Phytochemistry 173 (2020) 112256

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Lanostane triterpenoids with anti-inflammatory activities from *Ganoderma lucidum*

Hai-Guo Su[a,b], Xing-Rong Peng[a], Qiang-Qiang Shi[a,b], Yan-Jie Huang[a,b], Lin Zhou[a], Ming-Hua Qiu[a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming, 650201, China
[b] University of the Chinese Academy of Science, Beijing, 100049, China

ARTICLE INFO

Keywords:
Ganoderma lucidum
Ganodermataceae
Lanostane triterpenoids
Octonorlanostane carbon skeleton
Anti-inflammatory activity

ABSTRACT

Ganoderma lucidum is one of the most famous medicinal fungi and is traditional Chinese medicine with various biological activities in Asian countries. To clarify its pharmacodynamic material basis, 15 lanostane triterpenoidswere obtained from the fruiting bodies of *G. lucidum*, including 8 previously undescribed lanostanoids. Their structures, including absolute configuration, were established based on ultraviolet, infrared, high-resolution electrospray ionisation mass spectrometry, 1D and 2D nuclear magnetic resonance, and X-ray crystallographic analysis. Ganoluciduone A was an unusual octonorlanostane, which was isolated from *Ganoderma* for the first time. In addition, the anti-inflammatory activities of all isolates were evaluated by observing their inhibitory effects on nitric oxide production in RAW264.7 cells activated by a lipopolysaccharide. Ganoluciduone B exhibited moderate inhibitory activity on nitric oxide production, with an inhibition rate of 45.5% at a concentration of 12.5 μM.

1. Introduction

Ganoderma lucidum (Curtis) P. Karst (Ganodermataceae), a medicinal fungus called “Lingzhi” in China, is a highly regarded medicinal fungi and traditional Chinese medicine. It has been clinically used in China, Japan, and Korea for hundreds of years owing to its excellent health effects, such as in the prevention of cancer and delay of senility (Fatmawati et al., 2011; Xia et al., 2014). Ancient Chinese medical books, such as the Compendium of Materia Medica (Bencao Gangmu) and Shennong's Herbal Classic (Shennong Ben Cao Jing), describe *Ganoderma* as a fungus that can improve intellect and memory, extend lifespan, and relieve stress (Jiao et al., 2016). Ganodermataceae comprises more than 200 fungal species, but the Chinese Pharmacopoeia (2015 Edition) only recorded *G. lucidum* and *G. sinense* as sources of “Lingzhi” (Peng et al., 2014).

Currently, over 240 compounds have been isolated from *G. lucidum*. Triterpenoids are the major constituents of *G. lucidum* and possess extensive biological and pharmacological activities, such as cytotoxic (Cheng et al., 2010), α-glucosidase inhibitory (Zhao et al., 2015), anti-inflammatory (Jiao et al., 2016), and hepatoprotective (Wu et al., 2016) activities. Our research group has long focused on ingredients of *Ganoderma* fungi such as *G. applanatum* (Peng et al., 2019), *G. capense* (Peng et al., 2016), *G. hainanense* (Peng et al., 2015b), *G. cochlear* (Peng et al., 2014, 2015a), and *G. sinense* (Liu et al., 2012; Wang et al., 2010). As *G. lucidum* is one of the most popular medicinal fungi, we previously systematically investigated the ethyl acetate (EtOAc) extract of *G. lucidum* and eight previously undescribed lanostane triterpenoids (**1**–**8**), together with seven known compounds, were isolated. Compound **1** was an undescribed, highly degraded triterpenoid with an unusual 20,21,22,23,24,25,26,27-octonorlanostane carbon skeleton, which was isolated from *Ganoderma* for the first time. Compound **2** was an unusual lanostane nortriterpenoid with 29 carbons. Currently, only three compounds that possess the same skeleton as compound **2** have been reported (Huang et al., 2017; Su et al., 2018). The discovery of compounds **1** and **2** greatly enriched the types of lanostane nortriterpenoids known in *Ganoderma*. In this study, the anti-inflammatory activities of all isolates were evaluated by observing their inhibitory effects on nitric oxide production in RAW264.7 cells activated by a lipopolysaccharide.

2. Results and discussion

The powdered dry fruiting bodies of *G. lucidum* were extracted with CH_3OH. The extract was further partitioned with EtOAc to afford an EtOAc soluble extract (1456 g). Then, the EtOAc extract was separated

* Corresponding author.
E-mail address: mhchiu@mail.kib.ac.cn (M.-H. Qiu).

https://doi.org/10.1016/j.phytochem.2019.112256
Received 19 August 2019; Received in revised form 29 December 2019; Accepted 30 December 2019

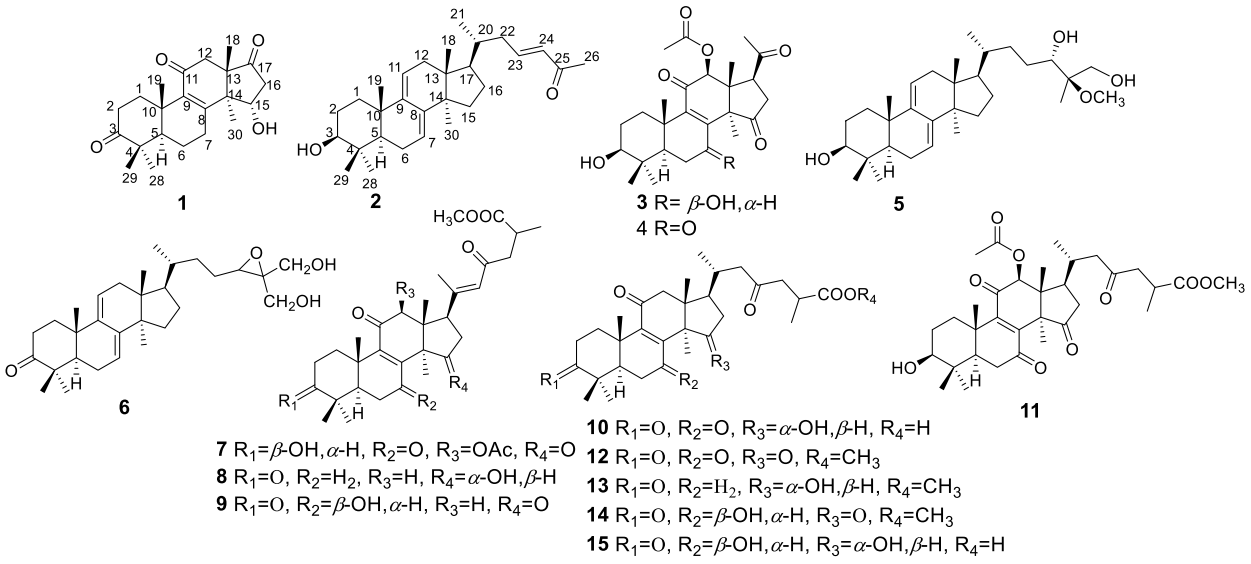

Fig. 1. Structures of compounds **1–15**.

using a silica gel column, Sephadex LH-20, an octadecylsilyl (ODS) column, preparative thin-layer chromatography (TLC), and semi-preparative high-performance liquid chromatography (HPLC) to yield 15 triterpenoids (Fig. 1). Seven known compounds were identified as 7α-hydroxy-3,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester (**9**) (Shim et al., 2004), ganoderic acid J (**10**) (Nishitoba et al., 1985b), methyl ganoderate H (**11**) (Kikuchi et al., 1985a), methyl ganoderate E (**12**) (Kikuchi et al., 1985a), methyl ganolucidate A (**13**) (Kikuchi et al., 1985b), methyl ganoderate D (**14**) (Kohda et al., 1985), and ganoderic acid A (**15**) (Kubota et al., 1982) via the comparison of their spectroscopic data with the literature.

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{22}H_{30}O_4$ by high-resolution electrospray ionisation mass spectrometry (HRESIMS) at *m/z* 359.2221 $[M + H]^+$ (calcd for $C_{22}H_{31}O_4$, 359.2217), implying eight degrees of unsaturation. Its infrared (IR) spectrum showed the presence of hydroxyl (3442 cm^{-1}) and α,β-unsaturated carbonyl (1640 cm^{-1}) groups. The ultraviolet (UV) absorption at 254 nm (logε 3.87) also supported the existence of an α,β-unsaturated carbonyl group. The ^{1}H nuclear magnetic resonance (NMR) spectrum of **1** showed resonances for five methyl signals [δ_H 1.05 (3H, s), 1.09 (3H, s), 1.14 (6H, s) and 1.24 (3H, s)] and one oxygenated methine [δ_H 4.80 (1H, m)]. The ^{13}C NMR and distortionless enhancement of polarisation transfer spectra displayed 22 carbons, corresponding to 5 methyl groups, 6 methylene groups, 2 methine groups (one oxygenated carbon at δ_C 69.4), 9 quaternary carbons (including one pair of olefinic carbons (δ_C 139.7, 160.2)), and 3 carbonyl carbons (δ_C 196.9, 211.9, and 218.0), which indicated that **1** was a lanostane nortriterpenoid without an eight-carbon side chain.

Furthermore, the core skeleton of this lanostane triterpenoid was demonstrated by the ^{1}H–^{1}H correlated spectroscopy (COSY) correlations of H_2-1/H_2-2, H-5/H_2-6/H_2-7, and H-15/H_2-16 (see Fig. 2). This was also supported by the heteronuclear multiple bond correlations (HMBCs) from H_3-18 to C-12, C-13, C-14, and C-17; from H_3-19 to C-1, C-5, C-9, and C-10; from H_3-28 to C-3, C-4, C-5, and C-29; from H_3-29 to C-3, C-4, C-5, and C-28; and from H_3-30 to C-8, C-13, C-14, and C-15. Additionally, the C-3 ketone was assigned based on the HMBC from H_2-1, H_2-2, H_3-28, and H_3-29 to this carbon (δ_C 218.0). The α,β-unsaturated ketone fraction was located at C-8, C-9, and C-11 based on the HMBC from H_2-12 to the carbonyl carbon (δ_C 196.9) and the downfield chemical shift of C-8 (δ_C 160.2). The HMBCs from the proton resonance of an oxymethine [δ_H 4.78 (1H, m)] to C-8 and C-30, as well as the ^{1}H–^{1}H COSY correlation of H-15/H_2-16 indicated the substitution of a hydroxyl at C-15 in **1**. The HMBC from H_3-18 and H_2-16 to another ketone (δ_C 211.9) illustrated that the ketone was connected to C-17.

The relative configuration of HO-15 was assigned as α-orientation by the nuclear overhauser effect spectroscopy (NOESY) correlation of H-15 with H_3-18 (see Fig. 3). In addition, the NOESY correlations of H-5 with H_3-28 and H_3-29 with H_3-19 suggested that H-5 was in normal α-orientation. Thus, the structure of compound **1** was determined to be 20,21,22,23,24,25,26,27-octo-*nor*-15α-hydroxylanosta-8-en-3,11,17-trione and named ganoluciduone A.

The molecular formula of compound **2** was assigned as $C_{29}H_{44}O_2$ by HRESIMS. Its ^{1}H NMR and ^{13}C NMR spectra revealed the presence of two olefinic carbons [δ_H 6.79 (1H, ddd, J = 15.6, 9.0, 7.2 Hz), 6.08 (1H, d, J = 15.6 Hz); δ_C 147.7, 132.8], a pair of conjugated double bonds [δ_H 5.48 (1H, br d, J = 5.4 Hz), 5.31 (1H, br d, J = 5.4 Hz); δ_C 146.1, 142.5, 120.6, 116.2], one oxymethine [δ_H 3.25 (1H, m); δ_C 79.1], and one ketone (198.7), which indicated that **2** was a 7,9(11)-dien lanostane triterpenoid and possessed the same side chain as (5α,23*E*)-27-nor-lanosta-8,23-dien-3,7,25-trione (Su et al., 2018). The structural elucidation of **2** was accomplished by the analysis of COSY and HMBC data. The location of the hydroxy group was revealed by the HMBCs from H-1, H_3-28, and H_3-29 to an oxygenated carbon (δ_C 79.1). The side chain structure was further determined by HMBC from H_3-21 to C-20, from H-23 to C-25, and from H_3-26 to C-25 and C-24, together with ^{1}H–^{1}H COSY of H_3-21/H-20/H_2-22/H-23/H-24. The NOESY correlation of H-3 with H_3-28 assigned the β-orientation for HO-3. The large coupling constant between H-23 and H-24 ($J_{23,24}$ = 15.6 Hz) suggested an *E* geometry for the double bond, which was further demonstrated by the NOESY correlation of H-22 with H-24. Thus, compound **2** was identified as (23*E*)-27-*nor*-3β-hydroxylanosta-7,9(11)-dien-25-one and named ganoluciduone B.

Compound **3** was isolated as white amorphous powder and its molecular formula was determined to be $C_{26}H_{36}O_7$ on the basis of HRESIMS data. The 1D NMR data showed 26 carbon resonances. Except for the typical acetyl signals, others belonged to a C24 lanostane nortriterpenoid skeleton, which was demonstrated by 1D and 2D NMR spectroscopic data. Compound **3** had a similar structure to lucidone A, except for an additional acetoxyl group (Nishitoba et al., 1985a). An acetoxyl group [δ_H 2.22 (3H, s), δ_C 170.4, 20.9] was located at C-12 based on the HMBC from the proton resonance of H-12 [δ_H 5.74 (1H, s)] to acetyl carbonyl (δ_C 170.4). The relative configurations of HO-3, HO-7, and AcO-12 were elucidated as 3β, 7β, and 12β based on NOESY correlations of H-3/H_3-28/H-5, H-7/H-5, and H-12/H-30. Finally, Compound **3** was named ganolucidoid A.

Compound **4** was assigned the molecular formula of $C_{26}H_{34}O_7$ by

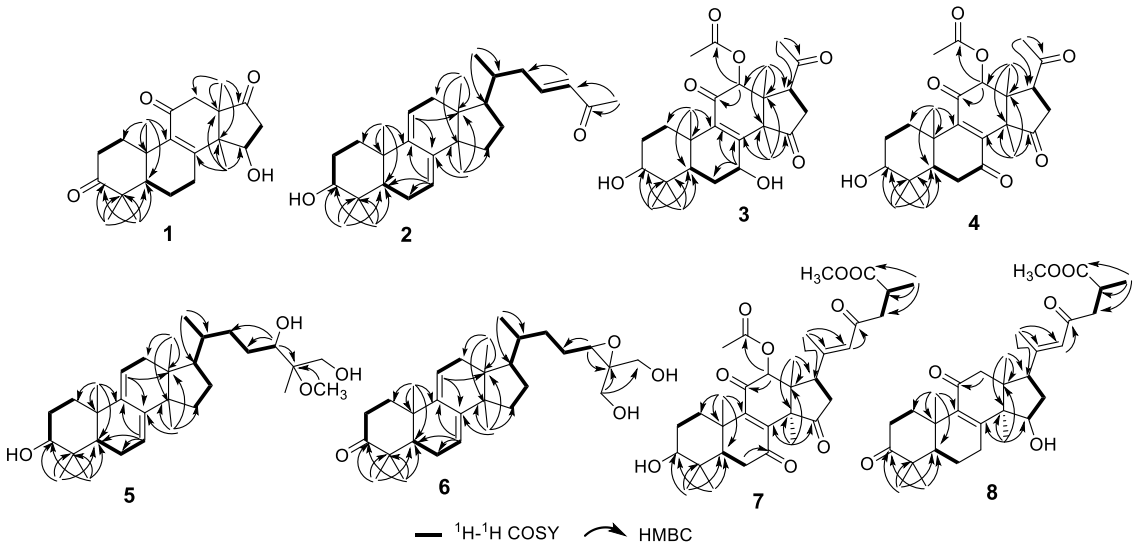

Fig. 2. Key HMBC and ^{1}H–^{1}H COSY correlations of compounds **1**–**8**.

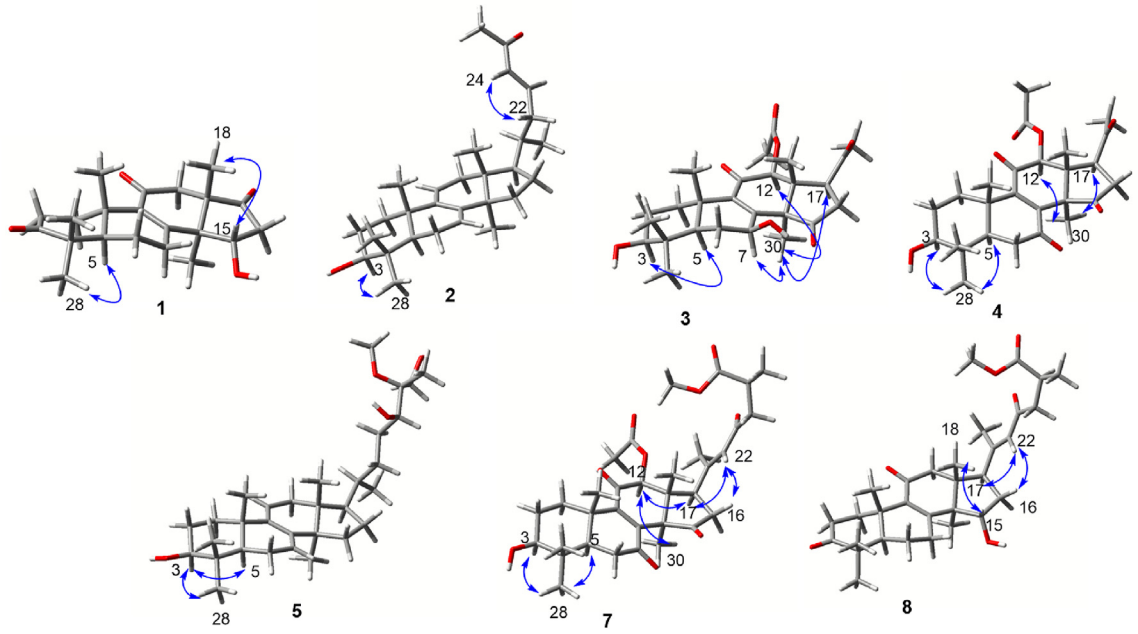

Fig. 3. Key NOESY correlations of compounds **1**–**5**, **7** and **8**.

HRESIMS data. The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **3** except that a carbonyl group (δ_C 198.6) in **4** replaced the oxymethine in **3** [δ_H 4.80 (1H, m); δ_C 66.2]. The HMBCs from H-5 and H_2-6 to this carbonyl carbon (δ_C 198.6) demonstrated that C-7 was the ketone carbonyl. Similarly, the relative configurations of OH-3 and AcO-12 were assigned as β-orientation. Compound **4** was named ganoluciidoid B.

The molecular formula of compound **5** was determined to be $C_{31}H_{52}O_4$ by HRESIMS. Analysis of the 1D and 2D-NMR data revealed that **5** had a similar structure as ganoderiol A (Sato et al., 1986), except for an extra methoxy group [δ_H 3.32 (3H, s); δ_C 49.5]. The location of a methoxy group was at C-25 based on the key HMBC from the methoxy proton signal [δ_H 3.32 (3H, s)] to C-25. The relative configuration of 3-OH was assigned as β-orientation by NOESY correlations of H-3 with H-5. The absolute configuration of **5** was determined as (24*S*, 25*R*) [Flack parameter = 0.06(6)] by X-ray diffraction (Fig. 4). Thus, the structure of compound **5** was established to be (24*S*, 25*R*)-25-methoxylanosta-

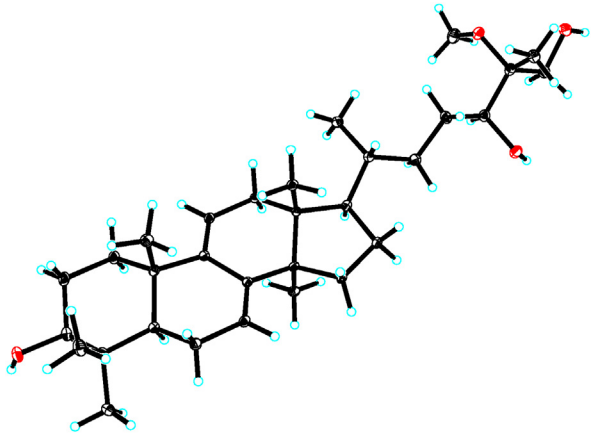

Fig. 4. Single-crystal X-ray structure of **5**.

7,9(11)-dien-3β,24,26-triol.

Compound **6** was obtained as a white amorphous powder. A molecular formula of $C_{30}H_{46}O_4$, with eight degrees of unsaturation, was assigned based on the HRESIMS ion at *m/z* 493.3294 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3288). The ^1H and ^{13}C NMR spectra of **6** indicated that it was a 7,9(11)-dien lanostane triterpenoid that possessed a carbonyl (δ_C 217.1), two oxygenated methylenes (δ_C 61.4, 64.2), an oxygenated methine (δ_C 62.2), and an oxygenated quaternary carbon (δ_C 62.8). The upfield chemical shift of the oxygenated methine [δ_H 3.05 (1H, m); δ_C 62.2] and oxygenated quaternary carbon (δ_C 62.8) indicated the existence of an epoxy group (Nishitoba et al., 1988). The unsaturation of compound **6** supported this speculation. The C-3 ketone was assigned by the HMBCs from H_3-28 and H_3-29 to the ketone carbon (δ_C 217.1). The location of the epoxy group was assigned to the C-24/C-25 position by HMBCs from H-24 [δ_H 3.05 (1H, m)] to C-23 and C-26. HMBCs from oxymethylene [δ_H 3.92 (1H, d, J = 12.0 Hz), 3.73 (1H, d, J = 12.0 Hz)] to C-27 and C-25 and from oxymethylene [δ_H 3.84 (2H, s)] to C-26 and C-25 demonstrated the substitution of the hydroxy groups at C-26 and C-27, respectively. We attempted to obtain a single crystal of compound **6** to determine the absolute configuration of C-24; however, the crystal state could not be used for single crystal diffraction no matter how much we accumulated and recrystallised it. Thus, compound **6** was determined to be 26,27-dihydroxy-24,25-epoxylanosta-7,9(11)-dien-3-one.

Compound **7** was obtained as a white amorphous powder. The molecular formula of $C_{33}H_{44}O_9$ was determined on the basis of a quasi-molecular ion peak at *m/z* 585.3062 $[M + H]^+$ (calcd for $C_{33}H_{45}O_9$, 585.3058) in HRESIMS. The UV and IR spectra suggested the presence of an α,β-unsaturated carbonyl group. The ^1H and ^{13}C NMR data of **7** were similar to those of methyl ganoderate H (Kikuchi et al., 1985a), except for the presence of an additional trisubstituted double bond [δ_H 6.11 (1H, s); δ_C 126.2, 154.3]. The location of the double bond was determined to be C-20/C-22 by the HMBCs from the proton (δ_H 6.11) to C-21, C-17, and C-23, together with the downfield chemical shift of the olefinic carbon (δ_C 154.3). The relative configurations of HO-3 and AcO-12 were determined to be β-oriented by NOESY correlations of H-3 with H-5 and H_3-28 and those of H-12 with H-17 and H_3-30. The NOESY correlations of H-22 with H-17 and H_2-16 suggested an *E* geometry for the $\Delta^{20(22)}$ (Guan et al., 2008; Shim et al., 2004). Consequently, compound **7** was determined to be 3β-hydroxy-12β-acetoxy-7,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester.

The molecular formula of compound **8** was determined by HRESIMS as $C_{31}H_{44}O_6$. The ^1H and ^{13}C NMR spectra of **8** revealed a similar structure to methyl ganolucidate A, except for an extra trisubstituted double bond [δ_H 6.07 (1H, s); δ_C 123.8, 198.3] (Kikuchi et al., 1985b). The position and configuration of the double bond were determined using the same method as described for **7**. HMBCs from the olefinic proton resonance [δ_H 6.07 (1H, s)] to C-21, C-17, and C-23 suggested that the trisubstituted double bond was located at C-20/C-22. The NOESY correlations of H-22 with H-17 and H_2-16 suggested an *E* geometry for the $\Delta^{20(22)}$ double bond. The relative configuration of HO-15 was assigned as α-orientation based on the NOESY correlation of H-15 with H_3-18. Compound **8** was determined to be 15α-hydroxy-3,11, 23-trioxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester.

Inflammation is associated with a variety of diseases such as cancer (Hanahan and Weinberg, 2011), diabetes (Donath and Shoelson, 2011), and other metabolic diseases (Saltiel and Olefsky, 2017). The increase in nitric oxide levels is associated with acute and chronic inflammatory diseases, indicating that nitric oxide is related to pro-inflammatory agents for inflammation-mediated pathogenesis (Jang et al., 2017). Research has shown that *Ganoderma* triterpenoids have anti-inflammatory activities (Jiao et al., 2016; Wu et al., 2019). Thus, the anti-inflammatory activities of all isolates were evaluated. Compound **2** exhibited moderate inhibitory activity on nitric oxide production, with an inhibition rate of 45.5% at a concentration of 12.5 μM. L-NMMA was used as a positive control, with a percentage inhibition 55.9% at a concentration of 50.0 μM. The cytotoxicity of all isolates on RAW264.7 cells was also measured to exclude the possibility that the inhibition of nitric oxide production was due to its cytotoxicity (See Table S1, Supplementary Data).

3. Conclusion

Triterpenoids are typical chemical constituents in *G. lucidum* and play an important role in the pharmacological effects of *G. lucidum*. In this study, 15 lanostane triterpenoids, including 8 previously undescribed compounds, were isolated from the fruiting bodies of *G. lucidum*. Among them, compound **1** was a highly degraded triterpenoid with an unusual 20,21,22,23,24,25,26,27-octonorlanostane carbon skeleton. This is the first natural product that has been discovered to possess such a carbon skeleton. Compound **2** was an unusual C29 triterpenoid that exhibited moderate inhibitory activity on nitric oxide production, with an inhibition rate of 45.5% at a concentration of 12.5 μM. The discovery of compounds **1** and **2** greatly enriched the types of lanostane nortriterpenoids known in *Ganoderma*. Single crystal diffraction analysis determined the absolute configuration of compound **5**, which was the first example of a single crystal of a 24,25,26-triol type *Ganoderma* triterpenoid.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured using a Rudolph Autopol VI automatic polarimeter. IR spectra were recorded using a Nicolet iS10 mid-infrared instrument (Thermo Fisher Scientific). UV spectra were recorded on a Shimadzu UV2401PC (Shimadzu). NMR spectra were obtained using a Bruker-AV-600 spectrometer with solvent peaks used as references. HRESIMS spectra were measured using an Agilent 1290 UPLC/6540 Q-TOF. Column chromatography was performed using silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, China). RP-HPLC separations were performed using an Agilent 1100 instrument with an Agilent Zorbax C_{18} column (9.4 × 250 mm, 5 μm). TLC was performed using glass precoated silica gel GF254 plates (Qingdao Puke Parting Materials Co., Ltd., Qingdao, China). Preparative TLC was performed using self-made glass plate, evenly spread with GF254 silica gel and 4‰ sodium carboxymethyl cellulose aqueous solution (1:3, m:v).

4.2. Fungal material

The fruiting bodies of *Ganoderma lucidum* (Curtis) P. Karst. [MB#148413] (Ganodermataceae) (http://www.mycobank.org/quicksearch.aspx) were purchased in August 2018 from the Kunming Luosiwan Chinese medicine market (Yunnan Province, China) and samples had been collected in Diantan town (25°31′N, 98°24′E), Tengchong county, Baoshan city, Yunnan Province, China. The fungal material was identified by Prof. Zhu-Liang Yang (Kunming Institute of Botany, Yunnan, China). A voucher specimen (GL-180827) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

4.3. Extraction, isolation, and characterization of compounds

The air-dried and powdered fruiting bodies of *G. lucidum* (39 kg) were extracted with CH_3OH under reflux three times to give a crude extract. The crude extract was suspended in hot water and partitioned with EtOAc to afford an EtOAc soluble extract (1456 g). The EtOAc soluble fraction was subjected to silica gel column chromatography (200–300 mesh) using a gradient elution of increasing MeOH (0–100%) in $CHCl_3$ to afford Fr.1–Fr.7. Fr.3 (47 g) was applied to an ODS column (MeOH–H_2O, 50%–100%) to obtain 23 subfractions (Fr.3.1–Fr.3.23).

Table 1
^{1}H and ^{13}C NMR data of compounds 1–4 in $CDCl_3$ at 600 and 150 MHz.

no.	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.97 ddd (13.8, 7.8, 5.4)	37.5	1.99 m	35.8	2.61 m	34.6	2.67 m	33.4
	1.61 m		1.44 ddd (15.6, 12.0, 3.6)		0.95 m		1.17 td (13.2, 4.2)	
2	2.57 m	35.0	2.07 m	27.9	1.62 m	27.6	1.70 m	27.4
	2.46 ddd (15.6, 7.8, 7.2)		1.68 m		1.45 m		1.71 m	
3		218.0	3.25 m	79.1	3.20 m	78.3	3.25 m	77.5
4		47.1		38.9		38.7		39.3
5	1.73 dd (12.6, 1.8)	51.7	1.08 dd (11.4, 4.2)	49.2	0.86 overlapped	49.3	1.55 dd (14.4, 1.8)	51.5
6	2.59 m	29.8	2.08 m	23.2	2.25 m	26.9	2.69 overlapped	36.7
	2.58 m		1.67 m		1.65 m		2.59 dd (14.4, 1.8)	
7	1.75 m	18.8	5.48 br d (5.4)	120.6	4.80 m	66.2		198.6
	1.52 m							
8		160.2		142.5		155.6		145.2
9		139.7		146.1		143.2		152.2
10		37.5		37.5		38.7		40.6
11		196.9	5.31 br d (5.4)	116.2		191.1		193.1
12	2.79 d (17.4)	44.2	2.18 m	37.8	5.74 s	79.3	5.72 s	78.9
	2.26 d (17.4)		2.08 m					
13		55.8		44.0		60.6		58.4
14		49.9		50.5		50.1		47.9
15	4.80 m	69.4	1.64 m	31.6		214.8		203.7
			1.41 m					
16	3.01 dd (19.2, 8.4)	43.1	2.13 m	28.1	2.95 dd (19.8, 9.6)	37.7	2.69 m	36.2
	2.13 dd (19.2, 7.2)		1.46 m		2.61 overlapped		2.65 m	
17		211.9	1.59 m	51.0	3.58 t (8.4)	51.9	3.64 t (8.4)	50.4
18	1.05 s	19.5	0.58 s	15.8	0.92 s	13.9	0.78 s	12.8
19	1.14 s	20.7	0.98 s	22.9	1.27 s	18.8	1.34 s	18.1
20			1.62	36.4		206.0		206.2
21			0.92 d (6.0)	19.0	2.23 s	31.9	2.24 s	32.0
22			2.37 m	39.7				
			1.99 m					
23			6.79 ddd (15.6, 9.0, 7.2)	147.7				
24			6.08 d (15.6)	132.8				
25				198.7				
26			2.25	27.1				
28	1.14 s	28.0	1.00 s	28.3	1.03 s	28.3	1.03 s	28.0
29	1.09 s	19.9	0.88 s	15.9	0.85 s	15.5	0.88 s	15.7
30	1.24 s	19.6	0,88 s	25.7	1.54 s	24.1	1.75	21.0
12-OAc						170.4		170.0
					2.22 s	20.9	2.22 s	20.8

Fr.3.6 was applied to a Sephadex LH-20 column using an isocratic elution with CH_3OH to produce eight subfractions. Fr.3.6.6 was subjected to preparative TLC to yield Fr.3.6.6a, Fr.3.6.6b, and Fr.3.6.6c. Compounds **3** (2.8 mg, t_R 27.4 min), **1** (1.5 mg, t_R 30.4 min), and **4** (8.1 mg, t_R 33.6 min) were isolated from Fr.3.6.6c by reverse-phase semi-preparative HPLC (31% MeCN in H_2O, 3 mL/min). Fr.3.12 was separated by silica gel column chromatography to afford Fr.3.12.1–Fr.3.12.10. Successive purification of Fr.3.12.2 by preparative TLC and reverse-phase semi-preparative HPLC (45% MeCN in H_2O, 4 mL/min) yielded **9** (5.4 mg, t_R 28.2 min), **14** (4.2 mg, t_R 31.3 min), and **12** (3.3 mg, t_R 33.8 min). Fr.3.12.9 was further purified by preparative TLC and reverse-phase semi-preparative HPLC (41% MeCN in H_2O, 4 mL/min) to yield compounds **7** (1.1 mg, t_R 32.7 min) and **11** (6.3 mg, t_R 33.9 min). Fr.3.14 was subjected to a Sephadex LH-20 column and silica gel column to yield three subfractions (Fr.3.14.1–Fr.3.14.3). Fr.3.14.1 was subjected to preparative TLC to yield Fr.3.14.1a and Fr.3.14.1b. Fr.3.14.1b was further purified by reverse-phase semi-preparative HPLC (62% CH_3OH in H_2O, 3 mL/min) to yield compounds **8** (2.9 mg, t_R 31.4 min) and **13**(11.7 mg, t_R 35.7 min). Fr.3.14.2 was subjected to preparative TLC and reverse-phase semi-preparative HPLC (42% MeCN in H_2O, 3 mL/min) to yield compound **15** (8.0 mg, t_R 37.3 min). Fr.3.21 was subjected to a silica gel column to obtain three subfractions (Fr.3.21.1–Fr.3.21.3). Fr.3.21.2 was further purified by reverse-phase semi-preparative HPLC (88% CH_3OH in H_2O, 3 mL/min) to yield compound **2** (1.2 mg, t_R 24.7 min). Fr.3.23 was separated by a silica gel column to obtain 14 subfractions (Fr.3.23.1–Fr.3.23.14). Fr.3.23.6 was further purified by reverse-phase semi-preparative HPLC (85% CH_3OH in H_2O, 3 mL/min) to yield compound **5** (12.0 mg, t_R 13.4 min). Fr.3.23.14 was further purified by preparative TLC and reverse-phase semi-preparative HPLC (90% CH_3OH in H_2O, 3 mL/min) to yield compound **6** (17.5 mg, t_R 13.4 min). Fr.4 was applied to an ODS column (MeOH–H_2O, 40%–100%) to obtain 17 subfractions (Fr.4.1–Fr.4.17). Compound **10** was isolated from Fr.4.8 by repeated recrystallisation.

4.3.1. Ganoluciduone A (1)

White amorphous powder; $[\alpha]_D^{28}$ + 212.5 (*c* 0.12, CH_3OH); UV (MeOH) λ_{max} (log ε) 254 (3.87) nm; IR (KBr) ν_{max} 3442, 2973, 2924, 2854, 1743, 1640, 1046, 981, and 877 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; (+)-HRESIMS *m/z* 359.2221 $[M + H]^+$ (calcd for $C_{22}H_{31}O_4$, 359.2217).

4.3.2. Ganoluciduone B (2)

White amorphous powder; $[\alpha]_D^{28}$ + 54.3 (*c* 0.13, CH_3OH); UV (MeOH) λ_{max} (log ε) 234 (4.41) nm; IR (KBr) ν_{max} 3444, 3039, 2961, 2928, 2886, 1661, 1634, 1454, 1383, and 1038 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; (+)-HRESIMS *m/z* 447.3228 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_2Na$, 447.3234).

Table 2
^{1}H and ^{13}C NMR data of compounds 5–8 in $CDCl_3$ at 600 and 150 MHz.

no.	5		6		7		8	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.99 m	35.8	2.22 m	37.9	2.73 m	33.4	2.96 m	35.1
	1.42 m		2.11 m		1.17 m		1.56 m	
2	1.64 m	28.4	2.77 td (14.4, 6.0)	35.0	1.68 m	27.5	2.51 m	34.3
	1.65 m		2.34 dt (14.4, 4.2)		1.66 m		1.62 m	
3	3.25 m	79.1		217.1	3.27 m	77.5		218.1
4		38.8		47.6		39.2		47.1
5	1.09 dd (11.4, 4.8)	49.2	1.56 dd (12.0, 3.6)	50.8	1.55 overlapped	51.3	1.66 overlapped	51.7
6	2.08 m	23.2	2.19 m	23.8	2.62 dd (14.4, 1.8)	36.6	1.67 m	18.8
	2.07 m		2.05 m		2.71 m		1.47 m	
7	5.47 br d (5.4)	120.4	5.50 d (6.6)	120.1		198.6	2.51m	29.7
							2.52 m	
8		142.7		142.9		146.0		126.7
9		146.1		144.6		152.1		138.8
10		37.5		37.3		33.4		37.3
11	5.31 br d (4.8)	116.4	5.38 d (6.0)	117.3		193.7		197.6
12	2.22 m	38.0	2.21 m	36.7	5.72 s	78.7	2.78 d (16.8)	50.7
	2.09 m		2.08 m				2.33 (16.8)	
13		43.9		43.9		48.8		48.2
14		50.4		50.4		58.0		53.3
15	1.61 m	31.6	1.64 m	31.6		204.7	4.49 m	73.2
	1.38 m		1.40 m					
16	1.97 m	27.9	2.02 m	28.0	2.75 overlapped	37.8	2.57 m	34.3
	1.47 m		1.33 m		2.39 dd (18.6, 9.0)		1.64 m	
17	1.58 m	51.2	1.55 m	50.9	3.33 t (9.0)	49.0	2.96 m	52.5
18	0.57 s	15.8	0.59 s	15.8	0.83 s	13.4	0.74 s	18.9
19	0.98 s	22.9	1.19 s	22.2	1.34 s	17.9	1.12 s	19.2
20		36.7	1.15 m	36.2		154.3		156.6
21	0.91 d (6.0)	18.8	0.91 d (6.6)	18.5	2.13 s	21.5	2.09 s	21.2
22	1.84 m	33.8	1.63 m	33.0	6.11 s	126.2	6.07 s	123.8
	1.83 m		1.42 m					
23	1.26 m	28.0	1.66 m	25.1		198.0		198.3
	1.25 m		1.53 m					
24	3.58 br d (10.2)	77.0	3.05 m	62.2	2.92 m	48.0	2.94 m	47.9
					2.51 br d (12.6)		2.50 m	
25		78.6		62.8	2.94 m	34.8	2.97 m	34.9
26	3.72 d (12.0), 3.62 d (12.0)	64.4	3.92 d (12.0); 3.73 d (12.0)	61.4		176.4		176.6
27	1.04 s	16.0	3.84 s	64.2	1.19 d (7.2)	17.3	1.19 d (7.2)	17.3
28	1.01 s	28.3	1.08 s	25.6	1.04 s	28.0	1.12 s	27.9
29	0.88 s	16.1	1.12 s	22.6	0.89 s	15.7	1.08 s	20.7
30	0.88 s	25.7	0.87 s	25.5	1.76 s	20.7	1.26 s	19.1
OCH_3	3.32 s	49.5			3.68 s	52.0	3.68 s	52.0
12-OAc						170.5		
					2.14	21.4		

4.3.3. Ganolucidoid A (3)

White amorphous powder; $[\alpha]_D^{28}$ + 110.37 (*c* 0.14, CH_3OH); UV (MeOH) λ_{max} (log ε) 252 (3.81) and 196 (3.74) nm; IR (KBr) ν_{max} 3439, 2926, 1736, 1632, 1384, 1069, and 895 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; (+)-HRESIMS *m/z* 483.2356 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_7Na$, 483.2353).

4.3.4. Ganolucidoid B (4)

White amorphous powder; $[\alpha]_D^{28}$ + 53.7 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 255 (3.60) and 196 (3.63) nm; IR (KBr) ν_{max} 3441, 2995, 2933, 1753, 1699, 1632, 1455, 1383, 1226, and 1060 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; (+)-HRESIMS *m/z* 459.2384 $[M + H]^+$ (calcd for $C_{26}H_{35}O_7$, 459.2377).

4.3.5. (24S, 25R)-25-methoxylanosta-7,9(11)-dien-3β,24,26-triol (5)

Colourless prism crystal (CH_3OH); $[\alpha]_D^{28}$ + 33.3 (*c* 0.14, CH_3OH); UV (MeOH) λ_{max} (log ε) 242 (4.16) and 196 (3.63) nm; IR (KBr) ν_{max} 3442, 2973, 1637, 1454, 1384, and 1045 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; (+)-HRESIMS *m/z* 511.3769 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_4$ Na, 511.3758).

4.3.6. 26,27-Dihydroxy-24,25-epoxylanosta-7,9(11)-dien-3-one (6)

White amorphous powder; $[\alpha]_D^{26}$ + 30.5 (*c* 0.32, CH_3OH); UV (MeOH) λ_{max} (log ε) 242 (3.23) nm; IR (KBr) ν_{max} 3428, 2964, 2933, 1711, 1635, 1466, and 1384 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; (+)-HRESIMS *m/z* 493.3294 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3288).

4.3.7. 3β-hydroxy-12β-acetoxy-7,11,15,23-tetraoxolanosta-8,20E(22)-dien-26-oic acid methyl ester (7)

White amorphous powder; $[\alpha]_D^{28}$ – 2.9 (*c* 0.09, CH_3OH); UV (MeOH) λ_{max} (log ε) 244 (3.71) nm; IR (KBr) ν_{max} 3443, 2977, 2925, 1746, 1637, 1455, 1045, and 877 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; (+)-HRESIMS *m/z* 585.3062 $[M + H]^+$ (calcd for $C_{33}H_{45}O_9$, 585.3058).

4.3.8. 15α-hydroxy-3,11,23-trioxolanosta-8,20E(22)-dien-26-oic acid methyl ester (8)

White amorphous powder; $[\alpha]_D^{28}$ + 101.29 (*c* 0.14, CH_3OH); UV (MeOH) λ_{max} (log ε) 248 (3.81) nm; IR (KBr) ν_{max} 3443, 2975, 2926, 1732, 1639, 1456, 1384, and 1046 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; (+)-HRESIMS *m/z* 535.3044 $[M + Na]^+$ (calcd for $C_{31}H_{44}O_6Na$, 535.3030).

4.4. Inhibitory activities on nitric oxide production

The nitrite concentration in the medium was measured as an indicator of nitric oxide production according to the Griess reaction. The Griess reaction was used to evaluate the inhibitory activity on nitric oxide production as described (Dirsch et al., 1998; Jang et al., 2017). Briefly, exponentially growing cells were harvested and seeded in 96-well plates, 1×10^5 cells per well. Then, the cells were cultured for 24 h. The cells were stimulated with 1 μg/mL lipopolysaccharide for 24 h in the presence or absence of different concentrations of tested compounds. L-NMMA was the positive control. The supernatant of the cell culture (100 μL) was mixed with Griess reagent, which was composed of 1% (w/v) sulphanilamide and 0.1% (w/v) N-(1-naphthyl) ethylenediamine in 5% (v/v) phosphoric acid, in a ratio of 1:1. Absorbance was measured in a microplate reader at 570 nm. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium inner salt. The inhibition rate = $(OD_{control} - OD_{test})/OD_{control}$. Experiments were in triplicate and data are represented by the mean ± SD of three independent experiments.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 21702209 and 81172940) and Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2010-ZZ14).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2019.112256.

References

Cheng, C.R., Yue, Q.X., Wu, Z.Y., Song, X.Y., Tao, S.J., Wu, X.H., Xu, P.P., Liu, X.A., Guan, S.H., Guo, D.A., 2010. Cytotoxic triterpenoids from *Ganoderma lucidum*. Phytochemistry 71, 1579–1585.

Dirsch, V.M., Stuppner, H., Vollmar, A.M., 1998. The Griess assay: suitable for a bio-guided fractionation of anti-inflammatory plant extracts? Planta Med. 64, 423–426.

Donath, M.Y., Shoelson, S.E., 2011. Type 2 diabetes as an inflammatory disease. Nat. Rev. Immunol. 11, 98–107.

Fatmawati, S., Shimizu, K., Kondo, R., 2011. Ganoderol B: a potent alpha-glucosidase inhibitor isolated from the fruiting body of *Ganoderma lucidum*. Phytomedicine 18, 1053–1055.

Guan, S.H., Xia, J.M., Yang, M., Wang, X.M., Liu, X., Guo, D.A., 2008. Cytotoxic lanostanoid triterpenes from Ganoderma lucidum. J. Asian Nat. Prod. Res. 10, 695–700.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Cell 144, 646–674.

Huang, S.Z., Ma, Q.Y., Kong, F.D., Guo, Z.K., Cai, C.H., Hu, L.L., Zhou, L.M., Wang, Q., Dai, H.F., Mei, W.L., Zhao, Y.X., 2017. Lanostane-type triterpenoids from the fruiting body of *Ganoderma calidophilum*. Phytochemistry 143, 104–110.

Jang, H.J., Lee, S., Lee, S.J., Lim, H.J., Jung, K., Kim, Y.H., Lee, S.W., Rho, M.C., 2017. Anti-inflammatory activity of eudesmane-type sesquiterpenoids from *Salvia plebeia*. J. Nat. Prod. 80, 2666–2676.

Jiao, Y., Xie, T., Zou, L.H., Wei, Q., Qiu, L., Chen, L.X., 2016. Lanostane triterpenoids from *Ganoderma curtisii* and their NO production inhibitory activities of LPS-induced microglia. Bioorg. Med. Chem. Lett 26, 3556–3561.

Kikuchi, T., Matsuda, S., Kadota, S., Murai, Y., Ogita, Z., 1985a. Ganoderic acid D, E F and H and lucidenic acid-D, E, and F, new triterpenoids from *Ganoderma lucidum*. Chem. Pharm. Bull. 33, 2624–2627.

Kikuchi, T., Matsuda, S., Murai, Y., Ogita, Z., 1985b. Ganoderic acid G and I and ganolucidic acid A and B, new triterpenoids from *Ganoderma lucidum*. Chem. Pharm. Bull. 33, 2628–2631.

Kohda, H., Tokumoto, W., Sakamoto, K., Fujii, M., Hirai, Y., Yamasaki, K., Komoda, Y., Nakamura, H., Ishihara, S., Uchida, M., 1985. The biologically active constituents of *Ganoderma lucidum* (Fr.) karst. histamine release inhibitory triterpenes. Chem. Pharm. Bull. 33, 1367–1374.

Kubota, T., Asaka, Y., Miura, I., Mori, H., 1982. Structures of ganoderic acid A and B, two new lanostane type bitter triterpenes from *Ganoderma lucidum* (Fr.) Karst. Helv. Chim. Acta 65, 611–619.

Liu, J.Q., Wang, C.F., Li, Y., Luo, H.R., Qiu, M.H., 2012. Isolation and bioactivity evaluation of terpenoids from the medicinal fungus *Ganoderma sinense*. Planta Med. 78, 368–376.

Nishitoba, T., Sato, H., Oda, K., Sakamura, S., 1988. Novel triterpenoids and a steroid from the fungus *Ganoderma lucidum*. Agric. Biol. Chem. 52, 211–216.

Nishitoba, T., Sato, H., Sakamura, S., 1985a. New terpenoids from *Ganoderma lucidum* and their bitterness. Agric. Biol. Chem. 49, 1547–1549.

Nishitoba, T., Sato, H., Sakamura, S., 1985b. New terpenoids, ganoderic acid J and ganolucidic acid C, from the fungus *Ganoderma lucidum*. Agric. Biol. Chem. 49, 3637–3638.

Peng, X.R., Liu, J.Q., Wang, C.F., Li, X.Y., Shu, Y., Zhou, L., Qiu, M.H., 2014. Hepatoprotective effects of triterpenoids from *Ganoderma cochlear*. J. Nat. Prod. 77, 737–743.

Peng, X., Li, L., Dong, J., Lu, S., Lu, J., Li, X., Zhou, L., Qiu, M., 2019. Lanostane-type triterpenoids from the fruiting bodies of *Ganoderma applanatum*. Phytochemistry 157, 103–110.

Peng, X., Li, L., Wang, X., Zhu, G., Li, Z., Qiu, M., 2016. Antioxidant farnesylated hydroquinones from *Ganoderma capense*. Fitoterapia 111, 18–23.

Peng, X., Liu, J., Wang, C., Han, Z., Shu, Y., Li, X., Zhou, L., Qiu, M., 2015a. Unusual prenylated phenols with antioxidant activities from Ganoderma cochlear. Food Chem. 171, 251–257.

Peng, X., Liu, J., Xia, J., Wang, C., Li, X., Deng, Y., Bao, N., Zhang, Z., Qiu, M., 2015b. Lanostane triterpenoids from *Ganoderma hainanense*. J. D. Zhao. Phytochem. 114, 137–145.

Saltiel, A.R., Olefsky, J.M., 2017. Inflammatory mechanisms linking obesity and metabolic disease. J. Clin. Investig. 127, 1–4.

Sato, H., Nishitoba, T., Shirasu, S., Oda, K., Sakamura, S., 1986. Ganoderiol-A and ganoderiol-B, new triterpenoids from the fungus Ganoderma lucidum (Reishi). Agric. Biol. Chem. 50, 2887–2890.

Shim, S.H., Ryu, J., Kim, J.S., Kang, S.S., Xu, Y.N., Jung, S.H., Lee, Y.S., Lee, S., Shin, K.H., 2004. New lanostane-type triterpenoids from *Ganoderma applanatum*. J. Nat. Prod. 67, 1110–1113.

Su, H.G., Zhou, Q.M., Guo, L., Huang, Y.J., Peng, C., Xiong, L., 2018. Lanostane triterpenoids from *Ganoderma luteomarginatum* and their cytotoxicity against four human cancer cell lines. Phytochemistry 156, 89–95.

Wang, C.F., Liu, J.Q., Yan, Y.X., Chen, J.C., Lu, Y., Guo, Y.H., Qiu, M.H., 2010. Three new triterpenoids containing four-membered ring from the fruiting body of *Ganoderma sinense*. Org. Lett. 12, 1656–1659.

Wu, J.G., Kan, Y.J., Wu, Y.B., Yi, J., Chen, T.Q., Wu, J.Z., 2016. Hepatoprotective effect of ganoderma triterpenoids against oxidative damage induced by tert-butyl hydroperoxide in human hepatic HepG2 cells. Pharm. Biol. 54, 919–929.

Wu, Y.L., Han, F., Luan, S.S., Ai, R., Zhang, P., Li, H., Chen, L.X., 2019. Triterpenoids from *Ganoderma lucidum* and their potential anti-inflammatory effects. J. Agric. Food Chem. 67, 5147–5158.

Xia, Q., Zhang, H.Z., Sun, X.F., Zhao, H.J., Wu, L.F., Zhu, D., Yang, G.H., Shao, Y.Y., Zhang, X.X., Mao, X., Zhang, L.Z., She, G.M., 2014. A comprehensive review of the structure elucidation and biological activity of triterpenoids from *Ganoderma spp*. Molecules 19, 17478–17535.

Zhao, X.R., Huo, X.K., Dong, P.P., Wang, C., Huang, S.S., Zhang, B.J., Zhang, H.L., Deng, S., Liu, K.X., Ma, X.C., 2015. Inhibitory effects of highly oxygenated lanostane derivatives from the fungus *Ganoderma lucidum* on P-glycoprotein and alpha-glucosidase. J. Nat. Prod. 78, 1868–1876.