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TWO NEW LANOSTANOIDS FROM *GANODERMA LUCIDUM*[1]

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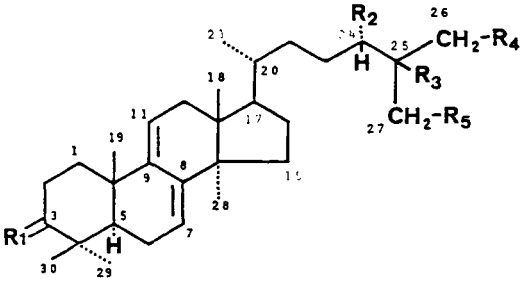
A Chinese crude drug "Ganoderma" has previously afforded many lanostane-type triterpenoids (1-6). Recently, we reported three new lanostanoids, ganodermenonol (**1**), ganodermadiol (**2**), and ganodermatriol (**3**), from the fresh fruiting body of the fungus, *Ganoderma lucidum* (Fr.) Karst (Polypolaceae) (7). Most recently, Moriwaki *et al.* (8) isolated several triterpenoids from the Chinese drug that inhibit angiotensin converting enzyme.

In a continuing investigation of the methanolic extract of the fruiting body, two new lanostanoids named ganodermanondiol (**4**) and ganodermanontriol (**5**) were isolated. We wish to report the structural elucidation of these two new compounds.

Column chromatographic separation of a fraction previously described (7) afforded two new lanostanoids, **4** and **5**. Lanostanoid **4** showed a positive Lieberman-Burchard (LB) reaction. The uv spectrum of the compound was similar to that of ganodermenonol (**1**), and its ir spectrum showed hydroxyl and ketone absorption (3390 and 1710 cm^{-1}). In the ms of **4** the presence of peaks at *m/z* 309 and 269 was characteristic of the suggested lanosta-7,9 (11)-diene-3-one skeleton. The ^{1}H-nmr spectrum of **4** resembled that of the ring system of ganodermenonol (**1**) but lacked the hydroxy methyl, vinylic methyl, and side chain olefinic protons and showed the signals of two tertiary methyl groups at δ 1.15 and 1.18 ppm and a methine proton at δ 3.30 ppm. Acetylation of **4** afforded a monoacetate (**4a**) in which a signal for the acetoxy methine proton at δ 4.76 (dd, J=2.6 and 10.0 Hz) ppm



	R_1	R_2	R_3	R_4	R_5
1	O	$\Delta^{24(25)}$		OH	H
	---H				
2	—OH	$\Delta^{24(25)}$		OH	H
	---H				
3	—OH	$\Delta^{24(25)}$		OH	OH
4	O	OH	OH	H	H
4a	O	OAc	OH	H	H
5	O	OH	OH	OH	H
5a	O	OAc	OH	OAc	H
	---H				
6	—OH	OH	OH	H	H

[1]Part of these data were presented at the 65th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, June, 1985.

was shifted by 1.46 ppm. The ^{13}C-nmr spectrum of **4** showed the presence of a carbonyl carbon at δ 216.92 ppm and two carbons attached to oxygen at δ 73.26 (s) and 79.58 (d) ppm. From these data hydroxyls were deduced to be at C-24 and C-25. The stereochemistry at C-24 was determined by the lanthanide complex method (9-13) on a reduction product of **4**. Compound **4** was reduced by $LiAlH_4$ to afford the 3β-alcohol (**6**). Complexation [1:1 ratio, substrate: $Eu(fod)_3$] of **6** in EtOH-free dry $CHCl_3$ gave a cd spectrum showing $\Delta\epsilon$ +10.17 at 304 nm indicating that the C-24 hydroxyl group had the *S* configuration. Therefore, the structure of **4** is determined to be 24(*S*),25-dihydroxy-5α-lanosta-7,9(11)-dien-3-one, and it was named ganodermanondiol (**4**).

The uv spectrum of lanostanoid **5** was similar to that of **4**. The ^{1}H-nmr spectrum of **5** closely resembled that of **4**, except for the appearance of hydroxymethyl signals at δ 3.48 and 3.84 (each, 1H, d, J=11.3 Hz) ppm and no signal for the C-26 methyl group. Acetylation of **5** afforded a diacetate (**5a**) which showed the signals at δ 4.90 (1H, dd, J=2.6 and and 10.0 Hz) ppm, 4.41 and 3.90 (each, 1H, d, J=11.4 Hz) ppm. The ^{13}C-nmr spectrum of **5** also closely resembled that of **4**, except for the appearance of a carbon signal at δ 67.56 (t) ppm instead of a signal for methyl carbon. From these spectral data one more hydroxyl was deduced to be at C-26; therefore, **5** was established as 24,25,26-trihydroxy-5α-lanosta-7,9(11)-dien-3-one. The similarity of the coupling constants for the acetoxy methine proton at C-24 suggests that the chiral center at C-24 has the *S* configuration as do biogenetic considerations based on the co-occurrence of **4** and **5**. Consequently, the structure of **5** is determined to be 24 (*S*), 25,26-trihydroxy-5α-lanosta-7,9(11)-dien-3-one, and it was named ganodermanontriol (**5**).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined with a

TABLE 1. ^{1}H-nmr Spectral Data of Lanostanoids (in $CDCl_3$, δ ppm, J=Hz)

Proton No.	Compounds				
	4	**4a**	**5**	**5a**	**6**
1β-H	2.77 (1H, m)	2.77 (1H, m)	2.80 (1H, m)	2.76 (1H, m)	
3α-H					3.25 (1H, m)
7-H	5.40 (1H, dd) (J=2.0, 6.0)	5.38 (1H, dd) (J=2.5, 5.5)	5.39 (1H, dd) (J=1.5, 5.0)	5.39 (1H, m)	5.32 (1H, m)
11-H	5.52 (1H, dd) (J=2.0, 6.0)	5.51 (1H, dd) (J=2.8, 6.0)	5.51 (1H, dd) (J=2.0, 6.1)	5.51 (1H, m)	5.47 (1H, m)
18-H	0.58 (3H, s)	0.58 (3H, s)	0.58 (3H, s)	0.58 (3H, s)	0.57 (3H, s)
19-H	1.07 (3H, s)	1.09 (3H, s)	1.09 (3H, s)	1.09 (3H, s)	0.89 (3H, s)
21-H	0.91 (3H, d) (J=6.4)	0.91 (3H, d) (J=6.4)	0.92 (3H, d) (J=6.1)	0.91 (3H, d) (J=6.3)	0.93 (3H, d) (J=6.4)
24-H	3.30 (1H, dd) (J=3.0, 8.0)	4.76 (1H, dd) (J=2.6, 10.0)	3.48 (1H, m)	4.90 (1H, dd) (J=2.6, 10.0)	3.31 (1H, m)
26-H	1.15 (3H, s)	1.20 (s)	3.48, 3.84 (each, 1H, d) (J=11.3)	3.90, 4.41 (each, 1H, d) (J=11.4)	1.16 (3H, s) 1.16 (3H, s)
27-H	1.18 (3H, s)	1.20 (s)	1.21 (3H, s)	1.21 (3H, s)	1.22 (3H, s)
28-H	0.86 (3H, s)	0.86 (3H, s)	0.88 (3H, s)	0.86 (3H, s)	0.88 (s)
29-H	1.11 (3H, s)	1.13 (3H, s)	1.11 (3H, s)	1.13 (3H, s)	1.00 (3H, s)
30-H	1.21 (3H, s)	1.20 (s)	1.20 (3H, s)	1.20 (3H, s)	0.88 (s)
OAc		2.12 (3H, s)		2.08, 2.09 (each, 3H, s)	

Yanagimoto micro melting point apparatus and are uncorrected. Uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer, and ir spectra were recorded with a Hitachi 260-10 infrared instrument with polystyrene calibration at 1601 cm^{-1}. Specific rotations were determined on JASCO D-IP-140 digital polarimeter, and cd curves were obtained with a JASCO J-500 C spectropolarimeter. ^1H- and ^{13}C-nmr spectra were taken with a Varian XL-200 spectrometer at 200 MHz and 50.3 MHz, respectively, and chemical shifts are given in δ (ppm) with TMS as an internal standard. Mass spectra were obtained with a JEOL JMS-D-200 mass spectrometer operating at 70eV.

EXTRACTION AND SEPARATION.—The extraction and separation of the fresh fruiting bodies of *G. lucidum* have been described previously (7). The $CHCl_3$ eluate from the silica gel column chromatography of the 90% MeOH extract was rechromatographed on a silica gel column by stepwise elution with a EtOAc/hexane solvent system to give **4** (25 mg) from 20% EtOAc/hexane. The 1% MeOH/$CHCl_3$ eluate from the 90% MeOH extract column chromatography was repeatedly separated by silica gel column chromatography ($CHCl_3$-EtOAc-Me_2CO, 14:1:1 and hexane-EtOAc-Me_2CO, 6:1:1) to afford **5** (45 mg).

CHARACTERIZATION OF GANODERMANONDIOL (**4**).—Colorless needles, mp 182-183° (MeOH); positive LB reaction; $[\alpha]^{23}D$ +45.8 (c=0.5, $CHCl_3$); ir ν max (KBr) 3390, 2960, 2930, 1710, 1460, 1370, 1165, 1110, 1080,

TABLE 2. ^{13}C-nmr Spectral Data of Lanostanoids (in $CDCl_3$, δ ppm)

Carbon No.	Compounds				
	4	**4a**	**5**	**5a**	**6**[a]
1	36.53, t	36.40, t	36.55, t	36.41, t	36.35, t
2	34.87, t	34.83, t	34.84, t	34.82, t	28.73, t
3	216.92, s	216.83, s	217.00, s	216.76, s	78.06, d
4	47.50, s	47.46, s	47.47, s	47.42, s	39.36, s
5	50.31, d	50.28, d	50.29, d	50.27, d	49.75, d
6	23.67, t	23.62, t	23.61, t	23.62, t	23.54, t
7	119.91, d	119.88, d	119.87, d	119.89, d	121.00, d
8	142.81, s	142.77, s	142.77, s	142.79, s	142.93, s
9	144.46, s	144.42, s	144.43, s	144.47, s	146.52, s
10	37.79, s	37.74, s	37.76, s	37.74, s	38.07, s
11	117.23, d	117.15, d	117.22, d	117.17, d	116.54, d
12	37.17, t	37.17, t	37.18, t	37.18, t	37.81, t
13	43.75, s	43.68, s	43.67, s	43.68, s	44.09, s
14	50.70, s	50.68, s	50.66, s	50.68, s	50.64, s
15	27.85, t	27.76, t	27.87, t	27.75, t	28.83, t
16	28.71, t	26.37, t	28.83, t	25.90, t	29.32, t
17	50.94, d	50.68, d	50.96, d	50.68, d	51.47, d
18	15.71, q	15.65, q	15.73, q	15.65, q	16.04, q
19	22.46, q	22.43, q	22.42, q	22.44, q	23.11, q
20	36.63, d	36.59, d	36.55, d	36.58, d	37.13, d
21	18.62, q	18.55, q	18.64, q	18.52, q	19.03, q
22	31.45, t	31.44, t	31.45, t	31.40, t	31.88, t
23	33.47, t	32.61, t	33.53, t	32.56, t	34.46, t
24	79.58, d	80.75, d	79.10, d	76.34, d	79.93, d
25	73.26, s	72.49, s	74.07, s	73.19, s	72.80, s
26	25.45, q[b]	25.36, q	67.56, t	68.40, t	26.11, q
27	25.32, q[b]	25.36, q	22.04, q	22.04, q	25.98, q[c]
28	23.18, q	24.95, q	25.42, q	25.40, 1	25.87, q[c]
29	26.57, q	26.76, q	25.42, q	25.30, q	28.18, q
30	22.06, q	22.03, q	20.88, q	20.86, q	16.67, q
OCOMe		171.31, s		170.63, s 171.12, s	
$OCOCH_3$		21.07, q	20.01, q	20.01, q 20.98, q	

[a]In pyridine-d_5.
[b, c]Interchangeable.

810 cm^{-1}; uv λ max (MeOH) (log ε) 235 (4.00), 243 (4.06), 251 nm (3.89); ^{1}H-nmr see Table 1; ^{13}C-nmr see Table 2; eims *m/z* (rel. int.) 456 ($M^{+\cdot}$, 7), 438 ($M^{+\cdot}$-H_2O, 6), 424 ($M^{+\cdot}$-OH-Me, 3), 309 (7), 269 (9), 69 (11), 55 (14), 32 (100); *Anal.* calcd. for $C_{30}H_{48}O_3$: 456.3603. Found (ms): 456.3610.

ACETYLATION OF **4**.—Compound **4** was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a monoacetate (**4a**). Colorless needles, mp 119-121° (MeOH); $[\alpha]^{23}D$ +51.81° (*c*=1.0, $CHCl_3$); uv λ max (MeOH) (log ε) 237 (3.91), 243 (3.97), 251 nm (3.81); ^{1}H-nmr see Table 1; ^{13}C-nmr see Table 2; eims *m/z* (rel. int.) 498 ($M^{+\cdot}$, 4), 480 ($M^{+\cdot}$-H_2O, 10), 438 ($M^{+\cdot}$-AcOH, 10), 421 (9), 405 (4), 309 (18), 269 (16), 149 (98), 71 (56), 57 (100); *Anal* calcd. for $C_{32}H_{50}O_4$: 498.3709. Found (ms): 498.3771.

REDUCTION OF **4** WITH $LiAlH_4$.—Lanostanoid **4** (20 mg) was dissolved in dry Et_2O (10 ml) and reduced with $LiAlH_4$ (1 mg) at 45° according to the reported procedure (14) to give **6** (13 mg). Colorless plates, mp 209-210° (MeOH); $[\alpha]^{23}D$ +53.96° (*c*=0.26, EtOH); ir ν max (KBr) 3425, 2975, 2930, 2880, 1460, 1370, 1330, 1140, 1075, 1030, 985, 815 cm^{-1}; uv λ max (MeOH) (log ε) 236 (3.97), 243 (4.03), 251 nm (3.86); ^{1}H-nmr see Table 1; ^{13}C-nmr see Table 2; eims *m/z* (rel. int.) 458 ($M^{+\cdot}$, 8), 440 ($M^{+\cdot}$-H_2O, 10), 422 ($M^{+\cdot}$-$2H_2O$, 58), 407 (57), 379 (46), 253 (35), 171 (37), 157 (53), 145 (45), 119 (37), 109 (73), 107 (44), 95 (49), 81 (57), 71 (51), 69 (62), and 55 (100); *Anal* calcd. for $C_{30}H_{50}O_3$: 458.3747. Found (ms): 458.3766.

CHARACTERIZATION OF GANODELMANONTRIOL (**5**).—Colorless needles, mp 161-162° (MeOH); positive LB reaction; $[\alpha]^{23}D$+35.70° (*c*=1.0, $CHCl_3$); ir ν max (KBr) 3400, 2960, 2925, 2875, 1700, 1460, 1445, 1370, 1110, 1040, 1000, 810 cm^{-1}; uv λ max (MeOH) (log ε) 236 (4.06), 243 (4.11), 251 nm (3.95); ^{1}H-nmr see Table 1; ^{13}C-nmr see Table 2; eims *mz* (rel. int.) 472 ($M^{+\cdot}$, 86), 454 ($M^{+\cdot}$-H_2O, 24), 439 ($M^{+\cdot}$-H_2O-Me, 14), 396 (46), 311 (62), 309 (72), 269 (90), 244 (32), 185 (38), 171 (40), 157 (50), 145 (42), 133 (52), 119 (48), 107 (44), 95 (54), 75 (80), 69 (72), and 55 (100); *Anal.* calcd. for $C_{30}H_{48}O_4$: 472.3552. Found (ms) 472.3551.

ACETYLATION OF **5**.—Using the acetylation procedure described for **4**, a diacetate (**5a**) was obtained as colorless needles, mp 156-158° (MeOH); $[\alpha]^{23}D$ +38.73 (*c*=1.0, $CHCl_3$); uv λ max (MeOH) (log ε) 236 (4.23), 243 (4.28), 251 nm (4.12); ^{1}H-nmr see Table 1; ^{13}C-nmr see Table 2; eims *m/z* (rel. int.) 556 ($M^{+\cdot}$, 12), 538 ($M^{+\cdot}$-H_2O, 20), 496 ($M^{+\cdot}$-AcOH, 16), 479 (16), 309 (66), 269 (41), 157 (30), 149 (44), 95 (46), 83 (56), 69 (96), 55 (100); *Anal.* calcd. for $C_{34}H_{52}O_6$: 556.3761. Found (ms): 556.3749.

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