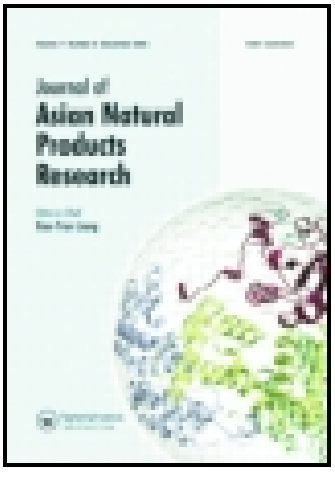

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Two new triterpenoids from fruiting bodies of fungus *Ganoderma lucidum*

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Two new triterpenoids, (24*E*)-9α,11α-epoxy-3β-hydroxylanosta-7,24-dien-26-al (**1**) and (22*Z*,24*Z*)-13-hydroxy-3-oxo-14(13 → 12)*abeo*-lanosta-8,22,24-trien-26,23-olide (**2**) were isolated from dried fruiting bodies of fungus *Ganoderma lucidum*. The structures of these two new compounds were elucidated on the basis of extensive spectroscopic analyses. Compound **1** possessed a lanostane skeleton, while compound **2** was based on a rare 14 (13 → 12)*abeo-lanostane* skeleton with a 26,23-olide moiety. Both of them were evaluated for their antifungal and cytotoxic activities. Neither of them displayed obvious inhibition on *Candida albicans* and five human cancer cell lines.

Keywords: *Ganoderma lucidum*; triterpenoid; 14 (13 → 12)*abeo-lanostane*

1. Introduction

The medicinal mushroom *Ganoderma lucidum* (Curtis.) P. Karst., which was thought to preserve human vitality and to promote longevity, has been utilized for centuries in East Asia [1]. It is a common species widely distributed in temperate to subtropical areas of China [2]; the species name of this prize medicinal fungus was clarified as "*G. lingzhi*" recently [3]. *G. lucidum* has been used to treat various human diseases such as allergy, arthritis, nephritis, neurasthenia, scleroderma, inflammation, and cancer [4,5]. The increasing interest in searching bioactive components from the genus *Ganoderma* has led to the discovery of large amounts of triterpenoids [6,7], polysaccharides [8,9], and proteins [10,11], most of which possessed apparent bioactivities. Our efforts in finding the bioactive components from *G. lucidum* resulted in the isolation of two new triterpenoids, (24*E*)-9α,11α-epoxy-3β-hydroxylanosta-7,24-dien-26-al (**1**) and (22*Z*,24*Z*)-13-hydroxy-3-oxo-14(13 → 12)*abeo*-lanosta-8,22,24-trien-26,23-olide (**2**) (Figure 1). Both of them were tested for their antifungal activity and cytotoxicity.

2. Results and discussion

Compound **1** was isolated as a white powder. The molecular formula of **1** was determined as $C_{30}H_{46}O_3$ by the HR-EI-MS at *m/z* 454.3456 (calcd for 454.3441). The maximum absorption at 241 nm in the UV spectrum, as well as IR absorption bands at 3439 and 1676 cm^{-1} revealed the existence of hydroxy and conjugated ketone groups. The ^1H NMR spectrum of compound **1** showed six methyl singlets at δ_H 0.73, 0.89, 1.02, 1.07, 1.08, and 1.75; a methyl doublet at δ_H 0.94 (3H, $J = 5.8$ Hz); two *O*-bearing methines at

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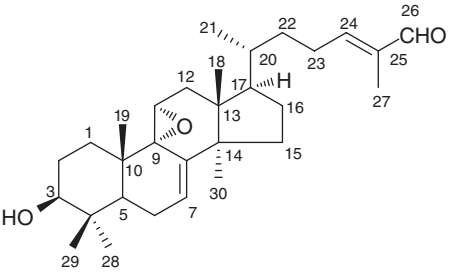

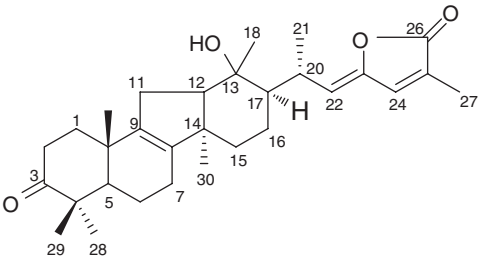

Figure 1. Structures of compounds **1** and **2**.

δ_H 3.24, 3.25; and two olefinic protons at δ_H 5.76 (1H, dd, $J = 5.0, 2.5$ Hz) and 6.48 (1H, t, $J = 7.1$ Hz), as well as an aldehyde at δ_H 9.39. The ^{13}C NMR and DEPT spectra of **1** showed 30 carbons resonances, which were ascribed to 7 methyls, 8 methylenes, 8 methines, and 7 quaternary carbons (Table 1). The chemical shift values of 1D NMR of **1** were similar to those of (24*E*)-3β-hydroxy-5α-lanosta-7,9(11),24-trien-26-al [12], except that the double bond between C-9 and C-11 was replaced by an epoxy moiety (δ_H 3.24, H-11; δ_C 55.8, C-11; δ_C 66.3, C-9) in **1**, which was supported by the HMBC correlations from H-11 at δ_H 3.24 (1H, overlapped) to C-9 at δ_C 66.3 (s) and C-12 at δ_C 35.4 (t), and the ^{1}H–^{1}H COSY correlation between H-11 and H-12 (δ_H 1.93–1.99, 2.06–2.09, respectively), as well as MS data analysis. These data indicated a planar structure of **1** as 9,11-epoxy-3-hydroxylanosta-7,24-dien-26-al. In the ROESY spectrum, a significant correlation between H-11 (δ_H 3.24, overlapped) and Me-19 (δ_H 1.08, s) was observed, which suggested that the configuration of 9,11-epoxy moiety should be an α configuration (Figure 2). To draw a conclusion, compound **1** was elucidated as (24*E*)-9α,11α-epoxy-3β-hydroxylanosta-7,24-dien-26-al.

Compound **2**, obtained as a white powder, had an $[M]^+$ peak at *m/z* 466.3084 ($C_{30}H_{42}O_4$) in the HR-EI-MS (calcd for 466.3078). 1D NMR spectra in combination with HSQC spectrum demonstrated 30 carbons, which were classified into 7 methyls, 7 methylenes, 6 methines (2 olefinic carbons), and 10 quaternary carbons (1 conjugated lactone carbon, 1 ketone, 1 oxygenated carbon, and 4 olefinic carbons) (Table 1). Detailed analyses of the HMBC spectrum found key correlations from Me-18 (δ_H 1.02, s) to an oxygenated quaternary carbon (δ_C 80.1) and two methines (C-12, δ_C 56.0; C-17, δ_C 42.2), as well as Me-30 (δ_H 0.96, s) to C-14 (δ_C 48.8), C-12 and C-15 (δ_C 32.9). This evidence combined with the cross peaks between H-15/H-16 and H-16/H-17 in the ^{1}H–^{1}H COSY spectrum suggested the presence of a six-member ring. On the other hand, only one proton of C-11 showed correlations in the ^{1}H–^{1}H COSY spectrum, which was H-11α (δ_H 2.38)/H-12 (δ_H 1.99). This evidence, as well as the HMBC correlations from H-12 to C-8 (δ_C 140.7) and C-9 (δ_C 141.0), proved that C-8, C-9, C-11, C-12 and C-14 formed a five-member ring. All these data suggested that compound **2** was a triterpenoid possessing a 14 (13 → 12) *abeo*-lanostaneskeleton, same as that of neokadsuranic acid A [13]. Further analyses of 2D NMR data suggested that the ketone group should be placed at C-3 as supported by the HMBC correlations from H-5 (δ_H 1.54, s) and Me-28 (δ_H 1.05, s) to C-3 (δ_C 218.3). The UV data at 294 nm indicated a conjugated moiety which can be readily identified as 22,24-diene-26-lactone, as supported by HMBC correlations from H-27 at δ_H 2.15

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of **1** and **2** (δ in ppm, J in Hz, $CDCl_3$).

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	28.6, t	1.13–1.15, m 1.60–1.64, m	34.9, t	1.87–19.2, m 1.55–1.63, m
2	26.6, t	1.99–2.02, m 1.59–1.64, m	34.4, t	2.47–2.52, m 2.51–2.56, m
3	78.5, d	3.25, overlapped	218.3, s	
4	38.9, s		47.1, s	
5	45.9, d	1.49, dd (11.4, 5.0)	51.9, d	1.54, overlapped
6	23.6, t	2.17–2.22, m 2.10–2.15, m	20.3, t	1.68–1.71, m 1.50–1.54, m
7	127.2, d	5.76, dd (5.0, 2.5)	23.2, t	1.99–2.03, m 1.82, overlapped
8	139.4, s		140.7, s	
9	66.3, s		141.0, s	
10	36.7, s		35.7, s	
11	55.8, d	3.24, overlapped	29.4, t	1.30, overlapped 2.38, overlapped
12	35.4, t	2.06–2.09, m 1.93–1.99, m	56.0, d	1.99, overlapped
13	45.2, s		80.1, s	
14	49.8, s		48.8, s	
15	33.3, t	1.60–1.66, m 1.37–1.40, m	32.9, t	1.88–1.93, m 1.32–1.37, m
16	26.8, t	1.98–2.02, m 1.27–1.31, m	24.2, t	1.54–1.58, m 1.16–1.20, m
17	50.7, d	1.51–1.56, m	42.2, d	1.74–1.79, m
18	17.4, q	0.73, s	14.6, q	1.02, s
19	19.3, q	1.08, s	19.3, q	1.07, s
20	35.6, d	1.40–1.44, m	30.1, d	2.36–2.41, m
21	18.6, q	0.94, d (5.8)	16.0, q	1.01, d (7.7)
22	34.8, t	1.60–1.66, m 1.18–1.27, m	117.9, d	5.14, d (4.8)
23	26.1, t	2.37–2.42, m 2.24–2.30, m	147.8, s	
24	155.5, d	6.48, t (7.1)	137.0, d	6.90, s
25	140.1, s		124.5, s	
26	195.4, q	9.39,s	173.2, s	
27	9.3, q	1.75, s	13.7, q	2.15, s
28	27.7, q	1.02, s	21.1, q	1.05, s
29	15.5, q	0.89, s	27.4, q	1.09, s
30	26.2, q	1.07, s	30.1, q	0.96, s

(s) to C-24 at δ_C 137.0 (s), C-25 at δ_C 124.5 (s), and C-26 at δ_C 173.2 (s), as well as ^{1}H–^{1}H COSY correlation between H-20 at δ_H 2.36–2.41 (m) and H-22 at δ_H 5.14 (d). In the ROESY spectrum, correlations of Me-30/H-12 and H-12/H-17 suggested both Me-30, H-12 and H-17 to be α-oriented. In addition, the ROESY correlation between H-22 at δ_H 5.14 and H-24 at δ_H 6.90 suggested that both double bonds of 22,24-diene were *Z* form. Consequently, compound **2** was characterized as (22*Z*,24*Z*)-13-hydroxy-3-oxo-14(13 → 12)*abeo*-lanosta-8,22,24-trien-26,23-olide (Figure 3).

Compounds **1** and **2** were evaluated for their antifungal and cytotoxic activities.

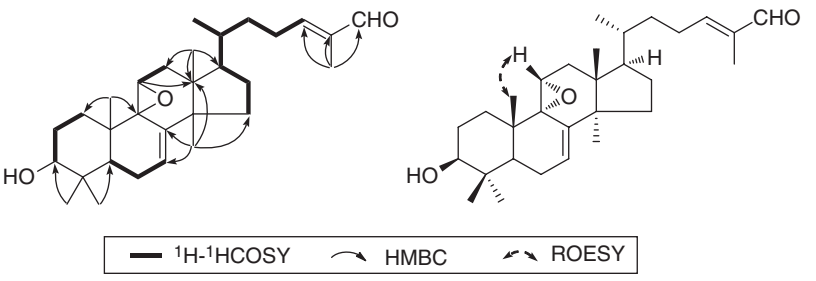

Figure 2. Key 2D NMR correlations of **1**.

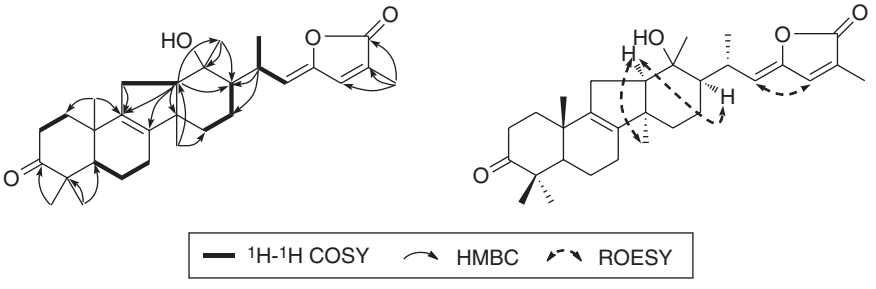

Figure 3. Key 2D NMR correlations of **2**.

However, no significant bioactivity was found.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). IR and UV spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Karlsruher, Germany) with KBr pellets and Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan), respectively. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance III 600 MHz spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany). ESI-MS and HR-EI-MS were measured on Waters Xevo TQ-S and Waters Autospec Premier P776 mass spectrometers (Waters, Milford, MA, USA), respectively. Silica gel 200–300 mesh (Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for column chromatography. Medium Pressure Liquid Chromatography (MPLC) was performed on a Büchi Sepacore System equipping with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan). Preparative High Performance Liquid Chromatography (Prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with a Zorbax SB-C18 column (5 μm, 9.4 mm × 150 mm) (Agilent Technologies, Santa Clara, CA, USA).

3.2. Fungus material

The fruiting bodies of *G. lucidum*, used as experimental material, were collected in Changjiang, Hainan province, China in September 2012, and identified by Prof. Yu-Cheng Dai (Institute of Microbiology, Beijing Forestry University). A voucher specimen of *G. lucidum* was deposited in the Herbarium of Kunming Institute of

Botany, Chinese Academy of Sciences (No. HFC20120912).

3.3. Extraction and isolation

The fresh fruiting bodies of *G. lucidum* (3.26 kg) were extracted four times by $CHCl_3$:MeOH (1:1) at room temperature. The extract was suspended in distilled water and partitioned successfully with EtOAc. The EtOAc layer was concentrated under reduced pressure to afford a crude extract (130 g), and the residue was subjected to silica gel column chromatography, eluting with a gradient of $CHCl_3$–MeOH (1:0–0:1) to obtain five fractions (A–E). Fraction B (18.9 g) was eluted with MeOH–H_2O (70–100%) by MPLC to obtained four subfractions (B1–B4). Fraction B2 (4.6 g) was subjected to silica gel column chromatography (petroleum ether–acetone, 4:1–2:1) to afford two subfractions (B21, B22). Subfraction B21 (21 mg) was separated by Prep-HPLC (45–90%, CH_3CN–H_2O, 10 ml/min, 50 min, detect wavelengths: 205, 240, and 290 nm) to yield compound **1** (0.9 mg, retention time: 25.3 min) and **2** (1.3 mg, retention time: 36.9 min).

3.3.1 (24E)-9α,11α-Epoxy-3β-hydroxylanosta-7,24-dien-26-al (*1*)

A white powder, $[\alpha]_D^{18} + 44.22$ ($c = 0.29$, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm ($\log \varepsilon$): 241.2 (3.89). IR (KBr) ν_{max} cm^{-1}: 3439, 2958, 2925, 1710, 1676, 1463, 1379, 1178, 1028. For ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data, see Table 1. HR–EI–MS *m/z*: 454.3456 $[M]^+$ (calcd for $C_{30}H_{46}O_3$, 454.3447).

3.3.2 (22Z,24Z)-13-Hydroxy-3-oxo-14(13 → 12)abeo-lanosta-8,22,24-trien-26,23-olide (*2*)

Amorphous white powder, $[\alpha]_D^{18}$-15.4 ($c = 0.17$, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm ($\log \varepsilon$): 294.8 (3.74), 240.2 (3.58). IR (KBr) ν_{max} cm^{-1}: 3441, 2955, 2925, 2855, 1706, 1634, 1459, 1380, 1253, 1198, 1030. For ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data, see Table 1. HR–EI–MS *m/z*: 466.3084 $[M]^+$ (calcd for $C_{30}H_{42}O_4$, 466.3083).

3.4. Bioactivity assay

3.4.1 Antifungal activity

Candida albicans (ATCC 32354) was purchased from Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College; Amphotericin B was purchased from Sigma-Aldrich (St. Louis, MO, USA). The test was performed in Potato Dextrose Agar (PDA). The samples were dissolved in dimethylsulfoxide (DMSO) and diluted to the highest concentrations (256 μg/ml). A volume of 100 μl aliquot from the stock solutions of the samples initially prepared, was added into the 96-well plates. Then 100 μl of the inoculum was added to achieve a final inoculum concentration of 2×10^5 CFU/ml in each well. The final volume in each well was 200 μl. Negative control and positive control were included in every experiment. Read plate at 625 nm after incubation at 30°C for 24 h, and calculate the MIC_{90} (minimal inhibitory concentration of 90% of the fungi). The assay was carried out in duplicate. Neither of them showed significant inhibition activities against *C. albicans.*

3.4.2 Cytotoxicity

The cytotoxicity assay was performed according to the MTT method in 96-well microplates. Five human cancer cell lines: human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and human colon cancer SW480 cells were used in the cytotoxicity assay. All the cells were cultured in Roswell Park Memorial Institute-1640 or Dulbecco's Modified Eagle Medium (Hyclone, Logan, UT,

USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37°C. Briefly, 100 μl of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1×105 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma) as a positive control (IC_{50}: SW480, 12.0 μmol; SMMC-7721, 10.2 μmol; HL-60, 3.1 μmol; MCF-7, 17.5 μmol; A-549, 9.1 μmol). After compound treatment, cell viability was detected and cell growth curve was graphed. Unfortunately, neither of them showed significant inhibition activities against above-mentioned five human cancer cell lines.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Y.H. Gao, Z.B. Lin, M. Huang, and S.F. Zhou, *Int. J. Med. Mushr.* **5**, 111 (2003).
[2] Y.C. Dai, *Mycoscience* **53**, 49 (2012).
[3] Y. Cao, S.H. Wu, and Y.C. Dai, *Fungal Divers* **56**, 49 (2012).
[4] Y.C. Dai, Z.L. Yang, B.K. Cui, C.J. Yu, and L.W. Zhou, *Int. J. Med. Mushr.* **11**, 287 (2009).
[5] D. Sliva, *Mini-Rev. Med. Chem.* **4**, 873 (2004).
[6] H.W. Kim and B.K. Kim, *Int. J. Med. Mushr.* **1**, 121138 (1999).
[7] J.L. Ríos, I. Andújar, M.C. Recio, and R.M. Giner, *J. Nat. Prod.* **75**, 2016 (2012).
[8] Z.B. Lin, *Modern research of* Ganoderma (Beijing Medical University-Peking Union Medical College Press, Beijing, 1996).
[9] C.H. Su, C.S. Sun, S.W. Juan, C.H. Hu, W.T. Ke, and M.T. Sheu, *Biomaterials* **18**, 1169 (1997).
[10] S. Tanaka, K. Ko, K. Kino, K. Tsuchiya, A. Yamashita, A. Murasugi, S. Sakuma, and H. Tsunoo, *J. Biol. Chem.* **264**, 16372 (1989).
[11] W.H. Lin, C.H. Hung, C.I. Hsu, and J.Y. Lin, *J. Biol. Chem.* **272**, 20044 (1997).
[12] J.J. Gao, B.S. Min, E.M. Ahn, N. Norio, H.K. Lee, and M. Hattori, *Chem. Pharm. Bull.* **50**, 837 (2002).
[13] K. Kunio, M. Toshio, K. Akira, I. Akiko, M. Taku, O. Sadafumi, L.N. Li, and H. Xue, *Planta Med.* **55**, 197 (1989).