Anti-HIV-1 Protease Activity of Lanostane Triterpenes from the Vietnamese Mushroom *Ganoderma colossum*

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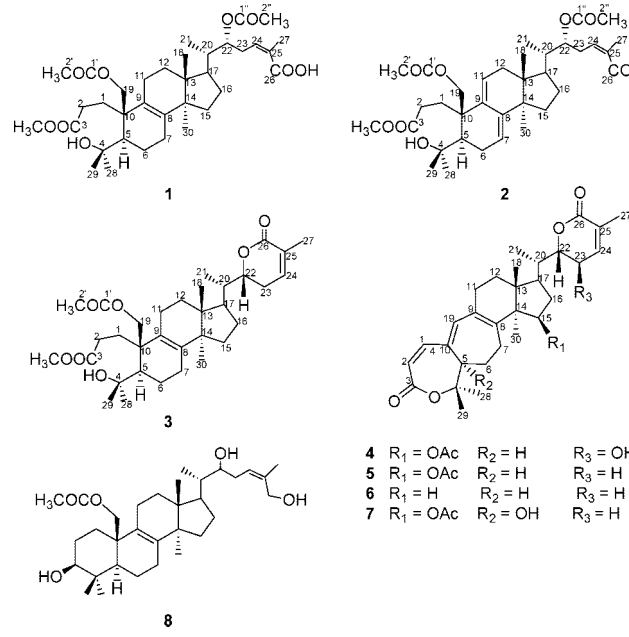
Received February 20, 2008

Four new lanostane triterpenes, colossolactone V (**1**), colossolactone VI (**2**), colossolactone VII (**3**), and colossolactone VIII (**4**), were isolated from the fruiting bodies of the Vietnamese mushroom *Ganoderma colossum*, together with the known compound colossolactone E (**5**). The structures of **1**−**4** were assigned on the basis of spectroscopic evidence, and their absolute configurations were determined by CD spectroscopy and the Mosher ester method. Compounds **1**−**5**, as well as two previously isolated compounds [schisanlactone A (**6**) and colossolactone G (**7**)] from the same mushroom, were evaluated for inhibition of HIV-1 protease, with IC_{50} values for the most potent compounds ranging from 5 to 13 μg/mL.

The human immunodeficiency virus type-1 (HIV-1), a member of the retrovirus family, is the causative organism for acquired immunodeficiency syndrome (AIDS). One of the important enzymes necessary for the replication of this virus is HIV-1 protease (HIV-1 PR), which belongs to the aspartyl protease class and functions as a dimer of 99 amino acids each. This enzyme cleaves the HIV gag and gag-pol polyprotein backbone at nine specific cleavage sites to produce shorter, functional proteins.[1] Drugs that inhibit protease and reverse transcriptase form the basis of highly active antiretroviral therapy (HAART), which has been successful in improving survival rates and quality of life for HIV-infected individuals.[2] The loss of sensitivity to protease inhibitors usually occurs because the resistant viral strains encode for protease molecules containing specific amino acid mutations that lower the affinity for the inhibitors yet maintain sufficient affinity for the substrate and sufficient catalytic efficiency.[2] The development of more powerful inhibitors with a lower susceptibility to mutations and reduced side effects remains a major task. Thus, searching for HIV-1 PR inhibitors from natural sources has become a promising approach. Triterpenes represent a structurally varied class of natural products existing in various plant species, and some naturally occurring triterpenes and their derivatives exhibit anti-HIV-1 activity.[3] The fruiting bodies of *G. colossum* were investigated previously for their triterpenoid content, and different colossolactones characterized by the presence of a six-membered lactone ring linked to ring D with or without a seven-membered lactone rings as A ring were isolated.[4] The fruiting bodies of this mushroom showed anti-inflammatory, cytotoxic,[4] and antimicrobial activities.[5,6] In this paper, the authors report the isolation of four new lanostane triterpenes (**1**−**4**) from the Vietnamese mushroom *G. colossum* as well as the anti-HIV-1 protease activity of these isolated compounds and some structural analogues.



Results and Discussion

Repeated column chromatography of the chloroform extract of the fruiting bodies of *G. colossum* after defatting led to the isolation of four new lanostane triterpenes, called colossolactones V−VIII (**1**−**4**), in addition to one known compound, colossolactone E (**5**). The known compound was identified by comparison of the spectroscopic data with reported values.[4]

Colossolactone V (**1**) was isolated as yellowish-white, amorphous powder. The HRFABMS indicated the molecular fomula $C_{35}H_{54}O_9$, which was in agreement with the ^{1}H and ^{13}C NMR data. The IR spectrum showed absorption bands due to a hydroxyl group (3449 cm^{-1}) and an aliphatic ester (1735 cm^{-1}). The ^{1}H NMR spectrum (Table 1) displayed a total of six methyl signals, i.e., one secondary methyl at δ 0.96 (d, J = 6.0 Hz), four tertiary methyls at δ 0.72, 0.91, 1.20, and 1.31, and one olefinic methyl at δ 1.92. In addition to two deshielded methyl singlets at δ 2.00 and 2.04, an oxymethine at δ 5.06, two doublets at δ 4.25 and 4.41 (1H each, J = 11.7 Hz) corresponding to an oxymethylene group, a methoxy signal at δ 3.66 (s), and one trisubstituted olefinic proton at δ 5.96 were observed. The ^{13}C NMR spectrum displayed 34 carbon signals, due to eight methyls, four methines (including one oxymethine at δ 75.6), 11 quaternary carbons (including four carbonyls at δ 175.6, 172.4, 170.9, and 170.6), and four olefinic carbons at δ 143.1, 141.0, 128.2, and 126.1. The long-range ^{1}H−^{13}C NMR correlations (Figure 1) indicated that **1** has the same ring B−D systems as colossolactone A (**8**), a known compound reported from the same mushroom.[4] The downfield shift of H-1 and H-2, the disappearance of the oxymethine at δ 3.20, and the presence of another hydroxyl group indicated structural differences in the A ring of **1** from that of **8**. The HMBC correlations indicated that two methyls were attached to an oxygenated carbon (C-4), since it showed correlations among the deshielded methyl signals at δ 1.31 (Me-28) and 1.20 (Me-29), an oxygenated quaternary carbon atom at δ 75.2 (C-4), and

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10.1021/np8001139 CCC: $40.75
Published on Web 06/12/2008

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3** ($CDCl_3$)

	1		**2**		**3**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	2.16, m (a), 1.61, m (b)	28.8	2.17, m, 1.70, m	28.8	2.14, m, 1.60, m	29.7
2	2.58, m (a), 2.26, m (b)	27.7	2.48, m, 1.80, m	25.5	2.54, m, 2.19, m	28.3
3		175.6		174.5		175.5
4		75.2		75.0		74.8
5	1.63, dd (12.0, 4.4)	47.9	1.90, m	48.0	1.39, m	47.8
6	1.00, 1.37, m	24.3	2.4, 2.18, m	21.5	1.81, m	24.8
7	1.42, m	24.3	5.24, br s	117.9	1.78, m	26.0
8		143.1		134.1		139.6
9		126.1		141.1		126.1
10		45.4		42.2		45.7
11	2.10, m	22.6	5.29, br s	120.4	1.79, m	22.5
12	1.8, 1.66, m	31.2	2.13, m	39.4	1.90, m	31.2
13		51.4		43.7		44.1
14		51.8		49.9		51.4
15	1.17, 1.50, m	30.5	1.68, m	31.5	1.62, m	30.8
16	1.49, 2.03, m	28.5	1.30, 2.03, m	26.0	1.23, 2.08, m	26.6
17	1.56, m	46.9	1.57, m	47.5	1.61, m	47.8
18	0.72, s	15.7	0.61,s	16.0	0.73, s	15.8
19	4.41, d (11.7)	67.2	4.53, d (11.1)	61.6	4.39, d (11.2)	67.2
	4.25, d (11.7)		4.31, d (11.1)		4.17, d (11.2)	
20	1.45, m	40.1	1.50, m	40.0	1.55, m	40.2
21	0.96 d (6.0)	12.9	0.95, d (6.6)	12.6	0.97, d (6.8)	13.3
22	5.06, t (13.5, 4.0)	75.6	5.03, t (13.5, 4.0)	75.5	4.43, dd (13.6, 2.8)	80.1
23	2.80, m	33.1	2.75, m	33.1	2.40, m	27.3
24	5.96, m	141.0	5.93, m	140.9	6.55, m	142.9
25		128.2		128.1		128.0
26		172.4		171.7		166.3
27	1.92, s	20.6	1.86, s	20.5	1.85, s	17.0
28	1.31, s	33.7	1.28, s	33.5	1.30, s	28.7
29	1.20, s	26.1	1.20, s	25.8	1.20, s	23.8
30	0.91,s	24.3	0.82, s	24.3	0.96, s	22.5
1′		170.6		170.5		170.4
2′	2.00, s	20.7	2.00, s	21.1	1.98, s	20.7
1″		170.9		170.8		
2″	2.04, s	21.1	2.04, s	21.2		
OCH_3	3.66, s	51.8	3.66, s	51.8	3.66, s	51.4

the tertiary carbon δ 47.9 (C-5). Furthermore, the proton signals of H-1 and H-2 exhibited correlations with the carbonyl ester at δ 175.6 (C-3) and two quaternary carbons, an olefinic carbon at δ 126.0 (C-9) for H-1 only, and a sp^3 carbon at δ 45.4 (C-10). Further coupling between the carbonyl at δ 175.6 (C-3) and the methoxyl protons at δ 3.66 confirmed the presence of a methyl ester moiety attached to C-2, suggesting that compound **1** is a derivative of a 3,4-seco-lanostene triterpenoid.[7−10] The presence of a free carboxylic group at C-26 was deduced from the correlation with the olefinic H-24 and methyl H-27 protons. The ^{1}H and ^{13}C NMR spectroscopic data confirmed the presence of two acetyl groups due to the two methyl group signals at δ 2.00 (δ 20.7) and 2.04 (δ 21.1). The positions of the two acetyl groups were assigned at C-19 and C-22 due to the HMBC correlations of H-19 (δ 4.25 and 4.41, each d, J = 11.7 Hz) with C-1′ (δ 170.6) and H-22 (δ 5.06, t) with C-1″ (δ 170.9). The partial structure from C-6 to C-12 (except C-8 and C-9) was deduced from COSY correlations of H-6/H-7 and H-11/H-12. In addition, the connectivities of C-15, C-16, and C-17 were confirmed by the COSY correlations of H-15/H-16 and H-16/H-17. The geometry of the double bond at C-24 of **1** was determined by selective NOE experiments. Irradiation of H-27 increased the intensity of H-24, which indicated that the double-bond geometry is in the *Z* configuration. The absolute configuration of the chiral center in **1** at C-22 was established using Mosher ester methodology after deacetylation of **1**.[10] The Mosher esters of the deacetylated compound **1a** indicated the *R* configuration at C-22 due to the negative difference values for H-23, H-24, and H-27 and the positive differences for H-20 (Table 3). Consequently, the absolute configuration of the stereogenic center of **1** could be deduced.[8] Therefore, the structure of compound **1** was assigned as (22*R*)-3,4-seco-19,22-diacetoxy-4-hydroxylanosta-8,24(*Z*)-dien-3,26-dioic acid 3-methyl ester.

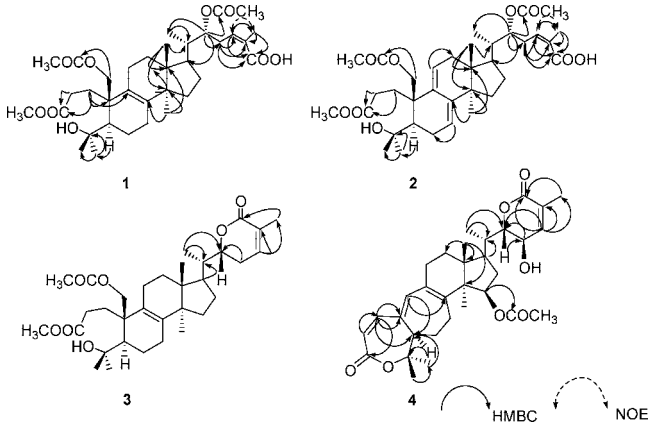


Figure 1. HMBC and NOE correlations of compounds **1**−**4**.

Colossolactone VI (**2**) was obtained as a white, amorphous powder, and its spectroscopic data were very similar to those of **1**. Detailed comparison of their 1D NMR data indicated that the differences between these compounds are in the positions and the number of double bonds. The molecular formula ($C_{35}H_{52}O_9$) of **2** was obtained by HRFABMS. The ^{1}H and ^{13}C NMR spectra showed similar signals to those in compound **1**, with two broad signals integrating as one proton each, at δ 5.24 and 5.29 (δ 117.9 and 120.4), corresponding to H-7 and H-11, respectively. The positions of the functional groups including the two additional olefinic carbons were confirmed by the HMBC experiment (Figure 1). The NOE correlations confirmed the *Z* configuration of C-24 and C-25 as the same as those in compound **1**. The absolute stereochemistry

Table 2. ^{1}H and ^{13}C NMR Data of Compound **4** ($CDCl_3$)

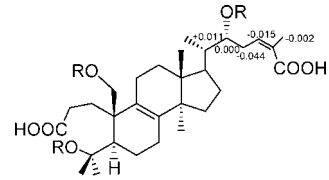
position	δ_H (J in Hz)	δ_C
1	6.68, d (12.4)	143.8
2	5.83, d (12.4)	118.0
3		167.0
4		77.8
5	2.60, m	48.9
6	2.40, m	35.8
7	2.08, m	27.1
8		146.0
9		147.0
10		142.8
11	1.97,m	26.3
12	1.78, m	31.1
13		44.3
14		54.9
15	4.88, dd (6.8, 1.3)	77.8
16	2.00, 2.70, m	38.5
17	2.28, m	44.8
18	1.03, s	16.7
19	6.23,s	142.8
20	2.03, m	35.8
21	1.06, d (5.6)	12.8
22	4.12, d (10.4)	84.0
23	4.45, dd (10.4, 1.2)	63.6
24	6.50, m	143.8
25		127.7
26		164.0
27	1.93, s	16.8
28	1.40, s	28.5
29	1.54, s	26.6
30	1.14, s	26.6
C=O (15)		170.4
CH_3 (15)	1.96,s	21.4

Table 3. Partial ^{1}H NMR Data of the (*S*)- and (*R*)-Mosher Ester Derivatives of Compound **1** in $CDCl_3$

	δ_H		
position	**1s**	**1r**	$\Delta\delta_{S-R}$
20	1.495	1.484	+0.011
23	2.529	2.573	−0.044
24	6.613	6.628	−0.015
27	1.917	1.919	−0.002

of **2** at C-22 was determined by comparing its CD spectrum with that of compound **1**,[10] showing a positive Cotton effect at 250 nm ($\Delta\epsilon$ +0.280, +0.238, respectively; $CHCl_3$), and its structure was assigned as (22*R*)-3,4-seco-19,22-diacetoxy-4-hydroxylanosta-7,9(11),24(*Z*)-trien-3,26-dioic acid 3-methyl ester.

Colossolactone VII (**3**) was obtained as colorless, cubic crystals. The ^{1}H and ^{13}C NMR data of this compound showed many similarities to those of compound **1** in the 3,4-seco-lanostane portion but displayed some differences in the aliphatic side chain. The ^{1}H and ^{13}C NMR spectra of compound **3** (Table 1) also showed nearly the same profiles in rings A−D as those of **1**, indicating that **3** has the same 3,4-seco-lanostane methyl ester backbone. Additionally, signals characteristic for the presence of a δ-lactone ring linked to C-20 as well as the absence of an acetyl group attached to C-22 were observed. The UV absorbance at 245 nm indicated the presence of an α,β-unsaturated lactone,[11] and the IR spectrum showed the occurrence of a hydroxyl group at 3470 cm^{-1} and one conjugated δ-lactone at 1736 cm^{-1}. Diagnostic ^{1}H NMR assignments at δ 4.43 (H-22), 6.55 (H-24), and 1.85 (H-27) as well as the ^{13}C NMR assignments at δ 166.3 (C-26) and 80.1 (C-22) indicated that compound **3** contains a methyl group in the α-position to the α,β-unsaturated δ-lactone ring. HMBC correlations confirmed this partial structure and indicated C_δ of the lactone ring to be linked at C-20.[4,12] The ^{1}H−^{1}H COSY spectrum confirmed this from the following correlations: H-23 (δ 2.40) with H-22 (δ 4.43) and H-24 (δ 6.55); H_3-27 (δ 1.85) with H-24 (δ 6.55); H_3-21 (δ 0.97, d, J = 6.8 Hz) with H-20 (δ 1.55); and H-20 (δ 1.55) with H-22 (δ 4.43).



1a R = **H**
1s R = **(*S*)-MTPA**
1r R = **(*R*)-MTPA**

Figure 2. $\Delta\delta = (\delta_S - \delta_R)$ values obtained from the MTPA esters of **1a** in $CDCl_3$ at 27 °C.

Table 4. IC_{50} Values of the Isolated Compounds against HIV-1 Protease

compound	IC_{50}[a]
1	9
2	>100
3	13.8
4	31.4
6	8
7	5
8	39
pepstatinA[b]	1×10^{-3}

[a] IC_{50} values in μg/mL. [b] Positive control.

In the HMBC spectrum, the H_3-27 protons were correlated with C-24 (δ 143.0), C-25 (δ 128.0), and C-26 (δ 166.4), the H_3-21 protons were correlated with C-17 (δ 47.8) and C-20 (δ 40.2), and the H-22 protons were correlated with C-20 (δ 40.2) and C-21 (δ 13.3). According to the COSY and HMQC correlations, all protons and carbons were finally assigned as shown in Table 1. The relative configurations of H-20 and H-18 were found to be β and those of H-5 and H-30 α by comparison with reported data.[12] For the determination of the absolute configuration at C-22 in compound **3**, a CD measurement was carried out. Since a strong negative Cotton effect at 258 nm ($\Delta\epsilon$ −4.1; $CHCl_3$) was observed, the absolute configuration at C-22 in **3** was consequently assigned with the *S* configuration.[12] Accordingly, compound **3** was determined as (22*S*)-3,4-seco-19-acetoxy-4-hydroxylanosta-8,24-dien-26,22-olide 3-methyl ester.

Colossolactone VIII (**4**) was obtained as a yellow, amorphous powder. A molecular formula of $C_{34}H_{46}O_6$, was determined from the HRFABMS. The UV spectrum showed λ_{max} at 245 and 332 nm, and the IR spectrum absorptions for two carbonyl groups at 1658 and 1724 cm^{-1}. The ^{1}H and ^{13}C NMR spectra (Table 2) showed nearly the same profiles as those of the known compound colossolactone E (**6**).[4] Additional signals at δ 4.45 (dd, J = 10.4, 1.2 Hz) and 63.6 were assigned to C-23. This was supported by the shift of the adjacent proton H-22 at δ 4.12 (dd, J = 10.4 Hz). A hydroxyl group was located at C-23, as the carbon and the attached proton were both shifted to lower field [δ 63.6 and 4.45 dd (J = 10.4, 1.2 Hz)]. The β-orientation of the hydroxyl group at C-23 was deduced from the multiplicities of H-23 (δ 4.45, dd, J = 10.4, 1.2 Hz) and H-24 at δ 6.50 (brs). The absolute configuration at C-22 was assigned as *S* by measuring the CD curve, which showed a strong negative Cotton effect at 273 nm ($\Delta\epsilon$ −0.398, $CHCl_3$).[12] Accordingly, compound **4** was established as (22*S*, 23*R*)-A,B-dihomo-19-nor-15-β-acetoxy-23-hydroxy-4-oxa-3-oxolanosta-1,8,19,24-tetraen-26,22-olide.

Compounds **1**−**5** as well as the previously isolated compounds **6** and **7** from the same mushroom[13] were tested for their inhibitory activity against HIV-1 protease. Schisanlactone A (**6**), colossolactone E (**5**), colossolactone V (**1**), and colossolactone VII (**3**) showed inhibitory activity against HIV-1 protease with IC_{50} values of 5.0, 8.0, 9.0, and 13.8 μg/mL, respectively (Table 4). Concerning the compounds containing seven-membered and six-membered lactone rings in rings A and E, respectively, the presence of a hydroxyl

group at C-23 or C-5 in the above-mentioned compounds markedly reduced the activity (i.e., **4** vs **5** and **7** vs **6**), suggesting that the hydrophobicity of the triterpene core in these compounds may play a significant role in mediating the anti-HIV-1 protease inhibitory activity. For the seco compounds (i.e., compounds with an opened ring A), colossolactone V (**1**), with unsaturation at C-8, exhibited higher activity than the related compound, colossolactone VI (**2**), with an unsaturation at C-7, C-8 and C-9, C-11. The significant influence of the unsaturation pattern on the activity may be due to the double bond(s) that alter the three-dimensional structures of compounds and consequently the spatial arrangement of pharmacophores in the structures. The presence of a lactone ring attached to ring D (compound **3**) did not markedly reduce the activity when compared to the compound with a side chain connected to ring D (compound **1**) (Table 4).

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto micro hot stage melting point apparatus. Optical rotations were measured with a DIP-360 automatic polarimeter (Jasco, Tokyo, Japan). UV spectra were measured with a UV 2200/UV−vis recording spectrophotometer (Shimadzu, Kyoto, Japan). CD spectra were recorded in $CHCl_3$ on a Jasco J-805 spectrometer. IR spectra were measured with a Fourier transform (FT)/Jasco IR-460 infrared spectrometer. ^1H NMR and ^{13}C NMR spectra were measured with JEOL JNA-LAA 400WB-FT (^1H, 400 MHz; ^{13}C, 100 MHz) NMR spectrophotometer. HRFABMS was measured with a JEOL JMX-AX 505 HAD mass spectrometer at an ionization voltage of 70 eV. ESIMS was carried out on an Esquire 3000 mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) system with ESI ionization source. Column chromatography was carried out on silica gel (Kieselgel 60, 70−230 mesh, Merck). Medium-pressure liquid chromatography (MPLC) was carried out on a LiChroprep Si 60 instrument (Merck, Darmstadt). Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates (0.25 mm, Merck) and RP-18 F_{254S} (0.25 mm, Merck), and spots were detected under a UV light and by spraying with *p*-anisaldehyde/H_2SO_4 followed by heating.

Fungal Material. The fruiting bodies of *Ganoderma colossum* were collected in July 2002 from Ho Chi Minh City and grown by Co Duc Trong and Phan Thi Nhieu at Linh Chi Vi Na Co., Ltd. in September 2003 in Vietnam and authenticated by Professor Co Duc Trong and Professor Leif Ryvarden. A voucher sample (TMPW 25804) has been preserved in the Museum for Materia Medica, Research Center for Ethnomedicine, Institute of Natural Medicine, University of Toyama, Toyama, Japan.

Enzymes and Chemicals. Rec HIV protease enzyme (expressed in *E. coli*) was purchased from Bachem Co., Japan. HIV substrate III [His-Lys-Ala-Arg-Val-Leu-(*P*-NO_2-Phe)-Glu-Ala-Nle-Ser-NH_2] and pepstatin A were purchased from Wako, Osaka, Japan.

Extraction and Isolation. The pulverized fruiting bodies of *G. colossum* (3.5 kg) were extracted with $CHCl_3$ (8 L × 4) at room temperature for 5 days. The combined extracts were filtered and concentrated to give a dark brown residue of 582 g. The chloroform extract was dissolved in MeOH (1 L) and defatted with hexane (2 L × 3). Then, the MeOH extract was chromatographed on silica gel (2 kg) with hexane−acetone mixtures (9:1 → 1:1). Fractions (200 mL each) were collected, and their homogeneity was monitored by TLC with solvent systems of increasing polarity (hexane−acetone, 9:1, 4:1, 7:3, and 1:1). The spots were visualized after spraying with *p*-anisaldehyde followed by heating. Those showing similar TLC profiles were combined to give eight pools (I−VIII). Pool VI (fractions 7.23 g) was further subjected to silica gel column chromatography (40 cm × 4 cm) eluted with a $CHCl_3$−CH_3OH mixture (9.9:0.1) to afford four subfractions, A1−A4. Subfraction A1 (574 mg) was chromatographed on a silica gel column (20 cm × 2.2 cm), eluting with hexane−acetone (8:2), to yield subfraction A1-1, which was further purified by using a MPLC silica gel 60 column (24 cm × 1 cm) with hexane−acetone (9:1) to yield compound **3** (64 mg). Fraction A1-2 was chromatographed over a silica gel column (20 cm × 1.5 cm) and yielded two subfractions, B1 and B2. Subfraction B1 was subjected to a silica gel column (20 cm × 1.5 cm) eluted with hexane−acetone (8.5:1.5) to yield compound **6** (38.5 mg). Subfraction B2 was finally purified by preparative HPLC with 60% MeOH in water containing 0.1% TFA as the mobile phase, to yield compound **4** (7 mg). Subfraction A2 (2.1 g) was subjected to silica gel column chromatography (35 cm × 2.5 cm) with increasing solvent polarity (hexane−acetone, 8:2 and 7.5:2.5). The fraction from 12 to 25 contained a major spot, which was rechromatographed on a silica gel column (20 cm × 2.1 cm) using hexane−acetone to afford compound **1** (380 mg). On the other hand, fraction 26−49 was separated by MPLC on a silica gel 60 column (24 cm × 1 cm) using a hexane−acetone mixture (8:2), affording compound **2** (17.3 mg).

Colossolactone V (1): yellowish-white, amorphous powder; $[\alpha]_D^{23}$ +60 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 243 (5.17) nm; CD $\Delta\epsilon_{250}$ + 0.238 ($CHCl_3$); IR (KBr) ν_{max} 3449, 2924, 2345, 1735, 1457, 1375, 1242, 1051 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 641.6 $[M + Na]^+$, 601 $[M + H - H_2O]^+$, 541 $[M - H_2O - OCOCH_3]^+$; positive HRFABMS *m/z* 641.36642 (calcd for $C_{30}H_{42}O_9$ Na, 641.36652).

Colossolactone VI (2): yellowish-white, amorphous powder; $[\alpha]_D^{23}$ +11.3 (*c* 0.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 246 (5.22) nm; CD $\Delta\epsilon_{250}$ +0.280 ($CHCl_3$); IR (KBr) ν_{max} 3434, 2926, 1734, 1382, 1245 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 639 $[M + Na]^+$, 598 $[M - H_2O]^+$, 539 $[M - H_2O - OCOCH_3]^+$; positive HRFABMS *m/z* 639.35091 (calcd for $C_{35}H_{52}O_9Na$, 639.35087).

Colossolactone VII (3): white, cubic crystals ($CHCl_3$); mp 165 °C; $[\alpha]_D^{23}$ +113 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 245 (5.91) nm; CD $\Delta\epsilon_{258}$ −4.1 ($CHCl_3$); IR (KBr) ν_{max} 3470, 2950, 1736, 1375, 1241, 1136, 1049 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 581 $[M + Na]^+$, 541 $[M + H - H_2O]^+$, 499 $[M + 2H - H_2O - COCH_3]^+$, 481 $[M + 2H - H_2O - COCH_3]^+$; positive HRFABMS *m/z* 581.34576 (calcd for $C_{33}H_{50}O_7Na$, 581.34542).

Colossolactone VIII (4): yellowish-white, amorphous powder, $[\alpha]_D^{23}$ +136 (*c* 0.5, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 245 (5.79), 332 (5.93) nm; CD $\Delta\epsilon_{273}$ −0.398, $\Delta\epsilon_{353}$ +0.928 ($CHCl_3$); IR (KBr) ν_{max} 3449, 2911, 1724, 1658, 1382, 1260, 1136 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) data, see Table 2; ESIMS *m/z* 539 $[M + H]^+$, 479 $[M + 2H - H_2O - COCH_3]^+$, 461 $[M + 2H - 2H_2O - COCH_3]^+$; positive HRFABMS *m/z* 539.30266 (calcd for $C_{35}H_{52}O_9$ +1, 539.30088).

Preparation of (*S*)- and (*R*)-MTPA Ester Derivatives of 1. Compound **1** (50 mg) was treated with 10 mL of 1 N NaOH for 1.5 h at room temperature followed by neutralization with 1 N HCl and extraction with EtOAc (20 mL × 3). The organic layer was washed with water and evaporated to yield a residue of 40 mg, which was purified by passage over a silica gel column (25 × 1 cm) eluted with $CHCl_3$−CH_3OH (95%), to yield a hydrolyzed product, **1a** (30 mg). To **1a** (6 mg in 0.5 mL of $CHCl_3$) were added sequentially pyridine (100 μL), 4-dimethylaminopyridine (0.5 mg), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 mg). The mixture was stirred for 4 h at room temperature and then passed through a disposable pipet (0.6 × 5 cm) packed with silica gel and eluted with 5 mL of $CHCl_3$. The solvent was removed in vacuo to obtain the *S*-Mosher ester, **1s**, as a white gum (5.8 mg, yield 69%). Treatment of **1** (6 mg) with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, in a similar manner, yielded the *R*-Mosher ester, **1r**, as a colorless oil (5.6 mg, yield 66%).

Assay for HIV-1 Protease Inhibitory Activity. HIV-protease assay buffer (10 μL; 50 mM NaOAc, pH 4.9) containing the substrate (0.1 mg/mL) was mixed with 2 μL of a compound solution (using DMSO as a solvent); then 8 μL of rec HIV-protease (0.02 mg/mL) was added. The reaction mixture was incubated for 30 min at 37 °C and then terminated by addition of 3 μL of 10% trifluoroacetic acid (TFA). The hydrolysates [*P*-NO_2-Phe-Glu-Ala-Nle-Ser-NH_2] and [His-Lys-Ala-Arg-Val-Leu] and the remaining substrate were quantitatively analyzed by reversed-phase HPLC.[14] HPLC conditions: column, TSK gel ODS-80Ts column (4.6 × 150 mm, Tosoh Co., Tokyo, Japan; solvent, gradient of acetonitrile (20−40%) in 0.1% TFA in water; flow rate, 1.0 mL/min; detector, UV 280 nm. The substrate was eluted at 9.5 min, while the hydrolysates were eluted at 6.3 and 7 min.

The HIV-protease inhibitory activity of a compound was calculated as follows:

$$\%\text{inhibition} = (A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}} \quad (1)$$

A is the relative peak area of the hydrolysates. Pepstatin A was used as a positive control, with its IC_{50} being 0.001 μg/mL. The IC_{50} values were calculated using the Microsoft Excel program.

Acknowledgment. The authors are grateful to Mr. N. Ide (Reishi-Sogo Kenkyusho Co., Tokyo) for providing the fruiting bodies of *Ganoderma colossum* cultivated in Vietnam.

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NP8001139