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Constituents of the Fungus *Ganoderma lucidum* (FR.) KARST. I. Structures of Ganoderic Acids C2, E, I, and K, Lucidenic Acid F and Related Compounds[1)]

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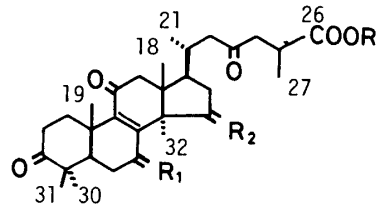
Sixteen new triterpene acids were isolated as the methyl esters from the gills of *Ganoderma lucidum* (Polyporaceae) along with five known triterpenes. The structures of seven new compounds among them, ganoderic acids C2, E, I, and K, compounds B8 and B9 and lucidenic acid F, were elucidated. Detailed analyses of their proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were also done by application of two-dimensional NMR techniques.

Keywords——*Ganoderma lucidum*; triterpene acid; ganoderic acid; lucidenic acid; ganolucidic acid; 2-D NMR; ^{1}H–^{1}H shift correlation NMR; ^{1}H–^{13}C shift correlation NMR; NOE; mass spectrum

Much attention has recently been paid to the biologically active constituents of the fungus *Ganoderma lucidum* (FR.) KARST. (Polyporaceae), which is widely used as a home remedy (Rëishi), and many bitter triterpenoids have been isolated from this fungus.[2-4)] In the course of our research on oriental medicines, we investigated the constituents of this fungus and isolated twelve new lanostane-type triterpenes, named ganoderic acids C2, E—I, and K, lucidenic acids D2, E2, and F, ganolucidic acids A and B and four related compounds tentatively named compounds B8, B9, C5′,[5)] and C6.[1,6)] More recently, Yamasaki *et al.*,[7)] Sakamura *et al.*,[8)] and Furuya *et al.*[9)] independently obtained several new triterpenes from the same fungus and some of the compounds isolated by the four groups were found to be identical.[6)] In this paper we wish to present full details of the isolation of the new triterpenes and of the structure elucidation of ganoderic acids C2 (**2a**), E (**1a**), I (**4a**), and K (**3a**), lucidenic acid F (**7a**), and compounds B8 (**5a**) and B9 (**6a**).[10)]

The surface part of the gills of dried fruit bodies of *Ganoderma lucidum* was scraped off with a small grinder and the powder[11)] obtained was extracted with ether. The extract was separated into an acidic fraction and a neutral one in the usual manner and the acidic fraction was methylated with diazomethane. The crude product was separated repeatedly by a combination of silica gel column chromatography and thin layer chromatography (TLC) to give sixteen new triterpenes (see the experimental section), together with several known compounds: methyl ganoderates A (**8b**), B (**10b**),[2)] and Cl (**9b**) and methyl lucidenates A (**11b**) and C (**13b**).[4)]

Prior to analyzing the structures of the new compounds, **1**—**7**, we carried out a re-examination of the proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra of the known compounds, **8b**—**11b**, by means of two-dimensional (2-D) NMR techniques; that is, assignments of the ^{1}H (400 MHz) and ^{13}C (100 MHz) signals were performed through measurements of the ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation spectra (Tables I and II).[12)] For example, the ^{1}H–^{1}H shift-correlated spectra of methyl ganoderate A (**8b**)

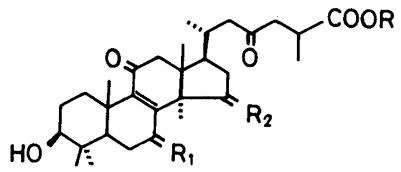


1: $R_1 = R_2 = O$

8: $R_1 = \alpha\text{-OH}$, $R_2 = \beta\text{-OH}$

9: $R_1 = \alpha\text{-OH}$, $R_2 = O$

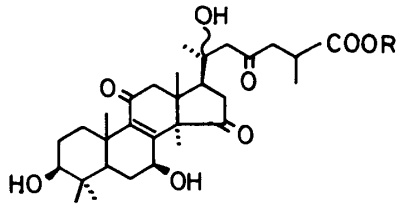
12: $R_1 = O$, $R_2 = \beta\text{-OH}$



2: $R_1 = \alpha\text{-OH}$, $R_2 = \beta\text{-H}$

3: $R_1 = O$, $R_2 = \beta\text{-OH}$

10: $R_1 = \alpha\text{-OH}$, $R_2 = O$



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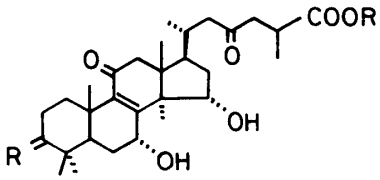
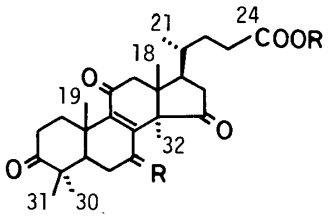


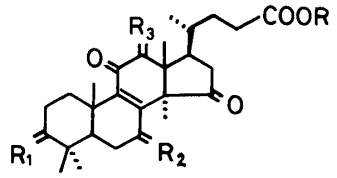
5: R = O

6: R = α-OH



7: R = O

11: R = α-OH



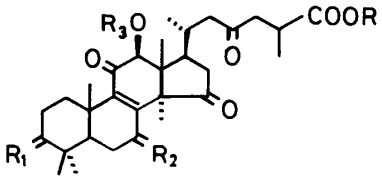
13: $R_1 = R_2 = \alpha\text{-OH}$, $R_3 = \alpha\text{-OH}$

19: $R_1 = R_2 = O$, $R_3 = \alpha\text{-OAc}$

20: $R_1 = \alpha\text{-OH}$, $R_2 = O$, $R_3 = \alpha\text{-OAc}$

21: $R_1 = R_2 = R_3 = O$

22: $R_1 = O$, $R_2 = \alpha\text{-OH}$, $R_3 = \beta\text{-OH}$



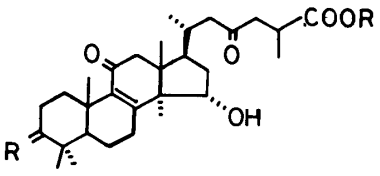
14: $R_1 = R_2 = O$, $R_3 = Ac$

15: $R_1 = R_2 = \alpha\text{-OH}$, $R_3 = H$

16: $R_1 = \alpha\text{-OH}$, $R_2 = O$, $R_3 = Ac$

17: $R_1 = \alpha\text{-OH}$, $R_2 = O$, $R_3 = H$

18: $R_1 = O$, $R_2 = \alpha\text{-OH}$, $R_3 = H$



23: R = O

24: R = α-OH

(**a**: R = H, **b**: $R = CH_3$)

Chart 1

allowed us to assign most of the ^{1}H-signals as illustrated in Figs. 1 and 2. In particular, the signals due to methyl groups, except the 30- and 31-methyls, were precisely assigned on the basis of the presence of long-range coupling between 19-CH_3 and 1α-H, 18-CH_3 and 12α-H, and 21-CH_3 and 22-H. The assignments of the 30- and 31-methyl signals were done by measurements of the nuclear Overhauser effect (NOE) difference spectra; *i.e.*, irradiation of the methyl signal at δ 1.12 gave appreciable NOE increases of the 5-H and 6α-H signals (δ 1.67 and 2.06, respectively), while irradiation of the methyl signal at δ 1.10 gave a small NOE

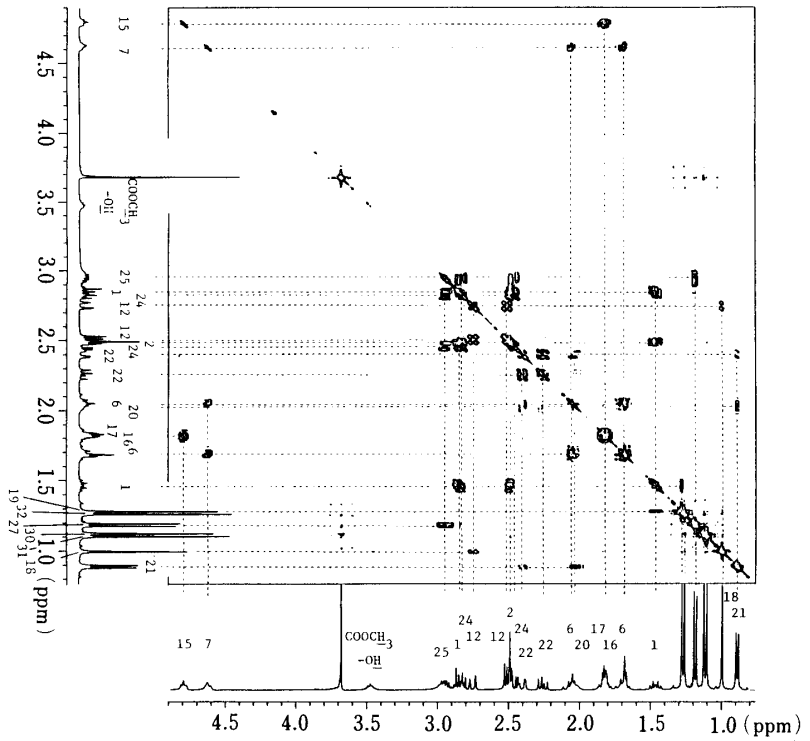


Fig. 1. Contour Map of the ^{1}H–^{1}H Shift-Correlated Spectrum of Methyl Ganoderate A (**8b**)

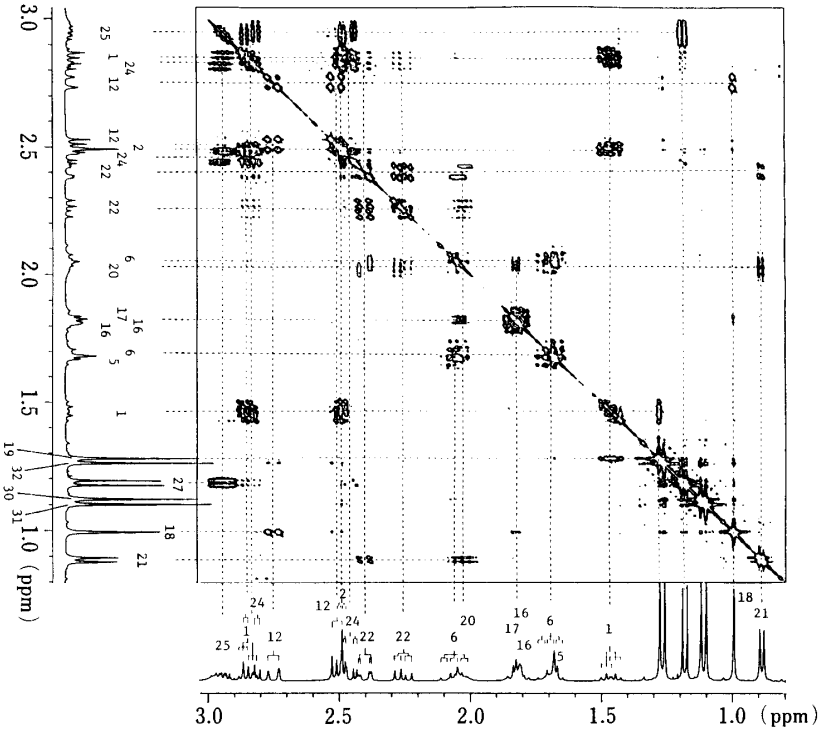


Fig. 2. Contour Map of the Highly Resolved ^{1}H–^{1}H Shift-Correlated Spectrum of Methyl Ganoderate A (**8b**) in the Upfield Region

increase of the 6β-H signal (δ 1.69).

The ^{1}H–^{13}C shift correlation spectrum of **8b** (Fig. 3) led readily to precise assignments of the ^{13}C-signals, but the assignments of quaternary carbon signals (C-4, -10, -13, and -14) were

TABLE I. ^{1}H-NMR Spectral Data for Lanostane-Type

Compd. Proton	**8b**[a]	**9b**[a]	**12b**[a]	**1b**[a]	**5b**[a]	**10b**[a]
1-H (α)	1.46[b] dt (14, 8)	1.47[b] dt (13.5, 8.5)	1.80[b] ddd (14.3, 9.4, 6.7)	1.74[b] ddd (14, 9.5, 6)	1.71[b] dt (15, 8)	0.98[b] td (14, 5.5)
1-H (β)	2.85 dt (14, 7)	2.95 ddd (13.5, 7.5, 5.5)	2.97 ddd (14.3, 8.6, 6)	2.87 ddd (14, 8, 5.5)	2.97 ddd (14, 8.5, 5.5)	2.83 dt (13.5, 3.5)
2-H (α)	2.45 dd (8, 7)	2.46 ddd (16, 8, 5.5)	2.52 ddd (15.2, 8.5, 7)	2.47 ddd (15, 8, 6.5)	2.45 ddd (15.5, 9, 7)	*ca.* 1.60
2-H (β)		2.54 ddd (16, 8.5, 7.5)	2.62 ddd (15.2, 9.1, 5.8)	2.61 ddd (15, 9.7, 6)	2.61 ddd (15.5, 9.5, 6)	*ca.* 1.66
3-H						3.20 dd (10.5, 5.5)
5-H	*ca.* 1.67	1.56 dd (13, 1.5)	2.26 dd (14.9, 2.7)	2.32 dd (14.8, 2.8)	2.09 dd (12.5, 3)	0.87 dd (13, 1.5)
6-H (α)	*ca.* 1.69	2.11 ddd (13, 8, 1.5)	2.47 dd (15, 2.5)	2.47 dd (13.4, 2.8)	*ca.* 1.72 m	2.18 ddd (13, 8, 1.5)
6-H (β)	*ca.* 2.06	1.67 td (13, 9.2)	2.62 t (15.2)	2.69 dd (15, 13.4)	*ca.* 1.78 m	1.61 td (13, 9.5)
7-H (α)	4.62 dd (10, 6.2)	4.87 ddd (9.2, 8, 4.5)				4.79 ddd (9.5, 8, 4.5)
7-H (β)					4.58 dd (5, 1.8)	
12-H (α)	2.75[c] br d (16)	2.77[c] br d (17.5)	2.82[c] br d (17.7)	2.87[c] br d (16.2)	2.75[c] br d (18)	2.77[c] br d (17)
12-H (β)	2.50 d (16)	2.72 d (17.5)	2.57 d (17.7)	2.75 d (16.2)	2.42 d (17.7)	2.69 d (17)
15-H	4.80 br t (8)		4.29 td (7.3, 1.5)		4.605 dd (9, 6)	
16-H	*ca.* 1.82	*ca.* 2.07	1.87 d-like	1.86 dd (18.2, 8.4)	*ca.* 1.82	2.05 m
16-H		2.68		2.74 dd (18.2, 9.1)	*ca.* 1.95	*ca.* 2.67 m
17-H	*ca.* 1.83 m	*ca.* 2.13 m	*ca.* 1.87	2.24 dt (10, 8.5)	*ca.* 1.95	*ca.* 2.12 m
20-H	*ca.* 2.03 m	*ca.* 2.16 m	2.01 m	2.10 m	2.00 m	*ca.* 2.14 m
22-H	2.26 dd (16.5, 9.2)	2.37 d (5)	2.265 dd (16.4, 9.4)	2.359 d (6.7)	2.27 dd (16.3, 8.5)	2.36 d (6.4)
22-H	2.40[d] dd (16.5, 2.8)		2.42[d] dd (16, 3)	2.361[d] d (4.9)	2.38[d] dd (16, 3)	2.362[d] d (4.6)
23-H						
23-H						
24-H	2.46 dd (17.5, 5.5)	2.44 dd (17.5, 5)	2.46 dd (17.7, 5.2)	2.43 dd (17.7, 5.2)	2.46 dd (17.3, 5)	2.43 dd (17.5, 5)
24-H	2.83 dd (17.5, 8.5)	2.85 dd (17.5, 8.8)	2.83 dd (17.5, 8.2)	2.83 dd (17.7, 9)	2.84 dd (17.5, 8.5)	2.85 dd (17.5, 8.5)
25-H	2.95 dqd (8.5, 7, 5.5)	2.96 dqd (9, 7, 5)	2.95 dqd (8.1, 7.2, 4.8)	2.96 dqd (9, 7, 5)	2.95 m	2.96 dqd (8.8, 7, 5)
18-H_3	0.99	1.03	0.90	0.89	0.89	1.00
19-H_3	1.28	1.26	1.27	1.28	1.03	1.22
21-H_3	0.89 d (6)	1.00 d (6)	0.87 d (6.4)	0.98 d (6.4)	0.86 d (5.8)	0.99 d (6)
27-H_3	1.18 d (7)	1.19 d (7)	1.18 d (7)	1.19 d (7)	1.18 d (7)	1.19 d (7)
30-H_3	1.12[e]	1.13	1.14	1.14[e]	1.16[e]	1.03[e]
31-H_3	1.10	1.11	1.12	1.12	1.08	0.85
32-H_3	1.26	1.34	1.19	1.64	1.30	1.34
$COOCH_3$	3.68	3.69	3.68	3.68	3.68	3.68
OH		4.055 d (4.5)	4.45 d (1.8)			4.03 d (4.5)

δ values in $CDCl_3$ and coupling constants in Hz. *a*) ^{1}H-^{1}H Correlation spectra were measured. *b*—*d*) Long-range coupling

Triterpenes from *Ganoderma lucidum* and Their Derivatives

2b[a]	**3b**[a]	**4b**[a]	**6b**[a]	**7b**[a]	**11b**[a]
ca. 0.93[b]	1.28 td	0.98[b] td	1.17[b] td	1.75[b] ddd	1.48[b] dt
	(13, 4.5)	(13, 5)	(13.5, 4.5)	(14, 9, 6)	(14, 7.5)
2.74 dt	2.84 dt	2.84 dt	3.00 dt	2.89 ddd	2.95 ddd
(13.5, 4)	(13.5, 3.5)	(13, 3.5)	(14, 3.5)	(13.5, 9, 6.5)	(13, 7.5, 5)
ca. 1.60	1.77 dq		*ca.* 1.65 m	2.48 ddd	2.53 ddd
	(13, 4.5)	1.65 m		(15.5, 9, 6)	(16, 8, 7)
ca. 1.66	1.69 tdd		*ca.* 1.72 br d-like	2.62 ddd	2.47 ddd
	(13, 11.5, 3.5)			(16, 9.8, 6.2)	(15.5, 8, 5.5)
3.20 dd	3.28 br dd	3.22 dd	3.31 dd		
(10.8, 5.8)	(11, 5)	(11, 6.5)	(11.5, 5)		
0.92 dd	1.54 dd	0.87 dd	1.28 dd	2.32 dd	1.57 ddd
(13, 1)	(12.5, 4.5)	(13.5, 1.5)	(13, 2)	(15, 2.5)	(13, 1.8)
2.14 ddd	2.54 dd	2.185 ddd	1.83 br d	2.48 dd	2.11 ddd
(12.5, 7.5, 1)	(16, 4.5)	(13.5, 8, 1.5)	(14)	(13.5, 2.5)	(13, 7.8, 1.8)
1.59 td	2.59 dd	1.62 td	1.73 td	2.70 dd	1.68 td
(12.5, 10.5)	(16, 13)	(13.5, 9.5)	(13.5, 5)	(15, 13.5)	(13, 9.5)
4.54 dd		4.80 ddd			4.85 ddd
(10.3, 7.5)		(9.5, 8, 4.5)			(9.5, 7.5, 4.5)
			4.56 br d		
			(5)		
2.76[c] br d	2.83[c] d	2.79[c]	2.75[c] br d	2.88[c] d	2.77[c] d
(15.5)	(17)	ABq	(17.5)	(16.5)	(17.5)
2.46 d	2.54 d	2.80 (17)	2.39 d	2.77 d	2.73 d
(15.5)	(17)		(17.5)	(16.5)	(18)
4.74 br t	4.335 td		4.57 dd		
(7.5)	(7.5, 1.8)		(9, 5.5)		
		2.50 dd	*ca.* 1.78	1.95 dd	2.16 dd
ca. 1.78	1.84 d-like	(19.5, 8.3)		(18, 8)	(19.5, 9.5)
		2.81 dd	*ca.* 1.94	2.84 dd	2.80 dd
		(19.5, 10)		(18.2, 9)	(19.5, 8.2)
ca. 1.80	*ca.* 1.85	2.25 dd	*ca.* 1.92 m	2.125 td	2.00 td
		(10, 9)		(9, 8)	(9.5, 8.5)
2.02 m	2.01 m		2.00 m	1.50 m	1.57 m
2.24 dd	2.245 dd	2.48[d] d	2.25 dd	1.35 dtd	1.36 dtd
(16.5, 9.5)	(16.5, 9.5)	(16.5)	(16.2, 9)	(14, 9, 5.5)	(14, 9, 5.5)
2.40[d] dd	2.41[d] dd	2.64 d	2.38[d] dd	1.78[d] dddd	1.79[d] dddd
(16.5, 3)	(16.5, 3)	(16.5)	(16.2, 3)	(13.5, 9, 7, 3)	(13.5, 9, 7, 2.5)
				2.27 ddd	2.29 ddd
				(16, 9, 7.5)	(16, 8.5, 7.5)
				2.40 ddd	2.41 ddd
				(16, 9, 5.5)	(16, 9, 5.5)
2.46 dd	2.455 dd	2.45 dd	2.46 dd		
(17.8, 5)	(17.5, 5)	(17.5, 4.5)	(17.5, 5)		
2.83 dd	2.823 dd	2.88 dd	2.83 dd		
(17.8, 9)	(17.5, 8.5)	(17.5, 9)	(17.5, 8.5)		
2.94 dqd	2.945 dqd	2.96 dqd	2.94 dqd		
(9, 7, 5)	(8.5, 7, 5)	(8.8, 7, 4.5)	(9, 7.5, 5.5)		
0.96	0.89	1.14	0.87	0.86	1.01
1.25	1.29	1.22	1.04	1.28	1.26
0.88 d (6.5)	0.87 d (6.5)	1.40 s	0.85 d (6)	0.96 d (6.7)	0.97 d (6.7)
1.18 d (7.5)	1.18 d (7)	1.20 d (7)	1.18 d (7)	—	—
1.02[e]	1.03	1.03	1.06	1.14	1.13
0.84	0.89	0.85	0.84	1.11	1.11
1.24	1.14	1.34	1.27	1.65	1.34
3.68	3.67	3.69	3.68	3.68	3.68
	4.485 d (2)	4.07 d (4.5)			4.11 d (4.5)

was observed with 19-Me, 18-Me, and 21-Me, respectively. *e*) Assignments were confirmed by the NOE experiment.

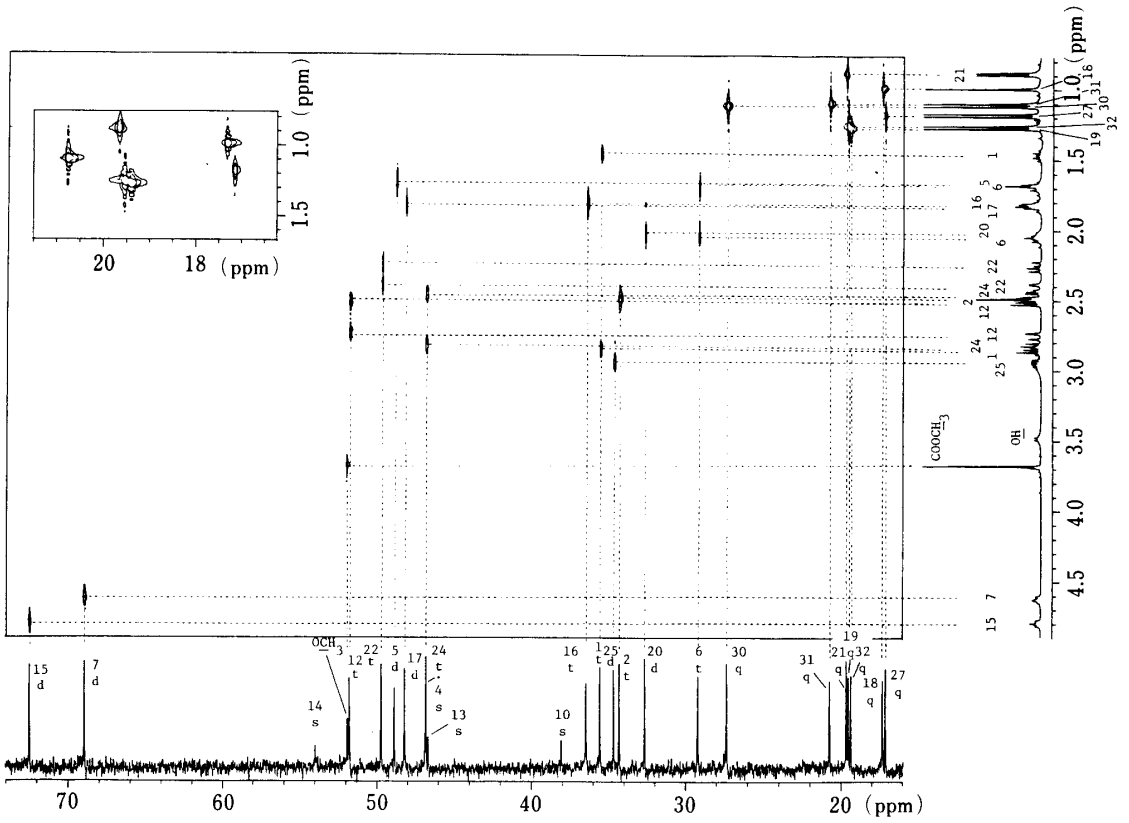


Fig. 3. Contour Map of the ^{1}H–^{13}C Shift-Correlated Spectrum of Methyl Ganoderate A (**8b**) in the Upfield Region

The ^{1}H shifts are shown on the ordinate and the ^{13}C shifts on the abscissa. The multiplicities of carbon signals were determined by means of off-resonance, and are indicated as s, d, t, and q.

based on the reported shift values[2] and comparisons of the ^{13}C-NMR patterns of **8b**—**11b** and the 3,7,11,23-tetraoxo ester (**12b**: methyl ganoderate J[8c]), prepared by allylic oxidation[13] of **8b**.

Methyl ganoderate E (**1b**), $C_{31}H_{42}O_7$, was obtained as yellow needles, mp 206—208.5 °C, $[\alpha]_D$ +167° ($CHCl_3$), and showed an ultraviolet (UV) absorption at 251.5 nm (log ε 3.93) and infrared (IR) absorptions at 1750 (five-membered ketone), 1720 (ester), 1710 (six-membered ketone), and 1680 (conj. ketone) cm^{-1}. It showed the molecular ion peak at *m/z* 526 along with significant fragment ion peaks[2] in the mass spectrum (MS), which was similar to that of **12b** (Table III). The ^{1}H- and ^{13}C-NMR spectra were also similar to those of **12b** and **8b**, and they were fully analyzed by measurements of the ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation spectra (Figs. 4 and 6) and NOE difference spectra (Fig. 5). Eventually this compound was found to be identical with the pentaoxo ester (**1b**)[2] obtained by oxidation of **8b**.

Methyl ganoderate C2 (**2b**), $C_{31}H_{48}O_7$, mp 199—202 °C, $[\alpha]_D$ +98° ($CHCl_3$), showed a UV absorption band at 253 nm (log ε 4.01) and IR absorptions at 3450 (hydroxyl), 1720 (ester), 1710 (ketone), 1700, and 1650 cm^{-1} (conj. ketone). In the MS it showed the molecular ion peak at *m/z* 532 together with several fragment ion peaks (Table III), and the spectral pattern resembled that of **8b** (see Chart 2). The ^{1}H-NMR spectrum of **2b** exhibited signals due to three carbinol methine protons (δ 3.20, 4.54, and 4.74), two *sec*-methyls, and five *tert*-methyls, which were assigned by measurements of the ^{1}H–^{1}H shift correlation (Figs. 7 and 8) and the NOE difference spectra. The ^{1}H–^{13}C shift correlation spectrum was also recorded (Fig. 9), and the results are given in Tables I and II. These data clearly indicated that **2b** is the 3β-hydroxy derivative of methyl ganoderate A (**8b**), or the 15α-hydroxy derivative of methyl

TABLE II. ^{13}C-NMR Spectral Data for Lanostane-Type Triterpenes from *Ganoderma lucidum* and Their Derivatives

Compd. ^{13}C	**8b**[a]	**9b**[a]	**12b**	**1b**[a]	**5b**	**10b**[a]	**2b**[a]	**3b**	**4b**	**6b**[d]	**7b**	**11b**[a]
1	35.5 t	35.5 t	35.2 t	34.6 t	34.8 t	34.9 t	34.6 t	34.3 t	34.9 t	34.2	34.6 t	35.7 t
2	34.3 t	34.1 t	34.0 t	33.8 t	34.1 t	27.7 t	27.8 t	27.7 t	27.8 t	27.3	33.9 t	34.3 t
3	217.3 s	217.3 s	214.8 s	217.2 s	217.8 s	78.3 d	78.2 d	77.5 d	78.4 d	78.5	215.3 s	217.9 s
4	46.8[b] s	46.7 s	46.6 s	47.0[b] s	47.2 s	38.9[b] s	38.6[b] s	40.2 s	38.9[b] s	39.1	47.0 s	46.8 s
5	48.7 d	48.7 d	49.3 d	50.9 d	45.2[c] d	49.2 d	49.1 d	49.8[c] d	49.2 d	47.7	51.0 d	49.0 t
6	29.0 t	27.6 t	37.0 t	37.3 t	27.9 t	26.7 t	28.2 t	36.5 t	26.7 t	28.0	37.3 t	27.7 t
7	68.9 d	66.1 d	204.5 s	199.3 s	66.7 d	66.9 d	69.5 d	205.3 s	66.9 d	68.0	199.5[b] s	66.3 d
8	159.3 s	157.7 s	152.6 s	149.7 s	159.3 s	156.9 s	158.1 s	154.6 s	156.6 s	158.8	149.7 s	157.9 s
9	140.1 s	141.0 s	150.9 s	146.8 s	140.0 s	142.7 s	141.9 s	149.8 s	142.3 s	141.6	146.7 s	141.2 s
10	38.0 s	38.1 s	39.3 s	39.4 s	38.0 s	38.7[b] s	38.5[b] s	38.9 s	38.7[b] s	38.6	39.4 s	38.3 s
11	199.6 s	197.4 s	201.0 s	199.3 s	199.1 s	197.8 s	199.9 s	201.3 s	197.8 s	199.4	199.4[b] s	197.7 s
12	51.7 t	50.0 t	51.9 t	48.9 t	51.8 t	50.3 t	51.9 t	52.3 t	50.7 t	52.2	49.0 t	50.3 t
13	46.6[b] s	44.8 s	47.7 s	43.9[b] s	46.4 s	45.3 s	47.1 s	48.0 s	45.7 s	46.1	43.9 s	45.0 s
14	54.0 s	59.2 s	52.8 s	57.2 s	53.4 s	59.4 s	54.0 s	52.8 s	59.7 s	53.5	57.2 s	59.4 s
15	72.4 d	216.3 s	72.1 d	206.8 s	72.4 d	217.4 s	72.5 d	72.1 d	217.7 s	72.3	207.3 s	216.5 s
16	36.2 t	40.8 t	36.4 t	39.8 t	37.8 t	40.9 t	36.1 t	36.3 t	36.1 t	37.8	39.9 t	41.1 t
17	48.1 d	45.6 d	48.1 d	44.5 d	49.0[c] d	45.6 d	48.1 d	48.2[c] d	49.3 d	49.0	45.2 d	46.3 d
18	17.3 q	17.5 q	17.5 q	16.1 q	17.5 q	17.4 q	17.1 q	17.4[c] q	19.0 q	17.3	16.1 q	17.7 q
19	19.4 q	18.0 q	17.8 q	18.6 q	17.5 q	18.5 q	19.6 q	17.6[c] q	18.4 q	17.3	18.6[c] q	18.2 q
20	32.7 d	31.8 d	32.4 d	32.0 d	32.5 d	32.0 d	32.7 d	32.4 d	73.0 s	32.5	35.4 d	35.2 d
21	19.6 q	19.5 q	19.5 q	19.8 q	19.3 q	19.7 q	19.6 q	19.5 q	26.7 q	19.3	18.3[c] q	18.1 q
22	49.7 t	48.9 t	49.5 t	49.1 t	49.6 t	49.1 t	49.7 t	49.5 t	52.7 t	49.6	30.8 t	30.7 t
23	208.4 s	207.6 s	208.2 s	207.6 s	208.3 s	207.7 s	208.7 s	208.2 s	210.4 s	208.3	31.0 t	30.9 t
24	46.8 t	46.6 t	46.8 t	46.7 t	46.9 t	46.8 t	46.7 t	46.8 t	47.7 t	46.9	173.8 s	173.9 s
25	34.6 d	34.4 d	34.7 d	34.7 d	34.7 d	34.7 d	34.6 d	34.7 d	34.5 d	34.7		
26	176.3 s	175.9 s	176.1 s	176.1 s	176.2 s	176.1 s	176.3 s	176.1 s	175.9 s	176.4		
27	17.1 q	16.9 q	17.1 q	17.1 q	17.1 q	17.1 q	17.1 q	17.1 q	17.0 q	17.1		
30	27.4 q	26.8 q	27.4 q	27.6 q	27.6 q	28.2 q	28.2 q	27.8 q	28.2 q	28.2	27.7 q	27.0 q
31	20.7 q	20.6 q	20.4[c] q	20.3 q	20.5 q	15.5 q	15.7 q	15.4[c] q	15.5 q	15.8	20.3 q	20.8 q
32	19.5 q	24.5 q	20.5[c] q	20.9 q	21.1[c] q	24.4 q	19.4 q	20.3 q	24.8 q	21.1	20.9 q	24.7 q
OCH_3	52.0 q	51.7 q	51.9 q	51.9 q	51.9 q	51.9 q	51.9 q	51.9 q	52.0 q	51.9	51.7 q	51.7 q

δ values in $CDCl_3$. The multiplicities of carbon signals are indicated as s, d, t, and q. *a*) ^{1}H–^{13}C Correlation spectra were measured. *b*) Assignments may be interchanged in each compound. *c*) Assignments were confirmed by the selective decoupling method. *d*) Only the complete decoupling spectrum was measured.

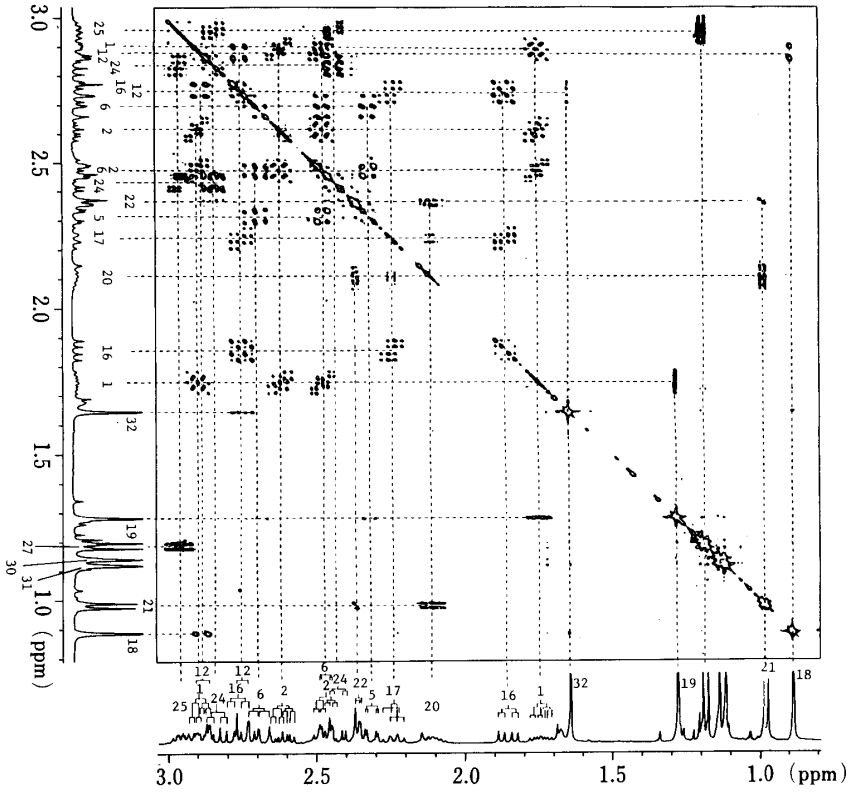


Fig. 4. Contour Map of the Highly Resolved ^1H–^1H Shift-Correlated Spectrum of Methyl Ganoderate E (**1b**) in the Upfield Region

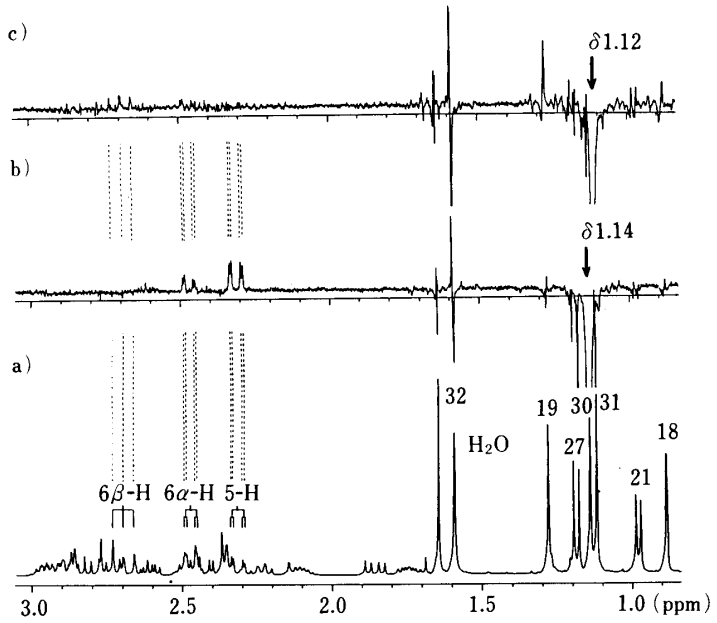


Fig. 5. ^1H-NMR (Normal and NOE) Spectra of Methyl Ganoderate E (**1b**)

a) Normal ^1H-NMR spectrum (400 MHz) of methyl ganoderate E (**1b**). b, c) NOE difference spectra of **1b** or irradiation at δ 1.14 and δ 1.12, respectively.

ganoderate B (**10b**). In addition, oxidation of **2b** with chromium trioxide in acetic acid afforded a pentaoxo ester (**1b**) which was identical with methyl ganoderate E (**1b**). Thus, methyl ganoderate C2 is methyl 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oate (**2b**).

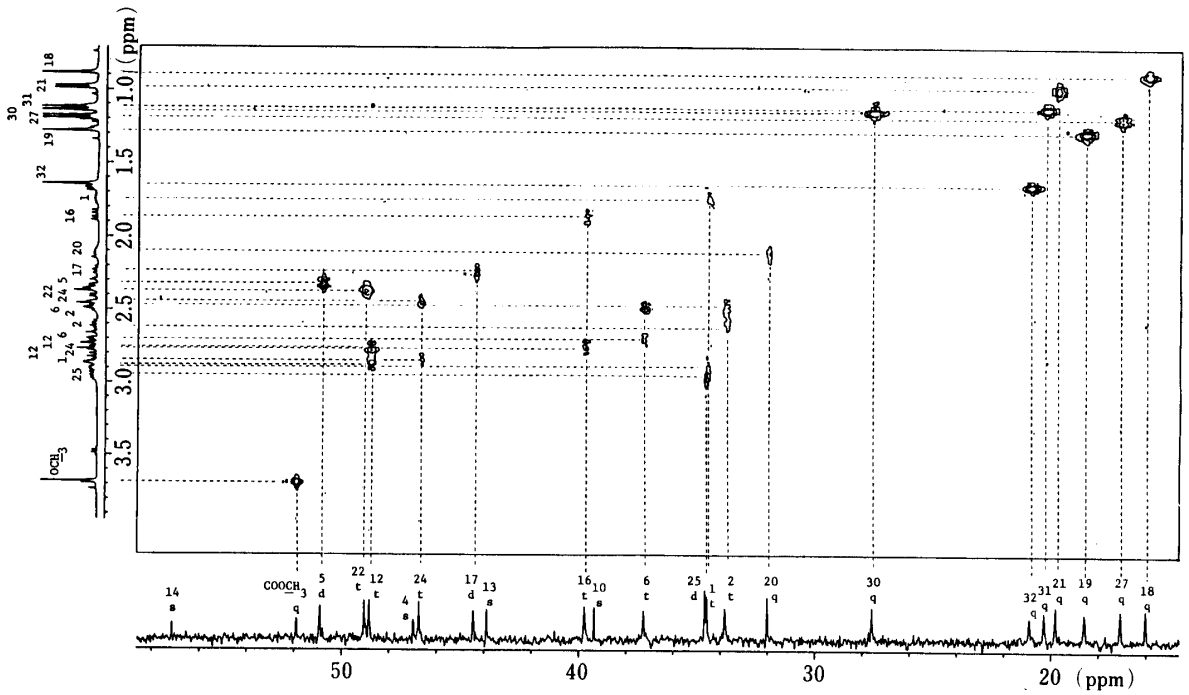
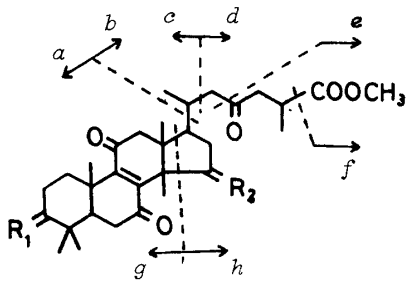
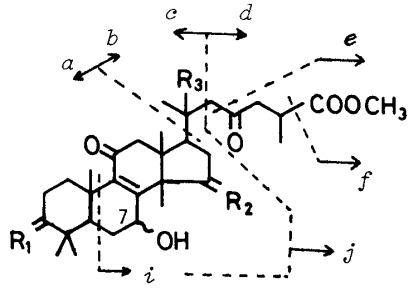


Fig. 6. Contour Map of the ^{1}H–^{13}C Shift-Correlated Spectrum of Methyl Ganoderate E (**1b**) in the Upfield Region

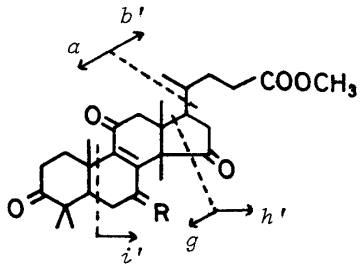
The ^{1}H shifts are shown on the ordinate and the ^{13}C shifts on the abscissa. The multiplicities of carbon signals were determined by means of off-resonance.



	R_1	R_2
1b:	O	O
3b:	OH (β)	OH (α)
12b:	O	OH (α)



	R_1	R_2	R_3	7
2b:	OH (β)	OH (α)	H	β
5b:	O	OH (α)	H	α
6b:	OH (β)	OH (α)	H	α
8b:	O	OH (α)	H	β
4b:	OH (β)	O	OH (ξ)	β
9b:	O	O	H	β
10b:	OH (β)	O	H	β



7b: R = O

11b: R = OH (β)

Chart 2

TABLE III. Mass Spectral Data for Triterpene Methyl Esters from *Ganoderma lucidum* (Relative Intensity in Parenthesis)

	1b	**3b**	**12b**	**2b**	**5b**	**6b**	**8b**	**4b**	**9b**	**10b**	**7b**	**11b**
M^+	526 (100)	530 (100)	528 (100)	532 (92)	530 (99)	532 (100)	530 (52)	546 (12)	528 (71)	530 (41)	470 (100)	472 (35)
M^+-CH_3	511 (2)								513 (7)	515 (3)	455 (5)	457 (10)
M^+-nH_2O	508 (7)	512 (12)		514 (52)	512 (24)	514 (29)	512 (33)	528 (10)	510 (16)	512 (10)		454 (7)
				496 (20)		496 (10)	494 (14)	510 (8)				
M^+-CO								500 (14)	500 (36)	502 (32)		444 (20)
M^+-H_2O–CO									482 (15)	484 (7)		
M^+-OCH_3	495 (20)	499 (8)	497 (10)								439 (8)	
a	355 (13)								357 (41)		355 (21)	
a-H									356 (87)	358 (49)		
a-H_2O		341 (13)										
a-CO	327 (5)							331 (32)	329 (44)	331 (24)	327 (4)	329 (100)
a-H_2O–CO								313 (28)	311 (10)	313 (5)		311 (7)
b (*b'*)	171 (8)	171 (26)	171 (24)	171 (37)	171 (34)	171 (35)	171 (39)		171 (26)	171 (15)	115 (13)	115 (16)
b-MeOH	139 (16)	139 (35)	139 (38)	139 (54)	139 (54)	139 (48)	139 (54)		139 (45)	139 (27)		
b'-HCOOMe											55 (65)	55 (39)
c	383 (27)						387 (12)					
c-H	382 (29)			388 (7)				402 (31)	384 (3)			
c-H–H_2O		368 (77)	366 (62)		368 (41)	370 (23)	368 (23)	384 (30)	366 (20)	368 (7)		
c-H–CO								374 (52)				
d+H	144 (8)	144 (6)	144 (14)	144 (30)	144 (50)	144 (25)	144 (42)	144 (18)	144 (10)	144 (5)		
e	129 (69)	129 (29)	129 (57)	129 (68)	129 (73)	129 (62)	129 (72)	129 (89)	129 (67)	129 (49)		
f	59 (79)	59 (41)	59 (67)	59 (100)	59 (100)	59 (96)	59 (100)	59 (100)	59 (100)	59 (100)		
g											300 (17)	
g+H	301 (18)	303 (11)	301 (24)								301 (14)	
h-H	225 (7)											
h'-H–CO											141 (65)	
h-H–MeOH	193 (12)											

TABLE III. (continued)

	1b	3b	12b	2b	5b	6b	8b	4b	9b	10b	7b	11b
h(*h'*)-H–CO–	165										109	
MeOH	(14)										(34)	
h'-H–CO–											81	
HCOOMe											(28)	
i (*i'*)				392	392	392	392		390	390		334
				(20)	(32)	(19)	(16)		(55)	(27)		(51)
i-CO				364	364	364	364		362			
				(39)	(47)	(33)	(32)		(3)			
j-H								262	246	246		
								(41)	(36)	(22)		
j-H–H_2O				230	230	230	230					
				(44)	(22)	(21)	(54)					

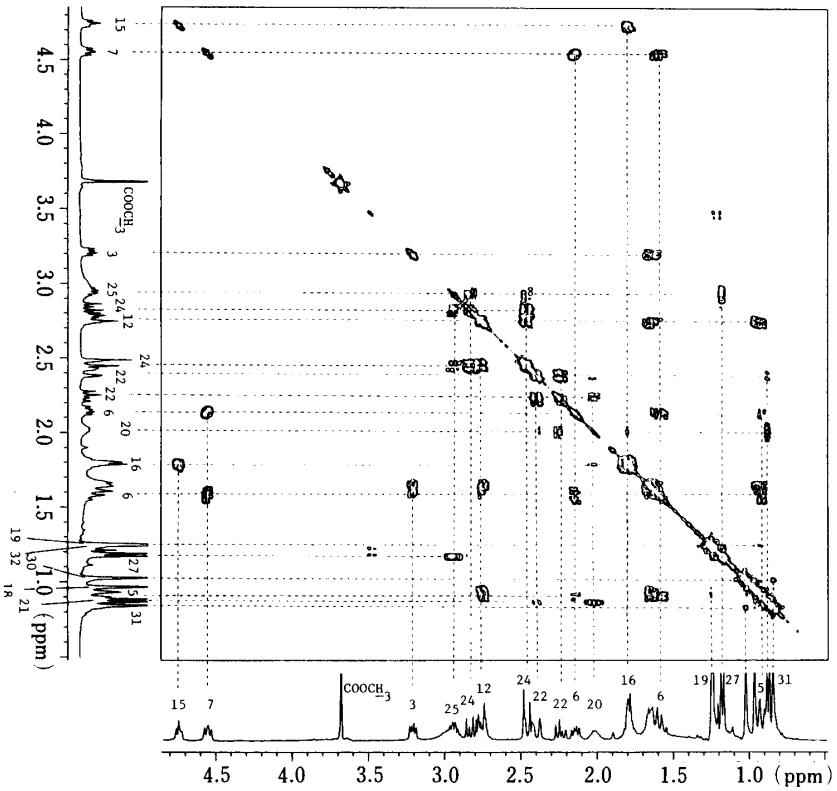


Fig. 7. Contour Map of the ^1H–^1H Shift-Correlated Spectrum of Methyl Ganoderate C2 (**2b**)

Methyl ganoderate K (**3b**), mp 166—167 °C, $[\alpha]_D$ +156° ($CHCl_3$), UV λ: 273 nm (log ε 3.68), and IR ν: 3450, 1720, 1710, and 1660 cm^{-1}, was obtained as a very minor component of *G. lucidum*. It showed the molecular ion peak at *m/z* 530 in the MS and its molecular formula was determined to be $C_{31}H_{46}O_7$ by high-resolution MS measurement. The ^1H-NMR spectrum of **3b** showed signals due to two carbinol methine protons (δ 3.28 and 4.34) and the whole spectral pattern resembled those of **2b** and **12b**. The ^{13}C-NMR spectrum was also similar to those of **2b** and **12b** (Tables I and II). Furthermore, the MS of **3b** exhibited a significant fragment peak at *m/z* 303 (*g*+H), which is suggestive of the 8-ene-7,11-dione structure (Chart 2). Finally, methyl ganoderate K was found to be identical with compound **3b** prepared by allylic oxidation of methyl ganoderate C2 (**2b**) by TLC and spectral

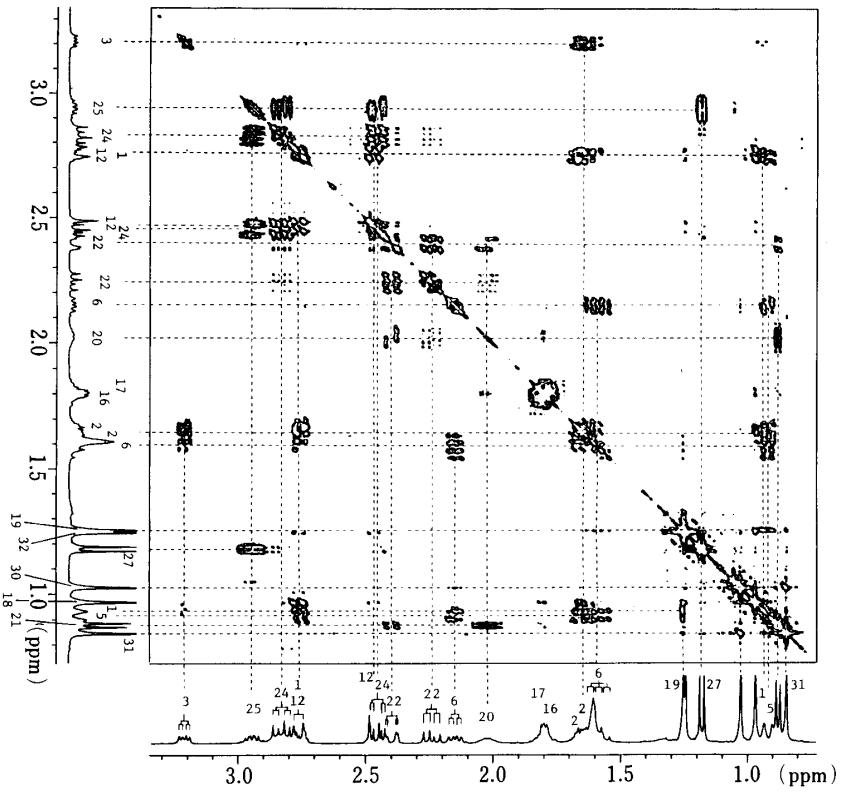


Fig. 8. Contour Map of the Highly Resolved ^{1}H–^{1}H Shift-Correlated Spectrum of Methyl Ganoderate C2 (**2b**) in the Upfield Region

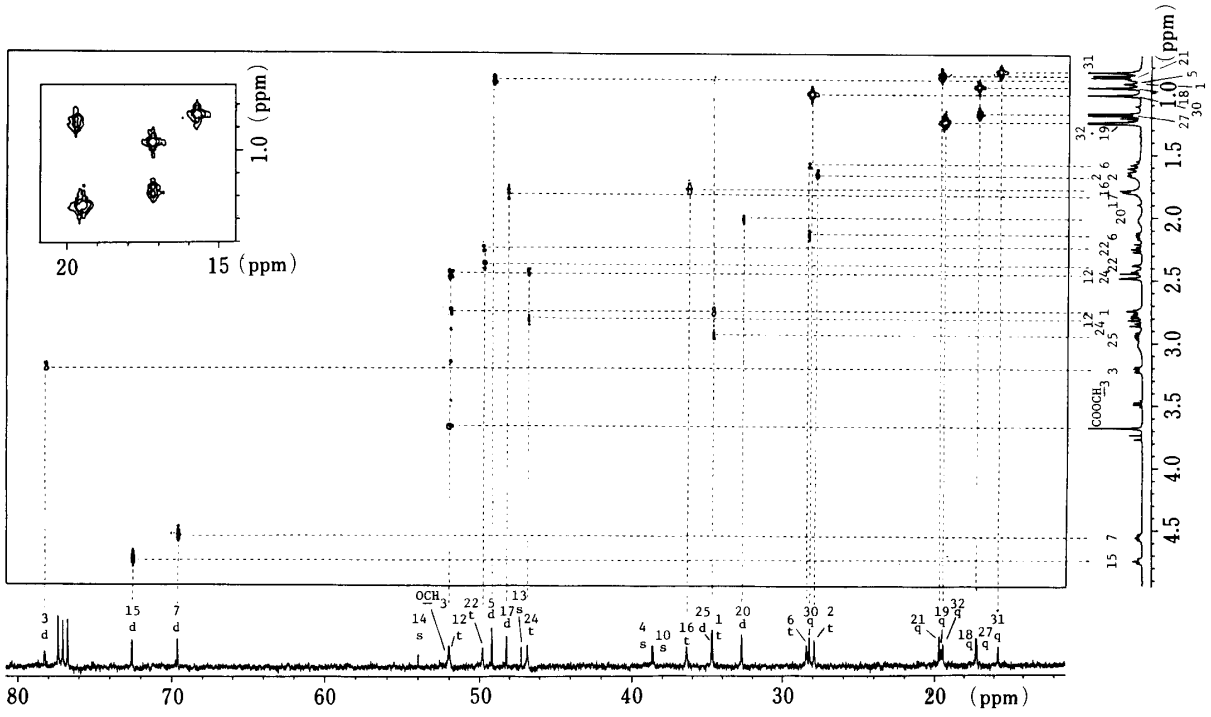


Fig. 9. Contour Map of the ^{1}H–^{13}C Shift-Correlated Spectrum of Methyl Ganoderate C2 (**2b**) in the Upfield Region

The ^{1}H shifts are shown on the ordinate and the ^{13}C shifts on the abscissa. The multiplicities of carbon signals were determined by means of off-resonance.

comparisons, and thus the structure was established.

Methyl ganoderate I (**4b**), a minor component, showed mp 279—281 °C, $[\alpha]_D$ +132° ($CHCl_3$), UV λ: 254.5 nm ($\log\varepsilon$ 3.86), and IR ν: 3450, 1730, 1710, and 1660 cm^{-1}. In the MS it gave the molecular ion peak at m/z 546(weak), corresponding to the formula $C_{31}H_{46}O_8$, and dehydrated ion peaks at m/z 528 and 510. The ^{1}H- and ^{13}C-NMR spectra of **4b** were similar to those of methyl ganoderate B (**10b**), but the doublet due to the 21-methyl protons changed to a singlet with a marked down-field shift (δ 1.40); the ^{13}C-signals assignable to C-17, C-20, C-21, and C-22 also shifted downfield (Tables I and II). From the above spectral data, the structure of methyl ganoderate I could be assigned as **4b** (20ξ-hydroxy derivative of **10b**). In accordance with this conclusion, fragment ion peaks were observed at m/z 402 ($c-H$), 384 ($c-H-H_2O$), 374 ($c-H-CO$), 331 ($a-CO$), 313 ($a-CO-H_2O$), 262 ($j-H$), 144 ($d+H$), 129 (e), and 59 (f) in the MS (Table III).

Compound B8 (**5b**), $C_{31}H_{46}O_7$, mp 158—163 °C, $[\alpha]_D$ +128° ($CHCl_3$), and compound B9 (**6b**), $C_{31}H_{48}O_7$, amorphous, are also very minor constituents of the fungus. The former (**5b**) showed a UV absorption at 254.5 nm ($\log\varepsilon$ 3.49) and IR absorptions at 3400, 1730, 1710, and 1650 cm^{-1}, while the latter (**6b**) showed a UV band at 255 nm ($\log\varepsilon$ 3.43) and IR bands at 3450, 1730, 1715, and 1665 cm^{-1}. The mass spectra of **5b** and **6b** exhibited the molecular ion peak at m/z 530 and 532, respectively, and their spectral patterns closely resembled those of methyl ganoderate A (**8b**) and methyl ganoderate C2 (**2b**), respectively (see Table III).

The ^{1}H-NMR pattern of **5b** resembled that of **8b**, but marked changes were observed in the chemical shifts and/or splitting patterns of ^{1}H-signals due to a carbinol methine at δ 4.58 (dd, J=5, 1.8 Hz), a methine at δ 2.09 (dd, J=12.5, 3 Hz), a methylene at around δ 1.7, and a methyl at δ 1.03, which were assignable to the 7-, 5-, 6-, and 19-protons, respectively (Table I). The ^{13}C-NMR spectrum of this compound (**5b**) was also similar to that of **8b**, except for the chemical shifts of several signals (C-5, C-6, C-7, C-19, and C-32) (Table II). In view of these data, the structure of compound B8 was assigned as **5b**, isomeric with **8b** at the C-7 position.

On the other hand, the ^{1}H-NMR spectrum of **6b** showed the signal due to an additional carbinol methine proton at δ 3.31 (dd, J=11.5, 5 Hz) along with the signals due to two carbinol methines similar to those of **5b**, accompanied with characteristic changes of the 1- and 2-methylene signals and the 30- and 31-methyl signals. Therefore, the structure of compound B9 should be represented by the formula **6b**, the 3β-hydroxy derivative of **5b**. This conclusion was supported by the ^{13}C-NMR spectrum, in which the signal of C-3 appeared at δ 78.5 (Table II).

Methyl lucidenate F (**7b**) was obtained as yellow needles, mp 208—211 °C, $[\alpha]_D$ +195° ($CHCl_3$), and its molecular formula was established to be $C_{28}H_{38}O_6$ by MS (M^+ ion at m/z 470) and high-resolution MS measurements. The UV, IR, and ^{1}H-NMR spectra of **7b** closely resembled those of **1b**, but the ^{1}H-NMR signals corresponding to the 24-methylene, 25-methine, and 27-methyl protons were not observed (Table I). In the ^{13}C-NMR spectrum, the chemical shifts of the carbons associated with the A—D rings were essentially identical with those of the corresponding carbons of **1b**, but the ^{13}C-signals arising from the side chain corresponded to only six carbons, reflecting the γ-substituted methyl pentanoate structure that appears in methyl lucidenate A (**11b**)[4] (Table II). Furthermore, the MS of **7b** exhibited significant fragment ion peaks at m/z 355 (a), 327 ($a-CO$), 301 ($g+H$), 300 (g), 141 ($h'-H-CO$), 109 ($h'-H-CO-MeOH$), 81 ($h'-H-CO-HCOOMe$), and 55 ($b'-HCOOMe$), which were reasonably explained by the fragmentations shown in Chart 2. On the basis of these data, the structure of methyl lucidenate F was determined to be **7b**.

Here we wish to discuss briefly the fragmentations in the MS of these compounds. In the case of the 7-oxo compounds (**1b**, **3b**, *etc.*), the ion $g+H$ arising from the fragmentation at the D-ring is characteristic, whereas the fragmentation at the B-ring proceeds predominantly in the 7-hydroxy compounds (**2b**, **8b**, **9b**, *etc.*), and the ions i and $j-H$ and related ions are

characteristic. In the case of the 15-oxo compounds (**1b**, **9b**, **10b**, *etc.*) the ion *a* and related ions produced by elimination of the side chain are usually observed, while they are too weak to be recognized in the MS of the 15-hydroxy compounds.

Throughout the structure elucidation of these triterpenes, the 2-D NMR methods were effectively used, and the assignments of some of the ^{1}H- and ^{13}C-signals reported in our previous communications[1] were revised. The structures of new triterpenes having a hydroxyl or an acetoxyl group at the C-12 position will be reported in the following paper.

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions on a JASCO DIP-4 automatic polarimeter at 22 °C. UV spectra were taken with a Shimadzu 202 UV spectrometer in EtOH solution and IR spectra with a JASCO IRA-2 spectrometer in $CHCl_3$ unless otherwise noted. MS and high-resolution MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX 400 spectrometer in $CDCl_3$ solutions with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. ^{1}H–^{1}H Shift-correlated NMR spectra were measured by the use of a ^{1}H–^{1}H shift correlation sequence with a 45° mixing pulse, and N-type peak selection. Data processing was carried out with the standard JEOL software. An f_2 spectral width of 1660—1700 Hz (4.15—4.35 ppm) over 1024 data points gave a digital resolution of 3.2—3.4 Hz. A total of 512 spectra, each of 16 transients, gave, with appropriate incrementing of the evolution delay, an f_1 width of 1660—1700 Hz and a digital resolution of 3.2—3.4 Hz (with zero filling). The sample concentration was 1—5 mg in 0.5 ml of $CDCl_3$; total acquisition time was about 12 h, and processing time about 20 min. In highly resolved spectral measurements, an f_2 spectral width of 900—1050 Hz (2.25—2.63 ppm) over 1024 data points gave a digital resolution of 1.8—2.0 Hz. A total of 512 spectra, each of 16 transients, gave, with appropriate incrementing of the evolution delay, an f_1 width of 900—1050 Hz and a digital resolution of 1.8—2.0 Hz (with zero filling). ^{1}H–^{13}C Shift-correlated NMR spectra were obtained with the usual pulse sequence and data processing was performed with the standard JEOL software. The spectral widths were 1700 Hz in f_1 and 6000 Hz in f_2, giving digital resolutions of 6.6 and 5.8 Hz with a 512 × 2048 data point matrix. The sample concentration was 3—5 mg in 0.5 ml of $CDCl_3$. Acquisition of 16 transients for each of the 256 FID's required about 13 h, and processing took about 1 h.

Column chromatography was done with Mallinckrodt silica gel. Preparative TLC was carried out on Merck Kieselgel GF_{254} plates and the plates were examined under UV light. Extraction of substances from silica gel was done with MeOH–CH_2Cl_2 (5 : 95 or 10 : 90) and solutions were concentrated *in vacuo*. TLC analyses were done on Merck Kieselgel GF_{254} plates developed with ether, acetone–$CHCl_3$ (10 : 90, 15 : 85, 30 : 70), and/or AcOEt–benzene (30 : 70),[14] and spots were detected by the use of 1% $Ce(SO_4)_2$–aq. H_2SO_4 (10%) reagent. For drying organic solutions, anhydrous $MgSO_4$ was employed.

Isolation and Properties of Triterpenes from *Ganoderma lucidum* (Fr.) Karst.——The surface part of the gills of dried fruit bodies of *G. lucidum* (2.56 kg), cultivated at Takayama, Gifu Prefecture, was scraped off with a small grinder and the powder (12.8 g) thus obtained was extracted with ether (100 ml × 6) at room temperature. The combined solutions were concentrated and the residue (7 g) was dissolved again in ether and extracted with saturated aq. Na_2CO_3 (100 ml × 3). The ether layer was washed with water, dried, and concentrated to give a neutral fraction (200 mg) as a yellow-brown syrup. The aqueous layer was acidified with 5% HCl and extracted with ether (100 ml × 3). The combined ether extracts were washed with water, dried, and concentrated to give an acidic fraction (6.40 g) as a yellow powder.

This acidic fraction was methylated with excess CH_2N_2 in ether and the crude product (6.40 g) was subjected to chromatography on a silica gel (200 g) column. The column was eluted successively with AcOEt–benzene mixtures (10 : 90, 400 ml; 20 : 80, 400 ml; 30 : 70, 800 ml; 40 : 60, 1000 ml; 50 : 50, 2300 ml; 70 : 30, 400 ml) with monitoring by TLC and the eluates were separated into thirteen fractions. Most of these fractions were further separated by rechromatography on silica gel (about 100 to 150 times the quantities of the substances) with AcOEt–benzene (10 : 90, 15 : 85, 20 : 80, 30 : 70) or acetone–$CHCl_3$ (2.5 : 97.5, 5 : 95, 7.5 : 92.5, 10 : 90, 20 : 80) as the eluent, and the following fractions were obtained.

AcOEt–benzene (30 : 70) eluate: Fract. 1 (430 mg)—Fr. 1-1 (70 mg), Fr. 1-2 (120 mg), Fr. 1-3 (230 mg); Fract. 2 (680 mg)—Fr. 2-1 (260 mg), Fr. 2-2 (420 mg); Fract. 3 (450 mg)—Fr. 3-1 (110 mg), Fr. 3-2 (280 mg).

AcOEt–benzene (40 : 60) eluate: Fract. 4 (290 mg); Fract. 5 (380 mg)—Fr. 5-1 (130 mg), Fr. 5-2 (190 mg); Fract. 6 (620 mg)—Fr. 6-1 (20 mg), Fr. 6-2 (140 mg), Fr. 6-3 (280 mg), Fr. 6-4 (90 mg); Fract. 7 (660 mg)—Fr. 7-1 (310 mg), Fr. 7-2 (230 mg); Fract. 8 (1100 mg).

AcOEt–benzene (50 : 50) eluate: Fract. 9 (270 mg); Fract. 10 (390 mg)—Fr. 10-1 (130 mg), Fr. 10-2 (190 mg); Fract. 11 (310 mg)—Fr. 11-1 (200 mg), Fr. 11-2 (40 mg); Fract. 12 (750 mg)—Fr. 12-1 (110 mg), Fr. 12-2 (50 mg), Fr.

12-3 (260 mg).

AcOEt–benzene (70 : 30) eluate: Fract. 13 (90 mg).

Treatment of Fract. 1—Fract. 4——Fraction 1-1 was recrystallized from EtOH to give methyl lucidenate F (**7b**) (3 mg), yellow needles, mp 208—211 °C, $[\alpha]_D$ +195° ($c=0.1$). UV λ_{max} nm ($\log\varepsilon$): 251.5 (3.35). IR ν_{max}^{KBr} cm^{-1}: 1750, 1737, 1702, 1700, 1675. ^1H- and ^{13}C-NMR: Tables I and II. MS data: Table III. High-resolution MS: Found 470.2689, Calcd for $C_{28}H_{38}O_6$ (M^+) 470.2668; Found 455.2423, Calcd for $C_{27}H_{35}O_6$ 455.2433; Found 439.2504, Calcd for $C_{27}H_{35}O_5$ 439.2485; Found 355.1852, Calcd for $C_{22}H_{27}O_4$ 355.1909; Found 327.1948, Calcd for $C_{21}H_{27}O_3$ 327.1959; Found 301.1826, Calcd for $C_{19}H_{25}O_3$ 301.1804; Found 300.1687, Calcd for $C_{19}H_{24}O_3$ 300.1724; Found 141.0921, Calcd for $C_8H_{13}O_2$ 141.0916; Found 115.0758, Calcd for $C_6H_{11}O_2$ 115.0758; Found 109.0641, Calcd for C_7H_9O 109.0653. The mother liquor (50 mg) was purified by preparative TLC with ether as the eluent to give methyl lucidenate D2 (**19b**) (32 mg), amorphous powder.

Fraction 1-2 was a complex mixture, which was subjected repeatedly to silica gel column chromatography with $CHCl_3$ as the eluent to give methyl lucidenate A (**11b**) (9 mg), colorless needles (from ether), mp 165—168 °C, $[\alpha]_D$ +230° ($c=0.4$). UV λ_{max} nm ($\log\varepsilon$): 254 (3.51). High-resolution MS: Found 472.2857, Calcd for $C_{28}H_{40}O_6$ (M^+) 472.2825; Found 457.2574, Calcd for $C_{27}H_{37}O_6$ 457.2589; Found 454.2728, Calcd for $C_{28}H_{38}O_5$ 454.2719; 444.2922, Calcd for $C_{27}H_{40}O_5$ 444.2876; Found 334.1794, Calcd for $C_{19}H_{26}O_5$ 334.1781; Found 329.2105, Calcd for $C_{21}H_{29}O_3$ 329.2116; Found 115.0756, Calcd for $C_6H_{11}O_2$ 115.0758. Its identity was confirmed by comparison of the MS and the ^1H- and ^{13}C-NMR spectra (Tables I, II, and III) with those reported in the literature.[4]

Fraction 1-3 and Fr. 2-1 were combined and chromatographed on a silica gel (50 g) column, eluting with benzene containing increasing amounts of ether. The ether–benzene (20 : 80) eluate (130 mg) was then subjected to preparative TLC with acetone–$CHCl_3$ (5 : 95) as the eluent and separated into two bands. The less polar band gave methyl ganoderate F (**14b**) (56 mg), amorphous powder. The constituents in the more polar band were not characterized.

Fraction 2-2 and Fr. 3-1 were combined and purified again by silica gel column chromatography. The later fractions were collected and then separated by preparative TLC with ether. The less polar band gave compound C5′ (**18b**)[5] (31 mg), colorless needles (from ether), mp 118.5—121.5 °C, while the more polar band gave methyl ganoderate E (**1b**) (48 mg), yellow needles (from EtOH), mp 206—208.5 °C, $[\alpha]_D$ +167° ($c=0.5$). UV λ_{max} nm ($\log\varepsilon$): 251.5 (3.93). IR ν_{max} cm^{-1}: 1750, 1720, 1710, 1680. ^1H- and ^{13}C-NMR: Tables I and II. MS data: Table III. *Anal.* Calcd for $C_{31}H_{42}O_7$: C, 70.16; H, 8.74. Found: C, 69.65; H, 8.67. High-resolution MS: Found 526.2926, Calcd for $C_{31}H_{42}O_7$ (M^+) 526.2930; Found 511.2668, Calcd for $C_{30}H_{39}O_7$ 511.2695; Found 508.2797, Calcd for $C_{31}H_{40}O_6$ 508.2824; Found 495.2726, Calcd for $C_{30}H_{39}O_6$ 495.2746; Found 494.2676, Calcd for $C_{30}H_{38}O_6$ 494.2669; Found 383.2218, Calcd for $C_{24}H_{31}O_4$ 383.2221; Found 382.2138, Calcd for $C_{24}H_{30}O_4$ 382.2144; Found 355.1952, Calcd for $C_{22}H_{27}O_4$ 355.1909; Found 301.1819, Calcd for $C_{19}H_{25}O_3$ 301.1804; Found 300.1730, Calcd for $C_{19}H_{24}O_3$ 300.1726; Found 225.1141, Calcd for $C_{12}H_{17}O_4$ 225.1127; Found 193.0865, Calcd for $C_{11}H_{13}O_3$ 193.0865; Found 171.1004, Calcd for $C_9H_{15}O_3$ 171.1021; Found 165.0922, Calcd for $C_{10}H_{13}O_2$ 165.0916; Found 144.0798, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0736, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0554, Calcd for $C_6H_9O_3$ 129.0552.

Fraction 3-2 and Fract. 4 were combined and recrystallized from EtOH to afford methyl ganoderate Cl (**9b**) (110 mg), colorless prisms, mp 171—173 °C, $[\alpha]_D$ +168° ($c=1.0$). *Anal.* Calcd for $C_{31}H_{44}O_7 \cdot 1/2H_2O$: C, 69.26; H, 8.44. Found: C, 69.30; H, 8.40. High-resolution MS: Found 528.3063, Calcd for $C_{31}H_{44}O_7$ (M^+) 528.3086; Found 500.3174, Calcd for $C_{30}H_{44}O_6$ 500.3138; Found 390.2033, Calcd for $C_{22}H_{30}O_6$ 390.2042; Found 384.2303, Calcd for $C_{24}H_{32}O_4$ 384.2301; Found 366.2195, Calcd for $C_{24}H_{30}O_3$ 366.2195; Found 362.2090, Calcd for $C_{21}H_{30}O_5$ 362.2092; Found 329.2096, Calcd for $C_{21}H_{29}O_3$ 329.2115; Found 246.1277, Calcd for $C_{15}H_{18}O_3$ 246.1256; Found 171.1007, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0791, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0772, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0557, Calcd for $C_6H_9O_3$ 129.0552. The identity of **9b** was established by comparison of the ^1H- and ^{13}C-NMR spectra and MS (Tables I, II, and III) with those reported in the literature.[4,7a] Further purification of the mother liquor (420 mg) is in progress.

Treatment of Fract. 5—Fract. 7——Fraction 5-1 and Fr. 6-1 were recrystallized from EtOH to give an additional crop of methyl ganoderate Cl (**9b**) (57 mg).

Fraction 5-2 was separated into three bands by preparative TLC with ether as the eluent. The residue from the least mobile band was recrystallized from ether to afford methyl lucidenate E2 (**20b**) (32 mg), yellow needles, mp 140—144 °C. The middle band was unidentified. Treatment of the most mobile band is described below.

Fraction 6-2 was subjected to preparative TLC with ether to give three bands, the most mobile one of which was combined with the most mobile band from Fr. 5-2 and recrystallized from EtOH to give methyl lucidenate C (**13b**)[4,8b] (7 mg), colorless prisms, mp 231—232 °C. MS *m/z*: 490 (M^+, 6%), 472 (3), 375 (1), 357 (1), 307 (20), 306 (100), 288 (10), 185 (1), 115 (2), 55 (10). Identification of **13b** was done by comparison of its MS and ^1H-NMR spectrum with those reported in the literature, and also by analysis of the ^{13}C-NMR spectrum. The middle band was still a mixture and its components were uncertain, while the least mobile band was recrystallized from ether to give methyl ganolucidate A (**23b**) (10 mg), colorless needles, mp 192—194 °C.

Fraction 6-3 and Fr. 7-1 were subjected again to preparative TLC with ether and divided into four fractions. Recrystallization of the most polar fraction from EtOH gave methyl ganoderate H (**16b**) (88 mg), yellow needles, mp 155—156 °C. Purification of the other fractions is under investigation.

Combined Fr. 6-4 and Fr.7-2 were also subjected to preparative TLC with ether and separated into three bands. The least polar band was recrystallized from ether to afford methyl ganoderate G (**15b**) (15 mg), colorless prisms, mp 134—135 °C. The most polar band gave a small amount of methyl ganoderate H (**16b**), but the middle band was unidentified.

Treatment of Fract. 8 and Fract. 9——Fraction 8 was recrystallized from EtOH to give methyl ganoderate B (**10b**) (110 mg), colorless prisms, mp 205—210 °C, $[\alpha]_D$ +125° (c=1.0). UV λ_{max} nm (log ε): 254.5 (4.01). *Anal.* Calcd for $C_{31}H_{46}O_7 \cdot 1/2H_2O$: C, 69.01; H, 8.78. Found: C, 69.26; H, 8.69. High-resolution MS: Found 530.3236, Calcd for $C_{31}H_{46}O_7$ (M^+) 530.3244; Found 515.3049, Calcd for $C_{30}H_{43}O_7$ 515.3089; Found 512.3160, Calcd for $C_{31}H_{44}O_6$ 512.3138; Found 502.3268, Calcd for $C_{30}H_{46}O_6$ 502.3293; Found 484.3175, Calcd for $C_{30}H_{44}O_5$ 484.3188; Found 390.2024, Calcd for $C_{22}H_{30}O_6$ 390.2042; Found 368.2352, Calcd for $C_{24}H_{32}O_3$ 368.2352; Found 331.2277, Calcd for $C_{21}H_{31}O_3$ 331.2274; Found 246.1236, Calcd for $C_{15}H_{18}O_3$ 246.1256; Found 171.1004, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0814, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0761, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0572, Calcd for $C_6H_9O_3$ 129.0552. The identity of **10b** was established by comparison of the ^{1}H- and ^{13}C-NMR spectra and MS (Tables I, II, and III) with those reported in the literature.[2,7a)]

The mother liquor (*ca.* 600 mg) was re-chromatographed on a silica gel (60 g) column with acetone–$CHCl_3$ (2.5 : 97.5, 5 : 95), giving three fractions. The first fraction was further purified by preparative TLC with ether,[14)] and the more polar band afforded an additional crop of methyl ganoderate H (**16b**) (32 mg), while the less polar band gave a small amount of methyl ganoderate B (**10b**). The second and the last fractions were also separated by preparative TLC using ether[14)] as the eluent into three bands, the least polar of which gave methyl ganoderate G (**15b**) (16 mg), while the most polar band afforded compound C6 (**17b**) (4 mg), pale yellow prisms (from ether), mp 146—148 °C.

Fraction 9 was subjected repeatedly to preparative TLC with ether as the eluent to give four bands, the most mobile of which gave a small amount of **15b** (5 mg). On the other hand, the next most mobile band gave methyl ganolucidate B (**24b**) (4 mg), colorless needles (from ether), mp 167—169 °C, and the third most mobile band gave another crop of methyl ganoderate B (**10b**) (15 mg), while the most polar band contained a small amount of methyl ganoderate A (**8b**).

Treatment of Fract. 10—Fract. 12——Fraction 10-1 was separated into two bands by preparative TLC with ether, and the less polar band gave an additional crop of methyl ganolucidate B (**24b**) (9 mg). The more polar fraction was combined with Fr. 10-2 and recrystallized from ether to give methyl ganoderate A (**8b**) (205 mg), colorless prisms, mp 189—193 °C, $[\alpha]_D$ +128° (c=1.0). UV λ_{max} nm (log ε): 252.5 (3.92). IR ν_{max} cm^{-1}: 3450, 1730, 1710, 1700, 1660. *Anal.* Calcd for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 69.65; H, 8.67. High-resolution MS: Found 530.3223, Calcd for $C_{31}H_{46}O_7$ (M^+) 530.3243; Found 512.3123, Calcd for $C_{31}H_{44}O_6$ 512.3138; Found 392.2185, Calcd for $C_{22}H_{32}O_6$ 392.2198; Found 387.2488, Calcd for $C_{24}H_{35}O_4$ 387.2494; Found 368.2335, Calcd for $C_{24}H_{32}O_3$ 368.2352; Found 364.2243, Calcd for $C_{21}H_{32}O_5$ 364.2249; Found 285.1852, Calcd for $C_{19}H_{25}O_2$ 285.1853; Found 230.1302, Calcd for $C_{15}H_{18}O_2$ 230.1307; Found 171.1026, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0783, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0761, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0567, Calcd for $C_6H_9O_3$ 129.0552. The identity of **8b** was confirmed by comparison of the ^{1}H- and ^{13}C-NMR spectra and MS (Tables I, II, and III) with those reported in the literature.[2,7)]

Fraction 11-1 was recrystallized from ether to give another crop of methyl ganoderate A (**8b**) (150 mg).

Fraction 11-2 was purified repeatedly by preparative TLC with ether and acetone–$CHCl_3$ (15 : 85), giving methyl ganolucidate B (**24b**) (2 mg) from the less polar band and compound B8 (**5b**) (10 mg) from the more polar band, colorless prisms (from ether), mp 158—163 °C, $[\alpha]_D$ +128° (c=0.5). UV λ_{max} nm (log ε): 254.5 (3.49). IR ν_{max} cm^{-1}: 3400, 1730, 1710, 1650. ^{1}H- and ^{13}C-NMR: Tables I and II. MS data: Table III. High-resolution MS: Found 530.3293, Calcd for $C_{31}H_{46}O_7$ (M^+) 530.3244; Found 392.2241, Calcd for $C_{22}H_{32}O_6$ 392.2198; Found 364.2248, Calcd for $C_{21}H_{32}O_5$ 364.2249; Found 230.1295, Calcd for $C_{15}H_{18}O_2$ 230.1307; Found 171.1033, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0795, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0776, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0538, Calcd for $C_6H_9O_3$ 129.0552.

Fraction 12-1 was recrystallized from ether to give an additional crop of **8b** (80 mg).

Fraction 12-2 was further purified by preparative TLC with acetone–$CHCl_3$ (10 : 90) and then recrystallized from ether to give methyl ganoderate I (**4b**) (8 mg), colorless prisms, mp 279—281 °C, $[\alpha]_D$ +132° (c=0.5). UV λ_{max} nm (log ε): 254.5 (3.86). IR ν_{max} cm^{-1}: 3450, 1730, 1710, 1660. ^{1}H- and ^{13}C-NMR: Tables I and II. MS data: Table III. High-resolution MS: Found 528.3149, Calcd for $C_{31}H_{44}O_7$ (M^+) 528.3188; Found 510.2967, Calcd for $C_{31}H_{42}O_6$ 510.2980; Found 402.2432, Calcd for $C_{24}H_{34}O_5$ 402.2406; Found 384.2348, Calcd for $C_{24}H_{32}O_4$ 384.2396; Found 374.2473, Calcd for $C_{23}H_{34}O_4$ 374.2457; Found 356.2380, Calcd for $C_{23}H_{32}O_3$ 356.2352; Found 331.2259, Calcd for $C_{21}H_{31}O_3$ 331.2274; Found 313.2184, Calcd for $C_{21}H_{29}O_2$ 313.2167; Found 262.1200, Calcd for $C_{15}H_{18}O_4$ 262.1205; Found 144.0776, Calcd for $C_7H_{12}O_3$ 144.0787; Found 129.0582, Calcd for $C_6H_9O_3$ 129.0552.

Fraction 12-3 was recrystallized from ether to give methyl ganoderate C2 (**2b**) (150 mg), colorless prisms, mp 199—202 °C, $[\alpha]_D$ +98° (c=1.0). UV λ_{max} nm (log ε): 253 (4.01). IR ν_{max} cm^{-1}: 3450, 1720, 1710, 1700, 1650. ^{1}H- and ^{13}C-NMR: Tables I and II. MS data: Table III. *Anal.* Calcd for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08. Found: C, 69.86; H, 9.20. High-resolution MS: Found 532.3395, Calcd for $C_{31}H_{48}O_7$ (M^+) 532.3400; Found 514.3294, Calcd for $C_{31}H_{46}O_6$ 514.3294; Found 496.3161, Calcd for $C_{31}H_{44}O_5$ 496.3187; Found 392.2153, Calcd for $C_{22}H_{32}O_6$ 392.2198;

Found 388.2641, Calcd for $C_{24}H_{36}O_4$ 388.2614; Found 230.1331, Calcd for $C_{15}H_{18}O_2$ 230.1307; Found 171.0983, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0782, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0748, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0592, Calcd for $C_6H_9O_3$ 129.0552.

Treatment of Fract. 13——Fraction 13 was subjected to preparative TLC with acetone–$CHCl_3$ (1:9) as the eluent, and the more mobile band contained methyl ganoderate C2 (**2b**) (18 mg). On the other hand, the substance from the less mobile band was a mixture, which was again separated by preparative TLC with ether to give compound B9 (**6b**) (2 mg), amorphous powder, from the lower band. UV λ_{max} nm ($\log\varepsilon$): 255 (3.43). IR ν_{max} cm^{-1}: 3450, 1730, 1715, 1665. ^1H- and ^{13}C-NMR: Tables I and II. MS data: Table III. High-resolution MS: Found 532.3413, Calcd for $C_{31}H_{48}O_7$ (M^+) 532.3400; Found 514.3300, Calcd for $C_{31}H_{46}O_6$ 514.3294; Found 496.3197, Calcd for $C_{31}H_{44}O_5$ 496.3187; Found 364.2215, Calcd for $C_{21}H_{32}O_5$ 364.2249; Found 230.1258, Calcd for $C_{15}H_{18}O_2$ 230.1307; Found 171.1039, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0783, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0761, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0561, Calcd for $C_6H_9O_3$ 129.0552.

The upper band gave methyl ganoderate K (**3b**) (2.2 mg), pale yellow prisms (from ether), mp 166—167 °C, $[\alpha]_D$ +156° (c=0.3). UV λ_{max} nm ($\log\varepsilon$): 273 (3.68). IR ν_{max} cm^{-1}: 3450, 1725, 1715, 1700, 1660. ^1H- and ^{13}C-NMR: Tables I and II. MS data: Table III. High-resolution MS: Found 530.3252, Calcd for $C_{31}H_{46}O_7$ (M^+) 530.3244; Found 499.3084, Calcd for $C_{30}H_{43}O_6$ 499.3080; Found 368.2418, Calcd for $C_{24}H_{32}O_3$ 368.2352; Found 341.2120, Calcd for $C_{22}H_{29}O_3$ 341.2117; Found 303.2000, Calcd for $C_{19}H_{27}O_3$ 303.1961; Found 209.1212, Calcd for $C_{12}H_{17}O_3$ 209.1178; Found 171.1042, Calcd for $C_9H_{15}O_3$ 171.1021; Found 144.0810, Calcd for $C_7H_{12}O_3$ 144.0787; Found 139.0750, Calcd for $C_8H_{11}O_2$ 139.0758; Found 129.0542, Calcd for $C_6H_9O_3$ 129.0552.

Chromium Trioxide Oxidation of Methyl Ganoderate A (8b)——A solution of CrO_3 (10 mg) in AcOH (0.5 ml) containing water (0.2 ml) was added dropwise under vigorous stirring to a solution of methyl ganoderate A (**8b**) (10 mg) in AcOH (0.5 ml) at room temperature. Stirring was continued for 2 h, then the reaction mixture was poured into ice-water, made alkaline by addition of aq. Na_2CO_3, and extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated. The crystalline residue (8 mg) was separated by preparative TLC with ether into two fractions. The more polar fraction gave the pentaoxo compound (**1b**) (5 mg), yellow needles (from EtOH), mp 206—208 °C, which was found to be identical with methyl ganoderate E (**1b**) by TLC and spectral comparisons. The less polar fraction (2.5 mg), amorphous, was identified as methyl ganoderate J (**12b**).

Allylic Oxidation of Methyl Ganoderate A (8b)——A solution of methyl ganoderate A (**8b**) (10 mg) in anhydrous CH_2Cl_2 (1.66 ml) containing benzotriazole (16.6 mg)[13] was added to pyridinium chlorochromate (7.55 mg) at −3 °C. Stirring was continued for 25 min under argon gas, then a saturated NaCl solution was added and the mixture was extracted thoroughly with $CHCl_3$. The extract was dried and concentrated, and the residue was subjected to preparative TLC with ether to give three bands. The most mobile band gave the tetraoxo compound (**12b**) (4.2 mg), amorphous, which was identified as methyl ganoderate J (**12b**). The middle band afforded methyl ganoderate E (**1b**) (2.8 mg), mp 205—207 °C, while the least mobile band gave the starting material (1.3 mg).

Chromium Trioxide Oxidation of Methyl Ganoderate C2 (2b)——Methyl ganoderate C2 (**2b**) (10 mg) was oxidized with CrO_3 in the same manner as described for **8b**. The crystalline product (9.5 mg) was separated by preparative TLC with ether into three bands. The most mobile band gave the tetraoxo compound (**12b**) (2.5 mg), amorphous, identical with methyl ganoderate J (**12b**). The middle band afforded the pentaoxo compound (**1b**) (3.2 mg), mp 205—206 °C, which was identified as methyl ganoderate E (**1b**) by spectral comparisons. The least mobile band gave the starting material (2.2 mg).

Allylic Oxidation of Methyl Ganoderate C2 (2b)——Methyl ganoderate C2 (**2b**) (4 mg) was stirred with activated MnO_2 (5 mg) in $CHCl_3$ for 5 h. After removal of MnO_2 by filtration, the $CHCl_3$ solution was concentrated to leave a crystalline residue. This was purified by preparative TLC developed with acetone–$CHCl_3$ (10:90) and then recrystallized from ether to afford the trioxo compound (**3b**) (2.5 mg), mp 166—167 °C. This was shown to be identical with methyl ganoderate K (**3b**) by TLC and spectral comparisons.

References and Notes

1) A part of this work was reported in our preliminary communications: *a*) T. Kikuchi, S. Matsuda, S. Kadota, Y. Murai, and Z. Ogita, *Chem. Pharm. Bull.*, **33**, 2624 (1985); *b*) T. Kikuchi, S. Matsuda, Y. Murai, and Z. Ogita, *ibid.*, **33**, 2628 (1985). A part of this work was presented at the 2nd Annual Meeting of the Medical & Pharmaceutical Society for Wakan-Yaku, Kyoto, September 1985, Abstr., p. 114.
2) T. Kubota, Y. Asaka, I. Miura, and H. Mori, *Helv. Chim. Acta*, **65**, 611 (1982).
3) J. O. Toth, B. Luu, and G. Ourisson, *Tetrahedron Lett.*, **24**, 1081 (1983); J. O. Toth, B. Luu, J. Beck, and G. Ourisson, *J. Chem. Research (S)*, **1983**, 299.
4) T. Nishitoba, H. Sato, T. Kasai, H. Kawagishi, and S. Sakamura, *Agric. Biol. Chem.*, **48**, 2905 (1984).
5) Compound C5′ was found to be identical with ganoderic acid D methyl ester (**18b**); see ref. 8*a*).
6) Because studies on the constituents of this fungus have been independently undertaken by four groups of workers in recent years, confusion has arisen regarding the trivial names of the compounds reported; *i.e.*, some identical compounds were named differently or some different compounds were named identically by different

workers. After discussions on this problem within these four groups, the following trivial names are newly proposed for the following compounds:

9a: ganoderic acid C1 (previously named ganoderic acid C[4] and D[7a]).
2a: ganoderic acid C2 (previously named ganoderic acid C,[7a] D,[1a] and D_2[8c]).
16a: ganoderic acid H (previously named ganoderic acid C[9] and H[1a]).
21a: lucidenic acid D1 (previously named lucidenic acid D[8a]).
19a: lucidenic acid D2 (previously named lucidenic acid D[1a,7b]).
22a: lucidenic acid E1 (previously named lucidenic acid E[8a]).
20a: lucidenic acid E2 (previously named lucidenic acid E[1a]).

The letter suffix of the name of each compound does not designate a substitution pattern, but a chronological sequence.

7) *a*) H. Kohda, W. Tokumoto, K. Sakamoto, M. Fujii, Y. Hirai, K. Yamasaki, Y. Komoda, H. Nakamura, S. Ishihara, and M. Uchida, *Chem. Pharm. Bull.*, **33**, 1367 (1985); *b*) Y. Komoda, H. Nakamura, S. Ishihara, M. Uchida, H. Kohda, and K. Yamasaki, *ibid.*, **33**, 4829 (1985).
8) *a*) T. Nishitoba, H. Sato, and S. Sakamura, *Agric. Biol. Chem.*, **49**, 1547 (1985); *b*) T. Nishitoba, H. Sato, T. Kasai, H. Kawagishi, and S. Sakamura, *ibid.*, **49**, 1793 (1985); *c*) T. Nishitoba, H. Sato, and S. Sakamura, *ibid.*, **49**, 3637 (1985).
9) M. Hirotani, T. Furuya, and M. Shiro, *Phytochemistry*, **24**, 2055 (1985).
10) Structure elucidation of the other new compounds will be reported in detail in forthcoming papers.
11) The ether extract of dried fruit bodies of this fungus showed an antiandrogenic activity in mice, and the extract from the surface part of the gills gave the most significant activity.
12) Some of the previous assignments of ^{1}H- and ^{13}C-signals were revised.
13) E. J. Parish and S. Chitrakorn, *Synth. Commun.*, **15**, 393 (1985).
14) In column chromatography and TLC of this series of compounds, the order of mobilities of several compounds is reversed by the change of developing solvent.