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TRITERPENES FROM *GANODERMA LUCIDUM*

MING-SHI SHIAO, LEE-JUIAN LIN and SHEAU-FARN YEH*

Department of Medical Research, Veterans General Hospital, Taipei, Taiwan 11217, R.O.C.; *Department of Biochemistry, National Yang Ming Medical College, Taipei, Taiwan 11217, R.O.C.

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

Abstract—The structures of five new lanostanoid triterpenes isolated from the fungus *Ganoderma lucidum* were determined by spectroscopic methods.

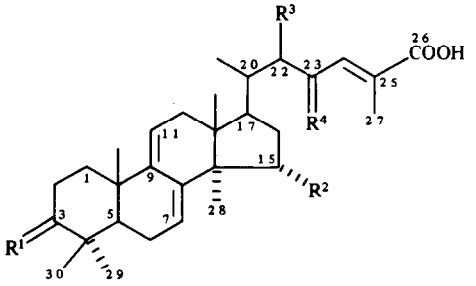
INTRODUCTION

Ganoderma lucidum (Fr.) Karst, a fungus which is widely used in traditional Chinese medicine, is rich in oxygenated lanosterol-derived triterpenoids. Over 80 new compounds have been isolated so far [1–20]. In a continuation of our study on hypocholesterolemic principles, we have now isolated from this fungus six further polyoxygenated triterpenes (**1**–**6**), five of which are new compounds.

RESULTS AND DISCUSSION

The UV spectra of compounds **1**–**6** revealed that all these triterpenes possessed a transoid heteroannular diene as part of their structures: they showed almost identical absorption at 252, 243 and 235 nm (MeOH), and, in the case of compounds **1**, **2** and **6**, end absorption near 210 nm. Compounds **1** and **2** both gave a molecular ion peak at *m/z* 612 ($C_{36}H_{52}O_8$) (EIMS, 12 eV) and three common fragment ion peaks at *m/z* 552 $[M-HOAc]^+$, 492 $[M-2HOAc]^+$ and 432 $[M-3HOAc]^+$. This demonstrated clearly that both compounds had three acetoxy groups. A prominent fragment ion peak at *m/z* 353 $[M-HOAc-C_{10}H_{15}O_4$ side-chain$]^+$ indicated they had the same side-chain at C-17 and that one of these acetoxy groups was located on the side chain. The close resemblance in mass fragmentation patterns strongly suggested that these compounds were either positional or stereo-isomers.

The ^{1}H NMR spectra (Bruker AM-400) of **1** and **2** (Table 1) also showed similar chemical shifts and coupling patterns for H-7, H-11, H-15 and H-24, but not for those signals adjacent to H-3. A singlet at δ4.65 in the spectrum of **1** suggested that one of its acetoxy groups was at C-3α. In compound **2**, a methine proton signal at δ4.48 (*dd*, $J=4.5$, 11.3 Hz) indicated the presence of C-3β acetoxy group. Similar chemical shifts and splitting patterns for H-15β in **1** (δ5.04, *dd*), **2** (δ5.06, *dd*) and other structurally related triterpenes [15] showed that the second acetoxy group was at C-15α. An additional methine proton signal appeared at δ4.99 (*t*, $J=6.6$ Hz) in **1** and δ5.00 (*t*, $J=7.1$ Hz) in **2**, but was not observed in the corresponding compounds **7** and **8**; this suggested that the remaining acetoxy group was most likely on the side-chain. This observation was in agreement with the mass spectral data. The 2D-homonuclear COSY spectrum of **1** showed that H-24 was coupled to two methylene protons assignable to H-23, which in turn was coupled to the H-22 methine proton. The attachment of the third acetoxy



	R¹	R²	R³	R⁴
1	OAc, H	OAc	OAc	H_2
2	H, OAc	OAc	OAc	H_2
3	OH, H	OAc	H	O
4	OAc, H	OAc	H	O
5	OAc, H	OH	H	O
6	OAc, H	OH	OH	H_2
7	OAc, H	OAc	H	H_2
8	H, OAc	OAc	H	H_2

Table 1. ^{1}H NMR spectral data of compounds **1–6** (400 MHz, $CDCl_3$)

H	1	2	3	4	5	6
3	4.65 *s*	4.48 *dd* (4.5, 11.3)*	3.44 *s*	4.66 *s*	4.62 *s*	4.64 *s*
7	5.45 *m*	5.45 *d* (4.9)	5.47 *m*	5.47 *m*	5.79 *d* (5.2)	5.82 *d* (4.4)
11	5.30 *d* (4.6)	5.29 *d* (5.8)	5.33 *d* (5.4)	5.31 *d* (5.6)	5.26 *d* (5.5)	5.28 *d* (5.4)
15	5.04 *dd* (5.0, 10.0)	5.06 *dd* (5.5, 10.6)	5.04 *dd* (5.4, 10.0)	5.05 *dd* (4.7, 9.1)	4.26 *dd* (4.8, 9.4)	4.25 *dd* (6.1, 8.9)
18	0.63 *s*	0.62 *s*	0.69 *s*	0.69 *s*	0.61 *s*	0.56 *s*
19	0.96 *s*†	0.96 *s*†	1.01 *s*†	1.04 *s*†	0.95 *s*†	0.96 *s*†
21	0.94 *d* (9.2)	0.94 *d* (7.7)	0.89 *d* (7.2)	0.90 *d* (6.3)	0.86 *d* (6.4)	0.93 *d* (6.5)
22	4.99 *t* (6.6)	5.00 *t* (7.1)	2.56 *d* (14.8)	2.57 *d* (14.9)	2.54 *d* (16.1)	4.55 *m*
24	6.69 *t* (7.0)	6.75 *t* (7.2)	7.08 *d* (1.3)	7.10 *d* (1.1)	7.08 *br s*	6.68 *d* (8.9)
27	1.80 *s*	1.84 *s*	2.19 *s*	2.18 *s*	2.16 *s*	1.86 *s*
28	1.00 *s*†	0.96 *s*†	0.97 *s*†	0.97 *s*†	0.93 *s*†	0.95 *s*†
29	0.86 *s*†	0.87 *s*†	0.91 *s*†	0.86 *s*†	0.82 *s*†	0.85 *s*†
30	0.95 *s*†	0.92 *s*†	0.97 *s*†	0.96 *s*†	0.94 *s*†	0.96 *s*†
OAc	2.05 *s*	2.06 *s*	2.07 *s*	2.07 *s*	1.99 *s*	2.07 *s*
OAc	2.03 s	2.04 s	—	2.03 *s*	—	—
OAc	2.02 *s*	2.04 *s*	—	—	—	—

*Values in parentheses are coupling constants in Hz.
†Tentative assignments.

group on the side chain was thus assigned to C-22. A comparison of the spectral data of **1** with those reported for ganoderic acids led to the conclusion that H-22 was in the α configuration [10].

DEPT experiments were carried out for **1** and **2**, and the spectra compared with those of compounds **7** and **8**. This showed that both compounds **1** and **2** had one more CH-type carbon signal at δ74.54 and 74.37, respectively, besides one additional carbonyl and CH_3 carbon, and one fewer CH_2-type carbon signal as compared with those of **7** and **8** [18]. Furthermore, an upfield shift of the C-24 (*ca* 6.6 ppm) signal and a downfield shift of the C-25 (*ca* 3.0 ppm) signal were also observed in **1** and **2**, which indicated that the side-chain acetoxy group was at C-22. Confirmation of the stereochemistry of the C-3 acetoxy group was based on the ^{13}C chemical shift of the C-3 signal, which was δ77.93 (α-actoxy) in **1** and 80.69 (β-acetoxy) in **2**. Compounds **1** and **2** were thus a pair of stereoisomers at C-3 and their structures were assigned as lanosta-7,9(11),24-trien-3α,15α,22β-triacetoxy-26-oic acid (**1**) and lanosta-7,9(11),24-trien-3β,15α,22β-triacetoxy-26-oic acid (**2**), respectively. Compound **1** was found to be identical with ganoderic acid T [10].

Compounds **3** and **5** had identical molecular ion peaks at *m/z* 526 ($C_{32}H_{46}O_6$). Three common fragment ion peaks at *m/z* 508 $[M-H_2O]^+$, 466 $[M-HOAc]^+$ and 448 $[M-HOAc-H_2O]^+$ indicated that these compounds possessed one hydroxy group and one acetoxy group. A prominent ion peak at *m/z* 293 $[M-HOAc-H_2O-C_8H_{11}O_3$ side-chain$]^+$ further revealed that both compounds had the same side-chain at C-17. However, a distinct fragment ion peak at *m/z* 257 was observed in **3** due to a facile D-ring cleavage not observed in **5**. This suggested that **3** had an acetoxy group on the D-ring. Examination of the ^{1}H NMR spectra of **3** and **5** confirmed that **3** had an acetoxy group at C-15α (δ5.04, *dd*) and **5** had a C-15 hydroxy group (δ4.26, *dd*) in the same configuration. Compared to **3** and **5**, compound **4** had a mass 42 units heavier ($C_{34}H_{48}O_7$, *m/z* 568) indicating that this compound possessed one additional acetoxy group. This was confirmed by the presence of two major fragment ion peaks at *m/z* 508 $[M-HOAc]^+$ and 448 $[M-2HOAc]^+$. A prominent fragment ion peak at *m/z* 353 $[M-HOAc-C_8H_{11}O_3$ side-chain$]^+$ indicated that **3–5** all had the same side-chain at C-17. Compounds **3** and **4** showed a unique fragment ion peak at *m/z* 299 [D-ring cleavage − Me]$^+$, denoting the presence of an acetoxy group on D-ring.

Comparison of the ^{1}H NMR data of **3–5** confirmed that these compounds all had the same configuration (β) for H-3, with a hydroxy group (δ3.44, *s*) for **3** and an acetoxy group for **4** (δ4.66, *s*) and **5** (δ4.62, *s*). Similar chemical shifts of H-15β in **3** (δ5.04) and **4** (δ5.05) indicated that both compounds bore an acetoxy group at C-15α. The upfield shift of H-15 to δ4.26 (*dd*, $J=4.8$, 9.4 Hz) in **5** clearly revealed that **5** had a hydroxy group at C-15α [19]. The presence of an additional conjugation in the side-chain of **3–5** was observed in their UV spectra (252, 243, 235 nm) and further confirmed by their NMR data. The downfield shifts of H-24 (δ7.08–7.10) by 0.3 ppm and H-27 by 0.4 ppm as compared with those of **7** and **8** suggested that a carbonyl functionality was most likely at C-23. Furthermore, the collapse of a triplet signal to a small doublet as observed in **3** (δ7.08, *d*, $J=1.3$ Hz) and **4** (δ7.10, *d*, $J=1.1$ Hz) and to a broad singlet in **5** (δ7.08, *br s*) for H-24 indicated that H-24 did not have any

Table 2. ^{13}C NMR spectral data of compounds **1**–**6** (100.6 MHz, $CDCl_3$)

C	1	2	3	4	5	6
1	30.47 *t*	35.36 *t*	29.88 *t*	30.51 *t*	30.62 *t*	30.54 *t*
2	22.09 *t*	24.17 *t*	25.54 *t*	23.02 *t*	23.14 *t*	23.05 *t*
3	77.93 *d*	80.69 *d*	76.68 *d*	78.01 *d*	78.06 *d*	78.00 *d*
4	36.39 *s*	37.55 *s*	37.33 *s*	36.43 *s*	36.52 *s*	36.43 *s*
5	43.76 *d*	48.91 *d*	42.91 *d*	43.84 *d*	44.03 *d*	43.94 *d*
6	22.69 *t*	22.83 *t*	22.95 *t*	22.73 *t*	22.79 *t*	22.70 *t*
7	121.22 *d*	121.32 *d*	121.49 *d*	121.26 *d*	121.37 *d*	121.23 *d*
8	139.87 *s*	140.00 *s*	140.02 *s*	139.95 *s*	140.61 *s*	140.56 *s*
9	145.85 *s*	145.75 *s*	145.98 *s*	145.84 *s*	146.11 *s*	146.05 *s*
10	37.19 *s*	37.28 *s*	37.33 *s*	37.25 *s*	37.33 *s*	37.22 *s*
11	115.27 *d*	115.85 *d*	115.46 *d*	115.41 *d*	115.54 *d*	115.42 *d*
12	37.84 *t*	37.95 *t*	37.85 *t*	37.76 *t*	38.33 *t*	38.33 *t*
13	43.82 *s*	43.87 *s*	44.14 *s*	44.09 *s*	44.51 *s*	44.26 *s*
14	51.28 *s*	51.31 *s*	51.68 *s*	51.42 *s*	52.18 *s*	51.98 *s*
15	77.12 *d*	77.00 *d*	77.00 *d*	77.22 *d*	74.57 *d*	74.51 *d*
16	36.42 *t*	36.63 *t*	37.17 *t*	37.05 *t*	40.05 *t*	40.15 *t*
17	45.33 *d*	45.39 *d*	48.74 *d*	48.64 *d*	48.84 *d*	49.23 *d*
18	15.62 *q*	15.73 *q*	15.99 *q*	15.89 *q*	15.97 *q*	15.74 *q*
19	22.51 *q*	22.83 *q*	22.64 *q*	22.53 *q*	22.66 *q*	22.54 *q*
20	39.48 *d*	39.55 *d*	32.80 *d*	32.75 *d*	32.95 *d*	33.42 *d*
21	12.60 *q*	12.63 *q*	19.37 *q*	19.30 *q*	19.57 *q*	19.41 *q*
22	74.54 *d*	74.37 *d*	51.51 *t*	51.54 *t*	51.88 *t*	67.02 *d*
23	31.80 *t*	31.88 *t*	201.57 *s*	201.44 *s*	201.75 *s*	43.57 *t*
24	137.62 *d*	139.03 *d*	133.83 *d*	133.92 *d*	134.09 *d*	144.80 *d*
25	130.27 *s*	129.17 *s*	139.48 *s*	139.36 *s*	139.34 *s*	128.31 *s*
26	172.90 *s*	171.28 *s*	171.21 *s*	171.82 *s*	170.99 *s*	171.95 *s*
27	12.31 *q*	12.31 *q*	14.09 *q*	13.92 *q*	14.09 *q*	12.64 *q*
28	18.31 *q*	18.38 *q*	18.51 *q*	18.33 *q*	17.23 *q*	17.12 *q*
29	27.62 *q*	28.07 *q*	28.18 *q*	27.66 *q*	27.77 *q*	27.65 *q*
30	22.30 *q*	16.91 *q*	22.64 *q*	22.33 *q*	22.46 *q*	22.34 *q*
AcCO	170.94 s	171.12 s	171.04 s	171.08 s	170.86 s	170.72 s
AcCO	170.67 *s*	170.63 *s*	—	170.72 *s*	—	—
AcCO	170.67 *s*	170.01 *s*	—	—	—	—
AcMe	21.25 *q*	21.41 *q*	21.39 *q*	21.23 *q*	21.30 *q*	21.16 *q*
AcMe	21.14 *q*	21.30 *q*	—	21.16 *q*	—	—
AcMe	20.89 *q*	21.01 *q*	—	—	—	—

vicinal coupling proton. The 2D-homonuclear COSY spectrum of **4** showed that H-24 was coupled only to H-27. A carbonyl signal appeared at δ201 in the ^{13}C NMR spectra of **3**–**5**, further confirming this assignment. Due to the presence of an α,β-unsaturated ketone group in the side-chain, a significant upfield shift of C-24 (*ca* 11 ppm) and a downfield shift of C-25 (*ca* 12 ppm) was observed in the spectra of compounds **3**–**5** when they were compared with those of compounds **7** and **8**. The structures of **3**–**5** were therefore determined to be lanosta-7,9(11),24-trien-15α-acetoxy-3α-hydroxy-23-oxo-26-oic acid, lanosta-7,9(11),24-trien-3α,15α-diacetoxy-23-oxo-26-oic acid and lanosta-7,9(11),24-trien-3α-acetoxy-15α-hydroxy-23-oxo-26-oic acid, respectively.

Compound **6** gave a molecular ion peak at *m/z* 528 ($C_{32}H_{48}O_6$) and four major fragment ion peaks at *m/z* 510 $[M-H_2O]^+$, 492 $[M-2H_2O]^+$, 468 $[M-HOAc]^+$ and 432 $[M-2H_2O-HOAc]^+$ indicating that this compound had two hydroxy groups and one acetoxy group. A fragment ion peak at *m/z* 353 $[M-H_2O-C_8H_{13}O_3$ side-chain$]^+$ further revealed that the acetoxy group was not located on the C-17 side-chain. A typical singlet at δ4.64 in the ^1H NMR spectrum of **6** indicated that the acetoxy group was at C-3α [15, 16]. The upfield shift of the H-15 (δ4.25) and H-22 (δ4.55) signals, when compared with those of **1**, clearly indicated that both C-15 and C-22 bore a hydroxy group [19]. A similar upfield shift of the C-15 and C-22 signals in the ^{13}C NMR spectrum of **6** confirmed the assignments. After alkaline hydrolysis of **6**, the corresponding triol was obtained, further supporting the structural assignment. Compound **6** was thus determined to be lanosta-7,9(11),24-trien-3α-acetoxy-15α,22β-dihydroxy-26-oic acid.

EXPERIMENTAL

Mycelia were harvested from a 30-day-old liquid culture (300 ml × 30, in 1 l culture flask) of *G. lucidum* (strain TP-1, collected locally and deposited at the Institute of Botany, Academia Sinica, R.O.C.). After filtration through 4 layers of cheese cloth and a gentle rinse with H_2O, the biomass (56 g) was ground to 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 percentages of MeOH in $CHCl_3$. The fractions containing **1** and **2** were combined and chromatographed by reversed phase high performance TLC (E. Merck HPTLC RP-18, F_{254}; 0.25 mm thickness; MeCN–HOAc, 100:0.1). Elution of the band at R_f 0.42 with MeOH yielded **1** (14.0 mg) (mp 138–140°) and the band at R_f 0.38 afforded **2** (3.5 mg) [21].

Purification of the more polar fractions by TLC (Merck Kieselgel 60 F_{254}; 0.25 mm thickness; *n*-hexane–Et_2O–EtOAc–HOAc, 400:200:200:1) gave two bands. The bands at R_f 0.14 and 0.18 were further purified separately by reversed phase HPTLC (MeCN–HOAc, 1000:1) to afford **3** (1.8 mg) and **4** (12.5 mg), respectively. Another column fraction, which migrated after compounds **3** and **4**, was subjected to TLC (*n*-hexane–Et_2O–EtOAc–HOAc, 200:200:200:1, triple development) three major bands were obtained. Purification of the band at R_f 0.20 by TLC ($CHCl_3$–Et_2O–MeOH, 9:1:1, triple development) yielded **5** (3.0 mg) and the band at R_f 0.14 in the same way afforded **6** (13.8 mg) (mp 198–199°).

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REFERENCES

1. Kubota, T., Asaka, Y., Miura, I. and Mori, H. (1982) *Helv. Chim. Acta* **65**, 611.
2. Toth, J. O., Luu, B., Beck, J.-P. and Ourisson, G. (1983) *J. Chem. Res.* (M) 2722.
3. Nishitoba, T., Sato, H., Kasai, T., Kawagishi, H. and Sakamura, S. (1984) *Agric. Biol. Chem.* **48**, 2905.
4. Nishitoba, T., Sato, H. and Sakamura, S. (1985) *Agric. Biol. Chem.* **49**, 1547.
5. Kohda, H., Tokumoto, W., Sakamoto, K., Fujii, M., Hirai, Y., Yamasaki, K., Komoda, Y., Nakamura, H., Ishihara, S. and Uchida, M. (1985) *Chem. Pharm. Bull.* **33**, 1367.
6. Kikuchi, T., Matsuda, S., Kadota, S., Murai, Y. and Ogita, Z. (1985) *Chem. Pharm. Bull.* **33**, 2624.
7. Komoda, Y., Nakamura, H., Ishihara, S., Uchida, M., Kohda, H. and Yamasaki, K. (1985) *Chem. Pharm. Bull.* **33**, 4829.
8. Nishitoba, T., Sato, H. and Sakamura, S. (1986) *Agric. Biol. Chem.* **50**, 809.
9. Sato, H., Nishitoba, T., Shirasu, S., Oda, K. and Sakamura, S. (1986) *Agric. Biol. Chem.* **50**, 2887.
10. Hirotani, M., Ino, C., Furuya, T. and Shiro, M. (1986) *Chem. Pharm. Bull.* **34**, 2282.
11. Morigiwa, A., Kitabatake, K., Fujimoto, Y. and Ikekawa, N. (1986) *Chem. Pharm. Bull.* **34**, 3025.
12. Arisawa, M., Fujita, A., Saga, M., Fukumura, H., Hayashi, T., Shimizu, M. and Morita, N. (1986) *J. Nat. Prod.* **49**, 621.
13. Fujita, A., Arisawa, M., Saga, M., Hayashi, T. and Morita, N. (1986) *J. Nat. Prod.* **49**, 1122.
14. Hirotani, M. and Furuya, T. (1986) *Phytochemistry* **25**, 1189.
15. Nishitoba, T., Sato, H., Shirasu, S. and Sakamura, S. (1987) *Agric. Biol. Chem.* **51**, 619.
16. Nishitoba, T., Sato, H. and Sakamura, S. (1987) *Agric. Biol. Chem.* **51**, 1149.
17. Nishitoba, T., Sato, H. and Sakamura S. (1987) *Phytochemistry* **26**, 1777.
18. Shiao, M.-S., Lin, L.-J., Yeh, S.-F. and Chou, C.-S. (1987) *J. Nat. Prod.* **50**, 886.
19. Shiao, M.-S., Lin, L.-J. and Yeh, S.-F. (1988) *Phytochemistry* **27**, 873.
20. Lin, L.-J., Shiao, M.-S. and Yeh, S.-F. (1988) *Phytochemistry* **27**, 2269.
21. Lin, L.-J. and Shiao, M.-S. (1987) *J. Chromatography* **410**, 195.