Cytotoxic triterpenoids from *Ganoderma lucidum*

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ABSTRACT

A systematic study of the metabolites in *Ganoderma lucidum* led to isolation of 43 triterpenoids, six of them (**1–6**) are hitherto unknown. The structures of the latter were elucidated on the basis of spectroscopic studies and comparison with the known related compounds. All of the compounds were assayed for their inhibitory activities against human HeLa cervical cancer cell lines. Some compounds exhibit significant cytotoxicity, and their structure–activity relationships are discussed.

1. Introduction

Ganoderma lucidum (Fr.) P. Karst. belongs to the family of Ganodermataceae (Basidiomyetes) (Ziegenbein et al., 2006), and whose clinical use can be traced back to 100 BC (Leung and Lin, 2002). With its beautiful legends, *G. lucidum* was believed by the ancient people to cure many kinds of diseases, and it was considered as an elixir that could revive the dead. In modern times, the mystery of *G. lucidum* arose the interest of scientists all over the world, and the publications and patents of *G. lucidum* have increased every year (Boh et al., 2007). It was reported that *G. lucidum* possessed activities including anti-tumor (Liu et al., 2009; Yue et al., 2007, 2008; Stanley et al., 2005; Sliva, 2006; Müller et al., 2006), antimicrobial (Yoon et al., 1994; Wang and Ng, 2006), antiviral (especially anti-HIV activities) (Min et al., 1998), and antiaging activities (Shieh et al., 2001).

Triterpenoids are typical chemical constituents in *G. lucidum*, and have an important role in the pharmacological effects described above. Furthermore, due to the unique structures, they were very important in the chemotaxonomy of genus Ganoderma. Since the first triterpenoid of ganoderic acid A was reported by Kubota et al. (1982), more than 150 compounds had been separated from Ganoderma spp. (Boh et al., 2007). Even today, the number of the new compounds identified from it seemed unendingly to increase. In order to search for bioactive metabolites, we launched a systematic study to investigate the chemical constituents in the CH$_2$Cl$_2$-soluble extract from *G. lucidum*. In the present study, 43 triterpenoids were isolated, including six new compounds. Herein, we described the structural elucidation and cytotoxic assay of these compounds.

2. Results and discussion

Phytochemical study of the CH$_2$Cl$_2$ extract of *G. lucidum* led to the isolation of 43 triterpenoids, including 6 new triterpenoids (Fig. 1) and 37 known compounds (Table 3). The known compounds were identified as ganodermadiol (**7**) (Arisawa et al., 1986), ganoderic acid DM (**8**) (Wang et al., 1997), ganoderenic acid F (**9**) (Nishitoba et al., 1989), ganodermanondiol (**10**) (Fujita et al., 1986), lucidadiol (**11**) (González et al., 1999), 15α-hydroxy-3-oxo-5α-lanosta-7,9,24(*E*)-triene-26-oic acid (**12**) (Li et al., 2006), 15α,26-dihydroxy-5α-lanosta-7,9,24(*E*)-trien-3-one (**13**) (González et al., 2002), lucidumol A (**14**) (Min et al., 1998), 3β-hydroxy-5α-lanosta-7,9,24(*E*)-trien-26-oic acid (**15**) (Li et al., 2006), 3β-hydroxy-7-oxo-5α-lanosta-8,24(*E*)-dien-26-oic acid (**16**) (Li et al., 2006), ganodermanontriol (**17**) (Fujita et al., 1986), ganoderiol F (**18**) (Nishitoba et al., 1988a), lucideric acid A (**19**) (Kikuchi et al., 1986a), ganoderic acid D (**20**) (Kohda et al., 1985), lucidone A (**21**) (Nishitoba et al., 1988b), ganolucidic acid E (**22**) (Nishitoba et al., 1988a), ganoderic acid F (**23**) (Komoda et al., 1985), ganoderenic acid D (**24**) (Komoda et al., 1985), ganoderic acid E (**25**) (Kikuchi et al., 1985a), ganoderic acid J (**26**) (Nishitoba et al., 1985c), ganoderic acid B (**27**) (Kubota et al., 1982), ganoderic acid A (**28**) (Kubota et al., 1982), 7β,12β-dihydroxy-3,11,15,23-
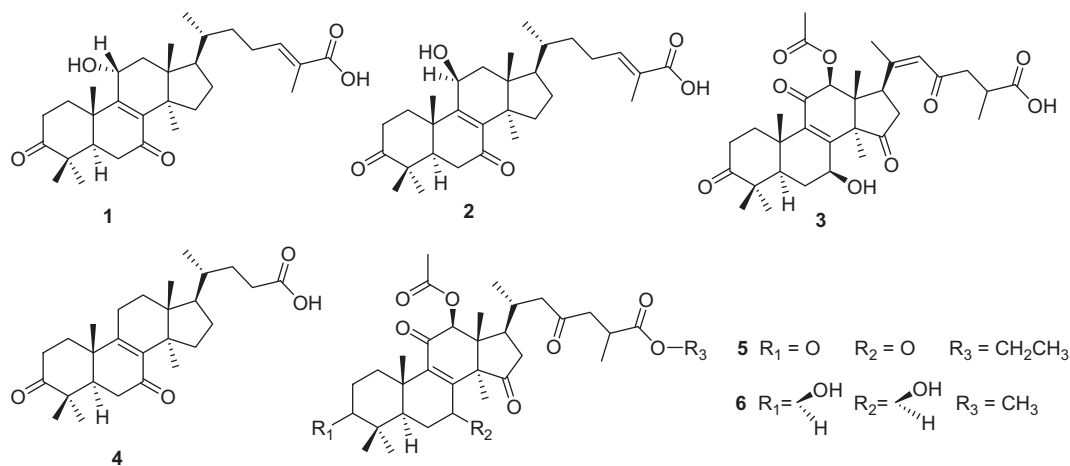
Fig. 1. Structures of compounds **1**–**6**.

tetraoxo-5α-lanosta-8-en-26-oic acid (**29**) (Nishitoba et al., 1985b), 12β-hydroxy-3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid (**30**) (Komoda et al., 1985), ganoderenic acid B (**31**) (Komoda et al., 1985), methyl ganoderate H (**32**) (Kikuchi et al., 1985a), methyl ganoderate B (**33**) (Kohda et al., 1985), 12β-acetoxy-3β,7β-dihydroxy-11,15,23-trioxo-5α-lanosta-8,20-dien-26-oic acid (**34**) (Yang et al., 2007), ganolucidic acid A (**35**) (Kikuchi et al., 1985b), methyl lucidenate C (**36**) (Nishitoba et al., 1985a), ganoderic acid H (**37**) (Kikuchi et al., 1985a), ganoderic acid AM (**38**) (Lin et al., 1993), ganoderic acid T-Q (**39**) (Lin et al., 1988), 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanosta-8-en-26-oic acid (**40**) (Hirotani and Furuya, 1986), ganoderic acid K (**41**) (Morigiwa et al., 1986), ganoderic acid G (**42**) (Kikuchi et al., 1985b), ganoderenic acid A (**43**) (Komoda et al., 1985). The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was isolated as a white powder, with $[\alpha]_D^{23}$ + 13.3 (c 0.33, CHCl$_3$). Its HREIMS spectrum gave a molecular ion peak at m/z 484.3187 corresponding to the molecular formula C$_{30}$H$_{44}$O$_5$. The ^{1}H NMR spectrum of **1** was indicative of five tertiary methyls (δ_H 0.69, 1.11, 1.12, 1.14, 1.41), a vinyl methyl (δ_H 1.84) and a secondary methyl [δ_H 0.96 (d, J = 6.0 Hz)] group, an oxymethine proton [δ_H 4.53 (dd, J = 5.2, 9.2 Hz)] and one vinyl proton [δ_H 6.89 (t, J = 7.2 Hz)], respectively. The ^{13}C NMR and DEPT spectra exhibited the presence of 23 sp^3 carbons due to seven methyls, eight methylenes, four methines including an oxymethine. Comparison of these spectroscopic data with those of ganoderic acid DM (Wang et al., 1997), suggested that the skeleton and side-chain moiety of **1** should be the same except for the presence of an extra hydroxy group. The UV spectrum of **1** exhibited an absorption maximum at 248 nm, suggesting the presence of an α, β-unsaturated carbonyl group. The location of the carbonyl group was confirmed by the analysis of its HMBC spectrum. In the HMBC spectrum, there were correlations between the proton signals at δ_H 2.59 (H-6, dd, J = 14.8, 15.2 Hz), 2.40 (H-6, dd, J = 3.2, 15.6 Hz) and the carbon resonance at δ_C 199.4 (C-7), and between the proton signals at δ_H 1.41 (H-19, 3H, s), 2.50 (H-12) and the carbon signal at δ_C 158.7 (C-9), and between the proton resonance at δ_H 1.14 (H-30, 3H, s) and the carbon signal at δ_C 142.1 (C-8). The above evidence indicated the presence of a 7-ketone-8-ene structural fragment. The attachment of the hydroxy group to C-11 was confirmed by the HMBC cross-peaks between the proton signal at δ_H 4.53 (H-11, dd, J = 5.2, 9.2 Hz) and the carbon resonances at δ_C 158.7 (C-9), δ_C 142.1 (C-8), δ_C 44.6 (C-12), δ_C 40.1 (C-10). The relative configuration of **1** was determined by analyzing the ROESY spectrum. Key ROESY correlations were observed between H-11

and H-19, H-18, indicating that H-11 was on the β-orientation same as H-19 and H-18. Thus, the structure of **1** was assigned as 11α-hydroxy-3,7-dioxo-5α-lanosta-8,24(E)-dien-26-oic acid.

Compound **2** was isolated as a white powder, with $[\alpha]_D^{23}$ − 14.0 (c 0.1, CHCl$_3$). Its HREIMS spectrum showed the molecular ion peak at m/z 484.3187 corresponding to the molecular formula C$_{30}$H$_{44}$O$_5$. The UV spectrum of **2** indicated the presence of an α, β-unsaturated carbonyl group [λ_{max} (CHCl$_3$) 248 nm]. The ^{1}H NMR spectrum of **2** showed the presence of a vinyl proton [δ_H 6.89 (t, J = 7.0 Hz)], a secondary methyl group [δ_H 0.99 (d, J = 6.0 Hz)], a vinyl methyl (δ_H 1.84) and five tertiary methyls (δ_H 0.87, 0.92, 1.09, 1.15, 1.59). The ^{13}C NMR spectrum of **2** suggested the presence of two carbonyl groups (δ_C 214.9 and δ_C 199.8) and a tetra-substituted double bond (δ_C 139.7 and 199.8). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for a minor difference. The HMBC and HSQC spectra suggested that compound **2** had the same overall skeleton as compound **1**. Analysis of the ROESY spectrum established that the stereochemistry of these two compounds was different at position 11. There was no correlation observed between H-11 and H-18, H-19, indicating that the H-11 might be in an α-orientation. Fortunately, we had established compound **1** had a β-orientation at position H-11, so H-11 in compound **2** must be in the α-orientation. The relative configuration at H-11 of compound **2** was also supported by comparing the ^{1}H NMR spectra of the two compounds. Since the hydroxyl group in compound **2** was on the same face as Me-18 and Me-19, the proton signals of H-18 and H-19 moved downfield. Therefore, compound **2** was established as 11β-hydroxy-3,7-dioxo-5α-lanosta-8,24(E)-dien-26-oic acid.

Compound **3** was isolated as a colorless gum, $[\alpha]_D^{22}$ + 85.0 (c 0.14, CHCl$_3$). Its HREIMS spectrum displayed a molecular ion peak at m/z 570.2829 corresponding to the molecular formula C$_{32}$H$_{42}$O$_9$. Its UV spectrum exhibited an absorption maximum at 246 nm, suggesting the presence of α, β-unsaturated carbonyl group. The ^{1}H and ^{13}C NMR spectra resembled ganoderenic acid D (Komoda et al., 1985), except for the proton (δ_H 5.71(s), 2.11(s)) and carbon (δ_C 78.5, 170.6, 20.6) resonances which was a characteristic of an acetoxyl group substituted on a carbon atom where two adjacent carbon atoms bear no proton (Komoda et al., 1985). The position of the acetoxyl group was further confirmed by the correlation between H-12 and C-31 in the HMBC spectrum. The position of the alkene in the side-chain was confirmed by analysis of the HMBC spectrum. In the HMBC spectrum, there were correlations between the proton signals at δ_H 3.25 (H-17, t, J = 10.4 Hz), 2.15 (H-21, s) and the carbon resonances at δ_C 154.1 (C-20), δ_C 126.1 (C-22), and between the proton signal at δ_H 6.12 (H-22, s) and the carbon