Steroidal Constituents of *Ganoderma applanatum* and *Ganoderma neo-japonicum*

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From the fruiting bodies of *Ganoderma applanatum* a new lanostanoid (**1**) and six known ergosteroids were isolated. Two known lanostanoids and five known steroids were isolated from the fruiting bodies of *Ganoderma neo-japonicum*. The new lanostanoid was characterized as 24ζ-methyl-5α-lanosta-25-one (**1**).

We previously reported the isolation of several new lanostanoids and steroids from *Ganoderma lucidum* and *Ganoderma amboinense*.[1−3] The new lanostanoid, ganoderic aldehyde A, and 2*β*,3α,9α-trihydroxyergosta-7,22-diene exhibited potent inhibition of human PLC/PRF/5 and KB cells in vitro.[2] In a continuation of studies on the bioactive principles of Formosan *Ganoderma* species, a new lanostanoid [24ζ-methyl-5α-lanosta-25-one (**1**)] and six known steroids [ergosta-4,6,8(14),22-tetraen-3-one (**2**); 5α,8α-epidioxyergosta-6,9(11),22-trien-3*β*-ol; ergosta-7,22-dien-3*β*-yl palmitate; ergosta-7,22-dien-3-one; ergosta-7,22-dien-3*β*-ol; and lucidone A] were isolated from *G. applanatum*. Two known lanostanoids (ganoderal A and ganodermadiol), four known ergosteroids [ergosta-7,22-dien-3*β*-yl palmitate; ergosta-7,22-dien-3-one; ergosta-7,22-dien-3*β*-ol; and ergosta-4,6,8(14),22-tetraen-3-one], and one known steroid (2*β*,3α,9α-trihydroxyergosta-7,22-diene) were isolated from *G. neo-japonicum*. Both of these plants are used in Formosan folk medicine. The characterization of **1** and the ^{1}H and ^{13}C NMR spectral assignments of **2**[4,5] are reported in the present paper.

Compound **1** gave a postive Libermann−Burchard reaction, and its IR spectrum indicated the presence of a carbonyl. The EIMS of **1** showed a molecular ion peak at *m*/*z* 428 and significant peaks at *m*/*z* 302, 205, 191, 179, 165, 123, 109, and 95 (Figure 1). The ^{1}H NMR spectrum of **1** showed signals for six tertiary methyl groups at *δ* 0.73, 0.96, 1.00, 1.01, 1.05, and 1.20 and two secondary methyl protons at *δ* 0.88 (6H, d, *J* = 6.4 Hz) as required by the lanostane skeleton.[6] The ^{13}C NMR spectrum of **1** indicated signals for a carbonyl carbon at *δ* 213.2 and eight methyl carbons at *δ* 6.8, 14.6, 17.9, 18.6, 20.3, 31.8, 32.1, and 35.0. In addition to the absence of a methine proton signal at C-3 and an oxygen-bearing C-3 carbon signal in the NMR spectrum of **1**, the above evidence indicated that **1** is a lanostane-type triterpenoid without any substituent, except for a keto group on the side chain. The HMBC spectrum of C-25 to H-24 and CH_3-24^1 and of C-24 to CH_3-24^1 confirmed that the C-24^1 and C-25 were linked to the C-24. Based on the above evidence, **1** was established as 24ζ-methyl-5α-lanosta-25-one (**1**). In addition to the above results, information from ^{1}H−^{1}H and ^{1}H−^{13}C COSY and long-range ^{13}C−^{1}H COSY spectra and from comparison of the ^{13}C NMR data of **1** with those of lanosterol,[7] 5α-cholestan-3*β*-ol,[8] and podocarpane[9] further supported the characterization of **1** as 24ζ-methyl-5α-lanosta-25-one (**1**)

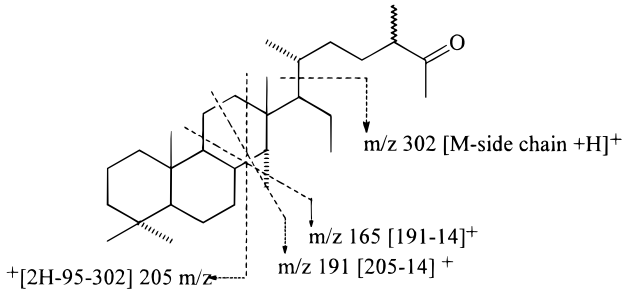
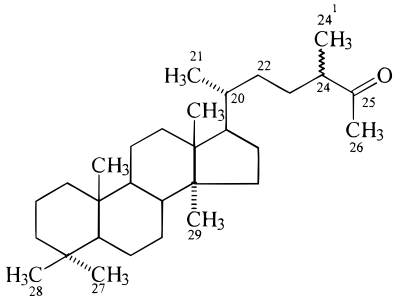


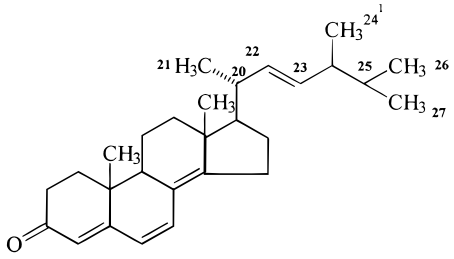
Figure 1. The mass spectral fragmentation of **1**.

and established the ^{1}H and ^{13}C NMR assignments (Experimental Section).

Compound **2** also gave a positive Libermann−Burchard reaction, and it was identified by UV, IR, $[\alpha]_D$, MS, and ^{1}H COSY and NOESY spectra, which further supported the characterization of **2** reported in the literature[5,6] and established the ^{1}H and ^{13}C NMR assignments (Experimental Section).



1



2

Experimental Section

General Experimental Procedures. The melting points are reported uncorrected. Optical rotation was

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obtained on a JASCO model DIP-370 digital polarimeter; UV spectra were obtained on a JASCO model 7800 UV/vis spectrophotometer; IR spectra were recorded on a Hitachi model 260−30 spectrophotometer; ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra (δ, ppm) were recorded on a Varian Unity-400 spectrometer; and MS were obtained on a JMS−HX 100 mass spectrometer.

Plant Material. *Ganoderma applanatum* was collected at Liu-Kuei Shian, Kaohsiung Hsieh, Taiwan, R. O. C., during February 1993. A voucher specimen (9501) is deposited in our laboratory. *Ganoderma neo-japonicum* was collected at Liu-Kuei Shian, Kaohsiung Hsieh, Taiwan, R. O. C., during February 1992. A voucher specimen (9201) is deposited in our laboratory.

Extraction and Isolation. Air-dried fruiting bodies (*G. applanatum*) (4.5 kg) were extracted with C_6H_{12}, $CHCl_3$, and MeOH successively and chromatographed on Si gel. Elution of the C_6H_{12} extract with C_6H_{12}−$CHCl_3$ (1:1) yielded **1** (0.010 g). Elution of the $CHCl_3$ extract with C_6H_{12}−$CHCl_3$ (4:1) yielded ergosta-7,22-dien-3-one (0.04 g) and ergosta-7,22-dien-3β-yl palmitate (0.120 g), and with $CHCl_3$ yielded ergosta-7,22-dien-3β-ol (1.350 g). Elution of the MeOH extract with $CHCl_3$ yielded **2** (0.045 g), with $CHCl_3$-MeOH (19:1) yielded 5α,8α-epidioxyergosta-6,9(11),-22-trien-3β-ol (0.010 g), and with $CHCl_3$−MeOH (9:1) yielded lucidone A (0.035 g). Air-dried fruiting bodies (*G. neo-japonicum*) (3 kg) were extracted with CH_2Cl_2 and chromatographed on Si gel. Elution of the CH_2Cl_2 extract with C_6H_{12}−CH_2Cl_2 (5:1) yielded ergosta-7,22-dien-3β-yl palmitate (0.125 g), with C_6H_{12}−CH_2Cl_2 (1:1) yielded ergosta-7,22-dien-3-one (0.164 g) and ergosta-7,22-dien-3β-ol (0.270 g), with C_6H_{12}−CH_2Cl_2 (1:4) yielded ganoderal A (0.010 g), with C_6H_{12}−CH_2Cl_2 (1:6) yielded ganodermadiol (0.248 g), with C_6H_{12}−MeOH (2:1) yielded 2β,3α,9α-trihydroxyergosta-7,22-diene (0.016 g), and with C_6H_{12}−Me_2CO (1:0.3) yielded ergosta-4,6,8(14),22-tetraen-3-one (**2**) (0.020 g). The known compounds were characterized by spectroscopic methods and a comparison of physical and spectroscopic data with those of authentic samples or literature.[1,2,4,10−12]

24ζ-Methyl-5α-lanosta-25-one (1): amorphorous powder (MeOH), mp 255−256 °C, $[\alpha]^{25}_D$ 40° ($CHCl_3$, 0.04); IR ν_{max} (KBr) 2940, 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.73 (3H, s, Me-18), 0.88 (6H, d, J = 6.4 Hz, Me-21 and 24^1), 0.96 (3H, s, Me-29), 1.00 (3H, s, Me-27), 1.01 (3H, s, Me-28), 1.05 (3H, s, Me-19), 1.20 (3H, s, Me-26), 2.24 (1H, m, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 6.8 (C-24^1), 14.6 (C-18), 17.9 (C-21), 18.2 (C-2), 18.6 (C-19), 20.3, (C-28) 22.3 (C-6, -11, and -23), 30.5 (C-16), 31.8 (C-27), 32.1 (C-26), 32.4 (C-7), 35.0 (C-29), 35.3 (C-22), 35.6 (C-15), 36.0 (C-20), 37.5 (C-10), 38.3 (C-4), 39.2 (C-1), 41.3 (C-3 or C-12), 41.5 (C-3 or C-12), 42.1 (C-13), 42.8 (C-8), 53.1 (C-9 and 17), 58.2 (C-24), 59.5 (C-5 and -14), 213.2 (C-25); EIMS (75 eV) *m*/*z* (rel int) 428 (9), 341 (3), 302 (1), 274 (10), 191 (17), 179 (23), 165 (34), 123 (64), 109 (72), 95 (100); HREIMS *m*/*z* 428.4012 (calcd for $C_{30}H_{52}O$, 428.4018).

Ergosta-4,6,8(14),22-tetraen-3-one (2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 (3H, d, J = 6.6 Hz, M-26), 0.84 (3H, d, J = 6.6 Hz, Me-27), 0.93 (3H, d, J = 6.6 Hz, Me-24^1), 0.94 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.04 (3H, d, J = 6.4 Hz, Me-21), 5.22 (2H, m, H-22 and 23), 5.73 (1H, s, H-4), 6.03 (1H, d, J = 9.5 Hz, H-6), 6.60 (1H, d, J = 9.5 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.6 (C-19), 17.6 (C-24^1), 18.9 (C-11), 19.0 (C-18), 19.7 (C-27), 20.0 (C-26), 21.2 (C-21), 25.4 (C-15), 27.7 (C-16), 33.1 (C-25), 34.2 (C-2 and -12), 35.6 (C-1), 36.8 (C-10), 39.3 (C-20), 43.0 (C-24), 44.0 (C-13), 44.3 (C-9), 55.7 (C-17), 122.7 (C-4), 124.5 (C-6), 132.6 (C-22), 134.1(C-5 and -7), 135.0 (C-23), 156.0 (C-8), 164.5 (C-14), 199.5 (C-3).

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