Lanostanoid Triterpenes from *Ganoderma lucidum*

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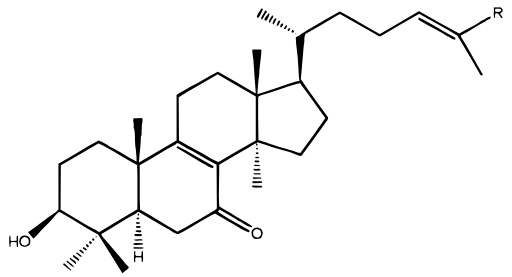
Two new lanostanoids lucidadiol (1) and lucidal (2), were isolated from an ethanolic extract of *Ganoderma lucidum*, together with the known compounds ganodermadiol; ganodermenonol; ganoderic acid DM, ergosterol, 22,23-dihydroergosterol; ergosta-7,22-dien-3-one; fungisterol; ergosta-4,6,8(14),22-tetraen-3-one; and ergosterol peroxide. The structures of **1** and **2** were determined based on spectral evidence.

A great number of sterols and triterpenoids have been isolated from the Polyporaceae (Basidiomycetes), some of which are bitter-tasting and/or show useful biological activity.[1−3] This paper describes the isolation and structure elucidation of two new lanostanoids, named lucidadiol (**1**) and lucidal (**2**), from the fresh fruiting body of the fungus *Ganoderma lucidum* (w. curt.: Fr.) Karst (Polyporaceae). In addition, three known lanostanoids, ganodermanodiol,[4,5] ganodermenonol,[4,6] and ganoderic acid[7] and ergosterol;[8] 22,23-dihydroergosterol;[8] ergosta-7,22-dien-3-one;[9] fungisterol;[10] ergosta-4,6,8(14),22-tetraen-3-one;[11] and ergosterol peroxide[12] were also isolated. The structures of the known compounds were confirmed by comparison of their spectroscopic data (MS, ^{1}H and ^{13}C NMR) with literature references.



1: R= CH_2OH
2: R= CHO

The HREIMS of **1** indicated a molecular ion peak at *m*/*z* 456.361145, which corresponded to the molecular formula $C_{30}H_{48}O_3$. Compound **1** showed a positive Lieberman−Burchard (LB) reaction. Hydroxyl (3500 cm^{-1}) and α,*β*-unsaturated ketone (1649 cm^{-1}) absorptions were observed in the IR spectrum. The UV spectrum displayed an absorption maximum at 253 (3.82) nm. The ^{1}H NMR spectrum of **1** (Table 1) showed signals for five tertiary methyl groups at *δ* 0.63, 0.85, 0.89, 0.97, and 1.14 and a secondary methyl group at *δ* 0.91 (d, *J* = 6.0 Hz) as required by the lanostane skeleton. Vinyl methyl, olefinic, and hydroxymethyl signals were observed at *δ* 1.64, 5.37, and 3.97, respectively. The ^{13}C NMR spectral assignments (Table 1) of **1** were made by performing ^{1}H decoupled, DEPT, and 2D NMR experiments. The chemical shift values of the carbon atoms of **1** were similar to those of the corresponding carbon atoms of 3*β*,26-dihydroxy-5α-lanosta-8,24-dien-11-one.[13] The signal of C-6 appeared at *δ* 36.52, due to the presence of a ketone group at C-7. Analysis of the HMBC spectrum established the connectivity of C-7 to C-6 and C-5 (Table 1).

Table 1. NMR Data for Compound **1** (125 and 500 MHz, $CDCl_3$)[a,b]

position	δ_C	δ_H	HMBC (C→H)
1α	34.70	1.39 m	H_2-2, Me-19
1*β*		1.81 dt (13.8, 3.3)	
2α	27.31	1.74 m	H-1
2*β*		1.67 m	
3α	77.83	3.25 dd (11.6, 4.4)	Me-30, Me-29, H_2-2
4	38.81		Me-29, Me-30, H-5, H-3
5α	49.73	1.60 dd (12.8, 4.4)	Me-19, Me-29, H_2-6
6α	36.52	2.38 dd (15.8, 3.97)	
6*β*		2.40 dd (15.8, 12.4)	
7	199.02		H_2-6, H-5
8	138.85		H_2-11, Me-28
9	164.77		H-5, Me-19, H_2-11
10	39.67		Me-19, H-5
11α	23.56	2.25 m	H_2-12
11*β*		2.28 m	
12α	30.02	1.72 m	Me-18, H_2-11
12*β*		1.70 m	
13	44.83		Me-18, H_2-12, Me-28
14	47.65		Me-18, Me-28
15α	31.88	2.04 dd (13.0, 2.6)	Me-28
15*β*		1.69 m	
16α	28.66	1.92 m	
16*β*		1.31 m	
17	48.87	1.43 m	Me-21, Me-18
18	15.69	0.63 s	H-17, H_2-12
19	18.24	1.14 s	H-5, H_2-1
20	36.07	1.39 m	H-17, H-22, Me-21
21	18.58	0.91 d (6.0)	H-17
22	35.81	1.44 m, 1.08 m	Me-21, H-24, H-17
23	24.37	2.10 m	H-24, H_2-22
		1.92 m	
24	126.83	5.37 t (6.7)	H2-26, Me-27, H_2-23
25	134.27		H_2-26, Me-27, H_2-23
26	68.96	3.97 s	Me-27, H-24
27	13.53	1.64 s	H-24, H_2-26
28	24.87	0.89 s	H-15
29	27.31	0.97 s	Me-30, H-5, H-3
30	15.18	0.85 s	Me-29, H-5, H-3

[a] Assignments confirmed by decoupling, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra. [b] *J* values are given in Hz.

Based on the above data, lucidadiol (**1**) was identified as 5α-lanosta-8,24-dien-3*β*,26-dihydroxy-7-one. The HMBC spectrum also supported this structure assignment.

The IR spectrum of compound (**2**) showed hydroxyl (3500 cm^{-1}) and conjugated ketone (1660 cm^{-1}) absorptions,

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Table 2. NMR Data for Compound **2** (125 and500 MHz, $CDCl_3$)[a,b]

position	δ_C	δ_H	HMBC (C→H)
1α	34.63	1.60 m	H_2-2, Me-19
		1.82 dt (12.9, 3.5)	
2α	27.32	1.69 m	Me-19
2β		1.64 m	
3α	77.80	3.25 dd (11.5, 4.4)	Me-30, Me-29
4	38.81		Me-29, Me-30
5α	49.76	1.60 dd (12.6, 4.6)	Me-19, Me-29, M-30
6α	36.51	2.39 dd (16.0, 3.8)	H-5
6β		2.41 dd (16.0, 12.5)	
7	198.85		H_2-6, H-5
8	138.76		Me-28
9	164.57		Me-19, H_2-11
10	39.68		Me-19, H-5
11α	23.50	2.26 m	H_2-12
11β		2.26 m	
12α	30.06	1.74 m	Me-18, H_2-11
12β		1.76 m	
13	44.91		Me-18, H_2-12, Me-28
14	47.67		Me-18, Me-28
15α	31.88	2.05 dd (11.6, 2.4)	Me-28, H-16
15β		1.73 m	
16α	28.68	1.91 m	
16β		1.30 m	
17	48.86	1.44 m	Me-21, Me-18, H_2-12
18	15.70	0.64 s	Me-18, H_2-12
19	18.24	1.14 s	H-5
20	36.14	1.42 m	H-17, H-22, Me-21
21	18.47	0.94 d (5.8)	H-17, H_2-22
22	34.74	1.55 m	Me-21, H_2-23
		1.21 m	H-17
23	25.88	2.24 m	H-24, H_2-22
		2.30 m	
24	155.10	6.45 t (7.1)	Me-27, H_2-23
25	139.10		H_2-26, Me-27, H_2-23
26	195.3	9.39 s	Me-27, H-24
27	9.02	1.72 s	H-24, H_2-26
28	24.86	0.89 s	H-15
29	27.32	0.97 s	Me-30, H-5, H-3
30	15.15	0.86 s	Me-29, H-5

[a] Assignments confirmed by decoupling, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra. [b] *J* values are given in Hz.

although there was also a signal for an unsaturated aldehyde (1685 cm^{-1}). The HREIMS of **2** showed a molecular ion peak at *m/z* 454.340553, corresponding to the molecular formula $C_{30}H_{46}O_3$. The ^1H NMR spectrum of **2** also indicated, as in **1**, the presence of five tertiary and one secondary methyl signals as required by a lanostane skeleton.

The hydroxymethyl signal in **1** disappeared, but a formyl proton at δ 9.39 appeared as a singlet in the ^1H NMR spectrum of **2**. In the ^{13}C NMR spectrum (Table 2), the chemical shift values were similar to those of the corresponding carbon atoms of **1** and the C-26 carbon resonated at δ 195.3. Based on the above data, lucidal (**2**) was identified as 5α-lanosta-8,24-dien-3β-hydroxy-7-on-26-al.

Experimental Section

General Experimental Procedures. Melting points were determined on a Ernst Leintz. GMBH Wetzlar apparatus and are uncorrected. Optical rotations were measured using a Perkin−Elmer model 241 polarimeter. UV spectra were recorded using a JASCO model V-560 spectrophotometer. IR spectra were recorded using a Bruker model IFS-55 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker model AMX-500 spectrometer with standard pulse sequences, operating at 500 MHz in ^1H and 125 MHz in ^{13}C. $CDCl_3$ was used as the solvent and TMS as the internal standard. EIMS were recorded on a spectrometer Micromass model Autospect (70 eV). Column chromatography was carried out over Si gel (70−230 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (Si gel $60F_{254}$), and preparative chromatography was carried out on Si gel 60 $PF_{254+366}$ plates (20 × 20 cm, 1 mm thick).

Plant Material. The fungus *G. lucidum* was gathered in the Parque Nacional Natural Los Farallones (Valle del Cauca, Colombia) in December 1997 and identified by Dr. Luis Henao and Dr. Jaime Uribe (Institute of Natural Science of the National University of Colombia). A sample is filed in the Colombian National Herbarium (no. 352016).

Extraction and Isolation. The dried fungi (300 g) were ground and steeped in EtOH (96%) for a week. The ethanol extract was concentrated in vacuo, to yield a brown crude extract (18 g). This was chromatographed on a Si gel column packed in *n*-hexane, then eluted with *n*-hexane and EtOAc mixtures of increasing polarity. The fraction obtained with a 9:1 mixture gave 22,23-dihydroergosterol[8] (150 mg), fungisterol[10] (120 mg), ergosterol[8] (210 mg), and ergosta-7,22-dien-3-one[9] (100 mg); a 4:1 mixture gave ergosterol peroxide[12] (350 mg), ergosta-4,6,8(14),22-tetraen-3-one[11] (80 mg), ganodermanodiol[4] (30 mg), and ganodermenonol[4] (10 mg); a 1:1 mixture gave ganoderic acid DM[7] (40 mg), lucidadiol (**1**, 20 mg), and lucidal (**2**, 8 mg). Sephadex LH-20 columns packed in *n*-hexane−$CHCl_3$−MeOH (2:1:1) and/or preparative TLC (Si gel in thicknesses ranging from 1 to 10 mm) developed with *n*-hexanes−EtOAc (7:3, 3:2, 1:1, and 2:3) were used in the further purification of the compounds.

Lucidadiol (1): obtained as a colorless solid (MeOH−EtOAc), mp 163−165 °C; $[\alpha]^{25}_D$ +80° (*c* 0.02, EtOH); UV (EtOH) λ_{max} (log ϵ) 253 (3.6) nm; IR (film) ν_{max} 3406 (OH), 2960 (unsaturated carbon), 1649 (C═C−C═O), 1583, 1371, 1027 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* 456 $[M]^+$ (34), 441 $[M - Me]^+$ (25), 438 $[M - H_2O]^+$ (29), 423 $[M - Me - H_2O]^+$ (69), 329 $[C_8H_{14}O]^+$ (36); HREIMS *m/z* 456.3611 (calcd for $C_{30}H_{48}O_3$, 456.3603).

Lucidal (2): obtained as colorless needles (MeOH−EtOAc); mp 106−108 °C; $[\alpha]^{25}_D$ +19° (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 nm (2.0), 256 (4.7) nm; IR ν_{max} (film) 3500 (OH), 2927, 2880 (unsaturated carbon), 1660 (C═C−C═O), 1685 (C═C−C═O, conjugated aldehyde), 1580, 1370 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS (70 eV) *m/z* 454 $[M]^+$ (37), 439 $[M - Me]^+$ (70), 436 $[M - H_2O]^+$ (3), 381 $[C_5H_9O]^+$ (14), 329 $[C_8H_{14}O]^+$ (8); HREIMS *m/z* 454.3405 (calcd for $C_{30}H_{46}O_3$, 454.3446).

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