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Structures of New Terpenoid Constituents of *Ganoderma lucidum* (Fr.) KARST (Polyporaceae)[1)]

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Eight new terpenoid constituents named ganoderenic acids A (**1**), B (**2**), C (**3**) and D (**4**), ganoderic acids E (**5**), F (**6**), and G (**7**), and lucidenic acid D (**8**) were isolated from dried fruiting bodies of the fungus, *Ganoderma lucidum* (Fr.) KARST (Polyporaceae) and their structures were determined on the basis of spectral data and some chemical interconversions.

Keywords——*Ganoderma lucidum*; Polyporaceae; lanostane; triterpene; C_{27}-terpene; ganoderenic acid; ganoderic acid; lucidenic acid

In the previous paper[2)] we reported the isolation of two histamine release-inhibitory triterpenes, ganoderic acids C (**13**) and D (**14**) from dried fruiting bodies of the fungus, *Ganoderma lucidum* (Fr.) KARST (Polyporaceae) (靈芝), along with known triterpenes, ganoderic acids A (**11**) and B (**12**), which had been isolated as bitter principles.[3)] Next, an attempt was made to isolate other structually related constituents contained in *G. lucidum* and eight new terpenes named ganoderenic acids A, B, C and D (**1**, **2**, **3** and **4**), ganoderic acids E, F and G (**5**, **6** and **7**), and lucidenic acid D (**8**) were isolated. In this paper we describe the isolation of these compounds and the determination of the structures on the basis of spectral analyses and some chemical interconversions.

Isolation of the Constituents

The 50% ethanolic extract obtained from dried fruiting bodies (200 g) of *G. lucidum* was partitioned between chloroform and water. The chloroform layer was treated with sodium bicarbonate solution to extract acidic compounds. Further fractionation of a mixture of the acidic compounds was carried out to isolate terpenoid acids as shown in Chart 1, guided by high-performance liquid chromatographic (HPLC) analyses.

The mixture was chromatographed over silica gel to give fractions 1—7. Fraction 2 was rechromatographed to afford fractions 2-1 and 2-2, which were further purified by preparative thin-layer chromatography (TLC) to give lucidenic acid D (**8**, 6 mg), and ganoderic acids E (**5**, 10 mg) and F (**6**, 33 mg), respectively. Ganoderenic acid D (**4**, 15 mg) and ganoderic acid D (**14**, 53 mg) from fraction 3, and ganoderenic acid B (**2**, 10 mg) and ganoderic acids B (**12**, 17 mg) and G (**7**, 16 mg) from fraction 4 were isolated by means of preparative HPLC. Repeated recrystallization of fraction 5 afforded ganoderic acid A (**11**, 307 mg), and the mother liquor was purified by preparative HPLC to give ganoderenic acid A (**1**, 29 mg). From fraction 6, ganoderic acid C (**13**, 140 mg) and ganoderenic acid C (**3**, 5 mg) were obtained by treatments similar to those used for fraction 5.

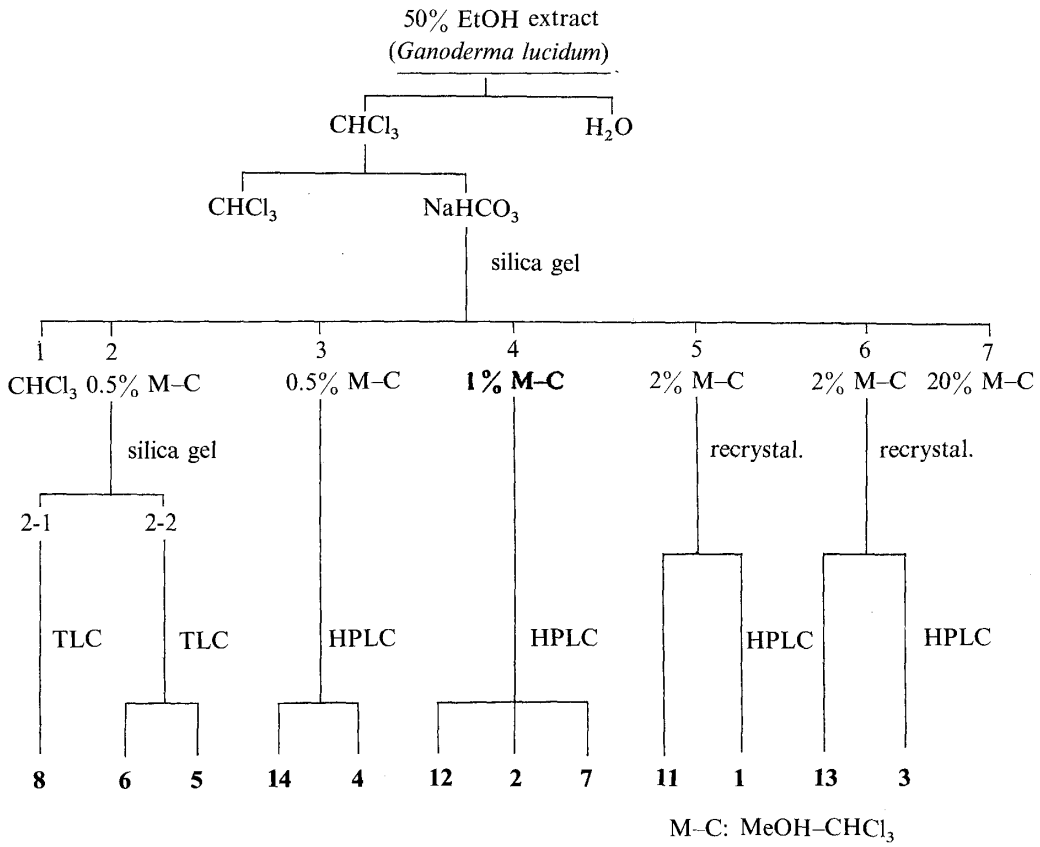


Chart 1

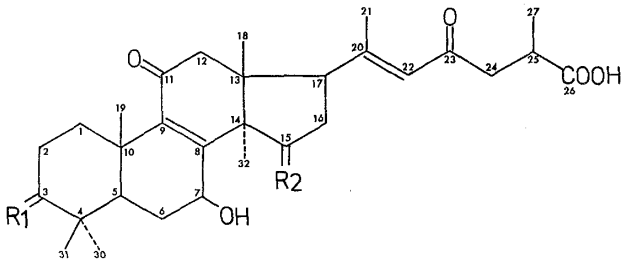
Structures of the Constituents

The signals of all carbons in the ^{13}C-nuclear magnetic resonance (NMR) spectra were assigned on the basis of the data obtained by off-resonance and, if necessary, selective proton-decoupling techniques, and the chemical shifts are shown in Tables I and II.

The molecular formula ($C_{30}H_{42}O_7$) of ganoderenic acid A (**1**), an amorphous powder, indicates that **1** is a dehydro derivative of ganoderic acid A (**11**),[2] which was eluted very closely after **1** in HPLC. The NMR spectra of **1** show the presence of an olefinic methyl group [2.10 (3H, s) and 6.12 ppm (1H, br s) in the ^1H-NMR; 21.3 (q), 124.3 (d) and 157.0 ppm (s) in the ^{13}C-NMR] on the side chain instead of the 21-secondary methyl group of **11**. The stereochemistry of the double bond was determined to be *E*, since the signal of the 21-methyl group in the ^1H-NMR spectrum is located at lower field as compared with those of normal olefinic methyl groups, due to the anisotropic effect of the 23-keto group. From a further comparison of the NMR spectra of **1** and **11**, it was concluded that the structure of **1** is (20*E*)-7β,15α-dihydroxy-3,11,23-trioxo-5α-lanost-8, 20-dien-26-oic acid.

It was also found on the basis of NMR spectral analyses that ganoderenic acids B (**2**), mp 211—214 °C, $C_{30}H_{42}O_7$, C (**3**), an amorphous powder, $C_{30}H_{44}O_7$ and D (**4**), mp 214—216 °C, $C_{30}H_{40}O_7$ have the structures with the 20*E*-double bond on the side chain of ganoderic acids B (**12**), C (**13**) and D (**14**), respectively, by analogy with that of **1** [**2** 2.16 (3H, s) and 6.04 ppm (1H, br s); 21.0 (q), 124.7 (d) and 153.8 ppm (s); **3** 2.10 (3H, s) and 6.16 (1H, br s); 21.3 (q), 124.5 (d) and 157.3 ppm (s); **4** 2.17 (3H, s) and 6.09 ppm (1H, br s); 20.9 (q), 124.7 (d) and 153.6 ppm (s)]. Therefore, the structures of **2**, **3** and **4** were determined to be (20*E*)-3β,7β-dihydroxy-11,15,23-trioxo-5α-lanost-8,20-dien-26-oic acid, (20*E*)-3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8,20-dien-26-oic acid and (20*E*)-7β-hydroxy-3,11,15,23-tetraoxo-5α-lanost-8,20-dien-26-oic acid, respectively.

The ^{13}C-NMR spectrum of ganoderic acid E (**5**), mp 117—119 °C, $C_{30}H_{40}O_7$ showed the



	R_1	R_2
1:	=O	H, OH
2:	OH, H	=O
3:	OH, H	H, OH
4:	=O	=O

Chart 2

TABLE I. Carbon-13 Chemical Shifts (1)[a]

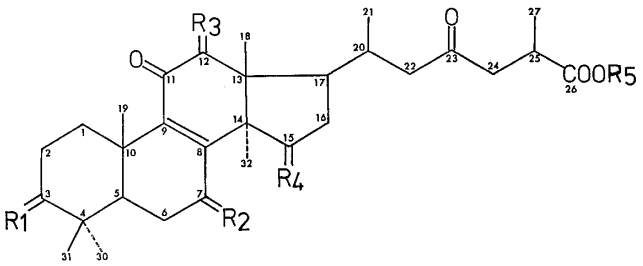
Carbon	1	11[2]	2	12[2]	3	13[2]	4	14[2]
1	35.5	35.6	34.8	34.8	34.8	35.0	35.6	35.7
2	34.3	34.3	27.7	27.6	27.8	28.0	34.2	34.3
3	217.4	217.3	78.3	78.3	78.1	77.5	216.5	216.6[b]
4	46.7	46.8	39.0	38.8	38.8	38.9	46.8	46.8
5	48.8	48.9	49.2	49.2	50.0	49.5	48.9	49.0
6	29.0	28.9	26.7	26.6	27.6	27.6	27.7	27.7
7	68.9	68.8	66.9	66.9	69.4	69.5	66.3	66.4
8	159.1	159.4	156.6	156.8	158.8	159.6	157.4	157.8
9	140.3	140.3	142.8	142.7	142.0	142.4	141.4	141.3
10	38.1	38.1	38.7	38.6	38.8	38.9	38.4	38.3
11	199.1	199.9	198.0	197.9	200.0	201.2	197.8	197.6
12	50.5	51.7	49.1	50.3	50.8	52.3	48.9	50.2
13	48.1	46.7	46.3	45.4	49.3	47.4	46.0	45.0
14	53.4	54.0	58.7	59.4	53.5	54.4	58.7	59.4
15	72.7	72.3	216.4	217.5	72.5	72.4	216.4	217.5[b]
16	31.8	35.9	37.7	40.9	31.6	35.9	37.9	41.0
17	52.2	48.0	49.7	45.6	52.4	48.5	49.7	45.7
18	19.0	17.3	18.8	17.4	18.8	17.2	19.0	17.7
19	19.9	19.6	18.4	18.5	19.7	19.6	18.1	18.2
20	157.0	32.7	153.8	32.0	157.3	33.0	153.6	32.0
21	21.3	19.8	21.0	19.6	21.3	19.7	20.9	19.6
22	124.3	49.7	124.7	49.0	124.5	50.0	124.7	49.0
23	198.6	209.1	197.2	207.6	199.6	210.0	196.9	207.5
24	47.5	46.6	47.6	46.6	48.1	46.9	47.5	46.6
25	35.1	34.7	34.8	34.6	34.8	35.0	34.8	34.5
26	180.2	179.4	180.0	180.3	179.0	178.5	180.6	180.3
27	17.1	17.0	17.1	16.9	17.3	17.2	17.0	16.9
30	27.5	27.4	28.2	28.2	28.2	28.3	27.0	27.0
31	20.7	20.8	15.5	15.5	15.8	15.9	20.8	20.8
32	19.4	19.4	24.4	24.4	19.5	19.5	24.7	24.7

a) The spectra were taken in $CDCl_3$ except for those of **1** and **2**, which were taken in $CDCl_3$–CD_3OD. The values are in ppm downfield from TMS. *b*) Assignments in reference 2 were reversed.

same chemical shift values within 0.3 ppm, except for that of C-26, as those of the methyl ester of the pentaoxo compound (**5a**) derived from **11** and **12**.[2] Thus, the structure of **5** was determined to be 3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oic acid.

Ganoderic acid F (**6**), an amorphous powder, has the molecular formula $C_{32}H_{42}O_9$. The ^{13}C-NMR spectrum of **6** shows the presence of five keto groups (194.1, 198.6, 205.5, 207.3 and 214.9 ppm) and one secondary acetoxyl group [20.9 (q), 79.0 (d) and 170.2 ppm]. Comparison of the ^{13}C-NMR spectrum of **6** with that of **5** indicates that all signals have essentially the same chemical shift values as those of **5** except for the signals of C-11, -12, -13, -14, -16, -18,

-20 and -21. The ^{1}H-NMR spectrum of **6** also shows the presence of a secondary acetoxyl group [2.25 (3H, s) and 5.68 ppm (1H, s)] on a carbon atom whose two adjacent carbon atoms bear no proton. From these findings, the location of the secondary acetoxyl group should be at the C-12 position. Stereochemistry of the acetoxyl group was determined by nuclear Overhauser effect (NOE) experiments. Observation of a 16.7% NOE enhancement of the C-12 proton signal at 5.68 ppm upon irradiation of the 32-methyl signal at 1.80 ppm indicated the β-configuration for the C-12 acetoxyl group. Consequently, it was concluded that the structure of **6** is 12β-acetoxy-3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oic acid. This was further confirmed by the reaction of **6** with sodium carbonate solution in aqueous methanol to give the hydrolysis product (**9**), which was oxidized with cupric acetate in methanol, affording the hexaoxo derivative (**10**).



	R_1	R_2	R_3	R_4	R_5
5:	=O	=O	H_2	=O	H
5a:	=O	=O	H_2	=O	CH_3
6:	=O	=O	/OAc \H	=O	H
7:	/OH \H	/OH \H	/OH \H	=O	H
7a:	/OH \H	/OH \H	/OH \H	=O	CH_3
9:	=O	=O	/OH \H	=O	H
10:	=O	=O	=O	=O	H
11:	=O	/OH \H	H_2	/H \OH	H
12:	/OH \H	/OH \H	H_2	=O	H
12a:	/OH \H	/OH \H	H_2	=O	CH_3
13:	/OH \H	/OH \H	H_2	/H \OH	H
14:	=O	/OH \H	H_2	=O	H

Chart 3

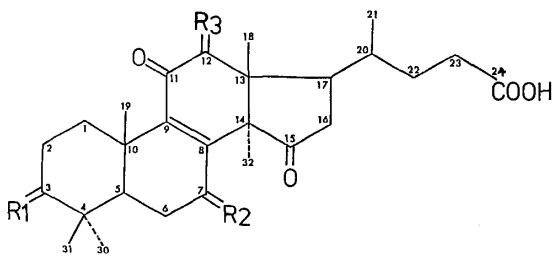
It was difficult to record the NMR spectrum of ganoderic acid G (**7**), mp 218—220 °C, $C_{30}H_{44}O_8$ in $CDCl_3$ because of its insolubility in this solvent. Therefore, **7** was treated with diazomethane to give the monomethyl ester (**7a**), whose NMR spectra taken in $CDCl_3$ were compared with those of the methyl ester (**12a**) of ganoderic acid B.[2] All ^{13}C-NMR signals of **7a** showed the same chemical shift values (differences of less than 0.7 ppm) as those in **12a** except for the signals of C-11, -12, -13, -14, -16, -18, -20, -21 and -32, and the presence of one additional secondary hydroxy group at the C-12 position was indicated [4.35 ppm (1H, s) in ^{1}H-NMR and 77.9 ppm (d) in the ^{13}C-NMR]. The configuration of the C-12 hydroxy group was determined to be β since irradiation of the 32-methyl signal (1.44 ppm) caused a 15.4% NOE enhancement of the C-12 proton signal (4.35 ppm). Thus, the structure of **7** was established as 3β,7β,12β-trihydroxy-11, 15, 23-trioxo-5α-lanost-8-en-26-oic acid. This was confirmed on the basis of transformation of **7** into the hexaoxo derivative (**10**) by oxidation with CrO_3–pyridine in dichloromethane.

The molecular formula ($C_{29}H_{38}O_8$) of lucidenic acid D (**8**), an amorphous powder, suggested that **8** does not belong to the group of triterpenoid constituents described previously. The ^{13}C-NMR spectrum of **8** showed the same chemical shift values (within 0.3 ppm) as those of ganoderic acid F (**6**) except for the value of C-17 and those corresponding

TABLE II. Carbon-13 Chemical Shifts (2)[a]

Carbon	5	5a[2]	6	8	9	10	7	7a	12a[2]
1	37.2	37.3	37.4	37.4	37.5	37.2	35.2	34.6	34.9
2	34.6	34.7	34.1	34.0	34.1	33.6	27.6	27.6	27.8
3	215.4	215.2	214.9	215.2	214.7	214.7	78.0	78.3	78.3
4	47.0	47.0[b]	46.9	46.9	47.0	47.0	39.1	38.6	38.9
5	50.9	51.0[b]	51.0	50.9	51.1	50.9	49.8	49.2	49.2
6	33.8	33.8	33.7	33.7	33.8	33.5	27.4	26.9	26.7
7	199.4	199.3	198.6	198.7	198.4	198.3	67.0	66.3	66.9
8	149.8	149.7	149.9	149.8	148.8	150.0	157.3	157.5	156.9
9	146.8	146.8	146.1	146.2	147.5	149.5	143.1	142.0	142.7
10	39.3	39.4	39.4	39.3	39.2	39.3	38.9	38.3	38.7
11	199.4	199.3	194.1	194.1	201.7	197.1	200.3	199.4	197.8
12	48.9	48.9[b]	79.0	79.1	77.4	192.6	78.4	77.9	50.3
13	43.9	43.9[b]	47.7	47.6	49.4	59.0	52.4	51.94	45.4
14	57.2	57.2	58.7	58.6	58.0	61.1	60.9	60.4	59.4
15	207.0	206.8	205.5	205.9	206.0	203.9	217.8	216.8	217.4
16	39.8	39.8	37.8	37.6	37.9	38.9	38.6	38.4	40.9
17	44.3	44.5	44.5	45.2	45.0	38.4	46.6	45.8	45.6
18	16.0	16.1[b]	12.1	12.0	10.8	12.5	12.3	12.0	17.4
19	18.6	18.6	18.7	18.7	18.6	18.6	19.1	18.8	18.5
20	32.1	32.0	29.5	33.1	29.6	32.2	29.1	28.7	32.0
21	19.8	19.8	21.6	20.0	21.2	23.3	21.8	21.4	19.7
22	48.8	49.1	48.4	29.9	48.8	48.6	48.7	48.5	49.1
23	207.6	207.6	207.3	31.6	208.0	207.5	210.3	208.2	207.7
24	46.5	46.8	46.4	178.7	46.4	46.5	46.9	46.4	46.8
25	34.6	34.7	34.6	—	34.5	34.6	35.4	34.7	34.7
26	180.9	176.1	180.8	—	180.3	181.0	178.8	176.2	176.1
27	16.9	17.1[b]	16.9	—	16.9	16.9	17.3	17.1	16.9
30	27.6	27.6	27.6	27.6	27.5	27.5	28.4	28.1	28.2
31	20.3	20.4[b]	20.4	20.4	20.6	20.3	15.8	15.4	15.4
32	21.0	21.0[b]	20.8	20.8	20.0	19.3	23.5	23.1	24.4
$COOC\underline{H}_3$	—	52.0	—	—	—	—	—	51.89	51.9
$OC\underline{O}CH_3$	—	—	170.2	170.1	—	—	—	—	—
$OCOC\underline{H}_3$	—	—	20.9	20.8	—	—	—	—	—

a) The spectra were taken in $CDCl_3$ except for that of **7**, which was taken in $CDCl_3$–CD_3OD. The values are in ppm downfield from TMS. *b*) Assignments in reference 2 were reversed.



	R_1	R_2	R_3
8:	=O	=O	/OAc, \H
15:	=O	/OH, \H	H_2
16:	=O	/OH, \H	/OH, \H
17:	/OH, \H	/OH, \H	/OH, \H

Chart 4

to the side chain. From further analyses of the NMR spectra, it was concluded that the structure of **8** is 12β-acetoxy-4,4,14α-trimethyl-3,7,11,15-tetraoxo-5α-chol-8-en-24-oic acid. The C_{27} terpenes, lucidenic acids A (**15**), B (**16**) and C (**17**) have been isolated from *G. lucidum* as bitter compounds.[4]

Studies are in progress on the biological activities of the constituents of *G. lucidum* described in this paper.

Experimental

All melting points were taken with a Yanagimoto microscope hot stage and are uncorrected. Mass spectra (MS) were obtained by the electron impact ionization method on a JEOL JMS-D300 mass spectrometer. Infrared (IR) spectra were measured on a Hitachi 285 spectrometer. Ultraviolet (UV) spectra were taken on a Hitachi 323 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained using a JEOL FX-270 spectrometer at 270 and 67.8 MHz, respectively. Chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, dd=double doublet, t=triplet and q=quartet. Column chromatography was carried out using silica gel (Wakogel C-200, Wako). TLC was performed on Silica gel 60 F_{254} plates (Merck). HPLC was carried out on Waters Associates ALC/GPC 204 and System 500 instruments employing NOVA-PAK C_{18} (8 mm × 10 cm) and PREPAK C_{18} columns, respectively, with CH_3CN–ammonium acetate buffer solution (10 mM, pH 4.0) as the eluent.

Isolation (Chart 1)——Dried fruiting bodies (200 g) of *G. lucidum* were crushed and extracted with 50% EtOH (2 l × 2) for 24 h at room temperature. The mixture was filtered and the combined filtrate was concentrated *in vacuo* at 35 °C to give an extract. The extract was partitioned between $CHCl_3$ and H_2O. The combined $CHCl_3$ layer was extracted with 5% $NaHCO_3$ solution. The combined $NaHCO_3$ layer was acidified to pH 3 with 2 N HCl under ice-cooling and extracted with $CHCl_3$, followed by concentration of the $CHCl_3$ layer to give a mixture of acidic compounds (6.3 g).

The mixture was chromatographed on a silica gel column, which was eluted with $CHCl_3$ (fraction 1), 0.5% MeOH–$CHCl_3$ (fractions 2 and 3), 1% MeOH–$CHCl_3$ (fraction 4), 2% MeOH–$CHCl_3$ (fractions 5 and 6) and 20% MeOH–$CHCl_3$ (fraction 7).

Fraction 2 was rechromatographed over silica gel to give fractions 2-1 and 2-2. Each fraction was further purified by preparative TLC to afford lucidenic acid D (**8**, 6 mg) from fraction 2-1, and ganoderic acids E (**5**, 10 mg) and F (**6**, 33 mg) from fraction 2-2. Fraction 3 was also purified by preparative HPLC and recrystallization to afford ganoderenic acid D (**4**, 15 mg) and ganoderic acid D (**14**, 53 mg). Fraction 4 was subjected to repeated preparative HPLC and each pure material obtained was recrystallized to give ganoderenic acid B (**2**, 10 mg), and ganoderic acids B (**12**, 17 mg) and G (**7**, 16 mg). Ganoderic acid A (**11**, 307 mg) was obtained by recrystallization of fraction 5 from 50% EtOH four times and the combined mother liquor was purified by preparative HPLC to afford ganoderenic acid A (**1**, 29 mg). Fraction 6 was recrystallized three times from EtOAc–MeOH to afford ganoderic acid C (**13**, 122 mg), and the combined mother liquor was purified by preparative HPLC giving an additional ganoderic acid C (28 mg) and ganoderenic acid C (**3**, 5 mg).

Ganoderenic Acid A (1)——A colorless amorphous powder. $[\alpha]_D^{22}$ +127.8° (c=0.43, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 248 (16400). IR ν_{max}^{KBr} cm^{-1}: 1710, 1665, 1610. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, 18CH_3), 1.09 (3H, s, 31CH_3), 1.12 (3H, s, 30CH_3), 1.22 (3H, d, J=6.6 Hz, 27CH_3), 1.25 (3H, s, 19CH_3), 1.34 (3H, s, 32CH_3), 2.10 (3H, s, 21CH_3), 4.67 (1H, dd, J=5.9, 6.9 Hz, 7CH), 4.90 (1H, dd, J=7.9, 9.2 Hz, 15CH), 6.12 (1H, br s, 22CH). MS m/z: 514.2951 (M^+, $C_{30}H_{42}O_7$=514.2931). *Anal.* Calcd for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 70.25; H, 8.29.

Ganoderenic Acid B (2)——Colorless plates, mp 211—214 °C (EtOAc–MeOH). $[\alpha]_D^{22}$ +102.9° (c=0.30, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 246 (16500). IR ν_{max}^{KBr} cm^{-1}: 1750, 1715, 1695, 1670, 1625. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (6H, s, 18 and 31CH_3), 1.04 (3H, s, 30CH_3), 1.21 (3H, s, 19CH_3), 1.23 (3H, d, J=6.6 Hz, 27CH_3), 1.40 (3H, s, 32CH_3), 2.16 (3H, s, 21CH_3), 3.22 (1H, dd, J=5.9, 10.2 Hz, 3CH), 4.84 (1H, dd, J=8.7, 8.7 Hz, 7CH), 6.04 (1H, br s, 22CH). MS m/z: 514.2938 (M^+, $C_{30}H_{42}O_7$=514.2931). *Anal.* Calcd for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 69.95; H, 8.31.

Ganoderenic Acid C (3)——A colorless amorphous powder. $[\alpha]_D^{22}$ +66.2° (c=0.15, $CHCl_3$–MeOH). UV λ_{max}^{MeOH} nm (ε): 249 (14800). IR ν_{max}^{KBr} cm^{-1}: 1710, 1660. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.79 (3H, s, 18CH_3), 0.84 (3H, s, 31CH_3), 1.02 (3H, s, 30CH_3), 1.20 (3H, d, J=6.6 Hz, 27CH_3), 1.25 (3H, s, 19CH_3), 1.31 (3H, s, 32CH_3), 2.10 (3H, s, 21CH_3), 3.19 (1H, dd, J=7.2, 9.2 Hz, 3CH), 4.53 (1H, dd, J=7.1, 10.0 Hz, 7CH), 4.80 (1H, dd, J=7.7, 8.8 Hz, 15CH), 6.16 (1H, br s, 22CH). MS m/z: 516.3102 (M^+, $C_{30}H_{44}O_7$=516.3087). *Anal.* Calcd for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found: C, 69.52; H, 8.60.

Ganoderenic Acid D (4)——Colorless needles, mp 214—216 °C (EtOH–H_2O). $[\alpha]_D^{22}$ +163.4° (c=0.34, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 245 (19600). IR ν_{max}^{KBr} cm^{-1}: 1735, 1715, 1670, 1620. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18CH_3), 1.11 (3H, s, 31CH_3), 1.13 (3H, s, 30CH_3), 1.24 (3H, d, J=6.9 Hz, 27CH_3), 1.25 (3H, s, 19CH_3), 1.41 (3H, s, 32CH_3), 2.17 (3H, s, 21CH_3), 4.89 (1H, dd, J=8.4, 8.4 Hz, 7CH), 6.09 (1H, br s, 22CH). MS m/z: 512.2778 (M^+, $C_{30}H_{40}O_7$=512.2775). *Anal.* Calcd for $C_{30}H_{40}O_7$: C, 70.29; H, 7.87. Found: C, 70.37; H, 7.63.

Ganoderic Acid E (5)——Pale yellow needles, mp 117—119 °C (MeOH). $[\alpha]_D^{22}$ +126.8° (c=0.49, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 253 (7160). IR ν_{max}^{KBr} cm^{-1}: 1740, 1730, 1700, 1680. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18CH_3), 0.98 (3H, d, J=6.3 Hz, 21CH_3), 1.12 (3H, s, 31CH_3), 1.14 (3H, s, 30CH_3), 1.23 (3H, d, J=7.3 Hz, 27CH_3), 1.28 (3H, s, 19CH_3), 1.64 (3H, s, 32CH_3). MS m/z: 512.777 (M^+, $C_{30}H_{40}O_7$=512.2775). *Anal.* Calcd for $C_{30}H_{40}O_7$: C, 70.29; H, 7.87. Found: C, 70.15; H, 7.92.

Ganoderic Acid F (6)——A pale yellow amorphous powder. $[\alpha]_D^{22}$ +114.1° (c=0.37, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 253 (7190). IR ν_{max}^{KBr} cm^{-1}: 1750, 1700. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s, 18CH_3), 0.99 (3H, d, J=6.3 Hz, 21CH_3), 1.12 (3H, s, 31CH_3), 1.14 (3H, s, 30CH_3), 1.22 (3H, d, J=6.9 Hz, 27CH_3), 1.34 (3H, s, 19CH_3), 1.80 (3H, s, 32CH_3), 2.25

(3H, s, $OCOCH_3$), 5.68 (1H, s, 12CH). MS *m/z*: 570.2833 (M^+, $C_{32}H_{42}O_9$ = 570.2828). *Anal.* Calcd for $C_{32}H_{42}O_9$: C, 67.35; H, 7.42. Found: C, 67.19; H, 7.51.

Ganoderic Acid G (7)——Colorless plates, mp 218—220 °C (EtOAc–MeOH). $[\alpha]_D^{22}$ +105.7° (*c* = 0.48, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 253 (6620). IR ν_{max}^{KBr} cm^{-1}: 1740, 1725, 1700, 1670. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.82 (3H, s, $18CH_3$), 0.87 (3H, s, $31CH_3$), 1.03 (3H, s, $30CH_3$), 1.13 (3H, d, *J* = 6.3 Hz, $21CH_3$), 1.19 (3H, d, *J* = 6.9 Hz, $27CH_3$), 1.31 (3H, s, $19CH_3$), 1.47 (3H, s, $32CH_3$), 3.17 (1H, dd, *J* = 5.3, 10.6 Hz, 3CH), 4.41 (1H, s, 12CH), 4.82 (1H, dd, *J* = 8.9, 8.9 Hz, 7CH). MS *m/z*: 532.3042 (M^+, $C_{30}H_{44}O_8$ = 532.3037). *Anal.* Calcd for $C_{30}H_{44}O_8$: C, 67.64; H, 8.33. Found: C, 67.73; H, 8.37.

Lucidenic Acid D (8)——A pale yellow amorphous powder. $[\alpha]_D^{22}$ +70.2° (*c* = 0.62, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 253 (6690). IR ν_{max}^{KBr} cm^{-1}: 1750, 1700. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, s, $18CH_3$), 1.02 (3H, d, *J* = 6.6 Hz, $21CH_3$), 1.12 (3H, s, $31CH_3$), 1.14 (3H, s, $30CH_3$), 1.34 (3H, s, $19CH_3$), 1.81 (3H, s, $32CH_3$), 2.22 (3H, s, $OCOCH_3$), 5.68 (1H, s, 12CH). MS *m/z*: 514.2577 (M^+, $C_{29}H_{38}O_8$ = 514.2563). *Anal.* Calcd for $C_{29}H_{38}O_8$: C, 67.68; H, 7.44. Found: C, 67.83; H, 7.47.

Hydrolysis of Ganoderic Acid F (6)——A solution of **6** (24 mg) in 80% MeOH (2 ml) was treated with 5% Na_2CO_3 solution (0.2 ml), and the mixture was stirred at room temperature for 3 h. The reaction mixture was acidified with 2 N HCl, MeOH was evaporated off *in vacuo*, and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue (21 mg) was purified by preparative TLC to give the hydrolysis product (**9**, 7 mg), 12β-hydroxy-3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oic acid, as a yellow amorphous powder. UV λ_{max}^{MeOH} nm (ε): 251 (7020). IR ν_{max}^{KBr} cm^{-1}: 1750, 1705. ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, $18CH_3$), 1.12 (3H, d, *J* = 6.3 Hz, $21CH_3$), 1.13 (3H, s, $31CH_3$), 1.14 (3H, s, $30CH_3$), 1.22 (3H, d, *J* = 6.9 Hz, $27CH_3$), 1.38 (3H, s, $19CH_3$), 1.75 (3H, s, $32CH_3$), 4.52 (1H, s, 12CH).

Synthesis of 3,7,11,12,15,23-Hexaoxo-5α-lanost-8-en-26-oic Acid (10)——(a) From **9**: Cupric acetate (4 mg) was added to a solution of **9** (5 mg) in MeOH (1 ml), and the mixture was stirred at room temperature for 2 h. Then water was added, and the whole was acidified with 2 N HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC to afford **10** (4 mg) as a yellow amorphous powder. UV λ_{max}^{MeOH} nm (ε): 266 (4680). IR ν_{max}^{KBr} cm^{-1}: 1760, 1740, 1710, 1695. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, d, *J* = 6.6 Hz, $21CH_3$), 1.15 (3H, s, $31CH_3$), 1.16 (3H, s, $30CH_3$), 1.20 (3H, s, $18CH_3$), 1.23 (3H, d, *J* = 7.3 Hz, $27CH_3$), 1.37 (3H, s, $19CH_3$), 1.55 (3H, s, $32CH_3$).

(b) From **7**: A solution of **7** (10 mg) in CH_2Cl_2 was treated with CrO_3–pyridine at room temperature for 1 h. The reaction mixture was treated with 2 N HCl, then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC then HPLC to give the oxidized product (2 mg), which was identical with **10** on the basis of comparisons of their ^{1}H-NMR and ^{13}C-NMR spectra.

Synthesis of Methyl Ganoderate G (7a)——Ganoderic acid G (**7**, 7 mg) was treated with ethereal diazomethane to afford **7a** as colorless needles, mp 144—146 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ; 0.80 (3H, s, $18CH_3$), 0.87 (3H, s, $31CH_3$), 1.04 (3H, s, $30CH_3$), 1.14 (3H, d, *J* = 6.3 Hz, $21CH_3$), 1.18 (3H, d, *J* = 6.9 Hz, $27CH_3$), 1.31 (3H, s, $19CH_3$), 1.44 (3H, s, $32CH_3$), 3.21 (1H, dd, *J* = 7.0, 9.0 Hz, 3CH), 4.35 (1H, s, 12CH), 4.77 (1H, dd, *J* = 8.6, 8.6 Hz, 7CH).

References and Notes

1) Parts of this work were presented at the 104th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April 1984 and at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985.
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