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Cytotoxic triterpenoids from *Ganoderma lucidum*

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ABSTRACT

A systematic study of the metabolites in *Ganoderma lucidum* led to isolation of 43 triterpenoids, six of them (**1**–**6**) are hitherto unknown. The structures of the latter were elucidated on the basis of spectroscopic studies and comparison with the known related compounds. All of the compounds were assayed for their inhibitory activities against human HeLa cervical cancer cell lines. Some compounds exhibit significant cytotoxicity, and their structure–activity relationships are discussed.

1. Introduction

Ganoderma lucidum (Fr.) P. Karst. belongs to the family of Ganodermataceae (Basidiomyetes) (Ziegenbein et al., 2006), and whose clinical use can be traced back to 100 BC (Leung and Lin, 2002). With its beautiful legends, *G. lucidum* was believed by the ancient people to cure many kinds of diseases, and it was considered as an elixir that could revive the dead. In modern times, the mystery of *G. lucidum* arose the interest of scientists all over the world, and the publications and patents of *G. lucidum* have increased every year (Boh et al., 2007). It was reported that *G. lucidum* possessed activities including anti-tumor (Liu et al., 2009; Yue et al., 2007, 2008; Stanley et al., 2005; Sliva, 2006; Müller et al., 2006), antimicrobial (Yoon et al., 1994; Wang and Ng, 2006), antiviral (especially anti-HIV activities) (Min et al., 1998), and antiaging activities (Shieh et al., 2001).

Triterpenoids are typical chemical constituents in *G. lucidum*, and have an important role in the pharmacological effects described above. Furthermore, due to the unique structures, they were very important in the chemotaxonomy of genus Ganoderma. Since the first triterpenoid of ganoderic acid A was reported by Kubota et al. (1982), more than 150 compounds had been separated from Ganoderma spp. (Boh et al., 2007). Even today, the number of the new compounds identified from it seemed unendingly to increase. In order to search for bioactive metabolites, we launched a systematic study to investigate the chemical constituents in the CH_2Cl_2-soluble extract from *G. lucidum*. In the present study, 43 triterpenoids were isolated, including six new compounds. Herein, we described the structural elucidation and cytotoxic assay of these compounds.

2. Results and discussion

Phytochemical study of the CH_2Cl_2 extract of *G. lucidum* led to the isolation of 43 triterpenoids, including 6 new triterpenoids (Fig. 1) and 37 known compounds (Table 3). The known compounds were identified as ganodermadiol (**7**) (Arisawa et al., 1986), ganoderic acid DM (**8**) (Wang et al., 1997), ganoderenic acid F (**9**) (Nishitoba et al., 1989), ganodermanondiol (**10**) (Fujita et al., 1986), lucidadiol (**11**) (González et al., 1999), 15α-hydroxy-3-oxo-5α-lanosta-7,9,24(*E*)-triene-26-oic acid (**12**) (Li et al., 2006), 15α,26-dihydroxy-5α-lanosta-7,9,24(*E*)-trien-3-one (**13**) (González et al., 2002), lucidumol A (**14**) (Min et al., 1998), 3β-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (**15**) (Li et al., 2006), 3β-hydroxy-7-oxo-5α-lanosta-8,24(*E*)-dien-26-oic acid (**16**) (Li et al., 2006), ganodermanontriol (**17**) (Fujita et al., 1986), ganoderiol F (**18**) (Nishitoba et al., 1988a), lucideric acid A (**19**) (Kikuchi et al., 1986a), ganoderic acid D (**20**) (Kohda et al., 1985), lucidone A (**21**) (Nishitoba et al., 1988b), ganolucidic acid E (**22**) (Nishitoba et al., 1988a), ganoderic acid F (**23**) (Komoda et al., 1985), ganoderenic acid D (**24**) (Komoda et al., 1985), ganoderic acid E (**25**) (Kikuchi et al., 1985a), ganoderic acid J (**26**) (Nishitoba et al., 1985c), ganoderic acid B (**27**) (Kubota et al., 1982), ganoderic acid A (**28**) (Kubota et al., 1982), 7β,12β-dihydroxy-3,11,15,23-

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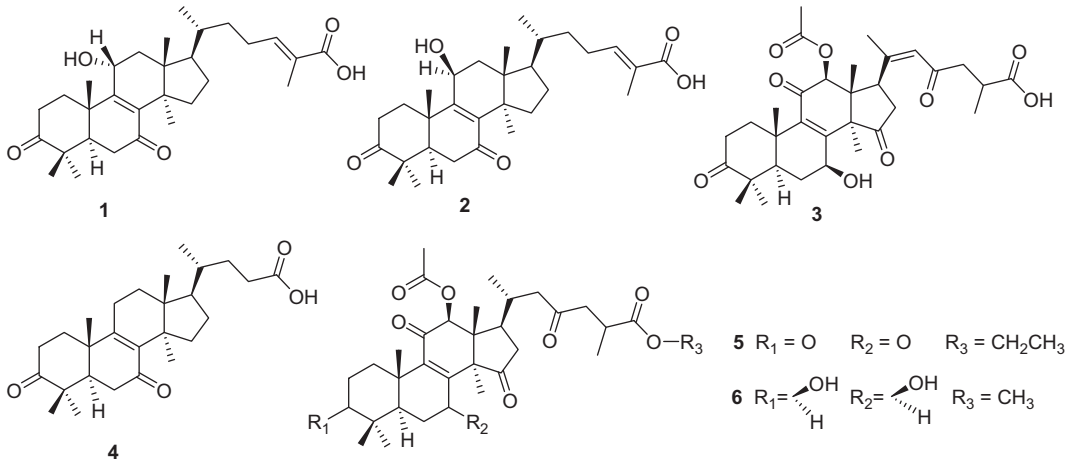


Fig. 1. Structures of compounds **1**–**6**.

tetraoxo-5α-lanosta-8-en-26-oic acid (**29**) (Nishitoba et al., 1985b), 12β-hydroxy-3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid (**30**) (Komoda et al., 1985), ganoderenic acid B (**31**) (Komoda et al., 1985), methyl ganoderate H (**32**) (Kikuchi et al., 1985a), methyl ganoderate B (**33**) (Kohda et al., 1985), 12β-acetoxy-3β,7β-dihydroxy-11,15,23-trioxo-5α-lanosta-8,20-dien-26-oic acid (**34**) (Yang et al., 2007), ganolucidic acid A (**35**) (Kikuchi et al., 1985b), methyl lucidenate C (**36**) (Nishitoba et al., 1985a), ganoderic acid H (**37**) (Kikuchi et al., 1985a), ganoderic acid AM (**38**) (Lin et al., 1993), ganoderic acid T-Q (**39**) (Lin et al., 1988), 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanosta-8-en-26-oic acid (**40**) (Hirotani and Furuya, 1986), ganoderic acid K (**41**) (Morigiwa et al., 1986), ganoderic acid G (**42**) (Kikuchi et al., 1985b), ganoderenic acid A (**43**) (Komoda et al., 1985). The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was isolated as a white powder, with $[\alpha]_D^{23} + 13.3$ (*c* 0.33, $CHCl_3$). Its HREIMS spectrum gave a molecular ion peak at *m*/*z* 484.3187 corresponding to the molecular formula $C_{30}H_{44}O_5$. The ^1H NMR spectrum of **1** was indicative of five tertiary methyls (δ_H 0.69, 1.11, 1.12, 1.14, 1.41), a vinyl methyl (δ_H 1.84) and a secondary methyl [δ_H 0.96 (*d*, *J* = 6.0 Hz)] group, an oxymethine proton [δ_H 4.53 (*dd*, *J* = 5.2, 9.2 Hz)] and one vinyl proton [δ_H 6.89 (*t*, *J* = 7.2 Hz)], respectively. The ^{13}C NMR and DEPT spectra exhibited the presence of 23 sp^3 carbons due to seven methyls, eight methylenes, four methines including an oxymethine. Comparison of these spectroscopic data with those of ganoderic acid DM (Wang et al., 1997), suggested that the skeleton and side-chain moiety of **1** should be the same except for the presence of an extra hydroxy group. The UV spectrum of **1** exhibited an absorption maximum at 248 nm, suggesting the presence of an α, β-unsaturated carbonyl group. The location of the carbonyl group was confirmed by the analysis of its HMBC spectrum. In the HMBC spectrum, there were correlations between the proton signals at δ_H 2.59 (H-6, *dd*, *J* = 14.8, 15.2 Hz), 2.40 (H-6, *dd*, *J* = 3.2, 15.6 Hz) and the carbon resonance at δ_C 199.4 (C-7), and between the proton signals at δ_H 1.41 (H-19, 3H, *s*), 2.50 (H-12) and the carbon signal at δ_C 158.7 (C-9), and between the proton resonance at δ_H 1.14 (H-30, 3H, *s*) and the carbon signal at δ_C 142.1 (C-8). The above evidence indicated the presence of a 7-ketone-8-ene structural fragment. The attachment of the hydroxy group to C-11 was confirmed by the HMBC cross-peaks between the proton signal at δ_H 4.53 (H-11, *dd*, *J* = 5.2, 9.2 Hz) and the carbon resonances at δ_C 158.7 (C-9), δ_C 142.1 (C-8), δ_C 44.6 (C-12), δ_C 40.1 (C-10). The relative configuration of **1** was determined by analyzing the ROESY spectrum. Key ROESY correlations were observed between H-11 and H-19, H-18, indicating that H-11 was on the β-orientation same as H-19 and H-18. Thus, the structure of **1** was assigned as 11α-hydroxy-3,7-dioxo-5α-lanosta-8,24(*E*)-dien-26-oic acid.

Compound **2** was isolated as a white powder, with $[\alpha]_D^{23} - 14.0$ (*c* 0.1, $CHCl_3$). Its HREIMS spectrum showed the molecular ion peak at *m*/*z* 484.3187 corresponding to the molecular formula $C_{30}H_{44}O_5$. The UV spectrum of **2** indicated the presence of an α, β-unsaturated carbonyl group [λ_{max} ($CHCl_3$) 248 nm]. The ^1H NMR spectrum of **2** showed the presence of a vinyl proton [δ_H 6.89 (*t*, *J* = 7.0 Hz)], a secondary methyl group [δ_H 0.99 (*d*, *J* = 6.0 Hz)], a vinyl methyl (δ_H 1.84) and five tertiary methyls (δ_H 0.87, 0.92, 1.09, 1.15, 1.59). The ^{13}C NMR spectrum of **2** suggested the presence of two carbonyl groups (δ_C 214.9 and δ_C 199.8) and a tetra-substituted double bond (δ_C 139.7 and 199.8). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for a minor difference. The HMBC and HSQC spectra suggested that compound **2** had the same overall skeleton as compound **1**. Analysis of the ROESY spectrum established that the stereochemistry of these two compounds was different at position 11. There was no correlation observed between H-11 and H-18, H-19, indicating that the H-11 might be in an α-orientation. Fortunately, we had established compound **1** had a β-orientation at position H-11, so H-11 in compound **2** must be in the α-orientation. The relative configuration at H-11 of compound **2** was also supported by comparing the ^1H NMR spectra of the two compounds. Since the hydroxyl group in compound **2** was on the same face as Me-18 and Me-19, the proton signals of H-18 and H-19 moved downfield. Therefore, compound **2** was established as 11β-hydroxy-3,7-dioxo-5α-lanosta-8,24(*E*)-dien-26-oic acid.

Compound **3** was isolated as a colorless gum, $[\alpha]_D^{22} + 85.0$ (*c* 0.14, $CHCl_3$). Its HREIMS spectrum displayed a molecular ion peak at *m*/*z* 570.2829 corresponding to the molecular formula $C_{32}H_{42}O_9$. Its UV spectrum exhibited an absorption maximum at 246 nm, suggesting the presence of α, β-unsaturated carbonyl group. The ^1H and ^{13}C NMR spectra resembled ganoderenic acid D (Komoda et al., 1985), except for the proton (δ_H 5.71(*s*), 2.11(*s*)) and carbon (δ_C 78.5, 170.6, 20.6) resonances which was a characteristic of an acetoxyl group substituted on a carbon atom where two adjacent carbon atoms bear no proton (Komoda et al., 1985). The position of the acetoxyl group was further confirmed by the correlation between H-12 and C-31 in the HMBC spectrum. The position of the alkene in the side-chain was confirmed by analysis of the HMBC spectrum. In the HMBC spectrum, there were correlations between the proton signals at δ_H 3.25 (H-17, *t*, *J* = 10.4 Hz), 2.15 (H-21, *s*) and the carbon resonances at δ_C 154.1 (C-20), δ_C 126.1 (C-22), and between the proton signal at δ_H 6.12 (H-22, *s*) and the carbon

resonances at δ_C 154.1 (C-20), δ_C 197.8 (C-23). Structure **3** was further confirmed by the reaction of **3** with NaOH solution (1%) in aqueous methanol to give the deacetylated product **29** (a known compound). While the absolute stereochemistry at position C-25 was uncertain, all of the natural products (confirmed by X-ray) with this kind of side-chain have been reported to possess *R* configuration in position C-25. Thus, compound **3** was provisionally assigned to be 12β-acetoxy-7β-hydroxy-3,11,15,23-tetraoxo-5α-lanosta-8,20-dien-26-oic acid.

Compound **4** was isolated as a white powder with a $[\alpha]_D^{22} + 23.0$ (*c* 0.14, $CHCl_3$). Its molecular formula of $C_{27}H_{40}O_4$ was established from HREIMS ($[M]^+$ at *m*/*z* 428.2923, calcd. 428.2927) with 8 degrees of unsaturation. The UV spectrum exhibited an absorption maximum at 251 nm, suggesting the presence of an α, β-unsaturated double bond. The ^1H NMR spectrum of **4** showed the presence of five tertiary methyl groups (δ_H 0.68, 0.93, 1.09, 1.12, 1.33) and a secondary methyl group [δ_H 0.93 (d, *J* = 6.0 Hz)]. The ^{13}C NMR spectrum exhibited two carbonyl groups (δ_C 214.7, 198.1), a tetra-substituted double bond (δ_C 139.5, 162.7) and a carboxylic acid group (δ_C 178.1). Comparing the NMR spectroscopic data with the known compounds, compound **4** had the same skeleton with that of ganoderic acid DM (Wang et al., 1997) and the same side-chain as that of lucidenic acid A (Kikuchi et al., 1986a). The two parts of the structures were connected at C-20 and C-17, this being supported by the correlation between H-17 and C-20 in the HMBC spectrum. Therefore, compound **4** was assigned as 4,4,14α-trimethyl-3,7-dioxo-5α-chol-8-en-24-oic acid.

Compound **5** was separated as a white powder, with $[\alpha]_D^{22}$+ 136.0 (*c*, $CHCl_3$). It possessed the molecular formula of $C_{34}H_{46}O_9$ (*m*/*z* 598.3145, calcd. 598.3142) as evidenced from analysis of the HREIMS. The UV spectrum exhibited maximum absorption at 251 nm. Comparing the ^1H NMR and ^{13}C NMR spectra with known compounds, compound **5** had almost the same chemical shifts as those of ganoderic acid F (Komoda et al., 1985; Kikuchi et al., 1986b), except for an additional *O*-CH_2CH_3 group and upfielded esterified carboxylic carbon at δ_C 175.6. This evidence indicated that **5** was the ethyl esterified derivative of ganoderic acid F. Thus, compound **5** was characterized as 12β-acetoxy-3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid ethyl ester.

Compound **6** was obtained as a white powder, with $[\alpha]_D^{25} + 68.0$ (*c* 0.32, $CHCl_3$). Its molecular formula was determined as $C_{33}H_{48}O_9$ by HREIMS (*m*/*z*, 588.3347, calcd. 588.3298). It showed an absorption band at 252 nm due to an α, β-unsaturated carbonyl group in the UV spectrum. The ^1H and ^{13}C NMR spectra of **6** were similar to those of ganoderic acid K (Kikuchi et al., 1986b; Morigiwa et al., 1986), except that an additional oxygenated methyl was present and the carboxylic carbon moved upfield. Thus evidence indicated that **6** was the methyl esterified derivative of ganoderic acid K. Thus, structure **6** was determined as 3β,7β-dihydroxy-12β-acetoxy-11,15,23-trioxo-5α-lanosta-8-en-26-oic acid methyl ester.

Compounds **1**–**43** were found only in the genus Ganoderma. Therefore, they could be used as marker compounds to distinguish genus Ganoderma from other genus, which is significant for the quality control of these herbal medicines.

The cytotoxicity of the isolated triterpenoids against human HeLa cervical cancer cells is summarized in Table 3. Among the compounds examined, 15α,26-dihydroxy-5α-lanosta-7,9,24(*E*)-trien-3-one (**13**), lucidadiol (**11**) and ganoderiol F (**18**) showed strong cytotoxic activities (IC_{50} 1, 5 and 8 μM, respectively). The new compounds (**2**, **4** and **5**) and the known compounds (**10**, **12**, **14**,

Table 1
^1H NMR spectroscopic data for compounds **1**–**6**.[a]

Position	1	2	3	4	5	6
1	2.18*m*, 2.30*m*	1.88*m*, 2.80*m*	1.38*m*, 2.80*m*	1.82*m*, 2.14*m*	1.72*ddd*(5.6, 8.8, 14.8), 2.73*ddd*(4.0, 9.6, 14.4)	0.92*m*, 2.61*ddd*(3.6, 7.2, 13.6)
2	2.46*m*, 2.70*ddd*(6.4, 14.4, 14.8)	2.42*m*, 2.82*m*	2.42*m*, 2.58*m*	2.50*m*, 2.70*ddd*(6.8, 10.4, 15.8)	2.46*m*, 2.47*m*	1.65*m*
3						3.19*dd*(5.2, 10.8)
5	2.26*dd*(3.2, 14.4)	2.07*dd*(3.2, 14.0)	1.52*m*	2.14*dd*(14.0, 3.2)	2.31*m*	0.89*m*
6	2.40*dd*(3.2, 15.6), 2.59*dd*(14.8, 15.2)	2.37*dd*(3.6, 16.8), 2.67*dd*(14.4, 16.8)	1.76*m*, 2.18*m*	2.38*m*, 2.54*dd*(16.0, 14.4)	2.78*m*, 2.49*m*	2.24*m*, 1.68*m*
7			4.88*t*(8.8)			4.79*t*(8.8)
11	4.53*dd*(5.2, 9.2)	4.61*d*(7.2)		2.36*m*		
12	1.86*dd*(5.2, 13.6), 2.50*dd*(4.0, 8.4)	1.91*d*(14.8), 2.30*dd*(15.2, 7.6)	5.71*s*	1.28*m*, 1.82*m*	5.66*s*	5.60*s*
15	1.61*m*, 2.08*m*	1.78*m*, 2.10*m*		1.42*m*, 2.02*m*		
16	1.98*m*, 1.28*m*	1.98*m*, 1.41*m*	2.67*dd*(8.4, 11.2)	1.72*m*, 2.10*m*	1.90*dd*(8.0, 18.0), 2.79*dd*(8.4, 18.8)	2.12*dd*(9.6, 19.6), 2.68*dd*(8.0, 19.2)
17	1.58*m*	1.46*m*	3.25*t*(10.4)	1.48*m*	2.57*m*	2.47*m*
18	0.69*s*	0.92*s*	1.02*s*	0.68*s*	0.85*s*	0.95*s*
19	1.41*s*	1.59*s*	1.34*s*	1.33*s*	1.32*s*	1.25*s*
20	1.40*m*	1.46*m*		1.46*m*	2.30*m*	2.44*m*
21	0.96*d*(6.0)	0.99*d*(6.0)	2.15*s*	0.93*d*(6.0)	0.98*d*(6.0)	0.96*d*(5.6)
22	1.18*m*, 1.56*m*	1.19*m*, 1.58*m*	6.12*s*	1.36*m*, 1.88*m*	2.31*m*, 2.41*m*	2.28*dd*(8.4, 16.0), 2.43*m*
23	2.10*m*, 2.23*m*	2.12*m*, 2.24*m*		2.30*m*, 2.41*m*		
24	6.89*t*(7.2)	6.89*t*(7.0)	2.55*m*, 2.92*m*		2.42*m*, 2.82*dd*(8.4, 17.6)	2.45*m*, 2.83*dd*(8.8, 17.6)
25			2.98*m*		2.92*m*	2.95*m*
27	1.84*s*	1.84*s*	1.23*d*(6.8)		1.17*d*(7.2)	1.17*d*(7.2)
28	1.12*s*	1.09*s*	1.12*s*	1.12*s*	1.13*s*	1.02*s*
29	1.11*s*	1.15*s*	1.11*s*	1.09*s*	1.11*s*	0.84*s*
30	1.14*s*	0.87*s*	1.54*s*	0.93*s*	1.80*s*	1.48*s*
CH$_3$CO			2.11*s*		2.24*s*	2.25*s*
–	OC*H*$_2$CH$_3$					4.12*q*(4.2)
–	OCH$_2$C*H*$_3$					1.24*t*(7.2)
–O*CH*$_3$						3.66*s*

[a] Recorded at 400 MHz in $CDCl_3$, δ_H in ppm, *J* in Hz.

15, **17**, **20**, **22** and **32**) exhibited moderate activities. The other compounds showed weak inhibition against the human HeLa cervical cancer cells.

After the summary of the cytotoxic assay results, it was found that the ganoderic alcohols showed stronger activities than ganoderic acids, hence an hydroxy group substituted in position 26 may be a very important structural feature for cytotoxic activity. However, the increase in number of the hydroxy groups in the side-chain, could result in a decrease of activity. This tendency also occurred in the ring skeleton. The more hydroxy groups, the lower the inhibitory activity.

Comparing the activities of compound **32** with **37**, compound **33** with **27**, compound **5** with **23**, it was found that esterified form of the carboxylic acid group in the side-chain would slightly increase the activity.

As shown in the Table 3, most of the active compounds contained a carbonyl group in position 3; hence, we might conclude that C-3 carbonyl group was crucial for the cytotoxic property. Generally, compounds with double bonds placed at positions 7 and 9 exhibited higher activities than that harboring the 7-ketone-8-ene structure. One strange phenomenon was that acetylation of 15-OH would result in a decrease in cytotoxicity (comparing compound **12** with **39**).

3. Conclusions

In conclusion, 43 triterpenoids were separated from *G. lucidum*, including 6 new compounds. The chemical structures of the new compounds were elucidated on the basis of spectroscopic studies. All of the compounds were assayed for their cytotoxicity against human HeLa cervical cancer cell lines, and the structure–activity relationships were discussed. The C-3 carbonyl group, the double bonds ($\Delta^{7,8}$, $\Delta^{9,11}$), the type of the side-chain and the number of hydroxy group played an important role in the structure–activity relationships.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined on a Perkin–Elmer 341 polarimeter, whereas UV spectra were recorded on a Shimadzu UV-2450 spectrometer. IR spectra were recorded on a Perkin–Elmer 577 spectrometer, whereas NMR spectra were measured on a Bruker AM-400 spectrometer for ^{1}H, ^{13}C, HSQC and HMBC spectra, and a Varian Inova-600 spectrometer for ROESY. EIMS (70 eV) analyses were carried out on a Finnigan-MAT 95 mass spectrometer. Analytical and semipreparative HPLC was performed on an Agilent 1100 with an Agilent DAD spectrophotometer and a SB-C_{18} column (4.6 × 250 mm, 5 μm) or Eclipse XDB-C_{18} column (10 × 250 mm, 5 μm). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (400–600 mesh, Qingdao Haiyang Chemical Co., Ltd.) and Sephadex LH-20 gel (Amersham Biosciences) were also used for column chromatography (CC). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

4.2. Plant material

The fruit bodies of *G. lucidum* were purchased in Huang Mountain (Anhui Province), People's Republic of China. The voucher specimen (No. 200508006) was deposited in Shanghai Research Center for TCM Modernization, National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

4.3. Extraction and isolation

The air-dried and powdered fruit bodies of *G. lucidum* (2 kg) were extracted with EtOH-H_2O (10 L × 3, 95:5 v/v). The crude extract (924 g) was washed with petroleum ether (60–90 C) to remove fatty acids, then extracted with CH_2Cl_2 (1.5 L × 5) to give the total triterpenoids fraction (54 g). An aliquot of the CH_2Cl_2 extract (35 g) was applied to a silica gel column (400–600 mesh) eluted successively with $CHCl_3$–MeOH (200:1–1:1 gradient system) to obtain 5 fractions. Fraction 2 was subjected to a silica gel column eluted with petroleum ether–EtOAc (10:1–1:1 gradient system) to afford six fractions, F21–F26. F21 was subjected to Sephadex LH-20 CC (petroleum ether–$CHCl_3$–MeOH, 2:1:1), then recrystallized to afford ganoderic acid DM (100 mg). F22 was applied to a Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 2:1:1), and then further purified by semipreparative HPLC (MeOH–H_2O, 90:10) to afford ganodermanondiol (20 mg) and ganoderic acid T-Q (25 mg). F23 was recrystallized to obtain lucidadiol (9 mg), F24 was extensively subjected to silica gel CC (petroleum ether–$CHCl_3$–Me_2CO, 8:1:1), Sephadex LH-20, and then further purified by semipreparative HPLC to give ganoderol B (18 mg), lucidumol A (30 mg) and 15α-hydroxy-3-oxo-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (23 mg). F25 was purified by semipreparative HPLC (MeOH-H_2O, 70:30, detection wavelength, 252 nm) to give 3β-hydroxy-5α-lanosta-7,9,24(*E*)-triene-26-oic acid (3 mg) and 15α,26-dihydroxy-5α-lanosta-7,9,24(*E*)-trien-3-one (6 mg). F26 was applied to a succession silica gel column (400–600 mesh, $CHCl_3$-MeOH, 100:1-10:1 gradient system) and Sephadex LH-20 (PE-$CHCl_3$-MeOH, 2:1:1) chromatography to afford 3β-hydroxy-7-oxo-5α-lanosta-8,24(*E*)-dien-26-oic acid (8 mg), ganodermanontriol (25 mg), **6** (10 mg), ganoderiol F (7 mg) and lucideric acid A

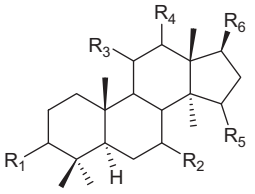
Table 2
^{13}C NMR spectroscopic data for compounds **1**–**6**.[a]

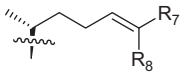
Position	1	2	3	4	5	6
1	34.8*t*	34.5*t*	35.1*t*	35.3*t*	33.9*t*	34.4*t*
2	34.6*t*	34.4*t*	34.1*t*	34.3*t*	33.6*t*	27.4*t*
3	214.4*s*	214.9*s*	215.9*s*	214.7*s*	215.1*s*	78.2*d*
4	47.5*s*	47.4*s*	46.8*s*	47.2*s*	46.8*s*	38.6*s*
5	50.8*d*	51.6*d*	49.2*d*	50.4*d*	50.8*d*	49.1*d*
6	37.5*t*	37.2*t*	27.6*t*	37.1*t*	37.4*t*	36.7*t*
7	199.4*s*	199.8*s*	65.8*d*	198.1*s*	198.6*s*	66.2*d*
8	142.1*s*	139.7*s*	156.7*s*	139.5*s*	149.8*s*	155.8*s*
9	158.7*s*	160.6*s*	141.6*s*	162.7*s*	145.9*s*	142.9*s*
10	40.1*s*	39.7*s*	38.2*s*	39.4*s*	39.2*s*	38.5*s*
11	65.8*d*	67.2*d*	191.8*s*	23.8*t*	194.1*s*	192.0*s*
12	44.6*t*	42.7*t*	78.5*d*	30.1*t*	78.9*d*	79.5*d*
13	47.6*s*	43.4*s*	50.3*s*	44.9*s*	47.6*s*	49.6*s*
14	48.1*s*	48.9*s*	59.9*s*	47.8*s*	58.7*s*	60.6*s*
15	32.6*t*	31.9*t*	215.1*s*	28.5*t*	205.6*s*	216.2*s*
16	27.9*t*	28.7*t*	38.4*t*	31.8*t*	37.8*t*	37.9*t*
17	49.7*d*	48.1*d*	50.1*d*	48.9*d*	44.3*d*	45.2*d*
18	16.9*q*	17.3*q*	14.4*q*	15.9*q*	12.0*q*	12.0*q*
19	19.2*q*	19.5*q*	18.1*q*	17.9*q*	18.7*q*	18.6*q*
20	36.0*d*	36.2*d*	154.1*s*	35.9*d*	29.4*d*	28.2*d*
21	18.4*q*	18.6*q*	20.3*q*	18.3*q*	21.6*q*	21.9*q*
22	34.6*t*	34.6*t*	126.1*d*	30.8*t*	48.4*t*	47.9*t*
23	25.8*t*	25.8*t*	197.8*s*	30.9*t*	207.6*s*	207.4*s*
24	145.3*d*	145.3*d*	47.5*t*	178.1*s*	46.6*t*	46.6*t*
25	126.8*s*	126.7*s*	34.4*d*		34.7*d*	34.6*d*
26	172.7*s*	172.7*s*	180.0*s*		175.6*s*	176.1*s*
27	11.9*q*	11.9*q*	17.0*q*		17.1*q*	17.1*q*
28	25.1*q*	25.1*q*	26.6*q*	25.3*q*	27.6*q*	28.0*q*
29	21.6*q*	21.8*q*	21.0*q*	21.4*q*	20.3*q*	15.4*q*
30	25.3*q*	24.9*q*	24.0*q*	24.9*q*	20.7*q*	24.0*q*
CH_3CO			170.6*s*		170.2*s*	170.4*s*
CH_3CO			20.6*q*		20.9*q*	20.9*q*
OCH_2CH_3					60.7*t*	
OCH_2CH_3					14.1*q*	
OCH_3						51.9*q*

[a] Recorded at 400 MHz in $CDCl_3$, δ_C in ppm.

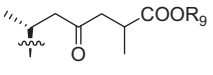
(30 mg). In the same manner, F3 (5.6 g) was applied to a silica gel column (400–600 mesh) eluted with PE (60–90 C)–Me_2CO (7:1–3:1) to afford 6 fractions, F31–F36. Each fraction was subjected to silica gel CC and repeated semipreparative HPLC to afford ganoderic acid D (50 mg), **1** (9 mg), **2** (5 mg), lucidone A (4 mg), ganolucidic acid E (9 mg), **4** (4 mg), ganoderic acid F (40 mg), ganoderenic acid D (35 mg), ganoderic acid E (22 mg), ganoderic acid J (20 mg), ganoderenic acid F (10 mg). F4 (8.9 g) was separated by repeated column chromatography ($CHCl_3$-MeOH, 100:1-5:1 gradient system) and semipreparative HPLC (MeOH-H_2O, 40:60, detection wavelength, 252 nm) to afford ganoderic acid B (35 mg), ganoderic acid A (40 mg), 7β,12β-dihydroxy-3,11,15,23-tetraoxo-5α-lanosta-8-en-26-oic acid (23 mg), 12β-hydroxy-3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid, ganoderenic acid B (20 mg), methyl ganoderate H (5 mg), **3** (5 mg), methyl ganoderic acid B (25 mg), 12β-acetoxy-3β,7β-dihydroxy-11,15,23-trioxo-5α-lanosta-8,20-dien-26-oic acid (6 mg), ganolucidic acid A (5 mg), methyl lucidenate C (4 mg), **5** (8 mg), ganoderic acid H (10 mg) and

Table 3
Cytotoxicity of compounds **1**–**43** against human HeLa cervical cancer cell lines.[a]

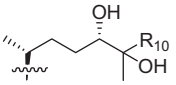




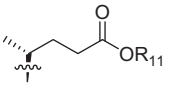
R61: R_7=CH_2OH, R_8=CH_3
R62: R_7=COOH, R_8=CH_3
R63: R_7=CH_2OH, R_8=CH_2OH



R64: R_9=H
R65: R_9=CH_2 CH_3
R66: R_9=CH_3



R67: R_{10}=CH_3
R68: R_{10}=CH_2OH



R69: R_{11}=H
R60: R_{11}=CH_3

	R1	R2	R3	R4	R5	R6	Double bond	IC_{50} (uM)
1	=O	=O	α-OH	H	H	R62	$\Delta^{8,9}$	123
2	=O	=O	β-OH	H	H	R62	$\Delta^{8,9}$	51
3	=O	β-OH	=O	β-OAc	=O	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	>300
4	=O	=O	H	H	H	R69	$\Delta^{8,9}$	48
5	=O	=O	=O	β-OAc	=O	R65	$\Delta^{8,9}$	63
6	β-OH	β-OH	=O	β-OAc	=O	R66	$\Delta^{8,9}$	166
7	β-OH	H	H	H	H	R61	$\Delta^{7,8}$, $\Delta^{9,11}$	147
8	=O	=O	H	H	H	R62	$\Delta^{8,9}$	167
9	=O	=O	=O	H	=O	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	258
10	=O	H	H	H	H	R67	$\Delta^{7,8}$, $\Delta^{9,11}$	32
11	β-OH	=O	H	H	H	R61	$\Delta^{8,9}$	5
12	=O	H	H	H	α-OH	R62	$\Delta^{7,8}$, $\Delta^{9,11}$	58
13	=O	H	H	H	α-OH	R61	$\Delta^{7,8}$, $\Delta^{9,11}$	1
14	=O	=O	H	H	H	R67	$\Delta^{8,9}$	21
15	β-OH	H	H	H	H	R62	$\Delta^{7,8}$, $\Delta^{9,11}$	59
16	β-OH	=O	H	H	H	R62	$\Delta^{8,9}$	>300
17	=O	H	H	H	H	R68	$\Delta^{7,8}$ $\Delta^{9,11}$	33
18	=O	H	H	H	H	R63	$\Delta^{7,8}$, $\Delta^{9,11}$	8
19	=O	β-OH	=O	H	=O	R69	$\Delta^{8,9}$	>300
20	=O	β-OH	=O	H	=O	R64	$\Delta^{8,9}$	22
21	β-OH	β-OH	=O	H	=O	β-Ac	$\Delta^{8,9}$	114
22	=O	H	=O	H	α-OH	R62	$\Delta^{8,9}$	31
23	=O	=O	=O	β-OAc	=O	R64	$\Delta^{8,9}$	>300
24	=O	β-OH	=O	H	=O	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	>300
25	=O	=O	=O	H	=O	R64	$\Delta^{8,9}$	108
26	=O	=O	=O	H	α-OH	R64	$\Delta^{8,9}$	>300
27	β-OH	β-OH	=O	H	=O	R64	$\Delta^{8,9}$	202
28	=O	β-OH	=O	H	α-OH	R64	$\Delta^{8,9}$	>300
29	=O	β-OH	=O	β-OH	=O	R64	$\Delta^{8,9}$	>300
30	=O	=O	=O	β-OH	=O	R64	$\Delta^{8,9}$	>300
31	β-OH	β-OH	=O	H	=O	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	204
32	β-OH	=O	=O	β-OAc	=O	R66	$\Delta^{8,9}$	64
33	β-OH	β-OH	=O	H	=O	R66	$\Delta^{8,9}$	114
34	β-OH	β-OH	=O	β-OAc	=O	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	137
35	=O	H	=O	H	α-OH	R64	$\Delta^{8,9}$	169
36	β-OH	β-OH	=O	H	=O	R60	$\Delta^{8,9}$	101
37	β-OH	=O	=O	β-OAc	=O	R64	$\Delta^{8,9}$	>300
38	β-OH	=O	=O	H	=O	R64	$\Delta^{8,9}$	213
39	=O	H	H	H	α-OAc	R62	$\Delta^{7,8}$, $\Delta^{9,11}$	>300
40	β-OH	β-OH	=O	H	α-OH	R64	$\Delta^{8,9}$	149
41	β-OH	β-OH	=O	β-OAc	=O	R64	$\Delta^{8,9}$	>300
42	β-OH	β-OH	=O	β-OH	=O	R64	$\Delta^{8,9}$	143
43	=O	β-OH	=O	H	α-OH	R64	$\Delta^{8,9}$, $\Delta^{20,22}$	>300

[a] The activity was shown as IC_{50} value, which was the concentration (uM) of tested compound that resulted in 50% inhibition of cell growth. Results were expressed as the mean value of triplicate data points. Adriamycin was used as a positive control (IC_{50} = 0.58um).

ganoderic acid AM1 (7 mg). Whereas fraction F5 (11.4 g) was separated by repeated column chromatography ($CHCl_3$-MeOH, 100:1-1:1 gradient system) and semipreparative HPLC (MeOH-H_2O, 30:70, detection wavelength, 252 nm) to give compounds 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanosta-8-en-26-oic acid (10 mg), ganoderic acid K (20 mg), ganoderic acid G (15 mg), ganoderenic acid A (18 mg).

4.3.1. 11α-Hydroxy-3,7-dioxo-5α-lanosta-8,24(E)-dien-26-oic acid (**1**)

White powder; $[\alpha]_D^{23} + 13.3$ (*c* 0.33, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 248 (2.33) nm; IR (dry film) ν_{max} 3323, 2962, 2919, 1711, 1645, 1587, 1383, 1261, 1099, 1022, 955, 800 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; positive EIMS *m/z* 484 $[M]^+$ (90), 466 (16), 332 (22), 258 (22), 125 (100); HREIMS *m/z* 484.3187 $[M]^+$ (calcd. for $C_{30}H_{44}O_5$, 484.3189).

4.3.2. 11β-Hydroxy-3,7-dioxo-5α-lanosta-8,24(E)-dien-26-oic acid (**2**)

White powder; $[\alpha]_D^{23} - 14.0$ (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 248 (2.36), nm; IR (KBr) ν_{max} 3523, 3421, 2962, 2925, 2852, 1709, 1658, 1645, 1574, 1458, 1419, 1380, 1261, 1097, 1022, 802 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 484 $[M]^+$ (100), 469 (26), 451 (42), 423 (27), 261 (18), 125 (28); HREIMS *m/z* 484.3187 $[M]^+$ (calcd. for $C_{30}H_{44}O_5$, 484.3189).

4.3.3. 12β-Acetoxy-7β-hydroxy-3,11,15,23-tetraoxo-5α-lanosta-8,20-dien-26-oic acid (**3**)

Colorless gum; $[\alpha]_D^{22} + 85.0$ (*c* 0.14, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (2.39) nm; IR (dry film) ν_{max} 3446, 2966, 2928, 1732, 1705, 1684, 1610, 1462, 1373, 1230, 1049, 951, 754 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 570 $[M]^+$ (8), 552 (10), 493 (32), 492 (92), 464 (24), 343 (32), 301 (28), 192 (100), 164 (48), 119 (38); HREIMS *m/z* 570.2829 $[M]^+$ (calcd. for $C_{32}H_{42}O_9$, 570.2829).

4.3.4. 4,4,14α-Trimethyl-3,7-dioxo-5α-chol-8-en-24-oic acid (**4**)

White powder; $[\alpha]_D^{22} + 23.0$ (*c* 0.14, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 252 (1.79) nm; IR (KBr) ν_{max} 3392, 2962, 2918, 1709, 1668, 1459, 1417, 1379, 1261, 1097, 1020, 800 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 428 $[M]^+$ (68), 413 (100), 395 (42), 377 (56), 327 (24), 285 (16), 271 (16), 195 (17), 135 (17), 55 (20); HREIMS *m/z* 428.2923 $[M]^+$ (calcd. for $C_{27}H_{40}O_4$, 428.2927).

4.3.5. 12β-Acetoxy-3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid ethyl ester (**5**)

White powder; $[\alpha]_D^{22} + 136.0$ (*c* 0.18, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 251 (2.15) nm; IR (KBr) ν_{max} 3435, 2979, 2935, 1751, 1701, 1462, 1377, 1229, 1175, 1041 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 598 $[M]^+$ (7), 556 (45), 538 (20), 510 (32), 495 (16), 441 (31), 398 (19), 380 (30), 353 (30), 318 (16), 316 (52), 302 (36), 288 (10), 255 (25), 237 (28), 209 (34), 191 (72), 158 (20), 143 (100), 115 (49), 95 (22), 69 (22); HREIMS *m/z* 598.3145 $[M]^+$ (calcd. for $C_{34}H_{46}O_9$, 598.3142).

4.3.6. 3β,7β-Dihydroxy-12β-acetoxy-11,15,23-trioxo-5α-lanosta-8-en-26-oic acid methyl ester (**6**)

Yellow gum; $[\alpha]_D^{25} + 68.0$ (*c* 0.32, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 254 (2.04) nm; IR (dry film) ν_{max} 3435, 2968, 2933, 2875, 1732, 1679, 1583, 1462, 1373, 1232, 1043, 758 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; EIMS *m/z* 588 $[M]^+$ (6), 560 (14), 417 (15), 306 (100), 241 (10), 191 (14), 139 (14), 129 (28); HREIMS *m/z* 588.3347 $[M]^+$ (calcd. for $C_{33}H_{48}O_9$, 588.3298).

4.4. Cytotoxicity assay

Cytotoxicity against the HeLa cells was evaluated by using the MTT (methyl thiazole tetrazolium) method according to the protocols described (Alley et al., 1988) with adriamycin as a positive control (IC_{50} = 0.58 μM against HeLa cells).

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