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Lancifodilactone G: A Unique Nortriterpenoid Isolated from *Schisandra lancifolia* and Its Anti-HIV Activity

Wei-Lie Xiao,[†,‡] Hua-Jie Zhu,[†] Yun-Heng Shen,[†,‡] Rong-Tao Li,[*,†] Sheng-Hong Li,[†] Han-Dong Sun,[*,†] Yong-Tang Zheng,[§] Rui-Rui Wang,[‡,§] Yang Lu,[||] Cheng Wang,[||] and Qi-Tai Zheng[||]

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, Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, 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

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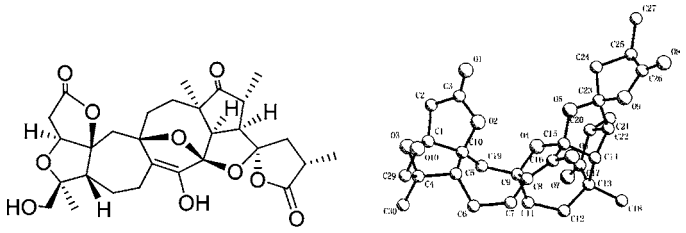
ABSTRACT



Lancifodilactone G (1)

Lancifodilactone G (1), a novel, highly oxygenated nortriterpenoid featuring a partial enol structure and a spirocyclic moiety, was isolated from the medicinal plant *Schisandra lancifolia*. Its structure and stereochemistry were determined from extensive one- and two-dimensional NMR and mass spectral data, coupled with single-crystal X-ray analysis. Compound 1 exerted minimal cytotoxicity against C8166 cells (CC_{50} > 200 μg/mL) and showed anti-HIV activity with EC_{50} = 95.47 ± 14.19 μg/mL and a selectivity index in the range of 1.82–2.46.

Previous studies of *Schisandra* species have reported lignans with various beneficial pharmacological effects such as antihepatitis, antitumor, and anti-HIV activities as typical of this genus.[1−3] Recent research showed that some triterpenoids isolated from this genus exhibited anti-HIV activities[4,5] and inhibitory activities toward cholesterol biosynthesis.[6−9]

Recently, we have reported the isolation and structure elucidation of several highly oxygenated nortriterpenoids with a new skeleton such as micrandilactone A,[10] lancifodilactones A−F,[11−13] and henridilactones A−D[14] from the

* Corresponding author. Phone: (86) 871-5223251. Fax: (86) 871-5216343.
[†] Kunming Institute of Botany.
[‡] Graduate School of the Chinese Academy of Sciences.
[§] Kunming Institute of Zoology.
[||] Institute of Materia Medica.

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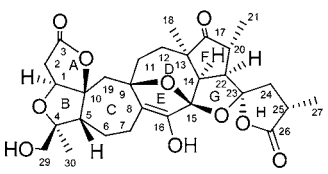
leaves and stems of *Schisandra micrantha* A. C. Smith, *Schisandra henryi* var. *yunnanensis* A. C. Smith, and *S. lancifolia* (Rehd. et Wils) A. C. Smith, respectively. Continued phytochemical investigation of the leaves and stems of *S. lancifolia* (Rehd. et Wils) A. C. Smith led to the isolation of a novel triterpene derivative, lancifodilactone G (**1**), which possesses a highly oxidized norcycloartane skeleton similar to that of lancifodilactone C (**3**)[12] and was determined to have a partial enol structure and a spirocyclic moiety. In addition, compound **1** was tested for its cytotoxic and anti-HIV-1 activities. Described herein are the isolation, structure elucidation, and biological activity of compound **1**.



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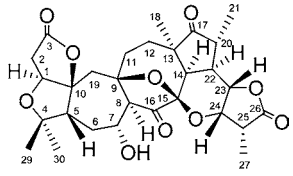

lancifodilactone C (**2**)

The leaves and stems of *S. lancifolia* were collected in Dali Prefecture of Yunnan Province, China, in August of 2002 and identified by Prof. Su-Gong Wu. The air-dried and powdered stems and leaves (5.7 kg) were extracted with 70% aqueous Me_2CO (4 × 15 L) at room temperature and concentrated in vacuo to give a crude extract (290 g), which was partitioned between H_2O and EtOAc. The EtOAc fraction (101 g) was subjected to column chromatography over silica gel and RP-18 repeatedly, followed by recrystallization from MeOH, yielding lancifodilactone G (**1**, 18.2 mg).

Lancifodilactone G (**1**),[15] $[\alpha]_D^{25.9}$ +75.00 (*c* 0.240, pyridine), crystallized as a colorless prism and has the molecular formula $C_{29}H_{36}O_{10}$ as determined by analysis of ^{1}H, ^{13}C, and DEPT NMR spectral data, which was verified by HR-ESIMS (found 567.2217, calcd 567.2206), requiring 12 degrees of unsaturation. The ^{1}H NMR spectrum displayed signals due to three tertiary methyls and a secondary methyl. The ^{13}C NMR spectrum of **1** exhibited signals for 29 carbons, including 2 ester groups, 1 carbonyl group, 8 quaternary carbons, 6 methines (including an oxygenated one), 8 methylenes (including an oxygenated one), and 4 methyls. Comparison of ^{1}H and ^{13}C NMR spectral data of **1** with those of lancifodilanctone C (**2**)[12] showed that some characteristic signals of this kind of nortriterpenoid skeleton still existed in compound **1** such as the proton signals at H-1 (δ 4.13, d, J = 5.3 Hz) and H-19 (δ 2.03 and 2.13, AB d, J = 15.9 Hz) and carbon signals at δ 80.8 (C-1), 97.0 (C-10), and 220.1 (C-17). All these data revealed that **1** possesses a structure partly similar to that of **2**. Considering that compound **1** and lancifodilanctone C (**2**) have similar structural parts, we first tentatively established the possible structure of **1** by comparison with the NMR spectral data of **2** together with detailed analysis of two-dimensional NMR spectral data of **1**. Uncertain structure details were established by single-crystal X-ray analysis.

The differences between **1** and **2** are as follows: The methyl group at C-29 in **2** was replaced by an oxygenated methylene in **1** with a downfield shift at δ 68.2. The oxygenated methine C-7 in **2** was replaced by a methylene in **1**, which was further confirmed by HMBC correlations from H-7 at δ 2.95 (m) to C-6 and C-9. Moreover, typical signals at C-8 and C-16 in **2** were not present in the spectra of **1**. In the HMBC spectrum, correlations observed between H-7 (δ 2.95) and C-8 (δ 111.8) and C-16 (δ 148.3) and between H-6 (δ 2.12) and C-7 and C-8 suggested that there was a double bond between C-8 and C-16 in **1**. The largely downfield chemical shift of C-16 to δ 148.3 hints that there should be a hydroxyl group or an oxygen bridge connected with it. Further comparison of ^{1}H and ^{13}C NMR data with those of **2** and analysis of two-dimensional NMR spectra of **1** (Table 1) allowed us to identify the existence of rings A−F, which led to the establishment of partial structure **1a** (Figure 1). HMBC cross-peaks (Table 1) observed from H-27 at δ 1.15 (d, J = 7.3 Hz) to C-24 and C-26 and from H-25 at δ 2.75 to C-23, C-26, and C-27, along with the proton spin system deduced from ^{1}H−^{1}H COSY correlations, H-24/H-25/H-27, established the partial structure **1b** (Figure 3). Furthermore, HMBC correlations from H-24 at δ 1.97 (d, J = 9.3 Hz, H-24α) and 2.47 (d, J = 9.3, 13.3 Hz, H-24β) to C-22 and from H-20 (δ 2.74) to C-23 required direct connection of C-22 with C-23 and permitted fragments **1a** and **1b** to be joined to obtain **1c** (Figure 1). Because C-15, C-16, C-23, and C-26 were quaternary carbons, it was not possible to determine the correct connections among these carbons. Luckily, a single crystal of compound **1** was achieved after repeated recrystallization, and the X-ray diffraction experiment[16] was successfully performed (Figure 2), which solved the problem. X-ray diffraction indicated

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(15) Lancifodilactone G (**1**): white crystals, mp 171−172 °C; $[\alpha]^{25.9}_D$ +75.00 (*c* 0.240, MeOH); UV (MeOH) λ_{max} (lg ϵ) 204 (3.87), 248 (2.44), 320 (2.15), 374 (1.92) nm; IR (KBr) υ_{max} 3606, 3547, 3420, 2925, 1774, 1732, 1457, 1175, 1108, 1055, 969, 921 cm^{-1}; NMR can be found in Table 1; positive ESIMS *m*/*z* (rel intensity) 567 (100, $[M + Na]^+$); HR-ESIMS found 567.2217, calcd for $C_{29}H_{36}O_{10}$ 567.2206.

(16) Crystallographic data for **1**: $C_{29}H_{36}O_{10}$, M = 544.22, monoclinic, space group $P2_1$, a = 7.348 (1) Å, b = 11.927(1) Å, c = 15.252(1) Å, β = 85.00(1)°, V = 1355.4 (2) Å^3, Z = 2, d = 1.379 g/cm^3, crystal dimensions 0.20 × 0.20 × 0.30 mm were used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max}$ = 50.0°), Mo Kα radiation. The total number of independent reflections measured was 2916, of which 2310 were observed ($|F|^2 \geq 8\sigma|F|^2$). Final indices: R_f = 0.044, R_w = 0.045 ($w = 1/\sigma\ |F|^2$). The crystal structure (**1**) was solved by direct methods using SHELX-86 (Sheldrich, G. M., University of Gottingen: Gottingen, Germany, 1985) and expanded using difference Fourier techniques, refined by the program and method NOMCS-DP (Lu, Y.; Wu, B. M. *Chin. Chem. Lett*. **1992**, *3*, 637−640) and full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 254748). Copies of these data can be obtained free of charge via the Internet at 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 e-mail: deposit@ccdc.cam.ac.uk).

Table 1. ^{1}H and ^{13}C NMR Assignments and Two-Dimensional NMR Correlations of **1**[a]

position	δ_H (mult, J, Hz)	δ_C (mult)	HMBC (H−C)	NOESY (H−H)
1	4.13 (d, 5.3)	80.8 (d)	2, 3, 10, 19	2α, 19α, 30
2 α	2.67 (overlap)	36.0 (t)	10	1, 2β
2 β	2.59 (d, 18.2)		1, 3, 10	2α
3		175.1 (s)		
4		87.6 (s)		
5	2.68 (overlap)	52.7 (d)	1, 4	19β, 29
6	2.12 (2H, m)	21.9 (t)	5, 7, 8, 16	7
7	2.95 (2H, m)	23.3 (t)	6, 8, 9, 16	6
8		111.8 (s)		
9		85.7 (s)		
10		97.0 (s)		
11 α	1.65 (m)	37.3 (t)	8, 12, 13	11β, 12
11 β	2.33 (m)		8, 9, 12, 13, 19	11α, 12, 19β
12	1.80 (2H, m)	29.6 (t)	9, 11, 13, 14, 17	11α, 11β, 14, 18
13		50.3 (s)		
14	3.24 (d, 6.7)	57.6 (d)	13, 18, 20, 22	12, 18, 22
15		114.1 (s)		
16		148.3 (s)		
17		220.1 (s)		
18	0.99 (3H, s)	26.8 (q)	12, 13, 14, 17	12, 14, 22
19 α	2.13 (AB d, 15.9)	43.3 (t)	1, 5, 8, 9, 10	1, 19β
19 β	2.03 (AB d, 15.9)		1, 9	5, 11β, 19α
20	2.74 (overlap)	43.6 (d)	17, 21, 22, 23	
21	1.27 (3H, d, 7.2)	17.8 (q)	17, 20, 22	22
22	2.68 (overlap)	53.7 (d)	14, 17, 20, 21, 23	14, 18, 21, 27
23		116.4 (s)		
24 α	1.97 (d, 9.3)	37.3 (t)	22, 23, 25, 26, 27	24β, 27
24 β	2.47 (dd, 9.3, 13.3)		22, 23, 25, 26, 27	24α, 25
25	2.75 (overlap)	36.0 (d)	23, 26, 27	24β, 27
26		180.0 (s)		
27	1.15 (3H, d, 7.3)	16.4 (q)	24, 25, 26	22, 24α, 25
29	3.63 (d, 11.6)	68.2 (t)	4, 5, 30	5, 30
	3.74 (d, 11.6)			
30	1.15 (3H, s)	17.1 (q)	4, 5, 29	1, 29

[a] Data were recorded in C_5D_5N on Bruker AM-400 MHz (^{1}H, ^{13}C) and Bruker DRX-500 MHz spectrometers (COSY, HMBC, NEOSY); chemical shifts (δ) are expressed in parts per million with reference to the most downfield signal of C_5D_5N (δ 8.71 ppm) for ^{1}H and to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) for ^{13}C.

that an enol structure existed between C-8 and C-16 and that a spirocyclic moiety (ring H) was located at C-23. Both are the first such examples in this series of highly oxidized novel nortriterpenoids[10−14] and are very rare in natural products.

Regarding the stereochemistry of lancifodilactone G (**1**), C-29 was biogenetically β-oriented, while Me-21 and Me-30 were in α-orientation. The cross-peaks observed between Me-30/H-1, H-29/H-5, H-18/H-21, H-14/H-18, and H-22/H-18 in the NOESY spectrum (Table 1) demonstrated that H-1, H-14, Me-18, and H-22 were α-oriented, while H-5 possessed β-orientation. The relative stereochemistry of the

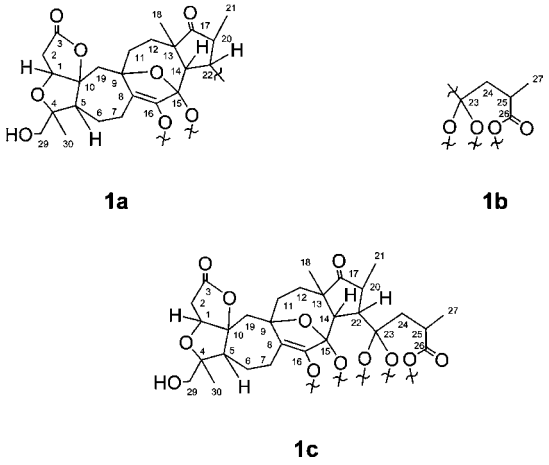


Figure 1. Structural fragments of **1**.

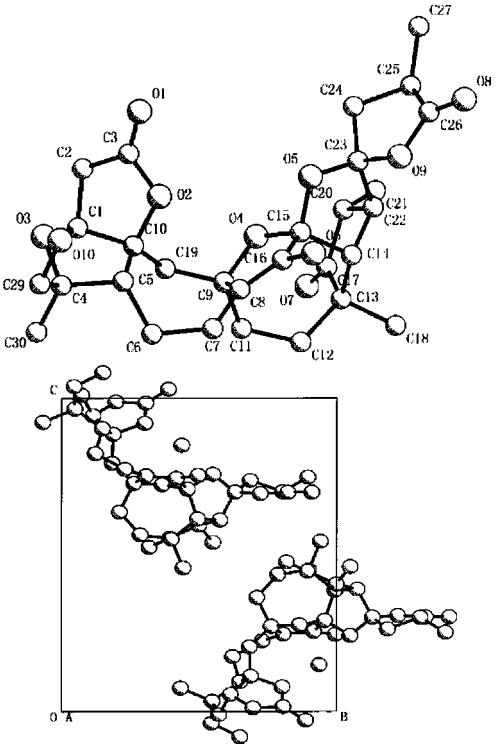


Figure 2. X-ray structure of **1** showing the relative configuration.

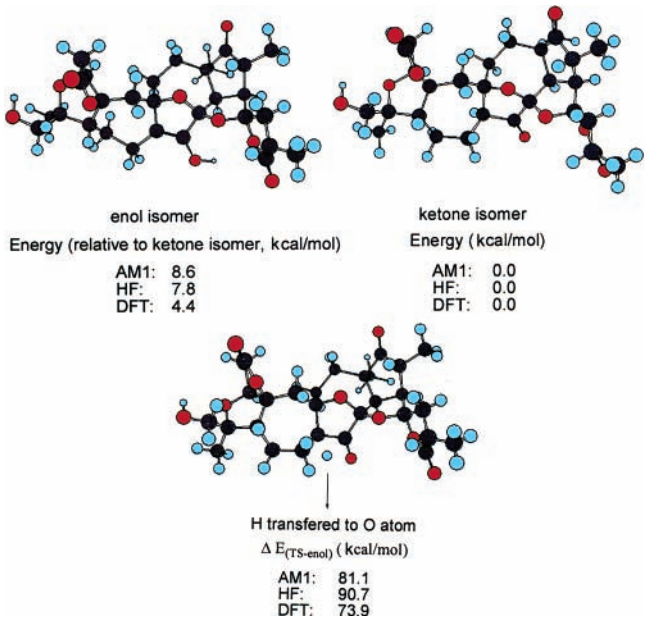
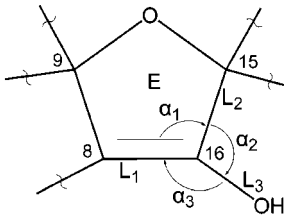


Figure 3. Relative energies of enol isomer, ketone isomer, and TS at different levels of theory.



X-ray: L_1 = 1.326, L_2 = 1.505, L_3 = 1.356
α_1 = 110.5, α_2 = 116.3, α_3 = 133.0
AM1: L_1 = 1.355, L_2 = 1.530, L_3 = 1.355
α_1 = 108.6, α_2 = 123.4, α_3 = 127.9
HF: L_1 = 1.314, L_2 = 1.509, L_3 = 1.338
α_1 = 109.8, α_2 = 122.3, α_3 = 127.6

Figure 4. Bond lengths and bond angles in ring E obtained by computations and X-ray experiment.

four quaternary carbons C-9, C-10, C-15, and C-23 was deduced as *R*, *S*, *S*, and *S*, respectively, by an X-ray diffraction experiment.

The confirmation by X-ray experiment that lancifodilactone G exists as an enol isomer might hint that this isomer may be more stable than the ketone isomer in energy in this special molecule. However, the computed relative energies in different levels of theory showed that enol isomers are 4.4−8.6 kcal/mol (Figure 3) higher in energy than the ketone isomer,[17] meaning that the enol isomer is less stable than the ketone isomer. Therefore, transition states (TSs) were investigated to explain this case. The TS computations were performed from the enol isomer to ketone at the AM1 and HF/6-31G(d,p) levels of theory; the single-point energy computations were performed using HF/6-31G(d,p)-optimized geometries at the B3LYP/6-31+G(d,p) level of theory. The calculated TS barriers are 81.1 kcal/mol using AM1, 90.7 kcal/mol using HF/6-31G(d,p), and 73.9 kcal/mol using single-point energy at the B3LYP/6-31+(d,p) (DFT method) level of theory. All barriers obtained are so high that it is almost impossible for such an isomerization to occur. The partial bond angles and bond lengths, which were obtained by X-ray and computations, are listed in Figure 4. These computed bond lengths and angles are close to those obtained by X-ray experiment. The above results suggest that lancifodilactone G is not an artifact. However, it remains unknown why the plant forms the enol isomer instead of the ketone structure.

(17) Frisch, M. J. et al. *Gaussian 03 User's Reference*; Gaussian, Inc.: Carnegie, PA, 2003 (see Supporting Information for complete reference).

Table 2. Summary of Cytotoxicity and Anti-HIV-1_{IIIB} Activity of Compound **1**

compound	cytotoxicity CC_{50} (μg/mL)[a]	anti-HIV-1_{IIIB} activitiy EC_{50} (μg/mL)	selectivity index CC_{50}/EC_{50}
lancifodilactone G (**1**)	>200	95.47 ± 14.19	>1.82−2.46

[a] Minimal cytotoxicity against C8166 cells when CC_{50} > 200 (μg/mL).

Compound **1** was tested for cytotoxicity against C8166 cells (CC_{50}) using the MTT method as reported previously,[18] and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1_{IIIB} (EC_{50})[19] (Table 2). Compound **1** exerted minimal cytotoxicity against C8166 cells (CC50 > 200 μg/ml) and showed anti-HIV-1_{IIIB} activity with EC_{50} = 95.47 ± 14.19 μg mL^{-1} and selectivity index over 1.82−2.46.

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Supporting Information Available: One- and two-dimensional NMR spectra of lancifodilactones F (**1**), the complete ref 17, and coordinates and optimized energies of computations. This material is available free of charge via the Internet at http://pubs.acs.org.

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