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TRITERPENES IN *GANODERMA LUCIDUM*

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Key Word Index—*Ganoderma lucidum*; Polyporaceae; structure determination; triterpenes.

Abstract—Four new oxygenated triterpenes, isolated from the mycelia of the fungus *Ganoderma lucidum*, were determined to be lanosta-7,9(11),24-trien-3α,15α-dihydroxy-26-oic acid, lanosta-7,9(11),24-trien-3β,15α-dihydroxy-26-oic acid, lanosta-7,9(11),24-trien-3α,22β-diacetoxy-15α-hydroxy-26-oic acid and lanosta-7,9(11),24-trien-15α,22β-diacetoxy-3β-hydroxy-26-oic acid by spectroscopic methods.

INTRODUCTION

We have reported previously the isolation and structural elucidation of ganodermic acids R (**1**), S (**2**), N (**5**), O (**6**) and Q (**7**), together with the known triterpenes, ganoderic acids X (**3**) [1, 2] and Mf (**4**) [3] from the mycelia of fungus *Ganoderma lucidum* (Fr.) Karst. Subsequent investigation of the more polar metabolites resulted in the isolation of four more new triterpenes. Based on spectral analysis their structures were determined to be lanosta-7,9(11),24-trien-3α,15α-dihydroxy-26-oic acid (**8**) (tentatively named ganodermic acid Ja), lanosta-7,9(11),24-trien-3β,15α-dihydroxy-26-oic acid (**9**) (ganodermic acid Jb), lanosta-7,9(11), 24-trien-3α,22β-diacetoxy-15α-hydroxy-26-oic acid (**10**) (ganodermic acid P1) and lanosta-7,9(11),24-trien-15α,22β-diacetoxy-3β-hydroxy-26-oic acid (**11**) (ganodermic acid P2).

RESULTS AND DISCUSSION

The UV spectra of compounds **8–11** showed almost identical absorption bands at 252, 243 and 235 nm (in MeOH) indicating that these compounds possessed a transoid heteroannular diene skeleton as a part of their structures. Compounds **8** and **9** both gave a molecular ion peak at *m/z* 470 ($C_{30}H_{46}O_4$) (EIMS, 12 eV). Two common fragment ion peaks at *m/z* 452 $[M-H_2O]^+$ and 419 $[M-2H_2O-Me]^+$ showed that both compounds had two hydroxy groups. A prominent fragment ion peak at *m/z* 311 $[M-H_2O-C_8H_{13}O_2$ side chain$]^+$ indicated that both compounds had the same side chain at C-17. The similarity in mass fragmentation patterns strongly suggested that these two triterpenes were either positional or stereo-isomers.

The ^{1}H NMR spectra (Bruker AM-400) of **8** and **9** (Table 1) showed similar chemical shifts and coupling patterns for H-7, H-11, H-15 and H-24 except for those signals adjacent to H-3. A singlet at δ3.44 in the spectrum of **8** strongly suggested that one of its hydroxy groups was a 3α-hydroxy group. A methine proton signal at δ3.06 (*dd*, $J = 6.3, 9.2$ Hz) in **9** was thus assigned to H-3α. An upfield shift of the H-15 signal in **8** and **9**, as compared with those of ganodermic acids R (**1**) and S (**2**) [1], indicated that the second hydroxy group was a 15α-hydroxy group. Com-

Table 1. Important ^{1}H NMR spectral data of compounds **8–11** (400 MHz, $CDCl_3$)

H	**8**	**9**†	**10**	**11**
3	3.44 *s*	3.06 *dd* (6.3, 9.2)*	4.65 *s*	3.23 *dd* (4.2, 11.3)
7	5.81 *m*	5.71 *d* (6.0)	5.86 *d* (4.4)	5.46 *d* (5.4)
11	5.30 *d* (5.7)	5.15 *d* (5.6)	5.29 *d* (5.1)	5.28 *d* (5.6)
15	4.26 *dd* (5.7, 9.6)	4.09 *dd* (5.9, 9.6)	4.24 *dd* (4.9, 9.8)	5.03 *dd* (5.0, 10.5)
18	0.59 *s*	0.46 *s*	0.59 *s*	0.62 *s*
19	0.96 *s*‡	0.83 *s*‡	0.98 *s*‡	0.99 *s*‡
21	0.88 *d* (6.5)	0.76 *d* (6.5)	§	§
22	§	§	5.02 *t* (6.6)	5.00 *t* (7.0
24	6.83 *t* (7.0)	6.64 *t* (7.1)	6.79 *t* (7.0)	6.75 *t* (7.2)
27	1.79 *s*	1.67 *s*	1.84 *s*	1.83 *s*
28	0.92 *s*‡	0.78 *s*‡	0.94 *s*‡	0.96 *s*‡
29	0.90 *s*‡	0.72 *s*‡	0.85 *s*‡	0.85 *s*‡
30	0.95 *s*‡	0.83 *s*‡	0.96 *s*‡	0.94 *s*‡
OAc	—	—	2.04 *s*	2.06 *s*
OAc	—	—	2.03 *s*	2.04 *s*

*Values in parentheses are coupling constants in Hz.

†Sample was dissolved in $CDCl_3/CD_3OD$ (≅ 3/1, v/v) due to its low solubility in $CDCl_3$.

‡Tentative assignments.

§Overlapped with other signals.

plete assignment of the ^{13}C NMR spectra of **8** and **9** (Table 2) was based on the trend of an upfield shift of the C-3 and C-15 signals and comparison with the spectral data of **1**–**3**, **5** and **7**. The assignments were compatible with those based on the ^1H NMR and DEPT experiments.

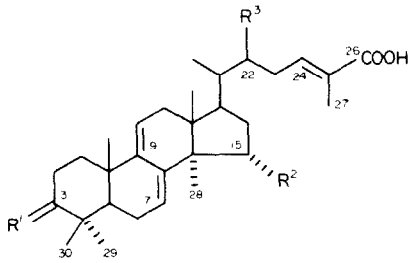
Alkaline hydrolysis of **1** afforded **8** further supporting the structure of **8** as lanosta-7,9(11),24-trien-3α,15α-dihydroxy-26-oic acid (ganodermic acid Ja). Upon hydrolysis of **2** in the same way, compound **9** was obtained. It was therefore confirmed that **9** was the C-3 stereoisomer of **8** and has the structure lanosta-7,9(11),24-trien-3β,15α-dihydroxy-26-oic acid (ganodermic acid Jb).

Triterpenes **10** and **11** also gave rise to the same molecular ion peak at *m/z* 570 ($C_{34}H_{50}O_7$). The mass spectra showed three common fragment ion peaks, *m/z* 552 $[M-H_2O]^+$, 510 $[M-HOAc]^+$ and 450 $[M-2HOAc]^+$, indicating the presence of one hydroxy and two acetoxy groups in these compounds. A prominent fragment ion peak at *m/z* 311 $[M-C_{10}H_{15}O_4$ side chain $-HOAc]^+$ suggested that both compounds possessed the same side chain. However, distinct fragment ion peaks

Table 2. ^{13}C NMR spectral data of compounds **8**–**11** (100.6 MHz, $CDCl_3$)

C	8	9*	10†	11
1	29.96 *t*	35.49 *t*	30.53 *t*	35.63 *t*
2	25.58 *t*	27.04 *t*	23.06 *t*	27.67 *t*
3	76.27 *d*	78.30 *d*	77.99 *d*	78.80 *d*
4	37.34 *s*	38.28 *s*	36.44 *s*	38.58 *s*
5	43.08 *d*	48.45 *d*	44.16 *d*	48.75 *d*
6	22.96 *t*	22.61 *t*	22.70 *t*	22.91 *t*
7	121.43 *d*	121.02 *d*	121.47 *d*	121.56 *d*
8	140.86 *s*	140.52 *s*	140.40 *s*	139.89 *s*
9	146.32 *s*	145.90 *s*	146.10 *s*	146.02 *s*
10	37.34 *s*	37.07 *s*	37.22 *s*	37.37 *s*
11	115.68 *d*	115.52 *d*	115.17 *d*	115.52 *d*
12	38.51 *t*	38.15 *t*	38.35 *t*	37.93 *t*
13	44.43 *s*	43.99 *s*	43.92 *s*	43.85 *s*
14	52.15 *s*	51.59 *s*	51.97 *s*	51.25 *s*
15	74.75 *d*	73.90 *d*	74.40 *d*	76.89 *d*
16	39.96 *t*	39.16 *t*	39.61 *t*	36.59 *t*
17	48.80 *d*	48.72 *d*	45.35 *d*	45.37 *d*
18	15.93 *q*	15.53 *q*	15.71 *q*	15.73 *q*
19	22.71 *q*	22.41 *q*	22.57 *q*	22.72 *q*
20	35.92 *d*	35.60 *d*	39.20 *d*	39.53 *d*
21	18.26 *q*	17.86 *q*	12.70 *q*	12.57 *q*
22	34.81 *t*	34.47 *t*	74.51 *d*	74.36 *d*
23	25.74 *t*	25.38 *t*	31.66 *t*	31.82 *t*
24	145.22 *d*	143.26 *d*	139.15 *d*	138.94 *d*
25	126.98 *s*	127.00 *s*	129.20 *s*	129.17 *s*
26	172.83 *s*	170.45 *s*	172.08 *s*	171.53 *s*
27	11.98 *q*	11.71 *q*	12.19 *q*	12.19 *q*
28	17.38 *q*	16.76 *q*	17.17 *q*	18.32 *q*
29	28.19 *q*	27.67 *q*	27.63 *q*	28.05 *q*
30	22.81 *q*	15.40 *q*	22.39 *q*	15.68 *q*
AcCO	—	—	170.76 *s*	170.96 *s*
AcCO	—	—	170.54 *s*	170.49 *s*
AcMe	—	—	21.20 *q*	21.29 *q*
AcMe	—	—	20.94 *q*	20.89 *q*

*Sample was dissolved in $CDCl_3/CD_3OD$ (≅ 3/1, v/v).
†Spectra were obtained at 50.3 MHz (Bruker MSL-200).



	R¹	R²	R³
1	OAc, H	OAc	H
2	H, OAc	OAc	H
3	OH, H	OAc	H
4	OAc, H	OH	H
5	H, OH	OAc	H
6	H, OAc	OH	H
7	=O	OAc	H
8	OH, H	OH	H
9	H, OH	OH	H
10	OAc, H	OH	OAc
11	H, OH	OAc	OAc

at *m/z* 314 (D-ring cleavage) in **10** and 257 (D-ring cleavage-Me) in **11** showed that the D-ring contained a hydroxy group in **10** and an acetoxy group in **11**. Examination of the ^1H NMR spectra (Table 1) confirmed that **10** contained a 15α-hydroxy group (δ4.24, *dd*, *J* = 4.9, 9.8 Hz) and that **11** a 3β-hydroxy group (δ3.23, *dd*, *J* = 4.2, 11.3 Hz). The strong coupling between the H-22 methine and H-23 methylene protons in the homonuclear COSY spectrum of **10** suggested that one of its acetoxy groups was at C-22. Assignment of the α-configuration to H-22 was based on a comparison of the spectral data of **10** and its acetate with those reported for ganoderic acid T [4]. The structure of **10** was therefore determined to be lanosta-7,9(11),24-trien-3α,22β-diacetoxy-15α-hydroxy-26-oic acid (ganodermic acid P1) and that of **11** was lanosta-7,9(11),24-trien-15α,22β-diacetoxy-3β-hydroxy-26-oic acid (ganodermic acid P2).

EXPERIMENTAL

Mycelia were harvested from a 30-day-old liquid culture (300 ml × 30, in 1 l culture flasks) of *G. lucidum* (strain TP-1, collected locally and deposited at the Institute of Botany,

Academia Sinica, R.O.C.) [5]. After filtration through 4 layers of cheese cloth and a gentle rinse with H_2O, the biomass (56 g) was ground into a powder and extracted with MeOH. The conc extract was partitioned between *n*-hexane and H_2O and the aq. layer was re-extracted with EtOAc. The EtOAc fractions were pooled and chromatographed on a silica gel column (45 × 2.5 cm) by stepwise elution with increasing percentage of MeOH in $CHCl_3$. Fractions containing compounds **8**–**11** were combined and subjected to TLC (Merck Kieselgel 60 F_{254}; 0.25 mm thickness; *n*-hexane–Et_2O–EtOAc–HOAc, 2:1:1:0.005). Elution of the band at R_f 0.19 with 5% MeOH in $CHCl_3$ yielded crystalline **9** (19.8 mg, mp 201–202°). Purification of the mother liquor by reversed phase high performance TLC (E. Merck HPTLC RP-18, F_{254}; 0.25 mm thickness; MeCN–HOAc, 100:0.1) resulted in the separation of compounds **10**/**11** from **8**/**9**. Elution of the band at R_f 0.44 with MeOH afforded a resinous compound, **11** (6.2 mg), and the band at R_f 0.35 gave another resinous compound, **10** (6.4 mg). The band at R_f 0.23 gave a mixture of **8** and **9** which was separated by HPLC on a semiprep reversed phase column (Lichrosorb C_{18}, 250 × 7 mm). Compounds **8** (2.9 mg) and **9** (2.0 mg) were well resolved from each other on elution with aq. 87% MeOH.

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