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GANODERIC ACID DERIVATIVES, HIGHLY OXYGENATED LANOSTANE-TYPE TRITERPENOIDS, FROM *GANODERMA LUCIDUM**

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Key Word Index—*Ganoderma lucidum*; Basidiomycetes; higher fungi; triterpenoids; lanostane derivatives; ganoderic acids C, E and F.

Abstract—The structures of two new compounds, ganoderic acids E and F, isolated in the form of their methyl esters from the fruit body of the fungus *Ganoderma lucidum* have been elucidated as 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oic acid and 3,7,11,15,23-pentaoxo-5α-lanost-8-en-26-oic acid, respectively.

INTRODUCTION

Recently we reported the isolation and structure determination of ganoderic acid D† [1], together with ganoderic acids A and B, from the methanol extract of the fruit body of *Ganoderma lucidum* (Japanese name: Mannen-take).

In this paper, we wish to report the isolation of the known ganoderic acid C [2] and two new ganoderic acids, named ganoderic acids E and F from the same material. ^{1}H NMR and ^{13}C NMR assignments of these compounds have been performed using proton–proton and proton–carbon shift correlation two dimensional NMR techniques.

RESULTS AND DISCUSSION

After treatment of the chloroform fraction (acidic fraction) from the methanol extract of *Ganoderma lucidum* with diazomethane, compounds **1c** and **1d** were isolated by silica gel column chromatography and reversed-phase HPLC.

Compound **1b**, methyl ganoderate E, analysed for $C_{31}H_{48}O_7$, and showed in the UV spectrum an absorption at 250 nm. Its value and intensity are characteristic of an α,β-unsaturated carbonyl group. The IR spectrum of **1c** showed the presence of hydroxyl groups, ester carbonyl and an α,β-unsaturated carbonyl groups. The ^{1}H NMR spectrum of **1c** revealed the presence of two secondary methyls, five tertiary methyls, a carbomethoxyl methyl and three hydroxymethine protons (δ3.20, 4.53 and 4.72) (Table 1).

Acetylation of **1c** gave **1g**, its ^{1}H NMR spectrum displayed signals for three acetoxyl methyls and the above three hydroxymethine proton signals were shifted downfield to δ4.46, 5.49 and 5.26, respectively (Table 1). In the mass spectra of compounds **1c** and **1g**, an *m/z* 129 fragment ion peak was observed resulting from cleavages between C-22 and C-23 and thus suggesting the presence of the same side chain, as found in methyl ganoderates A, B and D [1].

In the ^{13}C NMR spectrum of **1c**, three carbonyl carbon signals were observed at δ176.2 (C-26), 199.8 (C-11) and 208.5 (C-23) (Table 2). On the other hand, four carbonyl carbon signals were observed at δ176.2 (C-26), 199.4 (C-11), 208.6 (C-23) and 217.1 (C-3) in the spectrum of **1a** [1]. This suggested that the loss of one carbonyl carbon signal in the spectrum of **1c** is due to C-3. This was supported by the fact that the three carbon signals bearing hydroxyl groups were observed at δ69.3, 72.3 and 78.0 in **1c**, while two carbon signals in the ^{13}C NMR spectrum of **1a** were detected. Also, the loss of a carbonyl carbon signal in the spectrum of **1c** relative to that of **1b** [1], indicated that the lost signal was due to C-15. All these data indicated that the structure of compound **1c** is methyl 3,7,15-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oate. This was supported by its transformation into **2a** on reaction with pyridinium chlorochromate [3].

It was very easy to identify the cross peaks corresponding to the C-3, C-7 and C-15 carbon atom bands and the proton signals in the proton-carbon shift correlation diagram and so the three hydroxymethine proton signals at δ3.20, 4.53 and 4.72 were unambiguously assigned to the hydrogens attached at C-3, C-7 and C-15, respectively. The configuration of H-3 was revealed to be α by its coupling constants of 11 and 6.5 Hz with the protons of C-2 [4]. Also H-7 appeared to have an α-configuration [1]. The configuration of the last methine proton H-15 was revealed to be β by its coupling constants when referred to the ^{1}H NMR spectral data of compound **1a** [1]. Therefore, the structure of **1c** was elucidated as methyl 3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oate. It was named methyl ganoderate E and the parent acid is ganoderic acid E.

Compound **1d**, methyl ganoderate C, analysed for $C_{31}H_{44}O_7$, and showed in the UV spectrum an absorption at 250 nm. The IR spectrum of **1d** showed the presence of hydroxyl group, ester carbonyl and carbonyl groups. The ^{1}H NMR and ^{13}C NMR data of **1d** were very similar to those of **1b**, but the ^{1}H NMR spectrum of **1d** did

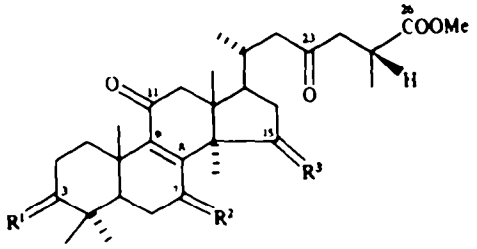
*Part 5 in the series "Studies on the Metabolites of Higher Fungi". For Part 4 see ref. [1].

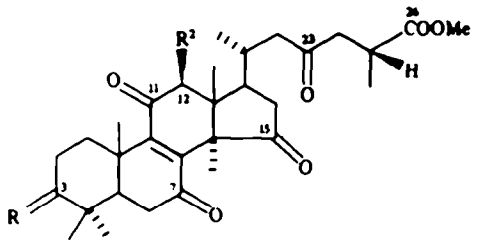
†In ref. [1] this compound was given the name ganoderic acid C. However, the latter name has already been assigned to a compound isolated by Nishitoba *et al.* [2].

Table 1. ^{1}H NMR spectral data of compounds **1c**, **1d**, **1g**, **1h** and **2a** (300 MHz, $CDCl_3$)

H	1c	1g	1d	1h	2a
1β	2.73 *ddd* (13.6, 3.6, 3.6)	2.81 *ddd* (13.5, 3.7, 3.7)	2.92 *m*	2.97 *m*	2.89 *ddd* (14, 9, 6)
1α		1.16 *m*	1.45 *ddd* (13.7, 8.2, 8.2)	1.57 *ddd* (13.7, 8.3, 8.3)	1.73 *ddd* (14, 10, 5.9)
2β	1.63 *m*	1.67 *m*	2.47 *dd* (13, 8)	2.50 *m*	2.61 *ddd* (15.2, 10, 6)
2α	1.63 *m*	1.67 *m*	2.45 *ddd* (8, 7, 6)	2.50 *m*	2.46 *ddd* (15.2, 9, 6)
3α	3.20 *dd* (11, 6.5)	4.46 *dd* (9.7, 6.5)	—	—	
5α	0.90 *dd* (12.5, 1.5)	0.99 *dd* (14, 2)	1.54 *dd* (13.5, 1)	1.64 *dd* (13.7, 1.6)	2.30 *dd* (15, 2.7)
6β	1.59 *ddd* (12.5, 12.5, 10.5)	1.39 *ddd* (14, 14, 8.5)	1.65 *ddd* (13.5, 12, 9.5)	1.40 *ddd* (13.7, 13.2, 8.8)	2.69 *dd* (15, 13.5)
6α	2.12 *ddd* (12.5, 7.5, 1.5)	2.40 *ddd* (14, 8.5, 2)	2.08 *ddd* (12, 7.4, 1)	2.39 *m*	2.46 *dd* (13.5, 2.7)
7α	4.53 *dd* (10.5, 7.5)	5.49 *dd* (8.5, 8.5)	4.82 *ddd* (9.5, 7.4, 4.5)	5.85 *dd* (8.8, 8.3)	—
12α	2.76 *br d* (15)	2.80 *d* (15.7)	2.76 *br d* (17.5)	2.76 *br d* (18)	2.88 *dd* (16.2, 0.8)
12β	2.45 *d* (15)	2.52 *d* (15.7)	2.68 *d* (17.5)	2.65 *d* (18)	2.74 *d* (16.2)
15β	4.72 *dd* (8, 7.5)	5.26 *dd* (9.5, 6)	-	—	—
16β	1.78 *m*	1.60 *ddd* (19, 10, 6)	2.05 *m*	2.04 *dd* (16.1, 9.8)	1.85 *dd* (18.2, 8)
16α	1.78 *m*	2.12 *ddd* (19, 9.5, 8)	2.68 *m*	2.47 *dd* (16.1, 9.7)	2.74 *dd* (18.2, 9.3)
17α	1.78 *m*	1.83 *ddd* (10, 10, 8)	2.10 *m*	2.00 *m*	2.23 *ddd* (11, 9.3, 8)
3H-18	0.94 *s*	1.01 *s*	1.00 *s*	1.03 *s*	0.88 *d* (0.8)
3H-19	1.22 *s*	1.24 *s*	1.23 *s*	1.15 *s*	1.27 *s*
20	2.00 *m*	2.00 *m*	2.15 *m*	2.18 *m*	2.10 *m*
3H-21	0.86 *d* (6.4)	0.86 *d* (6.4)	0.97 *d* (6)	0.96 *d* (6.2)	0.97 *d* (6.3)
22	2.23 *dd* (16.2, 9.5)	2.18 *dd* (16.1, 9.5)	2.35 *br d* (5.2)	2.35 *br d* (6.1)	2.35 *m*
22	2.39 *dd* (16.2, 3)	2.40 *dd* (16.1, 2.5)	2.35 *br d* (5.2)	2.35 *br d* (6.1)	2.35 *m*
24	2.46 *dd* (17.6, 5)	2.40 *dd* (17.5, 5.2)	2.42 *dd* (17.3, 4.5)	2.42 *dd* (17, 4.6)	2.42 *dd* 17.5, 4.8)
24	2.82 *dd* (17.6, 8)	2.79 *dd* (17.5, 8.5)	2.83 *dd* (17.3, 9)	2.84 *dd* (17, 8.5)	2.83 *dd* (17.5, 8.5)
25	2.92 *qdd* (7.1, 8, 5)	2.93 *qdd* (7, 8.5, 5.2)	2.93 *qdd* (7.2, 9, 4.5)	2.95 *qdd* (7, 8.5, 4.6)	2.95 *qdd* (7.1, 8.5, 4.8)
3H-27	1.16 *d* (7.1)	1.15 *d* (7)	1.16 *d* (7.2)	1.17 *d* (7)	1.18 *d* (7.1)
3H-28	1.23 s	1.27 s	1.31 s	1.23 s	1.63 s
3H-29	1.00 *s*	0.90 *s*	1.10 *s*	1.10 *s*	1.13 *s*
3H-30	0.82 *s*	0.87 *s*	1.08 *s*	1.03 *s*	1.11 *s*
OMe	3.67 *s*	3.67 *s*	3.64 *s*	3.67 *s*	3.67 *s*
OAc-3	—	2.03 s	—	—	—
-7	—	1.99 *s*	—	1.91 s	—
-15	—	2.00 *s*	—	—	—
OH-7	—	—	4.04 *d* (4.5)	—	—

Values in parentheses are coupling constants in Hz. — indicates no signal. Signals indicated as *m* were unresolved or overlapped multiplets.





1a R^1 = O, R^2 =  , R^3 = 

1b $R^1 = R^2$ =  , R^3 = O

1c $R^1 = R^2$ =  , R^3 = 

1d $R^1 = R^3$ = O, R^2 = 

1e R^1 = O, R^2 =  , R^3 = 

1f $R^1 = R^2$ =  , R^3 = O

1g $R^1 = R^2$ =  , R^3 = 

1h $R^1 = R^3$ = O, R^2 = 

2a R^1 = O, R^2 = H

2b R^1 =  , R^2 = OAc

2c R^1 =  , R^2 = OAc

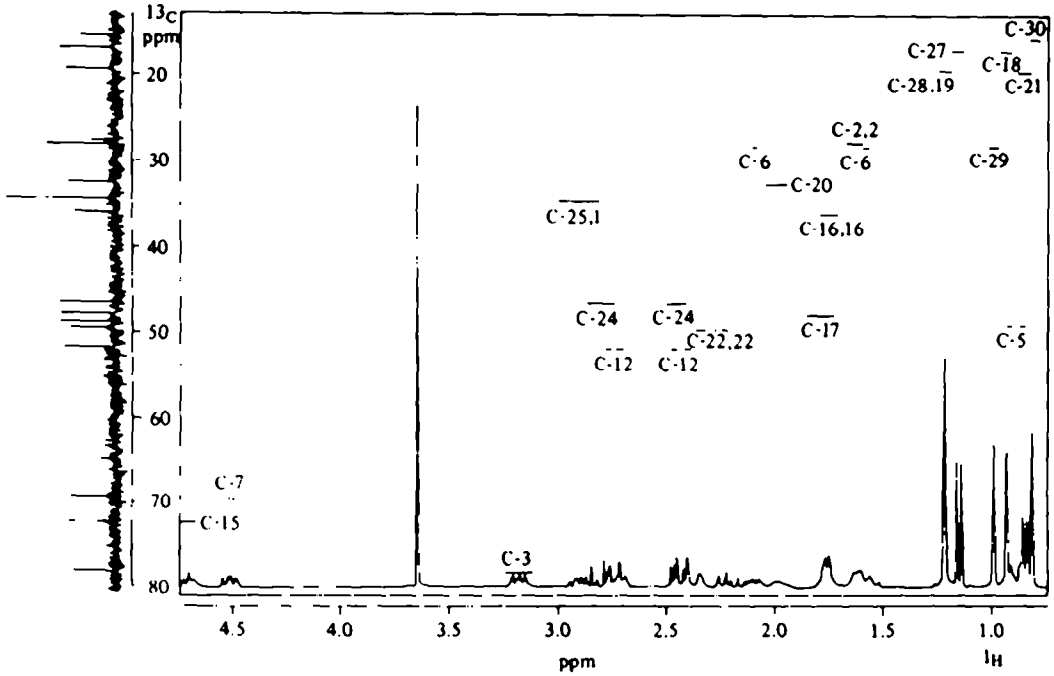


Fig. 1. 300/75.2 MHz ^1H-^{13}C chemical shift correction diagram for compound 1e.

Table 2. ^{13}C NMR spectral data of compound **1c**, **1d**, **1g**, **1h** and **2a** (75.2 MHz, $CDCl_3$)*

	1c	1g	1d	1h	2a
1	34.4	34.1	35.4	35.0	34.3
2	27.6	23.8	34.1	33.9	33.6
3	78.0	79.8	216.4	216.2	215.2
4	38.4	37.5	44.8	44.5	43.7
5	48.9	48.6	48.6	48.0	50.6
6	28.0	24.9	27.5	25.4	37.0
7	69.3	71.0	66.1	68.6	199.1
8	157.9	153.7	157.7	155.2	149.5
9	141.7	145.6	141.0	143.8	146.5
10	38.3	37.7	38.5	37.7	39.1
11	199.8	198.3	197.4	197.3	199.2
12	51.8	51.5	49.9	50.5	48.6
13	46.9	46.8	46.6	46.7	46.7
14	53.8	52.4	59.2	56.9	56.9
15	72.3	74.7	217.5	211.4	206.7
16	36.0	35.6	40.8	41.1	39.6
17	47.9	48.1	45.5	46.1	44.2
18	16.9	16.9	17.5	17.8	15.8
19	19.2	18.5	18.0	18.0	18.4
20	32.7	32.3	31.7	31.6	31.8
21	19.5	19.2	19.4	19.4	19.6
22	49.5	49.2	48.8	49.1	48.8
23	208.5	207.8	207.6	207.7	207.5
24	46.6	46.4	46.6	46.5	46.5
25	34.4	34.4	34.4	34.4	34.4
26	176.2	176.0	175.9	176.0	175.9
27	16.9	16.9	16.9	16.9	16.9
28	19.3	21.1	24.5	25.8	20.7
29	28.0	27.9	26.8	27.1	27.4
30	15.6	16.3	20.6	20.3	20.1
COOMe	51.8	51.7	51.8	51.8	51.7
3AcCO		170.8			
3AcMe		21.1			
7AcCO		170.4		169.7	
7AcMe		21.0		20.7	
15AcCO		171.3			
15AcMe		21.6			

*The number of directry atached protons to each individual carbon were verified by the experiments with the DEPT pulse sequence [5].

not show any signal around δ3.20 (H-3 of **1b**). The ^{13}C NMR spectrum also did not give any signal around δ78.1 and it showed a new signal at δ216.4. These observations indicated the presence of a carbonyl group at C-3. Therefore, compound **1d** was elucidated to be methyl 7β-hydroxy-3,11,15,23-tetraoxo-5α-lanost-8-en-26-oate. Its parent acid was identical with ganoderic acid C reported by Nishitoba *et al.* [2].

Compound **2a**, methyl ganoderate F, analysed for $C_{31}H_{42}O_7$, and showed in the UV spectrum the same absorptions as the other ganoderic acid derivatives. The IR spectrum of **2a** showed no absorption based upon the hydroxyl group. In the ^{13}C NMR spectrum of **2a**, six carbonyl carbon signals were observed at δ175.9, 199.1, 199.2, 206.7, 207.5 and 215.2 (Table 2). All the spectral data of compound **2a** were identical with those of the compound derived from **1a** previously reported [1, 6]. Compound **2a** was first isolated as a natural product from *G. lucidum*.

The assignments of the ^1H NMR and ^{13}C NMR spectral signals of ganoderic acid derivatives were based on the previous published data [1] and on the results of the HOMCOR and HETCOR [5] experiments. They are summarized in Tables 1 and 2.

Six new highly oxygenated triterpene acids, ganoderic acid derivatives, were isolated from the fruit bodies of *G. lucidum* [1, 4, 6]. They all possessed an α,β-unsaturated carbonyl group in the ring B and a terminal carboxylic acid group. While in the cultured mycelia of *G. lucidum*, six new ganoderic acid derivatives were isolated by Toth *et al.* [7], they had a terminal carboxylic acid group but not an α,β-unsaturated carbonyl group. Furthermore three of them all possessed a 3α-hydroxy group. And in our recent work [Hirotani, M. and Furuya, T., unpublished results], similar ganoderic acid derivatives to those reported by Toth *et al.* [7] were obtained from the cultured mycelia of the same fungus *G. lucidum*. They all possessed a 3α-hydroxyl group and a terminal carboxylic acid group.

In plant tissue cultures of *Isodon japonicus*, a 3α-hydroxytriterpenoid, 3-epimaslinic acid, is biosynthesized from its 3β-isomer maslinic acid via a 3-oxo-compound [8]. On the other hand, Rhomer *et al.* [9, 10] reported that in a cell free system from the bacteria *Acetobacter pasteurianum* and *Methylococus capsulantus*, 3α- and 3β-hydroxytriterpenoids were separately formed from (3*R*)- and (3*S*)-2,3-oxidosqualenes without involvement of 3-oxo-compounds.

It is very interesting that the triterpene acids of the differentiated fruit body such as ganoderic acids B and D have the 3β-hydroxyl group, whereas the main triterpene components in the cultured mycelia (undifferentiated) were 3α-hydroxytriterpene acids. It is hoped that a correlation between the biosynthesis of these triterpene acids and fungal differentiation will be revealed by further investigation.

EXPERIMENTAL

Mps were uncorr. MS were run on a direct insertion probe at 20 eV. NMR spectra were taken in $CDCl_3$ at 23°; ^{13}C NMR at 75.2 MHz and ^1H NMR at 300 MHz. HPLC of ganoderic acid derivatives was performed using a Unisil Q C18 (7.6 × 300 mm) column, coupled to a UV detector and a differential refractometer.

Extraction and separation procedure. The cultivated fruit bodies (1.9 kg dry wt) of *G. lucidum* were extracted with 90% MeOH in H_2O (13 l.) for 1 week at room temp. and filtered. The residue was homogenized with the same solvent (16 l.) in a Waring blender and allowed to stand for 1 week at room temp. The homogenate was filtered. The filtrates were combined and the organic solvent was removed under red. pres. The residue was made alkaline (pH 9) by the addition of 5% Na_2CO_3 aq. soln and extracted with $CHCl_3$. The aq. layer was acidified (pH 2) with 4 N H_2SO_4 and extracted with $CHCl_3$.

The $CHCl_3$ layer was washed with H_2O and dried with N_2SO_4 and evaporated to dryness (18.4 g). A part of the acid fraction (17.9 g) was methylated with ethereal CH_2N_2, subjected to CC over silica gel (600 g Wako gel C-200) eluted with EtOAc–C_6H_6, and separated as follows; fraction A C_6H_6 EtOAc (7:3, 2.0 l.) and C_6H_6–EtOAc (3:2, 0.3 l.); fraction B, C_6H_6 EtOAc (3:2, 1.4 l.); fraction C, C_6H_6 EtOAc (3:2, 0.7 l.); fraction D, C_6H_6–EtOAc (3:2, 0.3 l.) and C_6H_6 EtOAc (1:1, 1.2 l.); fraction E, C_6H_6 EtOAc (1:1, 2.25 l.); fraction F, C_6H_6 EtOAc (1:1,

0.75 l) and C_6H_6–EtOAc (2:3, 1.9 l.). Fraction B (7.45 g) contained a mixture of compounds **1d** and **2a**; fraction D (3.4 g) contained compounds **1b** and **2b**; fraction E (3.9 g) and fraction F (1.45 g) contained compounds **1a** and **1c**, respectively. The isolation and structure characterization of compounds **1a**, **1b** and **2b** have been reported in a previous paper [1].

Isolation of methyl ganoderate E (**1c**). Fraction F (1.45 g) was rechromatographed on a silica gel column (180 g Wako gel C-200) and eluted with a Me_2CO–C_6H_6 solvent system. Elution with C_6H_6–Me_2CO (9:1, 0.5 l.) and C_6H_6–Me_2CO (4:1, 1.25 l.) gave fraction F-1, a further 0.36 l. of the latter solvent gave fraction F-2 (0.80 g) which yielded the crude compound **1c**. Further purification of fraction F-2 was achieved by repeated HPLC (Unisil Q C18, solvent: 62% MeOH in H_2O, flow rate 3.5 ml/min) and compound **1c** was isolated from the fractions containing the peak at 13 min. Compound **1c** (307 mg), colourless prisms, mp 198–199.5°, $C_{31}H_{48}O_7$ (required 532.3395, [M]$^+$ *m/z* 532.3374); IR ν_{max}^{KBr} cm^{-1}: 3580 3300, 2960, 2920, 2870, 2840, 1725, 1630. UV λ_{max}^{EtOH} nm (log ε): 250 (3.9). For ^{1}H NMR and ^{13}C NMR spectra of **1c** see Tables 1 and 2. EIMS *m/z* (rel. int.) 532 [M]$^+$ (100), 514 [M – H_2O]$^+$ (28), 392 [$C_{22}H_{32}O_6$]$^+$ (16), 364 [$C_{21}H_{32}O_5$]$^+$ (30), 230 [$C_{15}H_{18}O_2$]$^+$ (19), 171 [side chain]$^+$ (9%), 129 [$C_6H_9O_3$]$^+$ (15).

Isolation of methyl ganoderates C (**1d**) *and F* (**2a**). Fraction B (7.45 g) was rechromatographed on a silica gel column (500 g Wako gel C-200) and eluted with a C_6H_6–Me_2CO solvent system. Elution with C_6H_6 (0.5 l.), C_6H_6–Me_2CO (95:5, 5.66 l.) and C_6H_6–Me_2CO (92:8, 0.85 l.) gave fraction B-1, C_6H_6–Me_2CO (92:8, 1.0 l.) (gave fraction B-2 (2.54 g) and C_6H_6–Me_2CO (92:8, 1.7 l.) gave fraction B-3 (1.64 g) which yielded the crude compounds **2a** and **1d**. The crude compound **2a** was repeatedly recrystallized from MeOH to give yellow needles (291.8 mg). These crystals were still impure on HPLC. Further purification of compound **2a** was achieved on HPLC (Unisil Q C18, solvent 62% MeOH in H_2O, flow rate: 3 ml/min) and compound **2a** was isolated from the fraction containing the peak at 18.9 min. Compound **2a** (114.2 mg) yellow needles, mp 211–213°, $C_{31}H_{42}O_7$ (required 526.2929, [M]$^+$ *m/z* 526.2925); IR ν_{max}^{KBr} cm^{-1}: 2900, 2850, 1730, 1710, 1690, 1670. UV λ_{max}^{EtOH} nm (log ε): 251 (3.9). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS, *m/z* (rel. int.) 526 [M]$^+$ (100), 495 [M – OMe]$^+$ (18), 494 [M – HOMe]$^+$ (22), 382 [$C_{24}H_{30}O_4$]$^+$ (46), 355 [M – side chain]$^+$ (12), 301 [$C_{19}H_{25}O_3$]$^+$ (42), 171 [side chain]$^+$ (9), 139 [$C_8H_{11}O_2$]$^+$ (16), 129 [$C_6H_9O_3$]$^+$ (36).

Compound **1d** (156 mg) was isolated from fraction B-3 by means of the same methods described above for compound **2a**. Compound **1d** (*R*, 20.0 min: HPLC), mp 173–174.5°, $C_{31}H_{44}O_7$ (required 528.3087 [M]$^+$ *m/z* 528.3110); IR ν_{max}^{KBr} cm^{-1}: 3420, 2970, 2930, 1730, 1720, 1700, 1650. UV λ_{max}^{EtOH} nm (log ε): 250 (3.9). For ^{1}H NMR and ^{13}C NMR spectral data of **1d** see Tables 1 and 2. EIMS *m/z* (rel. int.) 528 [M]$^+$ (100), 510 [M – H_2O]$^+$ (10), 500 [M – CO]$^+$ (65), 497 [M – OMe]$^+$ (37), 390 (37), 356 (67), 329 (48), 273 (30), 149 (30), 139 [$C_8H_{11}O_2$]$^+$ (25), 129 [$C_6H_9O_3$]$^+$ (42).

Methyl tri-O-acetyl ganoderate E (**1g**). This compound was prepared from **1c** (35.6 mg) in pyridine–Ac_2O in the usual way. After purification by HPLC, compound **1g** was recrystallized from MeOH–H_2O (27.0 mg), mp 145–146°, $C_{37}H_{54}O_{10}$ (required 658.3720, [M]$^+$ *m/z* 658.3744). IR ν_{max}^{KBr} cm^{-1}: 2960, 2940, 2840, 1730, 1650, 1370, 1240, 1025. UV λ_{max}^{EtOH} nm (lg ε): 249 (4.0). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS *m/z* (rel. int.): 658 [M]$^+$ (37), 616 [M – C_2H_2O]$^+$ (96), 538 [M – 2 × HOAc]$^+$ (8), 496 [M – 2 × HOAc – C_2H_2O]$^+$ (10), 412 [$C_{26}H_{36}O_4$]$^+$ (13), 411 [$C_{26}H_{35}O_4$]$^+$ (12), 171 [side chain]$^+$ (11), 129 [$C_6H_9O_3$]$^+$ (11).

Methyl O-acetyl ganoderate C (**1h**). Ac_2O–pyridine treatment of **1d** (26 mg) overnight at room temp. yielded the derivative **1h** (23.4 mg), mp 169–170°, $C_{33}H_{46}O_8$ (required 570.3192, [M]$^+$ *m/z* 570.3186). IR ν_{max}^{KBr} cm^{-1}: 2980, 2960, 2870, 1735, 1700, 1650. UV λ_{max}^{EtOH} nm (log ε): 251 (3.9). ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS *m/z* (rel. int.): 570 [M]$^+$ (26), 528 [M – C_2H_2O]$^+$ (94), 527 [M – Ac]$^+$ (100), 510 [M – HOAc]$^+$ (13), 480 [M – OAc – OMe]$^+$ (11), 366 [$C_{24}H_{29}O_3$]$^+$, 285 [$C_{19}H_{25}O_2$]$^+$ (22), 171 [side chain]$^+$ (12), 129 [$C_6H_9O_3$]$^+$ (20).

Oxidation of methyl ganoderates E (**1c**) *and C* (**1d**). Compound **1c** (34 mg) was reacted with pyridinium chlorochromate (200 mg) in CH_2Cl_2 (2.5 ml) for 24 hr at room temp. The reaction mixture was filtered and the filtrate was then further filtered through a small amount of silica gel to remove the last trace of chromium species. After removing the CH_2Cl_2, oxidized **1c** was recrystallized from MeOH to give yellow needles (16.3 mg) mp 207–209°, $C_{31}H_{42}O_7$ (required 526.2929, [M]$^+$ *m/z* 526.2941). Oxidized **1c** was identical in all respects to compound **2a**. Compound **1d** (22.4 mg) was oxidized in the same way as described above. Oxidized **1d** (4.4 mg), mp 210–211°, yellow needles, $C_{31}H_{42}O_7$ (required 526.2929, [M]$^+$ *m/z* 526.2927). Oxidized **1d** was identical in all respects to compound **2a**.

The conditions for the NMR measurements. The homo and hetero nuclear two-dimensional chemical shift correlation diagram was obtained according to the method described in a previous paper [1].

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