Cytotoxicity of *Ganoderma lucidum* Triterpenes

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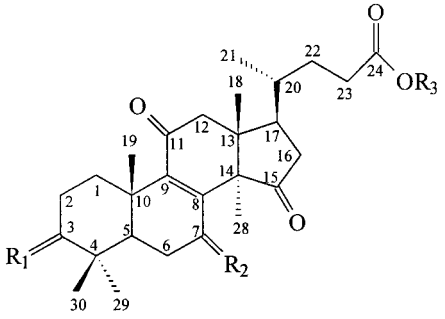
Two new triterpenoids, lucidenic acid N (**1**) and methyl lucidenate F (**2**), together with four known compounds, lucidenic acid A, lucidenolactone, lucidenic acid C, and ganoderic acid E, were isolated from the dried fruiting bodies of *Ganoderma lucidum*. Their structures were elucidated by spectral and chemical transformation studies. Among them, lucidenic acid N (**1**), lucidenic acid A, and ganoderic acid E showed significant cytotoxic activity against Hep G2, Hep G2,2,15, and P-388 tumor cells.

Ganoderma lucidum (Fr.) Krast (Polyporaceae) is a well-known Chinese crude drug, which has been used clinically in East Asia. The fruiting bodies are used for the treatment of hemorrhoids, fatigue symptoms, cancer, hepatitis, chronic bronchitis, and asthmatic conditions.[1] It is also used as a sedative and tranquilizer for dizziness and insomnia due to neurasthenia and hypertension and as a tonic for symptoms of weakness or debility.[1] *G. lucidum* has been reported to have the following pharmacological effects: anticancer, antihypertension, antihistamine, stabilization of mast cells, suppression of cough, hypoglycemic action, reduction of smooth muscle contraction, and antihepatotoxic action against CCl_4.[2]

In our continuing search for bioactive constituents from *G. lucidum*,[2] six triterpenoids were isolated from the ethanol extract of the fruiting bodies. In the present article, we describe the isolation and characterization of two new triterpenoids, lucidenic acid N (**1**) and methyl lucidenate F (**2**), and their cytotoxic activity.



1 R_1 = α-OH, R_2 = α-OH, R_3 = H

2 R_1 = O, R_2 = O, R_3 = CH_3

The HRMS of lucidenic acid N (**1**) revealed the molecular formula $C_{27}H_{40}O_6$. The UV absorbance at 251 nm indicated the presence of an α,β-unsaturated ketone system. Its IR absorption bands suggested the presence of hydroxyl (3449 cm^{-1}), carbonyl (1724 cm^{-1}), and carboxyl (1656 cm^{-1}) groups. The ^1H NMR spectrum showed signals for six methyls and two oxymethines [at δ 3.22 (1H, dd, J = 10.6, 5.0 Hz, H-3) and 4.80 (1H, dd, J = 9.2, 8.2 Hz, H-7)]. The ^{13}C NMR, combined with HMQC, showed that **1** had six methyls, seven methylenes, four methines (including two oxymethines), five quaternary carbons, two sp^2 carbons, and three carbonyls. These NMR data indicated that **1** was a lanostane-type triterpene close in structure to lucidenic acid A.[2] The major differences were the H-3 signal at δ 3.22, which had coupling with H-2 in the COSY spectrum, and the upfield shifts of C-2, C-3, and C-4 to δ 27.6, 78.3, and 38.6 from δ 34.1, 216.8, and 46.6, respectively. This indicated that a hydroxyl group was attached to C-3. It was further supported by an M^+ ion peak in the EIMS at m/z 460, which was two mass units larger than that of lucidenic acid A, and HMBC correlations between H-29/H-30 and C-3. The β-orientation of hydroxyl groups at C-3 and C-7 was deduced from the coupling constants of H-3 (J = 10.6, 5.6 Hz) and H-7 (J = 9.2, 8.2 Hz) and further confirmed by NOEs between H-3 and H-5, and H-5 and H-7. On the basis of the above data, the structure of lucidenic acid N (**1**) was established.

The HRMS of **2** exhibited a molecular ion peak (m/z 470) consistent with the molecular formula $C_{28}H_{38}O_6$. Its UV absorbance at 251 nm and its IR band at 1680 cm^{-1} were ascribable to a conjugated ketone. The compound **2** was identical in all respects to methyl lucidenate F,[3] which was reported earlier as a methylation product of lucidenic acid F by Kikuchi et al.[3]

To confirm the assignment, **1** was oxidized with K_2CrO_7 and concentrated H_2SO_4 in Me_2CO, which yielded lucidenic acid F, and on methylation with MeOH and H_2SO_4 afforded **2**.

The known compounds lucidenic acid A,[2] lucidenolactone,[2] lucidenic acid C,[4] and ganoderic acid E[4] were also isolated and identified by comparison of their spectroscopic data with literature values.

The compounds isolated from the fruiting bodies of *G. lucidum* were tested for their cytotoxicity against Hep G2, Hep G2,2,15, KB, CCM2, and P-388 tumor cell lines. The results (IC_{50} values) are summarized in Table 1. Lucidenic acids A and N and ganoderic acid E showed cytotoxic effects on Hep G2 and Hep G2,2,15 tumor cells.

Experimental Section

General Experimental Procedures. Melting points were uncorrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer, and IR spectra were determined in KBr disks on a Shimadzu FTIR-8501 spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AC-200 and AMX-400 spectrometers. The mass spectra were performed in the EI mode on a VG 70-250S spectrometer.

Plant Material. *G. lucidum* was purchased from Kodak Pharmaceutical Company and identified by C. S. Kuoh. A

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Table 1. Cytotoxicity of Compounds Isolated from the Fruiting Bodies of *G. lucidum* on Tumor Cell Lines, IC_{50} (μM)

compound	cell line				
	Hep G2	Hep G2,2,15	KB	CCM2	P388
lucidenic acid N (**1**)	2.06×10^{-4}	1.66×10^{-3}	26.69	35.49	1.20×10^{-2}
lucidenic acid A	1.64×10^{-4}	2.05×10^{-4}	16.97	27.51	1.70×10^{-2}
ganoderic acid E	1.44×10^{-4}	1.05×10^{-4}		31.25	5.012

voucher specimen (Wu 950012) is deposited in the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, Republic of China.

Extraction and Isolation. The air-dried fruiting bodies of *G. lucidum* (603 g) were cut into small pieces and soaked in EtOH (2.5 L) at room temperature overnight. They were then extracted twice with hot water (5 L). The H_2O extract was partitioned with $CHCl_3$. The $CHCl_3$ layer was concentrated to a brown syrup and chromatographed on silica gel by eluting with a gradient of $CHCl_3$ and MeOH to give eight fractions. Fraction 3 was rechromatographed on silica gel using Me_2CO−hexane (1:9) as an eluant to obtain lucidenic acid A (241 mg), lucidenolactone (32 mg), **2** (1.0 mg), and ganoderic acid E (19 mg), successively. Fraction 4 was repeatedly chromatographed on silica gel eluted with MeOH−$CHCl_3$ (1:15) to give **1** (23 mg) and lucidenic acid C (5 mg).

Lucidenic acid N (1): colorless powder ($CHCl_3$); mp 202−204 °C; $[\alpha]_D$ +119.5° (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 251(3.90) nm; IR (KBr) ν_{max} 3449, 2927, 1724, 1656, 1458, 1382, 1174, 1031, 750 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.80 (1H, dd, J = 9.2, 8.2 Hz, H-7), 4.37 (1H, br s, D_2O exchangeable, OH), 3.22 (1H, dd, J = 10.6, 5.6 Hz, H-3), 2.83 (1H, dt, J = 13.6, 3.7 Hz, H-1β), 2.78 (1H, dd, J = 19.7, 8.4 Hz, H-16α), 2.75 (1H, d, J = 16.6 Hz, H-12β), 2.71 (1H, d, J = 16.6 Hz, H-12α), 2.43 (1H, ddd, J = 16.3, 8.6, 5.5 Hz, H-23), 2.32 (1H, ddd, J = 16.3, 8.0, 8.0 Hz, H-23), 2.19 (1H, ddd, J = 12.0, 7.4, 1.2 Hz, H-6α), 2.13 (1H, dd, J = 19.7, 9.4 Hz, H-16β), 1.99 (1H, dt, J = 18.5, 9.4 Hz, H-17), 1.79 (1H, m, H-22), 1.60 (4H, m, H-2, 6β, 20), 1.35 (3H, s, H-28), 1.03 (3H, s, H-19), 0.98 (3H, d, J = 6.2 Hz, H-21), 0.96 (1H, m, H-1α), 0.96 (3H, s, H-18), 0.88 (1H, dd, J = 8.1, 1.2 Hz, H-5α), 0.85 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.5 (s, C-15), 198.0 (s, C-11), 178.2 (s, C-24), 156.8 (s, C-8), 142.7 (s, C-9), 78.3 (d, C-3), 66.9 (d, C-7), 59.4 (s, C-14), 50.3 (t, C-12), 49.1 (d, C-5), 46.1 (d, C-7), 45.3 (s, C-13), 41.0 (t, C-16), 38.8 (s, C-10), 38.6 (s, C-4), 35.1 (d, C-20), 34.8 (t, C-1), 30.7 (t, C-23), 30.4 (t, C-22), 28.1 (q, C-29), 27.6 (t, C-2), 26.6 (t, C-6), 24.4 (q, C-28), 18.4 (q, C-19), 18.0 (q, C-21), 17.4 (q, C-18), 15.4 (q, C-30); EIMS *m/z* 460 [M^+] (58), 432 (29), 331 (77), 320 (37), 55 (31); HREIMS *m/z* 460.2824 (calcd for $C_{27}H_{40}O_6$, 460.2824).

Methyl lucidenate F (2): pale yellow needles (MeOH); mp 205−207 °C; $[\alpha]_D$ +120.0° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 251, 224 nm; IR (KBr) ν_{max} 2956, 1747, 1701, 1680, 1641, 1456, 1386, 754 cm^{-1}; ^1H NMR($CDCl_3$, 400 MHz) δ 3.68 (3H, s, OCH_3), 2.89 (1H, ddd, J = 13.5, 9.0, 6.5 Hz, H-1β), 2.88 (1H, d, J = 16.5 Hz, H-12α), 2.84 (1H, dd, J = 18.2, 9.0 Hz, H-16), 2.77 (1H, d, J = 16.5 Hz, H-12β), 2.70 (1H, dd, J = 15.0, 13.5 Hz, H-6β), 2.62 (1H, ddd, J = 16.0, 9.8, 6.2 Hz, H-2β), 2.48 (1H, ddd, J = 15.5, 9.0, 6.0 Hz, H-2α), 2.48 (1H,dd, J = 13.5, 2.5 Hz, H-6α), 2.40 (1H, ddd, J = 16.0, 9.0, 7.5 Hz, H-23), 2.32 (1H, dd, J = 15.0, 2.5 Hz, H-5), 2.27 (1H, ddd, J = 16.0, 9.0, 7.5 Hz, H-23), 2.12 (1H, dt, J = 9.0, 8.0 Hz, H-17), 1.95 (1H, dd, J = 18.0, 8.0 Hz, H-16), 1.78 (1H, dddd, J = 14.0, 9.0, 5.0, 5.0 Hz, H-22), 1.75 (1H, ddd, J = 14.0, 9.0, 5.5 Hz, H-22), 1.28 (3H, s, H-19), 1.14 (3H, s, H-29), 1.12 (3H, s, H-30), 0.96 (3H, d, J = 6.2 Hz, H-21), 0.86 (3H, s, H-18); EIMS *m/z* 470 [M^+] (100), 455(5), 439(8), 355(14), 327(4), 300(12), 247(10), 215-(14), 159(17), 141(43), 109(34), 99(14), 95(22), 81(36), 69(53), 55(91); HREIMS *m/z* 470.2669 (calcd for $C_{28}H_{38}O_6$, 470.2668).

Oxidation of Lucidenic Acid A. Lucidenic acid A (18 mg) in Me_2CO (1 mL) was treated with K_2CrO_7 and concentrated H_2SO_4 at 0 °C and allowed to stand at room temperature overnight. After removal of the solvent, the residue was partitioned with 5 N HCl/CH_2Cl_2 (10 mL:40 mL × 4). The CH_2-Cl_2 layer was chromatographed on silica gel and eluted with a gradient of $CHCl_3$−MeOH (9:1) to give lucidenic acid F (14 mg).

Methylation of Lucidenic Acid F. Lucidenic acid F (10 mg) was treated with excess MeOH and 2 drops of H_2SO_4 and refluxed overnight. The solution was evaporated to dryness in vacuo. The residue was dissolved in H_2O (0 °C, 10 mL) and extracted with CH_2Cl_2 (20 mL × 4). The CH_2Cl_2 extract was chromatographed on a silica gel with $CHCl_3$−MeOH (9:1) eluant to obtain **2** (yellow oil, 8 mg), which was identical to **2** obtained previously.

Cytotoxicity Assay. The in vitro KB cytotoxicity assay was carried out according to the procedures by Geran et al.[5] and Ferguson et al.[6] The assay against P-388, CCM2, Hep G2, and Hep G2,2,15 tumor cells was based on a method reported by Lee et al.[7] and Cheng et al.[8]

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