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Steroids from the fungus *Fomitopsis pinicola*

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Abstract

Phytochemical examination of the *n*-hexane and methanol extracts of the crust of the wood-rotting fungus *Fomitopsis pinicola* led to the isolation of six new lanostanoid derivatives. Their structures were identified by one- and two-dimensional NMR spectroscopy and mass spectrometry. These new natural products are lanosta-7,9(11),24-trien-3-one, (−)-3,16-dioxolanosta-8,24-dien-21-oic acid, (+)-lanosta-7,9(11),24-trien-3β,15α,21-triol, (+)-21-(carboxyacetoxy)lanosta-7,9(11),24-trien-3-one, (+)-3,4-*seco*lanosta-4(28),8,24-trien-3,21-dioic acid and (−)-3β-hydroxy-16-oxolanosta-7,9(11),24-trien-21-oic acid. In addition, the known compounds (+)-pinicolic acid A, (+)-trametenolic acid B, (+)-21-hydroxylanosta-8,24-dien-3-one, 21-hydroxylanosta-7,9(11),24-trien-3-one, (+)-lanosta-7,9(11),24-trien-3β,21-diol and (+)-3α-acetoxylanosta-8,24-dien-21-oic acid were isolated from the crust.

Keywords: *Fomitopsis pinicola*; Polyporaceae; Basidiomycete; Crust; Steroids; Lanostane derivatives

1. Introduction

Since the early 1950s ten steroidal compounds were isolated from the wood-rotting basidiomycete *Fomitopsis pinicola* (Swartz ex Fr.) Karst Polyporaceae (syn. *Polyporus pinicola* Fr., *Fomes pinicola*) (Beereboom, Fazakereley & Halsall, 1957; Guider, Halsall & Jones, 1954; Halsall & Sayer, 1959; Keller, Maillard & Hostettmann, 1996; Sheth, Catalfomo & Sciuchetti, 1967; Yokoyama, Natori & Aoshima, 1975). The fact that at least five compounds show antimicrobial activity against *Bacillus subtilis* (Keller et al., 1996) indicates the importance of the progress in the isolation of unknown compounds from this fungus. *F. pinicola* is a brown-rot fungus that grows on coniferous and broad-leaved trees (in Germany mainly on spruces). Recently some cerebrosides (Striegler & Haslinger, 1996) have been reported to be constituents of this fungus. In the present paper, the isolation and the structure elucidation of twelve lanostane-type steroids are described. Furthermore it is shown that the steroids are concentrated in the crust of the fruiting bodies of *F. pinicola*.

2. Results and discussion

The crust of fresh fruiting bodies of *F. pinicola* was separated from the rest and both materials were crushed in liquid nitrogen. The resulting powder was in both cases extracted first twice with *n*-hexane and then with methanol. The methanol extracts were extracted again with dichloromethane to remove nucleic acids and proteins; 90% of the methanol extract of the crust but only 14% of the methanol extract of the rest of the fruiting bodies was soluble in dichloromethane. TLC assays and the following investigations indicated that all steroids were dissolved in dichloromethane. This led to the conclusion that the steroids are concentrated in the crust of *F. pinicola*. The *n*-hexane and the described dichloromethane extract were both submitted to column chromatography on silica gel and Sephadex LH-20 using chloroform-methanol and petrol-ethyl acetate step-gradients.

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Six compounds were identified as (+)-pinicolic acid A (**1**) (Ahmad, Hussain & Razaq, 1976; Guider et al., 1954; Keller et al., 1996; Sheth et al., 1967), (+)-trametenolic acid B (**2**) (Ahmad et al., 1976; Halsall et al., 1959; Keller et al., 1996; Sheth et al., 1967; Tai, Akahori & Shingu, 1993), 21-hydroxylanosta-8,24-dien-3-one (**3**) (Keller et al., 1996), 21-hydroxylanosta-7,9(11),24-trien-3-one (**4**) (Halsall et al., 1959; Sheth et al., 1967), (+)-lanosta-7,9(11),24-trien-3β,21-diol (**5**) (Halsall et al., 1959; Sheth et al., 1967) and (+)-3α-acetoxylanosta-8,24-dien-21-oic acid (**6**) (Keller et al., 1996) by comparison of the obtained mass spectra and NMR data with those given in the literature. Since 400 and 500 MHz NMR spectrometers were used in this work, almost each signal, especially in the ^{13}C spectra was fully resolved and the signal assignment of earlier publications could be completed. For compounds **4** and **5** the ^{13}C-NMR data are reported here for the first time.

In the mass spectrum compound **7** gave a signal at *m/z* 480.3615 (HR-MS) indicating a chemical composition of $C_{32}H_{48}O_3$. The ^{13}C- and ^{1}H-NMR showed good correspondence with the data of compound **4** with three additional carbon atoms at δ 169.02 (*s*), 167.47 (*s*) and 41.04 (*t*), an additional singlet at δ 3.41 in the ^{1}H-NMR spectrum and significantly different chemical shifts for the protons at C-21 (δ 4.30 and 4.07). The singlet at δ 3.41 represents a CH_2-group as indicated by the ^{1}H–^{1}H- and ^{1}H–^{13}C-COSY NMR spectra. The HMBC NMR spectrum showed long-range coupling between the protons at δ 3.41 and both mentioned quaternary carboxylic carbons. This indicated the existence of a carboxyacetoxy group attached to C-21. Furthermore, a long-range coupling between the carbon at δ 167.47 and the protons at C-21 was observed. All other carbon shifts could be assigned and were in accordance with the predicted structure. Thus, compound **7** proved to be (+)-21-(carboxyacetoxy)lanosta-7,9(11),24-trien-3-one for which we propose the name fomitopsic acid B. The signal in the EIMS at *m/z* 480 must result from easy thermal decarboxylation in position 2′.

The mass spectrum of compound **8** indicates a molecular ion of *m/z* 422. The ^{13}C-NMR contains 30 carbon signals and shows that the only functional group is a keto function (δ 216.88) and that there are six olefinic carbons present (δ 117.37, 119.85, 125.19, 131.04, 142.98, 144.52). The chemical shifts of these carbons and the shifts of the three olefinic protons are typical for a lanosta-7,9(11),24-triene partial structure. In addition, there are one tertiary and seven quaternary methyl groups in this molecule. Comparison of these data with the known shifts of lanosta-7,9(11),24-trien-3β-ol (agnosterol) (Emmons, Wilson & Schroepfer, 1989) leads to the conclusion that this compound is lanosta-7,9(11),24-trien-3-one or agnosterone.

The ^{13}C-NMR spectrum of compound **9** contains 30 carbon signals and includes signals of two secondary (δ 78.89 and 74.66) and one primary hydroxyl group (δ 62.45) as well as six olefinic carbons (δ 115.77, 121.61, 124.61, 131.73, 140.76 and 144.35). This and the olefinic protons in the ^{1}H-NMR spectrum again indicate the existence of a lanosta-7,9(11),24-triene skeleton. The HMBC spectrum showed long-range coupling between the proton at one hydroxymethylene group and the two carbon atoms in positions 28 and 29. Therefore one secondary hydroxyl group had to be in position 3. The other hydroxymethylene proton only showed long-range coupling with the carbons in position 30, 14 and 8. In addition, the NMR data of compound **9** are in good correspondence with the data of lanosta-7,9(11),24-trien-3β,15α-diol (polycarpol) which was previously isolated from the bark of *Melodorum fructicosum* (Jung, Pummangura, Chaichantipyuth, Patarapanich & McLaughlin, 1990). Thus, carbon 15 is the only possible position for this hydroxyl group and compound **9** corresponds to (+)-lanosta-7,9(11),24-trien-3β,15α,21-triol for which we propose the name pinicolol B.

The ^{13}C-NMR of compound **10** shows two keto functions (δ 217.37 and 215.60) and one carboxylic acid moiety (δ 176.85). The four olefinic carbons (δ 124.50, 131.42, 132.46 and 134.51) and the shape of the signal of the olefinic proton at C-24 is characteristic for a lanosta-8,24-diene structure. Comparison of the carbon shifts of rings A and B with those of compound **1** showed good correspondence, so that one keto function had to be in position 3. The shifts of the remaining carbons and the ^{1}H–^{13}C long-range couplings indicated that the second keto group had to be in position 16 and the acid function in position 21. Accordingly, compound **10** was (−)-3,16-dioxolanosta-8,24-dien-21-oic acid for which we propose the name pinicolic acid B.

Compound **11** showed six olefinic carbons (δ 113.70, 123.58, 129.44, 132.32, 138.98 and 147.46) in the ^{13}C-NMR spectrum and three olefinic protons in the ^{1}H-NMR spectrum [δ 4.69 (*s*), 4.90 (*s*) and 5.08 (*t*)]. This indicated the existence of a 8,24-diene with an additional exocyclic methylene double bond (C-28). From the 2D NMR spectra the presence of an isopropenyl group attached to carbon 5 could be derived. Careful analysis of all 2D spectra (^{1}H–^{1}H-COSY, HMQC, HMBC) lead to the conclusion that compound **11** was (+)-3,4-*seco*lanosta-4(28),8,24-trien-3,21-dioic acid for which we propose the name pinicolic acid C. In addition, comparison of the obtained spectral data with the data of 3,4-*seco*tirucalla-4(28),7,24-trien-3,21-dioic acid (delevoyin A) which was previously isolated from the bark of the Zambian tree *Entandrophragma delevoyi* (Mulholland, Osborne, Roberts & Taylor, 1994) showed good correspondence.

Furthermore lanostane derivatives of the 3,4-*seco*-type have previously been isolated and characterized from the fungus *Poria cocos* (Tai et al., 1993).

Compound **12** showed 30 signals in the ^{13}C-NMR spectrum. This and the ^{1}H-NMR indicated the existence of a lanosta-7,9(11),24-triene partial structure. In addition, from the HMBC spectrum the presence of a hydroxy group in position 3 in this molecule could be proved as the proton on the same carbon showed long-range coupling with the methyl carbons 28 and 29. Furthermore, a keto and an acid moiety could be detected and by comparison of the data with those of compound **10** it was concluded that the keto function, like in compound **10**, had to be in position 16 and the acid moiety in position 21. Thus, compound **12** had to be (−)-3β-hydroxy-16-oxolanosta-7,9(11),24-trien-21-oic acid for which we propose the name pinicolic acid D.

3. Experimental

3.1. Plant material

Fruiting bodies of *F. pinicola* were collected in September 1998 in Hamfelde near Hamburg and near Salzgitter (both in Northern Germany) (Gerhardt, 1995).

3.2. Extraction

The crust was separated from the fruiting bodies and both fractions (crust: 150 g fr. wt, rest: ~1.5 kg fr. wt) were crushed in liquid nitrogen. In both cases the resulting powder was extracted twice with *n*-hexane (crust: 2 × 11, rest: 2 × 21, 6 days each) and then with methanol (crust: 11, rest: 21, 6 days each). The methanol extracts (crust: 5.3 g of a dark red solid, rest: 8.6 g of an orange powder) were extracted with 250 ml of dichloromethane (each 1 day under stirring).

3.3. Isolation

The hexane extract of the crust (4.5 g of a dark orange oil) was submitted to flash column chromatography on silica gel with a step gradient of chloroform-methanol (from 49:1 to 1:1, vol/vol) and was rechromatographed several times on silica gel using step-gradients of petrol–ethyl acetate (from 39:1 to 0:1, vol/

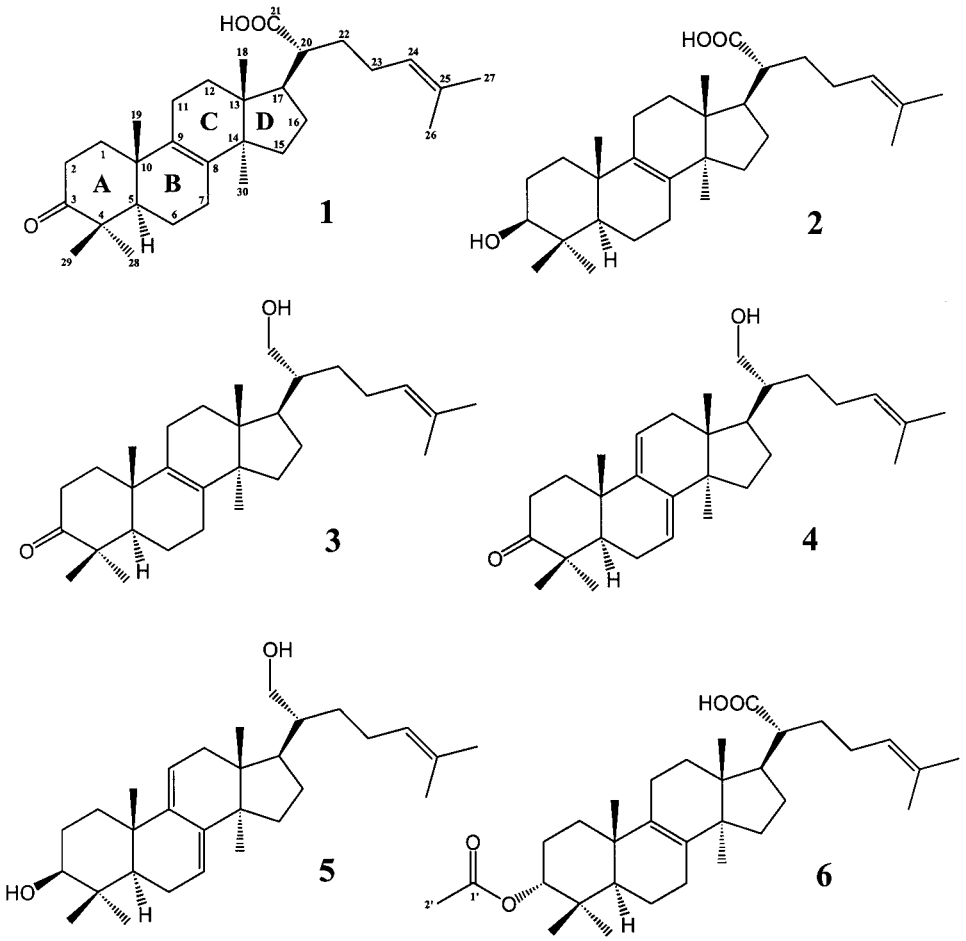


Table 1
^{13}C shifts for all identified compounds (pyr, pyridine-d_5)

C	**1** *(pyr)*	**2** (pyr)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** ($CDCl_3$)	**8** ($CDCl_3$)	**9** ($CDCl_3$)	**10** (pyr)	**11** ($CDCl_3$)	**12** (pyr)
1	35.78 *t*	35.72 *t*	36.02 *t*	36.56 *t*	35.73 *t*	30.39 *t*	36.61 *t*	36.66 *t*	35.75 *t*	35.49 *t*	31.48 *t*	35.69 *t*
2	34.50 *t*	28.31 *t*	34.53 *t*	34.76 *t*	27.79 *t*	23.42 *t*	34.84 *t*	34.89 *t*	27.78 *t*	34.26 *t*	29.34 *t*	28.19 *t*
3	215.78 *s*	77.60 *d*	217.73 *s*	216.66 *s*	78.98 *d*	77.93 *d*	216.90 *s*	216.88 *s*	78.89 *d*	215.60 *s*	181.38 *s*	77.56 *d*
4	47.33 *s*	39.14 *s*	47.32 *s*	47.41 *s*	38.68 *s*	36.80 *s*	47.49 *s*	47.52 *s*	38.70 *s*	47.00 *s*	147.46 *s*	38.95 *s*
5	50.90 *d*	50.50 *d*	51.16 *d*	50.63 *d*	49.11 *d*	45.39 *d*	50.69 *d*	50.75 *d*	48.95 *d*	50.78 *d*	47.25 *d*	49.32 *d*
6	19.22 *t*	18.31 *t*	19.39 *t*	23.63 *t*	23.04 *t*	17.99 *t*	23.67 *t*	23.70 *t*	22.98 *t*	19.12 *t*	24.00 *t*	23.13 *t*
7	26.14 *t*	26.42 *t*	26.29 *t*	120.06 *d*	120.53 *d*	26.00 *t*	120.20 *d*	119.85 *d*	121.61 *d*	26.56 *t*	26.62 *t*	122.45 *d*
8	133.25 *s*	133.89 *s*	133.10 *s*	142.73 *s*	142.52 *s*	134.54 *s*	142.59 *s*	142.98 *s*	140.76 *s*	132.46 *s*	138.98 *s*	139.65 *s*
9	134.77 *s*	134.77 *s*	135.23 *s*	144.61 *s*	146.14 *s*	133.92 *s*	144.54 *s*	144.52 *s*	144.35 *s*	134.51 *s*	129.44 *s*	146.38 *s*
10	36.70 *s*	37.00 *s*	36.87 *s*	37.17 *s*	37.41 *s*	36.93 *s*	37.22 *s*	37.23 *s*	37.46 *s*	36.83 *s*	40.33 *s*	37.59 *s*
11	20.93 *t*	20.86 *t*	21.04 *t*	117.00 *d*	115.96 *d*	20.88 *t*	117.04 *d*	117.37 *d*	115.77 *d*	20.05 *t*	21.67 *t*	116.05 *d*
12	28.93 *t*	29.00 *t*	30.42 *t*	37.12 *t*	37.21 *t*	28.99 *t*	36.92 *t*	37.86 *t*	37.88 *t*	28.67 *t*	28.49 *t*	35.10 *t*
13	44.46 *s*	44.50 *s*	44.19 *s*	43.48 *s*	43.55 *s*	44.29 *s*	43.48 *s*	43.78 *s*	44.16 *s*	43.47 *s*	44.10 *s*	42.64 *s*
14	49.52 *s*	49.45 *s*	49.95 *s*	50.35 *s*	50.43 *s*	49.60 *s*	50.38 *s*	50.34 *s*	52.06 *s*	44.11 *s*	50.32 *s*	44.13 *s*
15	30.52 *t*	30.48 *t*	30.81 *t*	31.36 *t*	31.45 *t*	30.92 *t*	31.38 *t*	31.62 *t*	74.66 *d*	46.35 *t*	30.62 *t*	46.45 *t*
16	27.06 *t*	27.10 *t*	27.64 *t*	27.43 *t*	27.53 *t*	27.06 *t*	27.52 *t*	27.91 *t*	39.63 *t*	217.37 *s*	25.99 *t*	216.81 *s*
17	46.96 *d*	47.34 *d*	44.38 *d*	44.87 *d*	44.92 *d*	47.20 *d*	45.19 *d*	50.95 *d*	42.98 *d*	57.46 *d*	47.47 *d*	57.32 *d*
18	16.04 *q*	15.99 *q*	16.11 *q*	15.97 *q*	15.80 *q*	16.01 *q*	15.94 *q*	15.73 *q*	16.30 *q*	16.86 *q*	15.97 *q*	16.24 *q*
19	18.28 *q*	19.01 *q*	18.64 *q*	21.99 *q*	22.75 *q*	18.87 *q*	22.03 *q*	22.09 *q*	22.87 *q*	18.15 *q*	22.44 *q*	22.54 *q*
20	48.64 *d*	48.63 *d*	42.81 *d*	42.66 *d*	42.73 *d*	47.68 *d*	39.67 *d*	36.07 *d*	42.36 *d*	44.85 *d*	47.07 *d*	44.64 *d*
21	178.22 *s*	178.24 *s*	62.47 *t*	62.43 *t*	62.59 *t*	182.93 *s*	65.88 *t*	18.46 *q*	62.45 *t*	176.85 *s*	184.04 *s*	176.76 *s*
22	32.90 *t*	32.91 *t*	29.74 *t*	29.71 *t*	29.77 *t*	32.53 *t*	30.15 *t*	36.30 *t*	29.76 *t*	31.57 *t*	32.15 *t*	31.58 *t*
23	26.35 *t*	26.34 *t*	24.99 *t*	24.93 *t*	25.00 *t*	25.94 *t*	24.72 *t*	24.97 *t*	25.01 *t*	26.44 *t*	25.92 *t*	26.40 *t*
24	124.49 *d*	124.50 *d*	124.85 *d*	124.82 *d*	124.85 *d*	123.62 *d*	124.41 *d*	125.19 *d*	124.61 *d*	124.50 *d*	123.58 *d*	124.45 *d*
25	131.36 *s*	131.32 *s*	131.31 *s*	131.36 *s*	131.51 *s*	132.27 *s*	131.63 *s*	131.04 *s*	131.73 *s*	131.42 *s*	132.32 *s*	131.41 *s*
26	17.35 *q*	17.33 *q*	17.67 *q*	17.67 *q*	17.74 *q*	17.67 *q*	17.68 *q*	17.67 *q*	17.77 *q*	17.40 *q*	17.68 *q*	17.36 *q*
27	25.40 *q*	25.38 *q*	25.67 *q*	25.67 *q*	25.66 *q*	25.71 *q*	25.71 *q*	25.76 *q*	25.74 *q*	25.47 *q*	25.70 *q*	25.53 *q*
28	20.83 *q*	15.95 *q*	21.24 *q*	22.40 *q*	28.16 *q*	21.89 *q*	22.46 *q*	22.49 *q*	15.84 *q*	20.99 *q*	113.70 *t*	28.43 *q*
29	26.00 *q*	28.24 *q*	26.17 *q*	25.32 *q*	15.98 *q*	27.59 *q*	25.32 *q*	25.38 *q*	28.17 *q*	25.96 *q*	22.83 *q*	16.49 *q*
30	24.11 *q*	24.11 *q*	24.39 *q*	25.48 *q*	25.72 *q*	24.58 *q*	25.45 *q*	25.47 *q*	17.25 *q*	24.59 *q*	25.40 *q*	25.42 *q*
1′						170.84 *s*	167.47 *s*					
2′						21.36 *q*	41.04 *t*					
3′							169.02 *s*					

vol) and on Sephadex LH-20 (chloroform–methanol 1:1, vol/vol). The following yields were obtained: compound **1** (500 mg), **2** (80 mg), **3** (30 mg), **6** (20 mg), **7** (20 mg) and **8** (11 mg). The dichloromethane extract (4.8 g of a dark red solid) was also submitted to flash column chromatography on silica gel with a step gradient of chloroform–methanol (from 49:1 to 1:1, vol/vol) and was rechromatographed several times using step-gradients of petrol–ethyl acetate (from 39:1 to 1:1, vol/vol). This yielded compound **1** (90 mg), **2** (40 mg), **3**, **4** (see the following), **5** (15 mg), **9** (7 mg), **10** (28 mg), **11** (4 mg) and **12** (8 mg). It should be noted, that compound **4** was only obtained as a 1:1 mixture with compound **3**. But due to the excellent resolution of the ^{13}C-NMR spectrum and with the knowledge of the chemical shift values of compound **3** (Keller et al., 1996) all carbon atoms could be assigned.

3.4. TLC

Silica gel $60F_{254}$ (Merck); eluent: chloroform–2-propanol 9:1, vol/vol and petrol–ethyl acetate, 1:1, vol/vol; detection with sulphuric acid (10% in ethanol).

3.5. Column chromatography

Various column sizes with silica gel 0.063–0.040 mm (Macherey–Nagel).

3.6. NMR spectroscopy

Bruker WM 400 at 400.16 (^1H) and 100.61 MHz (^{13}C) and Bruker DRX 500 at 500.13 (^1H) and 125.76 MHz (^{13}C). All NMR shifts are relative to TMS.

3.7. Mp

Apotech 'Schmelzpunkt-Bestimmer' (melting points uncorrected).

3.8. Polarimetry

Perkin–Elmer 241, $l = 1$ dm, $\lambda = 589$ nm.

3.9. EIMS

VG Analytical 70-250 S, 70 eV, exact mass measurement at resolution 10.000, direct inlet sample introduction.

3.10. Compound **1**, pinicolic acid A, 3-oxolanosta-8,24-dien-21-oic acid

White crystals from methanol–chloroform. Elemental analysis: Found: C 79.16%, H: 10.37%, O: 10.47% (calc. for $C_{30}H_{46}O_3$: C: 79.25%, H: 10.20%, O: 10.56%). Mp 197–201° (lit. (Guider et al.,1954) 197–202°, (Keller et al., 1996) 196–200°). $[\alpha]^{20}$ +63° ($CHCl_3$; *c* 0.66) (lit. (Guider et al.,1954) +68°, 21°C, $CHCl_3$; *c* 0.8, (Keller et al., 1996) +65°, 21°C, $CHCl_3$; *c* 1.0). ^{1}H-NMR (400.13 MHz, pyridine-d_5): δ 0.98 (3H, *s*, H-30), 0.99 (3H, *s*, H-19), 1.04 (3H, *s*, H-28), 1.05 (3H, *s*, H-18), 1.14 (3H, *s*, H-29), 1.62 (3H, *s*, H-26), 1.67 (3H, *s*, H-27), 2.64 (1H, *dt, J* = 11.1, 3.0 Hz, H-20), 5.33 (1H, *t*, H-24). ^{13}C-NMR (100.61 MHz, pyridine-d_5): see Table 1. EIMS *m/z* (rel. int): 454 $[M]^+$ (57), 439 $[M–CH_3]^+$ (100), 421 $[M–CH_3–H_2O]^+$ (33), 393 (43), 297 (48), 95 (38), 69 (43).

3.11. Compound **2**, trametenolic acid B, 3β-hydroxylanosta-8,24-dien-21-oic acid

Colorless needles from petrol–ethyl acetate, mp 255–261° (lit. (Ahmad et al., 1976) 252–258°, (Keller et al., 1996) 258–260°). $[\alpha]^{20}$ +39.7° (MeOH; *c* 0.23) (lit. (Keller et al., 1996) +40°, 21°C, MeOH; *c* 0.3). ^{1}H-NMR (400.13 MHz, pyridine-d_5): δ 1.01 (3H, *s*, H-18), 1.01 (3H, *s*, H-28), 1.07 (3H, *s*, H-19), 1.07 (3H, *s*, H-30), 1.24 (3H, *s*, H-29), 1.62 (3H, *s*, H-26), 1.66 (3H, *s*, H-27), 2.65 (1H, *dt, J* = 10.7, 3.6 Hz, H-20), 3.43 (1H, *dd, J* = 8.7, 7.1 Hz, H-3_a), 5.32 (1H, *t, J* = 7.1 Hz, H-24). ^{13}C-NMR (100.61 MHz, pyridine-d_5): see Table 1. EIMS *m/z* (rel. int): 456 $[M]^+$ (20), 441 $[M–CH_3]^+$ (31), 423 $[M–CH_3–H_2O]^+$ (61), 28 (26), 187 (42), 95 (100), 69 (96)

3.12. Compound **3**, 21-hydroxylanosta-8,24-dien-3-one

White powder, mp 105–110° (lit. (Keller et al., 1996) 92–96°, (Guider et al., 1954) 114–119°). $[\alpha]^{20}$ +66.2° ($CHCl_3$; *c* 0.19) (lit. (Keller et al., 1996) +59°, $CHCl_3$; *c* 1.0). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.73 (3H, *s*, H-18), 0.89 (3H, *s*, H-30), 1.05 (3H, *s*, H-29), 1.08 (3H, *s*, H-28), 1.10 (3H, *s*, H-19), 1.59 (3H, *s*, H-26), 1.67 (3H, *s*, H-27), 2.39 (1H, *ddd, J* = 15.8, 6.6, 3.6 Hz), 2.56 (1H, *ddd, J* = 15.8, 11.2, 7.2 Hz), 2.75 (1H, *dt, J* = 14.7, 6.1 Hz, H-20), 3.66 (1H, *dd, J* = 11.2, 4.6 Hz, H-21_a), 3.72 (1H, *dd, J* = 11.2, 3.0 Hz, H-21_b), 5.10 (1H, *t, J* = 6.6 Hz, H-24). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 440 $[M]^+$ (8), 425 $[M–CH_3]^+$ (11), 407 $[M–CH_3–H_2O]^+$ (14), 271 (14), 245 (15), 109 (100), 69 (63)

3.13. Compound **4**, 21-hydroxylanosta-7,9(11),24-trien-3-one

Obtained only as a mixture with compound **3**. ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1.

3.14. Compound **5**, lanosta-7,9(11),24-trien-3β,21-diol

White needles from petrol–ethyl acetate, mp 192–194° (lit. (Halsall et al., 1959) 194–197°). $[\alpha]^{20}$ +66.9° ($CHCl_3$; *c* 0.53) (lit. (Halsall et al., 1959) +72°, $CHCl_3$; *c* 1.06). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.59 (3H, *s*, H-18), 0.90 (3H, *s*, H-28), 0.99 (3H, *s*, H-19), 1.01 (3H, *s*, H-29), 1.26 (3H, *s*, H-30), 1.62 (3H, *s*, H-26), 1.69 (3H, *s*, H-27), 3.26 (1H, *dd, J* = 11.2, 4.6 Hz, H-3_a), 3.65 (1H, *dd, J* = 11.7, 5.1 Hz, H-21_a), 3.74 (1H, *dd, J* = 11.2, 2.6 Hz, H-21_b), 5.13 (1H, *t, J* = 6.6 Hz, H-24), 5.32 (1H, *d, J* = 6.1 Hz, H-11), 5.49 (1H, *d, J* = 5.1 Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 440 $[M]^+$ (28), 409 $[M–CH_2OH]^+$ (6), 311 (11), 271 (15), 109 (75), 69 (100).

3.15. Compound **6**, 3α-acetoxylanosta-8,24-dien-21-oic acid

White crystals from petrol–ethyl acetate. $[\alpha]^{20}$ +5.4° ($CHCl_3$; *c* 1.31) (lit. (Keller et al., 1996) +14.2°, $CHCl_3$; *c* 1.00). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.77 (3H, *s*, H-18), 0.86 (3H, *s*, H-28), 0.92 (3H, *s*, H-29), 0.94 (3H, *s*, H-30), 0.98 (3H, *s*, H-19), 1.59 (3H, *s*, H-26), 1.68 (3H, *s*, H-27), 2.07 (3H, *s*, H-2′), 4.67 (1H, *br s*, H-3_b), 5.10 (1H, *t, J* = 4.1 Hz, H-24). ^{13}C-NMR (125.76 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int.): 498 $[M]^+$ (19), 483 $[M–CH_3]^+$ (10), 437 $[M–H_2O–MeCO]^+$ (18), 424 (35), 423 $[M–CH_3–AcOH]^+$ (100), 281 (27), 187 (42), 95 (56), 69 (69), 40 (72).

3.16. Compound **7**, fomitopsic acid B, 21-(carboxyacetoxy)lanosta-7,9(11),24-trien-3-one

Amorphous white powder. $[\alpha]^{20}$ +36.2° ($CHCl_3$; *c* 1.36). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.61 (3H, *s*, H-18), 0.88 (3H, *s*, H-30), 1.08 (3H, *s*, H-29), 1.12 (3H, *s*, H-28), 1.19 (3H, *s*, H-19), 1.59 (3H, *s*, H-26), 1.68 (3H, *s*, H-27), 2.77 (1H, *dt, J* = 14.3, 5.6 Hz, H-2_a), 3.41 (2H, *s*, H-2′), 4.07 (1H, *dd, J* = 11.2, 5.6 Hz, H-21_a), 4.33 (1H, *dd, J* = 11.1, 2.6 Hz, H-21_b), 5.08 (1H, *t, J* = 6.7 Hz, H-24), 5.37 (1H, *d, J* = 5.6 Hz, H-

11), 5.51 (1H, *d*, $J = 6.1$ Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 480 $[M–CO_2]^+$ (42), 407 $[M–CO_2–C_3H_5O_2]^+$ (34), 309 (52), 269 (38), 109 (50), 95 (48), 69 (100). HR-MS *m/z*: 480.3615 $[M–CO_2]^+$ ($C_{32}H_{48}O_3$ requires 480.3603).

3.17. Compound **8**, agnosterone, lanosta-7,9(11),24-trien-3-one

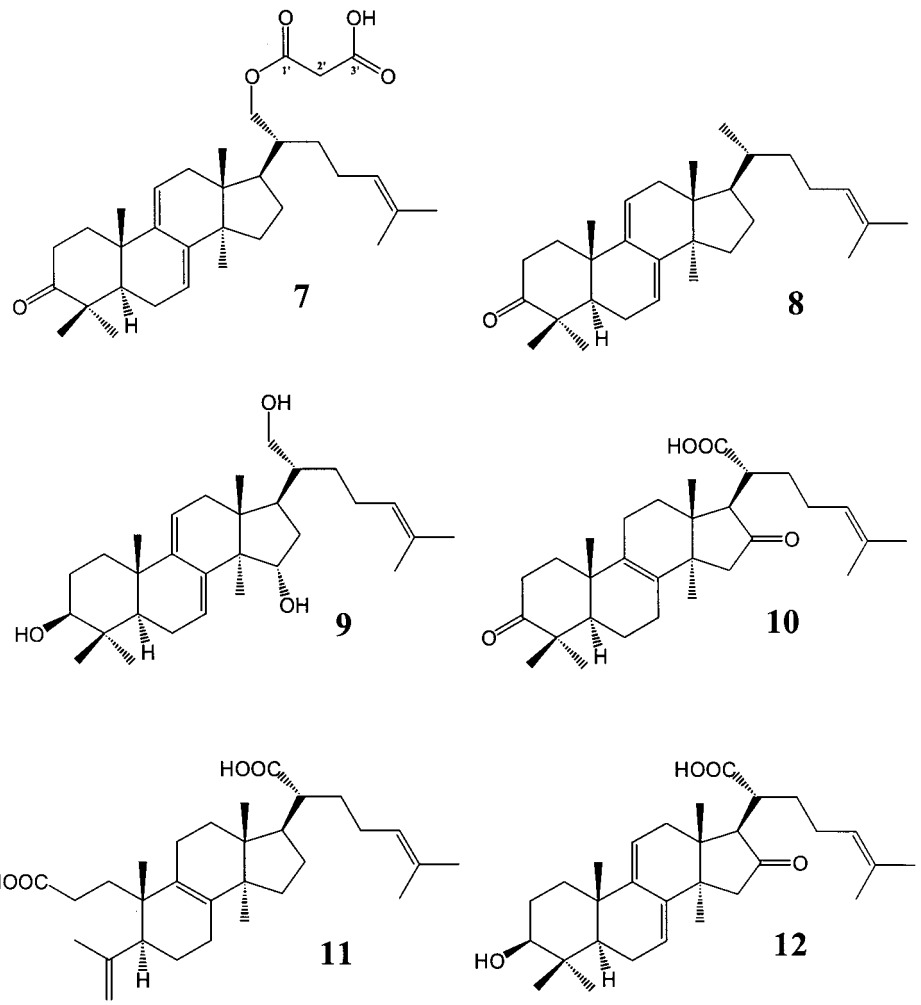
Amorphous white powder. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.60 (3H, *s*, H-18), 0.88 (3H, *s*, H-30), 0.92 (3H, *d*, $J = 6.6$ Hz, H-21), 1.10 (3H, *s*, H-29), 1.14 (3H, *s*, H-28), 1.21 (3H, *s*, H-19), 1.62 (3H, *s*, H-26), 1.70 (3H, *s*, H-27), 2.78 (1H, *dt*, $J = 14.3$, 5.6 Hz, H-2_a), 5.11 (1H, *t*, $J = 7.1$ Hz, H-24), 5.40 (1H, *d*, $J = 5.6$ Hz, H-11), 5.51 (1H, *d*, $J = 6.7$ Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 422 $[M]^+$ (28), 407 $[M–CH_3]^+$ (9), 309 (15), 269 (17), 257 (15), 69 (100).

3.18. Compound **9**, pinicolol B, lanosta-7,9(11),24-trien-3β,15α,21-triol

Amorphous powder. $[\alpha]^{20}$ +47.1° ($CHCl_3$; *c* 0.09). ^{1}H-NMR (500.13 MHz, $CDCl_3$): δ 0.64 (3H, *s*, H-18), 0.89 (3H, *s*, H-28), 0.96 (3H, *s*, H-30), 0.99 (3H, *s*, H-19), 1.01 (3H, *s*, H-29), 1.62 (3H, *s*, H-26), 1.69 (3H, *s*, H-27), 3.25 (1H, *dd*, $J = 11.4$, 4.4 Hz, H-3_a), 3.62 (1H, *dd*, $J = 11.4$, 3.5 Hz, H-21_a), 3.72 (1H, *dd*, $J = 11.3$, 2.2 Hz, H-21_b), 4.29 (1H, *dd*, $J = 9.1$, 6.0 Hz, H-15), 5.11 (1H, *t*, $J = 6.9$ Hz, H-24), 5.31 (1H, *d*, $J = 6.0$ Hz, H-11), 5.87 (1H, *d*, $J = 6.3$ Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 456 $[M]^+$ (18), 423 $[M–CH_2OH–H_2]^+$ (11), 327 (14), 185 (23), 119 (35), 109 (63), 69 (100).

3.19. Compound **10**, pinicolic acid B, 3,16-dioxolanosta-8,24-dien-21-oic acid

White crystals from methanol, mp 202–204°. $[\alpha]^{20}$



−34.6° ($CHCl_3$; *c* 0.15). ^{1}H-NMR (400.13 MHz, pyridine-d_5): δ 0.99 (3H, *s*, H-19), 1.04 (3H, *s*, H-28), 1.11 (3H, *s*, H-30), 1.12 (3H, *s*, H-18), 1.14 (3H, *s*, H-29), 1.62 (3H, *s*, H-26), 1.64 (3H, *s*, H-27), 2.95 (4H, *m*, H-17, H-20, H-22), 5.36 (1H, *t*, J = 7.1 Hz, H-24). ^{13}C-NMR (100.61 MHz, pyridine-d_5): see Table 1. EIMS *m/z* (rel. int): 468 $[M]^+$ (2), 453 $[M–CH_3]^+$ (2), 451 (4), 407 (26), 339 (36), 272 (22), 69 (100).

3.20. Compound **11**, pinicolic acid C, 3,4-secolanosta-4(28),8,24-trien-3,21-dioic acid

White powder. $[\alpha]^{20}$ +27.9° ($CHCl_3$; *c* 0.66). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.81 (3H, *s*, H-18), 0.97 (3H, *s*, H-30), 0.99 (3H, *s*, H-19), 1.59 (3H, *s*, H-26), 1.68 (3H, *s*, H-27), 1.77 (3H, *s*, H-29), 4.69 (1H, *br s*, H-28(*Z*)), 4.90 (1H, *br s*, H-28(*E*)), 5.08 (1H, *t*, J = 7.1 Hz, H-24). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EIMS *m/z* (rel. int): 470 $[M]^+$ (46), 397 $[M–CH_2CH_2COOH]^+$ (78), 255 (30), 161 (83), 69 (100).

3.21. Compound **12**, pinicolic acid D, 3β-hydroxy-16-oxolanosta-7,9(11),24-trien-21-oic acid

White crystals from methanol, mp 226–228°. $[\alpha]^{20}$ −67.5° (CH_2Cl_2; *c* 0.06). ^{1}H-NMR (400.13 MHz, pyridine-d_5): δ 1.03 (3H, *s*, H-19), 1.05 (3H, *s*, H-29), 1.12 (3H, *s*, H-18), 1.15 (3H, *s*, H-30), 1.22 (3H, *s*, H-28), 1.61 (3H, *s*, H-26), 1.63 (3H, *s*, H-27), 3.01 (2H, *m*, H-17, H-20), 3.45 (1H, *dd*, J = 9.1, 6.6 Hz, H-3$_a$), 5.37 (1H, *t*, J = 7.1 Hz, H-24), 5.41 (1H, *d*, J = 6.1 Hz, H-11), 5.51 (1H, *d*, J = 5.6 Hz, H-7). ^{13}C-NMR (100.61 MHz, pyridine-d_5): see Table 1. EIMS *m/z* (rel. int): 468 $[M]^+$ (2), 453 $[M–CH_3]^+$ (9), 435 $[M–CH_3–H_2O]^+$ (16), 407 (24), 339 (29), 132 (43), 69 (100).

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