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TWO NEW TRITERPENES OF THE FUNGUS *GANODERMA LUCIDUM*

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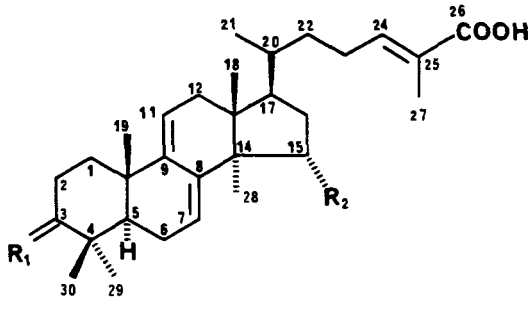
ABSTRACT.—Two new triterpenes isolated from the liquid culture of the Chinese medicinal fungus *Ganoderma lucidum* have been shown to be lanosta-7,9(11),24-trien-3α,15α-diacetoxy-26-oic acid [**1**] (ganodermic acid R) and lanosta-7,9(11),24-trien-3β,15α-diacetoxy-26-oic acid [**2**] (ganodermic acid S) by the spectral data.

Ganoderma lucidum (Fr.) Karst. (Polyporaceae) used in traditional Chinese medicine, has attracted recent attention (1-4). More than 40 polyisoprenoid natural products have now been isolated from this fungus, and many of them are new oxygenated triterpenoids (2,3, 5-15). Furthermore, six lanostane-type triterpenes were found to be cytotoxic to hepatoma cells (5). Some moderately polar, oxygenated triterpenes were responsible for the bitterness (2,3, 6,7). Most interestingly, several newly identified triterpenes exhibit unique oxygenation patterns and have structures analogous to known hypocholesterolemic sterols (16). We have cultured *G. lucidum* in liquid media and in polypropylene bags with solid nutrients for fruiting body formation and reported the identification of eight sterols and triterpenes from its mycelia (17,18).

We report in this paper the isolation and structural elucidation of two new lanostanoid triterpenes, namely, lanosta-7,9(11),24-trien-3α,15α-diacetoxy-26-oic acid [**1**] (ganodermic acid R) and lanosta-7,9(11),24-trien-3β,15α-diacetoxy-26-oic acid [**2**] (ganodermic acid S).



	R_1	R_2
1	OAc H	OAc
1a	OH H	OH
2	OAc H	OAc
2a	OH H	OH

RESULTS AND DISCUSSION

G. lucidum (strains TP-1 and AT-4) were cultured for mycelial growth in liquid

medium to secure a constant supply of materials and avoid seasonal variation of the metabolite production. The methanolic extract of dried mycelia was partitioned between *n*-hexane/H_2O so that the lipophilic metabolites, which includes ergosterol, could be drastically reduced. We chose not to adopt the methylation step of the C-26 carboxyl group because many of the interfering compounds could be removed from the desired metabolites by adjusting pH values in the extraction procedure. Lanosta-7,9(11),24-trien-3α,15α-diacetoxy-26-oic acid (named ganodermic acid R) [**1**] and lanosta-7,9(11),24-trien-3β,15α-diacetoxy-26-oic acid (ganodermic acid S) [**2**] were inseparable from each other by Si gel column chromatography or preparative tlc methods. Ganodermic acids R [**1**] and S [**2**] were marginally resolved by reversed phase hplc. To confirm that compounds **1** and **2** are natural products of *G. lucidum*, portions of fresh mycelia were immediately extracted with MeOH. The concentrated residues were cleaned over Sep-Pak C_{18} (Waters) cartridges and immediately analyzed by the hplc method.

On the basis of mass spectral data ganodermic acid R [**1**] (M^+,554, $C_{34}H_{50}O_6$) was categorized in the lanostanoid series. The fragment ion peaks (M^+-60, M^+-120) strongly confirmed the presence of two acetoxy groups. Other characteristic fragments (*m/z* 299 and 353) represented facile D-ring cleavage and confirmed the attachment of one of the acetoxy group to the D ring. The presence of a *transoid* heteroannular diene ($\Delta^{7,9(11)}$) and a *trans*-α,β-unsaturated acid skeleton was in agreement with the uv absorption bands (210, 235, 243, 251 nm) and ir absorption at 1695 and 1720 cm^{-1}.

Complete assignment of the stereochemistry and structure of **1** was established on the basis of ^{1}H- (200 and 400 MHz, Table 1) and ^{13}C-nmr (50 and 100 MHz, Table 2) spectral data. The ^{1}H-^{1}H (400 MHz) homonuclear and ^{1}H-^{13}C (400/100 MHz) heteronuclear shift correlation experiments and the DEPT pulse sequence (at $\sigma = \pi/4$, $\pi/2$ and $3\pi/4$) were also performed for the interpretation of ^{1}H- and ^{13}C-nmr chemical shifts. It is worth mentioning that the assignment of the upfield signal at δ 11.84 to C-27, which was based on the observation of a ^{1}H-^{13}C shift correlation (δ 1.79, s, 3H in ^{1}H-nmr for H-27), is somewhat unpredictable. Assignment of the 3α-acetoxy stereochemistry of ganodermic acid R [**1**] was facilitated by the characteristic chemical shift of the 3β-H at δ 4.64 (bs) which, upon alkaline hydrolysis, moved to δ 3.45 (bs), a trend commonly observed in related lanostanoid-type compounds (5). The structure of ganodermic acid R [**1**] was, thus, determined to be lanosta-7,9(11),24-trien-3α,15α-diacetoxy-26-oic acid.

Ganodermic acid S [**2**] was obtained as colorless needles. Its molecular formula, which was deduced from the mass spectral data performed in identical condition to that for ganodermic acid R [**1**], was also found to be $C_{34}H_{50}O_6$ (M^+,554). The fragment ion peaks at *m/z* 494 and 434, and base peak at *m/z* 419 were in agreement with the cleavage of two acetoxy groups. Upon alkaline hydrolysis of ganodermic acid S [**2**], a white crystalline compound [**2a**] was obtained that showed a molecular ion peak at *m/z* 470 and the characteristic fragment ion peaks at *m/z* 452, 437, 434, and 419 suggesting the subsequent removal of two H_2O molecules and methyl cleavage. The evidence supporting an identical D-ring side chain in compounds **1** and **2** came from the similar fragments at *m/z* 311 (M^+-$C_8H_{13}O_2$-H_2O) of compounds **1a** and **2a**.

The almost identical uv absorption bands at 210, 235, 243, 251 nm of ganodermic acid S [**2**] also confirmed the presence of a *transoid* heteroannular diene. All that remained for total assemblance of the complete structure of ganodermic acid S [**2**] was the assignment of a 3β-acetoxy group. The ^{1}H-nmr spectrum showed a chemical shift at δ 4.49 (dd, 4.5, 11 Hz) for the 3α proton. The hydrolyzed diol [**2a**], exhibiting a corresponding signal at δ 3.25 (dd, 5, 10 Hz), also supported this conclusion (11,14). Furthermore, the chemical shifts of H-15 in ganodermic acids R [**1**] and S [**2**] are almost

identical (δ 5.04, dd, 4.5, 10 Hz). Ganodermic acid S [**2**] was, therefore, concluded to be lanosta-7,9(11),24-trien-3β,15α-diacetoxy-26-oic acid. The presence of ganodermic acids R and S in substantial amounts in the liquid culture of *G. lucidum* strongly suggested oxidation-reduction processes at C-3 were involved in the biosynthesis of these metabolites.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on a Perkin Elmer 283B and a Lambda 5 spectrophotometers, respectively. ^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker MSL-200 and AM-400 nmr spectrometers and were reported as ppm downfield from TMS (δ=0). Mass spectra were recorded through the direct inlet of a JEOL JMS D-100 mass spectrometer at 12 and 70 eV and were recorded as *m/z*. All melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected.

CULTURE OF *GANODERMA LUCIDUM*.—*G. lucidum* of the strain TP-1 was collected from a local area, while AT-4 was selected from the subculture of ATCC 32471. Both strains are deposited at the Institute of Botany, Academia Sinica, ROC. These strains were maintained on potato-dextrose agar slants. For mycelial growth, fungi were inoculated in 1 liter culture flasks (×30) containing 300 ml sterilized medium, which consisted of 30 g dextrose, 20 g malt extract, and 1 g peptone per liter of distilled H_2O at pH 6.5. Stationary cultures were maintained at 28±1.5° for 30 days.

GENERAL PROCEDURE FOR ISOLATION AND PURIFICATION.—Mycelia were harvested by filtration through four layers of cheese cloth and gently rinsed with H_2O. After drying at 45° in darkness for 48 h, the biomass (TP-1, 56 g, AT-4, 90 g) was ground into powder for extraction with MeOH. The concentrated extracts were partitioned between *n*-hexane and H_2O. The aqueous layer was re-extracted with EtOAc. The pooled EtOAc fraction was chromatographed on a Si gel column (45×2.5 cm) by stepwise elution with increasing percentage of MeOH in $CHCl_3$. Fractions containing ganodermic acids R [**1**] and S [**2**], which were not separable from each other, were pooled and subjected to further separation by the hplc method. Resolution was achieved by hplc using a semipreparative reversed phase column (Lichrosorb C_{18}, 250×7 mm). Compounds **1** (100 mg) and **2** (150 mg) were eluted with 90% aqueous MeOH. Pure compounds were obtained through repeated recrystallization over $CHCl_3$/*n*-hexane.

CHARACTERIZATION OF GANODERMIC ACID R [**1**].—Amorphous powder, mp 126-129° ($CHCl_3$/*n*-hexane); ord (*c* 0.0001, MeOH), $[\phi]_{248}$+8200, $[\phi]_{223}$−7700, $[\phi]_{211}$−11600, $[\phi]_{201}$−20700; cd (*c* 0.0001, MeOH) $[\theta]_{230}$+17200; ir ν max ($CHCl_3$) 3025, 2945, 1715, 1680, 1240 cm^{-1}; uv λ max (MeOH) (log ε) 210 (4.08), 235 (4.14), 243 (4.16), 251 nm (3.97); ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; ms *m/z* (rel. int.) 554 (M^+, $C_{34}H_{50}O_6$, 100), 494 (M^+-CH_3COOH, 40), 479 (M^+-CH_3COOH-CH_3, 26), 434 (M^+-$2CH_3COOH$, 33), 419 (M^+-$2CH_3COOH$-CH_3, 90), 353 (M^+-CH_3COOH-$C_8H_{13}O_2$ side chain, 12), 299 (D-ring cleavage-CH_3, 14), 293 (M^+-$2CH_3COOH$-$C_8H_{13}O_2$ side chain, 19), 239 (*m/z* 299-CH_3COOH, 44).

TABLE 1. Partial ^{1}H-nmr Spectral Data of Compounds **1** and **2**

Proton	Compounds	
	1	**2**
H-3	4.64 (1H, bs)	4.49 (1H, dd, *J*=4.5, 11 Hz)
H-7	5.45 (1H, m)	5.44 (1H, m)
H-11	5.30 (1H, dd, *J*=5.7 Hz)	5.29 (1H, dd, *J*=5.7 Hz)
H-15	5.04 (1H, dd, *J*=4.5, 10 Hz)	5.04 (1H, dd, *J*=4.5, 10 Hz)
H-18	0.63 (3H, s)	0.62 (3H, s)
H-19	0.95 (3H, s)	0.97 (3H, s)
H-21	0.88 (3H, d, *J*=6.3 Hz)	0.88 (3H, d, *J*=6.5 Hz)
H-24	6.83 (1H, m)	6.84 (1H, m)
H-27	1.79 (3H, s)	1.80 (3H, s)
H-29	0.85 (3H, s)	0.86 (3H, s)
H-30	0.94 (3H, s)	0.92 (3H, s)
Ac*CH*$_3$	2.01 (3H, s)	2.03 (3H, s)
Ac*CH*$_3$	2.05 (3H, s)	2.06 (3H, s)

CHARACTERIZATION OF GANODERMIC ACID S [**2**].—Colorless needles, mp 123-124° ($CHCl_3$/*n*-hexane); ord (*c* 0.0001, MeOH), $[\phi]_{248}+7500$, $[\phi]_{225}-2700$, $[\phi]_{217}-10800$, $[\phi]_{209}-400$, $[\phi]_{204}-4400$; cd (*c* 0.0001, MeOH) $[\theta]_{230}+13700$; ir ν max ($CHCl_3$) 3030, 2950, 1720, 1695, 1245 cm^{-1}; uv λ max (MeOH) (log ϵ) 211 (4.17), 235 (4.21), 243 (4.22), 251 nm (4.04); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; ms *m/z* (rel. int.) 554 (M^+, $C_{34}H_{50}O_6$, 95), 494 (M^+-CH_3COOH, 35), 479 (M^+-CH_3COOH-CH_3, 25), 434 (M^+-$2CH_3COOH$, 23), 419 (M^+-$2CH_3COOH$-CH_3, 100), 353 (M^+-CH_3COOH-$C_8H_{13}O_2$ side chain, 36), 299 (D-ring cleavage-CH_3, 70), 293 (M^+-$2CH_3COOH$-$C_8H_{13}O_2$ side chain, 34), 239 (*m/z* 299-CH_3COOH, 62).

TABLE 2. ^{13}C-nmr Spectral Data of Compounds **1** and **2**

Carbon	Compounds	
	1	**2**
1	30.51 (t)	35.26 (t)
2	23.03 (t)	24.06 (t)
3	77.98 (d)	80.60 (d)
4	36.42 (s)	37.43 (s)
5	43.82 (d)	48.83 (d)
6	22.70 (t)	22.69 (t)
7	121.04 (d)	120.93 (d)
8	140.09 (s)	140.01 (s)
9	145.40 (s)	145.52 (s)
10	37.22 (s)	37.16 (s)
11	115.52 (d)	116.00 (d)
12	37.87 (t)	37.86 (t)
13	44.04 (s)	43.92 (s)
14	51.30 (s)	51.19 (s)
15	77.26 (d)	77.15 (d)
16	36.89 (t)	36.84 (t)
17	48.76 (d)	48.67 (d)
18	15.85 (q)	15.82 (q)
19	22.52 (q)	22.69 (q)
20	35.85 (d)	35.79 (d)
21	18.08 (q)	18.03 (q)
22	34.54 (t)	34.49 (t)
23	25.82 (t)	25.78 (t)
24	144.99 (d)	144.90 (d)
25	126.72 (s)	126.72 (s)
26	172.92 (s)	173.01 (s)
27	11.84 (q)	11.78 (q)
28	18.31 (q)	18.19 (q)
29	27.65 (q)	27.97 (q)
30	22.32 (q)	16.79 (q)
AcCH_3	21.15 (q)	21.06 (q)
AcCH_3	21.26 (q)	21.18 (q)
Ac*C*O	170.65 (s)	170.83 (s)
Ac*C*O	171.02 (s)	171.04 (s)

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