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TRITERPENES FROM *GANODERMA LUCIDUM*

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Key Word Index—*Ganoderma lucidum*; Polyporaceae; lanostane triterpenes.

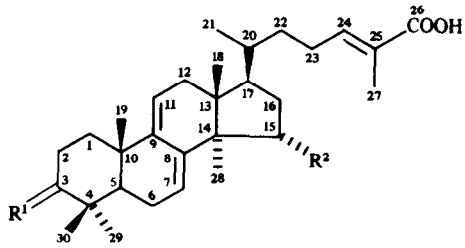
Abstract—Three new lanostanoid triterpenes, isolated from the mycelia of the fungus *Ganoderma lucidum*, were determined to be 3β-hydroxy-15α-acetoxy-lanosta-7,9(11),24-trien-26-oic acid, 3β-acetoxy-15α-hydroxy-lanosta-7,9(11),24-trien-26-oic acid and 3-oxo-15α-acetoxy-lanosta-7,9(11),24-trien-26-oic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

INTRODUCTION

Ganoderma lucidum (Fr.) Karst, a Polyporaceae species used in traditional Chinese medicine, has attracted great attention recently because of its production of many biologically active oxygenated triterpenes [1–4]. As a continuation of our study on their hypocholesterolemic activities and the previous identification of 3α,15α-diacetoxy-lanosta-7,9(11),24-trien-26-oic acid (ganodermic acid R) (**1**) and 3β,15α-diacetoxy-lanosta-7,9(11),24-trien-26-oic acid (ganodermic acid S) (**2**) [5], five more lanostanoid acids **3**–**7** were isolated. Among them, only **3** (ganoderic acid X) and **4** (ganoderic acid Mf) were previously reported [1, 6]. New compounds **5**, **6**, and **7** were tentatively named as ganodermic acids T-N, T-O and T-Q, respectively.

RESULTS AND DISCUSSION

The UV spectra (in MeOH) of compounds **3**–**7** showed almost identical absorption bands at 235, 243, and 252 nm, indicating the presence of a common heteronuclear conjugated diene skeleton. Identical molecular ion peaks at *m/z* 512 ($C_{32}H_{48}O_5$) (EIMS, 12 eV) and two common fragment ions at 494 $[M-H_2O]^+$ and 452 $[M-MeCO_2H]^+$ observed in compounds **3**–**6** suggested that they were stereo- or positional isomers and each compound possessed one hydroxy and one acetoxy substituents in the molecule. Furthermore, two fragment ions at *m/z* 311 $[M-MeCO_2H-C_8H_{13}O_2$ side chain$]^+$ and 257 (D-ring cleavage − Me) in **3** and **5**, which were not observed in **4** and **6**, indicated that **3** and **5** bear the acetoxy group at D-ring. The ^{1}H NMR spectra of **3**–**6** each showed a chemical shift at δ2.02–2.06 (*s*, 3H) (Table 1) confirming the presence of an acetoxy group. Comparison of the identical proton signals at δ5.04 (*dd*) in **3** and **5** with those of ganodermic acids R (**1**), S (**2**) and related triterpenoid metabolites [3, 7, 8] indicated that the acetoxy moieties were most likely attached to C-15 with the α-configuration. Compound **3** has a proton signal at δ3.43 (*br s*) and compound **5** a signal at 3.23 (*dd*) which supported the location of their hydroxy groups at C-3, with the α-configuration in **3** and the β-configuration in **5**. Acetylation of **3** and **4** afforded **1** finally confirming **3** as the reported 3α-hydroxy-15α-acetoxy-lanosta-7,9(11),24-trien-26-oic acid (ganoderic acid X) (mp 104–105°) [1] and **4** as its positional isomer. Compound **4** is therefore assigned the structure 3α-acetoxy-15α-hydroxy-lanosta-7,9(11),24-trien-26-oic acid (ganoderic acid Mf) [6].



	R¹	R²
1	3α-OAc, H	OAc
2	3β-H, OAc	OAc
3	3α-OH, H	OAc
4	3α-OAc, H	OH
5	3β-H, OH	OAc
6	3β-H, OAc	OH
7	=O	OAc

Table 1. Partial ^{1}H NMR spectral data of compounds 3–7 (400 MHz, $CDCl_3$)*

C	3	4	5	6	7
H-1	†	†	†	†	2.75 *ddd* (5.7, 14.6, 14.6)
H-3	3.43 *s*	4.65 *s*	3.23 *dd* (5.1, 10.2)	4.48 *dd* (5.2, 10.0)	
H-7	5.46 *m*	5.83 *m*	5.47 *m*	5.83 *m*	5.51 *m*
H-11	5.32 *d* (5.9)	5.30 *d* (5.3)	5.29 *d* (4.5)	5.28 *d* (4.7)	5.37 *d* (5.6)
H-15	5.04 *dd* (4.9, 9.2)	4.27 *dd* (5.2, 9.4)	5.04 *dd* (4.7, 9.4)	4.25 *dd* (5.1, 9.0)	5.06 *dd* (4.6, 9.4)
3H-18	0.64 *s*	0.60 *s*	0.63 *s*	0.58 *s*	0.66 *s*
3H-19	0.96 *s*‡	0.96 *s*‡	0.98 *s*‡	0.97 *s*‡	1.09 *s*‡
3H-21	0.90 *d* (6.2)	0.89 *d* (6.2)	0.89 *d* (6.2)	†	0.90 *d* (6.3)
H-24	6.84 *t* (7.0)	6.86 *t* (6.8)	6.83 *t* (7.1)	6.85 *t* (7.0)	6.82 *t* (6.9)
3H-27	1.81 *s*	1.82 *s*	1.80 *s*	1.80 *s*	1.81 *s*
3H-28	0.99 *s*‡	0.98 *s*‡	0.98 *s*‡	0.90 *s*‡	0.98 *s*‡
3H-29	0.91 *s*‡	0.85 *s*‡	0.85 *s*‡	0.86 *s*‡	1.07 *s*‡
3H-30	0.96 *s*‡	0.96 *s*‡	0.94 *s*‡	0.92 *s*‡	1.16 *s*‡
OAc	2.06 *s*	2.03 *s*	2.06 *s*	2.02 *s*	2.07 *s*

*Values in parentheses are coupling constants in Hz.
†Overlapped with other signals.
‡Signals were tentatively assigned.

Ganodermic acids T-N (**5**) and T-O (**6**), which upon acetylation both gave **2**, were also positional isomers. The attachment of an acetoxy substituent in **5** was deduced from the mass spectral data. Compound **5** gave a proton signal at δ3.23 (*dd*) which changed to 4.48 (*dd*) in **6** and both showed strong coupling with their C-2 protons. This suggested that their C-3 substituents were both in the β-configuration, with a hydroxy group in **5** and an acetoxy group in **6**. The close similarity of the ^{13}C NMR spectra between **5** and **3**, except for a characteristic downfield chemical shift pattern of **5** in the A-ring and particularly at C-3, also revealed that the hydroxy group of **5** was at the C-3β position (Table 2). Compound **5** was therefore delineated as 3β-hydroxy-15α-acetoxy-lanosta-7,9(11),24-trien-26-oic acid (ganodermic acid T-N) (prefixed with a T for Taipei) (mp 145–146°). Comparison of spectral data of **6** with those of **3**, **4** and **5** makes its structure become obvious. Compound **6** is concluded to be the epimer of **4** at C-3 and positional isomer of **5** at C-3/C-15. Chemical transformation and spectral analysis lead us to the structure of **6** as 3β-acetoxy-15α-hydroxy-lanosta-7,9(11),24-trien-26-oic acid (ganodermic acid T-O) (mp 160–162°).

Compound **7** is a minor component. It showed a prominent molecular ion peak at *m/z* 510 (100, $C_{32}H_{46}O_5$) and two fragment ions at *m/z* 450 [M $-$ $MeCO_2H]^+$ and 255 (D-ring cleavage-Me). ^{1}H NMR spectral data showed a proton signal at δ2.75 (*ddd*) for H-1β and a distinct ^{13}C signal at δ216.59 for a carbonyl, which are known to be characteristic of the 3-oxo-lanostanoid triterpenes [7, 8]. Oxidation of **5** with Jones reagent to yield **7** confirmed that **7** was 3-oxo-15α-acetoxy-lanosta-7,9 (11),24-trien-26-oic acid (ganodermic acid T-Q). The natural occurrence of oxygenated triterpenoid metabolites in *G. lucidum* as stereo- and positional isomers appears very interesting from the biosynthetic point of view.

In the course of our research on hypocholesterolemic constituents of *G. lucidum* we found that several triterpene isolates including compounds **4** and **6** exhibited an inhibitory activity on cholesterol synthesis.

EXPERIMENTAL

The extraction and fractionation of the mycelia of *G. lucidum* have been described [5]. Pooled EtOAc fractions were chromatographed on a silica gel column (45 × 2.5 cm) by stepwise elution with increasing percentage of MeOH in $CHCl_3$. Fractions containing compounds **3**–**7** were combined and chromatographed by reversed phase high performance TLC (E. Merck HPTLC RP-18, F_{254}; 0.25 mm thickness; 10 × 10 cm; MeCN–HOAc, 100:0.1). Eluting the band at R_f 0.28 with MeOH yielded resinous compound **7** (2.0 mg). Eluting the band at R_f 0.23 with the same solvent gave a mixture of **3** and **4** and eluting the band at R_f 0.20 afforded a mixture of **5** and **6**. Complete separation of **3** and **4** was achieved by normal phase TLC (Merck Kieselgel 60 F_{254}; 0.25 mm thickness) using *n*-hexane–Et_2O–EtOAc–HOAc (400:200:200:1, developed × 3). After elution the bands at R_f 0.24 and 0.19 separately with 5% MeOH in $CHCl_3$ compounds **3** (4.7 mg) and **4** (10.3 mg) were obtained, respectively. Resolution of **5** and **6** was achieved by normal phase TLC using $CHCl_3$–Et_2O–HOAc (380:20:1, developed three times). The bands at R_f 0.23 and 0.17 were eluted separately with 5% MeOH in $CHCl_3$ to afford compounds **5** (14.4 mg) and **6** (24.7 mg), respectively.

Table 2. ^{13}C NMR spectral data of compounds **3–7** (50.3 or 100.6 MHz, $CDCl_3$)

C	3	4*	5*	6*	7
1	29.78 *t*	30.53 *t*	35.61 *t*	35.28 *t*	36.60 *t*
2	25.44 *t*	23.04 *t*	27.62 *t*	24.09 *t*	37.48 *t*
3	75.97 *d*	78.00 *d*	78.80 *d*	80.66 *d*	216.59 *s*
4	37.20 *s*	36.41 *s*	38.54 *s*	37.43 *s*	47.42 *s*
5	42.81 *d*	43.93 *d*	48.74 *d*	48.94 *d*	50.39 *d*
6	22.85 *t*	22.69 *t*	22.88 *t*	22.63 *t*	23.63 *t*
7	121.17 *d*	121.10 *d*	121.28 *d*	121.02 *d*	121.04 *d*
8	140.08 *s*	140.66 *s*	140.04 *s*	140.64 *s*	140.37 *s*
9	145.86 *s*	145.98 *s*	145.83 *s*	145.73 *s*	145.00 *s*
10	37.23 *s*	37.21 *s*	37.33 *s*	37.15 *s*	37.25 *s*
11	115.45 *d*	115.52 *d*	115.76 *d*	116.01 *d*	116.92 *d*
12	37.85 *t*	38.34 *t*	37.89 *t*	38.34 *t*	37.99 *t*
13	43.99 *s*	44.33 *s*	43.99 *s*	44.21 *s*	44.06 *s*
14	51.29 *s*	51.95 *s*	51.19 *s*	51.83 *s*	51.31 *s*
15	77.29 *d*	74.57 *d*	77.25 *d*	74.48 *d*	77.23 *d*
16	36.91 *t*	39.89 *t*	36.89 *t*	39.77 *t*	36.97 *t*
17	48.74 *d*	48.71 *d*	48.74 *d*	48.67 *d*	48.85 *d*
18	15.85 *q*	15.84 *q*	15.85 *q*	15.82 *q*	16.00 *q*
19	22.66 *q*	22.56 *q*	22.71 *q*	22.74 *q*	22.44 *q*
20	35.85 *d*	35.81 *d*	35.84 *d*	35.76 *d*	35.92 *d*
21	18.06 *q*	18.17 *q*	18.09 *q*	18.12 *q*	18.16 *q*
22	34.55 *t*	34.62 *t*	34.54 *t*	34.67 *t*	34.62 *t*
23	25.83 *t*	25.75 *t*	25.83 *t*	25.70 *t*	25.92 *t*
24	145.00 *d*	145.13 *d*	144.94 *d*	145.01 *d*	144.53 *d*
25	126.64 *s*	126.77 *s*	126.74 *s*	126.81 *s*	126.76 *s*
26	172.52 *s*	172.86 *s*	172.86 *s*	172.93 *s*	172.10 *s*
27	11.88 *q*	11.89 *q*	11.89 *q*	11.86 *q*	12.04 *q*
28	18.39 *q*	17.13 *q*	18.27 *q*	16.99 *q*	18.24 *q*
29	28.07 *q*	27.66 *q*	28.06 *q*	27.95 *q*	25.40 *q*
30	22.55 *q*	22.35 *q*	15.70 *q*	16.81 *q*	22.14 *q*
AcCO	171.12 *s*	170.75 *s*	171.07 *s*	170.90 *s*	171.21 *s*
AcMe	21.31 *q*	21.19 *q*	21.30 *q*	21.16 *q*	21.40 *q*

*Spectra were obtained at 50.3 MHz.

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REFERENCES

1. Toth, J. O., Luu, B. and Ourisson G. (1983) *J. Chem. Res.* (M), 2722.
2. 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.
3. Morigiwa, A., Kitabatake, K., Fujimoto, Y. and Ikekawa, N. (1986) *Chem. Pharm. Bull.* **34**, 3025.
4. Hirotani, M., Ino, C., Furuya, T. and Shiro, M. (1986) *Chem. Pharm. Bull.* **34**, 2282.
5. Shiao, M.-S., Lin, L.-J., Yeh, S.-F. and Chou, C.-S. (1987) *J. Nat. Prod.* **50**, 886.
6. Nishitoba, T., Sato, H., Shirasu, S. and Sakamura, S. (1987) *Agric. Biol. Chem.* **51**, 619.
7. Kubota, T., Asaka, Y., Miura, I. and Mori, H. (1982) *Helv. Chim. Acta.* **65**, 611.
8. Hirotani, M., Furuya, T. and Shiro, M. (1985) *Phytochemistry* **24**, 2055.