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THE ISOLATION OF LANOSTA-7,9(11),24-TRIEN-3β,21-DIOL FROM THE FUNGUS *GANODERMA AUSTRALE*

AMOLAK C. JAIN and SUSHIL K. GUPTA

Department of Chemistry, University of Delhi, Delhi-110007, India

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Key Word Index—*Ganoderma australe*; Polyporaceae; wood-rotting fungus; ergosterol palmitate; ergosta-7,22-dien-3-one; ergosterol; lanosta-7,9(11),24-trien-3β,21-diol.

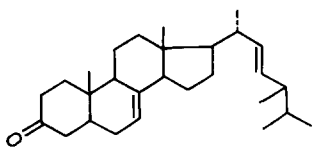
Abstract—The lipid-soluble fraction of the fungus *Ganoderma australe* belonging to the family Polyporaceae has yielded ergosterol palmitate, ergosta-7,22-dien-3-one, ergosterol and lanosta-7,9(11),24-trien-3β,21-diol. This fungus is the second reported natural source of the latter compound whose structure is now established on the basis of spectral data.

INTRODUCTION

The fungus infecting some local trees of a medicinally important host tree *Emblica officinalis*, and identified as *Ganoderma australe* (Specimen No. 8443, incorporated in Forest Research Institute Herbarium, Dehradun, India) belongs to the Polyporaceae. We now report the first chemical study of this fungus, although two other species of the genus *Ganoderma* have been studied recently in some detail. *G. lucidum* yielded L-mannitol, ergosterol and eight polyoxygenated triterpenic acids belonging to the lanostane series [1, 2] which all contain a terminal carboxylic group and some of them display cytotoxic activity *in vitro* on hepatoma cells [2]. The second fungus, *G. applanatum* afforded ergosta-4,6,8(14),22-tetraen-3-one [3], ergosta-7,22-dien-3β-ol, ergosta-7,22-dien-3-one [4], ergosterol and palmitic acid besides the two pentacyclic triterpenes, friedoolean-5-en-3-onc and friedelin [5].

RESULTS AND DISCUSSION

The petrol and benzene extracts of *G. australe* were found to have similar components on TLC and were therefore mixed. The total residue was 0.4% of the weight of the fungus. On column chromatography, four compounds were isolated. The first three compounds eluted were identified as ergosterol palmitate, ergosta-7,22-dien-3-one (**1**) and ergosterol, respectively. The identity of ergosterol palmitate was established by its mass spectrum and hydrolysis to ergosterol. The UV spectrum of compound **1** showed the absence of a conjugated chromophore and the IR band at 1710 cm^{-1} indicated the presence of a carbonyl group. The colour reaction and the general features of its ^1H NMR spectrum pointed out its steroidal nature. The mass ion at *m/z* 396 in the mass spectrum gave the molecular formula as $C_{28}H_{44}O$. Three important features were noteworthy in the mass spectrum; the intense peak at *m/z* 43 corresponding to an isopropyl group, the fragment of *m/z* 298 showing the presence of double bond in the C-22 position [6] and a peak at *m/z* 244 indicating the presence of a double bond in the C-7 position [7]. The ^1H NMR spectrum also supported the presence of three olefinic protons by a multiplet at δ5.12 and further, it showed the absence of typical signals of a 3α-proton at δ3.25. Finally, the mp of **1** agreed with the lit. mp [4].



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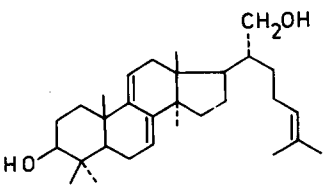

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The fourth compound showing a positive Liebermann-Burchard test was identified as lanosta-7,9(11),24-trien-3β,21-diol (**2**) which had previously been isolated from another fungus, *Fomes pinicola* (Polyporaceae) [8]. Since this structure was given earlier on the basis of its correlation with pinicolic acid A without the help of ^{1}H NMR and mass spectral techniques, we report now its spectral properties, and those of its diacetate (see Experimental), which fully confirm the structure originally proposed.

EXPERIMENTAL

Unless stated otherwise, mps are uncorr; the petrol used had a boiling range of 60–80°; silica gel was used for column chromatography and TLC; R_f values refer to TLC for which the solvent systems were: (A) C_6H_6, (B) $CHCl_3$. UV spectra were recorded in MeOH. ^{1}H NMR spectra were recorded at 90 MHz; chemical shifts are expressed in the δ scale downfield from TMS internal standard.

Extraction of G. australe. Air-dried fungus (800 g) was broken into small pieces and extracted exhaustively with petrol followed by C_6H_6. The two extracts upon evaporation yielded deep yellow oils (1.9 g and 1.4 g respectively). TLC showed that both extracts were similar with four main spots besides many minor ones. The two oils were therefore mixed and subjected to column chromatography when four fractions A, B, C and D were obtained successively.

Fraction A (*ergosterol palmitate*). This compound was obtained by eluting the column with petrol alone followed by petrol–C_6H_6 (9:1). It crystallized from MeOH–$CHCl_3$ mixture as white plates (110 mg), mp 109–111° (lit [9] mp 107–108°); R_f 0.9 (solvent A); IR ν_{max}^{KBr} cm^{-1}: 3370, 2840, 1730 (ester carbonyl), 1610, 1450, 1370 and 1170; MS *m/z*: 634.5604 $[M]^+$, 508, 377, 362, 255, 229, 213, 199, 159, 147, 133, 121, 109, 95, 81, 69, 57, 55, 43 (base ion). The above ester (50 mg) was hydrolysed with 7% ethanolic KOH (10 ml) and the sterol part extracted with Et_2O. The ethereal residue crystallised from MeOH to give ergosterol as colourless needles (25 mg), mp alone, and when mixed with the sample described in fraction C below, 163°.

Fraction B (*ergosta-7,22-dien-3-one*, **1**). This compound was obtained from the column by elution with petrol–C_6H_6 (1:1). It crystallized from MeOH as colourless crystals (50 mg), mp 178–179° (lit [4] mp 178°); R_f 0.25 (solvent A); IR ν_{max}^{nujol} cm^{-1}: 1710 (ketonic C=O); UV λ_{max}^{MeOH} nm: 217; ^{1}H NMR ($CDCl_3$): 0.55 (3H, *s*, H-18), 0.79 (3H, *s*, H-19), 0.85–1.05 (12H, 3*d*, 4 × sec. Me), 5.12 (3H, *m*, H-7, H-22, H-23). MS *m/z*: 396 $[M]^+$, 381 $[M-Me]^+$, 353 $(M-Me-CO)^+$, 298, 271, 269 (base ion), 244, 229, 213, 175, 161, 147, 133, 123, 119, 109, 105, 95, 81, 69, 55, 43, 41.

Fraction C (*ergosterol*). This was obtained from the column by elution with C_6H_6 alone. It crystallized from MeOH as colourless needles (180 mg), mp 163° (lit [9] mp 163°); R_f 0.35 (solvent B); IR, ^{1}H NMR and mass spectra were found to be superimposable with those of an authentic sample. Further, it formed an acetate as colourless crystals, mp 176° (lit [9] mp 175–176°).

Fraction D (*lanosta-7,9(11),24-trien-3β,21-diol*, **2**). Compound **2** was obtained by eluting the column with C_6H_6–EtOAc (4:1). It was repeatedly crystallized from MeOH until it showed homogeneity on TLC and HPLC when **2** was obtained as colourless needles (140 mg), mp 188–190° (lit [8], mp 195°); $[\alpha]_D$ +65° (*c* 1.47, $CHCl_3$) (lit [8], $[\alpha]_D$ +72° (*c* 1.06, $CHCl_3$)); R_f 0.25 (solvent B); IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 2845(s), 1610, 1445 *m*, 1370; UV λ_{max}^{MeOH} nm (log ε): 235.5 (4.37), 243 (4.43), 251.5 (4.27); ^{1}H NMR ($CDCl_3$): δ0.57 (3H, *s*, H-18), 0.90, 0.98, 1.02 (12H, 3*s*, 4 × Me), 1.68 (6H, *s*, 2 × olefinic Me), 3.25 (1H, *m*, H-3α), 4.0 (2H, *s*, H-21), 5.4 (3H, *br m*, H-7, H-11, H-24). MS *m/z*: 440 $[M]^+$, 425 $[M-Me]^+$, 422 $[M-H_2O]^+$, 407 $[M-Me-H_2O]^+$, 389 $[M-Me-2H_2O]^+$, 358, 339, 313 [M – side chain]$^+$, 311, 295, 282, 271 [M – side chain – 42]$^+$, 253, 240, 225, 211, 199, 185, 171, 157, 145, 131, 119, 107, 95, 81, 69, 55 (base ion). (Found C, 79.95; H, 10.73. $C_{30}H_{48}O_2 \cdot \frac{1}{2}H_2O$ requires C, 80.18; H, 10.91%.)

The above compound (40 mg) was acetylated at room temp. using pyridine–Ac_2O. Usual work up and crystallization from MeOH yielded the diacetate as colourless needles (35 mg), mp 121–122° (lit [8] mp 122°); $[\alpha]_D$ +65.4° (*c* 1.0 in $CHCl_3$), Lit [8] $[\alpha]_D$ +73° (*c* 0.8); R_f 0.3 (solvent A); IR ν_{max}^{KBr} cm^{-1}: 1730 (ester carbonyl); UV λ_{max}^{MeOH} nm: 236, 243, 251; ^{1}H NMR ($CDCl_3$): δ0.58 (3H, *s*, H-18), 0.87, 0.95, 1.00 (12H, 3*s*, 4 × Me), 1.65 (6H, *s*, 2 × olefinic Me), 2.02 (6H, *s*, 2 × OAc), 4.42 (2H, *s*, H-21), 4.5 (1H, *m*, H-3α), 5.35 (3H, *br m*, H-7, H-11, H-24); MS *m/z*: 524 $[M]^+$, 464 $[M-AcOH]^+$, 449 $[M-AcOH-Me]^+$, 404 $[M-2AcOH]^+$, 389 $[M-AcOH-Me]^+$, 355 [M – side chain]$^+$, 353, 313 [M – side chain – 42]$^+$, 282, 252, 239, 225, 213, 211, 199, 171, 157, 149, 131, 109, 95, 81, 69, 55.

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