Chem. Pharm. Bull.
33(4)1367—1374(1985)

The Biologically Active Constituents of *Ganoderma lucidum* (FR.) KARST. Histamine Release-Inhibitory Triterpenes

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(Received June 26, 1984)

The MeOH extract of *Ganoderma lucidum* has an inhibitory action on histamine release from rat mast cells. From the physiologically active fraction of the extract, along with the known triterpenes ganoderic acids A and B, two new triterpenes were isolated and named ganoderic acids C and D. The structures of ganoderic acids C and D were determined to be 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oic acid and 7β-hydroxy-3,11,15,23-tetraoxo-5α-lanost-8-en-26-oic acid respectively. Ganoderic acids C and D were shown to inhibit histamine release from rat mast cells. Quantitative analysis of these triterpenes was performed for the purpose of crude drug quality control.

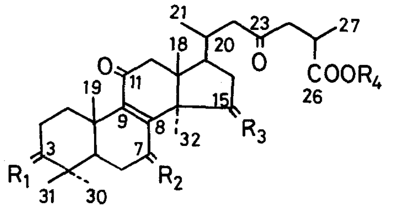
Keywords——*Ganoderma lucidum*; Polyporaceae; lanostane; triterpenoid; ganoderic acid C; ganoderic acid D; NMR; antiinflammatory activity; mast cell; histamine release

As a continuation of our studies to identify pharmacologically active constituents in crude drugs, we investigated the active constituents of a fungal crude drug, "ganoderma" by means of a screening test for inhibition of histamine release from rat mast cells.1)

"Ganoderma" (霊芝) is the dried fruit body of a fungus, *Ganoderma lucidum* (FR.) KARST. and has been used in Oriental medicine for a long time as a tonic and sedative, and for the treatment of chronic bronchitis, *etc.*2) Recently, several pharmacological activities of ganoderma have been experimentally verified. For example, administration of ganoderma extract to the CCl_4-intoxicated mouse caused a reduction of the serum glutamate pyruvate transferase activity.3) The fungus was proved to be effective for the treatment of muscular dystrophy.4)

Mannitol and ergosterol were isolated from this crude drug,5) and recently, two new lanostane type triterpenes, ganoderic acids A (**1**) and B (**2**) have been isolated as bitter principles by Japanese chemists6) (Chart 1). Several similar triterpenes, ganoderic acids T—Z, which all have a double bond at the C-24 position were isolated by a French group, from the same fungus, and were proved to have cytotoxic properties.7)

When our simple screening method for antiinflammatory activity was applied to more than 50 crude drugs, ganoderma showed activity to inhibit the histamine release from rat mast cells induced by compound 48/80 and concanavalin A.1) Therefore, ganoderma was extracted and fractionated by using several solvents, with monitoring of the bioactivity of each fraction. From the bioactive ethyl acetate-soluble fraction, four triterpenes were isolated. Two of them



	R_1	R_2	R_3	R_4
1	=O	OH H	H OH	H
1a	=O	OH H	H OH	CH_3
2	OH H	OH H	=O	H
2a	OH H	OH H	=O	CH_3
3	OH H	OH H	H OH	H
3a	OH H	OH H	H OH	CH_3
4	=O	OH H	=O	H
4a	=O	OH H	=O	CH_3
5a	=O	=O	=O	CH_3

Chart 1

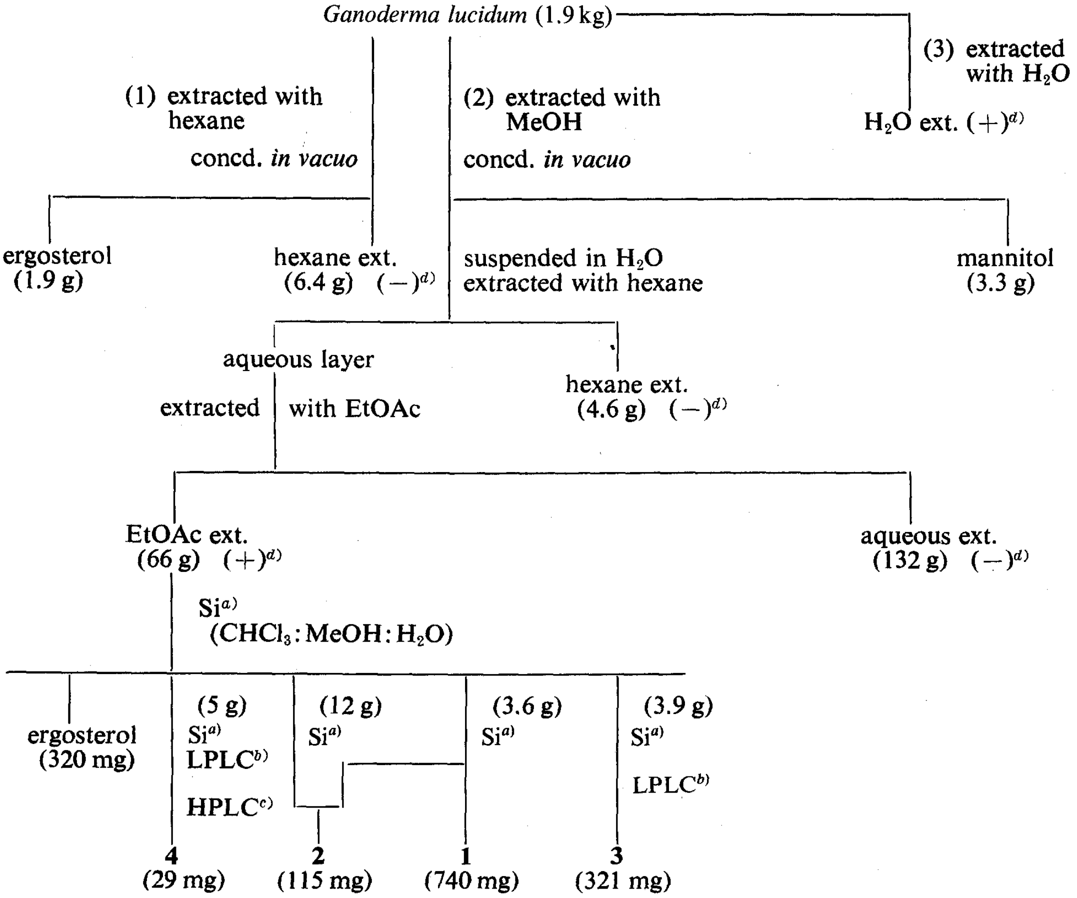


Fig. 1. Isolation and Biological Activity of Constituents of Ganoderma

a) Si: silica gel chromatography (open column). *b*) LPLC: low-pressure liquid chromatography over silica gel. *c*) HPLC: high-pressure liquid chromatography over ODS. *d*) Inhibitory activities on histamine release from rat mast cells: +, >50%; −, <50% (dose: 50 mg/ml).

were ganoderic acids A (**1**) and B (**2**). The other two are new compounds, having histamine release-inhibiting activity. Their structures were elucidated mainly by analysis of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra.

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Isolation of the Constituents

Ganoderma lucidum (Fr.) Karst. cultivated in Hiroshima was extracted with hexane, then with methanol. The methanol extract was suspended in water and extracted with ethyl acetate. Bioassay for inhibition of histamine release from rat mast cells was conducted for each fraction, and the ethyl acetate fraction showed significant activity (Fig. 1). From the hexane extract, ergosterol was isolated as colourless crystals, mp 151—155 °C; its identity was confirmed by comparison of its thin layer chromatographic (TLC) behavior and ^{13}C-NMR spectrum with those of an authentic sample.[8] From the MeOH extract, mannitol was isolated during the concentration procedure as colorless needles, mp 161 °C, identified from the TLC behavior and ^{13}C-NMR spectrum.[9]

From the biologically active ethyl acetate fraction, four crystalline substances were isolated by repeated silica-gel (Si gel) and reverse-phase chromatography, and tentatively designated as compound **1** (mp 233—236 °C), **2** (mp 233—235 °C), **3** (mp 231—232 °C) and **4** (mp 150—151 °C).

Structures of the Four Triterpenes

Compound **1** was identical with ganoderic acid A (**1**) on the basis of a comparison of its

Table I. ^{13}C-NMR Chemical Shifts of Ganoderic Acids Derivatives[a]

	1	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5a**
C-1	35.6 (35.7)[b]	35.5 (35.6)[b]	34.8	34.9 (34.8)[b]	35.0	34.7	35.7	35.7	37.3 (37.4)[b]
C-2	34.3 (34.4)	34.3 (34.4)	27.6	27.8 (28.3)	28.0	28.2	34.3	34.3	34.7 (34.7)
C-3	217.3 (208.4)	217.1 (208.7)	78.3	78.3 (78.5)	77.5	78.2	217.5	217.5	215.2 (208.4)
C-4	46.8 (47.0)	46.8 (46.7)	38.8	38.9 (39.0)	38.9	38.6	46.8	46.8	43.9 (44.1)
C-5	48.9 (49.2)	48.8 (48.8)	49.2	49.2 (45.7)	49.5	49.1	49.0	49.0	48.9 (44.8)
C-6	28.9 (29.2)	29.1 (29.1)	26.6	26.7 (27.8)	27.6	27.8	27.7	27.8	33.8 (33.8)
C-7	68.8 (69.1)	68.9 (68.9)	66.9	66.9 (67.0)	69.5	69.5	66.4	66.3	199.3 (200.0)
C-8	159.4 (159.6)	159.2 (159.5)	156.8	156.9 (155.0)	159.6	158.0	157.8	157.8	149.7 (150.4)
C-9	140.3 (140.6)	140.2 (140.1)	142.7	142.7 (142.9)	142.2	142.0	141.3	141.3	146.8 (147.7)
C-10	38.1 (46.8)	38.0 (46.8)	38.6	38.7 (45.4)	38.9	38.6	38.3	38.3	39.4 (47.1)
C-11	199.9 (200.0)	199.5 (199.7)	197.9	197.8 (200.0)	201.2	199.8	197.6	197.6	199.3 (200.2)
C-12	51.7 (51.9)	51.8 (51.8)	50.3	50.3 (51.0)	52.3	52.0	50.2	50.2	51.0 (51.3)
C-13	46.7 (38.2)	46.6 (38.0)	45.4	45.4 (38.8)	47.4	47.1	45.0	45.0	47.0 (39.6)
C-14	54.0 (54.2)	54.0 (54.1)	59.4	59.4 (58.5)	54.4	54.0	59.4	59.4	57.2 (57.4)
C-15	72.3 (72.6)	72.4 (72.4)	217.5	217.4 (207.5)	72.4	72.4	216.6	216.4	206.8 (207.5)
C-16	35.9 (36.2)	36.3 (36.3)	40.9	40.9 (41.0)	35.9	36.2	41.0	41.0	39.8 (39.9)
C-17	48.0 (48.3)	48.1 (48.2)	45.6	45.6 (49.2)	48.5	48.1	45.7	45.8	44.5 (49.1)
C-18	17.3 (17.4)	17.3 (17.3)	17.4	17.5 (17.2)	17.2	17.1	17.7	17.7	17.1 (17.0)
C-19	19.6 (19.6)	19.5 (19.4)	18.5	18.5 (17.6)	19.6	19.5	18.2	18.2	18.6 (18.5)
C-20	32.7 (32.8)	32.7 (32.8)	32.0	32.0 (32.1)	33.0	32.7	32.0	32.0	32.0 (32.1)
C-21	19.8 (19.4)	19.6 (19.7)	19.6	19.7 (19.8)	19.7	19.6	19.6	19.7	19.8 (19.8)
C-22	49.7 (49.8)	49.7 (49.7)	49.0	49.1 (49.3)	50.0	49.8	49.0	49.1	49.1 (49.3)
C-23	209.1 (217.3)	208.5 (217.4)	207.6	207.7 (215.9)	210.0	208.5	207.5	207.6	207.6 (215.9)
C-24	46.6 (46.7)	46.8 (46.8)	46.6	46.8 (46.9)	46.9	46.7	46.6	46.8	46.8 (46.9)
C-25	34.7 (34.8)	34.7 (34.8)	34.6	34.7 (34.9)	35.0	34.7	34.5	34.7	34.7 (34.8)
C-26	179.4 (27.4)	176.2 (27.5)	180.3	176.1 (26.8)	178.5	176.2	180.3	176.1	176.1 (27.6)
C-27	17.0 (180.1)	17.1 (176.3)	16.9	16.9 (176.2)	17.2	17.1	16.9	17.1	16.1 (176.8)
C-30	27.4 (17.0)	27.4 (17.2)	28.2	28.2 (15.5)	28.3	28.2	27.0	27.0	27.6 (16.5)
C-31	20.8 (20.8)	20.7 (20.8)	15.5	15.4 (24.5)	15.9	15.7	20.8	20.8	21.0 (21.0)
C-32	19.4 (19.8)	19.4 (19.7)	24.4	24.4 (18.7)	19.5	19.4	24.7	24.7	20.4 (20.4)
COOMe	—	51.9 (52.0)	—	51.9 (51.7)	—	51.9	—	51.9	52.0 (52.0)

a) δ (at 67.5 MHz) from tetramethylsilane (TMS), in $CDCl_3$ except **3** (in $CD_3OD+CDCl_3$). *b*) Reported values (100.61 MHz in $CDCl_3$).[6]

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proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR data with the reported values.[6)]

Compound **2** had the molecular formula $C_{30}H_{44}O_7$ and gave a monomethyl ester (**2a**), mp 203—205 °C, on methylation with diazomethane. The ^{1}H-NMR spectrum (270 MHz) recorded in deuterated chloroform ($CDCl_3$) was essentially identical to that of methyl ganoderate B (**2a**) in the literature (400 MHz) except for a singlet methyl signal at δ 0.85 instead of the described value of 1.84, which seemed to be a simple error.[6)] The ^{13}C-NMR spectrum of **2a** in $CDCl_3$ was almost superimposable on the actual spectrum of methyl ganoderate B provided by Dr. Y. Asaka, one of the authors of reference 6.[10)] Curiously enough, the reported δ_c values of this compound in $CDCl_3$ were very close to, but somewhat different from our data.[6)] Although most of the ^{13}C-signals of **2a** had δ-values less than 0.3 ppm different from the reported values, the signals corresponding to C-8, -11, -12, -14 and -15 showed differences of +1.9, −2.2, −0.7, +0.9 and +1.5 ppm, respectively.[11)] These differences seem to be too small for the compounds to be different, but at the same time are too large for the same compound in the same solvent. A probable reason for this discrepancy is that the reported data might have been obtained in a different solvent. Further, the ^{13}C-NMR assignments given in the literature should be corrected in some cases. The chemical shift values of C-10 (δ 45.4) and C-13 (δ 38.8) of methyl ganoderate B should be reversed according to the assignments for lanosterol.[12)] Assignments of the methyl groups should be corrected as shown in Table I based on the results of proton selective decoupling experiments. As regards the ketone signals, many precedents have shown that the 3-keto carbon resonates at *ca.* δ 215—218 instead of 208.4, assigned to C-3 of **1**.[13)] Further, the ^{13}C-signal of the C-23 keto group should appear at *ca.* δ 208 by analogy with diisobutyl ketone.[14)] Moreover, the chemical shift of the C-15 keto carbon signal is expected to be *ca.* δ 216 by analogy with 15-cholestanone.[15)] Accordingly, the assignments of C-15 and C-23 of methyl ganoderate B, and C-3 and C-23 of ganoderic acid A and its derivatives should be reversed (Table I). By the correction, including additional reversed assignments, compound **2** was finally concluded to have the structure of ganoderic acid B.

Compound **3** had the molecular formula $C_{30}H_{46}O_7$, and is not identical with any triterpene previously found in the same fungus.[6,7)] It also gave a monomethyl ester (**3a**), mp 187—190 °C, on reaction with diazomethane. Oxidation of **3a** with chromium trioxide (CrO_3)–pyridine gave a pentaketo methyl ester (**5a**) mp 189—194 °C, $C_{31}H_{42}O_7$, which is identical with the compound obtained from **2a** by the same reaction. The ^{13}C-NMR and ^{1}H-NMR chemical shifts of **5a** were also identical with the reported values of methyl 3,7,11,15,23-pentaoxo-5α-lanost-8-en-26 oate.[6)]

The ^{13}C-NMR spectrum of **3a** measured in $CDCl_3$ was compared with those of **1a** and **2a**. The spectrum of **3a** indicates the existence of two ketones (δ 199.8 and 208.5), one carboxylic acid methyl ester (176.2 and 51.9) and three secondary alcohol groups (69.5, 72.4 and 78.2). In general, the spectrum was closely related to those of **1a** and **2a**. The overlapped singlet peaks at δ 38.6 can be assigned to C-4 and C-10, supporting the existence of the 3β-OH group.[16)] If there were a 3-keto group, the C-4 singlet signal would appear at about δ 46 instead of 38.6. There was no peak between δ 55 and 69. If there were a 15-keto group, the C-14 singlet signal should appear at δ 58—60, which indicates the absence of a 15-keto group. Positive evidence for a 15 α-OH group is the doublet signal at δ 72.4, which is in good agreement with the C-15 signal of **1a**. From further analysis of the ^{13}C-NMR signals, the structure of **3a** was proved to be a combination of the partial structures of **1a** (C, D-ring and side chain carbons) and **2a** (A, B-rings) and was established as methyl 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oate. The chemical shifts of the corresponding signals were in good agreement (within 0.5 ppm) with the established structure, except in a few cases (C-6, 7, 8, 9 and 19). These differences can be explained in terms of a conformational change of the Bring caused by the interaction between the C7-OH and C15-OH groups. Compound **3** was named ganoderic acid C.

Compound **4** had the molecular formula $C_{30}H_{42}O_7$, and gave a monomethyl ester (**4a**), mp 171—172 °C, on reaction with diazomethane. The ^{13}C-NMR spectrum showed that **4a** has four keto (δ 197.6, 207.6, 216.4 and 217.5) and one secondary alcohol (δ 66.3) groups. Comparison of the spectrum of **4** with that of **2a** indicates that all signals of carbon atoms have essentially the same chemical shift values as those of **2a** except for the signals of C-1, 2, 3, 4, 30 and 31. The chemical shift values assignable to A-ring carbons are almost superimposable on those of the 3-keto compound **1a** or its derivatives.[6] Thus, it was concluded that the structure of **4** is 7β-hydroxy-3,11,15,23-tetraoxo-5α-lanost-8-en-26-oic acid; this compound was named ganoderic acid D.

Biological Activities of Ganoderic Acids

Inhibitory activity on histamine release from rat mast cells was tested as described previously.[1] This test is a simple *in vitro* assay for antiinflammatory or antiallergic drugs, and

TABLE II. Inhibitory Effects of Triterpenes Isolated from Ganoderma on Histamine Release from Mast Cells[a]

Compound	Dose (mg/ml)	Release	
		Concanavalin A	Compound 48/80
1	2	55	75
	0.4	5	20
	0.08	0	0
2	2	0	35
	0.4	0	25
3	2	85	70
	0.4	30	0
	0.08	0	0
4	2	80	80
	0.4	15	65
	0.08	0	25

a) Inhibition %, $n=4$—6, S.D. $= \pm 10\%$.

TABLE III. Contents of **2** and **3** in Various Ganoderma Samples

Origin (part)	Yield of EtOAc extract (%)	Contents (% of the sample)	
		2	**3**
Hiroshima[a] (whole)	5.8	0.18	0.21
Hiroshima[a] (caps)	6.5	0.25	0.34
Hiroshima[a] (stems)	4.1	0.05	0.07
Hiroshima[a] (spores)	1.3	0.04	0.05
Hiroshima[b] (whole)	9.75	0.30	0.47
Nagano (whole)	4.25	0.12	0.10
Kyoto (whole)	5.00	0.19	n.d.[c]
Kyoto in bottle	9.35	0.27	0.38
China (whole)	3.5	0.10	0.11

a) Cultivated at Yoshida-cho, Takata-gun, and grown on *Quercus variabilis* BLUME. *b*) Same Place, grown on *Prunus yedoensis* MATSUM. *c*) Not detected.

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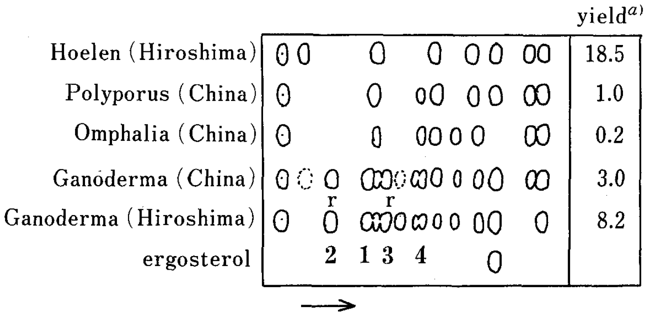

Fig. 2. TLC of MeOH Ext. of Some Chinese Fungal Drugs

Solvent, $CHCl_3$–MeOH–H_2O (30:4:1); plate, Merck Kieselgel 60 F_{254} (Aluminium sheets); detection, 10% H_2SO_4 with heating. Abbreviation: r, red.
a) Weight % of dried crude drugs.

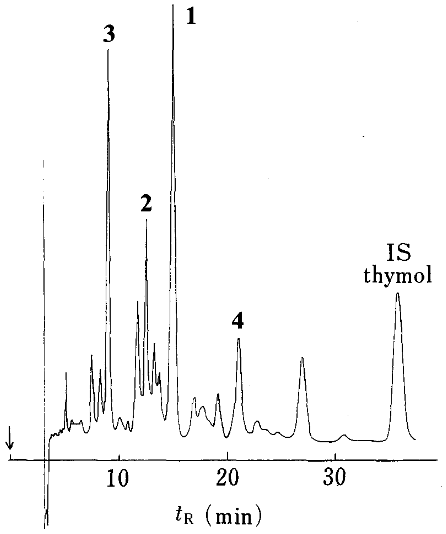

Fig. 3. High-Performance Liquid Chromatogram of the Ethyl Acetate Extract of Ganoderma Cultivated in Hiroshima

Conditions: see Experimental.

was proved to correlate well with the usual *in vivo* assay method. The physiological activities of pure compounds isolated from the active fraction were measured individually (Table II). The results indicate that the compounds **3** and **4** have distinct inhibitory activity on histamine release and they are at least partly responsible for one of the pharmacological activities of the crude drug, ganoderma.

Quantitative Determination of Ganoderic Acids

Several other fungal crude drugs are used in Oriental medicine. For example, hoelen (茯苓), omphalia (雷丸) and polyporus (猪苓) are frequently used in Chinese (Kampo) prescriptions. Although ergosterol is a common constituent of these three fungal drugs, **2** and **3** are unique constituents of ganoderma. On a TLC plate, these substances show characteristic bright red spots on heating after an H_2SO_4 spray (Fig. 2). This reaction can be used for qualitative detection of ganoderma extract. For the purpose of differentiation, detection or evaluation of crude drugs, quantitative determination of **2** and **3** was performed by means of high-pressure liquid chromatography (HPLC).

Ethyl acetate extract of ganoderma of various origins was analyzed by HPLC (conditions: see Experimental) with thymol as an internal standard. The peaks corresponding to **2** and **3** were sharp and well separated from other peaks, as shown in Fig. 3. The contents of **2** and **3** of each sample were calculated from the peak heights of the chromatogram (Table III). The results indicated that the maximum yield of extracts, as well as **2** and **3**, was obtained from the sample grown on cherry logs. However, as compared with cultivation of *Quercus variabilis*, the growth rate of the fruit body on cherry wood was slow and the yield was small. Bottle culture resulted in a fairly high content of active compounds, even though the shape of fruit body is poor. The caps were found to be the best source, followed by the stems, and the spores were the poorest source of these triterpene acids, although the HPLC or TLC patterns were essentially the same in all cases.

Experimental

Melting points are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a JEOL FX-270 instrument

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at 270 and 67.5 MHz, respectively (s, singlet; d, doublet; dd, double doublet).

Plant Material——Ganoderma (*Ganoderma lucidum*) was cultivated at Yoshida-cho, Hiroshima Prefecture, Japan.

Bioassay——The inhibiting effect of crude extract of ganoderma, and each fraction or isolated compound on histamine release from rat peritoneal mast cells was assayed by the method already described.[1]

Extraction and Separation of the Constituents of Ganoderma (Fig. 1)——Dried fruit bodies of ganoderma (1.9 kg) were crushed and extracted with *n*-hexane (18 l × 3), MeOH (18 l × 4), and H_2O (18 l × 3), successively. From the hexane extract, ergosterol (mp 151—155 °C) was isolated and shown to be identical with an authentic sample by TLC and ^{13}C-NMR comparisons.[8] From the MeOH extract, mannitol was isolated during the concentration procedure as colorless needles, mp 161 °C (identified by TLC and ^{13}C-NMR[9]). The MeOH extract was suspended in water and extracted with EtOAc. The EtOAc layer was evaporated to dryness and the residue (66 g) was chromatographed on silica (Si) gel. Elution with $CHCl_3$–MeOH–H_2O (300 : 40 : 3) gave five fractions. From the first fraction, ergosterol (320 mg) was obtained. The second fraction (5 g) was purified by Si gel chromatography, HPLC and low-pressure liquid chromatography (LPLC) to afford **4** (29 mg). A part (8 g) of the third fraction (12 g) was chromatographed on Si gel. Elution with benzene–MeOH (5 : 1) gave **2** (115 mg). The fourth fraction (3.6 g) was chromatographed on Si gel. Elution with $CHCl_3$–MeOH (12 : 1—1 : 1) afforded **1** (740 mg). The fifth fraction (3.9 g) was purified by repeated Si gel chromatography (benzene–MeOH–H_2O, 80 : 40 : 1 and $CHCl_3$–MeOH, 15 : 1) to give **3** (321 mg).

Ganoderic Acid A (1)——mp 233—236 °C (from MeOH). *Anal.* Calcd for $C_{30}H_{44}O_7$: C, 69.73; H, 8.59. Found: C, 69.51; H, 8.73. MS *m/z*: 516.3071 (Calcd 516.3087). $[\alpha]_D^{15}$ +150.0° (c=0.13, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 254 (3.70). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3400, 2700—2300, 1700, 1655, 1270, 1000. ^1H-NMR ($CDCl_3$) δ: essentially identical with the literature values.[6] ^{13}C-NMR data are shown in Table I.

Methyl Ganoderate A (1a)——Compound **1** (64 mg) was methylated with ethereal diazomethane to give **1a**, (55 mg), mp 199—201 °C (from EtOH) [lit. 196—197 °C].[6] High-resolution MS *m/z*: Calcd for $C_{31}H_{46}O_7$: 530.3243. Found: 530.3235. ^{13}C-NMR data are shown in Table I.

Ganoderic Acid B (2)——mp 233—235 °C (from MeOH). *Anal.* Calcd for $C_{30}H_{44}O_7$: C, 69.73; H, 8.59. Found: C, 69.19; H, 8.24. High resolution MS *m/z*: Calcd for $C_{30}H_{44}O_7$: 516.3087. Found: 516.3094. $[\alpha]_D^{15}$ +129.7° (c=0.12, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 254 (3.85). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3400, 2600—2400, 1720, 1700, 1640.

Methyl Ganoderate B (2a)——Compound **2** (115 mg) was methylated with ethereal diazomethane to give **2a**, (57 mg), mp 203—205 °C (from EtOAc) [lit. 202.5—203 °C].[6] High resolution MS *m/z*: Calcd for $C_{31}H_{46}O_7$: 530.3243. Found: 530.3217. ^1H-NMR ($CDCl_3$) δ: essentially identical with the literature values, except for δ 0.85 (s, 3H), instead of 1.84 (see text).[6] ^{13}C-NMR data are shown in Table I.

Ganoderic Acid C (3)——mp 231—232 °C (from MeOH). *Anal.* Calcd for $C_{30}H_{46}O_7$: C, 69.47; H, 8.94. Found: C, 69.81; H, 8.92. High-resolution MS *m/z*: Calcd for $C_{30}H_{46}O_7$: 518.3244. Found: 518.3245. $[\alpha]_D^{15}$ +95.5° (c=0.147, MeOH). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm (log ε): 254 (3.91).

Methyl Ganoderate C (3a)——Compound **3** (100 mg) was methylated with ethereal diazomethane followed by Si gel column chromatography to give **3a** (18 mg), mp 187—190 °C (from hexane–EtOAc). *Anal.* Calcd for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.69; H, 9.16. High resolution MS *m/z*: Calcd for $C_{31}H_{48}O_7$: 532.3400. Found: 532.3391. ^1H-NMR (in $CDCl_3$) δ: 4.74 (15C-H, dd, J=9.1, 6.6 Hz), 4.55 (7C-H, dd, J=7.2, 9.7 Hz), 3.20 (3C-H, dd, J=11, 5.5 Hz), 1.243 (32CH_3, s), 1.241 (19CH_3, s), 1.18 (27CH_3, d, J=7 Hz), 1.02 (30CH_3, s), 0.96 (18CH_3, s), 0.87 (21CH_3, d, J=7 Hz), 0.84 (31CH_3, s). ^{13}C-NMR data are shown in Table I.

Ganoderic Acid D (4)——mp 150—151 °C (from MeOH). $[\alpha]_D^{15}$ +175.4° (c=0.057, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 254 (3.87). IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3450, 2700—2300, 1720, 1700, 1655, 1455. ^1H-NMR (in $CDCl_3$) δ: 4.85 (7C-H, triplet like J=8.8 Hz). ^{13}C-NMR data are shown in Table I.

Methyl Ganoderate D (4a)——Compound **4** (21 mg) was methylated with ethereal diazomethane to give **4a** (18 mg) mp 171—172 °C (from EtOH). High resolution MS *m/z*: Calcd for $C_{31}H_{44}O_7$: 528.3084. Found: 528.3069. ^1H-NMR ($CDCl_3$) δ: 4.84 (7C-H, dd, J=9.1, 7.9 Hz), 1.34 (32CH_3, s), 1.26 (19CH_3, s), 1.19 (27CH_3, d, J=7 Hz), 1.11 (30CH_3, s), 1.10 (31CH_3, s), 1.03 (18CH_3, s), 1.00 (21CH_3, d, J=7 Hz). ^{13}C-NMR data are shown in Table I.

Synthesis of Methyl 3,7,11,15,23-Pentaoxo-5α-lanost-8-en-26-oate (5a)——(i) From **2a**: A solution of **2a** (15 mg) in pyridine was treated with CrO_3–pyridine at room temp. overnight. The reaction mixture was diluted with ether and washed with 1% KOH, 1% HCl and water successively. The ether layer was dried with Na_2SO_4 and evaporated. The residue was crystallized from MeOH to give **5a** (4 mg), mp 189—191 °C [lit. 182.5—183.5 °C].[6] High resolution MS *m/z*: Calcd for $C_{31}H_{42}O_7$: 526.2931. Found: 526.2936. $[\alpha]_D^{15}$ +142.1° (c=0.063, $CHCl_3$). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 254 (3.69). ^1H-NMR (in $CDCl_3$) essentially identical with the literature values.[6] ^{13}C-NMR data are shown in Table I.

(ii) From **3a**: Compound **3a** (76 mg) was oxidized as described above to give 15 mg of **5a**. mp 189—194 °C. High-resolution MS *m/z*: 526.2912. ^{13}C-NMR: superimposable on that of the compound obtained in (i).

Quantitative Determination of Ganoderic Acids——A sample of powdered ganoderma (100 mg) was extracted with boiling EtOAc (10 ml, 30 min × 4), and the combined extract was evaporated to dryness *in vacuo*. The concentrated extract was weighed and diluted with a standard solution of thymol in MeOH (1.0 mg/ml) to give about 3.0 mg ext./ml of sample solution. Ten μg of this solution was injected into the HPLC system.—Apparatus, HLC-803A (Toyo Soda Co., Ltd.); column, Prepacked column (300 × 4 mm i.d.) of 5 μm TSK gel LS-410 (Toyo Soda Co.,

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Ltd.); detector, UV-8 model II spectrometer (Toyo Soda Co., Ltd.) set at 252 nm; mobile phase, 2% AcOH–CH_3CN (2 : 1); flow rate, 1 ml/min at 41 °C. Amounts were calculated by the peak height ratio with respect to the internal standard, thymol.

Acknowledgement We thank Dr. A. Ogiso and Mr. T. Takagaki, Sankyo Co., Ltd. for the measurement of high-resolution mass spectra (MS) and elemental analysis. We are also grateful to Dr. Y. Asaka, School of Medicine, Kinki University for providing NMR charts of ganoderic acids and derivatives, and to Messrs. M. Ishikawa, K. Nakamae, H. Shimooku and T. Sumihara of Yoshida-cho, Hiroshima Prefecture for the cultivation of ganoderma.

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