Pergamon 0031-9422(95)00762-8 Phytochemistry, Vol. 41, No. 4, pp. 1041–1046, 1996

0031-9422/96 $15.00 + 0.00

# ANTIMICROBIAL STEROIDS FROM THE FUNGUS *FOMITOPSIS PINICOLA*

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(*Received in revised form* 10 *July* 1995)

**Key Word Index**—*Fomitopsis pinicola*; Polyporaceae; steroids; lanostane; 3α-(4-carboxymethyl-3-hydroxy-3-methylbutanoyloxy)-lanosta-8,24-dien-21-oic acid; fomitopsic acid.

**Abstract**—Phytochemical examination of the dichloromethane extract of the European fungus *Fomitopsis pinicola* led to the isolation of a new lanostanoid derivative identified from spectral and chemical evidences as 3α-(4-carboxymethyl-3-hydroxy-3-methylbutanoyloxy)-lanosta-8,24-dien-21-oic acid. In addition, seven known triterpenes, polyporenic acid C, 3α-acetyloxylanosta-8,24-dien-21-oic acid, ergosta-7,22-dien-3β-ol, 21-hydroxylanosta-8,24-dien-3-one, pinicolic acid A, trametenolic acid B and pachymic acid, were also isolated. Antimicrobial activity against *Bacillus subtilis* in a TLC bioassay was observed for five of the isolated steroids.

## INTRODUCTION

In the course of a screening of non-toxic indigenous mushrooms for biologically active constituents, it was found that the dichloromethane extract of *Fomitopsis pinicola* (Swartz ex Fr.) Karst Polyporaceae showed antibacterial activity against *Bacillus subtilis* in a TLC bioassay [1]. *F. pinicola* (syn. *Polyporus pinicola* Fr., *Fomes pinicola*) is a wood-rotting fungus growing on coniferous and broad-leaved trees of Europe. Only heterogalactans [2], fatty acids [3] and some ergostane and lanostane derivatives [4–7] have been reported from this fungus. In the present paper, the isolation and the structure elucidation of eight constituents of *F. pinicola* are described.

## RESULTS AND DISCUSSION

Fresh fruiting bodies of *F. pinicola* were submitted to lyophilization. The lyophilized mushroom (300 g) was extracted successively with dichloromethane and methanol. The dichloromethane extract (yield 13.7%) showed antibacterial activity against *B. subtilis* in a TLC bioassay [1], where large areas of growth inhibition were observed. The extract was then fractionated on a silica gel column with a step-gradient of petrol–ethyl acetate, ethyl acetate and finally methanol to give 19 fractions (A–S). Separation of the bioactive fractions H, I, K, M, N and P by various techniques such as low pressure and medium pressure liquid chromatography on reversed phase or DIOL supports or gel filtration on Sephadex LH-20 led to the isolation of eight sterols (**1**–**8**).

Seven compounds were identified as polyporenic acid C(**1**) [8, 9], 3α-acetyloxylanosta-8,24-dien-21-oic acid (**2**) [5, 6, 8, 10, 11], ergosta-7,22-dien-3β-ol (**3**) [6, 12], 21-hydroxylanosta-8,24-dien-3-one (**4**) [13, 14], pinicolic acid A (**5**) [4, 15, 16], trametenolic acid B (**6**) [6, 11, 15] and pachymic acid (**7**) [8], mainly by comparison of their $^1$H NMR and $^{13}$C NMR data with the known compounds. In addition, compound **1** was acetylated to afford the known monoacetate derivative **1a** and compound **2** was hydrolysed to give compound **2a** and methylated to yield derivative **2b**.

The molecular formula $C_{37}H_{58}O_7$ of compound **8** was established on the basis of $^{13}$C NMR and DEPT spectra, and confirmed by D/CI-MS (*m/z* 615 $[M + H]^+$, 632 $[M + NH_4]^+$). $^1$H NMR and $^{13}$C NMR data gave the following information: signals for an isoprenyl moiety (δ1.62, 1.66, 3H each, *s* and one proton *t* at δ5.32), five tertiary methyl groups (δ 0.86, 0.91, 0.95, 0.99 and 1.05), one carboxyl moiety (δ178.6), two olefinic quaternary carbons (δ 131.7, 134.3), one olefinic methine (δ124.8) and one oxymethine (δ 78.1). Comparison of these data with those recorded for compounds **2** and **6** confirmed the identification of a lanostan-8,24-dien-21-oic acid skeleton substituted at C-3 position by an oxygenated $C_7$ chain. Stereochemistry at position C-3 was proved to be α (typical shifts observed for carbons of the A-ring) [10]. Careful examination of the D/CI-MS fragmentation pattern observed for compound **8** brought the following information. Elimination of the side chain through proton transfer gave ions at *m/z* 456 $[(M + NH_4) - 176]^+$ and 439 $[(M + H) - 176]^+$ together with the corresponding ions *m/z* 177 $[chain + H]^+$ and 194 $[chain + NH_4]^+$. Thus, the side chain had the partial structure $C_7H_{11}O_5$ ($M_r$ 175). Four signals belonging to protons of the side chain were shown in the $^1$H NMR spectrum: one sharp signal at δ3.61 for a methyl ester moiety, two methylene groups at δ2.97 and 3.01 (*d*, $J$ = 13.9 Hz) adjacent to

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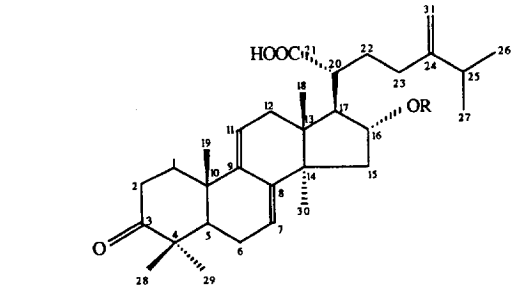
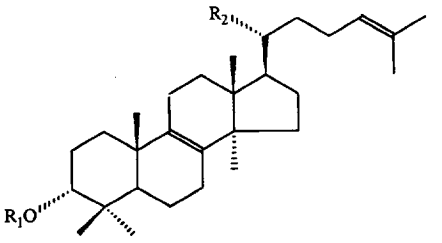


1: R = H

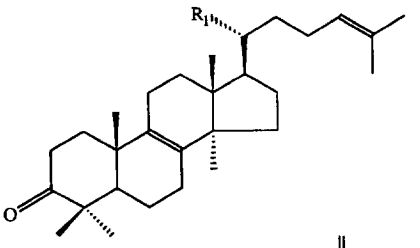
1a: R = Ac



2: $R_1$ = Ac, $R_2$ = COOH

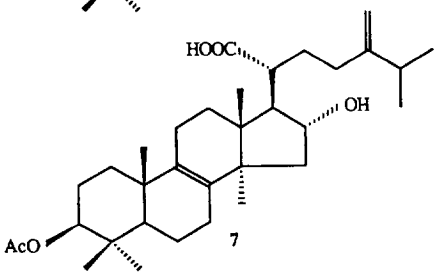
2a: $R_1$ = H, $R_2$ = COOH

2b: $R_1$ = Ac, $R_2$ = COO Me



4: $R_1$ = $CH_2OH$

5: $R_1$ = COOH



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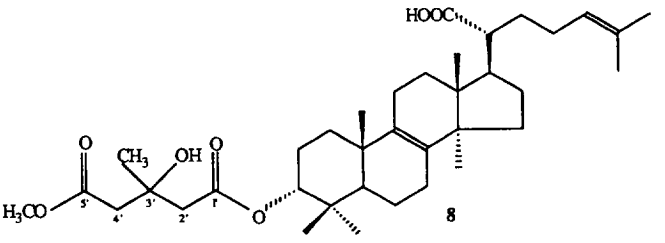

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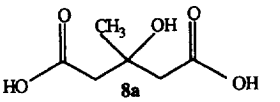
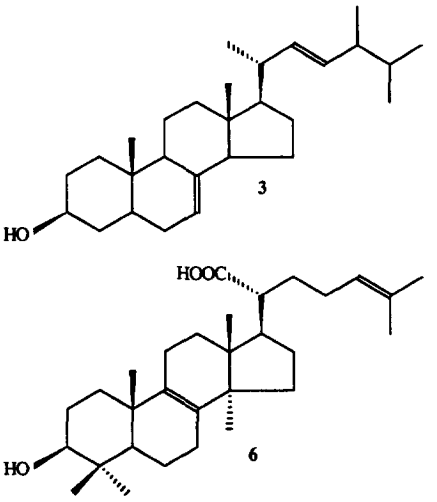


8a

a carboxyl group, and one signal at $\delta$ 1.66, due to a tertiary methyl group attached to a hydroxyl bearing carbon atom. The $^{13}C$ NMR spectrum showed signals attributable to two esterified carboxyl groups ($\delta$ 171.2, 171.8), one oxygen-bearing quaternary carbon ($\delta$ 69.8) and one low-field methyl group ($\delta$ 51.2). Comparison of these data with those reported for the carbons of the chain of fasciculic acid A [17] confirmed the presence of a methyl 3-hydroxy-3-methylglutaroyl group ($M_r$ 175) in compound **8**. This hypothesis was also supported by further mass fragmentation observed in the D/CI-MS spectrum. In addition, alkaline hydrolysis of compound **8** afforded the steroid **2a** and the diacid **8a**.

Thus, compound **8** was 3α-(4-carboxymethyl-3-hydroxy-3-methylbutanoyloxy)-lanosta-8,24-dien-21-oic acid, a new natural product for which we propose the name fomitopsic acid.

Phytochemical investigation of the lyophilized fruiting body of *F. pinicola* led to the isolation of eight steroids. Three of them (**2**, **3**, **5**) were steroids usually encountered in fungi, while compounds **1**, **4**, **6**, **7** and **8** were newly described as *F. pinicola* constituents. Compound **8** with its unusual substitution represents a new natural product. The $^{13}C$ NMR spectral data of the known compounds **1**, **2**, **4** and **5** have not yet been reported and are given in Table 1.




Five of the isolated compounds (**1**, **2**, **5**, **6** and **8**) showed antibacterial activity against *B. subtilis* in a TLC bioassay. The minimum quantities spotted on TLC plates required to inhibit *B. subtilis* were, respectively, 1 μg, 0.01 μg, 0.2 μg, 2.5 μg and 0.1 μg. Chloramphenicol, used as positive control, was active at 0.01 g. In addition, derivatives **1a** and **2a** were also active at 0.05 μg and 0.5 μg, respectively. However, when tested at different concentrations in a classical agar dilution assay using *B. subtilis* [18], none of the isolated compounds or their derivatives showed inhibition at concentrations up to 50 μg/ml.

## EXPERIMENTAL

*General.* TLC: Kieselgel $60F_{254}$ (Merck), detection with Godin reagent. Mp: Mettler-FP-80/82 hot stage

Table 1. $^{13}C$ NMR shifts for compounds **1** to **8** and derivatives, in pyridine $d_5$ (except **2**, **2b**, **3** and **4** in $CDCl_3$)

| C | 1 | 1a | 2 | 2a | 2b | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.8 | 36.7 | 30.3 | 30.7 | 30.5 | 37.1 | 35.9 | 36.1 | 36.1 | 35.2 | 30.7 |
| 2 | 34.9 | 34.9 | 23.4 | 26.8 | 23.3 | 29.6 | 34.4 | 34.6 | 28.7 | 24.4 | 23.5 |
| 3 | 215.1 | 215.0 | 77.9 | 75.0 | 78.0 | 71.0 | 217.0 | 216.0 | 78.0 | 80.5 | 78.1 |
| 4 | 47.5 | 47.4 | 36.8 | 37.4 | 36.9 | 38.0 | 47.2 | 47.3 | 39.5 | 37.9 | 36.8 |
| 5 | 51.1 | 50.9 | 45.2 | 44.5 | 45.2 | 40.2 | 51.0 | 51.2 | 50.9 | 50.6 | 45.8 |
| 6 | 23.9 | 23.8 | 17.9 | 18.5 | 18.0 | 31.5 | 19.3 | 19.5 | 18.7 | 18.3 | 18.2 |
| 7 | 120.7 | 121.2 | 26.9 | 26.5 | 26.0 | 117.4 | 26.2 | 26.5 | 26.8 | 26.7 | 26.7 |
| 8 | 142.9 | 141.8 | 134.4 | 134.0 | 134.5 | 139.6 | 133.0 | 134.6 | 134.3 | 134.9 | 134.2 |
| 9 | 144.8 | 144.7 | 133.8 | pyr | 133.8 | 49.4 | 135.1 | pyr | 135.2 | 134.3 | pyr |
| 10 | 37.6 | 37.5 | 36.9 | 37.4 | 36.7 | 34.2 | 36.8 | 37.0 | 37.4 | 37.0 | 37.1 |
| 11 | 117.6 | 117.4 | 20.8 | 21.3 | 20.8 | 21.5 | 20.9 | 21.3 | 21.3 | 20.8 | 21.1 |
| 12 | 36.3 | 30.9 | 28.9 | 29.3 | 28.7 | 39.4 | 29.6 | 29.2 | 29.4 | 29.6 | 29.2 |
| 13 | 45.1 | 44.4 | 44.2 | 44.8 | 44.1 | 43.3 | 44.1 | 44.8 | 44.9 | 46.1 | 44.8 |
| 14 | 49.4 | 48.9 | 49.5 | 49.8 | 49.4 | 55.1 | 49.8 | 49.8 | 49.9 | 48.6 | 49.8 |
| 15 | 44.4 | 41.4 | 30.8 | 30.8 | 30.8 | 22.9 | 30.7 | 30.9 | 30.9 | 43.6 | 30.9 |
| 16 | 76.4 | 79.1 | 27.0 | 27.5 | 27.0 | 28.1 | 27.5 | 27.4 | 27.5 | 76.6 | 27.4 |
| 17 | 57.6 | 54.0 | 47.1 | 47.7 | 47.8 | 55.9 | 44.2 | 47.7 | 47.8 | 57.2 | 47.6 |
| 18 | 17.7 | 17.3 | 16.0 | 16.3 | 16.0 | 12.1 | 16.0 | 16.8 | 16.4 | 16.7 | 16.3 |
| 19 | 22.4 | 22.3 | 18.8 | 19.3 | 18.9 | 13.0 | 18.6 | 18.6 | 19.4 | 19.1 | 19.0 |
| 20 | 48.5 | 47.4 | 47.7 | 49.0 | 47.3 | 40.5 | 42.7 | 49.0 | 49.1 | 48.7 | 49.0 |
| 21 | 178.6 | 177.8 | 183.3 | 178.6 | 170.9 | 21.1 | 62.3 | 178.6 | 178.7 | 178.7 | 178.6 |
| 22 | 37.6 | 35.9 | 32.4 | 33.3 | 32.6 | 135.1 | 30.3 | 33.2 | 33.4 | 33.2 | 33.2 |
| 23 | 31.5 | 32.4 | 25.9 | 26.7 | 25.9 | 131.9 | 24.8 | 26.7 | 26.8 | 31.5 | 26.2 |
| 24 | 156.1 | 155.8 | 123.6 | 124.9 | 123.7 | 42.8 | 124.8 | 124.9 | 124.9 | 156.0 | 124.8 |
| 25 | 34.2 | 34.4 | 132.2 | 131.7 | 132.1 | 33.1 | 132.2 | 131.6 | 131.7 | 34.0 | 131.7 |
| 26 | 21.9 | 22.0 | 17.6 | 17.7 | 17.6 | 17.6 | 17.6 | 17.7 | 17.8 | 17.7 | 17.6 |
| 27 | 22.0 | 22.0 | 25.7 | 25.8 | 25.7 | 19.6 | 25.6 | 25.8 | 25.8 | 25.3 | 25.7 |
| 28 | 22.3 | 22.3 | 21.8 | 21.8 | 21.9 | 19.9 | 21.2 | 21.3 | 16.4 | 21.8 | 21.8 |
| 29 | 26.4 | 25.8 | 27.5 | 29.1 | 27.5 | | 26.0 | 26.3 | 28.7 | 22.0 | 27.5 |
| 30 | 25.8 | 25.6 | 24.3 | 24.2 | 24.4 | | 24.3 | 24.5 | 24.5 | 27.9 | 24.2 |
| 31 | 107.1 | 106.9 | | | | | | | | 106.9 | |
| 1′ | | | | | | | | | | | 171.8 |
| 2′ | | | | | | | | | | | 45.9 |
| 3′ | | | | | | | | | | | 69.8 |
| 3′-Me | | | | | | | | | | | 28.4 |
| 5′ | | | | | | | | | | | 46.3 |
| 6′ | | | | | | | | | | | 171.2 |
| 5′-OMe | | | | | | | | | | | 51.2 |
| MeCO | | | 171.0 | | 170.9 | | | | | 170.9 | |
| MeCO | | | 21.3 | | 21.3 | | | | | 21.1 | |
| COOMe | | | | | 51.0 | | | | | | |

pyr: hidden by pyridine signal.

apparatus, uncorr. $\alpha_D$: Perkin-Elmer-241 polarimeter. UV: Varian DMS 100 spectrophotometer. IR: Philips PU 9716. $^1H$ and $^{13}C$-NMR: Varian VXR 200 at 200.06 and 50.03 MHz, respectively. EI-MS and D/CI-MS: Finnigan-MAT-TSQ-700 triple stage quadrupole instrument ($NH_3$, positive ion mode).

*TLC bioassay. Bacillus subtilis* strain ATCC 6633 (Sigma). The crude extracts of *F. pinicola* were tested at 100 $\mu$g. Dilutions of the test compounds were freshly prepared at 1 to $10^{-3}$ mg/ml$^{-1}$ in $CHCl_3$. Aliquots of these stock solns were spotted on glass-backed silica gel $GF_{254}$ TLC plates (Merck). Chloramphenicol was used as positive control (0.01 $\mu$g). The plates were developed in $CHCl_3$–*iso*-PrOH (9:1). After drying, an inoculum of bacteria (approx. $10^8$ cells ml$^{-1}$) in LB medium was distributed as a thin layer. The TLC plates were incubated overnight at 30° in a moist atmosphere in polyethylene boxes. The inhibition zones were revealed by spraying with an aqueous soln of methylthiazoyldiphenyltetrazolium bromide (2.5 mg ml$^{-1}$). Clear inhibition zones appeared against a blue background [1].

*Fungus material. Fomitopsis pinicola* was collected in June 1993 near Neuchâtel, Switzerland and identified by J. Keller, Institut de Botanique, Université de Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland.

*Extraction and isolation.* Lyophilized fruiting bodies (300 g) of *F. pinicola* were extracted successively with $CH_2Cl_2$ and MeOH (3 × 3 l. each). A portion (20 g) of the $CH_2Cl_2$ extract was sepd on a silica gel column Si 60 (65 × 7 cm) with a step-gradient of petrol–EtOAc (95:5–9: 1–7: 1–5:1–3:1–1:1), EtOAc and finally MeOH to give 19 frs (A–S). Fraction P (84 mg) afforded compound **1** (80 mg) as white needles by slow crystallization from petrol–EtOAc. Fr. I (280 mg) was submitted to low pressure liquid chromatography (LPLC) on silica gel with $CHCl_3$–*iso*-PrOH (98:2) giving two fractions (I1 and I2). Fraction I1 was further purified on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) to yield 260 mg of the non-UV active compound **2**. Fraction H (485 mg) was submitted to LPLC with petrol–EtOAc (5:1). Five frs were obtained (H1–H5). Further separation of H2 by LPLC with *n*-hexane–$CHCl_3$ (95:5) gave 11 mg of compound **3** and purification of H4 on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) afforded 110 mg of compound **4**. One portion (1.0 g) of fraction K (2.0 g) was separated by medium pressure liquid chromatography (MPLC) on RP-18 with MeOH-$H_2O$ (85:15), yielding 450 mg of compound **5**. Fraction M (350 mg) was separated by MPLC on a DIOL column with a step-gradient of *n*-hexane–$CHCl_3$ (2:1–1:1) yielding 3 frs (M1–M3). A further purification of M3 by MPLC on RP-18 with MeOH–$H_2O$ (85:15) afforded 14 mg of compound **6**. Fraction M2 yielded 79 mg of compound **7** after final purification on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1). 500 mg of fraction N (2.5 g) were submitted to MPLC on DIOL with a step-gradient of *n*-hexane:$CHCl_3$ (2:1–1:1) affording 3 fractions (N1–N3). A further purification of N3 on a Sephadex LH-20 column with $CHCl_3$–MeOH (1:1) yielded 35 mg of compound **8** as a white non-UV active powder.

*Compound* **1**, 16α-*hydroxy*-24-*methylene*-3-*oxolanosta*-7,9(11)-*dien*-21-*oic acid*. White needles, mp 265° (lit. [5] 260–263°). $[\alpha]^{21}$ + 29° ($CHCl_3$, *c* = 0.49). UV $\lambda_{max}^{MeOH}$ nm (log ε): 236 (4.06), 243 (4.11), 251 (3.95). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.51. $^1H$ NMR (pyridine-$d_5$): δ1.00, 1.01 (each 3H, *d*, *J* = 6.9 Hz, H-26, H-27), 1.04 (3H, *s*, H-28), 1.07 (3H, *s*, H-18), 1.13 (3H, *s*, H-19), 1.14 (3H, *s*, H-29), 1.44 (3H, *s*, H-30), 1.62 (1H, *dd*, *J* = 4.12 Hz, H-5), 1.90 (1H, *m*, $H_{eq}$-15), 2.45 (1H, *m*, $H_{ax}$-15), 2.88 (1H, *m*, $H_{ax}$-17), 2.90 (1H, *m*, H-20), 4.52 (1H, *dd*, *J* = 5.6, 7.8 Hz, H-16), 4.85, 4.97 (each 1H, *s*, H-31), 5.37 (1H, *d*, *J* = 5.9 Hz, H-11), 5.59 (1H, *d*, *J* = 6.0 Hz, H-7). $^{13}C$ NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 482 $[M]^+$ (2), 467 (12), 449 (24), 309 (40), 293 (100), 269 (24), 69 (26). D/CI-MS *m/z* (rel. int.): 500 $[M + NH_4]^+$ (100), 483 $[M + H]^+$ (52), 456 (18).

*Acetylation of* **1**. Compound **1** (15 mg) was stirred for 2 hr at 25° with $Ac_2O$ (1.5 ml) and pyridine (1.5 ml). The reaction was stopped by precipitation in ice water and the acetylated compound **1a** was then extracted with $CHCl_3$ (3 × 5ml). Compound **1a** was purified by chromatography on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to afford 15 mg.

*Compound* **1a**, 16α-*acetyloxy*-24-*methylene*-3-*oxolanosta*-7,9(11)-*dien*-21-*oic acid*. White powder, mp 183° (lit. [5] 177–180°). $[\alpha]^{21}$ − 8.5° ($CHCl_3$, *c* = 0.55). UV $\lambda_{max}^{MeOH}$ nm (logε): 236 (4.05), 242 (4.11), 250 (4.00). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.60. $^1H$ NMR (pyridine-$d_5$): δ0.99 (3H, *s*, H-18), 1.01, 1.01 (each 3H, *d*, *J* = 7.1 Hz, H-26, H-27), 1.06 (3H, *s*, H-28), 1.12 (3H, *s*, H-19), 1.13 (3H, *s*, H-29), 1.23 (3H, *s*, H-30), 2.15 (3H, *s*, acetoxy), 4.87, 4.91 (each 1H, *s*, H-31), 5.35 (1H, *d*, *J* = 6.1 Hz, H-11), 5.48 (1H, *m*, H-7). $^{13}C$ NMR: see Table 1. EI-MS *m/z* (rel. int.): 524 $[M]^+$ (20), 464 (26), 451 (28), 449 (27), 309 (100), 295 (36), 293 (36), 268 (25). D/CI-MS *m/z* (rel. int.): 542 $[M + NH_4]^+$ (100).

*Compound* **2**, 3α-*acetyloxylanosta*-8,24-*dien*-21-*oic acid*. White powder, mp 192–194°. $[\alpha]^{21}$ + 14° ($CHCl_3$, *c* = 1.0). IR: $\nu_{max}^{KBr}$ cm$^{-1}$: 3600–2750, 2920, 1715, 1680. TLC: EtOAc–petrol (1:1) $R_f$ = 0.54, EtOAc–petrol (1:3) $R_f$ = 0.24. $^1H$ NMR ($CDCl_3$): δ0.74 (3H, *s*, H-18), 0.83 (3H, *s*, H-29), 0.88 (3H, *s*, H-28), 0.92 (3H, *s*, H-30), 0.95 (3H, *s*, H-19), 1.56, 1.65 (each 3H, *s*, H-26, H-27), 2.04 (3H, *s*, acetoxy), 4.65 (1H, *br s*, H-3), 5.07 (1H, *t*, H-24). $^{13}C$ NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (rel. int.): 498 $[M]^+$ (45), 483 (18), 437 (17), 423 (100), 281 (27), 187 (23), 69 (23). D/CI-MS *m/z* (rel. int.): 516 $[M + NH_4]^+$ (100), 499 $[M + H]^+$ (6), 472 (21).

*Hydrolysis of* **2**. A soln of 40 mg of **2** in KOH 1N (20 ml) was refluxed for 10 hr. After cooling, the soln was neutralized with HCl 1N and the hydrolysis product **2a** was extracted with $CHCl_3$ (5 × 25 ml) and purified by Lobar LiChroprep Si 60 (40–63 $\mu$m, 31 × 2.5 cm), with *n*-hexane–*iso*-PrOH (95:5) to give 25 mg of **2a**.

*Compound* **2a**, 3α-*hydroxylanosta*-8, 24-*dien*-21-*oic acid*. White powder, mp 149–153°. $[\alpha]^{21}$ + 29° ($CHCl_3$, *c* = 1.0). TLC: EtOAc–petrol (1:1) $R_f$ = 0.38, $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.55. $^1H$ NMR (pyridine-$d_5$): δ0.92 (3H, *s*, H-18), 0.93 (3H, *s*, H-29), 1.04 (3H, *s*, H-30), 1.09 (3H, *s*, H-19), 1.23 (3H, *s*, H-28), 1.62, 1.67

(each 3H, *s*, H-26, H-27), 3.62 (1H, *s*, H-3), 5.33 (1H, *t*, H-24). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 456 $[M]^+$ (72), 441 (57), 423 (100), 281 (45), 187 (65), 69 (34). D/CI-MS *m/z* (rel. int.): 474 $[M + NH_4]^+$ (100).

*Methylation of* **2**. To a soln of **2** (30 mg) in $Et_2O$ (2 ml), excess $CH_2N_2$ was added (15 ml of a 35 mM solution in $Et_2O$) and the reaction mixture left at 25° for 1 hr. The resulting product was purified through a small column packed with silica gel using EtOAc–*n*-hexane (1:3) to give the monomethyl ester **2b**.

*Compound* **2b**, *methyl* 3α-*acetyloxylanosta*-8,24-*dien*-21-*oate*. White powder, mp 106° (lit. [5] 132°). $[\alpha]^{21}$ + 5.1° ($CHCl_3$, *c* = 0.45) (lit. [5] + 7°, $CHCl_3$, *c* = 1.5). TLC: EtOAc–petrol (1:3) $R_f$ = 0.53. $^1$H NMR ($CDCl_3$): δ0.71 (3H, *s*, H-18), 0.85 (3H, *s*, H-29), 0.89 (3H, *s*, H-28), 0.90 (3H, *s*, H-30), 0.96 (3H, *s*, H-19), 1.56, 1.67 (each 3H, *s*, H-26, H-27), 2.05 (3H, *s*, acetoxy), 3.65 (3H, *s*, COOMe), 4.64 (1H, *s*, H-3), 5.06 (1H, *t*, H-24). $^{13}$C NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (rel. int.): 512 $[M]^+$ (59), 497 (34), 451 (18), 437 (100), 281 (28). D/CI-MS *m/z* (rel. int.): 530 $[M + NH_4]^+$ (100), 513 $[M + H]^+$ (42).

*Compound* **3**, *ergosta* 7,22-*dien*-3β-*ol*. White powder, mp 165–170° (lit. [12] 168–170°). $[\alpha]^{21}$ − 11.5° ($CHCl_3$, *c* = 1.0) (lit. [12] − 25°, $CHCl_3$, *c* = 0.2). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.27. $^1$H NMR ($CDCl_3$): δ0.53 (3H, *s*, Me), 0.78 (3H, *s*, Me), 0.80 (3H, *d*, *J* = 8.1 Hz, Me), 0.82 (3H, *d*, *J* = 6.7 Hz, Me), 0.90 (3H, *d*, *J* = 6.8 Hz, Me), 1.00 (3H, *d*, *J* = 6.6 Hz, Me), 3.57 (1H, *m*, H-3), 5.17 (3H, *m*, H-7, H-22, H-23),. $^{13}$C NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (rel. int.): 398 $[M]^+$ (36), 273 (27), 271 (100), 255 (44). D/CI-MS *m/z* (rel. int.): 416 $[M + NH_4]^+$ (100), 414 (95), 399 $[M + H]^+$ (52), 397 (55), 273 (27).

*Compound* **4**, 21-*hydroxylanosta*-8,24-*dien*-3-*one*. White powder, mp 92–96° (lit. [14] 114–119°). $[\alpha]^{21}$ + 59° ($CHCl_3$, *c* = 1.0). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3250, 2905, 1685. TLC: EtOAc–petrol (1:3) $R_f$ = 0.25. $^1$H NMR ($CDCl_3$): δ0.69 (3H, *s*, H-18), 0.86 (3H, *s*, H-30), 1.01 (3H, *s*, H-28). 1.04 (3H, *s*, H-29), 1.07 (3H, *s*, H-19), 1.56, 1.63 (each 3H, *s*, H-26, H-27), 3.65 (2H, *m*, H-21), 5.07 (1H, *t*, H-24). $^{13}$C NMR ($CDCl_3$): see Table 1. EI-MS *m/z* (rel. int.): 440 $[M]^+$ (100), 425 (61), 407 (54), 271 (16), 257 (19), 245 (25), 109 (63), 69 (23). D/CI-MS *m/z* (rel. int.): 458 $[M + NH_4]^+$ (100).

*Compound* **5**, 3-*oxolanosta*-8,24-*dien*-21-*oic acid*. White powder, mp 196–200° (lit. [4] 197–202). $[\alpha]^{21}$ + 65° ($CHCl_3$, *c* = 1.0) (lit. [4] + 68°, $CHCl_3$, *c* = 0.8). TLC: EtOAc–petrol (1:1) $R_f$ = 0.38. $^1$H NMR (pyridine-$d_5$): δ0.99 (3H, *s*, H-18), 1.00 (3H, *s*, H-29), 1.06 (6H, *s*, H-19, H-30), 1.14 (3H, *s*, H-28), 1.63, 1.68 (each 3H, *s*, H-26, H-27), 5.34 (1H, *t*, H-24). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 454 $[M]^+$ (62), 439 (100), 421 (23), 393 (21), 297 (32). D/CI-MS *m/z* (rel. int.): 472 $[M + NH_4]^+$ (100), 428 (15).

*Compound* **6**, 3β-*hydroxylanosta*-8,24-*dien*-21-*oic acid*. White powder, mp 258–260° (lit. [15] + 252–258°). $[\alpha]^{21}$ + 40° (MeOH, *c* = 0.3). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.44. $^1$H NMR (pyridine-$d_5$): δ1.03 (6H, *s*, H-18, H-29), 1.08 (6H, *s*, H-19, H-30), 1.26 (3H, *s*, H-28), 1.63, 1.67 (each 3H, *s*, H-26, H-27), 3.43 (1H, *dd*, *J* = 7.6, 15.8 Hz, H-3), 5.34 (1H, *t*, H-24). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 456 (79), 441 (60), 423 (88), 69 (100). D/CI-MS *m/z* (rel. int.): 474 $[M + NH_4]^+$ (100).

*Compound* **7**, 3β-*acetyloxy*-16α-*hydroxy*-24-*methylene-lanosta*-8-*en*-21-*oic acid*. White powder, mp 251° (lit. [8] + 296–298°). $[\alpha]^{21}$ + 26° ($CHCl_3$, *c* = 1.0). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.58. $^1$H NMR (pyridine-$d_5$): δ0.92 (3H, *s*, H-28), 0.93 (3H, *s*, H-29), 0.96 (3H, *s*, H-19), 0.99, 1.00 (each 3H, *d*, *J* = 6.8 Hz, H-26, H-27), 1.13 (3H, *s*, H-18), 1.48 (3H, *s*, H-30), 2.06 (3H, *s*, acetoxy), 4.52 (1H, *dd*, *J* = 6.0, 7.6 Hz, H-16), 4.67 (1H, *dd*, *J* = 5.0, 10.8 Hz, H-3), 4.85 (each 1H, *s*, H-31). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 528 $[M]^+$ (27), 495 (100), 435 (50), 316 (35), 187 (30), 147 (30), 69 (24). D/CI-MS *m/z* (rel. int.): 546 $[M + NH_4]^+$ (100), 529 $[M + H]^+$ (39), 511 (26).

*Compound* **8**, 3α-(4-*carboxymethyl*-3-*hydroxy*-3-*methylbutanoyloxy*)-*lanosta*-8,24-*dien*-21-*oic acid*. White powder, mp 147°. $[\alpha]^{21}$ + 5° ($CHCl_3$, *c* = 1.0). TLC: $CHCl_3$–*iso*-PrOH (9:1) $R_f$ = 0.60. $^1$H NMR (pyridine-$d_5$): δ0.86 (3H, *s*, H-18), 0.91 (3H, *s*, H-29), 0.95 (3H, *s*, H-28), 0.99 (3H, *s*, H-30), 1.05 (3H, *s*, H-19), 1.62, 1.66 (each 3H, *s*, H-26, H-27), 1.66 (3H, *s*, 3′-methyl), 2.97 (2H, *s*), 3.01 (*d*, *J* = 13.9 Hz), 3.61 (3H, *s*, 5′-methoxy), 4.93 (1H, *s*, H-3), 5.32 (1H, *t*, H-24). $^{13}$C NMR (pyridine-$d_5$): see Table 1. EI-MS *m/z* (rel. int.): 614 $[M]^+$ (0.4), 540 (1.6), 438 (56), 423 (100), 296 (32), 281 (24), 187 (29), 177 (60), 159 (39), 117 (30), 69 (17). D/CI-MS *m/z* (rel. int.): 632 $[M + NH_4]^+$ (50), 615 $[M + H]^+$ (16), 474 $[M$ − side chain + $NH_4]^+$ (17), 458 (22), 439 $[M$ − side chain + $H]^+$ (68), 194 [side chain + $NH_4]^+$ (100).

*Hydrolysis of* **8**. 0.5 ml of NaOH 2% was added to a soln of 10 mg of compound **8** in 0.5 ml EtOH at 0°, and stirred at 25° for 4 hr. The reaction was then stopped with 0.5 ml $H_2O$ and the mixture extracted with $Et_2O$ (3 × 2 ml). After concentration under red. pres., the hydrolysis product was identified as **2a** by D/CI-MS [*m/z* (rel. int.): 474 $[M + NH_4]^+$ (100)] and TLC (($CHCl_3$–*iso*-PrOH 9:1) $R_f$ = 0.55 with the reference compounds **8** ($R_f$ 0.60) and **2a** ($R_f$ 0.55)). The aq. layer was neutralized by HCl 1N to yield compound **8a** [D/CI-MS *m/z* (rel. int.): 180 $[M + NH_4]^+$ (100)], identified as the diacid 3-hydroxy-3-methyl-glutaric acid.

*Acknowledgements*—Financial support was provided by the Swiss National Science Foundation. Thanks are due to PD Dr Jean Keller, University of Neuchâtel, Switzerland, for collecting the fungus.

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