October 1998 Chem. Pharm. Bull. 46(10) 1607—1612 (1998) 1607

Triterpenes from the Spores of *Ganoderma lucidum* and Their Inhibitory Activity against HIV-1 Protease

Byung-Sun MIN,[a] Norio NAKAMURA,[a] Hirotsugu MIYASHIRO,[a] Ki-Whan BAE,[b] and Masao HATTORI*,[a]

Research Institute for Traditional Sino-Japanese Medicines, Toyama Medical and Pharmaceutical University,[a] 2630 Sugitani, Toyama, 930–0194, Japan, and College of Pharmacy, ChungNam National University,[b] Taejon 305–764, Korea.

Received May 14, 1998; Accepted June 18, 1998

Two new lanostane-type triterpenes, lucidumol A and ganoderic acid β, were isolated from the spores of *Ganoderma (G.) lucidum*, together with a new natural one and seven that were known. The structures of the new triterpenes were determined as (24*S*)-24,25-dihydroxylanost-8-ene-3,7-dione and 3β,7β-dihydroxy-11,15-dioxo-lanosta-8,24(*E*)-dien-26-oic acid, respectively, by chemical and spectroscopic means. The quantitative analyses of 5 fruiting bodies, antlered form and spores of *G. lucidum* were performed by high performance liquid chromatography and demonstrated that ganoderic alcohol and acid contents were quite high in the spore. Of the compound isolated, ganoderic acid β, (24*S*)-lanosta-7,9(11)-diene-3β,24,25-triol (called lucidumol B), ganodermanondiol, ganodermanontriol and ganolucidic acid A showed significant anti-human immunodeficiency virus (anti-HIV)-1 protease activity with IC_{50} values of 20—90 μM.

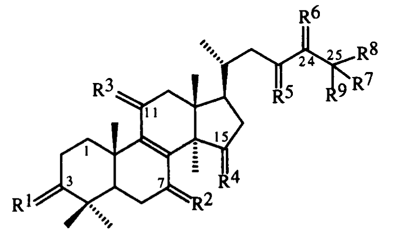
Key words ganoderic acid β; *Ganoderma lucidum*; human immunodeficiency virus-1 protease; lucidumol A; Polyporaceae; spore

The fruiting bodies of *Ganoderma* (*G.*) *lucidum* KARST (Polyporaceae) are widely used in China, Japan and Korea as a valuable crude drug. Over one hundred oxygenated triterpenes have been isolated from this mushroom[1—24] and these compounds were proved to display cytotoxic,[25] histamine release inhibiting,[26] angiotensin converting enzyme inhibiting[27] and cholesterol synthetic inhibiting effects.[28,29] We earlier reported the isolation of a new triterpene acid, ganoderic acid α, together with twelve known compounds from the fruiting bodies of *G. lucidum*. Of these, ganoderiol F and ganodermanontriol were found to have anti-human immunodeficiency virus (anti-HIV) activity.[30]

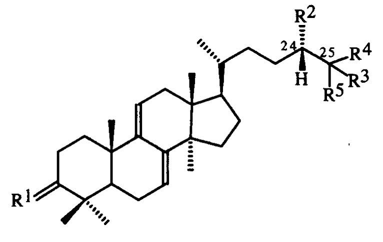
As a part of our continuing studies to find novel anti-HIV agents from natural products, we found that the $CHCl_3$-soluble fraction of the MeOH extract of the spores of *G. lucidum* had appreciable anti-HIV-1 protease activity (47% inhibition at 100 μg/ml). This paper describes the characterization of 10 lanostane-type triterpenes isolated from the spores, as well as the HPLC analysis of the triterpene compounds in the five fruiting bodies produced at different areas (specimens I—V), the antlered form and the spores of *G. lucidum*. The anti-HIV-1 protease activity is also described for these compounds.

Results and Discussion

Isolation and Structure Determination Repeated column chromatography (CC) of a $CHCl_3$-soluble fraction of the MeOH extract of the spores of *G. lucidum* led to the isolation of ten compounds (**1**—**10**). Seven of the compounds were identified as ganodermanondiol (**4**),[12] ganoderiol F (**5**),[21] ganodermanontriol (**6**),[12] ganoderic acid A (**7**),[1,13] ganoderic acid B (**8**),[1,13] ganoderic acid C1 (**9**)[13] and ganolucidic acid A (**10**)[4] by comparison with the reported data (Chart 1). Compound **3** was identified as (24*S*)-lanosta-7,9(11)-dien-3β,24,25-triol (lucidumol B) previously synthesized from ganodermanondiol.[12] This is the first example of the isolation of **3** as a natural product.



	R1	R2	R3	R4	R5	R6	R7	R8	R9
1	O	O	H_2	H_2	H_2	H OH	OH	CH_3	CH_3
1a	O	O	H_2	H_2	H_2	H O-(R)-MTPA	OH	CH_3	CH_3
1b	O	O	H_2	H_2	H_2	H O-(S)-MTPA	OH	CH_3	CH_3
2	OH H	OH H	O	O	H_2	$\Delta^{24\,(25)}$		COOH	CH_3
7	O	OH H	O	H OH	O	H_2	H	COOH	CH_3
8	OH H	OH H	O	O	O	H_2	H	COOH	CH_3
9	O	OH H	O	O	O	H_2	H	COOH	CH_3
10	O	H_2	O	H OH	O	H_2	H	COOH	CH_3



	R1	R2	R3	R4	R5
3	OH H	OH	OH	CH_3	CH_3
4	O	OH	OH	CH_3	CH_3
5	O	$\Delta^{24\,(25)}$		CH_2OH	CH_2OH
6	O	OH	OH	CH_2OH	CH_3

Chart 1. Structures of Compounds Isolated from the Spores of *G. lucidum*

The structures of the new compounds were determined as follows. Compound **1**, obtained as colorless needles (C_6H_6–

---

* To whom correspondence should be addressed.

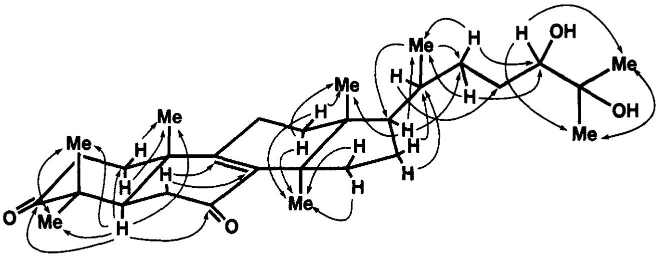


Fig. 1. HMBC Correlations of Compound **1**

EtOAc), mp 185—187 °C. The molecular formula of $C_{30}H_{48}O_4$ was estimated from the high resolution electron impact mass spectrometric (HR-EIMS) spectrum ($[M]^+$ at *m/z* 472.3516, Calcd for $C_{30}H_{48}O_4$, 472.3554). The ultraviolet (UV) absorption at 251 nm (log ε 4.0) was characteristic of an α,β-unsaturated carbonyl group. The infrared (IR) spectrum showed the presence of hydroxyl groups (3500 cm^{-1}) and ketone groups (1710, 1650 cm^{-1}). The proton nuclear magnetic resonance (^1H-NMR) spectrum (Table 1) showed signals for seven tertiary methyls, a secondary methyl (d, $J=6.2$ Hz) at δ 0.94 and an oxymethylene (t, $J=10.1$ Hz) at δ 3.30. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table 2) including distortionless enhancement by polarization transfer (DEPT) and ^1H-detected multiple quantum coherence (HMQC) experiments showed signals for eight methyls, nine methylenes, four sp^3 methines including an oxymethylene at δ 79.5, seven quaternary carbons including two sp^2 carbons at δ 139.5 and 162.8, and sp^3 carbon at δ 73.2, and two carbonyl carbons at δ 214.8 and 198.2 indicating **1** to be an oxygenated lanostane-type triterpene with conjugated enone. Significant heteronuclear multiple-bond correlations (HMBC, see Fig. 1) among the signal of δ_H 2.15 (H-5) and δ_C 214.8, 198.2, and 162.8, among those of δ_H 2.34 and 2.55 (H_2-6) and δ_C 198.2 and 139.5 confirmed the substitutions at C-3, C-7, C-8 and C-9 as shown in formula **1**. Long-range correlations between H_2-12 and H-17, and C-18 (δ 15.9) together with the upfield shift of H_3-18 (δ 0.69), when compared with that of **7**—**10**, confirmed the position and resonance of C-18, and eliminated the possibility of an oxygen substitution at either C-11 or C-12. The presence of the same side chain as that of **3** and **4** containing a glycol moiety in the terminal position was confirmed by the HMBC (Fig. 1) and EIMS spectrum, which showed prominent fragment ion peaks at *m/z* 327 $[M-\text{side chain}]^+$ and 285 $[M-\text{side chain}-C_3H_6]^+$.[13]

The A/B *trans* and C/D *trans* ring junctions were established by the spatial correlations observed between H_{ax}-5 and H-28 (δ 1.10), H-19 (δ 1.34) and H-29 (δ 1.20), H-18 and H-19 (δ 1.34), and H-30 (δ 0.94) and H_{ax}-16 (δ 2.07) in the nuclear Overhauser effect spectroscopy (NOESY) spectrum. The absolute configuration at C-24 was confirmed by the application of modified Mosher's method.[31,32] The (*R*)-(+)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) and (*S*)-(−)-MTPA esters of **1** were prepared. In the ^1H-NMR spectrum of (*R*)-(+)-MTPA ester (see Experimental) proton signals assigned for H-20, H_3-21, H_2-22 and H_2-23 were observed at a lower field than those in the (*S*)-(−)-MPTA ester, while signals due to H_3-26 and H_3-27 in the former ester were shifted to a higher field than those in the latter ester (Fig. 2). Therefore, the absolute configuration at C-24

Table 1. ^1H-NMR Spectral Data of Compounds **1**—**3** (500 MHz, in $CDCl_3$)

H	**1**	**2**	**3**
1α	1.83 m	0.98 m	1.44 m
1β	2.11 m	2.84 dt (13.5, 3.6)	1.99 dt (13.0, 3.1)
2α	2.47 ddd (15.8, 6.2, 3.4)	1.68 m	1.72 ddd (16.4, 7.8, 3.9)
2β	2.71 ddd (15.8, 12.3, 6.7)	1.66 m	1.76 m
3α	—	3.22 dd (11.0, 5.1)	3.25 dd (11.4, 4.4)
5α	2.15 dd (14.5, 3.2)	0.88 m	1.09 d (10.8, 4.6)
6α	2.34 dd (15.8, 3.2)	2.20 m	2.10 m
6β	2.55 dd (15.8, 14.5)	1.63 m	2.10 m
7	—	4.80 t (9.3)	5.48 d (6.0)
11	2.32 m	—	5.32 d (6.4)
12	1.81 m	2.75 m	2.21 br d (17.5)
15α	2.00 m	—	1.39 m
15β	1.38 m	—	1.62 m
16α	2.07 m	2.70 m	1.65 m
16β	1.69 m	2.16 m	1.67 m
17	1.47 m	2.01 m	1.56 m
18	0.69 s	0.98 s	0.57 s
19	1.34 s	1.22 s	0.98 s
20	1.43 m	1.54 m	2.07 m
21	0.94 d (6.2)	1.01 d (6.6)	0.91 d (6.4)
22	1.05, 1.80	1.26 m	1.08 m
23	1.59	2.15, 2.28	1.58 m
24	3.30 d (10.1)	6.83 t (7.4)	3.30 d (10.3)
26	1.17 s	—	1.17 s
27	1.22 s	1.84 s	1.22 s
28	1.10 s	1.04 s	1.01 s
29	1.20 s	0.86 s	0.88 s
30	0.94 s	1.35 s	0.88 s

δ Values in ppm and coupling constants (in parentheses) in Hz.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**3** (125 MHz, $CDCl_3$)

C	**1**	**2**	**3**
1	35.3 t	34.7 t	35.7 t
2	34.4 t	27.6 t	27.9 t
3	214.8 s	78.3 d	78.9 d
4	47.2 s	38.8 s	38.7 s
5	50.3 d	49.1 d	49.1 d
6	37.1 t	26.6 t	23.0 t
7	198.2 s	66.8 d	120.2 s
8	139.5 s	156.9 s	142.6 s
9	162.8 s	142.7 s	145.9 s
10	39.4 s	38.6 s	37.3 s
11	23.8 t	198.0 s	116.2 s
12	30.1 t	50.3 t	37.8 t
13	44.9 s	45.3 s	43.7 s
14	47.4 s	59.3 s	50.3 s
15	28.6 t	217.9 s	31.5 t
16	31.8 t	41.1 t	27.8 t
17	49.0 d	46.1 d	50.9 d
18	15.9 q	17.4 q	15.7 q
19	24.9 q	18.4 q	22.7 q
20	36.6 d	35.5 d	36.5 d
21	18.9 q	18.2 q	18.6 q
22	33.4 t	37.4 t	33.5 t
23	28.6 t	25.6 t	28.7 t
24	79.5 d	143.9 d	79.6 d
25	73.2 s	127.5 s	73.2 d
26	23.2 q[a)]	172.3 s	23.6 q[a)]
27	26.5 q[a)]	12.1 q	26.5 q[a)]
28	25.3 q	28.1 q	28.1 q
29	21.4 q	15.4 q	15.8 q
30	17.9 q	24.4 q	25.6 q

a) Assignments may be interchangeable.

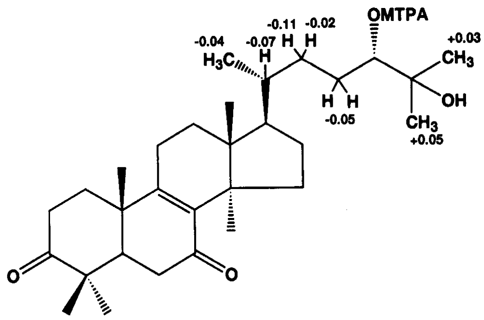


Fig. 2. Chemical Shift Difference for the (*S*)-(−)-MTPA Ester (**1b**) and (*R*)-(+)-MTPA Ester (**1a**) in ppm at 500 MHz

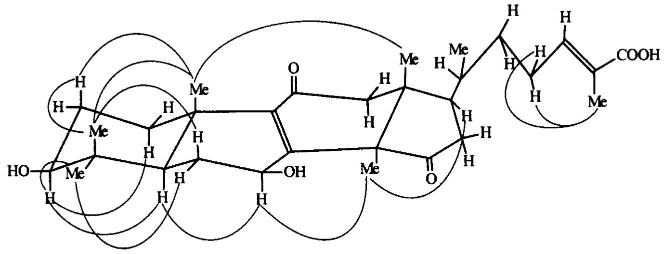


Fig. 3. NOE Correlations in NOESY Spectrum of **2**

was proved to be *S*. The structure of lucidumol A (**1**) was then determined to be (24*S*)-24,25-dihydroxylanost-8-ene-3,7-dione.

Compound **2** showed the molecular formula $C_{30}H_{44}O_6$ (HR-EIMS). The UV and IR spectra exhibited absorption at 252 nm (conjugated C=O), and 3430 (OH), 1710, 1700, and 1650 (C=O) cm^{-1}. The ^{1}H-NMR spectrum of **2** (Table 1) exhibited proton signals for six tertiary methyls, a secondary methyl at δ 1.01 (d, J=6.6 Hz), an olefinic methine at δ 6.83 (t, J=7.4 Hz), and two oxymethylenes at δ 3.22 and 4.80. The ^{13}C-NMR spectrum (Table 2) showed signals for three carbonyl carbons (δ 217.9, 198.0 and 172.3), an sp^2 methine carbon (δ 143.9), and two carbinol carbons (δ 78.3 and 66.8). Signals for seven methyl carbons, seven methylenes, three methines, and seven quaternary carbons (four sp^3 and three sp^2) were also observed. With the aid of ^{1}H–^{1}H correlation spectroscopy (COSY) and HMQC experiments, the two carbinols at δ 78.3 and 66.8 were assigned to C-3 and C-7, respectively. The β orientation of the hydroxyl groups at C-3 and C-7 was deduced from the multiplicity of H-3 (dd, J=11.0 and 5.1 Hz) and H-7 (t, J=9.3 Hz),[33] which was supported by the NOE correlations observed from H-3 to H_{ax}-1 and H_{ax}-5, and H-7 to H_{ax}-5 and H-30 in the NOESY spectrum (Fig. 3). The olefinic methine at δ 143.9 (δ_H 6.83) was assigned to C-24.[24] The conjugated carbonyl signal at δ 198.0 was assigned to C-11 by comparison with those of **7**—**9** as well as signals for C-8 (δ 156.9), C-9 (δ 142.7) and C-15 (δ 217.9).[1,13] This finding was supported by the HMBC correlations from H_2-12 (δ 2.75) to C-11, H_2-6 and H_3-30 to C-8, and H-7 and H-19 to C-9. Signals of an olefinic quaternary carbon and a carboxyl carbon were also assigned to C-25 and C-26, respectively. This was supported by the fragment ion peak at *m/z* 399 $[M-C_5H_9O_2]^+$ resulting from partial cleavage of the side chain. Similar to that in **1**, the side chain of **2** was assigned on the basis of the HMBC and the nuclear Overhauser effect (NOE) correlations in the NOESY spectrum. The configuration at C-24 was confirmed to have *E* form by NOE observed between H_2-23 (δ 2.15 and 2.28) and H_3-27 (δ 1.84) in the NOESY spectrum. No similar correlation has been observed between H-24 and H_3-27 (Fig. 3). Hence, the structure of ganoderic acid β (**2**) was determined as 3β,7β-dihydroxy-11,15-dioxolanosta-8,24(*E*)-dien-26-oic acid.

Table 3. Yields (mg/g of Dry Specimen) of Alcohol and Acid Fractions of Various *G. lucidum* Specimens

	$CHCl_3$ extract (mg/g)	Fractions (mg/g)	
		Alcohol	Acid
I	27.9±1.1[a]	7.5±0.3	16.7±1.5
II	25.9±0.4	6.0±0.2	14.0±0.1
III	20.3±1.7	3.2±0.0	8.6±0.7
IV	23.8±0.7	3.8±0.6	12.2±0.1
V	24.9±2.6	4.0±0.4	12.9±0.2
Antlered[b]	42.2	11.5	22.8
Spore	136.4±1.7	16.0±1.5	99.6±2.1

a) Standard deviation (*n*=3). *b*) *n*=1.

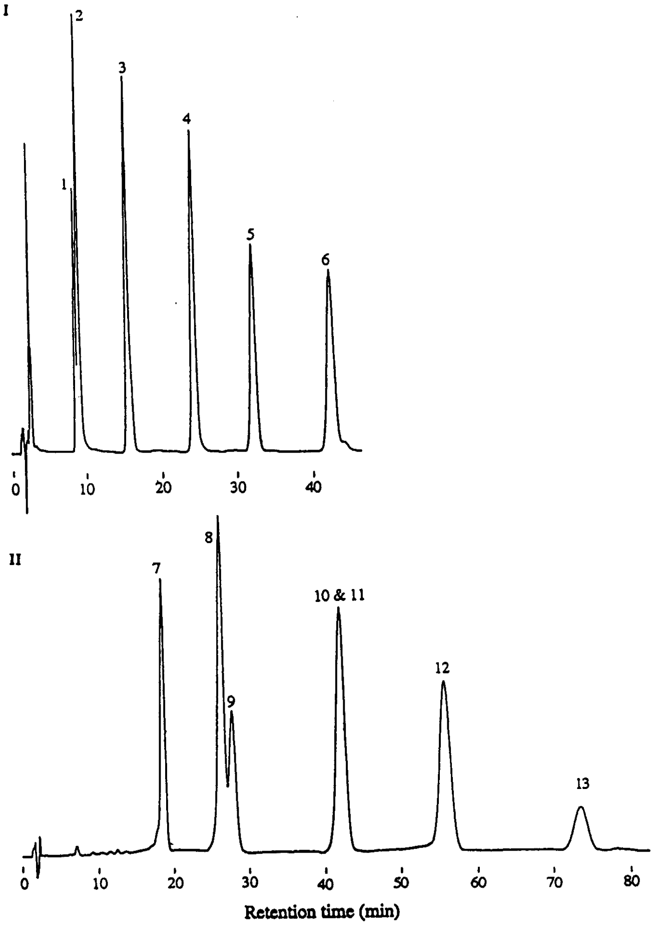


Fig. 4. Elution Profiles of a Mixture of Standard Compounds Isolated from the Spores and Fruiting Bodies of *G. lucidum*

I. Ganoderic alcohols: 1, lucidumol A; 2, ganoderiol A; 3, ganodermanontriol; 4, lucidumol B; 5, ganoderiol F; 6, ganodermanondiol.

II. Ganoderic acids: 7, ganoderic acid B; 8, ganoderic acid A; 9, ganoderic acid α; 10, ganoderic acid H; 11, ganoderic acid C1; 12, ganolucidic acid A; 13, ganoderic acid β.

Quantitative Determination of Triterpenes The alcohol and acid fractions were separated from the respective $CHCl_3$ extracts of 7 specimens of the fruiting bodies of *G. lucidum* (I—V), the antlered form and the spores by Florisil

Table 4. Contents of Ganoderic Alcohols and Acids of Various *G. lucidum* Specimens

	Fruiting body						Spore
	I	II	III	IV	V	Antlered[c]	
Alcohol							
Lucidumol A	27.8[a]±0.2[b]	31.0±0.6	17.3±2.5	230.6±13.	203.6±8.6	34.9	376.7±2.6
Ganoderiol A	67.7±6.9	27.9±1.0	27.7±2.5	—[d]	—	81.8	17.4±40.9
Ganodermanontriol	117.6±18.2	48.2±5.6	57.0±8.9	1.8±0.1	6.8± 0.3	78.4	94.9±2.2
Lucidumol B	30.0±0.4	26.9±0.2	20.4±2.0	16.0±1.4	18.4±0.6	22.0	85.1±0.6
Ganoderiol F	78.2±2.3	82.4±0.6	100.3± 9.0	14.6±0.9	12.8±0.4	83.1	122.4±6.0
Ganodermanondiol	40.9±0.3	27.1±0.7	25.4±1.4	88.3±6.1	92.2±4.3	14.5	313.1±6.6
Total	362.5±40.4	243.8±6.1	248.4± 3.8	351.±31.4	333.9±20.4	314.9	1109.6±66.6
Acid							
Ganoderic acid B	778.5±33.9	213.5±11.2	254.4±33.5	387.3±35.9	389.7±20.5	501.6	3092.8±82.2
Ganoderic acid A	1170.6±52.5	249.5±10.9	445.7±54.5	949.1±44.4	977.9±82.0	1934.3	8408.3±250.0
Ganoderic acid *α*	796.8±17.3	235.5±4.2	219.5± 28.4	783.8±28.1	779.4±17.7	44.0	5577.1±131.4
Ganoderic acid H and C1	738.5±6.7	213.9±6.8	348.5±39.8	629.9±39.4	670.1±29.5	1822.8	6583.5±228.9
Ganolucidic acid A	119.9±4.4	28.2±1.1	77.2± 9.1	89.6±3.5	84.2±26.2	392.2	736.3±36.9
Ganoderic acid *β*	—	75.7±4.4	—	—	—	—	179.3±18.3
Total	3604.3±91.4	1016.5±32.4	1345.4±234.1	2839.9±214.3	2901.5±208.2	4694.9	24577.3±234.1

a) μg/g (dry weight of the sample). *b*) Standard deviation (*n*=3). *c*) *n*=1. *d*) Not detected.

column chromatography (Table 3). The quantitative analysis of the alcohols and acids was carried out by HPLC with a reversed-phase column. The chromatograms of a standard mixture containing the purified ganoderic alcohols and acids from *G. lucidum* are shown in Fig. 4. The peaks were well separated except for ganoderic acid C1 and H under the conditions used. Calibration lines showed good linearity with authentic samples in a range of 0—5 mg/ml, respectively (data not shown). Table 3 shows that the acid content was appreciably higher than the alcohol content in both fruiting bodies and spores. The alcohol content of the spore was 3—5 times higher than that of the fruiting bodies, while the acid content of the former was 5—24 times higher.

The composition of triterpenes among the 7 specimens differed quite greatly. The major alcohols were ganodermanontriol in specimen I, ganoderiol F in specimens II, III and the antlered form, and lucidumol A in specimens IV and V and the spores. The major acid was ganoderic acid A in all the specimens (Table 4). Ganoderic acid *β* (**2**) was detected only in specimen II and the spores, while no ganoderiol A was found in specimens IV and V. The triterpene contents and their fruiting body compositions varied appreciably in the specimens obtained from different areas. The total triterpene content of the spores was 5—20 times that of the fruiting bodies. Thus, the spores were the best source of these triterpenes.

Inhibitory Effects of Compounds on HIV-1 Protease The isolated compounds were tested for their inhibitory activities on HIV-1 protease. Ganoderic acid *β* (**2**), lucidumol B (**3**), ganodermanondiol (**4**), ganodermanontriol (**6**) and ganolucidic acid A (**10**) showed significant inhibitory activity against HIV-1 protease with IC$_{50}$ values of 20, 50, 90, 70 and 70 μM, respectively (Table 5). Of the tested compounds, **2** was found to be the most active on HIV-1 protease. Ganoderic acid A (**7**), ganoderic acid B (**8**) and ganoderic acid C1 (**9**) moderately inhibited the enzyme activity (IC$_{50}$ values, 140—430 μM). As to HIV-1 protease, we could reach no conclusion on the structure–activity relationship of isolated compounds. However, in the present study, hydroxyl groups at C-3 or C-24 and C-25 of lanostane-type triterpene are essential parts showing activity against HIV-1 protease.

Table 5. HIV-1 Protease Inhibitory Activities of Compounds **1**—**10**

Compound	IC$_{50}$ (μM)
Lucidumol A (**1**)	>500
Ganoderic acid *β* (**2**)	20
Lucidumol B (**3**)	50
Ganodermanondiol (**4**)	90
Ganoderiol F (**5**)	>500
Ganodermanontriol (**6**)	70
Ganoderic acid A (**7**)	430
Ganoderic acid B (**8**)	140
Ganoderic acid C1 (**9**)	240
Ganolucidic acid A (**10**)	70
Acetyl pepstatin[a]	0.24

a) Positive control.

Experimental

Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus and are uncorrected. Optical rotations were measured with a DIP-360 automatic polarimeter (JASCO Co., Tokyo, Japan). UV spectra were measured with a UV-2200 UV-VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan). IR spectra were measured with a Fourier transform (FT)/IR-230 infrared spectrometer (JASCO Co., Tokyo). ^{1}H- and ^{13}C-NMR spectra were measured with UNITY 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrophotometer (Varian Co., Palo Alto, U.S.A.), the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. HR-EIMS and EIMS were measured with a JMX-AX 505 HAD mass spectrometer (JEOL Co., Tokyo) at an ionization voltage of 70 eV. Column chromatography was carried out on silica gel (Kieselgel 60, 70—230 mesh, Merck Co., Darmstadt, Germany), Florisil (100—200 mesh, Nacalai Tesque Co., Kyoto) and Diaion HP-20 (Mitsubishi Chemical Co., Tokyo). Medium pressure liquid chromatography (MPLC) was carried out on a LiChroprep Si 60 and LiChroprep RP-18 (both size A from Merck Co., Darmstadt). Analytical HPLC was carried out on a Tosoh CCP 8020 system (Tosoh Co., Tokyo). Thin layer-chromatography (TLC) was carried out on pre-coated Silica-gel 60 F$_{254}$ plates (0.25 mm, Merck Co.), and spots were detected under a UV light and by spraying 10% H_2SO_4 followed by heating. Preparative TLC was carried out on pre-coated Silica-gel 60 F$_{254}$ plates (0.5 mm, Merck Co.).

Plant Materials Five specimens I—V were the fruiting bodies of *G. lucidum* collected from different countries: specimen I was produced in China. specimens II, IV and V were from Vietnam and specimen III was produced in Nagano, Japan. The 5 fruiting bodies and spores of *G. lucidum* were provided by Linzhi General Institute Co., Ltd. (Tokyo). The fruiting bodies in an antler shape were provided by Seizen Co., Kyoto.

Enzyme Recombinant HIV-1 PR (purity 95% by sodium dodecyl sulfate–polyacrylamide gel electrophoresis) was purchased from Bachem (Feinchemikalien AG, Bubendort, Switzerland).

Isolation Procedure The spores of *G. lucidum* KARST (250 g) were extracted with MeOH (1.5 l×3) by refluxing for 3 h to give 35.7 g of a solid extract. The MeOH extract (30 g) was suspended in 90% MeOH (300 ml) and extracted with hexane (150 ml×2). The resulting MeOH solution was concentrated *in vacuo* and suspended in H_2O (300 ml). The suspension was extracted with $CHCl_3$ (150 ml×2) to give a $CHCl_3$-soluble fraction (23.2 g). The $CHCl_3$ fraction (20 g) was chromatographed on a column of silica gel. Elution was started with hexane–acetone (3 : 2, 1 : 1, 2 : 3) and then $CHCl_3$–MeOH (4 : 1) yielded 6 fractions (fr. A—F; 1.3, 5.5, 10.1, 1.9, 0.7 and 0.4 g, respectively). Repeated column chromatography of Fr. A on silica gel (hexane–Me_2CO, 4 : 1) and Florisil (hexane–Me_2CO, 3 : 2), followed by MPLC on silica gel (hexane–Me_2CO, 4 : 1) afforded **1** (15 mg), **3** (7 mg), **4** (20 mg) and **5** (7 mg). Repeated column chromatography of fr. B using silica gel (C_6H_6–Me_2CO, 4 : 1), Florisil (hexane–Me_2CO, 1 : 1), MPLC/silica gel (hexane–Me_2CO, 7 : 3) and reversed phase C-18 (50% aq. MeOH and 70% aq. CH_3CN) furnished **2** (6 mg), **6** (30 mg), **7** (19 mg), **8** (23 mg), **9** (52 mg) and **10** (11 mg).

(24*S*)-24,25-Dihydroxylanost-8-ene-3,7-dione (1, Lucidumol A) Colorless needles (C_6H_6–EtOAc), mp 185—187 °C, $[\alpha]_D$ +35° (c=0.1, EtOH). IR ν_{max} cm^{-1}: 3500 (OH), 1710, 1650 (C=O). UV λ_{max} nm (log ε): 251 (4.0). EI-MS (rel. int.) *m/z*: 472 $[M]^+$ (74), 454 $[M-H_2O]^+$ (43), 439 $[M-H_2O-CH_3]^+$ (100), 413 $[M-C_3H_7O]^+$ (35), 327 $[M-C_8H_{17}O_2]^+$ (98), 285 $[M-C_{11}H_{23}O_2]^+$ (24). HR-EIMS *m/z*: 472.3516 (M^+, Calcd for $C_{30}H_{48}O_4$: 472.3554). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

(*R*)-(+)-MTPA Ester of 1 (1a) A solution of (*R*)-(+)-MTPA chloride (5 mg) in pyridine (0.2 ml) was added to a mixture of **1** (2 mg) in CCl_4 (0.2 ml). After stirring at room temperature for 20 h, the mixture was poured into water (10 ml), and extracted with $CHCl_3$ (10 ml×2). The $CHCl_3$ extract was concentrated *in vacuo* and purified by preparative thin layer chromatography (TLC) [C_6H_6–Me_2CO (5 : 1)] to give an (*R*)-(+)-MTPA ester (2 mg) as a colorless oil, $[\alpha]_D$ +40.0° (c=0.02, MeOH). ^{1}H-NMR ($CDCl_3$): δ 1.90 (1H, H-1α), 2.11 (1H, H-1β), 2.47 (1H, ddd, J=15.8, 6.1, 3.4 Hz, H-2α), 2.70 (1H, ddd, J=15.8, 12.3, 6.7 Hz, H-2β), 2.15 (1H, dd, J=14.3, 3.2 Hz, H-5α), 2.35 (1H, dd, J=15.8, 3.2 Hz, H-6α), 2.55 (1H, dd, J=15.8, 14.3 Hz, H-6β), 2.32 (2H, H-11), 1.84 (2H, H-12), 2.05 (1H, H-15α), 1.41 (1H, H-15β), 2.07 (1H, H-16α), 1.78 (1H, 16β), 1.48 (1H, H-17), 0.66 (3H, s, H-18), 1.34 (3H, s, H-19), 1.43 (1H, H-20), 0.91 (3H, d, J=7.0 Hz, H-21), 1.79 (1H, H-22a), 1.07 (1H, H-22b), 1.67 (2H, H-23), 4.96 (1H, dd, J=9.7, 2.1 Hz, H-24), 1.15 (3H, s, H-26), 1.19 (3H, s, H-27), 1.10 (3H, s, H-28), 1.12 (3H, s, H-29), 0.94 (3H, s, H-30), 3.58 (3H, s, MTPA-OC$\underline{H}_3$), 7.39—7.43 (3H, MTPA-$\underline{Ph}$), 7.59—7.61 (2H, MTPA-$\underline{Ph}$). EI-MS, (rel. int.) *m/z*: 688 $[M]^+$ (16), 674 $[M-CH_3-H]^+$ (16), 455 $[M-OMTPA]^+$ (33), 327 $[M-\text{side chain}-MTPA]^+$ (40), 189 $[MTPA-CO]^+$ (100).

(*S*)-(−)-MTPA Ester of 1 (1b) (*S*)-(−)-MTPA chloride (5 mg) and pyridine (0.2 ml) were added to a mixture of **1** (2 mg) in CCl_4 (0.2 ml). Workup as mentioned above gave the (*S*)-(−)-MTPA ester (1.5 mg) as a colorless oil, $[\alpha]_D$ +10.0° (c=0.03, MeOH). ^{1}H-NMR ($CDCl_3$): δ 1.82 (1H, H-1α), 2.11 (1H, H-1β), 2.46 (1H, ddd, J=15.7, 6.1, 3.3 Hz, H-2α), 2.70 (1H, ddd, J=15.7, 12.2, 6.7 Hz, H-2β), 2.15 (1H, dd, J=15.7, 3.1 Hz, H-5α), 2.34 (1H, dd, J=15.9, 3.1 Hz, H-6α), 2.54 (1H, dd, J=15.9, 14.2 Hz, H-6β), 2.32 (2H, H-11), 1.80 (2H, H-12), 2.01 (1H, H-15α), 1.35 (1H, H-15β), 2.03 (1H, H-16α), 1.70 (1H, H-16β), 1.42 (1H, H-17), 0.63 (3H, s, H-18), 1.33 (3H, s, H-19), 1.37 (1H, H-20), 0.87 (3H, d, J=6.4 Hz, H-21), 1.77 (1H, H-22a), 0.95 (1H, H-22b), 1.62 (2H, H-23), 4.95 (1H, dd, J=9.7, 2.1 Hz, H-24), 1.18 (3H, s, H-26), 1.24 (3H, s, H-27), 1.10 (3H, s, H-28), 1.12 (3H, s, H-29), 0.92 (3H, s, H-30), 3.57 (3H, s, MTPA-OC$\underline{H}_3$), 7.41—7.42 (3H, MTPA-$\underline{Ph}$), 7.61—7.63 (2H, MTPA-$\underline{Ph}$). EI-MS (rel. int.) *m/z*: 688 $[M]^+$ (43), 674 $[M-CH_3-H]^+$ (36), 455 $[M-OMTPA]^+$ (44), 327 $[M-\text{side chain}-MTPA]^+$ (44), 189 $[MTPA-CO]^+$ (100).

3*β*,7*β*-Dihydroxy-11,15-dioxolanosta-8,24(*E*)-dien-26-oic acid (2, Ganoderic Acid *β*) Colorless needles (EtOH–MeOH–H_2O), mp 187—189 °C, $[\alpha]_D$ +60.0° (c=0.1, EtOH). IR ν_{max} cm^{-1}: 3430 (OH), 1710, 1700, 1650 (C=O). UV λ_{max} nm (log ε): 215 (4.1), 252 (3.8). EI-MS (rel. int.) *m/z*: 500 $[M]^+$ (73), 472 $[M-CO]^+$ (51), 399 $[M-C_5H_9O_2]^+$ (41), 360 $[M-C_8H_{12}O_2]^+$ (47), 331 $[M-C_9H_{12}O_3]^+$ (53), 306 $[M-C_{11}H_{18}O_2]^+$ (100). HR-EIMS *m/z*: 500.3128 (M^+, Calcd for $C_{30}H_{44}O_6$: 500.3139). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

(24*S*)-Lanost-7,9(11)-diene-3*β*,24,25-triol (3, Lucidumol B)[12] Colorless plates ($CHCl_3$), mp 209—211 °C, $[\alpha]_D$ +53.0° (c=0.04, MeOH). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

Quantitative Determination of Triterpene The samples of powdered fruiting bodies (50 g) and spores (20 g) were extracted with $CHCl_3$ (300 ml×3) refluxing for 3 h, and the combined extracts were evaporated to dryness *in vacuo*. Half of the $CHCl_3$ extracts was chromatographed on Florisil (10 g). Elution was started with hexane–acetone (9 : 1, 50 ml), hexane–acetone (7 : 3, 300 ml) and then $CHCl_3$–MeOH (1 : 2, 600 ml) to give three fractions. Fractions 2 and 3, corresponding to ganoderic alcohol and acid fractions, respectively, were evaporated *in vacuo* and then dissolved again in MeOH (80 mg/ml of fraction 2 and 20 mg/ml of fraction 3). Twenty μl of each sample solution was injected into the HPLC system under the following conditions: pump, CCPM-II (Tosoh Co., Tokyo); column, packed column (150×4.6 mm i.d.) of TSKgel ODS-80 TS (Tosoh); detector, UV-8020 (Tosoh) set at 243 nm (fraction 2) and 250 nm (fraction 3); mobile phase,[26] 2% AcOH–CH_3CN (4 : 6 for fraction 2 and 7 : 3 for fraction 3). Amounts of the alcohols and acids were calculated by the peak area ratio with respect to the standards.

Chemicals Ganoderiol A and ganoderic acids α and H were obtained from the fruiting bodies of *G. lucidum* by the method described previously.[30]

Protease Assay Twenty five μl of HIV-1 PR assay buffer (Bachem HIV protease assay Kit S-1000) containing 2.5 μg of a substrate, His–Lys–Ala–Arg–Val–Leu–(*p*-NO_2–Phe)–Glu–Ala–NLe–Ser–NH_2, was mixed with 2.5 μl of a dimethyl sulfoxide (DMSO) solution of test compound and then 2.5 μl of recHIV 1-PR (0.175 μg protein) was added to the mixture. After incubation at 37 °C for 15 min, the reaction was stopped by addition of 2.5 μl of 10% trifluoroacetic acid (TFA). The hydolysate and the remained substrate were quantitatively analyzed by HPLC under the following conditions: column, RP-C18 (150×4.6 mm i.d., YMC Co., Kyoto); elution, a linear gradient of CH_3CN (15—35%) in 0.1% TFA; injection volume, 5 μl; flow rate, 1.0 ml/min; detection, 280 nm. The hydrolysate and substrate were eluted at 5.3 and 11.6 min, respectively. The inhibitory activity of the compound in the HIV-1-PR reaction was calculated as follows: % inhibition$=100\times(A_{control}-A_{sample})/(A_{control})$; where A is a relative peak area of the hydrolysate. Acetyl pepstatin was used as a positive control with an IC_{50} of 0.24 μM under the above conditions.

Acknowledgments Part of this study was financially supported by Linzhi General Institute Co., Ltd. (Tokyo), and the Human Science Promoting Foundation (Tokyo).

References and Notes

1) Kubota T., Asaka Y., Miura I., Mori H., *Helv. Chim. Acta*, **65**, 611—619 (1982).
2) Nishitoba T., Sato H., Kasai T., Kawagishi H., Sakamura S., *Agric. Biol. Chem.*, **48**, 2905—2907 (1984).
3) Kikuchi T., Matsuda S., Kadota S., Murai Y., Ogita Z., *Chem. Pharm. Bull.*, **33**, 2624—2627 (1985).
4) Kikuchi T., Matsuda S., Murai Y., Ogita Z., *Chem. Pharm. Bull.*, **33**, 2628—2631 (1985).
5) Hirotani M., Furuya T., Shiro M., *Phytochemistry*, **24**, 2055—2061 (1985).
6) Komoda Y., Nakamura H., Ishihara S., Uchida M., Kohda H., Yamasaki K., *Chem. Pharm. Bull.*, **33**, 4829—4835 (1985).
7) Hirotani M., Asaka I., Ino C., Furuya T., Shiro M., *Phytochemistry*, **26**, 2797—2803 (1987).
8) Arisawa M., Fujita A., Saga M., Fukumura H., Hayashi T., Shimizu M., Morita N., *J. Nat. Prod.*, **49**, 621—625 (1986).
9) Hirotani M., Ino C., Furuya T., Shiro M., *Chem. Pharm. Bull.*, **34**, 2282—2285 (1986).
10) Nishitoba T., Sato H., Shirasu S., Sakamura S., *Agric. Biol. Chem.*, **51**, 619—622 (1987).
11) Sato H., Nishitoba T., Shirasu S., Oda K., Sakamura S., *Agric. Biol. Chem.*, **50**, 2887—2890 (1986).
12) Fujita A., Arisawa M., Saga M., Hayashi T., Morita N., *J. Nat. Prod.*, **49**, 1122—1125 (1986).
13) Kikuchi T., Kanomi S., Kadota S., Murai Y., Tsubono K., Ogita Z., *Chem. Pharm. Bull.*, **34**, 3695—3712 (1986).
14) Kikuchi T., Kanomi S., Murai Y., Kadota S., Tsubono K., Ogita Z., *Chem. Pharm. Bull.*, **34**, 4018—4029 (1986).
15) Nishitoba T., Sato H., Sakamura S., *Phytochemistry*, **26**, 1777—1784 (1987).
16) Nishitoba T., Sato H., Oda K., Sakamura S., *Agric. Biol. Chem.*, **52**, 211—216 (1988).
17) Shiao M. S., Lin L. J., Yeh S. F., *Phytochemistry*, **27**, 873—875 (1988).

18) Shiao M. S., Lin L. J., Yeh S. F., Chou C. S., *J. Nat. Prod.*, **50**, 886—890 (1987).
19) Shiao M. S., Lin L. J., Yeh S. F., *Phytochemistry*, **27**, 2911—2914 (1988).
20) Lin L. J., Shiao M. S., Yeh S. F., *Phytochemistry*, **27**, 2269—2271 (1988).
21) Nishitoba T., Oda K., Sato H., Sakamura S., *Agric. Biol. Chem.*, **52**, 367—372 (1988).
22) Lin L. J., Shiao M. S., Yeh S. F., *J. Nat. Prod.*, **51**, 918—924 (1988).
23) Chen R. Y., Yu D. Q., *Yaoxue Xuebao*, **26**, 267—273 (1991).
24) Hirotani M., Ino C., Furuya T., *Phytochemistry*, **32**, 379—382 (1993).
25) Lin C. N., Tome W. P., Won S. J., *J. Nat. Prod.*, **54**, 998—1002 (1991).
26) Kohda H., Tokumoto W., Sakamoto K., Fujii M., Hirai Y., Yamasaki K., Komoda Y., Nakamura H., Ishihara S., Uchida M., *Chem. Pharm. Bull.*, **33**, 1367—1374 (1985).
27) Morigiwa A., Kitabatake K., Fujimoto Y., Ikekawa N., *Chem. Pharm. Bull.*, **34**, 3025—3028 (1986).
28) Sonoda Y., Sekigawa Y., Sato Y., *Chem. Pharm. Bull.*, **36**, 966—973 (1988).
29) Komoda Y., Shimizu M., Sonoda Y., Sato Y., *Chem. Pharm. Bull.*, **37**, 531—533 (1989).
30) El-Mekkawy S., Meselhy M. R., Nakamura N., Tezuka Y., Hattori M., Kakiuchi N., Shimotohno K., Kawahata T., Otake T., *Phytochemistry*, **37**, in press (1998).
31) Dale J. A., Mosher H. S., *J. Am. Chem. Soc.*, **95**, 512—519 (1973).
32) Ohitani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4096 (1991).
33) Hishitoba T., Sato H., Kasai T., Kawagishi H., Sakamura S., *Agric. Biol. Chem.*, **49**, 1793—1798 (1985).