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THREE NEW LANOSTANOIDS FROM *GANODERMA LUCIDUM*[1]

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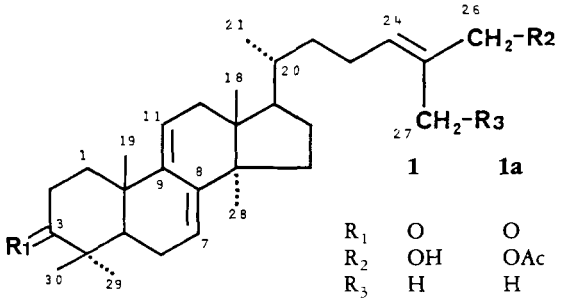
ABSTRACT.—Three new lanostanoids—ganodermenonol (**1**), ganodermadiol (**2**), and ganodermatriol (**3**) [isolated as its triacetate derivative (**3a**)]—were isolated from the MeOH extract of *Ganoderma lucidum*, together with ergosterol and its peroxide. The new compounds were identified as 26-hydroxy-5α-lanosta-7,9(11),24-trien-3-one (**1**), 5α-lanosta-7,9(11),24-triene-3β,26-diol (**2**), and 5α-lanosta-7,9(11),24-triene-3β,26,27-triol (**3**) by their respective spectral data.

A Chinese drug "Ganoderma" is the dried fruiting body of *Ganoderma lucidum* (Fr.) Karst (Polyporaceae), and it has been prescribed in Chinese medicine as a tonic and sedative drug, and has been used to treat hepatopathy, hypertension, arthritis, neurasthenia, and bronchitis among other things (1). Recently, many lanostane type triterpenoids have been isolated from this drug (2-7). Some of them have been shown to inhibit histamine release in rat mast cells (3) and to have cytotoxic activity against hepatoma cells in vitro (5).

In this paper we wish to report the isolation and structural elucidation of three new lanostanoids, named ganodermenonol (**1**), ganodermadiol (**2**), and ganodermatriol (**3**), the latter isolated as a triacetate (**3a**) from the fresh fruiting body of the fungus *G. lucidum* together with ergosterol and ergosterol peroxide.



	1	1a	2	2a	3	3a
			H	H	H	H
R_1	O	O	OH	OAc	OH	OAc
R_2	OH	OAc	OH	OAc	OH	OAc
R_3	H	H	H	H	OH	OAc

RESULTS AND DISCUSSION

The MeOH extract of the fresh fruiting body of *G. lucidum* was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ fraction was partitioned between aqueous MeOH and petroleum ether. Chromatography of the aqueous MeOH fraction, as described in the experimental, afforded three new lanostaniods (**1-3**). The petroleum ether fraction afforded two known compounds, ergosterol and ergosterol peroxide.

Ganodermenonol (**1**) showed a positive Liebermann-Burchard (LB) reaction, and hydroxyl (3425 cm^{-1}) and ketone (1700 cm^{-1}) absorptions were observed in its ir spectrum. An uv spectrum of **1** was similar to that of methyl-3β-hydroxy-5α-lanosta-7,9(11),24-triene-21-oic acid (8), indicating the presence of a heteroannular diene system in the molecule. The ms of **1** showed a molecular ion peak at *m/z* 438 and promi-

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nent peaks at *m/z* 309 (M^+-side chain-2H) and 269 (H^+-side chain-C_3H_4). The ^{1}H-nmr spectrum of **1** showed signals for five tertiary methyl groups at δ 0.59, 0.88, 1.09, 1.13, and 1.20 ppm and a secondary methyl group at δ 0.92 (d, J=6.2 Hz) ppm as required by the lanostane skeleton. Three olefinic proton signals were observed at δ 5.41 (2H) and 5.51 (1H) ppm. Furthermore, a vinyl methyl signal at δ 1.67 ppm and a hydroxy methyl signal at δ 4.00 ppm appeared as singlets.

When **1** was acetylated, a monoacetate derivative was produced, showing a molecular ion at *m/z* 480. In its ^{1}H-nmr spectrum, the signal at δ 4.00 (2H) ppm was shifted downfield to 4.46 ppm. A ^{13}C-nmr spectrum of **1** showed the presence of three methine and nine methylene carbons, including one methylene carbon attached to an oxygen, as well as six olefinic and fourfully substituted carbons and a carbonyl carbon (Table 1). The carbon signals at δ 216.95 (s), 69.01 (t), and 13.62 (q) ppm were assignable to C-3, C-26, and C-27, respectively (9-12). From these spectral data, compound **1** was determined to be 26-hydroxy-5α-lanosta-7,9(11),24-triene-3-one.

TABLE 1. ^{13}C-nmr Spectral Data of Isolated Compounds (δ ppm)

Carbon No.	Compounds		
	1	**2**	**3a**
1	36.59(t)	35.76(t)	35.40(t)
2	34.82(t)	28.17(t)	28.10(t)
3	216.95(s)	78.98(d)	80.82(d)
4	47.43(s)	38.73(s)	37.95(s)
5	50.66(d)	50.35(d)	50.32(d)
6	23.62(t)	23.13(t)	24.33(t)
7	119.83(d)	120.25(d)	120.01(d)
8	142.81(s)	142.69(s)	142.63(s)
9	144.47(s)	145.93(s)	145.64(s)
10	37.17(s)	37.36(s)	37.22(s)
11	117.24(d)	116.26(d)	116.50(d)
12	37.76(t)	37.85(t)	37.82(t)
13	43.68(s)	43.84(s)	43.81(s)
14	50.27(s)	49.15(s)	49.27(s)
15	27.86(t)	27.95(t)	27.91(t)
16	31.44(t)	31.53(t)	31.50(t)
17	50.86(d)	50.94(d)	50.83(d)
18	15.65(q)	15.71(q)	15.69(q)
19	22.43(q)	22.79(q)	22.87(q)
20	36.01(d)	36.12(d)	36.07(d)
21	18.39(q)	18.44(q)	18.41(q)
22	35.87(t)	35.98(t)	35.78(t)
23	24.50(t)	24.57(t)	24.81(t)
24	126.86(d)	126.99(d)	128.64(d)
25	134.34(s)	134.33(s)	137.38(s)
26	69.01(t)	69.11(t)	66.83(t)
27	13.62(q)	13.65(q)	59.84(t)
28	25.28(q)	25.60(q)	25.56(q)
29	25.38(q)	27.82(q)	28.16(q)
30	22.03(q)	15.80(q)	16.97(q)

Ganodermadiol (**2**) also showed a positive LB reaction. The uv spectrum of **2** was similar to that of **1**, and its ir spectrum was also similar to that of **1**, except for the absence of a carbonyl absorption. Acetylation of **2** afforded a diacetate which had a molecular ion peak at *m/z* 524. The ^{1}H- and ^{13}C-nmr spectra of **2** closely resembled those of **1**, except for the appearance of a proton signal at δ 3.25 ppm and a ^{13}C signal at

δ 78.98 ppm as expected for the proton and carbon parts of a secondary hydroxy group instead of a signal for a carbonyl carbon at δ 216.95 ppm. The signal at δ 78.98 ppm suggested that the hydroxy group at C-3 is in the β-configuration (10). From these spectral data, compound **2** was established as 5α-lanosta-7,9(11),24-triene-3β,26-diol.

Ganodermatriol triacetate (**3a**) was obtained from an acetylated crude fraction. Its uv spectrum also suggested the presence of a heteroannular diene system. In its mass spectrum, the molecular ion peak appeared at *m/z* 582, and prominent peaks were observed at *m/z* 567, 522, 507, 462, 447, 429, 387, 353, 279, 253, 167, and 149, suggesting that **3a** contained one additional acetoxy group relative to **2a**. The ^{1}H-nmr spectrum of **3a** resembled that of **2a** except for the appearance of a signal for an acetoxy group overlapped with the C-26 acetoxy group signal at δ 2.07 (6H, s) ppm and an acetoxy methyl group at δ 4.66 (2H, s) ppm instead of the signal of a vinyl methyl for C-27. The ^{13}C-nmr spectrum of **3a** verified the presence of three carbons attached to oxygen at δ 80.82, 66.83, and 59.84 ppm, three carbonyl carbons for acetyl moieties at δ 170.75, 170.78, and 170.97 ppm, and three acetyl methyl carbons 21.02, 21.42, and 22.87 ppm. From these spectral data, the structure of this acetyl derivative was deduced to be 3β,26,27-triacetoxy-5α-lanosta-7,9(11),24-triene, presumably originating from 5α-lanosta-7,9(11),24-triene-3β,26,27-triol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. Uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer and ir spectra were obtained on a Hitachi 260-10 ir spectrometer with polystyrene calibration at 1601 cm^{-1}. Specific rotations were determined on a JASCO DIP-140 digital polarimeter. ^{1}H- and ^{13}C-nmr spectra were taken with a Varian XL-200 spectrometer at 200 MHz and 50.3 MHz, respectively, in $CDCl_3$ solutions with TMS as an internal standard and are recorded in δ (ppm) units. Mass spectra were obtained on a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

EXTRACTION AND SEPARATION.—The fruiting bodies of *G. lucidum* (8.0 kg) were cultured at Yatsuo, Toyama, Japan. A voucher specimen is deposited in the Herbarium of our university. These fruiting bodies were cut into small pieces and extracted three times with MeOH (4 liters) at room temperature for 3 days.

The MeOH extract (180 g) was partitioned between H_2O and $CHCl_3$, and the $CHCl_3$ fraction (54 g) was partitioned between petroleum ether and MeOH-H_2O (90:10). The 90% MeOH extract (36 g) was chromatographed on a silica gel column (700 g) by stepwise elution with $CHCl_3$, 1% MeOH/$CHCl_3$, 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, and MeOH. The $CHCl_3$ elution (4.5 g) was rechromatographed on a silica gel column by stepwise elution with a EtOAc/hexane solvent system to give **1** (32 mg) from 5% EtOAc/hexane and **2** (33 mg) from 10% EtOAc/hexane. The 1% eluate (12 g) 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 a mixture of colorless crystals. The mixture was treated overnight with Ac_2O and pyridine at room temperature, and was separated by plc to give **3a** (7 mg).

The petroleum ether extract (15.8 g) was chromatographed on a silica gel column (300 g) by stepwise elution with a EtOAc/hexane solvent system to give ergosterol (8 g) from 15% EtOAc/hexane and ergosterol peroxide (150 mg) from 25% EtOAc/hexane. Ergosterol was identified by direct comparison with an authentic sample and ergosterol peroxide was also identified from physical and spectral data (13).

CHARACTERIZATION OF GANODERMENONOL (**1**).—Colorless needles, mp 109-111° (MeOH); positive to LB reaction; $[\alpha]^{23}D$ +38.96° (*c*=1.0, $CHCl_3$); ir ν max (KBr) 3425, 2930, 2880, 1700, 1450, 1375, 1110, 1000, 815 cm^{-1}; uv λ max (MeOH) (log ε) 236 (4.17), 243 (4.23), 251 nm, (4.06); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 438 (M^+, 80), 423 (M^+-Me, 14), 420 (M^+-H_2O, 10), 405 (M^1-Me-H_2O, 12), 309 (M^+-side chain-2H, 100), 269 (M^+-side chain-42, 50), 244 (16), 199 (16), 185 (20), 171 (30), 157 (40), 145 (34), 133 (36), 119 (36), 109 (38), 95 (44), 81 (54), 69 (64), 55 (100); *Anal* calcd for $C_{30}H_{46}O_2$: 438.3498. Found (ms): 438.3507.

ACETYLATION OF **1**.—Compound **1** was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a monoacetate as colorless needles (**1a**), mp 50-53°; ^{1}H nmr see Table 2; eims *m/z* (rel. int.) 480 (M^+, 6), 438 (5), 420 (9), 405 (8), 309 (100), 282 (28), 269 (20), 69 (64), 55 (96).

TABLE 2. ^{1}H-nmr Spectral Data of Isolated Lanostanoids and Their Acetates (δ ppm, J=Hz)

Proton No.	Compounds				
	1	**1a**	**2**	**2a**	**3a**
18-H	0.59 (3H, s)	0.59 (3H, s)	0.57 (3H, s)	0.56 (3H, s)	0.56 (3H, s)
19-H	1.09 (3H, s)	1.09 (3H, s)	1.00 (3H, s)	1.01 (3H, s)	1.01 (3H, s)
21-H	0.92 (3H, d) (J=6.2)	0.92 (3H, d) (J=6.3)	0.92 (3H, d) (J=6.3)	0.91 (3H, d) (J=7.8)	0.92 (3H, d) (J=7.4)
28-H	0.88 (3H, s)	0.88 (3H, s)	0.88 (s)	0.87 (3H, s)	0.87 (3H, s)
29-H	1.13 (3H, s)	1.13 (3H, s)	0.98 (3H, s)	0.96 (3H, s)	0.96 (3H, s)
30-H	1.20 (3H, s)	1.20 (3H, s)	0.88 (s)	0.89 (3H, s)	0.89 (3H, s)
26-H	4.00 (2H, s)	4.46 (2H, s)	4.00 (2H, s)	4.45 (2H, s)	4.57 (2H, s)
27-H	1.67 (3H, s)	1.66 (3H, s)	1.67 (3H, s)	1.66 (3H, s)	4.66 (2H, s)
OAc	—	2.07 (3H, s)	—	2.06 (3H, s) 2.07 (3H, s)	2.06 (3H, s) 2.07 (3H, s)
3-H	—	—	3.25 (1H, dd) (J=5.4, 10.1)	4.51 (1H, dd) (J=5.2, 10.4)	4.50 (1H, m)
7-H	5.41 (m)	5.45 (1H, m)	5.34 (1H, m)	5.34 (1H, dd) (J=0.9, 3.9)	5.33 (1H, m)
11-H	5.51 (1H, dd) (J=6.3)	5.53 (m)	5.47 (1H, m)	5.46 (m)	5.46 (1H, m)
24-H	5.41 (m)	5.53 (m)	5.42 (1H, m)	5.46 (m)	5.78 (1H, dd) (J=7.3, 7.4)

CHARACTERIZATION OF GANODERMADIOL (**2**).—Colorless needles, mp 168-170° (MeOH); positive to LB reaction; $[\alpha]^{23}$D +53.0°±1.0, $CHCl_3$); ir ν max (KBr) 3340, 2930, 1440, 1430, 1370, 1070, 1040, 1010 cm^{-1}; uv λ max (MeOH) (log ε) 236 (4.08), 243 (4.12), 251 nm (3.95); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 440 (M^+, 100), 425 (M^+-Me, 12), 422 (M^+-H_2O, 8), 407 (M^+-Me-H_2O, 12), 311 (M^+-side chain, 50), 271 (M^+-side chain-40, 44), 253 (30), 171 (28), 157 (32), 145 (34), 119 (38), 107 (40), 95 (48), 81 (54), 69 (70), 55 (98); *Anal.* calcd for $C_{30}H_{48}O_2$: 440.3654. Found (ms): 440.3612.

ACETYLATION OF **2**.—Using acetylation as described for **1**, colorless needles (**2a**) were obtained with mp 69-70°; $[\delta]^{23}$D +61.09 (c=1.0, $CHCl_3$); ^{1}H nmr see Table 2; eims *m/s* (rel. int.) 524 (M^+, 10), 509 (1), 464 (12), 449 (9), 405 (4), 389 (7), 353 (100), 313 (8), 282 (3), 253 (30), 149 (26), 69 (44), 55 (48).

CHARACTERIZATION OF GANODERMATRIOL ACETATE (**3a**).—Colorless needles, mp 98-100° (MeOH); positive to LB reaction; $[\alpha]^{23}$D+59.91° (c=0.14, $CHCl_3$); uv λ max (log ε) 236 (3.89), 243 (3.94), 251 nm (3.78); ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 582 (M^+, 3), 567 (M^+-Me, 1), 522 (M^+-AcOH, 3), 507 (M^+-Me-AcOH, 2), 462 (M^+-2AcOH, 4), 447 (3), 429 (1), 387 (2), 353 (60), 279 (10), 253 (10), 167 (28), 149 (100), 55 (90); *Anal.* calcd for $C_{36}H_{54}O_6$; 582.3920. Found (ms): 582.3945.

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