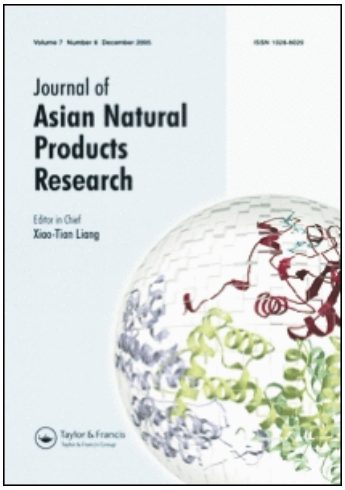


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new lanostane-type triterpene from the fruiting bodies of Ganoderma lucidum

Jun Luo[a]; Yu-Ying Zhao[b]; Zhi-Bin Lin[a]
[a] Department of Pharmacology, School of Basic Medical Science, Peking University, Beijing, China [b] Division of Natural Medical Chemistry, School of Pharmaceutical Science, Peking University, Beijing, China

Online publication date: 09 September 2010

To cite this Article Luo, Jun , Zhao, Yu-Ying and Lin, Zhi-Bin(2002) 'A new lanostane-type triterpene from the fruiting bodies of Ganoderma lucidum', Journal of Asian Natural Products Research, 4: 2, 129 — 134
To link to this Article: DOI: 10.1080/10286020290027416
URL: http://dx.doi.org/10.1080/10286020290027416

Journal of Asian Natural Products Research, 2002 VOL. 4 (2), pp. 129–134

A NEW LANOSTANE-TYPE TRITERPENE FROM THE FRUITING BODIES OF *GANODERMA LUCIDUM*

JUN LUO[a], YU-YING ZHAO[b] and ZHI-BIN LIN[a,*]

[a]*Department of Pharmacology, School of Basic Medical Science, Peking University, Beijing 100083, China;* [b]*Division of Natural Medical Chemistry, School of Pharmaceutical Science, Peking University, Beijing 100083, China*

(Received 28 August 2001; Revised 20 September 2001; In final form 24 October 2001)

A new lanostane-type triterpene, named ganoderic acid LM_2 (**5**), was isolated from the fruiting bodies of *Ganoderma lucidum*. Its structure was characterized as (23S) 7β, -dihydroxy-3, 11, 15-trioxo-5α-lanosta-8, 24-dien-26-oic acid by 1D- and 2D-NMR spectra. In addition, a known triterpene, ganoderic acid ε (**4**), was obtained. Both of them exhibited potent enhancement of ConA-induced mice splenocytes proliferation *in vitro*.

Keywords: *Ganoderma lucidum*; Fruiting body; Triterpene; 2D-NMR; Biological activity

INTRODUCTION

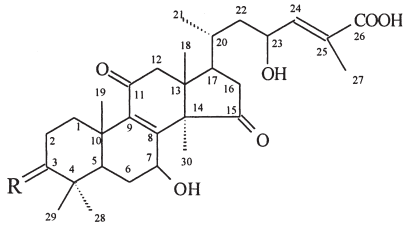
Ganoderma lucidum (Leyss. ex Fr.) Karst, a well-known traditional Chinese medicine, has been used as a tonic drug for thousands of years. In the past two decades, more than 100 triterpenes from *G. lucidum* have been reported [1]. Some of them show inhibition of HIV-1 and HIV-1 PR [2,3] and some have AEC-inhibitory activity [4]. From the 95% ethanol extract of fruiting bodes of *G. lucidum*, we have isolated and characterized a new triterpene, named ganoderic acid LM_2 (**5**) and a known triterpene, ganoderic acid ε (**4**) using spectroscopic methods. In this paper, we describe the structure elucidation and the biological activities of enhancing concanavalin A (ConA)-induced mice splenocytes proliferation *in vitro*. (Fig. 1)

RESULTS AND DISCUSSION

Powdered fruiting bodies of *G. lucidum* were extracted with 95% hot ethanol. The EtOAc acidic portion from the ethanol extract was chromatographed on silica gel column with $CHCl_3$–MeOH (10:0–6:4) gradiently giving six fractions (A–F). Purification of fraction E

*Corresponding author. Tel./Fax: +86-10-62091686. E-mail: linzb@public3.bta.net.cn

ISSN 1028-6020 print/ISSN 1477-2213 online
DOI: 10.1080/10286020290027416



	4	5
R	H / OH	O

FIGURE 1 Structures of **4** and **5**.

by rechromatography on silica gel column with $CHCl_3$–MeOH (10:0–6:4) as gradient eluents yielded compounds **4** and **5**.

Compound **5**, obtained as white amorphous powder (acetone–$CHCl_3$), mp 228–230°C, showed a positive Lieberman–Burchard reaction. The molecular formula $C_{30}H_{42}O_7$ was deduced from a molecular ion at *m/z* 514$[M]^+$ in its EI-MS and confirmed by HR-MS (514.2950 $[M]^+$, calcd. 514.2931). The EI-MS showed some prominent peaks at *m/z* 496 ($[M - H_2O]^+$), 478 ($[M - 2H_2O]^+$), 358 ($[M -$ side chain $+ H]^+$), 357 ($[M -$ side chain$]^+$), and 157 ([side chain]$^+$). The hydroxy (3369 cm^{-1}) and ketone (1647 cm^{-1}) absorptions were observed in its IR spectrum. The UV spectrum showed characteristic peak (210, 253 nm) of an α,β-unsaturated carbonyl group. The ^{1}H-NMR spectrum (Table I) exhibited seven methyl groups at δ 1.09, 1.10, 1.13, 1.32, 1.38, 2.14(3H each, s) and 1.15(3H, d, $J = 6.0$ Hz). Two hydroxymethine protons (δ_H 5.12, 1H, t, $J = 8.0$ Hz; δ_H 5.01, 1H, dd, $J = 6.0$, 15.0 Hz) were also seen in the low field region. Finally, one doublet of an olefinic proton (δ_H 7.38, 1H, d, $J = 8.5$ Hz) completed the spectrum. The ^{1}C-NMR spectrum, similar to that of the known compound ganoderic acid ε [5], showed 30 carbon signals, including three carbonyl groups (δ_C 217.0, 215.9 and 198.2), a carboxyl group (δ_C 170.7), two pairs of olefinic carbons (δ_C 159.7, 145.0, 140.9 and 128.8) and two carbons bearing hydroxy group (δ_C 66.5 and 66.0). The signal for C-3 of Compound **5** was changed to a carbonyl carbon (δ_C 215.9) instead of the signal of a carbon bearing a hydroxy group (δ_C 78.7) in ganoderic acid ε. In addition, compared with those of ganoderic acid ε the downfield shift of its adjacent carbons such as C-2 ad C-4 in the ^{13}C-NMR spectrum of **5** was observed. Comparison of the spectral data with the known compound permitted the identification of a derivative of dihydroxy-trioxo-lanosta-8, 24-diene. The final structure was established by extensive 2D-NMR experiments: ^{1}H–^{1}H COSY, HMQC and HMBC.

With the aid of ^{1}H–^{1}H COSY and HMQC experiments, the two carbinols at δ_C 66.0 and 66.5 were assigned to C-7 and C-23, respectively, and supported by the HMBC correlations between the signals of H-7 with C-8 and C-9, and H-23 with C-22 and C-25. The β orientation of the hydroxy groups at C-7 and *S* configuration of C-23 were determined by comparison with the multiplicities of H-7 (δ_H 5.12 t, $J = 8.0$ Hz) and H-23 (δ_H 5.01 dd, $J = 6.0$ and 15.0 Hz) of ganoderic acid ε, which was confirmed by the NOESY spectrum and a modification of Mosher's method. The olefinic methane at δ_C 145.0 (δ_H 7.38) was assigned to C-24, and the conjugated carbonyl signal at δ_C 198.2 to C-11, as well as signals for C-8 (δ_C 159.7), C-9 (δ_C 140.9) and C-15 (δ_C 217.0). This finding was supported by the HMBC correlations between the signal of H-12 with C-11, H-7 and H-30 with C-8, H-7 and H-19 with C-9, and H-16, H-17 and H-30 with C-15. Signal of a carboxyl carbon was also assigned to C-26, which was also supported by the HMBC correlations between the signal of H-24 and H-27 with C-26. Therefore, the structure of Compound **5** was determined as (23S) 7β, -dihydroxy-3, 11, 15-trioxo-5α-lanosta-8, 24-dien-26-oic and named ganoderic acid LM_2 (Fig. 2).

TABLE I The NMR spectral data of ganoderic acid LM_2 (**5**) and ganoderic acid ε

	Ganoderic acid ε (*in* $CDCl_3 + CD_3OD$)		*Ganoderic acid* LM_2 (**5**) (*in* C_2D_6CO)		*Ganoderic acid* LM_2 (**5**) (*in* C_5D_5N)	
No.	δ_C	δ_H	δ_C	δ_H (300 MHz)	δ_C	δ_H (500 MHz)
1	35.7	0.98 (1H, m) 2.78 (dt 9.9, 3.5)	36.3		36.0	1.59 (α-H, m) 3.16 (β-H, m)
2	28.0	1.68 (2H, m)	34.6		34.5	2.54 (2H, t, 8.0)
3	78.7	3.16 (dd 11.2, 5.0)	215.9		215.9	
4	39.7		47.1		47.0	
5	50.0	0.91 (1H, m)	48.9		48.7	1.72 (1H, d, 13.5)
6	27.6	2.19 (1H, m) 1.55 (1H, m)	28.6		28.8	2.19 (α-H, m) 1.86 (β-H, m)S
7	67.7	4.84 (1H, t, 9.4)	66.9	4.84 (1H, m)	66.0	5.12 (1H, t, 8.0)
8	158.4		158.9		159.7	
9	143.9		145.2		140.9	
10	39.4		38.8		38.4	
11	200.0		198.4		198.2	
12	51.2	2.90 (1H, d, 16.7) 2.66 (1H, d, 16.7)	48.9		50.9	2.97 (α-H, d, 17.0) 2.85 (β-H, d, 17.0)
13	46.4		45.8		45.2	
14	60.3		60.1		59.0	
15	218.5		218.8		217.0	
16	42.0	2.83 (1H, m) 2.07 (1H, dd, 19.3, 9.7)	41.1		42.0	3.01 (α-H, m) 2.34 (β-H, m)
17	47.2	2.18 (1H, m)	46.9		46.8	2.28 (1H, m)
18	17.6	0.95 (3H, s)	17.8	0.97 (3H, s)	17.8	1.09 (3H, s)
19	18.8	1.22 (3H, s)	18.5	1.21 (3H, s)	18.2	1.32 (3H, s)
20	34.0	1.60 (1H, m)	34.0		33.9	1.82 (1H, m)
21	20.0	1.07 (3H, d, 6.1)	20.0	1.10 (3H, d, 6.3)	19.8	1.15 (3H, d, 6.0)
22	43.7	1.55 (2H, m)	43.7		43.8	1.84 (2H, m)
23	66.8	4.56 (1H, m)	66.8	4.62 (1H, d, 8.7)	66.5	7.38 (1H, d, 8.5)
24	144.2	6.59 (1, dd, 1.5, 9.2)	145.2	6.62 (1H, d, 8.7)	145.0	7.38 (1H, d, 8.5)
25	129.6		128.1		128.8	
26	171.2		169.3		170.7	
27	13.1	1.88 (3H, d, 1.5)	13.0	1.84 (3H, s)	13.4	2.14 (3H, s)
28	28.6	1.03 (3H, s)	27.1	1.04 (3H, s)	27.0	1.10 (3H, s)
29	16.1	0.84 (3H, s)	20.9	1.08 (3H, s)	20.8	1.13 (3H, s)
30	24.9	1.38 (3H, s)	24.8	1.40 (3H, s)	25.1	1.38 (3H, s)

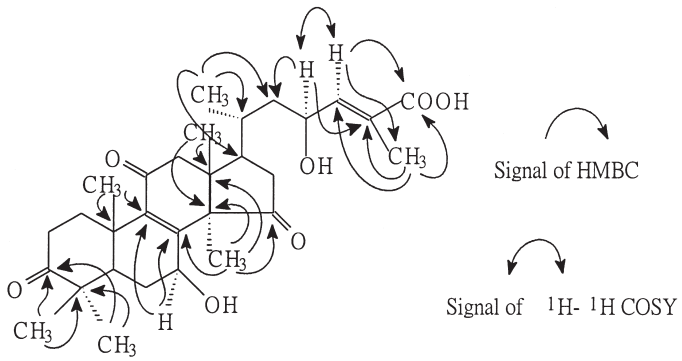


FIGURE 2 Key HMBC and $^1H-^1H$ COSY correlations for compound **5**.

EXPERIMENTAL SECTIONS

General Experimental Procedures

Melting points were determined on X_4 micro hot-stage melting point apparatus and are uncorrected. UV spectra were measured on Beckman DV-7 spectrometer in methanol. IR spectra were taken on Perkin–Elmer 983 spectrometer in KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded in C_5N_5D, 500 and 125 MHz, respectively, with INOVA-500 spectrometers using tetramethylsilane (TMS) as in internal standard. HR-EIMS was measured with ZAB-HS and EI-MS (70 eV) with AEI MS-50 mass spectrometer. Thin layer and column chromatography were carried out on pre-coated silica gel GF_{254} plates (10–40μ) and silica gel (100–200 mesh) purchased from QingDao Marine Chemical Factory. Spots were detected under a UV light by spraying 5% phosphomolybdic acid (in ethanol) followed by heating.

Fungal Material

The fruiting bodies of *G. lucidum* was given as a present by Edible Mushroom Company of Fujian Province and identified by Professor Xiao-Lan Mao, Microbiology Institute, Science Academy of China. A voucher specimen was deposited in the Department of Pharmacology, School of Basic Medial Science, Peking University, Beijing, China.

Extraction and Isolation

Powdered fruiting bodies of *G. lucidum* (13.5 kg) was extracted with 95% hot EtOH. The concentrated extracts were suspended in H_2O and the suspension was extracted with petroleum ether and EtOAc, successively. The EtOAc extract was dissolved in 2% NaOH and extracted with EtOAc. The aqueous layer was acidified with 1% HCl and reextracted with EtOAc to yield an EtOAc acidic portion, which was chromatographed on silica gel with $CHCl_3$–MeOH (10:0–6:4) as gradient eluents to give six fractions (A–F). Purification of fraction E by rechromatography on silica gel columns with $CHCl_3$–MeOH (10:0–6:4) gradiently gave compounds **4** and **5**. Pure compounds were obtained through repeated crystallization over acetone–$CHCl_3$.

Compound (**4**): white amorphous powder, mp 255–257°C (acetone–$CHCl_3$), $[\alpha]_D^{25}$ + 142 (*c* 0.1, Me_2CO); UV λ_{max} (MeOH) nm: 215, 254; IR (KBr) ν_{max} cm^{-1}: 3547, 3327, 1709, 1650, 1065, 1040; MS: *m/z* (rel. int.) 516($[M]^+$, 16), 498 ($[M - H_2O]^+$, 17), 480 ($[M - 2H_2O]^+$, 10), 359 ([M − side chain]$^+$, 10), 358 ([M − side chain − H]$^+$, 20), 343, 331, 277, 189, 157 ([side chain]$^+$, 19) and 55 (base peak). HR-EIMS *m/z*: 516.3102 $[M]^+$ ($C_{30}H_{44}O_7$, calcd. 516.3087). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 1.20 (1H, m, H-1), 3.21 (1H, m, H-1), 1.85 (2H, m, H-2), 3.47 (1H, dd, J = 5.0 and 11.5 Hz, H-3), 1.12 (1H, m, H-5), 2.38 (1H, m, H-6), 1.90 (1H, m, H-6), 5.15 (1H, t, J = 8.5 Hz, H-7), 3.00 (1H, d, J = 17.0 Hz, H-12), 2.86 (1H, d, J = 17.0, H-12), 2.98 (1H, m, H-16), 2.30 (1H, m, H-16), 2.28 (1H, m, H-17), 1.09 (3H, s, H-18), 1.43 (3H, s, H-19), 1.80 (1H, m, H-20), 1.14 (3H, d, J = 6.0 Hz, H-21), 1.83 (2H, m, H-22), 5.01 (1H, dd, J = 6.5 and 15.0 Hz, H-23), 7.38 (1H, dd, J = 1.5 and 8.5 Hz, H-24), 2.14 (3H, s H-27), 1.25 (3H, s, H-28), 1.08 (3H, s, H-29), 1.40 (3H, s, H-30); ^{13}C-NMR (125 MHz, C_5D_5N) δ: 35.4 (C-1), 28.5 (C-2), 77.5 (C-3), 39.2 (C-4), 49.6 (C-5), 28.0 (C-6), 66.8 (C-7), 158.7 (C-8), 142.7 (C-9), 39.2 (C-10), 198.5 (C-11), 51.1 (C-12), 45.6 (C-13), 59.1 (C-14), 217.0 (C-15), 41.8 (C-16), 46.8 (C-17), 17.6 (C-18), 18.7 (C-19), 33.8 (C-20), 19.8 (C-21), 43.8 (C-22), 66.5 (C-23), 144.9 (C-24), 128.8 (C-25), 170.7 (C-26), 13.4 (C-27), 28.7 (C-28), 16.4 (C-29), 24.8 (C-30). All data were identical to ganoderic acid ε.

TABLE II Effects of **4** and **5** on ConA-induced BALB/c mice splenocytes proliferation *in vitro*

Groups	*OD* ($\bar{x} \pm$ SD)	*Increment* (%)
Cell control	0.28 ± 0.01	
Vehicle	0.75 ± 0.03*	
ConA	0.85 ± 0.04*	
Compound **4**		
250 μg	0.61 ± 0.03**	−23.0
100 μg	0.83 ± 0.04	9.6
50 μg	0.86 ± 0.05**	12.8
10 μg	0.90 ± 0.05**	16.7
1 μg	0.87 ± 0.07**	13.8
Compound **5**		
250 μg	0.68 ± 0.03	−10.3
100 μg	0.80 ± 0.04	6.2
50 μg	0.84 ± 0.03**	10.7
10 μg	0.91 ± 0.05**	17.6
1 μg	0.86 ± 0.06**	12.8

$*p < 0.05$ vs. cell control group.
$**p < 0.05$ vs. vehicle control group; $n = 6$.

Compound (**5**): white amorphous powder, mp 228–230°C (acetone–$CHCl_3$), $[\alpha]_D^{25}$ + 132 (*c* 0.1, Me_2CO); UV λ_{max} (MeOH) nm: 210, 253; IR (KBr) ν_{max} cm^{-1}: 3369, 2977, 1705, 1647; MS: *m/z* (rel. int.) 514 ($[M]^+$, 14), 496 ($[M - H_2O]^+$, 15), 478 ($[M - 2H_2O]^+$, 14), 468, 396, 358 ($[M -$ side chain + H$]^+$, 20), 357 ($[M -$ side chain$]^+$, 6), 329, 157 ([side chain$]^+$, 17) and 55 (base peak). HR-EIMS *m/z*: 514.2950 $[M]^+$ ($C_{30}H_{42}O_7$, calcd. 514.2930). ^{1}H- and ^{13}C-NMR data are shown in Table I.

The experiment of mice splenocytes proliferation *in vitro* showed that both compounds **4** and **5** at concentrations of 1–250 μg ml^{-1} can be significantly enhanced ConA-induced mice splenocytes proliferation compared with the control group (Table II). The result hinted that compounds **4** and **5** might possess immunomodulation effect. In addition, the inhibitory activity of the two compounds against mice sarcoma 180 (S_{180}), hepatoma 22 (H_{22}) and human colon tumor (HCT) cells were studied *in vitro*. Both **4** and **5**, however, have no directive cytotoxic effect on the above tumor cells.

Bioassays

The preparation of splenocytes was the same as described by Kashida [6]. The BALB/c mice splenocytes were suspended at a density of 2×10^6 cells ml^{-1} in RPMI 1640 medium supplemented with 10% fetal bovine serum. Spleen cells (200 μl per well) was seeded in 96-well plates containing ConA (1 μg ml^{-1}) and various concentrations (1–250 μg ml^{-1}) of the isolated compounds, the latter were dissolved in dimethyl sulphoxide (DMSO) and diluted to available concentrations with RPMI 1640. The vehicle control contained the same cells, DMSO and ConA except any tested compounds. The cells were cultured for 72 h and the spleen cells proliferation was determined with an MTT colorimetric method described by Mosmann [7].

Acknowledgements

Shanghai Green Valley Group Co., Ltd., China and Lingzhi General Institute Co., Ltd., Japan, financially supported this study.

References

[1] Chen, R.Y. and Yu, D.Q. (1990), *Acta Pharm. Sin.* **25**(12), 940–953.
[2] El-Mekkawy, S., Meselhy, M.R., Nakamura, N., Tezuka, Y., Masao, H., Kakiuchi, N., Shimotohno, K., Kawahata, T. and Otake, T. (1998), *Phytochemistry* **49**(6), 1651–1657.
[3] Min, B.-S., Nakamura, N., Hirotsugu, M., Ki-Whan, B. and Masao, H. (1998), *Phytochemistry* **46**(10), 1607–1612.
[4] Morigiwa, A., Kitabatake, K., Fujimoto, Y. and Ikekawa, N. (1986), *Chem. Pharm. Bull.* **34**(7), 3025–3028.
[5] Min, B.-S., Gao, J.J., Nakamura, N. and Masao, H. (2000), *Chem. Pharm. Bull.* **48**(7), 1026–1033.
[6] Kashida, T., Narasake, N., Tsujihara, K., Naito, K. and Takeyama, S. (1996), *Int. J. Immunopharmacol.* **18**, 311–319.
[7] Mosmann, T. (1983), *J. Immunol. Method* **65**, 55–63.