Anti-human Immunodeficiency Virus-1 Protease Activity of New Lanostane-Type Triterpenoids from *Ganoderma sinense*

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Five new highly oxygenated lanostane-type triterpenoids [ganoderic acid GS-1 (1), ganoderic acid GS-2 (2), ganoderic acid GS-3 (3), 20(21)-dehydrolucidenic acid N (4) and 20-hydroxylucidenic acid A (5)] were isolated from the fruiting body of *Ganoderma sinense*, together with known compounds including 6 triterpenoids and 3 sterols. The structures of the new triterpenoids determined by spectroscopic means including 2D NMR were 7β-hydroxy-3,11,15-trioxo-lanosta-8,24(*E*)-dien-26-oic acid (1), 7β,15α-dihydroxy-3,11-dioxo-lanosta-8,24(*E*)-dien-26-oic acid (2), 12β-acetoxy-3β,7β-dihydroxy-11,15-dioxo-lanosta-8,24(*E*)-dien-26-oic acid (3), 3β,7β-dihydroxy-11,15-dioxo-25,26,27-trinorlanosta-8,20-dien-24-oic acid (4), and 7β,20ξ-dihydroxy-3,11,15-trioxo-25,26,27-trinorlanost-8-en-24-oic acid (5), respectively. Among these, ganoderic acid GS-2, 20-hydroxylucidenic acid N, 20(21)-dehydrolucidenic acid N and ganoderiol F inhibited human immunodeficiency virus-1 protease with IC_{50} values of 20—40 μM.

Key words ganoderic acid; human immunodeficiency virus-1 protease inhibition; AIDS; herbal drug; *Ganoderma sinense*

Ganoderma species have long been used in China, Japan, Korea and other Asian countries as a source of valuable herbal drugs. *Ganoderma lucidum* Karst. (Polyporaceae) is particularly well known and its constituents have been studied for several decades. Over 140 highly oxygenated triterpenoids have been isolated and characterized from *G. lucidum*[1—3] over the past 30 years and some of them have biological activities of interest.[1,4—10] In contrast, little is known about *G. sinense*, which is similar to *G. lucidum*.[11—13] *G. sinense* is registered in the Chinese Pharmacopoeia together with *G. lucidum*, and it is available on the Chinese crude drug market. A preliminary HPLC analysis of bitter principles during our ongoing evaluation of Ganoderma species revealed that the fruiting bodies of *G. sinense* contain some triterpenoids that differ from those of *G. lucidum*.[1,14] We therefore searched for pharmacologically active constituents generated by this mushroom. We already reported that some triterpenes appreciably inhibit human immunodeficiency virus (HIV)-protease,[1,9,10,15] possibly through dimerization inhibition.[15] Here, we elucidated the structures of five new lanostane-type triterpenoids (**1**—**5**), and compared the anti-HIV-1 protease activities of novel lanostane-type triterpenoids isolated from *G. sinense* with those from *G. lucidum*.

Results and Discussion

Isolation and Structure Determination Repeated column chromatography of a chloroform extract of the fruiting body of *G. sinense* resulted in the isolation of eleven triterpenoids and three sterols. A comparison of their spectroscopic data with reported ones revealed the known compounds, ganoderic acid β (**6**),[10] 20(21)-dehydrolucidenic acid A (**7**),[16] 20-hydroxylucidenic acid N (**8**),[16] lucidenic acid D2 (**9**),[17] ganodermanontriol (**10**),[18] ganoderiol F (**11**),[19] cerevisterol (**12**), 5,6-dihydroergosterol (**13**) and ergosterol peroxide (**14**). These compounds have been isolated from *G. lucidum*.

The structures of the new compounds were determined as follows. Ganoderic GS-1 (**1**) was obtained as a white amorphous powder; $[\alpha]_D$ +130.4 ($CHCl_3$). The molecular formula of **1** was $C_{30}H_{42}O_6$, estimated from the high-resolution electron impact mass spectrometric (HR-EI-MS) spectrum (*m*/*z* 498.2932 $[M]^+$, Calcd for $C_{30}H_{42}O_6$ 498.2981). The UV absorption at 253 nm (log ε 3.9) and the IR band at 1660 cm^{-1} suggested an α,β-unsaturated carbonyl group. The ^{1}H-NMR spectrum (Table 1) showed signals for six tertiary methyls, a secondary methyl (δ_H 1.02 d, J=6.0 Hz), an oxymethine (δ_H 4.86 dd, J=9.0, 7.5 Hz) and an olefinic methine (δ_H 6.85 t, J=8.0 Hz). The ^{13}C-NMR spectrum (Table 2), analyzed with the aid of distortionless enhancement by polarization transfer

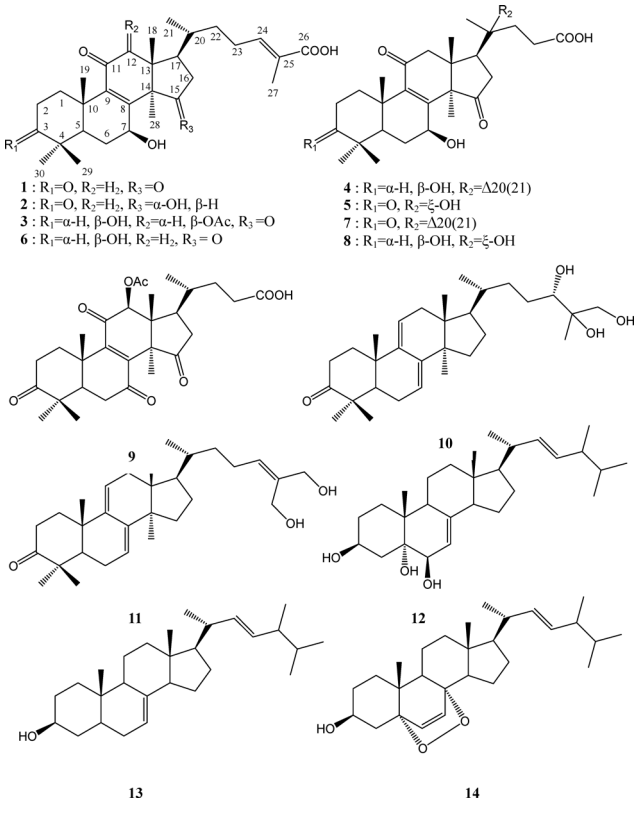

Fig. 1. Structures of Compounds **1**—**14** Isolated from *G. sinense*

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Table 1. ^{1}H-NMR Data (500 MHz) of **1**—**5** (in $CDCl_3$)

Position	1	2	3	4	5
1	1.47 m	1.47 m	0.97 m	0.96 d-like, J=10.0 Hz	1.48 d-like, J=10.0 Hz
	2.95 m	2.95 ddd, J=13.0, 7.0, 5.5 Hz	2.63 m	2.79 m	2.96 m
2	2.44 m	2.49 m	1.63 m	1.63 m	2.45 m
	2.54 m	2.54 m			2.52 m
3	—	—	3.21 dd, J=10.5, 5.0 Hz	3.21 dd, J=10.5, 5.0 Hz	—
5	1.57 d, J=13.0 Hz	1.77 m	0.86 m	0.86 br d	1.55 d, J=13.0 Hz
6	1.68 m	1.69 m	1.64 m	1.61 m	1.61 like s
	2.11 m	2.04 m	2.24 m	2.68 m	2.09 m
7	4.86 dd, J=9.0, 7.5 Hz	4.64 dd, J=10.0, 7.5 Hz	4.81 dd, J=9.0, 7.5 Hz	4.82 dd, J=9.5, 8.0 Hz	4.83 dd, J=9.5, 8.0 Hz
12	2.77 s (2H)	2.54 d, J=16.0 Hz	5.63 s	2.58 d, J=9.0 Hz	2.81 s (2H)
		2.76 d, J=16.0 Hz		2.83 d, J=9.0 Hz	
15	—	4.79 dd, J=9.0, 6.5 Hz	—		
16	2.08 m	1.81 m	2.26 m	2.61 m	2.49 m
	2.76 m	1.89 m	2.68 m		2.61 m
17	2.02 m	2.48 m	2.46 m	2.99 dd, J=9.0, 9.0 Hz	2.50 m
18	1.01 s	0.97 s	0.97 s	0.88 s	1.18 s
19	1.26 s	1.28 s	1.27 s	1.13 s	1.26 s
20	1.56 m	1.43 m	1.69 m		
21	1.02 d, J=6.0 Hz	0.92 d, J=6.5 Hz	1.00 d, J=6.0 Hz	4.88 s, 5.03 s	
22	1.51 m	1.53 m	1.47 m	2.28 m	2.02 m
				2.42 m	2.08 m
23	2.17 m	2.14 m	2.19 m	2.56 m	2.49 t J=7.5 Hz
					2.61 m
24	6.85 t, J=8.0 Hz	6.87 t, J=8.0 Hz	6.85 t, J=7.0 Hz		
27	1.86 s	1.84 s	1.85 s		
28	1.35 s	1.26 s	1.49 s	1.38 s	1.37 s
29	1.11 s	1.11 s	0.86 s	0.83 s	1.10 s
30	1.13 s	1.12 s	1.03 s	0.99 s	1.10 s
MeCO	—	—	2.22 s	0.99 s	1.13 s

Table 2. ^{13}C-NMR Data (500 MHz) of **1**—**5** (in $CDCl_3$)

Position	1	2	3	4	5
1	35.6	35.7	34.4	34.7	35.8
2	34.4	34.5	27.4	27.5	34.1
3	216.8	218.0	78.2	78.3	216.6
4	46.2	47.0	38.5	38.6	46.7
5	48.8	49.0	49.1	49.1	48.8
6	27.6	29.2	26.6	26.5	27.6
7	66.3	69.2	66.2	66.9	66.2
8	157.8	159.2	155.9	156.8	157.4
9	141.2	140.6	142.0	142.6	141.1
10	38.2	38.2	38.5	38.8	38.2
11	197.7	200.1	192.1	197.8	196.9
12	50.1	52.0	79.8	49.1	49.9
13	44.9	46.9	49.9	46.0	45.0
14	59.3	54.1	60.5	58.8	59.2
15	218.1	72.9	216.4	217.7	215.8
16	41.2	36.7	37.3	38.4	35.6
17	46.7	48.7	46.1	46.1	49.5
18	17.7	17.4	13.4	18.4	19.0
19	18.2	19.6	18.6	18.5	18.1
20	35.5	36.2	35.5	143.9	85.9
21	18.2	18.5	20.8	112.2	25.9
22	34.5	34.8	33.1	31.3	34.2
23	25.6	25.9	26.5	32.3	27.4
24	144.1	145.3	143.2	177.3	175
25	127.0	127.2	127.1		
26	171.2	172.0	171.0		
27	12.1	12.3	12.1		
28	24.7	19.7	24.1	24.2	25.0
29	26.9	27.6	28.1	28.1	26.9
30	20.7	20.9	15.4	15.4	20.7
MeCO			170.1		
MeCO			20.7		

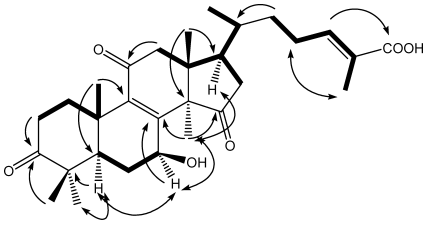


Fig. 2. Selected 2D Correlations for Ganoderic Acid GS-1 (**1**)

Bold lines, ^{1}H–^{1}H COSY; single arrows (selected), HMBC; double arrows (selected) NOESY.

(DEPT) and ^{1}H-detected multiple quantum coherence (HMQC) studies, revealed signals for seven methyls, seven methylenes, five methines (including an oxymethine at δ_C 66.3 and an sp^2 methine at δ_C 144.1) and eleven quaternary carbons (including three olefinic carbons at δ_C 127.0, 141.2, and 157.8, and four carbonyl carbons at δ_C 171.2, 197.7, 216.8, and 218.1). These data suggested a highly oxygenated lanostane-type triterpene similar to ganoderic acid β (**6**).[10] However, the MS spectrum showed that the molecular weight of **1** was 2 mass units less than that of **6**. Heteronuclear multiple bond correlations (HMBC) of **1** showed a carbonyl carbon (δ_C 216.8) that had long range correlations with two methyl protons at δ_H 1.11 and 1.13 (CH_3-29, 30) (Fig. 2). These findings indicated that a carbonyl group in **1** was located at C-3. The β orientation of a hydroxyl group at C-7 was deduced from multiplicity of H-7 (δ_H 4.86 dd, J=9.0, 7.5 Hz),[20] which was also supported by NOE correlations between H-7 to H_{ax}-5 and between H_{ax}-5 and H-30 in the nuclear Overhauser effect spectroscopy (NOESY) study. The

geometry of the double bond at C-24 was *entgegen* (*E*) according to the NOE observed between H_2-23 and H_3-27 in the NOESY spectrum. No such correlation was evident between H-24 and H_3-27. Hence, the structure of ganoderic acid GS-1 (**1**) was 7β-hydroxy-3,11,15-trioxo-lanosta-8,24(*E*)-dien-26-oic acid.

Ganoderic acid GS-2 (**2**) was obtained as a white amorphous powder; $[\alpha]_D$ +112.9 ($CHCl_3$). The molecular formula of **2** was estimated as $C_{30}H_{44}O_6$ from the HR-EI-MS spectrum (*m/z* 500.3149 $[M]^+$, Calcd for $C_{30}H_{44}O_6$ 500.3138). The spectral data suggested that **2** had a similar structure to **1**, but the MS spectra showed that the molecular weight of **2** was 2 mass units higher than **1**, and ^1H–^1H correlation spectroscopy (COSY) indicated an oxymethine proton at δ_H 4.79 assignable to H-15. The α orientation of a hydroxyl group at C-15 was deduced from the multiplicity of H-15 (δ_H 4.79 dd, J=9.0, 6.5 Hz), which was also supported by an NOE correlation between H-15 and H_3-18 in the NOESY spectrum. Hence, the structure of ganoderic acid GS-2 (**2**) was as 7β,15α-dihydroxy-3,11-dioxo-lanosta-8,24(*E*)-dien-26-oic acid.

Ganoderic acid GS-3 (**3**) was obtained as white amorphous powder; $[\alpha]_D$ +69.1 ($CHCl_3$). The molecular formula of **3** was estimated as $C_{32}H_{48}O_8$ from the HR-EI-MS spectrum (*m/z* 558.3184 $[M]^+$, Calcd for $C_{32}H_{48}O_8$ 558.3193). The ^1H- and ^{13}C-NMR spectra suggested that the structure of **3** was close to **6** and that it possessed an acetoxy group. The HMBC experiment assigned an oxymethine proton at δ_H 5.63 to H-12. The β orientation of the acetoxy group at C-12 was deduced from NOE correlations between H-12 and H_{ax}-17 or H_3-28 in the NOESY experiment. Hence, the structure of ganoderic acid GS-3 (**3**) was 12β-acetoxy-3β,7β-dihydroxy-11,15-dioxo-lanosta-8,24(*E*)-dien-26-oic acid.

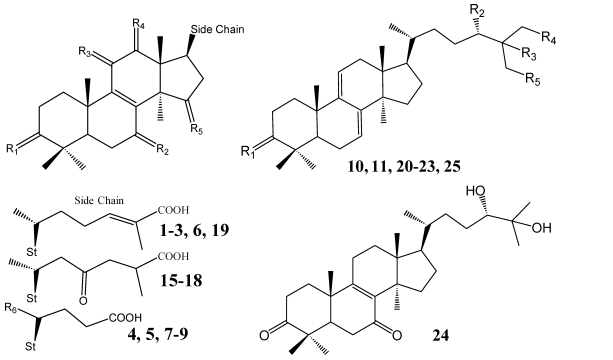
We obtained 20(21)-dehydrolucidenic acid N (**4**) as a white amorphous powder; $[\alpha]_D$ +108.3 ($CHCl_3$). Its molecular formula was $C_{27}H_{38}O_6$ as estimated from the HR-EI-MS spectrum (*m/z* 458.2666 $[M]^+$, Calcd for $C_{27}H_{38}O_6$ 458.2668). The ^1H-NMR spectra suggested that **4** was structurally similar to 20(21)-dehydrolucidenic acid A (**7**),[16] which is a C_{27} triterpenoid. However, the MS spectra showed that the molecular weight of **4** was 2 mass units higher than that of **7**, and the ^1H–^1H COSY experiment assigned an oxymethine proton at δ_H 3.21 to H-3. The β orientation of a hydroxyl group at C-3 was deduced from the multiplicity of H-3 (δ_H 3.21 dd, J=10.5, 5.0 Hz),[20] which was supported by NOE correlations between H-3 and H_{ax}-5 or H-30 in the NOESY experiment. Hence, the structure of **4** was 3β,7β-dihydroxy-11,15-dioxo-25,26,27-trinorlanosta-8,20(21)-dien-24-oic acid.

We obtained 20-hydroxylucidenic acid A (**5**) as a white amorphous powder; $[\alpha]_D$ +184.4 ($CHCl_3$). The molecular formula of **5** was estimated as $C_{27}H_{38}O_7$ from the HR-EI-MS spectrum (*m/z* 474.2676 $[M]^+$, Calcd for $C_{27}H_{38}O_7$ 474.2618). The ^1H-NMR spectra suggested the structure of **5** was similar to that of 20-hydroxylucidenic acid N (**8**).[16] However, the MS spectra showed that the molecular weight of **5** was 2 mass units less than that of **8**. The HMBC experiment showed that a carbonyl carbon (δ_C 216.6) had long-range correlations with two methyl protons at δ_H 1.10 and 1.13 (H_3-29, 30). These findings indicated that a carbonyl group was located at C-3 in **5**. The absolute configuration at C-20 remains undetermined as well as that of **8**. The structure of **5** was subsequently determined as 7β,20ξ-dihydroxy-3,11,15-trioxo-25,26,27-trinorlanost-8-en-24-oic acid.

Anti-HIV-1 Protease Activities of Compounds Isolated from *Ganoderma* Species Our previous publications addressing the development of naturally occurring anti-viral agents,[21,22] indicate that some triterpenes, such as ganoderic acid B and ganoderiol B,[9] *N*-(3β-hydroxyolean-12-en-28-oyl)-6-aminohexanoic acid,[15] hemiesters of ursolic acid,[23] 3-oxotirucalla-7,24-dien-21-oic acid,[24] 16β-hydroxy-2,3-seco-lup-20(29)-en-2,3-dioic acid,[25] colossolactone V,[26] and 2-*O*-acetyldryopteric acid A,[27] potently inhibit HIV-1 PR, which is an essential enzyme for HIV proliferation.[21,22]

Here we compared the abilities of 11 triterpenoids isolated from the fruiting body of *G. sinense* and 11 [ganoderic acid A (**15**), B (**16**), C1 (**17**), G (**18**), DM (**19**), ganoderiol A (**20**), ganodermadiol (**21**), ganodermanondiol (**22**), ganodermatriol (**23**), lucidumol A (**24**), and lucidumol B (**25**)] isolated from *G. lucidum* to inhibit HIV-protease. Table 3 shows the IC_{50} values of these compounds. Of 24(25) unsaturated ganoderic acids (such as **1**, **2**, **3**, **6** and **19**), which are the major compounds of *G. sinense*, the 3-oxo compounds were more inhibitory than the 3-hydroxy compounds. Among the lucidenic acids (such as **4**, **5**, **7**, **8** and **9**), the 3-hydroxy com-

Table 3. Anti-HIV-1 Protease Activities of Ganoderma Triterpenes



No.	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50} (μM)
1	O	β-OH	O	H_2	O		58
2	O	β-OH	O	H_2	α-OH		30
3	β-OH	β-OH	O	β-OAc	O		>200
4	β-OH	β-OH	O	H_2	O	Δ20(21)	48
5	O	β-OH	O	H_2	O	CH_3, OH	>200
6	β-OH	β-OH	O	H_2	O		116
7	O	β-OH	O	H_2	O	Δ20(21)	>200
8	β-OH	β-OH	O	H_2	O	CH_3, OH	25
9	O	O	O	β-OAc	O	α-CH_3	>200
10	O	OH	OH	OH	H		65
11	O	Δ24(25)		OH	OH		22
15	O	β-OH	O	H_2	α-OH		>200
16	β-OH	β-OH	O	H_2	O		>200
17	O	β-OH	O	H_2	O		>200
18	β-OH	β-OH	O	β-OH	O		>200
19	O	O	H_2	H_2	H_2		38
20	β-OH	OH	OH	OH	H		80
21	β-OH	Δ24(25)		OH	H		29
22	O	OH	H	OH	H		>200
23	β-OH	Δ24(25)		OH	OH		>200
24							99
25	β-OH	OH	OH	H	H		>200
Pepstatin A							2.1

pounds were more inhibitory than the 3-oxo compounds. Among the ganoderma alcohols (such as **10**, **11** and **20**—**25**), 24(25) unsaturated compounds were more inhibitory than 24-hydroxy compounds. The 23-oxo ganoderic acids (such as **15**—**18**), which are the major compounds of *G. lucidum*, had no activity against HIV-protease. Ganoderiol F (**11**), 20-hydroxylucidenic acid N (**8**), ganoderic acid GS-2 (**2**), and 20(21)-dehydrolucidenic acid N (**4**) were significantly inhibitory against HIV-1 protease, with IC_{50} values of 22, 25, 30 and 48 μM, respectively.

Experimental

Optical rotation was measured using a JASCO DIP-360 automatic polarimeter and UV spectra were measured with a UV-2200 UV–VIS recording spectrophotometer (Shimadzu Co., Kyoto, Japan). We measured IR spectra with a Jasco FT/IR-230 infrared spectrometer and NMR spectra with a Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) NMR spectrometer. The internal standard was tetramethyl silane (TMS) and *J* values are reported in Hertz. The HR-EI-MS spectra were measured on a Jeol JMS-AX505HAD apparatus at an ionization voltage of 70 eV. Preparative HPLC proceeded on a Tosoh CCPM-II system (Tosoh Co., Tokyo, Japan) equipped with a UV 8020 detector and a Cholester Waters HPLC column (20×250 mm). Column chromatography proceeded on BW-820MH silica gel (Fuji Silica Chemical Co., Aichi, Japan) or Wakogel 50C18 (38—63 μm, Wako Pure Chemical Industries, Ltd.).

Plant Materials *Ganoderma sinense* was purchased in Hehauchi market for medicinal herbs (Chengdu, China) and identified by Dr. De-Yuan Chen (Guiyang College of Traditional Chinese Medicine, Guiyang, China). The specimen has been deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama.

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

Extraction and Isolation Dried and chopped *G. sinense* (275 g) was extracted three times with $CHCl_3$ (2 l) by refluxing for 3 h to afford 6.8 g of a solid extract. The crude extract (6.5 g) was eluted through a silica gel column (4×40 cm) with hexane–acetone (9 : 1; Fr. 1, 300 mg), hexane–acetone (7 : 3; Fr. 2; 3.4 g), and $CHCl_3$–MeOH (1 : 2; Fr. 3, 1.8 g). Fraction 2 was separated by silica gel column chromatography (CC) into six fractions using a hexane–acetone gradient. Fraction 2-1 was separated by silica gel CC with a hexane–acetone gradient to yield ergosterol peroxide (**14**, 80 mg) and 5,6-dihydroergosterol (**13**, 25 mg). Fraction 2-2 was similarly separated to yield ganoderiol F (**11**, 50 mg). Fraction 2-3 was separated by octadecyl silica (ODS) CC with an H_2O–MeOH gradient to yield 20(21)-dehydrolucidenic acid A (**7**, 35 mg), ganodermanontriol (**10**, 150 mg), and cerevisterol (**12**, 5 mg). Fraction 2-4 was separated by preparative HPLC (ODS column; MeCN–0.1% trifluoroacetic acid (TFA)/H_2O, 5 ml/min) to yield ganoderic acid GS-1 (**1**, 15 mg) and ganoderic acid GS-2 (**2**, 10 mg). Fraction 2-5 was separated by ODS CC with an H_2O–MeOH gradient to yield 20(21)-dehydrolucidenic acid N (**4**, 30 mg) and 20-hydroxylucidenic acid A (**5**, 30 mg). Fraction 2-6 was resolved by preparative HPLC (Cholester column; MeOH–0.1% TFA/H_2O, 5 ml/min) to yield ganoderic acid GS-3 (**3**, 5 mg) and ganoderic acid β (**6**, 5 mg). Fraction 3 was separated by ODS CC with an H_2O–MeOH gradient to yield 20-hydroxylucidenic acid N (**8**, 40 mg) and lucidenic acid D2 (**9**, 10 mg).

7β-Hydroxy-3,11,15-trioxo-lanosta-8,24(*E*)-dien-26-oic Acid (1, Ganoderic Acid GS-1) Amorphous powder. $[\alpha]_D^{23}$ +130.4° (c=0.276, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 252 (3.9) nm. IR (KBr) ν_{max}: 3440 (OH), 2880, 1700 (carbonyl), 1660 (carboxyl), 1480, 1390, 1270, 1140, and 1060 cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra: see Tables 1 and 2. HR-EI-MS *m*/*z*: 498.2982 $[M]^+$ (Calcd for $C_{30}H_{42}O_6$, 498.2981).

7β,15α-Dihydroxy-3,11-dioxo-lanosta-8,24(*E*)-dien-26-oic Acid (2, Ganoderic Acid GS-2) Amorphous powder. $[\alpha]_D^{23}$ +112.9° (c=0.403, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 253 (3.8) nm. IR (KBr) ν_{max}: 3440 (OH), 2880, 1700 (carbonyl), 1660 (carboxyl), 1470, 1420, 1390, 1270, 1140, and 1060 cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra: see Tables 1 and 2. HR-EI-MS *m*/*z*: 500.3139 $[M]^+$ (Calcd for $C_{30}H_{44}O_6$, 500.3138).

12β-Acetoxy-3β,7β-dihydroxy-11,15-dioxo-lanosta-8,24(*E*)-dien-26-oic Acid (3, Ganoderic Acid GS-3) Amorphous powder. $[\alpha]_D^{23}$ +69.1° (c=0.857, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 253 (3.8) nm. IR (KBr) ν_{max}: 3440 (OH), 2880, 1730 (acetyl), 1700 (carbonyl), 1660 (carboxyl), 1450, 1380, 1230, 1170, 1040 and 755 cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra: see Tables 1 and 2. HR-EI-MS *m*/*z*: 558.3194 $[M]^+$ (Calcd for $C_{32}H_{48}O_8$, 558.3193).

3β,7β-Dihydroxy-11,15-dioxo-25,26,27-trinorlanosta-8,20(21)-dien-24-oic Acid [4, 20(21)-Dehydrolucidenic Acid N] Colorless needles. $[\alpha]_D^{23}$ +108.3° (c=0.411, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 255 (3.8) nm. IR (KBr) ν_{max}: 3450 (OH), 2880, 1730, 1700 (carbonyl), 1660 (carboxyl), 1460, 1380, 1230, 1170, 1040 and 900 cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra: see Tables 1 and 2. HR-EI-MS *m*/*z*: 458.2666 $[M]^+$ (Calcd for $C_{27}H_{38}O_6$, 458.2668).

7β,20ξ-Dihydroxy-3,11,15-trioxo-25,26,27-trinorlanost-8-en-24-oic Acid (5, 20-Hydroxylucidenic Acid A) Colorless needles. $[\alpha]_D^{23}$ +184.4° (c=0.373, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 254 (3.8) nm. IR (KBr) ν_{max}: 3480 (OH), 2880, 1770, 1700 (carbonyl), 1660, 1460, 1420, 1390, 1210, 1170, 1130 and 1070 cm^{-1}. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra: see Tables 1 and 2. HR-EI-MS *m*/*z*: 474.2616 $[M]^+$ (Calcd for $C_{27}H_{38}O_7$, 474.2618).

Chemicals The purity of *Ganoderma* triterpenes (**15**—**25**) isolated in our laboratory from the fruiting bodies of *G. lucidum* (provided by the Institute of Linzi Co., Ltd., Tokyo, Japan) determined by HPLC was >99%. A previously described procedure[14] was employed for the purity check, using a CCP 8020 system (Tosoh Co., Tokyo, Japan), a TSK gel ODS-80 Ts (Tosoh) column (150—4.6 mm i.d.), a mobile phase of 1% AcOH/H_2O–CH_3CN (0 min, 45 : 55; 40 min, 40 : 60, for ganoderma alcohols) and 2% AcOH/H_2O–CH_3CN (0 min, 75 : 25; 50 min, 70 : 30; 70 min, 60 : 40, for ganoderma acids). The flow rate was set at 1.0 ml/min with detecting wavelengths of 243 and 250 nm for ganoderma alcohols and acids, respectively.

HIV-1 Protease Inhibitory Assay We assayed anti-HIV-1 protease activity following the procedure described in previous papers.[23,26] Briefly, 10 μl of 50 mM NaOAc buffer containing 1 μg of substrate was mixed with 2 μl of test compounds (7 ganoderma alcohols and 15 ganoderma acids) in DMSO and then 8 μl of HIV-1 protease was added to the mixture. After incubation at 37 °C for 30 min, the reaction was terminated with 3 μl of 10% TFA. The hydrolysate and the remaining substrate were quantified by HPLC. The anti-HIV-1 protease activity of the compound was calculated as:

$$\% \text{ inhibition} = 100 \times (A_{control} - A_{sample})/A_{control}$$

where A is the relative peak area of the hydrolysate. Potency is expressed as 50% inhibitory concentration (IC_{50}).

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