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# Triterpenoids from *Ganoderma lucidum* and their cytotoxic activities

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From the ethyl acetate fraction of the fruiting body of *Ganoderma lucidum*, a new triterpenoid, ethyl 7$\beta$-hydroxy-4,4,14$\alpha$-trimethyl-3,11,15-trioxo-5$\alpha$-chol-8-en-24-oate (**4**), named ethyl lucidenates A, along with three known compounds, ganodermanondiol (**1**), lucidumol B (**2**) and methyl lucidenates A (**3**) were isolated by silica gel column, ODS column chromatography and PHPLC. Their structures were established on the basis of spectroscopic analysis and chemical evidence. The isolated compounds were tested using *in vitro* MTT assay for their cytotoxic activities against the K562, HL-60, CA46, HepG2, SW480 and SMMC-7221 cancer cell lines. Among them, compound **4** showed cytotoxicity against HL-60 and CA46 cancer cell lines with $IC_{50}$ values of 25.98 and 20.42 $\mu g\,mL^{-1}$, respectively.

**Keywords:** *Ganoderma lucidum*; triterpenoid; cytotoxic activity

## 1. Introduction

*Ganoderma lucidum* (Leyss. ex Fr.) Karst. is a species of basidiomycetes that belongs to Ganodermataceae of Aphyllophorales. *Ganoderma lucidum*, a traditional Chinese medicine called Lingzhi, is one of the most highly ranked herbal medicines by Asian people, whose fruiting body, mycelia and spores were traditionally used as a folk medicine for treatment of debility and weakness, insomnia, hepatitis, cardiovascular diseases, cancer, etc. (Gao & Zhou, 2004; Lin & Zhang, 2004; Liu & Zhang, 2005; Yuen & Gohel, 2005). Extensive studies have been made on the constituents of *G. lucidum*, including triterpenes, polysaccharides, nucleosides, steroids, fatty acids, alkaloids, proteins, peptides, amino acids and inorganic elements (Chang, 1996; Chen, Xie, & Gong, 2007; Jong & Birmingham, 1992). Previous studies have reported that the triterpenoids possess the bioactivities of antioxidation and hepatoprotection, suppress angiogenesis, have anticancer property, etc. (Harvey, Slivova, Jiang, & Sliva, 2005; Stanley, Hong, Dunn, Shen, & Pence, 2004; Wang, Liu, Che, & Lin, 2000; Zhu, Chang, Wong, Chong, & Li, 1999). Over 130 triterpenoids have been isolated from the fruiting body, cultured mycelia and spores during the past two decades (Chen & Yu, 1990; Luo & Lin, 2002).

This article deals with the isolation and structure elucidation of the new compound, ethyl 7$\beta$-hydroxy-4,4,14$\alpha$-trimethyl-3,11,15-trioxo-5$\alpha$-chol-8-en-24-oate (**4**), named ethyl lucidenates A, together with three known compounds isolated from the fruiting body of

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*G. lucidum* (Figure 1). We also describe the cytotoxic activity against K562, HL-60, CA46, HepG2, SW480 and SMMC-7221 cancer cell lines of the isolated compounds.

## 2. Results and discussion

### 2.1. *Identification of compounds*

New and three known compounds were isolated from the fruiting body of *G. lucidum*. The known compounds were identified as ganodermanondiol (**1**), lucidumol B (**2**) and methyl lucidenates A (**3**) on the basis of spectroscopic analysis, chemical evidence and comparison of spectral data with literature data (Arisawa et al., 1988; Kikuchi et al., 1986).



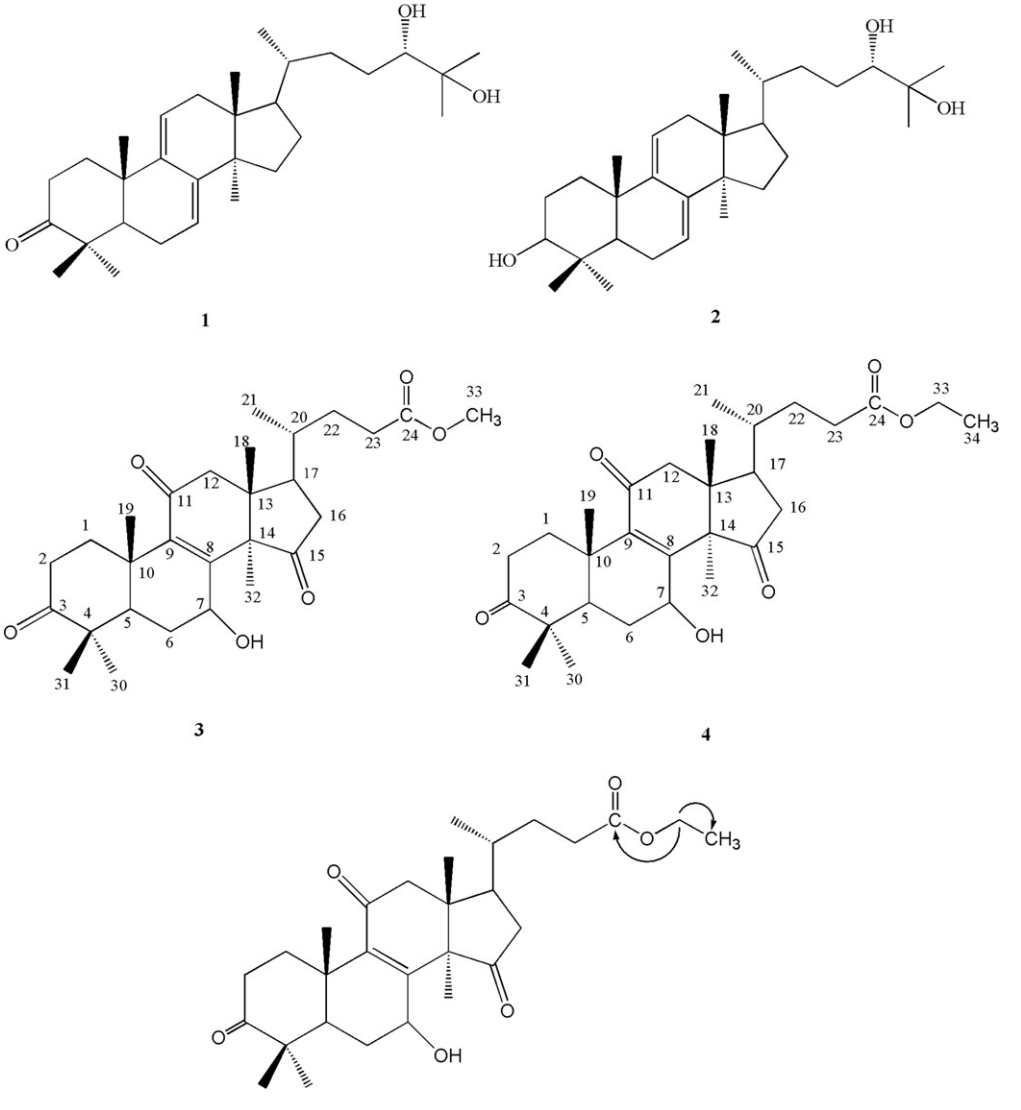


Figure 1. The structures of compounds **1**–**4** isolated from *G. lucidum* and key HMBC correlations of compound **4**.

Compound **4**, obtained as a white amorphous powder, gave a positive red colouration in the Liebermann–Burchard reaction. UV $\lambda_{max}$ (EtOH) nm: 254. The molecular formula was established as $C_{29}H_{42}O_6$ on the basis of HR–ESI–MS (*m*/*z* 485.2908 $[M-H]^-$). The IR spectrum exhibited the presence of hydroxy (3427 $cm^{-1}$) and carbonyl (1725, 1703 and 1652 $cm^{-1}$) groups. The $^1$H-NMR spectrum showed signals for seven methyl groups at $\delta$ 0.96 (3H, s, H-18), 1.06 (3H, s, H-31), 1.08 (3H, s, H-30), 1.21 (3H, s, H-19), 1.31 (3H, s, H-32), 0.94 (3H, d, $J = 6.5$ Hz, H-21), 1.22 (3H, t, $J = 7.0$ Hz, H-34), one oxymethine proton at $\delta$ 4.81 (1H, m, H-7) and one oxymethene proton at $\delta$ 4.09 (2H, *q*, $J = 7.0$ Hz, H-33). The $^1$H-NMR spectrum further showed an ethoxyl proton at $\delta$ 4.09 (2H, *q*, $J = 7.0$ Hz, H-33) and $\delta$ 1.22 (3H, t, $J = 7.0$ Hz, H-34). The $^{13}$C-NMR spectrum and distortionless enhancement by polarisation transfer spectra revealed three carbonyl carbon at $\delta$ 197.8 (C-11), 216.8 (C-15) and 218.1 (C-3), an ester carbonyl carbon at $\delta$ 173.5 (C-24), two oxygenated carbon at $\delta$ 66.2 (C-7) and 60.5 (C-33), one carbon–carbon double bond at $\delta$ 157.9 (C-8) and 141.1 (C-9) and seven methyl carbons at $\delta$ 14.2 (C-34), 17.6 (C-18), 18.0 (C-21), 18.2 (C-19), 20.7 (C-31), 24.6 (C-32) and 27.0 (C-30). The other carbon signals were observed and assigned to eight methylenes, four methines and four quaternary carbons. The NMR data of compound **4** were very similar to those of methyl lucidenates A except for the absence of an ethyl group. The full NMR assignments and connectivities were determined by the $^1$H–$^1$H correlation spectroscopy (COSY) and heteronuclear multiple bond coherence (HMBC) spectroscopic data analyses. The HMBC correlations between H-33 ($\delta$ 4.09) and C-34 ($\delta$ 14.2), C-24 ($\delta$ 173.5) and between H-34 ($\delta$ 1.22) and C-33 ($\delta$ 60.5) showed that the ethoxyl group was linked at C-24. Based on the above evidence analysis, the structure of compound **4** was determined as ethyl 7$\beta$-hydroxy-4, 4,14$\alpha$-trimethyl-3,11,15-trioxo-5$\alpha$-chol-8-en-24-oate, which we named ethyl lucidenates A.

### 2.2. *Cytotoxic activity*

Using the MTT assay method, the isolates were evaluated for *in vitro* cytotoxic activity against cancer cell lines K562, HL-60, CA46, HepG2, SW480 and SMMC-7221. It can be observed from the results presented in Table 1 that compounds **3** and **4** showed cytotoxic activity against CA46 and HL-60 cell lines with $IC_{50}$ values of 17.13, 20.51 $\mu g\,mL^{-1}$ and 20.42, 25.98 $\mu g\,mL^{-1}$, respectively, and weak inhibitory activity on K562, HepG2, SW480 and SMMC-7221 cell lines. Compound **1** exhibited cytotoxic activity against HL-60 cell lines with an $IC_{50}$ value of 22.49 $\mu g\,mL^{-1}$ and weak inhibitory activity on K562 cell lines with an $IC_{50}$ value of 33.71 $\mu g\,mL^{-1}$. In addition, compound **2** showed weak cytotoxic activity in the inhibition of K562 and HL-60 cancer cell lines with $IC_{50}$ values of 37.25 and 40.12 $\mu g\,mL^{-1}$.

Table 1. Cytotoxicity of compounds isolated from *Ganoderma lucidum* on tumour cell growth.

| | $IC_{50}$ ($\mu g\,mL^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Sample | K562 | HL-60 | CA46 | HepG2 | SW480 | SMMC-7221 |
| Compound | | | | | | |
| **1** | 33.71 | 22.49 | >100 | >100 | >100 | >100 |
| **2** | 37.25 | 40.12 | >100 | >100 | >100 | >100 |
| **3** | 31.15 | 20.51 | 17.13 | 49.82 | 41.02 | 70.45 |
| **4** | 37.62 | 25.98 | 20.42 | 55.15 | 50.45 | 73.98 |

## 3. Experimental

### 3.1. *General experimental procedures*

UV spectra were measured with a Varian Cary 50 Bio UV–visible spectrophotometer. IR spectra were measured with an Avatar 330 FT–IR spectrometer. A SHIMADZU LC-6AD HPLC was used for purification and isolation with an Aglient ZORBAX 300SB-C18 HPLC column (21.2 × 250 mm, 7 μm). NMR spectra were recorded on a Bruker Ultrashield Plus 500 MHz spectrometer ($^1$H, 500 MHz; $^{13}$C, 125 MHz). Conventional pulse sequences were used for COSY and HMBC. All chemical shifts ($\delta$) were given in ppm units with reference to tetramethylsilane as an internal standard and the coupling constants ($J$) were in Hz. HR–ESI–MS was measured on an API Qstar Pulsar instrument. TLC was carried out on precoated silica gel 60 F254 (Marine Chemical Factory, Qingdao, China). Chromatography suppliers were used for isolation: silica gel (200–300 mesh) (Marine Chemical Factory, Qingdao, China) or RP-$C_{18}$ (YMC*GEL ODS-A 50 μm). Optical density (OD) values in the cytotoxic activity by MTT assays were read on a Bio-Rad Microplate Reader.

### 3.2. *Materials*

The fruiting body of *G. lucidum* was collected in March 2010 at Fujian Xianzhilou Biological Science and Technology Co., Ltd, Fuzhou, China, and identified by Professor Yong-Hong Zhang, School of Pharmacy, Fujian Medical University, Fuzhou, China. A voucher specimen (no. GL-20100309) has been deposited at the Pharmacognosy Laboratory, School of Pharmacy, Fujian Medical University.

### 3.3. *Extraction and isolation*

The air-dried fruiting body of *G. lucidum* (30 kg) was extracted with EtOH at room temperature for 72 h and the process was repeated three times. After the evaporation of the solvent under reduced pressure, the crude EtOH extract (944 g) was obtained. This extract was suspended in water and partitioned with petroleum ether, EtOAc and *n*-BuOH, successively. The EtOAc solution was concentrated and the residue (400 g) was dissolved again in EtOAc and extracted with saturated aqueous $NaHCO_3$ (500 mL × 4). The EtOAc layer was washed with water, dried and concentrated to give a neutral fraction (280 g) as a yellow–brown syrup. This neutral fraction (250 g) was applied to a silica gel column (10 × 75 cm) eluted with $CHCl_3$–MeOH (99:1 to 0:1, V/V) to give 10 fractions (E1–E10). Subfraction E1 (7 g) was subjected to a silica gel column (5 × 65 cm), eluted with petroleum ether–EtOAc (9:1 to 1:1, V/V) to give five subfractions (E1.1–E1.5). Subfraction E1.3 (5.4 g) was subjected to a RP-$C_{18}$ column (2.6 × 61 cm, 50 μm), eluted with MeOH–$H_2O$ (85 : 15, V/V) to give subfraction E1.3.1 (1.3 g), compound **1** (120 mg) and compound **2** (75 mg). Subfraction E1.3.1 (1.3 g) was purified by HPLC {mobile phase: MeOH–$H_2O$ (65 : 35, V/V); UV: 254 nm; flow rate: 6 mL min$^{-1}$; column: ZORBAX 300SB $\phi$ 21.2 × 250 mm, 7 μm} to obtain compound **3** (72.0 mg) and compound **4** (11.0 mg).

#### 3.3.1. *Ethyl 7β-hydroxy-4,4,14α-trimethyl-3,11,15-trioxo-5α-chol-8-en-24-oate (**4**)*

White amorphous powder. IR $\nu_{max}$ (KBr) cm$^{-1}$: 3427, 1725, 1703 and 1652. HR–ESI–MS: $[M-H]^-$ $m/z$: 485.2908 (Calcd for $C_{29}H_{42}O_6$, 485.2903). $^1$H-NMR (500 MHz, $CDCl_3$): $\delta_H$ 1.52, 2.90 (each 1H, m, H-1), 2.50, 2.45 (each 1H, m, H-2), 1.55 (1H, m, H-5), 2.11, 1.64 (each 1H, m, H-6), 4.81 (1H, m, H-7), 2.79, 2.75 (each 1H, d, $J$ = 8.5 Hz, H-12), 2.12 (1H, dd, $J$ = 19.5, 9.5 Hz, H-16), 2.77 (1H, dd, $J$ = 19.5, 8.5 Hz, H-16), 1.97 (1H, m, H-17), 0.96 (3H, s, H-18), 1.21 (3H, s, H-19), 1.57 (1H, m, H-20), 0.94 (3H, d, $J$ = 6.5 Hz, H-21), 1.35,

1.75 (each 1H, m, H-22), 2.30, 2.45 (each 1H, m, H-23), 1.08 (3H, s, H-30), 1.06 (3H, s, H-31), 1.31 (3H, s, H-32), 4.09 (2H, *q*, $J = 7.0$ Hz, H-33) and 1.22 (3H, t, $J = 7.0$ Hz, H-34). $^{13}$C-NMR (125 MHz, $CDCl_3$): $\delta_C$ 35.6 (C-1), 34.2 (C-2), 218.1 (C-3), 46.7 (C-4), 48.7 (C-5), 27.6 (C-6), 66.2 (C-7), 157.9 (C-8), 141.1 (C-9), 38.2 (C-10), 197.8 (C-11), 50.2 (C-12), 44.9 (C-13), 59.3 (C-14), 216.8 (C-15), 41.1 (C-16), 46.2 (C-17), 17.6 (C-18), 18.2 (C-19), 35.2 (C-20), 18.0 (C-21), 30.6 (C-22), 31.1 (C-23), 173.5 (C-24), 27.0 (C-30), 20.7 (C-31), 24.6 (C-32), 60.5 (C-33) and 14.2 (C-34). Key HMBC: H-34 [C-33] and H-33 [C-24, C-34].

### 3.4. *Cytotoxic activity*

The cancer cell lines (K562, HL-60, CA46, HepG2, SW480 and SMMC-7221) were maintained in RPMI and Dulbecco's modified eagle's medium that included L-glutamine with 10% FBS and 2% penicillin–streptomycin. Cells were cultured at 37°C in a 5% $CO_2$ incubator. Cytotoxic activity was measured using a modified MTT assay (Chen, Ales, Baenziger, & Wiemer, 1983). Viable cells were seeded in the growth medium (100 μL) into 96-well microtitre plates ($1 \times 10^4$ cells per well) and incubated at 37°C in a 5% $CO_2$ incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations of 6.25, 12.5, 25, 50 and 100 μg mL$^{-1}$ by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to $<0.1\%$. After allowing the test sample to stand for 24 h, 10 μL of it was added to each well. The same volume of DMSO was added to the control wells. Then, after removing the medium of the test sample treatment after 48 h, 10 μL of MTT was also added to the each well (final concentration, 5 mg mL$^{-1}$). After 4 h of incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μL). The OD was measured at 570 nm. The $IC_{50}$ value was defined as the concentration of sample which reduced absorbance by 50% relative to the vehicle-treated control.

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