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NOTE

# Lanostane-type triterpenes from the sporoderm-broken spores of *Ganoderma lucidum*

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The fruiting bodies of *Ganoderma lucidum* are widely used in China, Japan and Korea as medicine for a variety of diseases, such as chronic bronchitis inflammation, hyperlipidemia, hypertension, neurasthenia, hepatitis, leukopenia and adjuvant treatment of cancers.[1,2] *Ganoderma* spores is the fungus's reproductive cells ejected from the cap of *G. lucidum* after the fruiting bodies become mature. In recent years, with the development of spores collection, sporoderm-broken technology and application of modern analytical instruments, there has been some progress on chemical constituents, pharmacological effects and mechanisms of action of *Ganoderma* spores. Moreover, the increasing publications in domestic and international suggest the important bioactivities of the spores of *G. lucidum*.[3,4]

The chemical constituents and bioactivities of the fruiting bodies of *G. lucidum* have been fully investigated, and >150 triterpenes have been reported from the fruiting bodies of *G. lucidum* representing five major structural classes.[5–7] However, the chemical investigation of the spores of *G. lucidum* can only be traced to 1988.[8] Up to now, a series of new lanostane-type triterpenes, such as ganosporelactones A, B,[9] lucidumol A, ganoderic acid β,[10] ganoderic acid γ, δ, ε, ξ, η, θ[11] and lucidenic acid SP,[12] were isolated from the spores of *G. lucidum*.

Sporoderm-broken technology recently has been applied to *Ganoderma* spores to improve the extraction rate and improve the remedy effect in the clinical trials.[13] However, to the best of our knowledge, an article on the chemistry of the compounds isolated from the sporoderm-broken spores of *G. lucidum* has not been prepared. As a part of our continuing studies to find novel bioactive compounds from mushroom, we found a new highly oxygenated lanostane-type triterpene aldehyde, lucialdehyde E (**1**) from the sporoderm-broken spores of *G. lucidum*, together with seven known triterpenes, lucialdehyde D (**2**), ganodermanondiol (**3**), ganoderic acid $C_1$ (**4**), ganodermanontriol (**5**), genoderic acid ξ (**6**), ganoderic acid A (**7**) and lucidumol A (**8**) (Figure 1). The structural elucidations of all the compounds were based on the spectroscopic evidences and comparing with literature data. Lanostane-type triterpene aldehydes are very few in the Ganodermataceae, only lucialdehydes A–C and lucialdehyde D were isolated from the fruiting bodies of *G. lucidum* and *G. pfeifferi*, respectively.[14,15] Compound **1** is a lanostane-type triterpene aldehyde isolated from the sporoderm-broken spores of *G. lucidum* for the first time. The cytotoxicity of compounds **1**–**8** was tested *in vitro* against esophageal tumor EC109 cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Compounds **1**, **2**, **3**, **4** and **7** showed significant cytotoxic activity with $IC_{50}$ values of 18.7, 21.4, 16.2 and 20.1 μg ml$^{-1}$, respectively. In addition, the other four triterpenes only showed weak cytotoxic activity with $IC_{50}$ values of 124.8, 162.6, 148.1 and 121.5 μg ml$^{-1}$, respectively.

Lucialdehyde E (**1**) was obtained as a white amorphous powder, with a positive optical rotation, $[\alpha]_D^{20}$ + 137° ($CHCl_3$). The presence of two conjugated carbonyl groups (1668 and 1660 cm$^{-1}$) was suggested by IR spectrometry. The high-resolution electron impact mass (HR-EIMS) spectrum of **1** give an ion peak at *m/z* 484.3106 (calculated for 484.3114) and corresponded to a molecular formula of $C_{30}H_{44}O_5$. $^1$H-NMR spectrum of **1** showed signals for seven methyls, and a singlet at $\delta$ 9.41 for an aldehyde proton (Table 1). The $^{13}$C-NMR spectrum of **1** demonstrated signals characteristic for seven methyls, four olefinic carbons, two oxymethylene carbons ($\delta$ 68.6, 72.1), and an aldehyde carbon ($\delta$ 194.4). The $^{13}$C-NMR spectrum of **1** was close to the structure of ganoderic acid γ,[11] except for chemical shifts of C-26 ($\delta$ 194.4 in lucialdehyde E vs $\delta$ 170.2 in ganoderic acid γ) and C-23 ($\delta$ 26.0 in lucialdehyde E vs $\delta$ 65.9 in ganoderic acid γ). HMBC correlations were observed between signals of H-5 and C-7; H-30 and C-8; H-19 and C-9, 11; H-17 and C-15; H-18 and C-11, 12. In addition, HMBC correlations between signals of H-22 and C-24 (an olefinic carbon at $\delta$ 156.3), and between signals of H-24 and C-26, as well as those of H-24 and C-27 revealed a conjugated aldehyde at C-24–C-26 in the side chain. As the signals of H-28 and H-29 were shift-correlated with that of C-3, a carbonyl group was concluded to be at C-3 (Figure 2). Two equatorial hydroxyl groups at C-7 (β-orientation) and C-15 (α-orientation) were deduced from the

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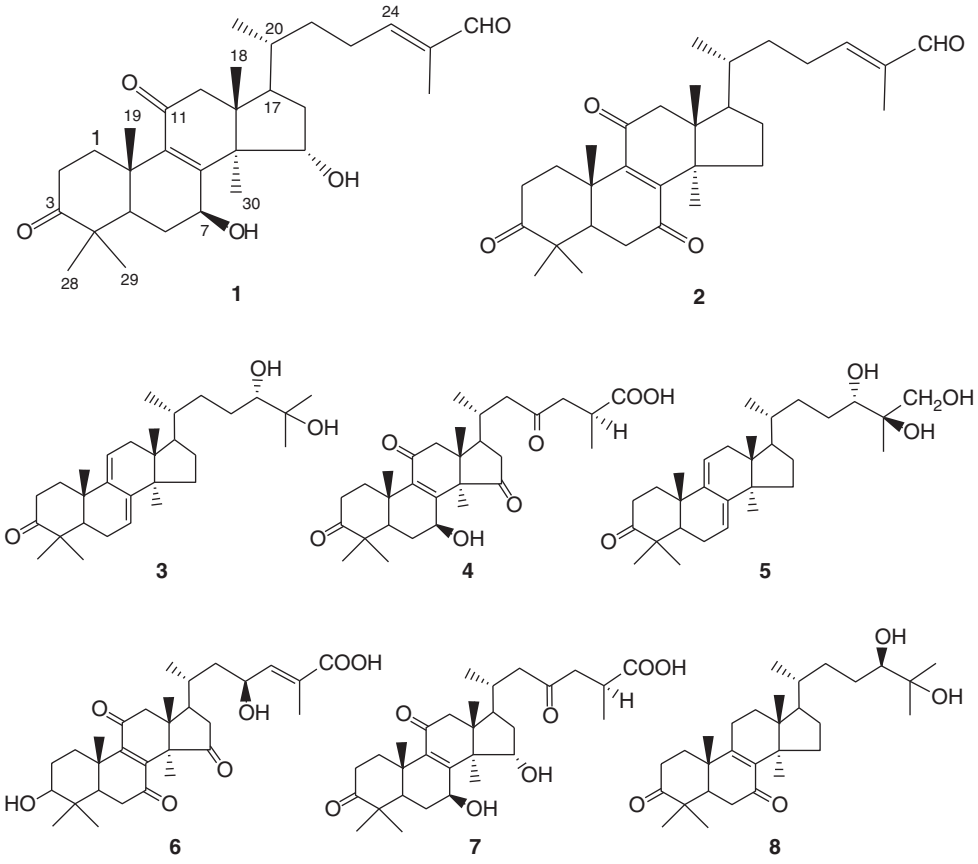


**Figure 1** Structures of triterpenes **1**–**8**.

multiplicities of H-7 and H-15, which was supported by NOE correlations observed from H-7 to H-5, H-29 and H-30. NOE correlations were also observed from H-15 to H-18, H-19, and H-19 to H-28. As NOE correlations were observed between H-24 and H-26, the configuration of C-24 was suggested to be *E* with respect to an aldehyde group, which was also supported by comparing to the NMR spectral data of ganoderal A having a (24*E*)-26-al moiety.[16] These data above suggested a highly oxygenated lanostane-type *Ganoderma* triterpene, consequently, the structure of **1** was determined as 7β, 15α-dihydroxyl-3, 11-dioxo-5α-lanosta-8, 24-dien-26-al.

Some progresses of chemical and pharmacological research have been made on the spores of *G. lucidum*. In some cases, extracts of partly purified preparations and pure compounds from *Ganoderma* spores have been used for *in vitro* or *in vivo* testing.[17,18] However, as the biological activities of *Ganderma* spores are determined by the active ingredients contained, the levels of active ingredients vary from the origin, cultivation, acquisition time and extraction methods.[19]

At present, the spores of *G. lucidum* have been widely used in China as a home remedy for the adjuvant treatment of cancers, and its medical value has been widely recognized, however, its biological activities still need further assessment before they can be accepted not only by the traditional Asian medicine, but also by the west science and medicine.

## EXPERIMENTAL PROCEDURE

### General

$[\alpha]_D$ was carried out on JASCO-20 polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 IR spectrometer in KBr pellets (Bio-Rad, Philadelphia, PA, USA). $^1$H, $^{13}$C NMR and 2D NMR spectra were recorded on a Bruker AM-400 and DRX-500 instruments (Bruker, Fällanden, Switzerland). Chemical shifts are given as $\delta$ in p.p.m. relative to tetramethylsilane as internal standard and coupling constants in Hz. Mass spectra were measured with a VG Autospec 3000 mass spectrometer (VG, Manchester, UK).

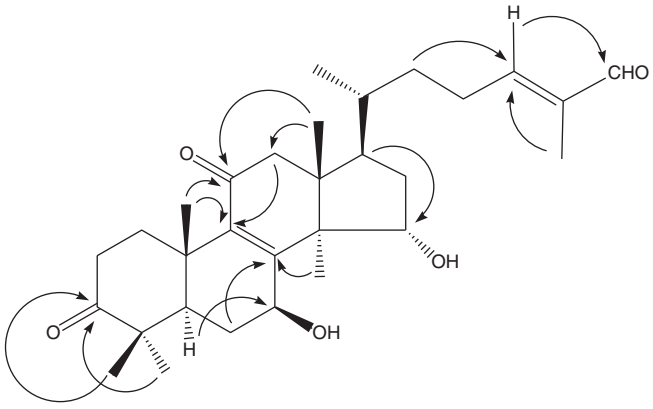

**Figure 2** Key HMBC correlations of lucialdehyde E.

### Mushroom material

The sporoderm-broken spores of *G. lucidum* were purchased from Zhengzhou market of Henan Province, People's Republic of China, in August 2010. The fungal identification was made by Professor Jin-Wen Shen, Henan Agricultural University. A dried specimen was deposited in the Herbarium of Henan Agricultural University.

**Table 1** $^{1}H$ and $^{13}C$ NMR data of lucialdehyde E in $CDCl_3$

| *Position* | δ *C* | δ *H* |
|---|---|---|
| 1 | 35.2 | 1.63 (1H, m), 2.72 (1H, m) |
| 2 | 34.1 | 2.34 (1H, m), 2.86 (1H, m) |
| 3 | 218.4 | |
| 4 | 45.9 | |
| 5 | 48.6 | 1.99 (1H, m) |
| 6 | 28.8 | 1.82 (1H, m), 2.45 (1H, m) |
| 7 | 68.6 | 4.93 (1H, dd, 9.8, 6.8) |
| 8 | 159.8 | |
| 9 | 140.3 | |
| 10 | 37.5 | |
| 11 | 199.8 | |
| 12 | 51.6 | 2.23 (1H, m), 2.75 (1H, m) |
| 13 | 46.2 | |
| 14 | 53.7 | |
| 15 | 72.1 | 4.68 (1H, m) |
| 16 | 36.2 | 2.01 (2H, m) |
| 17 | 47.5 | 1.50 (1H, m) |
| 18 | 17.0 | 0.92 (3H, s) |
| 19 | 19.3 | 1.27 (3H, s) |
| 20 | 35.9 | 1.78 (1H, m) |
| 21 | 18.4 | 1.13 (3H, d, 6.3) |
| 22 | 34.4 | 1.46 (2H, m), 1.82 (1H, m) |
| 23 | 26.0 | 2.10 (1H, m), 2.34 (1H, m) |
| 24 | 156.3 | 6.33 (1H, m) |
| 25 | 138.6 | |
| 26 | 194.4 | 9.41 (1H, s) |
| 27 | 9.2 | 1.93 (3H, s) |
| 28 | 20.8 | 1.25 (3H, s) |
| 29 | 27.5 | 0.95 (3H, s) |
| 30 | 19.1 | 0.92 (3H, s) |

### Chemicals

Column chromatography was carried out on silica gel (200–300 mesh), and TLC was carried out on plates precoated with silical gel $F_{254}$ (Qingdao Marine Chemical Ltd., Qingdao, PR China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). Fractions were monitored by TLC and spots were detected by spaying 10% sulfuric acid in ethanol followed by heating.

### Isolation procedure

The sporoderm-broken spores of *G. lucidum* (8.0 kg) was extracted with a mixed solvent of $CHCl_3$–MeOH (50 : 50 v/v, 20 L×3) at room temperature, and the combined solutions were evaporated *in vacuo* to give a residue (462.8 g). The residue was suspended in 90% MeOH (1000 ml) and extracted with hexane (600 ml×2). The residual MeOH solution was concentrated *in vacuo* to give a residue (414.2 g), which was applied to a column chromatography of silica gel. Elution was started with $CHCl_3$, and then $CHCl_3$–MeOH (9 : 1, v/v) to yield two fractions (A: 122.0 g, B: 85.5 g). Column chromatography of fraction A on silica gel (petroleum ether–EtOAc, 5 : 1→1 : 1, v/v) yielded five subfractions (A1-A5; 9.8, 13.5, 25.4, 24.6 and 16.8 g, respectively). The subfraction A1 was submitted to repeated column chromatography on silica gel by eluting with hexane–acetone (5 : 1, v/v) and repeated Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 1, v/v), followed by preparative HPLC (a linear gradient of $CH_3CN$ (75%→95%) in 2% AcOH, flow rate 5 ml $min^{-1}$, detection at 235 nm)to afford compounds **2** (14.6 mg), **3** (9.8 mg), **1** (15.9 mg) and **5** (10.2 mg). The subfraction A2 was further purified by column chromatography on silica gel (hexane–acetone, 4 : 1, v/v) and repeated Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 1, v/v), followed by preparative HPLC (a linear gradient of $CH_3CN$ (72%→90%) in 2% AcOH, flow rate 6 ml $min^{-1}$, detection at 230 nm) to afford compounds **8** (11.7 mg), **6** (20.1 mg), **4** (22.7 mg) and **7** (19.6 mg).

### Cell growth inhibition assay

The growth inhibitor concentrations of compounds **1–8** on esophageal cancer EC109 cell line were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.[20] The EC109 cell was exposed to compounds at 0.01, 0.1, 1, 10 and 100 mg $l^{-1}$ concentrations and each concentration was tested in triplicate. The OD was measured with a microplate reader at 570 nm.

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