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STEROIDS OF FORMOSAN *GANODERMA TSUGAE*

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Key Word Index—*Ganoderma tsugae*; Polyporaceae; lanostanoid; steroid; 3 α-acetoxy-5α-lanosta-8,24-dien-21-oic acid; 3β-hydroxy-5α-lanosta-8,24-dien-21-oic acid; 3α-acetoxy-16α-hydroxy-24ξ-methyl-5α-lanosta-8, 25-dien-21-oic acid.

Abstract—Two new lanostanoids together with two known lanostanoids, 3β-hydroxy-5α-lanosta-8,24-dien-21-oic acid and 3-oxo-5α-lanosta-8,24-dien-21-oic acid, and a known steroid, ergosta-7,22-dien-3β-ol have been isolated from formosan *Ganoderma tsugae*. The new lanostanoids were characterized as 3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid, named tsugaric acid A, and 3α-acetoxy-16α-hydroxy-24ξ-methyl-5α-lanosta-8,25-dien-21-oic acid, named tsugaric acid B. © 1997 Elsevier Science Ltd

INTRODUCTION

The crude extracts of *Ganoderma tsugae*, a traditional Chinese medicine, was demonstrated to enhance splenic natural killer cell activity and serum interferon production in mice [1]. However, no work has been done on the constituents of Formosan *Ganoderma tsugae*. In addition, our previous studies showed that new steroids and lanostanoids and several known compounds have been isolated and characterized from Formosan *G. lucidum* and *G. amboinense* [2–4] and ganoderic aldehyde A and 2β, 3α, 9α-trihydroxy-ergosta-7,22-diene exhibited potent inhibition of KB cells and human PLC/PRF/5 cells *in vitro* [3]. In a continuation of studies on the bioactive principles of Formosan *Ganoderma*, two new lanostanoids, tsugaric acids A (**1**) and B (**4**), two known lanostanoids, 3β-hydroxy-5α-lanosta-8,24-dien-21-oic acid (**2**) and 3-oxo-5α-lanosta-8,24-dien-21-oic acid (**3**) and a known steroid, ergosta-7, 22-dien-3β-ol were obtained. The characterization of **1**, **4** and **2** with more detailed spectral methods, and assignment of ^{13}C NMR spectra signals of **3** [5–7] are reported in this paper.

RESULTS AND DISCUSSION

Compound **1** showed a positive Liebermann–Burchard reaction, and ester (1745 cm^{-1}) and carboxylic acid (1719 cm^{-1}) absorptions were observed in the IR spectrum. The EI mass spectrum of **1** showed a $[M]^+$ peak at *m/z* 498 and significant peaks at *m/z* 483 $[M\text{-}a]^+$, 437 $[M\text{-}b]^+$, 423 $[M\text{-}c\text{-}H]^+$ and 281 $[423\text{-}d]^+$ (Scheme 1). The ^1H NMR spectrum of **1** showed signals for seven tertiary methyl groups at δ 0.75, 0.85, 0.91, 0.93, 0.97, 1.59 and 1.63, an acetyl group at δ 2.06, HCO— [δ 4.67 (1H, *bs*)] and =C=C*H*—CH_2— [δ 5.09 (1H, *t*, *J* = 6.8 Hz)]. The signal at δ 4.67 moved to δ 3.43 after alkaline hydrolysis, as observed in related lanostanoid-type compound [8] and was due to the methine group bearing the α-acetoxyl group at C-3. In addition to the above evidence, the absence of two secondary methyl signals in the ^1H NMR spectrum of **1** [9], and a tertiary carbon signal at δ 123.6 and four quaternary carbon signals at δ 132.2, 133.8, 134.6 and 182.0 in the ^{13}C NMR spectra of **1**, clearly indicated that **1** was a 3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid (**1**) [10].

In addition to the above results, information from ^1H-^1H and ^1H-^{13}C COSY, long range ^{13}C-^1H COSY spectra (Scheme 2), and by comparing the ^{13}C NMR data of **1** with those of lanosterol [10] further supported the characterization of tsugaric acid A (**1**) as 3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid (**1**) and established the ^1H and ^{13}C NMR spectral assignment (Table 1).

Compound **4** also showed a positive Liebermann–Burchard reaction, and hydroxyl (3399 cm^{-1}), ester (1743 cm^{-1}) and carboxylic acid (1705 cm^{-1}) absorptions were observed in the IR spectrum. The EI mass spectrum of **4** showed a molecular ion peak at *m/z* 528 and significant peaks at 513 $[M\text{-}a]^+$, 495 $[513\text{-}b\text{-}H]^+$, 453 $[495\text{-}c+1]^+$, 451 $[495\text{-}d+1]^+$, 435 $[453\text{-}H_2O]^+$, 435 $[495\text{-}e\text{-}H]^+$, 280 $[435\text{-}f]^+$ (Scheme 1). The ^1H NMR spectrum of **4** showed signals for six tertiary methyl groups at δ 0.76, 0.87, 0.92, 0.99, 1.02 and

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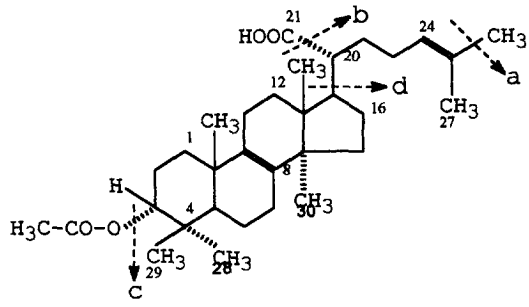

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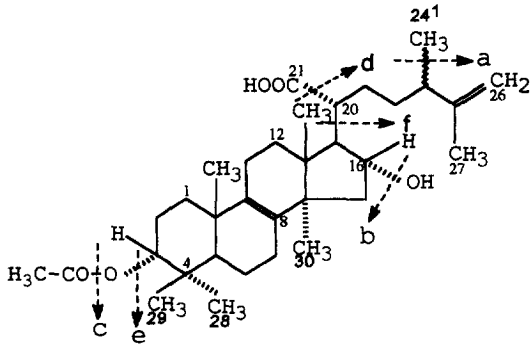

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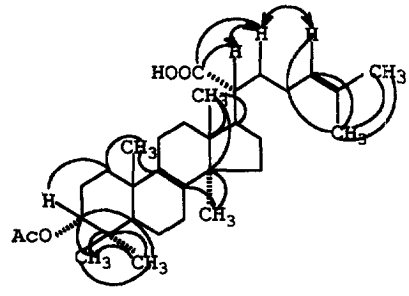
Scheme 1.



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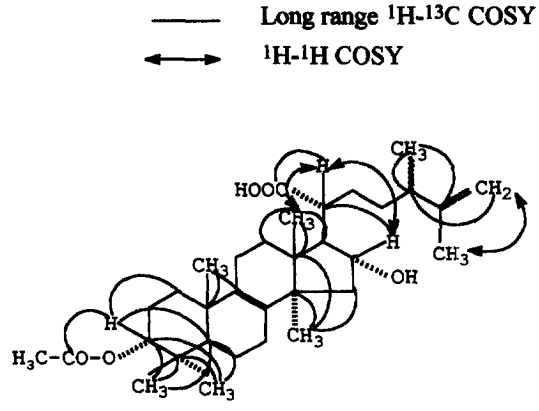

4

Long range ^{1}H-^{13}C COSY

^{1}H-^{1}H COSY

HMBC

NOESY

Scheme 2.

1.17, a secondary methyl group at δ 1.03 (*d*, $J = 6.8$ Hz), and an acetyl group at δ 2.00. Two proton signals at δ 4.15 (*t*, $J = 6.4$ Hz) and 4.65 (*bs*) were due to the methine group bearing the α-hydroxy group at C-16 [11] and the methine group bearing the α-acetoxyl group at C-3 as that of **1**, respectively. Two olefinic protons at δ 4.72 (*s*) and 4.76 (*s*) supported the Δ^{25}-double bond [12] in this compound. The HMBC of C-24 to H-24^{1} and H-26 and C-25 to H-24^{1} confirmed that the C-24^{1} was linked to the C-24 (Scheme 1). Based on the above evidence, **4** was established as 3α-acetoxy-16α-hydroxy-24ξ-methyl-5α-lanosta-8,25-dien-21-oic acid.

In addition to the above results, information from ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY spectra (Scheme 2) and comparison of ^{13}C NMR spectrum with those of **1** further supported the characterization of tsugaric acid B as **4** and established the ^{1}H and ^{13}C NMR spectral assignment (Table 1).

Compound **2** was acetylated because it was difficult to purify. The IR and EI mass spectra of **2** acetate (**2a**) showed the same signals as those of **1** except for the methine proton signal at δ 4.49 (1H, *dd*, $J = 4.8$ and 7.6 Hz). This signal indicated a high field shift compared with that of **1**. Based on the above evidence, Compound **1** was characterized as 3β-hydroxy-5α-lanosta-8,24-dien-21-oic acid (**2**). The ^{13}C NMR spectra of **2a** and **3** (Table 1) were assigned by ^{1}H-decoupled spectra, DEPT pulse sequence, and comparison of chemical shifts with those of **1**. The ^{13}C NMR spectrum of **2a** also supported the characterization of this compound.

EXPERIMENTAL

Extraction and separation. Ganoderma tsugae was collected at Liu-Kuei Shian, Kaohsiung Hsien, Taiwan, Republic of China, during July 1995. A voucher specimen (9501) is deposited in our laboratory. It was identified by Dr Ming-Hong Yen, School of Pharmacy, Kaohsiung Medical College. Air-dried fruit bodies (10 kg), were extracted with $CHCl_3$ and MeOH, respectively. The $CHCl_3$ extract was chromatographed on a silica gel column. Elution with $CHCl_3$ and $CHCl_3$–MeOH (19:1) yielded frs A and B. Frs A and B were rechromatographed on silica gel, respectively. Elution of the fr. A with cyclohexane–$CHCl_3$ (1:40) and $CHCl_3$–EtOAc (25:1) yielded **1** and ergosta-7, 22-dien-3β-ol, respectively. Elution of the fr. B with cyclohexane–$CHCl_3$–MeOH (2:40:3),

Table 1. ^{13}C NMR and ^{1}H NMR chemical shift assignments for compounds **1**, **1**-OH, **2a**, **3** and **4***

C	**1** ($CDCl_3$)+		**1**-OH ($CDCl_3$)		**2a** ($CDCl_3$)		**3** ($CDCl_3$)		**4** ($CDCl_3$–CD_3OD)‡	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	30.4		30.3		35.4		36.2		30.5	
2	23.4		25.7		24.2		36.9		23.0	
3	78.0	4.67 (*bs*)(β)	76.1	3.43 (*bs*)(β)	80.7	4.49 (*dd*)(α)	217.5		78.2	4.65 (*bs*)(β)
4	36.8		37.5		37.8		47.4		36.5	
5	45.3		44.2[a]		50.5		51.2		45.1	
6	18.0		18.1		18.1		26.3		17.7	
7	26.0		26.0		26.3		26.2		25.8	
8	133.8		133.6		134.1		133.1		133.9	
9	134.6		134.8		134.3		135.0		134.3	
10	36.9		36.9		36.9		34.6		36.6	
11	20.8		20.8		20.9		20.9		21.0	
12	30.8		30.3		30.3		30.4		28.6	
13	44.3		44.3[a]		44.3		44.3		45.6	
14	49.6		49.5		49.5		49.6		49.3	
15	27.0		27.0		27.1		27.0		42.1	
16	29.0		28.7		28.9		28.8		76.6	4.13 (*t*)
17	47.2		47.2		47.2		47.2		56.1	
18	16.0	0.75 (*s*)	15.9	0.77 (*s*)	16.0	0.75 (*s*)	16.1	0.78 (*s*)	17.0	0.76 (*s*)
19	18.9	0.97 (*s*)	18.9	0.96 (*s*)	19.2	0.98 (*s*)	18.6	1.10 (*s*)	21.4[b]	1.02 (*s*)
20	47.5	2.28 (*m*)	47.6	2.28 (*m*)	47.5	2.28 (*m*)	47.7	2.29 (*m*)	47.9	2.42 (*m*)
21	182.0		182.6		182.0		182.9		179.7	
22	32.5	1.57 (*m*)	32.3	1.57 (*m*)	32.5	1.57 (*m*)	32.4	1.57 (*m*)	32.0	
23	25.9		26.0		26.0		25.9		24.9	
24	123.6	5.10 (*t*)	123.6	5.10 (*m*)	123.6	5.09 (*t*)	123.5	5.10 (*m*)	33.6	
24[1]									21.5[b]	1.03 (*d*)
25	132.2		132.2		132.2		132.2		155.1	
26	17.6	1.59 (*s*)	17.6	1.59 (*s*)	17.6	1.58 (*s*)	17.6	1.59 (*s*)	106.4	4.72 (*s*)
										4.76 (*s*)
27	25.7	1.67 (*s*)	25.7	1.68 (*s*)	25.7	1.69 (*s*)	25.7	1.68 (*s*)	18.6	0.99 (*s*)
28	27.6	0.85 (*s*)	28.0	0.96 (*s*)	27.9	0.87 (*s*)	21.2	1.06 (*s*)	27.3	0.87 (*s*)
29	21.8	0.91 (*s*)	22.2	0.85 (*s*)	16.5	0.89 (*s*)	19.4	1.10 (*s*)	21.5	0.92 (*s*)
30	24.3	0.93 (*s*)	24.5	0.90 (*s*)	24.3	0.95 (*s*)	24.4	0.90 (*s*)	24.8	1.17 (*s*)

1 OCOMe 21.3, 2.06 (*s*); OCOMe 170.8. **2a** OCOMe 21.3, 2.05 (*s*); OCOMe 170.9.
4 OCOMe 21.0, 2.00 (*s*); OCOMe 171.3.

* The number of directly attached protons to each individual carbons was verified with DEPT pulse sequence.
† These signals were obtained by Long range ^{1}H-^{13}C COSY and ^{1}H-^{1}H COSY.
‡ These signals were obtained by HMBC, HMQC and NOESY techniques.
[a,b] Assignments may be interchanged in each compound.

cyclohexane–$CHCl_3$–MeOH (1:50:1) and cyclohexane–$CHCl_3$–MeOH (2:40:1) yielded **2**, **3** and **4**, respectively. The characterizations of known compounds were achieved by spectral methods.

Tsugaric acid A (3α-*acetoxy*-5α-*lanosta*-8,24-*dien*-21-*oic acid* (**1**). Colourless needles (MeOH–$CHCl_3$), mp 181–182°, $[\alpha]_D^{27}$ +6° ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 1745, 1719, 1658; ^{1}H NMR ($CHCl_3$): see text and Table 1; EIMS (70 eV) *m/z* (rel. int.): 498 $[M]^+$ (26), 483 (15), 437 (3), 423 (61), 281 (15), 187 (27), 119 (44), 69 (77), 43 (100). Anal. Calcd for $C_{32}H_{50}O_4$: 498.3709. Found (MS): 498.3716. Saponification of **1** by the usual method gave 3α-hydroxy-5α-lanosta-8,24-dien-21-oic acid (**1**-OH). Colourless powder (MeOH–$CHCl_3$), mp 198–200°, IR ν_{max}^{KBr} cm^{-1}: 3404, 1700; ^{1}H NMR ($CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 1; EIMS (70 eV) *m/z* (rel. int.): 456 $[M]^+$ (34), 441 (26), 423 (53), 281 (16), 187 (30), 119 (6), 69 (100).

3β-*Acetoxy*-5α-*lanosta*-8,24-*dien*-21-*oic acid* (**2a**). Acetylation of **2** by the usual method gave **2a**. Colourless needles (MeOH–$CHCl_3$), mp 194–195°, $[\alpha]_D^{27}$ +59° ($CHCl_3$; *c* 0.0435); IR ν_{max}^{KBr} cm^{-1}: 1735, 1711, 1649; ^{1}H NMR ($CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$): see Table 1; EIMS (70 eV) *m/z* (rel. int.): 498 $[M]^+$ (37), 483 (45), 437 (6), 423 (100), 281 (22), 187 (31), 119 (50), 69 (92). Anal. Calcd for $C_{32}H_{50}O_4$: 498.3709. Found (MS): 498.3705.

Tsugaric acid B (3α-*acetoxy*-16α-*hydroxy*-24ξ-*methyl*-5α-*lanosta*-8,25-*dien*-21-*oic acid*) (**4**). Colourless powder (MeOH–$CHCl_3$), mp 240–242°, $[\alpha]_D^{27}$ −15° ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3400, 1743, 1705, 1641; ^{1}H NMR ($CDCl_3$–CD_3OD): see text and Table

1; ^{13}C NMR ($CDCl_3$–CD_3OD): see Table 1; EIMS (70 eV) *m/z* (rel. int.): 528 $[M]^+$ (4), 513 (1), 495 (16), 453 (6), 451 (1), 435 (25), 280 (3), 279 (10), 187 (26), 119 (33), 69 (70), 43 (100). Anal. Calcd for $C_{33}H_{52}O_5$: 528.3815. Found (MS): 528.3813.

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