



Phytosterols and alkaloids from the fruiting bodies of *Ganoderma lucidum*

Neil Patrick Uy¹ · Kung-Woo Nam² · Sanghyun Lee^{1,3}

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Abstract Mushrooms have long been recognized for their bioactive and medicinal properties. Using repeated column chromatography, three phytosterols and two alkaloids were isolated from the fruiting bodies of *Ganoderma lucidum*. The structures were identified as (22*E*)-23-methylergosta-5,7,22-trien-3 β -ol (**1**), (22*E*)-ergosta-5,7,22-trien-3 β -ol (**2**), 5 α ,8 α -epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3 β -ol (**3**), 2,4-dihydroxypyrimidine (**4**), and 5-hydroxypyrrolidin-2-one (**5**) by analyses of ¹H-NMR and ¹³C-NMR spectroscopy in combination with mass spectrometry. Compounds **1** and **5** were isolated from *G. lucidum* for the first time.

Keywords Alkaloid · Column Chromatography · *Ganoderma lucidum* · Phytosterol

Introduction

The genus *Ganoderma*, belonging to the Ganodermataceae family, grows on the stump of broadleaf trees such as *Colophospermum mopane* and *Grewia bicolor* [1,2]. This genus comprises

approximately 80 species and is found naturally in many subtropical regions [3]. Among *Ganoderma* species, *G. lucidum* is one of the famous medicinal mushrooms. In East Asian countries, this mushroom is referred to as Youngji (Korea), Reishi (Japan), or Lingzhi (China) and has been used in traditional medicine for centuries [4].

According to the Compendium of Materia Medica, *G. lucidum* is classified into six categories based on shape and color (red, purple, yellow, green, black, and white), which are believed to target different parts of the body [5]. Reddish and dark varieties of *G. lucidum* are widely used in Korea [6]. These mushrooms and their derivatives are used not only as dietary supplements but also as therapeutic agents for preventing and treating various diseases such as hepatitis, nephritis, neurasthenia, leucopenia, *etc* [7,8]. Phytochemical studies on the spores, fruiting bodies, and cultured mycelia of *G. lucidum* have identified polysaccharides [9,10], proteoglycans [11], and terpenoids [12–14]. Among these, terpenoids have demonstrated remarkable biological activities, including antitumor [11], anti-androgenic [15,16], anti-oxidant, and antimutagenic properties [17].

Despite its extensive use worldwide for thousands of years, limited research on the phytochemical constituents of edible mushrooms like *G. lucidum* [18]. Hence this study aimed to screen and isolate the phytochemical compounds of the *G. lucidum* fruiting bodies.

Materials and Methods

Plant materials

The fruiting bodies of *G. lucidum* were obtained from the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Republic of Korea. A voucher specimen was deposited at the Natural Product Institute of Science and Technology.

Apparatus and reagents

Electron ionization-mass spectrometry (EI-MS) spectra were recorded using a JEOL JMS-600W mass spectrometer (Tokyo,

Sanghyun Lee (✉)
E-mail: slee@cau.ac.kr

Kung-Woo Nam (✉)
E-mail: kwnam1@sch.ac.kr

¹Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

²Industry-Academic Cooperation Foundation, Soonchunhyang University, Asan 31538, Republic of Korea

³Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

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Japan). ^1H - and ^{13}C -NMR spectroscopy was performed using a Bruker AVANCE 500 NMR spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts were reported as δ -values (ppm) relative to tetramethylsilane as the internal standard, and coupling constants (J) are expressed in hertz. Thin-layer chromatography (TLC) was conducted on precoated silica gel 60 F₂₅₄ plates (Art. 5715, Merck Co., Darmstadt, Germany) for the analysis. The spot colors of the compounds were detected by spraying the TLC plate with 10% sulfuric acid in methanol (MeOH) and heated at 100 °C. Silica gel (200–400 mesh ASTM; Merck Co.) was used as the stationary phase for the repeated column chromatography.

Isolation and fractionation

Dried fruiting bodies of *G. lucidum* (4.0 kg) were extracted with MeOH (8 L $\times$ 4) under reflux. After filtration and *in vacuo* solvent removal, the crude MeOH extracts were collected. A portion (26.6 g) of the total extract (164.2 g) was recrystallized with MeOH to yield compound **1**. The remaining residues were suspended in distilled water and sequentially fractionated with *n*-hexane, chloroform (CHCl_3), ethyl acetate (EtOAc), and *n*-butanol. A portion of the *n*-hexane fraction (15.0 g) was chromatographed on a silica gel column (No. 7734, 6 $\times$ 80 cm) using a gradient solvent system of *n*-hexane- CHCl_3 and CHCl_3 -MeOH to obtain 6 sub-fractions. Subfraction 6 (CHCl_3 :MeOH=1:1) resulted in the isolation of compound **2**. A portion (5.0 g) of the EtOAc fraction was chromatographed into 143 sub-fractions using a gradient solvent system (from 100% *n*-hexane to 100% MeOH). Compound **3** was recrystallized with MeOH and isolated from the subfraction 43 (*n*-hexane : EtOAc =95:5). Finally, a portion of the *n*-butanol fraction (20.0 g) was subjected to silica gel column chromatography with stepwise CHCl_3 -MeOH gradient elution, yielding 99 sub-fractions. Subfractions 12–24 obtained with a gradient of CHCl_3 and MeOH (97:2) yielded compounds **4** and **5** after recrystallization with MeOH.

Compound **1** was obtained as a white powder. $\text{C}_{29}\text{H}_{46}\text{O}$; EI-MS

(rel. int., %): m/z 410 $[\text{M}]^+$ (7.8), 384 (100), 370 (15.0), 339 (13.7), 294 (4.7); ^1H -NMR (500 Hz, CDCl_3 - d); and ^{13}C -NMR (125 MHz, CDCl_3 - d) (Tables 1 and 2, respectively).

Compound **2** was obtained as colorless crystals. $\text{C}_{28}\text{H}_{44}\text{O}$; EI-MS (rel. int., %): m/z 396 $[\text{M}]^+$ (100), 355 (3.9), 337 (27.4), 271 (40.8), 253 (26.7); ^1H -NMR (500 Hz, CDCl_3 - d); and ^{13}C -NMR (125 MHz, CDCl_3 - d) (Tables 1 and 2, respectively).

Compound **3** was obtained as a white powder. $\text{C}_{28}\text{H}_{44}\text{O}_3$; EI-MS (rel. int., %): m/z 428 $[\text{M}]^+$ (9.5), 410 (15.8), 396 (100), 363 (35.3), 251 (15.8); ^1H -NMR (500 Hz, CDCl_3 - d); and ^{13}C -NMR (125 MHz, CDCl_3 - d) (Tables 1 and 2, respectively).

Compound **4** was obtained as a brown powder. $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$; EI-MS (rel. int., %): m/z 112 $[\text{M}]^+$ (100), 69 (47.2); ^1H -NMR (500 Hz, CDCl_3 - d); and ^{13}C -NMR (125 MHz, CDCl_3 - d): see Table 3.

Compound **5** was obtained as a white powder. $\text{C}_4\text{H}_7\text{NO}_2$; EI-MS (rel. int., %): m/z 101 $[\text{M}]^+$ (25.4), 84 (100), 68 (2.3); ^1H -NMR (500 Hz, CDCl_3 - d); and ^{13}C -NMR (125 MHz, CDCl_3 - d): see Table 3.

Results and Discussion

Column chromatography of the MeOH extracts of *G. lucidum* fruiting bodies led to the isolation of compounds **1–5** (Fig. 1).

Compound **1** was isolated as a white powder from the MeOH extracts. The molecular formula $\text{C}_{29}\text{H}_{46}\text{O}$ was derived from the molecular-ion peak at m/z 410. The ^1H -NMR spectrum, showed an ergostan-type steroid skeleton by multiplet signal patterns at δ 0.8–2.5. Seven methyl singlet signals at δ 0.55, 0.63, 0.83, 0.92, 0.95, 1.04, and 1.30 corresponding to five secondary methyl groups and two tertiary methyl groups were observed. Three olefinic proton signals of H-6, -7, and -22 were observed at δ 5.57, 5.39, and 5.21, respectively. Furthermore, one multiplet proton signal at δ 3.62 indicated a proton (H-3), and a nearby hydroxyl group. The ^{13}C -NMR spectrum of compound **1** showed 29

Table 1 ^1H -nuclear magnetic resonance spectral data for compounds **1**, **2**, and **3**

No.	1	2	3
3	3.62 (m)	3.49 (m)	3.96 (m)
6	5.57 (dd, $J=2.7$, 5.0)	5.57 (dd, $J=2.8$, 5.0)	6.59 (d, $J=8.5$)
7	5.38 (q, $J=2.7$)	5.38 (q, $J=2.8$)	6.24 (d, $J=8.5$)
18	0.63 (s)	0.63 (s)	0.81 (s)
19	0.95 (s)	0.93 (s)	0.88 (s)
21	1.03 (d, $J=6.6$)	1.03 (d, $J=6.5$)	1.00 (d, $J=6.5$)
22	5.18	5.15	5.14 (dd, $J=8.0$, 15.2)
23	-	5.19	5.22 (dd, $J=8.0$, 15.2)
26	0.80 (d, $J=7.0$)	0.82 (d, $J=7.0$)	0.82 (d, $J=5.0$)
27	0.83 (d, $J=5.0$)	0.83 (d, $J=5.0$)	0.83 (d, $J=5.0$)
28	0.91 (d, $J=5.0$)	0.91 (d, $J=5.0$)	0.91 (d, $J=6.9$)
29	1.50 (d, $J=1.0$)	-	-

Chemical shifts are reported as parts per million (d), and coupling constants (J) are presented

Table 2 ^{13}C -nuclear magnetic resonance spectral data for compounds **1**, **2**, and **3**

No.	1	2	3
1	38.4	38.2	34.9
2	32.0	32.2	30.4
3	70.5	70.7	66.7
4	40.8	41.0	37.2
5	139.7	140.0	82.4
6	119.5	119.7	135.6
7	116.3	117.7	131.7
8	141.4	141.6	79.7
9	46.3	46.5	51.4
10	37.0	37.3	37.2
11	21.1	21.8	23.6
12	39.1	39.3	39.6
13	42.8	43.0	44.8
14	54.5	54.8	51.9
15	23.0	23.1	20.9
16	27.7	21.6	28.9
17	56.6	56.0	56.5
18	12.2	12.3	13.1
19	16.3	16.5	18.3
20	34.8	40.7	40.0
21	20.8	21.3	21.1
22	135.4	135.8	135.4
23	131.4	132.2	132.6
24	50.2	43.0	43.0
25	30.8	33.3	33.3
26	21.7	19.9	19.9
27	20.1	20.2	20.2
28	16.9	17.8	17.8
29	13.2	-	-

Chemical shifts are reported as parts per million (d), and coupling constants (J) are presented

resonances, and three olefinic carbons (C-5, -7, and -22) of the structure are indicated at the δ 116.5–141.6 range. Examination of the spectral data and a comparison with data from previous literature [19] revealed that compound **1** can be confirmed as (22*E*)-23-methylergosta-5,7,22-trien-3 β -ol. Compound **1** has been isolated from *Lentinula edodes* and *Tricholoma matsutake*, which are also edible mushrooms [19].

Compound **2** was isolated as a white powder from the *n*-hexane fraction. The molecular formula $\text{C}_{28}\text{H}_{44}\text{O}$ was derived from the molecular-ion peak at m/z 396. The ^1H -NMR spectrum showed an ergostan-type steroid structure that was similar to the structure of compound **1**. However, singlet signals of six methyl groups at δ 0.55, 0.63, 0.83, 0.92, 0.95, and 1.04 were observed. In addition, the ^{13}C -NMR spectrum showed 28 resonances. However, the three olefinic carbons (C-5, -7, and -22) of the structure are similar

Table 3 ^1H - and ^{13}C -nuclear magnetic resonance spectral data for compounds **4** and **5**

No.	4		5	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2	-	153.8	-	177.4
3	-	-	2.23 (m, eq and ax)	27.9
4	-	166.4	1.81 (m, eq), 2.03 (m, ax)	28.1
5	7.55 (d, $J=7.5$)	101.8	5.04 (d, $J=5.0$)	82.6
6	5.82 (d, $J=7.5$)	142.8	-	-

Chemical shifts are reported as parts per million (d), and coupling constants (J) are presented

to those in compound **1**. Accordingly, the structure of compound **2** was elucidated as (22*E*)-ergosta-5,7,22-trien-3 β -ol by comparing the spectral data with an authentic sample from the literature [20,21]. Compound **2** was previously isolated from the fruiting bodies of *Phellinus linteus* [22].

Compound **3** was isolated as a white powder from the EtOAc fraction. The molecular formula $\text{C}_{28}\text{H}_{44}\text{O}_3$ was derived from the molecular-ion peak at m/z 428. In the ^1H -NMR spectrum, it was observed to possess an ergostan sterol pattern and was also found to be similar to compound **1**. However, singlet signals of six methyl groups at δ 0.81, 0.82, 0.83, 0.88, 0.91, and 1.00 were observed as well as two doublet signals of the methylene group at δ 6.59 (H-7) and 6.24 (H-6). Furthermore, 28 resonances in the ^{13}C -NMR spectrum were observed. Two methylene groups in C-3 and -4 of the structure are indicated at δ 27.9 and 28.1, respectively. Accordingly, the structure of compound **3** was elucidated as 5 α ,8 α -epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3 β -ol by comparing the spectral data with an authentic sample in the literature [24]. Compound **3** has previously been isolated from *Locicera caulis* [24].

Compound **4** was isolated as a brown powder from the *n*-butanol fraction. The molecular formula of $\text{C}_4\text{H}_4\text{N}_2\text{O}_2$ was derived from the molecular-ion peak at m/z 112. In the ^1H -NMR spectrum, it showed only two doublet signals at δ 5.82 and 7.55 for the proton signals of the double bond. The ^{13}C -NMR spectrum of compound **4** showed four resonances. Among them, two signals at δ 153.8 and 166.4 were characterized as carbonyl groups. However, these peaks shifted upfield due to the presence of two nitrogen groups. Examination of the spectral data and a comparison with data from the literature [25] revealed that compound **4** was 2,4-dihydroxypyrimidine. Compound **4** has been isolated from the leaves of *Peucedanum japonicum* [26].

Compound **5** was isolated as colorless crystals from the *n*-butanol fraction. The molecular formula $\text{C}_4\text{H}_7\text{NO}_2$ was derived from the molecular ion peak at m/z 101. Two proton signals from the methylene groups of H-3 and -4 at δ 2.23 (H-3_{ax} and 3_{eq}), 1.81 (H-4_{ax}), and 2.03 (H-4_{eq}) were observed in the ^1H -NMR spectrum. The ^{13}C -NMR spectrum showed four resonances. Two methylene groups in C-3 and -4 of structure are indicated at δ 27.9 and 28.1,

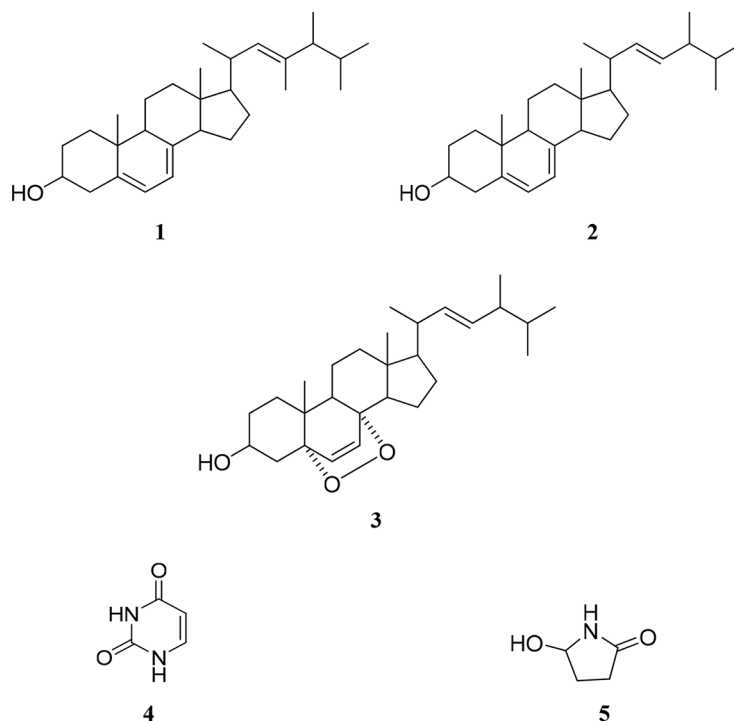


Fig. 1 Chemical structures of the three phytosterols (**1**, **2**, and **3**) and two alkaloids (**4** and **5**)

respectively. In addition, a signal at δ 177.4 was characterized as a carbonyl group. Accordingly, the structure of compound **5** was determined to be 5-hydroxypyrrolidin-2-one by a comparison of the spectral data with an authentic sample from the literature [27]. Compound **5** has been previously isolated from the aerial parts of *Paris verticillata* [28].

Previous studies have isolated sterols from *Ganoderma* spp. and *G. lucidum* [29,30]. Like the present study, these investigations detected and isolated ergosterols from *G. lucidum*, compounds commonly found in mushrooms. However, while our study identified ergosterols as compounds **2** and **3**, we also discovered an ergosterol derivative, compound **1**, which features a methyl group at position 23. Although similar in structure to ergosterol, this derivative is not a true ergosterol due to the methyl substitution. Alkaloids have also been isolated from *G. lucidum* [31]. However, these compounds are less well-documented compared to compounds **1-3** [32]. To our knowledge, this is the first report of compounds **1** and **5** isolated from *G. lucidum*.

These compounds have several industrial applications. Compounds **1-3**, being ergosterol or ergosterol derivatives, are primarily used in the pharmaceutical and nutraceutical industries [33]. In the pharmaceutical industry, the cholesterol-lowering effects of ergosterol have been studied, as it contributes to vitamin D2 production in response to UV radiation [34]. Additionally, ergosterol derivatives may offer anti-inflammatory, antifungal, and anticancer benefits [35]. In the nutraceutical industry, ergosterol fortification

is utilized in developing dietary supplements [36]. In cosmetics, ergosterol derivatives are incorporated into skincare products for their potential moisturizing and skin-rejuvenating properties [37].

Uracil and 5-hydroxypyrrolidin-2-one also have diverse industrial applications, primarily in the pharmaceutical and chemical industries, due to their unique chemical properties and versatile synthesis processes. Uracil, or 2,4-dihydropyrimidine is frequently used as a biochemical research reagent in molecular biological procedures, such as RNA production, DNA sequencing, and polymerase chain reaction [38]. In agriculture, uracil derivatives serve as herbicides and plant growth regulators [39]. Similarly, 2-pyrrolidinone and 2-pyrrolidone are used in the pharmaceutical industry for their analgesics and antipsychotic properties [40]. 5-Hydroxypyrrolidin-2-one, is used in some skincare products as humectant to retain moisture or in formulations for specific skin conditions [41]. These represent only some of the current and potential applications of these compounds across various industries.

Mushrooms have different metabolic profiles compared to plants due to their unique environmental conditions compared to plants. Consequently, growth conditions can influence the synthesis and accumulation of bioactive compounds in mushrooms. This study contributes to the understudied field of metabolic profiling in edible mushrooms and offers valuable insights for the pharmaceutical and nutraceutical industries in developing drugs and functional foods. Additionally, this study can also serve as a reference for studies in this area.

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