Triterpenes from the Spores of *Ganoderma lucidum* and Their Cytotoxicity against Meth-A and LLC Tumor Cells

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Received January 28, 2000; accepted March 16, 2000

Six new highly oxygenated lanostane-type triterpenes, called ganoderic acid γ (1), ganoderic acid δ (2), ganoderic acid ε (3), ganoderic acid ζ (4), ganoderic acid η (5) and ganoderic acid θ (6), were isolated from the spores of *Ganoderma lucidum*, together with known ganolucidic acid D (7) and ganoderic acid C2 (8). Their structures of the new triterpenes were determined as (23*S*)-7β,15α,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid (1), (23*S*)-7α,15α,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid (2), (23*S*)-3β,7β,23-trihydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid (3), (23*S*)-3β,23-dihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid (4), (23*S*)-3β,7β,12β,23-tetrahydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid (5) and (23*S*)-3β,12β,23-trihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid (6), respectively, by chemical and spectroscopic means, which included the determination of a chiral center in the side chain by a modification of Mosher's method. The cytotoxicity of the compounds isolated from the *Ganoderma* spores was carried out *in vitro* against Meth-A and LLC tumor cell lines.

Key words ganoderic acids γ—θ; lanostane-type triterpene; *Ganoderma lucidum*; spore; cytotoxicity

The fruiting body of *Ganoderma* (*G.*) *lucidum* (Reishi in Japanese) is a well known Chinese crude drug which has been used clinically in East Asia and gives much attention as a home remedy. Over one hundred highly oxygenated and pharmacologically active lanostane-type triterpenoids have been isolated from the fruiting bodies and the mycelium of *G. lucidum*.[1—24] Some of them have been shown to have cytotoxicity against hepatoma cells *in vitro* (ganoderic acids U, V, W, X and Y),[25] anti-histamine releasing activity in rat mast cells (ganoderic acids C and D),[26] inhibitory activity against angiotensin converting enzyme (ganoderic acid F),[27] hepatoprotective activity (ganoderic acid A),[28] and an inhibitory effect on farnesyl protein transferase (ganoderic acid A and methyl ganoderate A).[29] Earlier and more recently, we reported the isolation of new triterpenoids, ganoderic acids α and β, and lucidumols A and B, with several known compounds from the spores and fruiting bodies of this mushroom.[30,31] Of the compounds isolated, ganoderiol F and ganodermanontriol were found to have anti-human immunodeficiency virus (anti-HIV-1) activity, and ganoderic acid β, lucidumol B, and ganolucidic acid A showed an inhibitory effect on HIV-1 protease.

As part of our continuing research to find pharmacologically active constituents from *G. lucidum*, we have isolated six new lanostane-type triterpenes, called ganoderic acids γ (**1**), δ (**2**), ε (**3**), ζ (**4**), η (**5**) and θ (**6**), from a chloroform-soluble fraction of the spores of *G. lucidum* and examined their cytotoxicity against Meth-A (sarcoma) and LLC (Lewis lung carcinoma) mouse tumor cell lines. In this paper, we describe the characterization of these new lanostane-type triterpenes (**1**—**6**), as well as cytotoxic activity of triterpenes isolated from the spores of this mushroom.

Results and Discussion

Silica gel column chromatography (CC) and prep. HPLC with an ODS-80T$_S$ column of a $CHCl_3$-soluble fraction of the MeOH extract of spores of *G. lucidum* resulted in the isolation of eight triterpenes (**1**—**8**). The structures of known compounds were identified as ganolucidic acid D (**7**)[10] and ganoderic acid C2 (**8**),[13] which had previously been isolated from the same mushroom, by comparison with the reported data.

Ganoderic acid γ (**1**) was obtained as colorless needles (MeOH–H_2O), mp 243—245 °C, with a positively optical rotation ($[\alpha]_D$ +156°). The ultraviolet (UV) absorbance at 252 nm (log ε 3.82) and infrared (IR) band at 1660 cm^{-1} suggested the presence of a conjugated carbonyl group. The high-resolution electron impact mass (HR-EIMS) spectrum revealed the molecular formula of **1** to be $C_{30}H_{44}O_7$.

The ^{1}H-NMR spectrum showed signals for seven methyls including a doublet at δ 0.96 (J=6.3 Hz) and an allylic methyl at δ 1.88 (J=1.5 Hz), three oxymethylenes at δ 4.53 (dt, J=9.2, 5.1 Hz), 4.59 (dd, J=9.8, 6.6 Hz) and 4.70 (dd, J=9.2, 6.3 Hz), and an olefinic methine at δ 6.61 (dd, J=9.2, 1.5 Hz) (Table 1). The ^{13}C-NMR, in combination with distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected multiple quantum coherence (HMQC) experiments, showed signals for seven methyls, six methylenes, seven methines (including three oxymethylenes at δ 65.9, 68.2 and 71.6, and an sp^2 methine at δ 143.2), seven quaternary carbons (including three sp^2 carbons at δ 128.4, 139.6 and 160.4), and three carbonyls at δ 170.2, 200.3 and 218.6 (Table 2). On the basis of spectroscopic evidence by ^{1}H–^{1}H-correlation spectroscopy (COSY) and HMQC experiments, all protons and carbons were assigned as shown in Tables 1 and 2, respectively. These ^{1}H- and ^{13}C-NMR spectral data indicated a highly oxygenated lanostane-type triterpene close to the structure of ganoderic acid A (**9**),[1] isolated from the same mushroom, except for those of C-22 to C-27. The higher-field shifts of C-22, C-23, C-26 and C-27 by 6.8, 142.5, 6.3 and 4.3 ppm, respectively, and the lower-field shifts of C-24 and C-25 by 96.4 and 93.8 ppm, respectively, compared with those of **9** suggested that an allylic alcohol group may be located at C-23—25 in the side chain. The presence of the allylic alcohol group in the side chain of **1** supported by prominent fragment ions at m/z 401 $[e]^+$, 359 $[a]^+$ and 157 $[b]^+$ corresponding to a loss of the side chain molecule in the EIMS spectrum (Fig. 1). The fragmentation

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Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**7** (400 MHz, in $CDCl_3$+CD_3OD)

^{1}H	**1**	**2**	**3**	**4**
1-H (α)	1.53 dt (13.5, 7.0)	1.70 td (13.9, 4.6)	0.98 m	1.22 m
1-H (β)	2.81 ddd (13.5, 8.3, 5.9)	2.94 ddd (13.9, 8.2, 5.9)	2.78 dt (9.9, 3.5)	2.82 m
2-H (α)	2.45 dd (15.5, 7.0)	2.45 m	1.68 m	1.71 m
2-H (β)	2.53 ddd (15.5, 9.2, 5.9)	2.62 ddd (15.5, 9.6, 5.9)		
3-H (α)			3.16 dd (11.2, 5.0)	3.23 dd (10.8, 6.3)
5-H	1.72 m	2.09 dd (12.8, 2.4)	0.91 m	1.59 m
6-H (α)	2.03 m	1.74 m	2.19 m	2.62 d (15.5)
6-H (β)	1.71 m		1.55 m	2.56 dd (15.5, 3.0)
7-H (α)	4.59 dd (9.8, 6.6)		4.84 t (9.4)	
7-H (β)		4.56 br d (3.4)		
12-H (α)	2.79 d (15.7)	2.77 d (17.9)	2.90 d (16.7)	2.90 d (16.0)
12-H (β)	2.49 d (15.7)	2.40 d (17.9)	2.66 d (16.7)	2.69 d (16.0)
15-H (β)	4.70 dd (9.2, 6.3)	4.53 br t (5.1)		
16-H (α)	1.77 m	1.90 m	2.83 m	2.84 m
16-H (β)			2.07 dd (19.3, 9.7)	2.80 m
17-H	1.87 m	1.92 m	2.18 m	2.22 m
18-H_3	0.94 s	0.82 s	0.95 s	0.81 s
19-H_3	1.26 s	1.02 s	1.22 s	1.27 s
20-H	1.26 m	1.31 m	1.60 m	1.68 m
21-H_3	0.96 d (6.3)	0.91 d (6.5)	1.07 d (6.1)	1.04 d (6.2)
22-H	1.45 m	1.48 m	1.55 m	1.50 m
22-H	1.62 m	1.58 br t (11.1)		
23-H	4.53 td (9.2, 5.1)	4.51 m	4.56 m	4.53 dd (13.2, 9.2)
24-H	6.61 dd (9.2, 1.5)	6.53 br d (8.7)	6.59 dd (9.2, 1.5)	6.57 br d (9.2)
27-H_3	1.88 d (1.5)	1.87 br s	1.88 d (1.5)	1.87 br s
28-H_3	1.13 s	1.16 s	1.03 s	1.02 s
29-H_3	1.11 s	1.08 s	0.84 s	0.88 s
30-H_3	1.26 s	1.28 s	1.38 s	1.54 s

^{1}H	**5**	**6**	**7**
1-H (α)	0.92 m	1.10 m	1.55 m
1-H (β)	2.57 dt (13.3, 3.9)	2.72 m	2.97 ddd (13.8, 8.3, 5.6)
2-H (α)	1.65 m	1.73 m	2.56 m
2-H (β)			
3-H (α)	3.19 dd (8.9, 7.3)	3.22 dd (8.9, 7.2)	
5-H	0.89 m	1.55 dd (14.5, 2.2)	
6-H (α)	2.25 m	2.72 m	
6-H (β)	1.64 m	2.57 dd (14.5, 2.2)	
7-H (α)	4.76 t (8.7)		
7-H (β)			
12-H (α)	4.40 s	4.51 s	2.73 d (17.2)
12-H (β)			2.41 d (17.2)
15-H (β)			4.31 dd (8.4, 5.6)
16-H (α)	2.68 dd (19.6, 8.7)	2.75 dd (18.0, 9.8)	2.44 ddd (16.6, 8.5, 6.8)
16-H (β)	2.21 dd (19.6, 9.8)	2.63 m	1.69
17-H	2.50 dd (8.7, 4.0)	1.99 dd (18.0, 8.2)	1.72 m
18-H_3	0.75 s	0.62 s	0.85 s
19-H_3	1.29 s	1.37 s	1.01 s
20-H	2.00 m	1.78 m	1.26 s
21-H_3	1.16 d (6.8)	1.15 d (6.5)	0.92 d (6.3)
22-H	1.66 m	1.46 m	1.85 m
22-H		1.62 m	
23-H	4.56 ddd (12.6, 8.9, 5.1)	4.54 ddd (13.8, 8.9, 4.8)	4.51 m
24-H	6.57 br d (8.9)	6.52 br d (8.9)	6.54 d (8.9)
27-H_3	1.88 br s	1.87 br s	1.88 br s
28-H_3	1.02 s	1.05 s	1.08 s
29-H_3	0.86 s	0.89 s	1.13 s
30-H_3	1.43 s	1.68 s	1.18 s

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

ions *m/z* 498 $[M-H_2O]^+$, 480 $[M-2H_2O]^+$ and 462 $[M-3H_2O]^+$, due to the successive losses of 18 mass units, indicated the presence of three hydroxyl groups.

The connectivities of **1** were established by interpretation of the significant heteronuclear multiple bond correlation (HMBC) spectrum. Correlations among the signals H-5 and C-3/C-7/C-9; H_3-28/H_3-29 and C-3; H_2-6 and C-8; H_3-19 and C-9; and H_2-12 and C-11, confirmed that the positions of ke-

tone and hydroxyl, and α,β-unsaturated carbonyl groups were at C-3 and C-7, and C-8, C-9 and C-11, respectively (Fig. 2). Long-range correlations between H-15/C-17 and H_3-30/C-15 indicated the presence of another hydroxyl group at C-15. On the other hand, the connectivity of an allylic alcohol group in the side chain moiety at C-23, C-24 and C-25 as shown in formula **1** was revealed by the ^1H–^1H correlations between H_2-22 and H-23; H-23 and H-24; and H-24 and H_3-27. This was further supported by long-range correlations between H-20 and C-23; H_2-22 and C-24; H-23 and C-25; and H-24 and C-26/C-27 in the HMBC spectrum.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**7** (100 MHz, in $CDCl_3+CD_3OD$)

C	1	2	3	4	5	6	7
1	35.2	34.9	35.7	33.6	34.4	33.2	34.9
2	33.9	34.3	28.0	26.9	27.1	26.7	34.0
3	218.6	219.5	78.7	77.2	78.0	76.8	219.6
4	46.4	46.6	39.7	39.0	38.4	40.0	46.8
5	48.3	45.3	50.0	50.8	49.0	51.2	51.3
6	28.1	27.7	27.6	36.2	26.5	36.4	18.4
7	68.2	66.6	67.7	199.9	66.2	199.5	29.3
8	160.4	160.9	158.4	148.6	156.6	146.2	165.1
9	139.6	139.7	143.9	151.8	142.0	151.0	137.9
10	37.6	37.9	39.4	40.4	38.1	38.8	36.8
11	200.3	200.1	200.1	200.1	199.5	201.4	199.1
12	51.5	52.0	51.2	49.6	78.2	77.6	51.6
13	46.3	47.0	46.4	44.2	51.7	49.5	46.4
14	53.7	53.4	60.3	57.0	60.1	57.5	53.3
15	71.6	71.7	218.5	209.0	217.4	207.6	72.0
16	35.6	37.5	42.0	45.6	36.8	36.7	38.1
17	48.5	49.7	47.2	42.7	46.2	45.5	49.0
18	16.6	17.3	17.6	15.9	11.9	10.6	16.7
19	18.9	17.5	18.8	17.6	18.6	17.6	18.6
20	33.1	33.5	34.0	33.1	28.5	29.4	33.1
21	19.0	19.2	20.0	19.3	22.0	21.3	18.8
22	42.9	43.3	43.7	40.4	41.3	41.8	42.9
23	65.9	66.4	66.8	65.9	67.0	66.5	66.0
24	143.2	142.4	144.2	142.8	142.7	142.2	142.4
25	128.4	130.2	129.6	128.8	129.3	129.0	129.5
26	170.2	172.0	171.2	170.8	170.8	175.0	171.5
27	12.3	13.1	13.1	12.6	12.8	12.6	12.7
28	27.0	27.5	28.6	27.5	27.9	27.4	27.5
29	20.1	20.5	16.1	15.3	15.2	15.2	20.2
30	19.0	21.0	24.9	21.6	22.9	20.1	18.7

Two equatorial hydroxyl groups at C-7 (β-orientation) and C-15 (α-orientation) were deduced from the multiplicities of H-7 (δ_H 4.59, dd, J=9.8 and 6.6 Hz) and H-15 (δ_H 4.70, dd, J=9.2 and 6.3 Hz), which was supported by nuclear Overhauser effect (NOE) correlations observed from H-7 to H-5 and H_3-30, and H-15 to H_3-18 in the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 2). The configuration of the double bond at C-24 and C-25 was confirmed to be an *E* form by ^1H-NMR chemical shift of H-24 (δ_H 6.61), which resembles those of tiglic acid[32] and ganoderic acids U—Z.[33] Determination of the absolute configuration at C-23 was examined by a modification of Mosher's method.[34] Under the standard reaction conditions with (*R*)-(+)- and (*S*)-(−)-MTPA for 20 h, **1** gave 15,23-di-(*R*)-(+)-MTPA ester and 7,15,23-tri-(*S*)-(−)-MTPA ester, respectively. However, **1** gave 15,23-di-(*R*)-(+)-MTPA ester (**1a**) and 15,23-di-(*S*)-(−)-MTPA ester (**1b**), respectively, when stirred for 6 h. In the ^1H-NMR spectrum of (*S*)-(−)-MTPA ester (**1b**), proton signals assigned for H-20, H_3-21 and H_2-22 were observed at a higher-field than those in the (*R*)-(+)-MTPA ester (**1a**), while signals due to H-24 and H_3-27 in the former ester were shifted to a lower-field than those in the latter ester (Fig. 3). Therefore, the absolute configuration at C-23 was concluded to be 23*S*. Consequently, the structure of **1** was determined as (23*S*)-7β,15α,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid.

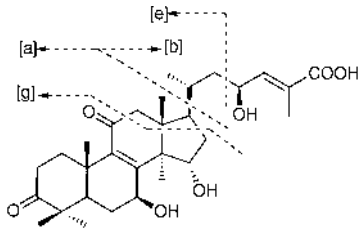

Fig. 1. Proposed Mass Fragmentation Pattern of **1**

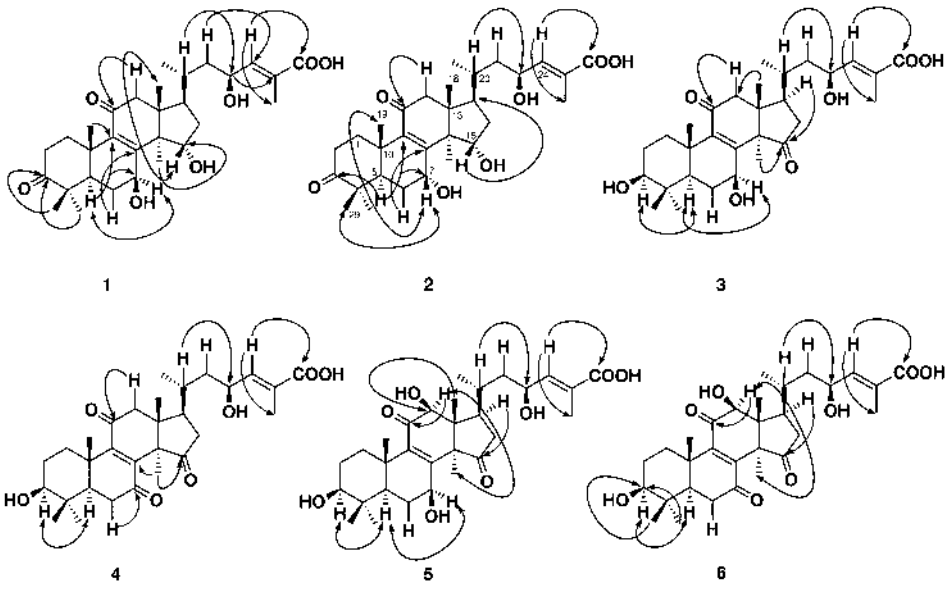

Fig. 2. Long-range and NOE Correlations Observed in the HMBC and NOESY Spectra of **1**—**6** (HMBC →, NOE ↔)

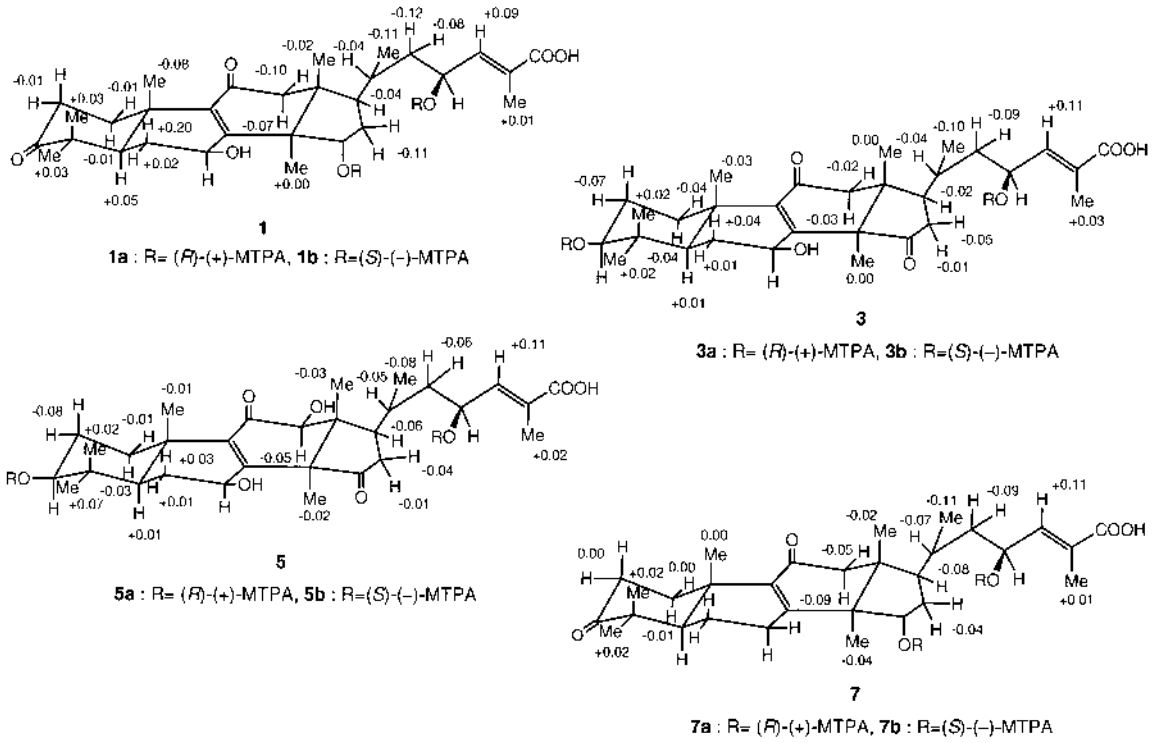

Fig. 3. Chemical Shift Difference for the (*S*)-(−)-MTPA Esters (**1b**, **3b**, **5b** and **7b**) and (*R*)-(+)-MTPA Esters (**1a**, **3a**, **5a** and **7a**) in ppm at 400 MHz

Ganoderic acid δ (**2**) was isolated as white amorphous powder, $[\alpha]_D$ +160°, and possessed the same molecular formula as that of **1**, HR-EIMS. Its UV and IR spectra exhibited absorptions at 252 nm (log ε 3.70) and 1657 cm^{-1}, ascribable to a conjugated ketone. The ^{1}H- and ^{13}C-NMR spectra of **2** were quite similar to those of **1**. Careful examination of the spectral data, however, revealed several significant differences. The most noticeable change were higher-field shifts of H-7 (δ_H 4.56, br d, J=3.4 Hz) and H-15 (δ_H 4.53, br t, J=5.1 Hz) by 0.3 and 1.3 ppm, respectively, and a higher-field shift of C-7 by 1.6 ppm, compared with the corresponding signals of **1** in the ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2). In the HMBC spectrum of **2**, long-range correlations were also similar to those of **1** (Fig. 2). A detailed comparison of spectral data of **1** and **2** with ganoderic acid A (**9**) and B8 (**10**)[13] showed that **2** was a stereoisomer of **1** with regard to a hydroxyl group at C-7. This was further supported by a NOESY experiment, which showed NOE correlations between H-7 and H_3-19/H_3-29, and H-15 to H_3-18 (Fig. 2). The configurations of C-23 and C-24 were assigned as 23*S* and 24*E* on the basis of the proton and carbon signals of the side chain moiety (C-20—27), which were very similar to those of **1**. Consequently, the structure of **2** was determined to be (23*S*)-7α,15α,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid.

Ganoderic acid ε (**3**) was also obtained as colorless needles (MeOH–H_2O) of mp 249—251 °C. The molecular formula was determined as $C_{30}H_{44}O_7$ by HR-FABMS.

Inspection of spectral data of **3** revealed the presence of the same functional groups as in **1**, including three oxymethylenes at δ 3.16 (dd, J=11.2, 5.0 Hz), δ 4.84 (t, J=9.4 Hz) and δ 4.56 (m), and an sp^2 methine at δ 144.2 and three carbonyls (δ 171.2, 200.1 and 218.5). In the ^{13}C-NMR spectrum, most of the signals in **3** were superimposable over those of **1**, except signals C-2, C-3, C-4, C-14, C-15 and C-16; the signals of C-2, C-3 and C-4 were shifted to a higher-field by 5.9, 139.9 and 6.7 ppm, respectively, while those of C-14, C-15 and C-16 shifted to a lower-field by 6.6, 146.9 and 6.4 ppm, respectively, compared with those of **1**, indicating a hydroxyl group at C-3 and a carbonyl group at C-15. This was further supported by HMBC correlations observed between signals of H_3-28 (δ_H 1.03)/H_3-29 (δ_H 0.84) and C-3 (δ_C 78.7), as well as signals between H_3-30 (δ_H, 1.38) and C-15 (δ_C, 218.5) (Fig. 2). Furthermore, the proton and carbon signals in **3** were also superimposable on those of ganoderic acid B (**11**),[1] except for the signals due to the side chain moiety (C-20—27), where the former possesses an allylic alcohol group at C-23—25 and the latter possesses an isolated carbonyl group.

The β orientation of hydroxyl groups at C-3 and C-7 was deduced from the multiplicities of H-3 (δ_H 3.16, dd, J=11.2, 5.0 Hz) and H-7 (δ_H 4.84, t, J=9.4 Hz). This was further confirmed by NOE correlations observed between H-3 and H-5 and between H-5 and H-7 in the NOESY spectrum (Fig. 2). For determination of absolute configuration at C-23, **3** was treated with (*R*)-(+)- and (*S*)-(−)-MTPA chloride to give 3,23-di-(*R*)-(+)-MTPA ester (**3a**) and 3,23-di-(*S*)-(−)-MTPA ester (**3b**), respectively. The chemical shift difference of **3a** and **3b** were observed in the same direction as **1a** and **1b** in the ^{1}H-NMR spectrum (Fig. 3). Consequently, the absolute configuration at C-23 in **3** was assigned as 23*S*. The structure of **3** was established as (23*S*)-3β,7β,23-trihydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid.

Ganoderic acid ζ (**4**) was isolated as colorless needles (MeOH–H_2O), mp 143—145 °C. Its IR spectrum exhibited absorption bands due to a five-membered carbonyl (1747 cm^{-1}) and α,β-unsaturated carbonyl (1698 cm^{-1}) groups, and the UV spectrum showed an absorption at

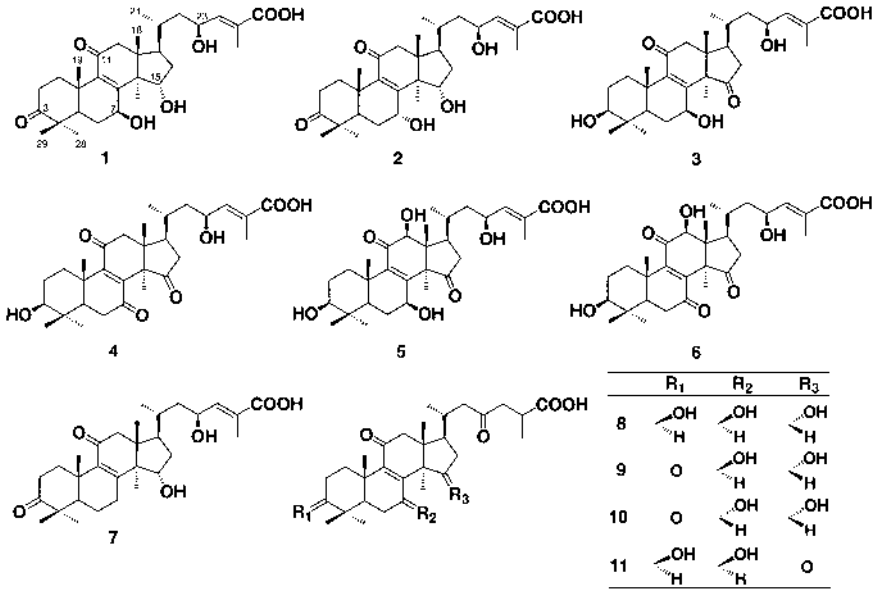

Chart 1. Structures of Compounds Isolated from the Spores of *Ganoderma lucidum*

257 nm (log ε, 3.81) due to an α,β-unsaturated carbonyl group. In the positive-mode FABMS of **4**, quasimolecular ion peaks were observed at *m/z* 515 $[M+H]^+$ and 537 $[M+Na]^+$, and the molecular formula, $C_{30}H_{42}O_7$, was determined by HR-FABMS.

The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3**. However, an oxymethylene signal (δ_H 4.84/δ_C 67.7) observed in the spectrum of **3** was absent in that of **4**. Instead, a new signal at δ_C 199.9 was observed, indicating that **4** has a carbonyl group at C-7. The connectivity of the carbonyl group was confirmed by HMBC correlations observed between H_2-6 (δ_H 2.56 and δ_H 2.62) and C-7 (δ_C 199.9) (Fig. 2). The carbon signals at δ_C 146.8 and 151.8 assigned as C-8 and C-9 correlated with H_3-30 and H_3-19 methyl protons, respectively, in the HMBC spectrum. The carbon signal of C-8 was more shielded than that of C-9. The configuration of a hydroxyl group at C-3 was assigned as β on the basis of multiplicity (δ_H 3.22, dd, J=10.8, 6.3 Hz). The configurations of C-23 and C-24 were assigned as 23*S* and 24*E* on the basis of NMR data, which were similar to those of **3**. Ganoderic acid ζ (**4**) was accordingly determined as (23*S*)-3β,23-dihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid.

Ganoderic acid η (**5**) was isolated as yellow needles (MeOH–H_2O), mp 212—214 °C, with a positive optical rotation ($[\alpha]_D$ +128°, EtOH). The molecular formula, $C_{30}H_{44}O_8$, of **5** was determined by positive-mode FABMS.

The ^{1}H- and ^{13}C-NMR spectra of **5** exhibited signals due to four oxymethylenes (δ_H 3.19/δ_C 78.0, δ_H 4.40/δ_C 78.2, δ_H 4.56/δ_C 67.0 and δ_H 4.76/δ_C 66.2) and an olefinic methine (δ_H 6.57/δ_C 142.7). The ^{1}H-NMR spectral feature of **5** closely resembled that of **3** except for a singlet signal at δ 4.40 due to the oxymethylene proton, instead of a pair of doublets arising from 12-methylene protons of **3** and appreciable higher-field and lower-field shifts of H_3-18 and H_3-19 signals, respectively, indicating that **5** may be a 12-hydroxy derivative of **3**. This was supported by the ^{13}C-NMR spectrum analyzed by means of the HMQC spectrum. The signals of C-12 and C-13 were shifted to the lower-field by 27.0 and 5.3 ppm, respectively, and the signal due to C-18 was shifted to the higher-field by 5.7 ppm, compared with the corresponding signals in **3**. This was confirmed by the HMBC correlations observed between signals of H_3-18 and C-12, and those of H-12 and C-11 (Fig. 2).

The β-configuration of a hydroxyl group at C-12 was inferred from NOE correlations observed between H-12 (δ 4.40) and H_3-30 (δ 1.43) in the NOESY spectrum. The absolute stereochemistry at C-23 was determined by a modification of Mosher's method. The signals due to H-20, H_3-21 and H_2-22 in 3,23-di-(*S*)-(−)-MTPA ester (**5b**) were observed at higher fields, compared to those of 3,23-di-(*R*)-(+)-MTPA ester (**5a**), while the signals due to H-24 and H_3-27 in **5b** were observed at lower-fields (Fig. 3). Thus, the absolute configuration at C-23 was assigned to be *S*. The structure of **5** was determined as (23*S*)-3β,7β,12β,23-tetrahydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid.

Ganoderic acid θ (**6**) was obtained as yellow needles (MeOH–H_2O), mp 131—132 °C, with a positive optical rotation ($[\alpha]_D$ +71.3°). In the positive-mode FABMS of **6**, quasimolecular ion peaks were observed at *m/z* 531 $[M+H]^+$ and 553 $[M+Na]^+$, and the molecular formula, $C_{30}H_{42}O_8$, was determined by HR-MS measurement.

The ^{1}H-NMR spectrum of **6** was quite similar to that of **5**, except for absence of an oxymethylene signal at δ 4.76 (t, J=8.7 Hz) and lower-field shifts of H-5, H_α-6 and H_β-6 by 0.66, 0.93 and 0.47 ppm, respectively (Table 1); this suggested the presence of a 7-oxo group in **6**, instead of a 7-hydroxyl group in **5**. It was supported by ^{13}C-NMR spectrum evidence that signals due to C-6 and C-7 (δ 9.9 and 133.3, respectively) shifted to the lower-field, reflecting the structural change in ring B. Furthermore, the HMBC spectrum showed correlation peaks between H-5 and C-7/C-9; H_2-6 and C-8; and H_3-19 and C-9, which led to the conclusion that the structure of **6** was (23*S*)-3β,12β,23-trihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid.

Ganolucidic acid D (**7**) was isolated from the fruiting body of *G. lucidum* by Nishitoba *et al.*,[21] who determined the absolute configuration at C-23 as 23*S*, by converting **7** to a mono-*p*-dimethylaminobenzoate derivative and measuring its

Table 3. Cytotoxicity of Compounds Isolated from the Spores of *Ganoderma lucidum* on Tumor Cell Growth

Compound	ED_{50} (μg/ml)	
	Meth-A	LLC
Ganoderic acid γ (**1**)	15.6	>20
Ganoderic acid δ (**2**)	>20	>20
Ganoderic acid ε (**3**)	12.2	>20
Ganoderic acid ζ (**4**)	>20	>20
Ganoderic acid η (**5**)	>20	>20
Ganoderic acid θ (**6**)	5.7	15.2
Ganolucidic acid D	>20	>20
Ganoderic acid C2	>20	>20
Lucidumol A	4.2	2.3
Lucidumol B	8.5	16.6
Ganodermanondiol	3.4	12.5
Ganoderiol F	4.4	6.0
Ganodermanontriol	5.4	9.6
Ganoderic acid A	>20	>20
Ganoderic acid B	>20	>20
Ganoderic acid C1	>20	17.0
Ganolucidic acid A	>20	15.5
Lucideric acid α	>20	17.8
Ganoderic acid C6	>20	>20
Ganoderic acid G	6.8	>20
Adriamycin[a)]	0.01	0.15

a) Positive control.

CD and UV spectra.[35)] Herein, **7** was converted to 15,23-di-(*R*)-(+)-MTPA ester (**7a**) and 15,23-di-(*S*)-(−)-MTPA ester (**7b**) using a modification of Mosher's method. The chemical shift difference between **7a** and **7b** was found to be the same as those observed in the respective derivatives of **1**, **3** and **5** in the ^{1}H-NMR spectra (Fig. 3). Consequently, the absolute configuration at C-23 of **7** was confirmed to be 23*S*.

The compounds isolated from the spores of *G. lucidum* were tested for their cytotoxicity against Meth-A and LLC tumor cell lines. The results (ED_{50} values) are summarized in Table 3. The ganoderic alcohols lucidumols A and B, ganodermanondiol, ganoderiol F, and ganodermanontriol showed cytotoxic effects on both tumor cells. Of these, lucidumol A exhibited the most potent cytotoxicity (ED_{50} value, 2.3 μg/ml) against LLC tumor cells and ganodermanondiol (ED_{50}, 3.4 μg/ml) against Meth-A cells. However, ganoderic acids, including ganoderic acids γ—θ isolated in the present experiment were inactive to both tumor cells.

Experimental

Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus and are not corrected. Optical rotations were measured with a DIP-360 automatic polarimeter (JASCO). UV spectra were measured with a UV-2200 UV-VIS recording spectrophotometer (Shimadzu), and IR spectra were measured with a FT/IR-230 infrared spectrometer (JASCO). ^{1}H- and ^{13}C-NMR spectra were measured with a JNA-LAA 400 WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz; JEOL) spectrophotometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. HR-FABMS and FABMS were measured with a JMX-AX 300L spectrometer (JEOL) using glycerol as a matrix. HR-EIMS and EIMS were measured with a JMX-AX 505 HAD mass spectrophotometer (JEOL). Prep. HPLC was carried out on a Gilson HPLC system; pump: model 305 and 306, detector: 119 UV/VIS detector. Column chromatography was carried out on silica gel (Kieselgel 60, 70—230 mesh, Merck). Thin layer chromatography (TLC) was carried out on pre-coated Silica gel 60 F_{254} plates (0.25 mm, Merck) and RP-18 F_{254}S (0.25 mm, Merck), and spots were detected under a UV light and by spraying 10% H_2SO_4 followed by heating.

Plant Materials The spore of *G. lucidum* was provided by Linzhi General Institute Co., Ltd. (Tokyo). The voucher specimen is deposited in the authors' laboratory.

Chemicals Lucidumols A and B, ganodermanondiol, ganoderiol F, ganodermanontriol, ganoderic acids A, B, C1, C6 and G, ganolucidic acid A, and lucidenic acid α were obtained from the spores of *G. lucidum* by a method described previously.[31)]

Cells Meth-A cells (mouse sarcoma) and LLC (mouse lung carcinoma) were purchased from RIKEN Cell Line Bank (Tsukuba, Japan). The cells were maintained as monolayer cultures in RPMI 1640 medium supplemented with 10% fetal bovine serum, sodium bicarbonate, penicillin G and streptomycin.

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 fraction (20 g) was chromatographed on a column of silica gel. Elution was started with hexane–acetone (3 : 2, 1 : 1 and 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). Column chromatography of fr. D on silica gel ($CHCl_3$–MeOH, 19 : 1) was separated into five subfractions (subfr. D1—D5; 0.12, 0.26, 0.24, 0.30 and 0.63 g, respectively). Purification of subfr. D4 by the prep. HPLC, a linear gradient of CH_3CN (25%→65%) in 3% AcOH, afforded **1** (11.4 mg, R_t 81.0 min), **2** (1.0 mg, R_t 82.8 min), **3** (18.6 mg, R_t 72.6 min), **4** (1.5 mg, R_t 74.4 min), **5** (12.5 mg, R_t 70.4 min), **6** (2.4 mg, R_t 67.0 min), **7** (5.0 mg, R_t 91.0 min) and **8** (12.0 mg, R_t 78.6 min).

(23*S*)-7β,15α,23-Trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic Acid (1, Ganoderic Acid γ) Colorless needles (MeOH–H_2O), mp 243—245 °C. $[\alpha]_D$ +155.3° (c=0.1, EtOH). IR ν_{max} cm^{-1}: 3373 (OH), 1706, 1675, 1660 (C=O). UV λ_{max} nm (log ε): 208 (4.05), 252 (3.82). EIMS *m/z* (rel. int.): 516 $[M]^+$ (3), 498 $[M-H_2O]^+$ (15), 480 $[M-2H_2O]^+$ (27), 462 $[M-3H_2O]^+$ (15), 401 $[e]^+$ (8), 370 $[M-C_6H_2O_4]^+$ (27), 359 $[a]^+$ (7), 306 $[g+H]^+$ (10), 252 $[M-C_6H_2O_4]^+$ (40), 157 $[b]^+$ (22), 69 (100). HR-EIMS *m/z*: 516.3092 (M^+, Calcd for $C_{30}H_{44}O_7$: 516.3088). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-2,19; C-2/H-1; C-3/H-1,2,5,28,29; C-4/H-2,5,6,28,29; C-5/H-6; C-6/H-5,7; C-7/H-5,6; C-8/H-6,30; C-9/H-5,19; C-10/H-5,6,19; C-11/H-12; C-12/H-18; C-13/H-12,15,16,20; C-14/H-12,16,18; C-15/H-30; C-16/H-20; C-17/H-15,18,21,22; C-18/H-12,30; C-19/H-1,5,6; C-20/H-21,22; C-21/H-20,22; C-23/H-20,27; C-24/H-22,23,27; C-25/H-23,27; C-26/H-24,27; C-27/H-24; C-28/H-5,29; C-29/H-5,28; C-30/H-12.

(*R*)-(+)-MTPA Ester of 1(1a) (*R*)-(+)-MTPA chloride (15 mg, 59 μmol) in pyridine (0.2 ml) was added to a solution of **1** (2.0 mg, 3.9 μmol) in CCl_4 (0.2 ml). After stirring at room temperature for 6 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) [hexane–acetone (1 : 1)] to give a 15,23-di-(*R*)-(+)-MTPA ester (**1a**, 1.5 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.37 (1H, m, H_α-1), 2.89 (1H, ddd, J=13.7, 7.8, 5.2 Hz, H_β-1), 2.45 (2H, m, H-2), 1.41 (1H, m, H-5), 1.39, 1.63 (each 1H, m, H-6), 3.63 (1H, t, J=7.9 Hz, H-7), 2.71 (1H, d, J=16.9 Hz, H_α-12), 2.51 (1H, d, J=16.9 Hz, H_β-12), 5.89 (1H, dd, J=9.7, 5.8 Hz, H-15), 1.82 (1H, m, H-16), 1.85 (1H, m, H-17), 0.92 (3H, s, H-18), 1.17 (3H, s, H-19), 1.37 (1H, m, H-20), 0.96 (3H, d, J=6.3 Hz, H-21), 1.80, 1.62 (each 1H, m, H-22), 5.79 (1H, ddd, J=18.7, 9.7, 5.1 Hz, H-23), 6.51 (1H, dd, J=9.7, 1.5 Hz, H-24), 2.01 (3H, d, J=1.5 Hz, H-27), 1.04 (3H, s, H-28), 1.03 (3H, s, H-29), 1.22 (3H, s, H-30), 3.52, 3.62 (each 3H, s, MTPA-OC$\underline{H}_3$).

(*S*)-(−)-MTPA Ester of 1 (1b) (*S*)-(−)-MTPA chloride (15 mg) in pyridine (0.2 ml) was added to a mixture of **1** (2 mg) in CCl_4 (0.2 ml). Workup as described above gave a 15,23-di-(*S*)-(−)-MTPA ester (**1b**, 1.0 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.36 (1H, m, H_α-1), 2.88 (1H, ddd, J=13.7, 7.8, 5.2 Hz, H_β-1), 2.44 (2H, m, H-2), 1.46 (1H, m, H-5), 1.41, 1.83 (each 1H, m, H-6), 3.82 (1H, t, J=8.4 Hz, H-7), 2.61 (1H, d, J=17.2 Hz, H_α-12), 2.44 (1H, d, J=17.2 Hz, H_β-12), 5.93 (1H, dd, J=10.1, 5.8 Hz, H-15), 1.71 (1H, m, H-16), 1.80 (1H, m, H-17), 0.90 (3H, s, H-18), 1.09 (3H, s, H-19), 1.33 (1H, m, H-20), 0.85 (3H, d, J=6.3 Hz, H-21), 1.68, 1.54 (each 1H, m, H-22), 5.77 (1H, ddd, J=17.8, 9.4, 5.9 Hz, H-23), 6.62 (1H, dd, J=9.4, 1.5 Hz, H-24), 2.02 (3H, d, J=1.5 Hz, H-27), 1.07 (3H, s, H-28), 1.06 (3H, s, H-29), 1.22 (3H, s, H-30), 3.46, 3.54 (each 3H, s, MTPA-OC$\underline{H}_3$).

(23*S*)-7α,15α,23-Trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic Acid (2, Ganoderic Acid δ) White amorphous powder, $[\alpha]_D$ +160.0° (c=0.074, EtOH). IR ν_{max} cm^{-1}: 3419 (OH), 1763, 1657 (C=O). UV λ_{max} nm (log ε): 207 (3.95), 252 (3.70). EIMS *m/z* (rel. int.): 516 $[M]^+$ (4), 498

$[M-H_2O]^+$ (21), 480 $[M-2H_2O]^+$ (29), 370 $[M-C_6H_2O_4]^+$ (29), 306 $[M-C_{12}H_{20}O_3]^+$ (12), 252 $[M-C_6H_2O_4]^+$ (46), 106 (100). HR-EIMS *m/z*: 516.3056 (M^+, Calcd for $C_{30}H_{44}O_7$: 516.3088). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-2,19; C-2/H-1; C-3/H-1,2,5,28,29; C-4/H-5,6,28,29; C-5/H-6,19,28,29; C-6/H-5,7; C-7/H-5,6; C-8/H-6,30; C-9/H-1,5,19; C-10/H-2,5,6,19; C-11/H-12; C-12/H-18; C-13/H-12,18,30; C-4/H-7,12,16,18; C-15/H-30; C-16/H-20; C-17/H-15,18,21; C-18/H-12,30; C-19/H-1,5,6; C-20/H-21,22; C-21/H-20,22; C-22/H-21,23; C-23/H-20,27; C-24/H-22,27; C-25/H-23,27; C-26/H-24,27; C-27/H-24; C-28/H-5,29; C-29/H-6,28; C-30/H-12.

(23*S*)-3β,7β,23-Trihydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic Acid (3, Ganoderic Acid ε) Colorless needles (MeOH–H_2O), mp 249—251 °C. $[\alpha]_D$ +153.3° (*c*=0.1, EtOH). IR ν_{max} cm^{-1}: 3553, 3335 (OH), 1714, 1695, 1658 (C=O). UV λ_{max} nm (log ε): 212 (4.13), 253 (4.03). Positive-mode FABMS *m/z*: 517 $[M+H]^+$, 539 $[M+Na]^+$. HR positive-mode FABMS *m/z*: 517.3142 ($[M+H]^+$, Calcd for $C_{30}H_{45}O_7$: 517.3165). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-19; C-2/H-1; C-3/H-1,28,29; C-4/H-5,6,28,29; C-5/H-6,19,28,29; C-6/H-5,7; C-7/H-5,6; C-8/H-6,7,30; C-9/H-5,7,12,19; C-10/H-1,5,6,19; C-11/H-12; C-12/H-18; C-13/H-12,16,17,18,30; C-14/H-12,18,30; C-15/H-17,30; C-16/H-18,30; C-17/H-16,18,21; C-18/H-12,17; C-19/H-1,5; C-20/H-16,17,21,23; C-21/H-17,20,22; C-22/H-20,21,23; C-23/H-20,27; C-24/H-27; C-25/H-23,24,27; C-26/H-24,27; C-27/H-24; C-28/H-3,29; C-29/H-3,5,28.

(*R*)-(+)-MTPA Ester of 3 (3a) (*R*)-(+)-MTPA chloride (15 mg, 59 μmol) in pyridine (0.2 ml) was added to a solution of **3** (2.0 mg, 3.9 μmol) in CCl_4 (0.2 ml). After stirring at room temperature for 6 h, workup as mentioned above gave a 3,23-di-(*R*)-(+)-MTPA ester (**3a**, 1.8 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.11 (1H, m, H_α-1), 2.91 (1H, dt, *J*=14.0, 3.6 Hz, H_β-1), 1.82 (2H, m, H-2), 4.71 (1H, dd, *J*=11.6, 5.1 Hz, H-3), 0.98 (1H, m, H-5), 2.16 (1H, m, H_α-6), 1.61 (1H, m, H_β-6), 4.79 (1H, t, *J*=9.0 Hz, H-7), 2.76 (1H, d, *J*=16.8 Hz, H_α-12), 2.68 (1H, d, *J*=16.8 Hz, H_β-12), 2.75, 2.05 (each 1H, m, H-16), 1.94 (1H, m, H-17), 0.86 (3H, s, H-18), 1.24 (3H, s, H-19), 1.50 (1H, m, H-20), 1.06 (3H, d, *J*=6.5 Hz, H-21), 1.70 (2H, m, H-22), 5.78 (1H, m, H-23), 6.49 (1H, dd, *J*=9.2, 1.2 Hz, H-24), 2.00 (3H, d, *J*=1.2 Hz, H-27), 0.93 (3H, s, H-28), 0.85 (3H, s, H-29), 1.26 (3H, s, H-30), 3.49, 3.56 (each 3H, s, MTPA-OC$\underline{H}_3$).

(*S*)-(−)-MTPA Ester of 3 (3b) (*S*)-(−)-MTPA chloride (15 mg) in pyridine (0.2 ml) was added to a solution of **3** (2 mg) in CCl_4 (0.2 ml). Workup as mentioned above gave a 3,23-di-(*S*)-(−)-MTPA ester (**3b**, 1.0 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.07 (1H, m, H_α-1), 2.87 (1H, dt, *J*=13.7, 3.7 Hz, H_β-1), 1.75 (2H, m, H-2), 4.69 (1H, dd, *J*=11.8, 4.4 Hz, H-3), 0.99 (1H, m, H-5), 2.17 (1H, ddd, *J*=12.7, 7.9, 1.1 Hz, H_α-6), 1.65 (1H, m, H_β-6), 4.79 (1H, t, *J*=8.9 Hz, H-7), 2.73 (1H, d, *J*=16.6 Hz, H_α-12), 2.67 (1H, d, *J*=16.6 Hz, H_β-12), 2.70, 2.04 (each 1H, m, H-16), 1.92 (1H, m, H-17), 0.86 (3H, s, H-18), 1.21 (3H, s, H-19), 1.46 (1H, m, H-20), 0.96 (3H, d, *J*=6.8 Hz, H-21), 1.61 (2H, m, H-22), 5.79 (1H, m, H-23), 6.60 (1H, dd, *J*=9.7, 1.2 Hz, H-24), 2.03 (3H, d, *J*=1.2 Hz, H-27), 0.95 (3H, s, H-28), 0.87 (3H, s, H-29), 1.26 (3H, s, H-30), 3.52, 3.54 (each 3H, s, MTPA-OC$\underline{H}_3$).

(23*S*)-3β,23-Dihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic Acid (4, Ganoderic Acid ζ) Colorless needles (MeOH–H_2O), mp 143—145 °C. $[\alpha]_D$ +213.3° (*c*=0.015, EtOH). IR ν_{max} cm^{-1}: 3421 (OH), 1747, 1698, 1681, 1654 (C=O). UV λ_{max} nm (log ε): 210 (4.13), 257 (3.81). Positive-mode FABMS *m/z*: 515 $[M+H]^+$, 537 $[M+Na]^+$. HR positive-mode FABMS *m/z*: 515.3044 ($[M+H]^+$, Calcd for $C_{30}H_{43}O_7$: 515.3009). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-19; C-3/H-28,29; C-4/H-19,28,29; C-5/H-6,19,28,29; C-7/H-6; C-8/H-30; C-9/H-19; C-10/H-19; C-11/H-12; C-12/H-18; C-13/H-18,30; C-14/H-12,18,30; C-15/H-30; C-16/H-18,20; C-17/H-12,16,18; C-18/H-12,16,30; C-19/H-1,5; C-20/H-21; C-22/H-21; C-23/H-20; C24/H-22,27; C25/H-27; C26/H-24,27; C-27/H-24; C28/H-29; C29/H-29.

(23*S*)-3β,7β,12β,23-Tetrahydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic Acid (5, Ganoderic Acid η) Yellow needles (MeOH–H_2O), mp 212—214 °C. $[\alpha]_D$ +128.0° (*c*=0.1, EtOH). IR ν_{max} cm^{-1}: 3405 (OH), 1725, 1697, 1673 (C=O). UV λ_{max} nm (log ε): 209 (4.10), 257 (3.80). Positive-mode FABMS *m/z*: 533 $[M+H]^+$, 555 $[M+Na]^+$. HR positive-mode FABMS *m/z*: 555.2791 ($[M+Na]^+$, Calcd for $C_{30}H_{44}O_8Na$: 555.2812). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-2,19; C-2/H-1; C-3/H-1,2,28,29; C-4/H-2,3,6,28,29; C-5/H-6,19,28,29; C-6/H-7; C-7/H-5,6; C-8/H-6,7,30; C-9/H-5,7,19; C-10/H-1,19,28,29; C-11/H-12; C-12/H-18; C-13/H-12,16,17,18,30; C-14/H-7,12,18,30; C-15/H-17; C-16/H-17,20; C-17/H-16,18,21; C-18/H-17; C-19/H-1,5; C-20/H-16,17,23; C-21/H-17; C-22/H-21; C-23/H-20; C-24/H-22,27; C-25/H-23,27; C-26/H-24; C-27/H-24; C-28/H-3,29; C-29/H-3,6,28.

(*R*)-(+)-MTPA Ester of 5 (5a) (*R*)-(+)-MTPA chloride (15 mg, 59 μmol) in pyridine (0.2 ml) was added to a solution of **5** (2.0 mg, 3.8 μmol) in CCl_4 (0.2 ml). The mixture was stirred at room temperature for 6 h and worked up as described above to give a 3,23-di-(*R*)-(+)-MTPA ester (**5a**, 1.2 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.01 (1H, m, H_α-1), 2.68 (1H, dt, *J*=13.8, 3.7 Hz, H_β-1), 1.83 (2H, m, H-2), 4.70 (1H, dd, *J*=11.0, 5.2 Hz, H-3), 0.95 (1H, m, H-5), 2.20 (1H, m, H_α-6), 1.67 (1H, m, H_β-6), 4.77 (1H, t, *J*=10.9 Hz, H-7), 4.33 (1H, s, H-12), 2.62, 2.13 (each 1H, m, H-16), 2.51 (1H, m, H-17), 0.72 (3H, s, H-18), 1.25 (3H, s, H-19), 2.04 (1H, m, H-20), 1.19 (3H, d, *J*=6.8 Hz, H-21), 1.85 (2H, m, H-22), 5.79 (1H, m, H-23), 6.48 (1H, dd, *J*=9.2, 1.5 Hz, H-24), 2.01 (3H, d, *J*=1.5 Hz, H-27), 0.87 (3H, s, H-28), 0.86 (3H, s, H-29), 1.32 (3H, s, H-30), 3.52, 3.55 (each 3H, s, MTPA-OC$\underline{H}_3$).

(*S*)-(−)-MTPA ester of 5 (5b) (*S*)-(−)-MTPA chloride (15 mg) and pyridine (0.2 ml) were added to a solution of **5** (2 mg) in CCl_4 (0.2 ml). Workup as mentioned above gave a 3,23-di-(*S*)-(−)-MTPA ester (**5b**, 0.8 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.00 (1H, m, H_α-1), 2.65 (1H, dt, *J*=14.0, 2.9 Hz, H_β-1), 1.71 (2H, m, H-2), 4.67 (1H, dd, *J*=9.4, 4.4 Hz, H-3), 0.96 (1H, m, H-5), 2.23 (1H, m, H_α-6), 1.68 (1H, m, H_β-6), 4.77 (1H, dd, *J*=8.3, 4.1 Hz, H-7), 4.28 (1H, s, H-12), 2.58, 2.12 (each 1H, m, H-16), 2.45 (1H, m, H-17), 0.69 (3H, s, H-18), 1.24 (3H, s, H-19), 1.99 (1H, m, H-20), 1.11 (3H, d, *J*=6.8 Hz, H-21), 1.79 (2H, m, H-22), 5.81 (1H, m, H-23), 6.59 (1H, dd, *J*=8.6, 1.5 Hz, H-24), 2.03 (3H, d, *J*=1.5 Hz, H-27), 0.94 (3H, s, H-28), 0.88 (3H, s, H-29), 1.30 (3H, s, H-30), 3.51, 3.53 (each 3H, s, MTPA-OC$\underline{H}_3$).

(23*S*)-3β,12β,23-Trihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic Acid (6, Ganoderic Acid θ) Yellow needles (MeOH–H_2O), mp 131—133 °C. $[\alpha]_D$ +71.3° (*c*=0.1, EtOH). IR ν_{max} cm^{-1}: 3404 (OH), 1747, 1685, 1653 (C=O). UV λ_{max} nm (log ε): 212 (4.08), 251 (3.75). Positive-mode FABMS *m/z*: 531 $[M+H]^+$, 553 $[M+Na]^+$. HR positive-mode FABMS *m/z*: 531.2933 ($[M+Na]^+$, Calcd for $C_{30}H_{43}O_8$: 531.2958). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2. In the HMBC spectrum, the following correlations were observed: C-1/H-19; C-3/H-1,28,29; C-4/H-6,19; C-5/H-6,19,28,29; C-6/H-5; C-7/H-6; C-8/H-6,30; C-9/H-5,19; C-10/H-1,19; C-11/H-12; C-12/H-18; C-13/H-12,16; C-14/H-12,18,30; C-15/H-16,17,30; C-17/H-16,18,21; C-19/H-5; C-20/H-21,22; C-21/H-23; C-22/H21; C-23/H-20,27; C-24/H-22,27; C-25/H-23,27; C-26/H-24,27; C-27/H-24; C-28/H-3,29; C-29/H-3,28.

(*R*)-(+)-MTPA Ester of 7 (7a) (*R*)-(+)-MTPA chloride (10 mg, 40 μmol) in pyridine (0.1 ml) was added to a solution of **7** (1.0 mg, 2 μmol) in CCl_4 (0.1 ml). After stirring at room temperature for 20 h, workup as mentioned above gave a 15,23-di-(*R*)-(+)-MTPA ester (**7a**, 0.7 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.46 (1H, m, H_α-1), 2.96 (1H, ddd, *J*=15.1, 9.0, 6.0 Hz, H_β-1), 2.45 (2H, m, H-2), 2.68 (1H, d, *J*=17.2 Hz, H_α-12), 2.43 (1H, d, *J*=17.2 Hz, H_β-12), 5.20 (1H, dd, *J*=9.7, 5.5 Hz, H-15), 1.81 (2H, m, H-16), 1.91 (1H, m, H-17), 0.87 (3H, s, H-18), 1.05 (3H, s, H-19), 1.36 (1H, m, H-20), 0.95 (3H, d, *J*=6.3 Hz, H-21), 1.62, 1.75 (each 1H, m, H-22), 5.77 (1H, m, H-23), 6.50 (1H, dd, *J*=9.4, 1.5 Hz, H-24), 2.01 (3H, d, *J*=1.5 Hz, H-27), 1.06 (3H, s, H-28), 1.05 (3H, s, H-29), 1.13 (3H, s, H-30), 3.51, 3.63 (each 3H, s, MTPA-OC$\underline{H}_3$).

(*S*)-(−)-MTPA Ester of 7 (7b) (*S*)-(−)-MTPA chloride (10 mg) in pyridine (0.1 ml) were added to a solution of **7** (1 mg) in CCl_4 (0.1 ml). After workup as usual, a 15,23-di-(*S*)-(−)-MTPA ester (**7b**, 0.6 mg) was obtained as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.46 (1H, m, H_α-1), 2.95 (1H, ddd, *J*=18.0, 7.7, 5.7 Hz, H_β-1), 2.45 (2H, m, H-2), 2.59 (1H, d, *J*=17.3 Hz, H_α-12), 2.38 (1H, d, *J*=17.3 Hz, H_β-12), 5.21 (1H, dd, *J*=9.4, 5.6 Hz, H-15), 1.77 (2H, m, H-16), 1.83 (1H, m, H-17), 0.85 (3H, s, H-18), 1.05 (3H, s, H-19), 1.29 (1H, m, H-20), 0.84 (3H, d, *J*=5.8 Hz, H-21), 1.53, 1.66 (each 1H, m, H-22), 5.77 (1H, m, H-23), 6.61 (1H, dd, *J*=8.0, 1.5 Hz, H-24), 2.02 (3H, d, *J*=1.5 Hz, H-27), 1.08 (3H, s, H-28), 1.07 (3H, s, H-29), 1.09 (3H, s, H-30), 3.45, 3.54 (each 3H, s, MTPA-OC$\underline{H}_3$).

Cytotoxicity Assay The *in vitro* Meth-A tumor cell assay was carried out according to the procedure by Geran *et al.*[36] and LLC cells, by a sulforhodamin B (SRB) method[37] as described previously.[38]

Acknowledgment Part of this study was financially supported by Linzin General Institute Co., Ltd. (Tokyo).

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