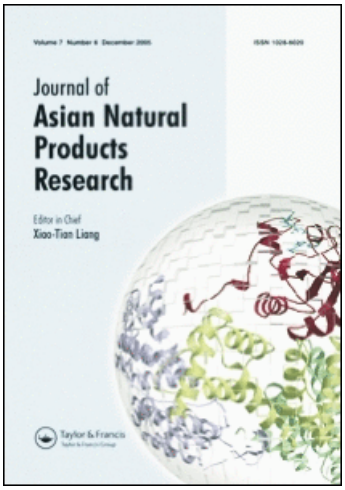

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A new ganoderic acid from *Ganoderma lucidum* mycelia

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NOTE

A new ganoderic acid from *Ganoderma lucidum* mycelia

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A new ganoderic acid (GA), 7-*O*-ethyl ganoderic acid O (GA-O) (**1**), together with two known compounds, GA-T (**2**) and GA-Me (**3**), was isolated and purified from fermented mycelia of *Ganoderma lucidum*. The structure of the new triterpenoid was elucidated on the basis of the interpretation of extensive spectroscopic data (HR-MS, IR, UV, 1D and 2D NMR) as 3α,15α,22-triacetoxy-7α-ethoxy-5α-lanost-8,24*E*-dien-26-oic acid. The new compound was found to contain a rare ethoxyl group at C-7. In addition, its cytotoxicity against 95D and HeLa human cancer cell lines was also evaluated.

Keywords: *Ganoderma lucidum*; Ganodermataceae; 7-*O*-ethyl GA-O; ethoxyl; cytotoxicity

1. Introduction

Ganoderma lucidum (Fr.) Karst, a therapeutic fungal biofactory [1], has been used as a tonic and sedative folk medicine for thousands of years in China and other Asian countries [2–4]. Ganoderic acids (GAs), known to be a major kind of bioactive compound in this fungus [5], were claimed to possess anti-cancer [6–9], anti-inflammatory [5,10], and anti-HIV effects [5] by various modern pharmaceutical studies. A two-stage culture process combining conventional shaking culture (first-stage) with static culture (second-stage) is a prospective method to produce bioactive GAs from mycelia of *G. lucidum* [11,12]. Our efforts in finding the bioactive components from this resource resulted in the isolation of a new highly oxidized triterpenoid, 7-*O*-ethyl ganoderic acid O (GA-O) (**1**), together with two known compounds, GA-T (**2**) and GA-Me (**3**). The structures of the known compounds were identified by comparison of the NMR spectral data with those reported in the literature [13]. The structural elucidation of the new compound is presented herein. Additionally, the cytotoxicity of 7-*O*-ethyl GA-O against 95D and HeLa human cancer cell lines is also described in this study.

2. Results and discussion

The molecular formula of compound **1** was determined as $C_{38}H_{58}O_9$ by the HR-ESI-MS at *m/z* 629.3687 $[M-CH_2CH_3]^-$ and ^{13}C NMR spectral data. The absence of the molecular ion peak might be the result of structural fragility. The UV spectrum of this compound showed absorption maximum at 225 nm and hydroxyl (3450 cm^{-1}), α,β-unsaturated carbonyl (1725 cm^{-1}), and ether bond (1250 cm^{-1}) absorptions were seen in the IR spectrum. The ^1H NMR spectrum of compound **1** showed the presence of eight methyl singlets at δ 0.70, 0.88, 0.90, 0.94, 1.14, 2.03, 2.04, and 2.06, a

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methyl doublet at δ 0.94, a methyl triplet at δ 1.18, a vinyl methyl singlet at δ 1.86, a methylene quartet at δ 3.46, four *O*-bearing methine signals at δ 4.09, 4.66, 5.02, 5.12, and an olefinic proton double of doublets at δ 6.73. The ^{13}C NMR spectrum and DEPT measurement of **1** (Table 1) showed the presence of 11 methyls, 8 methylenes, 6 methines, 5 sp^3 quaternary carbons, 4 olefinic carbons, and 4 carboxyl carbons. The ^{1}H, ^{13}C NMR, and HMBC (Figure 1) spectral patterns of **1** were very similar to those of 3α,15α,22-triacetoxy-7α-hydroxy-5α-lanost-8,24*E*-dien-26-oic acid (also

Table 1. NMR spectral data of 7-*O*-ethyl GA-O in $CDCl_3$ (δ in ppm).

Position	δ_H (*J*, Hz)	δ_C (DEPT)	HMBC (H → C)
1α	1.41 (1H, overlapping)	30.2t	C-3, C-28
1β	1.49 (1H, overlapping)		C-3
2α	1.90 (1H, overlapping)	23.2t	C-4, C-30
2β	1.65 (1H, overlapping)		C-4
3β	4.66 (1H, br s)	77.4d	C-1, C-4, C-5, C-29, 3-*Ac*Me
4	–	36.2s	–
5	1.49 (1H, m)	39.9d	C-3, C-28
6α	1.85 (1H, overlapping)	27.3t	C-7
6β	1.66 (1H, overlapping)		C-8, C-29, C-5
7β	4.09 (1H, br s)	66.5d	–
8	–	133.6s	–
9	–	141.8s	–
10	–	38.4s	–
11α	2.09 (1H, overlapping)	20.6t	C-9
11β	2.06 (1H, overlapping)		C-9
12α	1.86 (1H, overlapping)	31.3t	C-9, C-10
12β	1.61 (1H, overlapping)		C-10
13	–	45.2s	–
14	–	51.2s	–
15β	5.12 (1H, dd, $J = 9.6, 5.8$)	76.1d	C-8, C-14, 15-*Ac*Me
16α	2.11 (1H, br d, $J = 5.0$)	36.2t	C-12, C-14
16β	1.88 (1H, overlapping)		C-12, C-13, C-20
17	1.75 (1H, overlapping)	45.8s	C-11, C-13, C-18
18	0.70 (3H, s)	16.2q	C-12, C-14, C-17
19	0.94 (3H, s)	17.4q	C-12, C-13, C-15, C-16, C-18
20	1.66 (1H, overlapping)	39.8d	C-16, C-21
21	0.94 (3H, d, $J = 6.4$)	12.6q	C-17
22	5.02 (1H, t, $J = 7.1$)	74.3d	C-20, C-23, 22-*Ac*Me
23α	2.30 (1H, m)	31.8t	C-24, C-25
23β	2.54 (1H, m)		C-20, C-24, C-25
24	6.73 (1H, dd, $J = 7.5, 6.4$)	138.7d	C-22, C-23, C-26, C-27
25	–	129.6s	–
26	–	171.9s	–
27	1.86 (3H, s)	12.3q	C-24, C-25, C-26
28	0.88 (3H, s)	27.3q	C-1, C-8, C-10, C-11
29	0.90 (3H, s)	21.9q	C-3, C-4, C-5, C-6, C-30
30	1.14 (3H, s)	20.2q	C-3, C-4, C-5, C-30
C═O	–	171.0	–
C═O	–	170.6	–
C═O	–	170.5	–
Ac*Me*	2.03 (3H, s)	21.4	3-*Ac*Me
Ac*Me*	2.04 (3H, s)	21.1	15-*Ac*Me
Ac*Me*	2.06 (3H, s)	21.0	22-*Ac*Me
7-O*CH*$_2$CH$_3$	3.46 (2H, q, $J = 7.0$)	65.8t	C-7
7-OCH$_2$*CH*$_3$	1.18 (3H, t, $J = 7.1$)	15.2q	C-7, 7-O*CH*$_2$CH$_3$

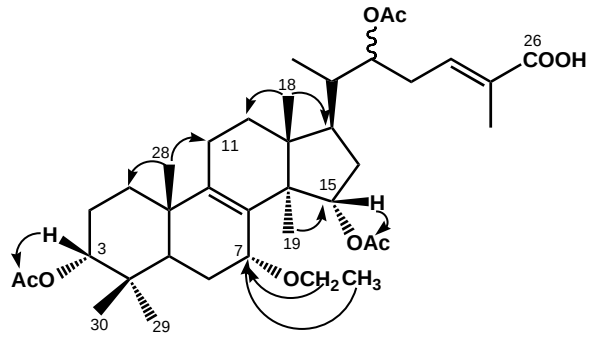

Figure 1. Chemical structure and key HMBC correlations (H → C) of compound **1**.

called GA-Mb) [14] except for an additional *O*-ethyl signal (δ_C 65.8, 15.2; δ_H 3.46, 1.16). The HMBC correlations from C-7 to the proton of the ethoxyl group suggested that C-7 of compound **1** was substituted by OCH_2CH_3 instead of OH. Therefore, the structure of compound **1** was elucidated as 3α,15α,22-triacetoxy-7α-ethoxy-5α-lanost-8,24*E*-dien-26-oic acid. In addition, 7-*O*-ethyl GA-O exhibited cytotoxicity against 95D and HeLa human cancer cell lines with IC_{50} values (48 h) of 46.7 and 59.1 μM, respectively [hydroxycamptothecine as the reference substance: 21.3 μM (95D) and 29.5 μM (HeLa)].

Generally, triterpenoids contain substituted groups as hydroxyl, acetyl, ketone groups, and glycosides [15–17]; however, this novel triterpenoid was found to have a rare ethoxyl group at C-7. The fact that ethanol was not used in the purification procedures could exclude the possibility that it is an artifact. Furthermore, the total yield of 1.1 g of purified 7-*O*-ethyl GA-O after only one-step silica gel column chromatography is rather a high production that has not ever been reported in the GA-related literature. Therefore, further research on its biosynthetic pathway and mass production will be of great interest.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a JASCO automatic polarimeter. UV spectra on a Thermo Evolution 300 spectrometer and IR spectra on a Bruker Equinox 55 IR spectrometer were recorded in MeOH and KBr disks, respectively. NMR spectra were recorded on a Bruker Avance-III spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ with TMS (^{1}H NMR) as the internal reference. HR-ESI-MS were recorded on a Waters QTOFMS Premier mass spectrometer (70 eV) using a direct inlet system. An Agilent 1200 HPLC system was used for GA separation and determination. Silica gel (200–300 mesh) was purchased from Qingdao Haiyang Chemical Group Co. (Qingdao, China).

3.2 Fungal material

The strain of *G. lucidum* CGMCC 5.616 from Chinese General Microbiological Fermentation Collection Center (Beijing, China) was maintained on potato-dextrose agar slants. A two-stage culture of *G. lucidum* by combining conventional shaking culture (first-stage) with static culture (second-stage) was applied to obtain the mycelia for GA separation and purification [11].

3.3 Extraction and isolation

GAs were extracted from dried mycelia of *G. lucidum* (200 g). The dried mycelia were powdered and extracted with chloroform (2 liters) twice, each for 1 h by ultrasonic-

assisted extraction. The total crude extract was filtered through an ashless filter paper, and then evaporated to dryness by a rotary evaporator under vacuum at 30°C. The resulting extract (14.2 g) was subjected to chromatography on silica gel (4 × 40 cm), eluted with ethyl ether–petroleum ether (9:1) to afford three fractions (1–3). Fraction 2 (8.6 g) was further purified by reversed-phase preparative high-performance liquid chromatography eluted with 85% methanol to give 100 mg of compound **2**, and 50 mg of compound **3**. Fraction 3 (2.3 g) was crystallized in ethyl ether at −20°C to yield 1.1 g of compound **1**.

3.3.1 *Compound **1***

An amorphous solid; $[\alpha]_D^{25}$ −7 ($c = 0.1$, MeOH); UV (MeOH) λ_{max} (log ε): 225 (4.02) nm; IR (KBr film) ν_{max}: 3450, 2965, 1725, 1647, 1450, 1372, 1250, 1184, 1029, 967 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS: *m/z* 653 $[M-CH_2CH_3+H+Na]^+$(100), 613 $[M-CH_3CH_2OH+H]^+$(47), 553 (33), 493 (44), 433 (47), 293 (6), 201 (5), 113 (5); HR-ESI-MS: *m/z* 629.3687 $[M-CH_2CH_3]^-$ (calcd for $C_{36}H_{53}O_9^-$, 629.3690).

3.4 *Cytotoxicity assay*

The cytotoxicity of 7-*O*-ethyl GA-O and hydroxycamptothecine (reference substance) was evaluated and identified on human highly metastatic lung tumor cell line 95D and human cervical cancer cell line HeLa (Cell Bank of Chinese Academy of Sciences, Shanghai, China), as described in our previous work [8].

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