Development of a rapid and confirmatory method to identify ganoderic acids in *Ganoderma* mushrooms

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**INTRODUCTION**

In our ongoing investigations of Traditional Chinese Medicine (TCM) for dietary supplements we are particularly interested in the medicinal mushroom *Ganoderma lucidum* (W. Curt.:Fr.) Karst because it is a rich producer of lanostanoids. This class of triterpenes has been reported to have distinct pharmacological activities. These include angiotensin-converting enzyme inhibition, anti-HIV action, antinociceptive action, histamine release inhibition, HMG-CoA reductase inhibition, and liver function stimulation (Mekenna, 1998). The prevalent usage of *G. lucidum* by cancer patients in Asia also persuaded us to further investigate the active components of *G. lucidum*. Indeed, we found that the extract of *G. lucidum* prepared in our labs induced the modulation of secretion from normal human peripheral blood mononuclear leukocytes of cytokines IL-2, IL-4, and IFN-γ (Ma et al., 2002).

Solvent partitioning and repeated chromatography, followed by crystallization resulted in the isolation of 32 lanostanoids from the lipophilic extract of the fruiting body of *G. lucidum*. These include six previously unknown oxygenated lanostanoids: 8,9α-dihydroganoderic acid J (1), methyl 8β, 9α-dihydroganoderate J (2), 20-hydroxyganoderic acid G (3) (Ma et al., 2002), ganoderic acid S (4) (Li et al., 2005), 7-oxo-ganoderic acid Z (5), and 15-hydroxy-ganoderic acid S (6) (Figure 1). Compounds 5 and 6 both exhibited inhibitory activities against HMG-CoA reductase and acyl CoA-acyltransferase (Li et al., 2006). We have also isolated 26 known lanostanoids, including ganoderic acids I (7) and C2 (8), 12-deacetylganoderic acid H (9), ganoderic acid G (10), ganoderenic acid B (11), ganoderic acids B (12), AM1 (13), A (14), and H (15), ganoderenic acid D (16), ganoderic acids C (17), F (18), J (19), DM (20), S and Y, methyl ganoderate I, ganoderiols B and F, ganoderol B, ganodermic acid Q, ganodermonanol, ganodermanondiol, ganodermanontriol, lucidone A, and lucidenic A (Ma et al., 2002; Li et al., 2005, 2006; and references cited therein). Their structures were determined by using 1D and 2D NMR and MS spectroscopic methods. *Ganoderma lucidum* has usually been taken orally as mushroom powder or in its extract form, whether as prescribed by TCM doctors or in modern pill form. There is a need to understand the composition of the natural material ingested. Liquid chromatography–mass spectrometry (LC–MS) has been used wildly to characterize mass spectrometry (LC–MS) has been used wildly to characterize the composition of lanostanoids from *G. lucidum* without requiring the reference standards of these ganoderic acids. Subsequently, only the HPLC–UV method would be needed to analyze routine samples of *G. lucidum*.

**METHODS ARTICLE**

To examine the composition of lanostanoids in *Ganoderma lucidum*, we have developed a liquid chromatography–mass spectrometry (LC–MS) method by using the ganoderic acids isolated in our laboratory as reference standards. The identity of 14 peaks in the high-performance liquid chromatogram (HPLC) of *G. lucidum* has been confirmed. By using the HPLC retention times of these ganoderic acids and their mass fragmentation patterns established in this paper, one can use LC–MS to analyze *G. lucidum* without requiring the reference standards of these ganoderic acids. Subsequently, only the HPLC–UV method would be needed to analyze routine samples of *G. lucidum*.

**Keywords:** mushroom, *Ganoderma lucidum*, lanostanoids, triterpenes, ganoderic acids, LC–MS

**MATERIALS AND METHODS**

**GENERAL EXPERIMENTAL PROCEDURES**

Melting points were recorded on a Fisher-Johns melting point apparatus. Optical rotations were recorded on a WZZ-1S automatic polarimeter. UV spectra were recorded on a HP-8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna
FTIR-750 spectrometer. One- and two-dimensional NMR spectra were recorded on a Bruker AMX 400 spectrometer. Acetonitrile used in LC–MS analysis was supplied by Sigma-Aldrich Co. LLC. Other reagents were all supplied by Sinopharm Chemical Reagent Co. Ltd (Shanghai).

**FUNGAL MATERIAL**
The mushroom of *G. lucidum* was identified by Professor Guanyun Gu, School of Pharmacy, Fudan University, Shanghai, People’s Republic of China. A voucher specimen (GL-9807) has been deposited at the Department of Pharmacognosy, Fudan University, Shanghai, People’s Republic of China.

**IL-2, IL-4, AND IFN-γ ASSAYS**
The sample was dissolved in a 0.4% DMSO solution, then incubated with normal human peripheral blood mononuclear leukocytes in a RPMI 1640 buffer containing 10% FBS, 50 unit/mL penicillin, and 50 μg/mL streptomycin at 37°C for 16 h. Levels of

![FIGURE 1 | Structures of oxygenated lanostanoids.](image1)

![FIGURE 2 | High performance liquid chromatogram of the extract of *G. lucidum*.](image2)
IL-2, IL-4, and IFN-γ were measured using ELISA (Welker et al., 1996; Ma et al., 2002).

**HMG-CoA REDUCTASE ASSAY**

A phosphate buffer solution contains 100 mM potassium phosphate (pH 7.5), 8 mM G-6-P, 1 mM NADP, 4 mM EDTA, 2 mM DTT, and 0.6 U G-6-P-DH was prepared. The compound was dissolved in a 1% DMSO solution and then pre-incubated with microsomal protein in the phosphate buffer solution at 37°C for 15 min. The reaction was started by the addition of 2.5 μM [14C]HMG-CoA reductase and the reaction was run at 37°C for 15 min. [14C]mevalonate was then quantified. It was determined...
that compounds 1 and 2 inhibited HMG-CoA reductase with IC50 = 22.3 and 21.7 μM, respectively (Heller and Gould, 1973; Kubo and Strott, 1987; Li et al., 2006).

**ACYL CoA-CHOLESTEROL ACYLTRANSFERASE ASSAY**

The compound was dissolved in a 1% DMSO solution and pre-incubated with Wistar rat hepatic microsomes in the 0.2-M
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FIGURE 3 | Mass spectra of the major ganoderic acids (7–20) in G. lucidum.

phosphate buffer at 37°C for 15 min. The reaction was started by the addition of 18 μM \([^{14}\text{C}]\)palmitoyl CoA-acyltransferase and the reaction was run at 37°C for 15 min. \([^{14}\text{C}]\)cholesterol ester was then quantified. The inhibitory activities, IC50, of compounds 1 and 2 against CoA-acyltransferase were determined to be 5.5 and 47.3 μM, respectively (Largis et al., 1989; Li et al., 2006).
Table 1 | Mass fragments and HPLC retention times of ganoderic acids (7–20).

| Compound | tR  | MW  | [M + H]+ | [M-nH2O + H]+ | [M-nH2O + H-130]+ |
|----------|-----|-----|----------|--------------|-------------------|
| 7        | 14.3| 532 | –        | 515(26) 497(100) 479(25) | 385(27) 367(85) 349(30) |
| 8        | 17.5| 518 | –        | 501(35) 483(100) 465(35) | 371(35) 353(31) 335(26) |
| 9        | 20.1| 530 | 531(4)   | 495(11) 477(22)       | 383(100) 365(50) 347(18) |
| 10       | 22.1| 532 | –        | 497(31) 479(40) 461(50) | 367(62) 349(100) 331(29) |
| 11       | 22.8| 514 | 515(2)   | 479(100) 461(50)       | –                  |
| 12       | 24.3| 516 | –        | 499(20) 481(100) 463(45) | 369(35) 351(27)   |
| 13       | 25.8| 514 | 515(4)   | 497(7) 479(22)         | 367(100)          |
| 14       | 30.4| 516 | –        | 499(100) 481(53) 463(49) | 387(47) 351(75)   |
| 15       | 31.1| 572 | 573(4)   | 495(14) 477(35) 459(20) | 383(51) 365(100) |
| 16       | 37.6| 512 | –        | 495(68) 477(100)       | –                  |
| 17       | 40.4| 514 | 515(4)   | 497(100) 479(60) 461(45) 427(45) | 367(22) 349(22) |
| 18       | 46.6| 570 | –        | 511(3) 493(8) 475(9) 447(11) | 381(34) 363(100) |
| 19       | 47.3| 514 | 515(2)   | 497(8) 479(18) 461(50)   | 403(37) 367(100) |
| 20       | 70.2| 468 | 469(100) | –                   | –                  |
lab has used the HPLC chromatograms as sample fingerprints for the cGMP compliant purpose. We believe this method could also be an effective tool for researchers in future studies of other related *Ganoderma* species.

**REFERENCES**

Adamec, J., Jannasch, A., Sudhgaonkar, S., Jednak, A., Sedlak, M., and Sliva, D. (2009). Development of a new method for improved identification and relative quantification of unknown metabolites in complex samples: determination of a triterpenoid metabolic fingerprint for the in situ characterization of *Ganoderma* bioactive compounds. *J. Sep. Sci.* 32, 4052–4058.

Heller, R. A., and Gould, R. G. (1973). Solubilization and partial purification of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochem. Biophys. Res. Commun.* 50, 859–865.

Kubo, M., and Strott, C. A. (1987). Differential activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in zones of the adrenal cortex. *Endocrinology* 120, 214–221.

Largis, E. E., Wang, C. H., DeVries, V. G., and Schaffer, S. A. (1989). Cl. 277,082: a novel inhibitor of ACAT-catalyzed cholesterol esterification and cholesterol absorption. *J. Lipid Res.* 30, 681–690.

Li, C., Li, Y., and Sun, H. H. (2006). New ganoderic acids, bioactive triterpenoid metabolites from the mushroom *Ganoderma lucidum*. *Nat. Prod. Res.* 20, 983–991.

Li, C., Yin, J., Guo, G., Zhang, D., and Sun, H. H. (2005). Ganoderic acid Sz, a new lanostanoid from the mushroom *Ganoderma lucidum*. *Nat. Prod. Res.* 19, 461–465.

Liu, Y., Liu, Y., Qiu, F., and Di, X. (2011) Sensitive and selective liquid chromatography-tandem mass spectrometry method for the determination of five ganoderic acids in *Ganoderma lucidum* and its related species. *J. Pharm. Biomed. Anal.* 54, 717–721.

Ma, J., Ye, Q., Huai, Y., Zhang, D., Cooper, R., Chang, M. N., Chang, J. Y., and Sun, H. H. (2002). New lanostanoids from the mushroom *Ganoderma lucidum*. *J. Nat. Prod.* 65, 72–75.

Mekenna, D. J. (ed.). (1998). *Natural Dietary Supplements: A Desktop Reference*. St. Croix: Institute for Natural Products Research.

Welker, P., Lippert, U., Nurnberg, W., Kruger-Krasagakes, S., Moller, A., and Czarnetzki, B. (1996). Glucocorticoid-induced modulation of cytokine secretion from normal and leukemic human myelomonocytic cells. *Int. Arch. Allergy Immunol.* 109, 110–115.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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