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A GANODERIC ACID DERIVATIVE, A HIGHLY OXYGENATED LANOSTANE-TYPE TRITERPENOID FROM *GANODERMA LUCIDUM**

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Key Word Index—*Ganoderma lucidum*; Basidiomycetes; higher fungi; triterpenoids; ganoderic acid A, B and C.

Abstract—Ganoderic acid C, a new lanostane-type triterpenoid was isolated from the fruit body of *Ganoderma lucidum*. The structure of ganoderic acid C was elucidated by spectroscopic data and X-ray analysis of methyl ganoderate C acetate.

INTRODUCTION

Species of fungi of the Polyporaceae family (Basidiomycetes) are known to produce lanostane type compounds [1]. In continuation of our studies on the triterpenoids from Polyporaceae [2, 3], a new ganoderic acid derivative has been found in the methanol extract of the fruit body of *Ganoderma lucidum* (Japanese name: Mannen-take). Previous chemical investigation of this fungus [4] and its cultured mycelia [5] led to the isolation of eight polyoxygenated triterpenic acids belonging to the lanostane series which all contain a terminal carboxylic acid group. Some of them were proved to have the cytotoxicity against hepatoma cells *in vitro* [5].

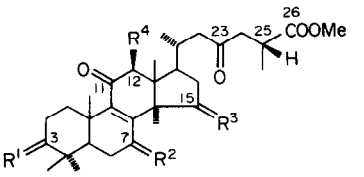
In this paper, we wish to report the isolation of two known ganoderic acids A and B [4] and the structure elucidation of a new ganoderic acid, named ganoderic acid C, by spectroscopic and X-ray analysis, and the assignments of the ^{1}H NMR and ^{13}C NMR spectral signals of ganoderic acid derivatives. The ^{1}H NMR and ^{13}C NMR assignments were performed using proton–proton and proton–carbon shift correlation by two dimensional NMR techniques.

RESULTS AND DISCUSSION

After methylation by ethereal diazomethane, the acidic fraction of the extract from *G. lucidum* was chromatographed on silica gel and separated into fractions A–F (see Experimental). Each fraction was rechromatographed on silica gel and/or HPLC, and compounds **1**, **2** and **3** were isolated from fractions E and D respectively.

Compound **1**, methyl ganoderate A, analysed for $C_{31}H_{46}O_7$ and showed in the UV spectrum an absorption at 249 nm (log ε3.9) characteristic of an α,β-unsaturated carbonyl group. The IR spectrum of **1** showed the presence of hydroxy groups (3540 and 3300 cm^{-1}), an ester carbonyl group (1735 cm^{-1}) and an α,β-unsaturated carbonyl group (1705 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra of **1** were very similar to ganoderic acid A [4] except for the presence of a methyl ester group in compound **1**. The acetylated **1**, mp 199–200°, was identical in all respects (mp, MS, ^{1}H NMR and ^{13}C NMR) to methyl di-*O*-acetyl ganoderate A [4]. Thus, compound **1** was identified as methyl ganoderate A. Compound **2**, methyl ganoderate B, $C_{31}H_{46}O_7$, mp 202–203° was identical in all respects to the reference compound (lit. mp 202.5–203° [4]).

Compound **3**, a new ganoderic acid derivative, mp 91–93°, showed an absorption in the UV spectrum at 251 nm (log ε 3.9), suggesting the presence of an α,β-unsaturated carbonyl system similar to that in compounds **1** and **2**. The molecular formula of compound **3** was established as $C_{33}H_{46}O_9$ by high resolution mass spectrometry. A base peak at m/z 129 in the mass spectrum resulted from cleavage between C-22 and C-23 suggesting the presence of the side chain as in compounds **1** and **2**. The IR spectrum of compound **3** showed a hydroxy band at 3400 cm^{-1}, ester carbonyl at 1735 cm^{-1}



1 R^1 = O, R^2 = α-OH, R^3 = β-H, α-OH, R^4 = H

2 R^1 = R^2 = α-OH, β-H, R^3 = O, R^4 = H

3 R^1 = α-OH, β-H, R^2 = R^3 = O, R^4 = OAc

4 R^1 = O, R^2 = α-OAc, β-H, R^3 = β-H, α-OAc, R^4 = H

5 R^1 = R^2 = α-OAc, β-H, R^3 = O, R^4 = H

6 R^1 = α-OAc, β-H, R^2 = R^3 = O, R^4 = OAc

7 R^1 = R^2 = R^3 = O, R^4 = H

*Part 4 in the series "Studies on the Metabolites of Higher Fungi", For Part 3 see ref. [3].

Table 1. ^{1}H NMR spectral data of compounds 1–7 (300 MHz, $CDCl_3$)*

	1	2	3	4	5	6	7
H-1β	2.82 *ddd* (14, 7.3, 6)	2.83 *ddd* (13.3, 3.4, 3.4)	2.70 *ddd* (14, 3.9, 3.9)	2.91 *ddd* (13.5, 7.5, 5.3)	2.93 *ddd* (13, 4, 4)	2.73 *ddd* (14, 3.8, 3.8)	2.89 *ddd* (14, 9, 6)
H-1α	1.45 *ddd* (14, 8, 8)		1.17 *m*	1.42 *ddd* (13.5, 9.4, 7.7)	1.16 *m*	1.16 *m*	1.73 *ddd* (14, 10, 5.9)
H-2β		1.58 *m*	*ca* 1.68 *m*	2.53 *ddd* (16, 9.4, 7.5)	*ca* 1.68 *m*	1.71 *m*	2.61 *ddd* (15.2, 10, 6)
H-2α		1.67 *m*	*ca* 1.68 *m*	2.43 *ddd* (16, 7.7, 5.3)	*ca* 1.68 *m*	1.71 *m*	2.46 *ddd* (15.2, 9, 6)
H-3α	—	3.20 *ddd* (10.8, 5, 4)	3.24 *ddd* (10.6, 5.5, 5)	—	4.47 *dd* (11, 5.3)	4.50 *dd* (11.5, 5)	—
H-5α		0.87 *dd* (13, 1.5)	1.53 *dd* (13.8, 3)	1.56 *dd* (13.4, 1.4)	1.02 *dd* (13.3, 2)	1.25 *dd* (13, 4.5)	2.30 *dd* (15, 2.7)
H-6β	1.68 *ddd* (13, 13, 9)	1.60 *ddd* (13, 13, 9.3)	2.65 *dd* (13.8, 13.8)	1.44 *ddd* (13.4, 12, 8.1)	1.39 *ddd* (13.3, 13.3, 8.2)	2.65 *dd* (15, 13)	2.69 *dd* (15, 13.5)
H-6α	2.04 *m*	2.18 *ddd* (13, 8, 1.5)	2.53 *dd* (13.8, 3)	2.30 *ddd* (12, 8, 1.4)	2.47 *ddd* (13.3, 8.2, 2)	2.54 *dd* (15, 3)	2.46 *dd* (13.5, 2.7)
H-7α	4.60 *ddd* (11.7, 6.3, 3.5)	4.79 *ddd* (9.3, 8, 4.5)	—	5.55 *dd* (8.1, 8)	5.85 *dd* (8.2, 8.2)	—	—
H-12α	2.74 *dd* (16, 0.8)	2.77 *br d* (17)	5.61 *s*	2.78 *dd* (17, 1)	2.78 *br d* (17.4)	5.63 *s*	2.88 *dd* (16.2, 0.8)
H-12β	2.49 *d* (16)	2.68 *d* (17)	—	2.55 *d* (17)	2.66 *d* (17.4)	—	2.74 *d* (16.2)
H-15β	4.77 *ddd* (7.2, 7.2, 4.5)	—	—	5.34 *dd* (9.5, 6)	—	—	—
H-16β		2.03 *dd* (14.7, 9)	1.90 *dd* (18.2, 8.4)	1.62 *ddd* (14, 10, 6)	2.03 *m*	1.90 *dd* (18, 8.2)	1.85 *dd* (18.2, 8)
H-16α		2.67 *dd* (14.7, 6.4)	2.74 *dd* (18.2, 9.5)	2.14 *ddd* (14, 9.5, 7.5)	2.47 *m*	2.75 *dd* (18, 10)	2.74 *dd* (18.2, 9.3)
H-17		2.09 *m*	2.50 *m*	1.84 *ddd* (10, 10, 7.5)	2.03 *m*	2.51 *ddd* (10, 8.2, 6.5)	2.23 *ddd* (11, 9.3, 8)
3H-18	0.97 *d* (0.8)	1.00 *s*	0.79 *s*	1.03 *s*	1.01 *s*	0.80 *s*	0.88 *d* (0.8)
3H-19	1.24 *s*	1.21 *s*	1.31 *s*	1.28 *s*	1.17 *s*	1.33 *s*	1.27 *s*
H-20	2.00 *m*	2.15 *m*	2.28 *m*	1.99 *m*	2.20 *m*	2.31 *m*	2.10 *m*
3H-21	0.87 *d* (6.3)	0.98 *d* (6.5)	0.96 *d* (6.5)	0.86 *d* (6.3)	0.96 *d* (6.3)	0.96 *d* (6)	0.97 *d* (6.3)
H-22	2.24 *dd* (16, 9.3)	2.36 *br d* (5.0)	2.28 *m*	2.36 *dd* (15.6, 2.5)	2.35 *br d* (5.5)	2.31 *m*	2.35 *m*
H-22	2.38 *dd* (16, 3.2)	2.36 *d* (5.0)	2.28 *m*	2.19 *dd* (15.6, 9.5)	2.35 *br d* (5.5)	2.31 *m*	2.35 *m*
H-24	2.45 *dd* (17, 4.7)	2.44 *dd* (17, 5)	2.40 *dd* (17, 3.5)	2.40 *dd* (17.5, 5.5)	2.43 *dd* (17.2, 4.4)	2.41 *dd* (17.5, 5)	2.42 *dd* (17.5, 4.8)
H-24	2.84 *dd* (17, 8.8)	2.85 *dd* (17, 8.6)	2.81 *dd* (17.3, 9)	2.79 *dd* (17.5, 8.3)	2.84 *dd* (17.2, 8.6)	2.81 *dd* (17.5, 8.5)	2.83 *dd* (17.5, 8.5)
H-25	2.93 *qdd* (7.2, 8.8, 4.7)	2.96 *qdd* (7.3, 8.6, 5)	2.93 *qdd* (7.2, 9, 5)	2.92 *qdd* (7, 8.3, 5.5)	2.94 *qdd* (7, 8.6, 4.4)	2.94 *qdd* (7.2, 8.5, 5)	2.95 *qdd* (7.1, 8.5, 4.8)
3H-27	1.16 *d* (7.2)	1.18 *d* (7.3)	1.16 *d* (7.2)	1.15 *d* (7)	1.17 *d* (7)	1.16 *d* (7.2)	1.18 *d* (7.1)
3H-28	1.26 *s*	1.33 *s*	1.71 *s*	1.28 *s*	1.24 *s*	1.71 *s*	1.63 *s*
3H-29	1.10 *s*	1.03 *s*	1.01 *s*	1.10 *s*	0.90 *s*	0.94 *s*	1.13 *s*
3H-30	1.08 *s*	0.84 *s*	0.86 *s*	1.04 *s*	0.87 *s*	0.89 *s*	1.11 *s*
OMe	3.66 *s*	3.67 *s*	3.65 *s*	3.67 *s*	3.67 *s*	3.65 *s*	3.67 *s*
OAc-3	—	—	—	—	2.04 *s*	2.03 *s*	—
OAc-7	—	—	—	2.00 *s*	1.91 *s*	—	—
OAc-12	—	—	2.22 *s*	—	—	2.22 *s*	—
OAc-15	—	—	—	2.01 *s*	—	—	—
OH-3	—	1.37 *d* (4)	1.43 *d* (5.5)	—	—	—	—
OH-7	3.66 *d* (6.3)	4.04 *d* (4.5)	—	—	—	—	—
OH-15	3.16 *d* (4.5)	—	—	—	—	—	—

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

and several carbonyl groups (1710, 1690 and 1660 cm^{-1}). The ^1H NMR spectrum further showed the presence of an acetoxy methyl group (δ2.14) and two methine protons (δ3.24, *ddd*, J = 10.6, 5.5, 5 Hz and δ5.61, *s*) attached to carbon atoms bearing oxygen functions (Table 1). The apparent singlet signal at δ5.61 corresponds to the acetoxy methine proton which was placed on the position unable to couple with any other proton. The presence of the acetoxy group was supported by the bands (δ21.2 and 170.7) of the ^{13}C NMR spectrum (Table 2).

The hydroxy group of compound **3** is secondary and equatorially oriented, because its geminal proton appeared at δ3.24 (*ddd*, J = 10.6, 5.5, 5 Hz) in the ^1H NMR spectrum of **3** and was shifted to 4.50 and transformed into a doublet doublet (J = 11.5, 5 Hz) in the derivative **6**. In the ^{13}C NMR spectrum of **3**, six carbonyl carbon signals were observed at δ170.1, 176.0, 193.8, 198.8, 205.6 and 207.4. Two of them (δ170.1 and 176.0) were unambiguously assigned to the ester carbonyl carbons. Two (δ 193.8 and 198.8) of the remaining carbonyl carbons were also assigned to the α,β-unsaturated carbonyl carbons (C-11 and C-7) in comparison with the spectral data of compound **7**. The last remaining two carbonyl carbon signals (δ205.6 and 207.4) were assigned to the isolated carbons (C-15 and C-23) by comparison with the published data [4]. All these data indicated that compound **3** was a 3β-hydroxy-7,11,15,23-tetra oxo-Δ^8-lanostane derivative with an acetoxy group in the lanostane skeleton. The relative configurations at the respective asymmetric centres of compound **6** and the position of the acetoxy group were determined by X-ray analysis. Crystals of compound **6** were obtained as a monohydrate. Crystal data: orthorhombic, space group $P2_12_12_1$, a = 15.779 (3), b = 20.177 (3), c = 10.995 (2) Å, Z = 4. Three-

Table 2. ^{13}C NMR spectral data of compounds **1**–**7** (75.2 MHz, $CDCl_3$)*

	1	**2**	**3**	**4**	**5**	**6**	**7**
1	35.4	34.7	33.1	35.2	34.0	32.8	34.3
2	34.1	27.5	27.1	34.1	23.7	23.5	33.6
3	217.1	78.1	77.2	216.0	79.7	78.8	215.2
4	46.5	38.7	39.0	46.3	37.4	37.8	43.7
5	48.6	49.0	51.2	48.2	48.8	51.1	50.6
6	28.9	26.5	36.3	25.9	24.5	36.3	37.0
7	68.7	66.8	198.8	70.5	69.2	198.3	199.1
8	159.0	157.1	151.5	154.7	154.5	151.4	149.5
9	140.1	143.0	145.6	144.2	145.3	145.8	146.5
10	37.8	38.5	40.2	37.4	38.3	40.1	39.1
11	199.4	198.3	193.8	198.1	197.5	193.9	199.2
12	51.6	50.1	79.0	51.3	50.7	79.0	48.6
13	46.6	45.2	47.8	46.6	45.0	47.9	46.7
14	53.8	59.2	58.3	52.4	56.9	58.3	56.9
15	72.3	217.8	205.6	74.7	211.3	205.6	206.7
16	36.2	40.8	37.7	35.5	41.0	37.8	39.6
17	47.9	45.4	44.5	48.2	45.9	44.6	44.2
18	17.1	17.2	12.0	17.1	17.6	12.1	15.8
19	19.2	18.3	17.8	18.1	17.9	17.8	18.4
20	32.5	31.8	29.2	32.3	31.6	29.2	31.8
21	19.4	19.5	21.5	19.2	19.5	21.5	19.6
22	49.5	48.9	48.3	49.1	49.2	48.3	48.8
23	208.6	208.3	207.4	207.8	207.7	207.4	207.5
24	46.6	46.8	46.5	46.4	46.7	46.5	46.5
25	34.5	34.5	34.5	34.4	34.5	34.5	34.4
26	176.2	176.3	176.0	176.0	176.0	176.0	175.9
27	17.0	17.0	16.9	16.9	17.0	16.9	16.9
28	19.5	24.3	21.1	21.9	25.5	21.0†	20.7
29	27.2	28.0	27.7	26.7	28.0	27.7	27.4
30	20.6	15.3	15.4	20.6	16.3	16.5	20.1
$COO\underline{C}H_3$	51.8	51.8	51.8	51.7	51.8	51.8	51.7
$3Ac\underline{C}O$	—	—	—	—	170.8†	170.5	—
$3Ac\underline{C}H_3$	—	—	—	—	21.1	21.2†	—
$7Ac\underline{C}O$	—	—	—	170.3	169.8†	—	—
$7Ac\underline{C}H_3$	—	—	—	20.9	20.7	—	—
$12Ac\underline{C}O$	—	—	170.1	—	—	170.0	
$12Ac\underline{C}H_3$	—	—	20.7	—	—	20.7	—
$15Ac\underline{C}O$	—	—	—	171.2	—	—	—
$15Ac\underline{C}H_3$	—	—	—	21.1	—	—	—

*The number of directly attached protons to each individual carbon were verified by the experiments with the DEPT pulse sequence [6].

†Values in any vertical column may be interchanged.

dimensional intensity data were collected on a Rigaku AFC-5UD diffractometer with graphite monochromated Cu Kα radiation ($\lambda = 1.54178$ Å) using a crystal of dimensions 0.5 × 0.3 × 0.2 mm. 3113 of 3344 unique reflections in the range $\theta \leqslant 65°$ were observed [$|F_o| \geqslant \sigma(F_o)$]. The structure was solved by the direct methods, and refined by the block-diagonal least-squares technique to an R value of 0.039 for 2850 reflections with $|F_c| \geqslant \sigma_1(F_o)$ and $|\Delta F| < 3\sigma_1(F_o)$, where $\sigma_1(F_o) = [\sigma^2(F_o) + 0.00197|F_o|^2]^{1/2}$. The positions of the hydrogen atoms, except those of the C-32 methyl group and the water molecule, were located in a difference electron density map and refined by the least-squares method. There are no unusual bond lengths and angles. The absolute configuration of the molecule is based on the CD spectrum of compound **6** which showed a negative Cotton effect ($[\theta]_{248} -17210$) like that of the known compound **7** derived from compound **1**. Thus **3** and **6** are methyl 12β-acetoxy-3β-hydroxy-7,11,15,23-tetraoxo-5α-lanost-8-en-26-oate and the corresponding acetate, respectively. The former was named methyl ganoderate C and the parent acid ganoderic acid C.

The assignments of the ^{13}C NMR spectral signals of compound **3** were made from the results of the DEPT [6], HOMCOR and HETCOR [7] experiments and according to the published data [4, 8]. The nine carbon side chain bands were assigned as shown in Table 1 on the basis of the published data [4]. The six carbonyl carbon signals at δ198.8, 193.8, 205.6, 207.4, 176.0 and 170.1 were easily assigned to C-7, C-11, C-15, C-23, C-26 and the acetoxy carbonyl carbon relative to the data of ganoderic acids A and B, respectively (Table 2). Also two olefinic carbon signals at δ145.6 and 151.5 were assigned to C-9 and C-8 respectively.

The assignments of the ring A carbon atom bands were facilitated by the presence of the 3β-hydroxy group. The hydroxy-bearing C-3 atom gives a band well removed from the main group of bands characterizing the remaining saturated carbon atoms and so it is easily identified. A study of the spectra given by the 3β-alcohol acetate (**6**) and 3-oxo compounds (**1** and **4**) enabled the C-2, C-4, C-29 and C-30 bands to be assigned [8]. The remaining sp^3 quaternary carbon atom bands were assigned on the basis of the data of ganoderic acids A and B.

The remaining bands were assigned from the comparative study of the proton–proton and proton–carbon two dimensional NMR [7] spectral data. The assignments of the two methyl proton signals (C-21 and C-27 methyl) of **3** were facilitated by their doublet signals. It was very easy to identify the cross-peaks corresponding to the C-21 and C-27 carbon atom bands and the proton signals in the proton–carbon shift correlation diagram (Fig. 2), and so the two methyl carbon bands at δ16.9 and 21.5 were unambiguously assigned to C-27 and C-21. Also two (H-20 and H-25) of the four methine proton signals were identified as the signals at δ2.28 and 2.93 by the proton-proton shift correlation diagram (Fig. 3).

The signal of the H-5 methine proton was assigned by the chemical shift value (δ1.53) and coupling constants (*dd*, J = 13.8, 3 Hz). Three (δ29.2, 34.5 and 51.2) of the four methine carbon atom bands were therefore assigned to C-20, C-25 and C-5 respectively. Consequently, the remaining CH carbon atom band (δ44.5) must be assigned to C-17. In the same way, the remaining five methylene carbon atom bands, except for C-2 in the spectrum of **3**, were assigned and they are listed in Table 2.

The off-resonance decoupling (SFORD) was used to provide the number of directly attached protons to each individual carbon. The multiplets are often broad due to uncollapsed long range couplings and are difficult to interpret in complex molecules. A more complete analysis with respect to quarternary carbons (CH, CH_2 and Me groups) was possible using spectral editing with the DEPT pulse sequence [6]. As shown in Fig. 4, the band at δ48.8 in the ^{13}C NMR spectrum of compound **7** was apparently assigned to a CH_2 carbon. However, in a previous paper [4], this band was assigned to C-17 which was a CH carbon atom.

The assignments of the ^1H NMR and ^{13}C NMR spectral signals of the other ganoderic acid derivatives were also performed by the techniques described above and the results are listed in Tables 1 and 2. These results showed that the modern NMR techniques, for example proton–proton and proton–carbon two dimensional NMR are powerful methods for the assignments of the ^1H NMR and ^{13}C NMR spectral signals.

EXPERIMENTAL

Mps were uncorr. Mass spectra were run on a direct insertion probe. NMR spectra were taken in $CDCl_3$ at 23°; ^{13}C NMR at

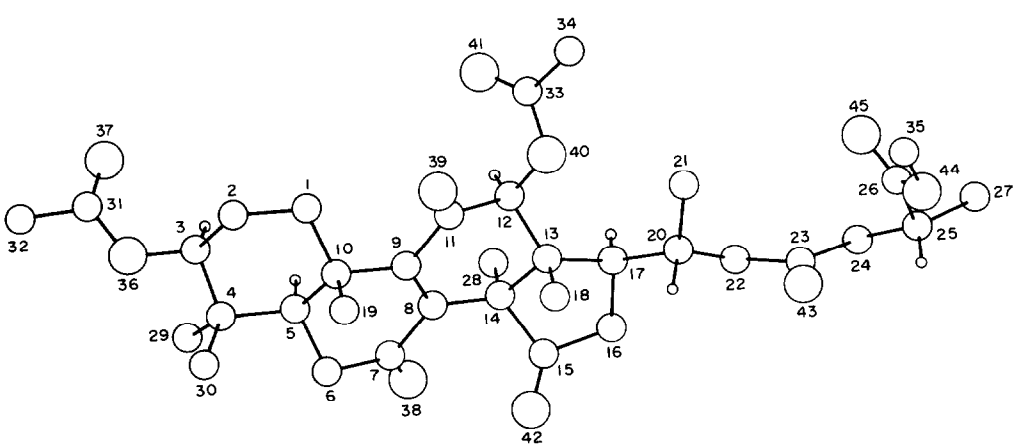


Fig. 1. A computer-generated perspective drawing of **6**.

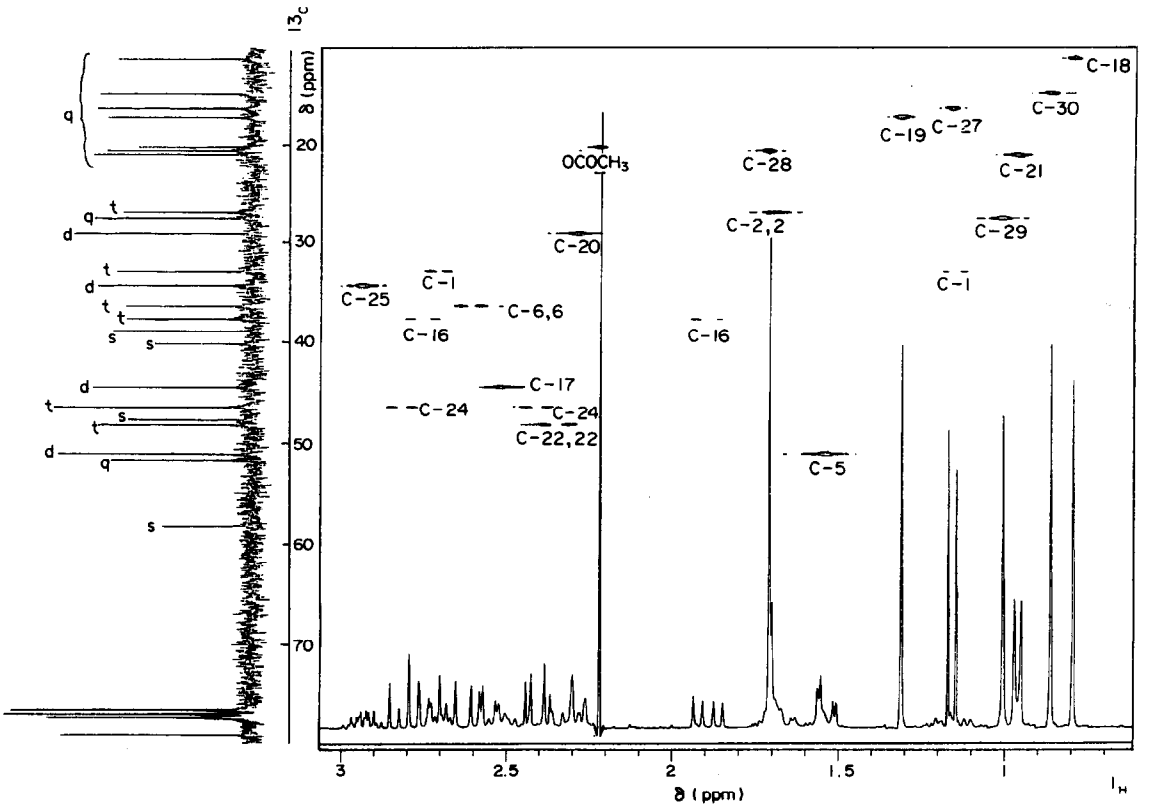


Fig. 2. 300/75.2 MHz ^{1}H–^{13}C chemical shift correlation diagram for compound 3.

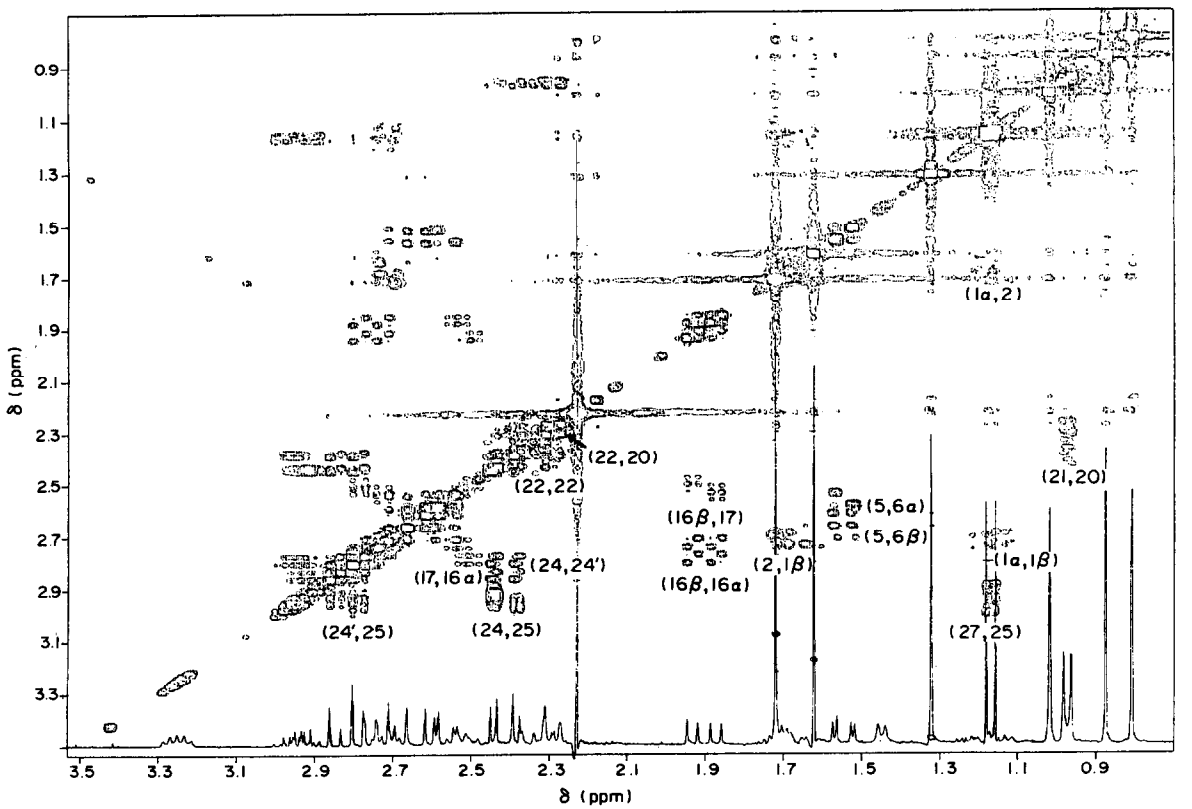


Fig. 3. Homonuclear ^{1}H–^{1}H chemical shift correlation diagram for compound 3.

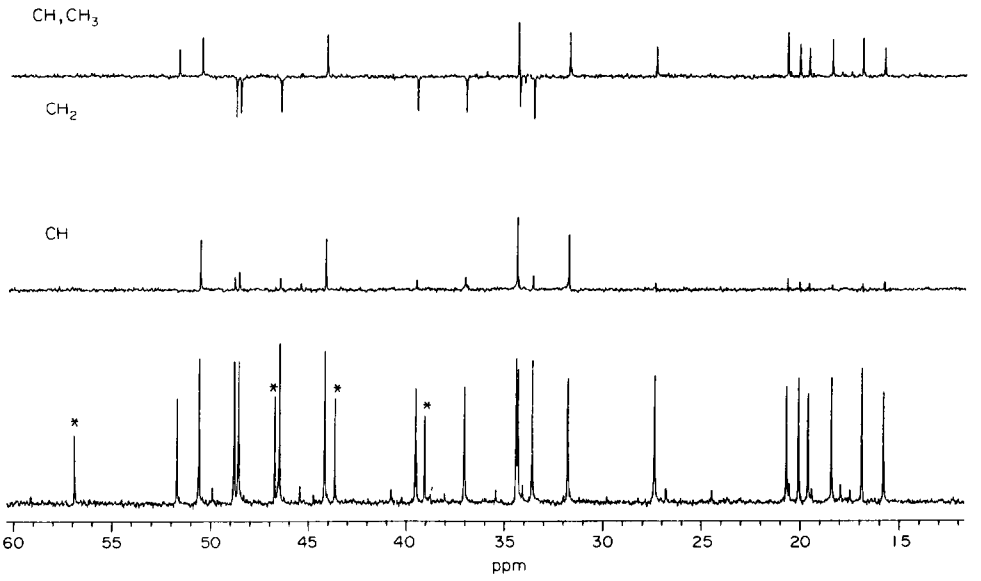


Fig. 4. ^1H decoupled ^{13}C NMR spectrum of compound 7 (sp^3 region only) and subspectra for CH, CH_2 and Me groups obtained from experiments with the DEPT pulse sequence; quaternary carbons are marked by asterisks.

75.2 MHz and ^1H NMR at 300 MHz. All the spectra were recorded on a Varian XL-300.

Extraction and separation procedure. The cultivated fruit bodies (1.9 kg dry wt) of *G. lucidum* were cut into small pieces and extracted with 90% MeOH in H_2O (13 l.) for one week at room temp. and filtered. The residue was homogenized with 90% MeOH in H_2O (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% aq. Na_2CO_3 and extracted × 4 with $CHCl_3$ (in total 2 l.). The aq. layer was acidified (pH 2) with 4 N H_2SO_4 and extracted × 5 with $CHCl_3$ (in total 3 l.). The $CHCl_3$ layer was washed with H_2O and dried with Na_2SO_4 and evaporated to dryness (18.35 g). A part of the acid fraction (17.88 g) was methylated with ethereal CH_2N_2 in the usual way. The methylated acid fraction (17.42 g) was subjected to chromatography over silica gel (600 g Wako gel C-200). Elution with 30% EtOAc in C_6H_6 (2.0 l.) and 40% EtOAc in C_6H_6 (0.3 l.) gave fraction A; 40% EtOAc in C_6H_6 (1.4 l.) gave fraction B and a further 0.7 l. gave fraction C; 40% EtOAc in C_6H_6 (0.3 l.) and 50% EtOAc in C_6H_6 (1.2 l.) gave fraction D and after 2.25 l. yielded fraction E; 50% EtOAc in C_6H_6 (0.75 l.) and 60% EtOAc in C_6H_6 (1.9 l.) gave fraction F. A mixture of compounds **2** and **3** was in fraction D (3.4 g) and compound **1** was in fraction E (3.9 g).

Isolation and identification of methyl ganoderate A (**1**). Fraction E (3.9 g) was rechromatographed on a silica gel column (200 g Wako gel C-200) and eluted with a C_6H_6–Me_2CO to yield three fractions (fractions E-1, 2 and 3). After evaporation of the solvent of fraction E-2, compound **1** was recrystallized from C_6H_6 to yield colourless needles (1.10 g), mp 199.5–200.5°, $C_{31}H_{46}O_7$ (required 530.3244, $[M]^+$ *m/z* 530.3268); IR ν_{max}^{KBr} cm^{-1}: 3540, 3300 (OH), 2980, 2960, 2800 (CH), 1735 (COO), 1705, 1660, 1635 (CO); UV λ_{max}^{EtOH} nm (log ε): 249 (3.9). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 530 $[M]^+$ (83), 512 $[M-H_2O]^+$ (50), 494 $[M-2\times H_2O]^+$ (18), 392 (33), 387 (50), 386 (22), 368 (50), 364 (67), 240 (100), 171 $[C_9H_{15}O_3]^+$ (44), 144 $[C_7H_{12}O_3]^+$ (93), 129 $[C_6H_9O_3]^+$ (50).

Isolation and structure elucidation of methyl ganoderate B (**2**) *and C* (**3**). Fraction D (3.4 g) was rechromatographed on a silica gel column (250 g Wako gel C-200) and eluted with C_6H_6–EtOAc mixtures. Elution with 20% EtOAc in C_6H_6 (0.5 l.) and 30% EtOAc in C_6H_6 (2.4 l.) gave fraction D-1; a further 0.9 l. gave fraction D-2 (2.12 g). Fraction D-2 was rechromatographed on a silica gel column and gave three fractions (D-2-1, D-2-2 and D-2-3). Fraction D-2-2, after evaporation of the solvent, was recrystallized from C_6H_6–*n*-hexane to yield colourless crude crystals (629 mg). Further purification was achieved by repeated HPLC and compounds **2** and **3** were isolated from the fractions containing the peak at 14.5 min and 16.0 min, respectively. Compound **2** (164.3 mg), colourless needles, mp 203–204°, $C_{31}H_{46}O_7$ (required 530.3244, $[M]^+$ *m/z* 530.3252); IR ν_{max}^{KBr} cm^{-1}: 3560, 3540 (OH), 2960, 2920, 2870 (CH), 1730 (COO), 1705, 1640 (CO); UV λ_{max}^{EtOH} nm (log ε): 250 (3.7). ^1H NMR and ^{13}C NMR spectra of **2**: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 530 $[M]^+$ (100), 512 $[M-H_2O]^+$ (23), 502 $[M-CO]^+$ (74), 390 (58), 358 (95), 129 $[C_6H_9O_3]^+$ (30).

Compound **3** (115.5 mg), pale yellow needles, mp 91–93°, $C_{33}H_{46}O_9$ (required 586.3142, $[M]^+$ *m/z* 586.3156); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2960, 2920, 2870 (CH), 1735 (COO), 1710, 1690, 1660 (CO); UV λ_{max}^{EtOH} nm (log ε): 251 (3.8). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 586 $[M]^+$ (16), 568 $[M-H_2O]^+$ (37), 544 $[C_{31}H_{44}O_8]^+$ (79), 526 $[M-HOAc]^+$ (68), 508 $[M-H_2O-HOAc]^+$ (21), 493 $[M-H_2O-HOAc-Me]^+$ (24), 415 [M − side chain]$^+$ (16), 129 $[C_6H_9O_3]^+$ (100).

Methyl di-O-acetyl ganoderate A (**4**). This compound was prepared from **1** (56 mg) in pyridine–Ac_2O by warming to about 80° on a water bath for 3 hr. After purification, compound **4** was recrystallized from MeOH as colourless prisms (24 mg), mp 199–200°, $C_{35}H_{50}O_9$ (required 614.3454, $[M]^+$ *m/z* 614.3447). IR ν_{max}^{KBr} cm^{-1}: 2915, 2850 (CH), 1735 (COO), 1710, 1660 (CO); UV λ_{max}^{EtOH} nm (log ε): 248 (4.0). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 614 $[M]^+$ (12), 573 $[C_{33}H_{49}O_8]^+$ (28), 572 $[M-C_2H_2O]^+$ (75), 554 $[M-HOAc]^+$ (24), 513 $[C_{31}H_{35}O_6]^+$ (35), 512 $[M-HOAc-CHO]^+$ (100), 480 $[M-HOAc-OAc-Me]^+$ (16), 411

$[C_{26}H_{35}O_4]^+$ (14), 368 (19), 367 (18), 171 [side chain]$^+$ (19), 129 $[C_6H_9O_3]^+$ (29).

Methyl di-O-acetyl ganoderate B (**5**). Ac_2O–pyridine treatment of **2** (59 mg) overnight at room temp. yielded the derivative **5** (50 mg) after crystallization, mp 159.5–160.5°, $C_{35}H_{50}O_9$ (required 614.3454, $[M]^+$ *m/z* 614.3444); IR ν_{max}^{KBr} cm^{-1}: 2960, 2880 (CH), 1730 (COO), 1650 (CO); UV λ_{max}^{EtOH} nm (log ε): 249 (3.9). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 614 $[M]^+$ (10), 571 $[M-Ac]^+$ (100), 512 $[M-Ac-AcO]^+$ (13), 410 (13), 139 $[C_8H_{11}O_2]^+$ (8), 129 $[C_6H_9O_3]^+$ (12).

Methyl-O-acetyl ganoderate C (**6**) *and X-ray data.* Compound **3** (31 mg) was acetylated by reacting with Ac_2O–pyridine and allowing the mixture to stand at room temp. overnight. The acetate was recrystallized from MeOH–H_2O to afford pale yellow needles (27.8 mg), mp 135–136°, $C_{35}H_{48}O_{10}$ (required 628.3246, $[M]^+$ *m/z* 628.3242); IR ν_{max}^{KBr} cm^{-1}: 2970, 2940 (CH), 1750, 1730 (COO), 1720, 1690 (CO); UV λ_{max}^{EtOH} nm (log ε): 253 (3.7); CD curve $[\theta]_{221}$ +38 380, $[\theta]_{248}$ −17 210, $[\theta]_{273}$ +30 000, $[\theta]_{304}$ −27 448, $[\theta]_{395}$ +4187 (EtOH, *c* = 0.054). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 628 $[M]^+$ (11), 586 $[M-CH_2CO]^+$ (26), 568 $[M-HOAc]^+$ (22), 457 $[M-$side chain$]^+$ (26), 397 $[M-$side chain $-HOAc]^+$ (15), 346 (89), 191 $[C_{12}H_{15}O_2]^+$ (87), 171 [side chain]$^+$ (13), 129 $[C_6H_9O_3]^+$ (100). X-ray data are described in the Results and Discussion, and atomic coordinates have been deposited at the Cambridge Crystallographic Data Centre.

Synthesis of compound **7**. Compound **1** (30 mg) was reacted with pyridinium chlorochromate (100 mg) in 2 ml CH_2Cl_2 for 10 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 Cr species. After removing the CH_2Cl_2 and purification by HPLC, compound **7** [9] was recrystallized from MeOH to give yellow needles (6.5 mg), mp 212–213°, $C_{31}H_{42}O_7$ (required 526.2929, $[M]^+$ *m/z* 526.2921), IR ν_{max}^{KBr} cm^{-1}: 2900, 2850 (CH), 1730 (COO), 1710, 1690, 1670 (CO). UV λ_{max}^{EtOH} nm (log ε): 252 (3.9), CD curve $[\theta]_{223}$ +22330, $[\theta]_{249}$ −5700, $[\theta]_{272}$ +13650, $[\theta]_{303}$ −13900, $[\theta]_{398}$ +4720 (EtOH, *c* = 0.021). ^1H NMR and ^{13}C NMR: see Tables 1 and 2. EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 526 $[M]^+$ (100), 384 $[C_{24}H_{31}O_4]^+$ (15), 355 $[M-$side chain$]^+$ (15), 301 $[C_{19}H_{25}O_3]^+$ (45), 225 (13), 171 [side chain]$^+$ (8), 139 $[C_8H_{11}O_2]^+$ (14), 129 $[C_6H_9O_3]^+$ (45).

Conditions for HPLC and data of ganoderic acid derivatives. HPLC analyses were run on a Waters liquid chromatograph 5000 A instrument with a Shimadzu absorbance detector model SPD-SA. A column (7.6 × 300 mm) packed with Unisil Q C18 was operated under the following conditions: 60% MeOH in H_2O 3.5 ml/min, compound **1** 20.0 (min), compound **2** 14.5 min, compound **3** 16.0 min, compound **7** 13.7 min; 75% MeOH in H_2O 3.5 ml/min, compound **5** 10.4 min, compound **6** 6.9 min; 80% MeOH in H_2O 3.0 ml/min, compound **4** 8.9 min.

The conditions for NMR measurements. All the spectra were recorded on a Varian XL-300. The heteronuclear two-dimensional ^1H–^{13}C chemical shift correlation diagram was obtained using the 5 mm sample, spectral widths of 5197.5 Hz (^{13}C, F_2) and ±500 Hz (^1H, F_1) and a data acquisition of 128 scans, 128 increments in t_1 to provide, after zero filling, a matrix of 2048 × 512 (t_2, t_1) which was transformed into (F_2, t_1) and then to (F_2, F_1) using shifted sine bell functions for weighting in both dimensions. This provided digital resolutions of 5.08 and 0.98 Hz per point in the F_2 and F_1 domains, respectively. The refocusing delay was 6.3 msec, the relaxation delay 1 sec.

The homonuclear ^1H–^1H chemical shift correlation two-dimensional diagram was obtained using the 5 mm sample by application of the HOMCOR. The spectral widths were F_2 1210.2 Hz and F_1 ±605.1 Hz, allowing a digital resolution of 2.368 Hz per point after zero filling and weighting with shifted sine bell functions to obtain a matrix of 1024 × 1024 (t_2, t_1), which was transformed in both dimensions as in the previous case. The number of increments in t_1 was 256, scans 16 and the relaxation delay 0.6 s.

For spectral editing with the DEPT pulse sequence spectral widths of 3671.1 Hz and 8192 data point were used, amounting 8192 zero filling to a digital resolution 0.896 Hz per point. The DEPT sequence was performed with 4.0 ms for the $(2J)^{-1}$ delay, 2 sec relaxation delay and an overall repetition time of 3.12 sec. Pulse widths were 14.7 μs for the 90° ^{13}C and 15 μsec for the 90° ^1H pulse. Two DEPT spectra with $(^1H) = \pi/2$ (128 transients) I and $(^1H) = 3\pi/4$ (128 transients) II were recorded.

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