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A LANOSTANOID OF FORMOSAN *GANODERMA LUCIDUM*

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Key Word Index—*Ganoderma lucidum*; Polyporaceae; fungi; ergosta-7,22-dien-3β-yl palmitate; lanostanoid; ganoderic aldehyde A, 3β-hydroxy-26-oxo-5α-lanosta-8,24-dien-11-one.

Abstract—A new steryl ester, ergosta-7,22-dien-3β-yl palmitate, a novel lanostanoid, named ganoderic aldehyde A (3β-hydroxy-26-oxo-5α-lanosta-8,24-dien-11-one, ergosta-7,22-dien-3β-ol, ergosta-7,22-dien-3-one, and ergosterol peroxide, were isolated from the fruit bodies of Formosan *Ganoderma lucidum* and characterized by chemical degradation, synthesis, and spectral data.

INTRODUCTION

While working on the chloroform extract of Formosan *Ganoderma lucidum* (Fr.) Karst (Polyporaceae), a new steryl ester, ergosta-7,22-dien-3β-yl palmitate (**1**), a novel lanostanoid, ganoderic aldehyde A (**5**), and three known steroids, ergosta-7,22-dien-3-one (**2**), ergosta-7,22-dien-3β-ol (**3**) and ergosterol peroxide (**7**) were obtained. In this paper we report the isolation and structure elucidation of these two new compounds.

The Chinese drug, *Ganoderma lucidum*, has been used to treat hepatopathy [1]. The isolated principles from this Formosan fungus, grown on *Acacia confusa* Merr. (Leguminosae), is being screened for cytotoxic effect against human hepatoma PLC/PRF/5 cell *in vitro*.

RESULTS AND DISCUSSION

The new steryl ester, ergosta-7,22-dien-3β-yl palmitate (**1**), $C_{44}H_{76}O_2$ ($[M]^+$ at *m/z* 636), was obtained as granules, mp 92°. Its IR spectrum (KBr) showed an absorption band at 1735 cm^{-1} (ester of CO). The EI mass spectrum of **1** showed peaks of characteristic fragmentation of Δ^7-monoene steroid at *m/z* 255, 229 and 213 [2] and a peak at 256 corresponding to a $C_{16}H_{32}O_2$ moiety from the molecular ion. On alkaline hydrolysis, **1** yielded palmitic acid and the steroid, ergosta-7-22-dien-3β-ol (**3**), thus establishing **1** as ergosta-7,22-dien-3β-yl palmitate (**1**). Compound **5** showed a positive Liebermann–Burchard reaction, and a hydroxyl (3300 cm^{-1}) α,β-unsaturated aldehyde (1685 cm^{-1}) [3] and conjugated ketone (1640 cm^{-1}) absorption were observed in its IR spectrum. The EI mass spectrum of **5** showed a molecular ion peak at *m/z* 454 and significant peaks at *m/z* 439 [M − Me], 436 [M − H_2O], 407 [436 − CHO], 353 [**a** + H], 329 [c]. The ^{1}H NMR spectrum of **5** (Table 1) showed signals for five tertiary methyl groups at δ0.83 (Me × 2), 1.03, and 1.13 (Me × 2), and a secondary methyl group at δ0.94 (*d*, *J* = 6 Hz) as required by the lanostane skeleton. A vinyl methyl, an olefinic, and a formyl proton signals were observed at 1.75, 6.48 and 9.39, respectively. In the ^{13}C NMR spectrum of **5** (Table 2), in addition to the chemical shift values of C-1 to C-13, C-18, C-19, C-29 and C-30 were almost superimposable with those of corresponding data of methyl ganolucidate B [4], and showed two olefinic carbon signals at δ139.2 and 154.9, a formyl carbon signal at δ195.2 and a high field methyl carbon signal at δ9.1.

Table 1. ^{1}H NMR spectral data of compounds **5** and **6** (δppm, *J* = Hz)

H	5	6
18	0.83 (3H, *s*)	0.76 (3H, *s*)
19	1.13 (3H, *s*)	1.06 (3H, *s*)
21	0.94 (3H, *d*) (*J* = 6)	0.83 (3H, *d*) (*J* = 6)
28	1.13 (3H, *s*)	1.06 (3H, *s*)
29	1.03 (3H, *s*)	0.95 (3H, *s*)
30	0.83 (3H, *s*)	0.76 (3H, *s*)
26	9.39 (1H, *s*)	3.93 (2H, *s*)
27	1.75 (3H, *s*)	1.60 (3H, *s*)
3	3.48 (1H, *s*)	3.41 (1H, *m*)
24	6.48 (1H, *m*)	5.31 (1H, *m*)

Compound **5** was reduced by sodium borohydride to give **6**, which also showed a hydroxy (3300 cm^{-1}) and a conjugated ketone (1650 cm^{-1}) absorption, but the α,β-unsaturated aldehyde (1685 cm^{-1}) absorption in **5** disappeared in its IR spectrum. The EIMS of **6** showed a moleculor ion peak at *m/z* 456 and significant peaks at *m/z* 441 [M − Me], 423 [441 − H_2O], 355 [**a** + H], 290 [**b** + 2H]. The ^{1}H NMR spectrum of **6** (Table 1) also showed five tertiary and a secondary methyl signals as required by the lanostane skeleton. The formyl proton signal in **5** disappeared but a hydroxy methyl signal at δ3.93 appeared as singlet in the ^{1}H NMR spectrum of **6**. In the ^{13}C NMR spectrum of **6** (Table 2), the chemical shift values of C-1 to C-13, C-18, C-19, C-29 and C-30 were almost superimposable with those of corresponding data of **5** and the chemical shift values of C-14 to C-17, C-20 to

Table 2. ^{13}C NMR spectral data of compounds **1**, **5** and **6**

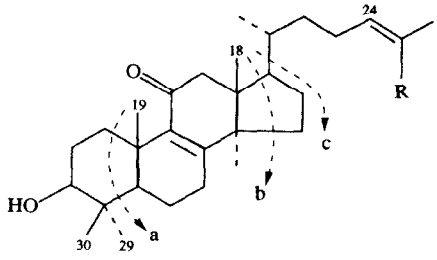
C	1	5	6
1	37.0	34.3	34.3
2	28.1	27.9	28.0
3	73.2	78.6	78.7
4	36.9	39.0	39.0
5	42.9	50.0	50.1
6	27.6	17.3	17.4
7	117.4	29.9	29.9
8	139.5	164.0	164.1
9	49.3	139.4	139.5
10	34.5	37.6	37.7
11	21.4	199.0	199.2
12	39.4	51.8	51.8
13	43.0	47.2	47.2
14	55.1	51.6	51.6
15	22.7	27.0	27.0
16	28.1	30.9	31.0
17	56.0	51.6	51.6
18	12.1	16.6	16.6
19	13.0	18.9	19.0
20	40.4	36.0	35.9
21	21.1	18.2	18.3
22	135.7	34.5	35.7
23	132.0	25.9	24.4
24	42.9	154.9	126.5
25	33.1	139.2	134.6
26	20.0	195.2	68.9
27	19.7	9.1	13.6
28	17.6	25.7	25.7
29		28.3	28.3
30		15.6	15.6

*The number of directly attached protons to each individual carbon was verified with the DEPT pulse sequence.

Palmitoyl carbon: 14.1, 22.7, 25.1, 29.2, 29.4, 29.5, 29.7, 31.9, 35.0, 173.5.

C-27, and C-28 showed good agreement with those of the corresponding data of ganodermenonol (Table 2) [1].

Based on the above data, **6** was elucidated as 3β,26-dihydroxy-5α-lanosta-8,24-dien-11-one (**6**). Therefore the structure of **5** was determined as 3β-hydroxy-26-oxo-5α-lanosta-8,24-dien-11-one (**5**).



5 R = CHO
6 R = CH_2OH

EXPERIMENTAL

Mps: uncorr.

Extraction and separation. Ganoderma lucidum, grown on the stem of *Acacia confusa* Merr., was collected at Liu-Kuei Shian, Kaohsiung Hsien, Taiwan, R.O.C., during June 1987. A voucher specimen is deposited in our laboratory. Air-dried fruit bodies (10 kg), were chipped and extracted for 1 week at room temp. The $CHCl_3$ extract was chromatographed over silica gel. Elution cyclohexane–C_6H_6 (4:1), C_6H_6, C_6H_6–EtOAc (4:1), and C_6H_6–EtOAc (2:1), yielded **1**–**3**, **5** and **7**, respectively. Compounds **2**, **3** and **7** were identical from physical, spectral data, and chemical reaction [2, 5].

Ergosta-7,22-dien-3β-yl palmitate. Granules (Me_2CO), mp 92°, $[\alpha]_D^{25}$ −75.6° ($CHCl_3$; *c* 0.045); IR ν_{max}^{KBr} cm^{-1}: 1735 (CO), 1175 (CO–O). ^1H NMR ($CDCl_3$): δ 0.54 (3H, *s*, 18-H_3), 0.82 (3H, *d*, *J* = 6.6 Hz, 27-H_3), 0.84 (3H, *d*, *J* = 6.6 Hz, 26-H_3), 0.88 (3H, *s*, 19-H_3), 0.92 (3H, *d*, *J* = 6.6 Hz, 28-H_3), 1.02 (3H, *d*, *J* = 6.6 Hz, 21-H_3), 4.68 (1H, *br s*, $W_{1/2}$ = 20 Hz, 3-H), 5.19 (3H, *m*, olefinic proton). ^{13}C NMR ($CDCl_3$): see Table 1. EIMS (direct inlet) 15 eV, *m/z* (rel. int.): 636 $[M]^+$ (17), 509 $[M-C_9H_{17}-2H]^+$ (17), 378 $[M-C_{16}H_{32}O_2-2H]^+$ (100), 255 $[M-C_{16}H_{32}O_2-C_9H_{17}]^+$ (27), 229 $[M-C_{16}H_{32}O_2-C_{11}H_{19}]^+$ (10), 213 $[378-C_{12}H_{21}]^+$ (9), 69 $[C_5H_9]^+$ (67), 57 (63), 43 (75).

Hydrolysis of **1**. Compound **1** (50 mg) was refluxed with 5% alcoholic KOH (20 ml) for 4 hr. The solvent was reduced to half and it was then diluted with H_2O, extracted with Et_2O, washed with H_2O, and dried (Na_2SO_4). Removal of solvent furnished an alcohol **3**, 20 mg and it was acetylated to yield **3** acetate (**4**), needles, mp 179°, it was identical to **4** by comparison of mmp, IR, $[\alpha]_D^{25}$, MS, and NMR with those of **4**. The mother liquor from the above extraction was acidified with dil. HCl and then extracted with Et_2O, washed with H_2O, and dried (Na_2SO_4). Removal of solvent furnished palmitic acid, $C_{16}H_{32}O_2$, $[M]^+$ at *m/z* 256, 8 mg, mp 62° (mmp, MS).

Ganoderic aldehyde A (**5**). Needles, mp 131–133° (MeOH); positive to Liebermann–Burchard reaction; $[\alpha]_D^{20}$ +248.4° ($CHCl_3$; *c* 0.095); IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1685, 1640, 1580, 1450, 1375, 1100, 1000; ^1H NMR see Table 1; ^{13}C NMR see Table 2; EIMS *m/z* (rel. int.) 454 ($[M]^+$, 100), 439 ($[M-Me]^+$, 8), 436 ($[M-H_2O]^+$, 10), 407 ($[M-H_2O-CHO]^+$, 8), 353 (**a** + H, 3), 329 (**c**, 3), 290 (**b** + 2H, 30); Anal. calcd. for $C_{30}H_{46}O_3$: 454.3498, Found (MS): 454.3418.

Reduction of ganoderic aldehyde A (**5**). Compound **5** (30 mg) was reduced with $NaBH_4$ (100 mg) in MeOH at room temp. The reaction product was purified by chromatography on silica gel to give needles (**6**), mp 155–156° (MeOH); positive to Liebermann–Burchard reaction; $[\alpha]_D^{20}$ +209.5° ($CHCl_3$; *c* 0.105); IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1650, 1590, 1460, 1380, 1030; ^1H NMR see Table 1; ^{13}C NMR see Table 2; EIMS *m/z* (rel. int.): 456 ($[M]^+$, 100), 441 ($[M-Me]^+$, 16), 423 ($[M-Me-H_2O]$, 8), 355 (**a** + H, 3), 329 (**c**, 16), 290 (**b** + 2H, 24); Anal. calcd. for $C_{30}H_{48}O_3$: 456.3658. Found (MS): 456.3610.

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ISOKAEROPHYLLIN, A BUTYROLACTONE FROM *BUPLEURUM SALICIFOLIUM*

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Key Word Index—*Bupleurum salicifolium*; Umbelliferae; lignans; benzylidene-benzyl-γ-butyrolactones; kaerophyllin; isokaerophyllin.

Abstract—From the leaves of *Bupleurum salicifolium* two benzylidene-benzyl-γ-butyrolactone-type lignans were isolated. One was identified as kaerophyllin, the other as its *cis*-isomer, hitherto unreported. The latter was characterized as 2(3,4-dimethoxy)-benzylidene-3-(3,4-dioxymethyl)-phenylmethyl-3*S*-γ-butyrolactone by spectroscopic and chemical methods, and from a study of its derivatives.

INTRODUCTION

Salicifoliol [1], a new lignan from *Bupleurum salicifolium* has already been reported. Further study of the minor chemical components of the same plant has now yielded two benzylidene-benzyl-γ-butyrolactone lignans, one of which is new and has been called isokaerophyllin. Its structure was determined as 2-veratrylidenyl-3-piperonylmethyl-3*S*-γ-butyrolactone (**1**).

RESULTS AND DISCUSSION

Column chromatography of ethanol extracts of the leaves of *B. salicifolium*, a species endemic to the Canary Islands, yielded two lignans with a benzylidene-benzyl-γ-butyrolactone skeleton. One, with mp 148°, $[M]^+$ *m/z* 368, $C_{21}H_{20}O_6$, was identified as kaerophyllin (**2**) [2] by spectroscopy and a study of its derivatives. The other, with mp 130°, had the same $[M]^+$ and formula as **2**, in addition to comparable spectral data. It was thus given the name isokaerophyllin.

The ^{1}H NMR for **1** showed signals for six aromatic protons, two methoxy groups and one $-OCH_2O-$ group. These data are characteristic of veratrylidenyl and piperonylmethyl groups, lactone ring substituents at C-1 and C-2, respectively. The spectroscopic data which most clearly exhibit the *cis* disposition of the veratryl and carbonyl groups in **1** as compared to kaerophyllin (**2**) and other lignans with a similar structure and *trans* disposition are as follows. (i) IR and UV: the bands for the carbonyl group have lower values for **1** than for **2** (15 cm^{-1} and 7 nm, respectively). (ii) in the ^{1}H NMR (see Table 1) signals for the vinylic proton are 18.2 ppm further upfield in **1** than in **2**. The H-2′ signals, on the other hand, are 21.0 ppm further downfield. (iii) in the ^{13}C NMR (see Table 1) the signals for C-2, C-3, C-4 and C-5 are shifted downfield and those for C-1 upfield in **1** compared with **2**. The strong paramagnetic shift of the aromatic H-2′ proton indicates that it must be close to the carbonyl group. COSY experiments show the β orientation of the H-3 proton coupled with the H-4 protons while NOE DIF experiments show that the irradiation of the H-3 affects the H-4β and, to a lesser extent, the aromatic H-6″.

The optical activity of **1** is similar to that of kaerophyllin and other lignans which have structures with H-3β [3–5]. The absorption spectra show the conjugated carbonyl with an olefinic double bond (IR, ν_{max} 1735 cm^{-1} (C=O) and 1630 cm^{-1} (C=C olefin); UV λ_{max} 337 nm). In the EI mass spectrum the peak at *m/z* 151 (characteristic of the veratrylmethyl grouping) is insignificant compared