. Apart from four double bonds and two lactones, the remaining elements of the unsaturation in **1** were assumed to be a hexacyclic skeleton.

Interpretation of ^1H – ^1H COSY, HMBC, and HSQC spectral data provided substructures **1a**–**c** as described as follows (Figure 1). In HMBC spectrum (Figure 2), the

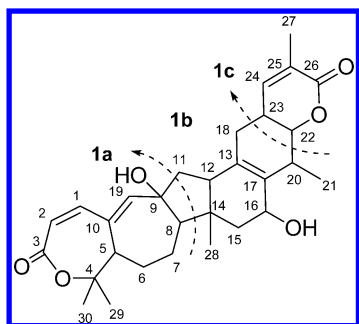


Figure 1. Structure and substructures (**1a**–**c**) of **1**.

correlations of Me-30 (δ_{H} 1.43, s) with C-4, C-5, and C-29, and of Me-29 (δ_{H} 1.41, s) with C-4 and C-30, required that

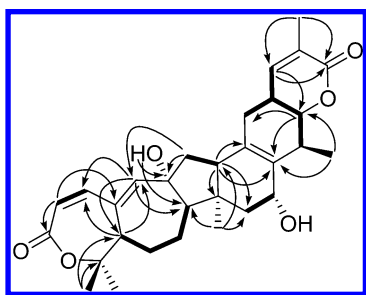


Figure 2. Key COSY (—), and HMBC (---) correlations of **1**.

both methyls be attached to the same oxygenated quaternary carbon (δ_{C} 80.3, s, C-4). In addition, the signal at δ_{H} 5.96 (d, J = 12.2 Hz, H-2) showed HMBC correlations with C-3 and C-10, H-5 (δ_{H} 4.09, d, J = 9.3 Hz) with C-1, C-4, C-6, and C-19, and 9-OH (δ_{H} 6.53, s) with C-8 and C-9. These facts, along with two proton spin systems (H-1–H-2 and H-5–H-8) deduced from the ^1H – ^1H COSY (Figure 2) correlations, the IR spectral carbonyl group absorption at 1694 cm^{-1} , and lack of absorption bands due to carboxylic acid groups, led to the establishment of substructure **1a**. HMBC cross-peaks from Me-28 (δ_{H} 1.54) to C-12, C-14, and C-15, from H-12 (δ_{H} 2.70) to C-11, C-13, C-14, C-15, C-17, and C-28, and from Me-21 (δ_{H} 1.57, d, J = 7.3 Hz) to C-17 and C-20 determined the existence of partial structure **1b**. Likewise, the third fragment, **1c**, was assigned by a continuous sequence from C-22 to Me-27 as deduced from COSY, HMBC, and IR spectra. Moreover, HMBC cross-peaks of H-11 β (δ_{H} 1.37, overlap) with C-9 and C-19 and of Me-28 (δ_{H} 1.54, s) with C-8 required the combination of **1a** and **1b**. In addition, HMBC correlations from H-23 (δ_{H} 2.26, overlap) to C-18 and from H-22 (δ_{H} 4.48, dd, J = 2.0, 4.4 Hz) to C-18, C-20, and C-17, in conjunction with ^1H –

^1H COSY correlations of H-18/H-23 and H-22/H-20, determined the direct connections of **1b** and **1c**. The final X-ray crystallographic diffraction analysis (Figure 3) of **1** revealed

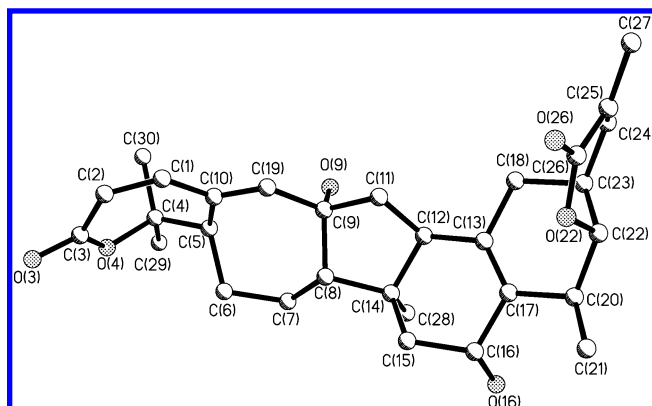


Figure 3. X-ray crystal structure of **1** showing relative configuration.

its structure and relative stereochemistry and directly supported the structural assignment of kadlongilactone A.¹⁶

HR-ESIMS analysis of kadlongilactone B (**2**)¹⁷ indicated that it has the molecular formula $\text{C}_{30}\text{H}_{36}\text{O}_6$ (13 unsaturations), differing from **1** by the loss of two hydrogen atoms. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**, except for the presence of a carbonyl group at δ_{C} 200.5 and the absence of an oxygenated methine assigned to C-16 (δ_{C} 64.1) of **2**, indicating that C-16 of **1** was replaced by a α,β -unsaturated ketone in **2**. This assignment was in accord with the observation of remarkable downfield shifts of the C-13, C-14, and C-15 signals from δ_{C} 133.2, 45.7, and 40.9 in **1** to δ_{C} 151.1, 52.0, and 43.7 in **2**, respectively. This was further confirmed by the HMBC correlations observed between H₂-15 and C-16. The relative stereochemistry of **2** was in agreement with that of **1** due to the similar ROESY

(16) Crystallographic data for **1**: $\text{C}_{30}\text{H}_{38}\text{O}_6$, M = 494.60, monoclinic, space group $P1$, a = 6.824(1) Å, b = 7.733(1) Å, c = 14.387(3) Å, α = 77.63(3)°, β = 84.79(3)°, γ = 64.36(3)°, V = 668.6 (2) Å³, Z = 1, crystal dimensions $0.50 \times 0.50 \times 0.50\text{ mm}^3$ was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω cans, $2\theta_{\text{max}}$ = 50.0°), Mo K α radiation. The total number of independent reflections measured was 1752, of which 1751 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_{F} = 0.0478, wR_2 = 0.1299, S = 1.155, $(\Delta/\sigma)_{\text{max}}$ = 0.000, $(\Delta/\rho)_{\text{min}}$ = $-0.154\text{ e}/\text{\AA}^3$, $(\Delta/\rho)_{\text{max}}$ = $0.219\text{ e}/\text{\AA}^3$. The crystal structure (**1**) was solved by the direct method SHELX-86 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1985) and expanded using difference Fourier techniques, refined by the program and method NOMCSDP (Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, 3, 637–640) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 278406). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(17) Kadlongilactone B (**2**): colorless crystals, mp 236–238 °C; $[\alpha]_{\text{D}}^{25}$ -227.6 (c 0.52, $\text{C}_5\text{H}_5\text{N}$); negative FABMS m/z 583 $[\text{M} + \text{Gly} - 1]^-$, 491 $[\text{M} - 1]^-$, 474 $[\text{M} - \text{H}_2\text{O}]^-$; negative HR-ESIMS $[\text{M} - 1]^-$ (found 491.2431, calcd for $\text{C}_{30}\text{H}_{36}\text{O}_6$ 491.2433). UV (MeOH) λ_{max} (log ϵ): 278 (4.63), 255 (4.41), 248 (4.37), 215 (4.40) nm. IR (KBr) ν_{max} 3471, 2940, 2865, 1714, 1655, 1625, 1130, 1121 cm^{-1} . NMR data can be found in Table 1.

correlations and by the comparison of NMR data with both compounds.

Compounds **1** and **2** were tested for cytotoxicity against human tumor K562 cells by MTT method as previously reported, and *cis*-platin was used as the positive control.¹⁸ Both compounds showed significant inhibitory activity against K562 cells with IC₅₀ values of 1.40 and 1.71 μg/mL, respectively (Table 2), which were nearly comparable to the activity of *cis*-platin. Further biological evaluation of **1** and **2** is in progress.

Supporting Information Available: This project was supported by grants from the National Natural Science Foundation of China (no. 20402016 to R.-T.L.). We express our thanks to Ms. Li-Li Ban (Kunming Medical College) for assistance with cytotoxicity experiments.

(18) Niu, X. M.; Li, M. L.; Zhao, Q. S.; Mei, S. X.; Na, Z.; Wang, S. J.; Lin, Z. W.; Sun, H. D. *Planta Med.* **2002**, *68*, 528–33.

Table 2. Inhibitory Effects of **1** and **2** against Human Tumor K562 Cells

compound	K562		
	IC ₅₀ (μg/mL)	95% creditable zone	response coefficient (r)
<i>cis</i> -platin	0.90	0.19–4.15	0.92
1	1.40	0.45–4.34	0.88
2	1.71	0.55–5.30	0.89

^a Minimal cytotoxicity against K562 cells when IC₅₀ > 10 (μg/mL).

Supporting Information Available: 1D and 2D NMR spectra of kadlongilactones A (**1**) and B (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL052161J