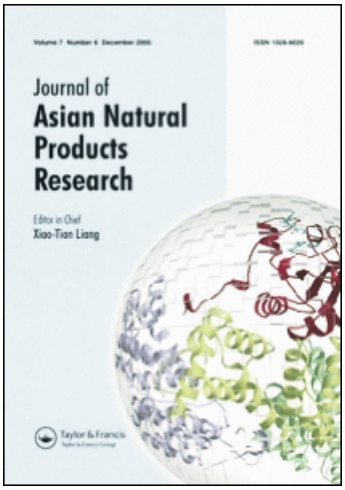

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Two new lanostane triterpenoids from *Poria cocos*

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Two new lanostane triterpenoids, 29-hydroxypolyporenic acid C (**4**) and 25-hydroxypachymic acid (**5**), together with three known compounds, ergosta-7,22-dien-3β-ol (**1**), polyporenic acid C (**2**) and pachymic acid (**3**), were isolated from the 95% ethanolic extract of the sclerotium of *Poria cocos* (Schw.) Wolf. Their structures were determined by extensive spectroscopic analyses, including IR, UV, ESITOF–MS, HRESI–MS, 1D and 2D NMR data (^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC).

Keywords: *Poria cocos*; lanostane triterpenoids; 29-hydroxypolyporenic acid C; 25-hydroxypachymic acid

1. Introduction

Dried sclerotium of *Poria cocos* (Schw.) Wolf (Polyporaceae) is combined in many traditional Chinese medicine prescriptions or solely used as a diuretic, sedative and tonic.[1] It has been reported that the sclerotium of *P. cocos* contains various triterpenoids of lanostane type,[2–8] some of which possess biological activities such as anti-inflammatory,[9] anti-emetic[10] and cytotoxic activity.[8] This biological importance motivated us to further investigate the chemical constituents of the sclerotium of *P. cocos*. Present studies led to the isolation of two new lanostane triterpenoids named as 29-hydroxypolyporenic acid C (**4**) and 25-hydroxypachymic acid (**5**), along with three known compounds, ergosta-7,22-dien-3β-ol (**1**),[11] polyporenic acid C (**2**)[12] and pachymic acid (**3**)[6] by repeated column chromatography.

2. Results and discussion

Five compounds were obtained from the sclerotium of *P. cocos*. Compounds **2–4** all reacted positively to Liebermann–Burchard test for triterpenes[13] and bromocresol green test for free carboxylic acids.[14] Three known compounds, ergosta-7,22-dien-3β-ol (**1**),[11] polyporenic acid C (**2**)[12] and pachymic acid (**3**),[3,6] were identified by comparing their spectral data with those reported in the literature, and their structures are shown in Figure 1.

Compound **4** was obtained as white amorphous powder with $[\alpha]_D^{20} + 27.4$ (*c* 0.0732, MeOH). The molecular formula was inferred as $C_{31}H_{46}O_5$ from HRESI–MS, HSQC and ^{13}C NMR (Table 1) spectral data. The IR spectrum showed an absorption band at 1642 cm^{-1} and the UV spectrum showed an absorption maximum at 243 nm (log ε, 2.55), suggesting the presence of a $\Delta^{7,9(11)}$ diene moiety in **4**.[5,15] The strong IR bands at 3424 and 1707 cm^{-1} were indications of the carboxyl group in **4**. The ^{1}H NMR spectrum (Table 1) of **4** showed signals of two secondary methyl groups at δ_H 0.96 (3H, d, $J = 6.5$ Hz) and 0.97 (3H, d, $J = 6.5$ Hz), four tertiary methyl groups at δ_H 1.04, 1.06, 1.12, 1.40 (3H each, s each), an oxygen-bearing methine at δ_H 4.51 (1H, dd, $J = 6.0, 9.0$ Hz) and a hydroxymethyl at δ_H 3.56 (1H, d, $J = 11.0$ Hz) and 4.15 (1H, d, $J = 11.0$ Hz), an exocyclic methylene group at δ_H 4.82 (1H, s) and 4.93 (1H, s), and two olefinic methines at δ_H 5.42 (1H, d, $J = 5.4$ Hz) and 5.57 (1H, br s). Plus, the ^{13}C NMR and HSQC spectra of **4** confirmed the presences of a ketone carbon at δ_C 215.5 (C-3), a free carboxylic acid carbon at δ_C 178.8 (C-21), two exocyclic methylene carbons at δ_C 106.9 (C-31) and 156.1 (C-24), four olefinic carbons at δ_C 117.7 (C-11), 120.7 (C-7), 142.8 (C-8) and 144.2 (C-9), two oxygenated carbons at δ_C 66.7 (C-29) and 76.4 (C-16), and six methyl carbons at δ_C 17.6 (C-18), 18.6 (C-28), 21.8 (C-27), 22.0 (C-26), 22.4 (C-19) and 26.1 (C-30). The signals observed in the ^{1}H NMR and ^{13}C NMR spectra closely resembled those of compound **2** (polyporenic acid C),[12] indicating that **4** also had a similar lanostane structure as same as **2**, except for the presence of the hydroxyl group at C-29. The HMBC (Figure 2) spectrum showed the correlations between H-29 and C-3, C-4, C-5, and C-28, and the NOESY correlations from Me-28α at δ_H 1.04 (3H, s) to H-5α at

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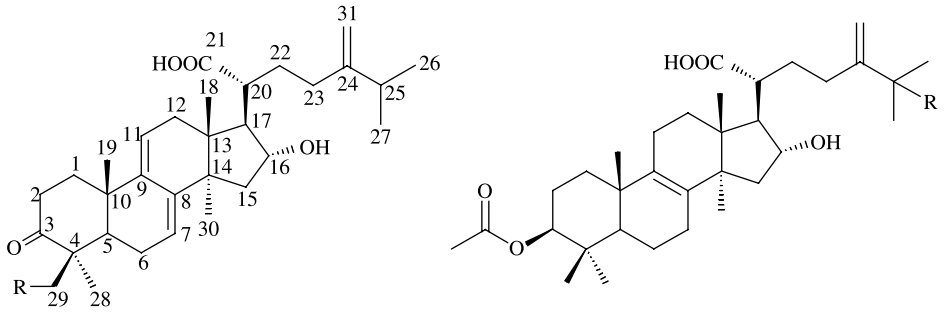

Figure 1. Structures of compounds **2**–**5**.

Table 1. ^{1}H NMR and ^{13}C NMR spectral data for compounds **2**, **3**, **4** and **5** (500 MHz for ^{1}H; 125 MHz for ^{13}C, in C_5D_5N).

Position	**4** δ_H[†]	**4** δ_C[‡]	**2** δ_C	**5** δ_H[¶]	**5** δ_C[‡]	**3** δ_C
1	2.47 (m), 2.68 (m)	36.1 t	36.7	1.06 (m), 1.48 (m)	35.3 t	35.3
2	1.82 (m), 2.10 (m)	35.2 t	34.9	1.60 (m), 1.70 (m)	24.4 t	24.4
3		215.5 s	215.2	4.66 (dd, 4.0, 12.0)	80.6 d	80.6
4		52.7 s	47.4		38.0 s	38.0
5	2.67 (dd, 3.0, 12.0)	43.0 d	51.0	1.12 (br d, 12.0)	50.6 d	50.7
6	2.13 (m)	23.7 t	23.8	1.44 (m), 1.61 (br d, 12.0)	18.3 t	18.3
7	5.57 (br s)	120.7 d	120.5	2.05 (m), 2.12 (m)	26.7 t	26.7
8		142.8 s	142.9		134.9 s	134.9
9		144.2 s	144.6		134.3 s	134.4
10		37.1 s	37.5		37.1 s	37.1
11	5.42 (d, 5.4)	117.7 d	117.7	1.91 (m), 2.09 (m)	20.9 t	20.9
12	2.40 (br d, 17.0)	36.3 t	36.2	1.96 (m), 2.17 (m)	29.6 t	29.6
	2.65 (dd, 5.4, 17.0)					
13		45.1 s	45.0		46.2 s	46.2
14		49.3 s	49.3		48.7 s	48.7
15	1.87 (d, 13.0)	44.4 t	44.4	1.68 (br d, 13.0)	43.6 t	43.6
	2.49 (dd, 9.0, 13.0)			2.36 (dd, 8.0, 13.0)		
16	4.51 (dd, 6.0, 9.0)	76.4 d	76.4	4.52 (dd, 6.0, 8.0)	76.4 d	76.7
17	2.86 (dd, 6.0, 9.0)	57.7 d	57.7	2.80 (dd, 6.0, 10.0)	57.4 d	57.4
18	1.06 (s)	17.6 q	17.6	1.10 (s)	17.7 q	17.7
19	1.12 (s)	22.4 q	22.3	0.94 (s)	19.1 q	19.2
20	2.95 (m)	48.5 d	48.5	2.99 (m)	48.7 d	48.4
21		178.8 s	178.8		178.3 s	178.9
22	2.45 (m), 2.63 (m)	31.7 t	31.7	2.57 (m), 2.79 (m)	30.0 t	31.6
23	2.39 (m), 2.49 (m)	33.3 t	33.3	2.66 (m), 2.81 (m)	30.3 t	33.2
24		156.1 s	156.2		158.0 s	156.1
25	2.26 (m)	34.1 d	34.1		72.5 s	34.1
26	0.96 (d, 6.5)	22.0 q	22.0	1.53 (s)	30.0 q	22.0
27	0.97 (d, 6.5)	21.8 q	22.0	1.54 (s)	30.0 q	21.9
28	1.04 (s)	18.6 q	25.6	0.90 (s)	27.9 q	28.0
29	3.56 (d, 11.0)	66.7 t	21.9	0.91 (s)	16.7 q	16.8
	4.15 (d, 11.0)					
30	1.40 (s)	26.1 q	26.4	1.46 (s)	25.4 q	25.4
31	4.82 (s), 4.93 (s)	106.9 t	106.9	5.15 (s), 5.46 (s)	106.9 t	107.0
OAc-CH_3				2.03 (s)	21.1 q	21.1
OAc-COOR					170.6 s	170.6

[†] Assignments were made by COSY, HSQC, HMBC and NOESY spectra.
[‡] Assignments were made by HSQC spectrum.
[¶] Assignments were made by COSY, HSQC and HMBC spectra.

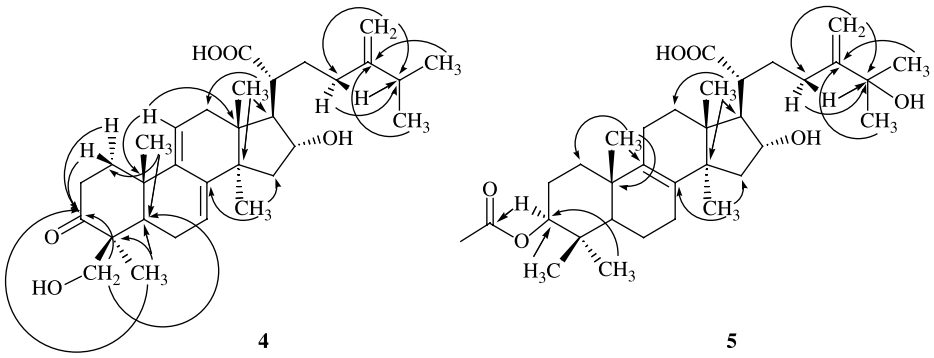

Figure 2. Key HMBC correlations of **4** and **5** (from H to C).

δ_H 2.67 (1H, dd, $J = 3.0, 12.0$ Hz) and Me-19β at δ_H 1.12 (3H, s) to H_2-29 at δ_H 3.56 (1H, d, $J = 11.0$ Hz) and 4.15 (1H, d, $J = 11.0$ Hz) revealed that the oxygenated C-29 took the β orientation. The detailed assignment of ^{1}H NMR and ^{13}C NMR spectral data (Table 1) was based on ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC spectra. Thus, the structure of **4** was concluded to be 3-oxo-16α,29β-dihydroxylanost-7,9(11),24-trien-21-oic acid, named as 29-hydroxypolyporenic acid C.

Compound **5** was obtained as white amorphous powder with $[\alpha]_D^{20} + 20.1$ (*c* 0.0995, MeOH). Its UV spectrum showed an absorption maximum at 200 nm (log ε, 2.42), suggesting the absence of conjugated unsaturated bonds. The HRESI–MS (*m/z* 543.3691 calcd. for $C_{33}H_{51}O_6$, 543.3686 $[M - H]^-$) indicated a molecular formula of $C_{33}H_{52}O_6$. Its ^{1}H NMR (Table 1) spectrum showed the signals of eight methyl groups at δ_H 0.90, 0.91, 0.94, 1.10, 1.46, 1.53, 1.54 and 2.03, two oxygen-bearing methines at δ_H 4.52 (1H, dd, $J = 6.0, 8.0$ Hz) and 4.66 (1H, dd, $J = 4.0, 12.0$ Hz), and an exocyclic methylene group at δ_H 5.15 (1H, s) and 5.46 (1H, s). Additionally, its ^{13}C NMR and HSQC (Table 1) spectra showed eight methyl carbons (δ_C 16.7, 17.7, 19.1, 21.1, 25.4, 27.9, 30.0 and 30.0), three oxygenated carbons (δ_C 72.5, 76.4 and 80.6), two tetrasubstituted olefinic carbons (δ_C 134.3 and 134.9), an exocyclic methylene carbon (δ_C 106.9 and 158.0), an acetyl carbon (δ_C 170.6), as well as a free carboxylic acid carbon (δ_C 178.3). The signals observed in the ^{1}H NMR and ^{13}C NMR spectra (Table 1) closely resembled those of compound **3** (pachymic acid)[3,6] except for the partial signals of C-17 side-chain, indicating that **5** also had a lanostane skeleton the same as **3**. On the basis of the HMBC correlations between δ_H 2.66, 2.81 (H_2-23) and δ_C 72.5; δ_H 5.15, 5.46 (H_2-31) and δ_C 30.3 (C-23), 72.5, therefore, the signal at δ_C 72.5 was assigned to C-25. In addition, the presence of hydroxy group at C-25 caused down-field shifting of C-24 signals and high-field shifting of C-23 signal. Thus, the structure of **5** was determined to be 3-*O*-acetyl-16α,25-dihydroxylanost-8-en-21-oic acid, named as 25-hydroxypachymic acid.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 243B polarimeter with MeOH as solvent. UV spectra were obtained on a Varian Cary-300 ultraviolet-visible photometer in MeOH solution. IR spectra were taken on a Thermo Nicolet Nexus 470 FT-IR spectrometer. Mass spectra were recorded on a TRACE 2000 GC–MS (for EI–MS), a MDS SCIEX API ASTAR (for ESITOF–MS) and a APEX II FTICR–MS (for HRESI–MS) spectrometer. 1D and 2D NMR spectra were recorded on a Varian INOVA-500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) using C_5D_5N as solvent and TMS as internal standard. Preparative high-performance liquid chromatography was performed on a P680 chromatograph (Dionex Co., CA, USA) equipped with UVD170U detector using a Phenomenex Luna 10 C18 (2) column (250 × 21.2 mm, 10 μm) at a flow rate 10 ml/min. Open column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China) as stationary phase. TLC was conducted on silica gel GF_{254} plates (Merck) and reversed-phase C_{18} silica gel plates (Merck).

3.2 Plant material

The sclerotia of *Poria cocos* were collected from The China National GAP Base of Chinese Materia Medica for *Poria cocos* at Luotian county of Hubei province of China, in September 2002. The fungus was identified at the site by Professor Xiu-wei Yang who is a co-author of this paper, and a voucher specimen (No. 20020920) has been deposited in the State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, China.

3.3 Extraction and isolation

Dried sclerotia of *P. cocos* (10 kg) were powdered and extracted with 95% ethanol (40 L × 5 times, 1 h/time) under reflux. The ethanolic extract was concentrated under reduced pressure to afford an extract (130.7 g, yield 1.31%), which was suspended in water (1.25 L) and partitioned successively with cyclohexane (2.5 L × 7 times), EtOAc (2.5 L × 5 times) and *n*-BuOH (2.5 L × 5 times). The cyclohexane solution was concentrated *in vacuo* to yield a green residue (16.2 g, 0.162%), which was chromatographed on a silica gel column and eluted with cyclohexane/EtOAc mixture of increasing polarity. A total of 151 fractions (*ca.* 100 ml each) were collected and combined on the basis of TLC analysis. The fractions 62–69 were chromatographed on a silica gel column eluted with cyclohexane/EtOAc gradient mixtures to yield **1** (40 mg).

The EtOAc solution was concentrated *in vacuo* to give a brown residue (36.8 g, yield 0.368%), and fractionated on a silica gel column eluting with EtOAc/MeOH gradient mixtures to give 258 fractions (*ca.* 100 ml each), which were combined on the basis of TLC analysis leading to four main fractions (A–D). Fraction A was subjected to preparative reverse-phase HPLC eluting with MeOH/H_2O/HCOOH (80:20:0.05) at a flow rate of 10 ml/min to give **2** (24 mg). Fraction B was purified by preparative reverse -phase HPLC eluting with MeOH/H_2O/HCOOH (80:20:0.05) at a flow rate of 10 ml/min to give **3** (240 mg). Compounds **4** (11 mg) and **5** (12 mg) obtained from fraction D were separated by preparative reverse-phase HPLC eluting with MeOH/H_2O/HCOOH (60:40:0.05) at a flow rate of 10 ml/min.

3.3.1 Ergosta-7,22-dien-3β-ol (*1*)

White needles (MeOH); $C_{28}H_{46}O$; NMR data were in agreement with the reported data for ergosta-7, 22-dien-3β-ol.[11]

3.3.2 Polyporenic acid C (*2*)

White amorphous powder; $C_{31}H_{46}O_4$; $[\alpha]_D^{20}$ + 7.1 (*c* 0.14, MeOH); IR, UV and NMR spectral data were in agreement with the reported data for polyporenic acid C;[12] positive ESITOF–MS *m/z* 483.3 $[M + H]^+$.

3.3.3 Pachymic acid (*3*)

$[\alpha]_D^{20}$ + 34.3 (*c* 0.12, MeOH); IR and NMR spectral data were in agreement with the reported data for pachymic acid;[3,6] negative ESITOF–MS *m/z* 527.3 $[M - H]^-$.

3.3.4 29-Hydroxypolyporenic acid C (*4*)

White amorphous powder; $C_{31}H_{46}O_5$; $[\alpha]_D^{20}$ + 27.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} nm (log ε): 235 (2.51), 243 (2.55), 251 (2.39); IR (KBr) ν_{max} cm^{-1}: 3734, 3424, 2959, 2926, 1707, 1681, 1642, 1613, 1453, 1381, 1259, 1169, 1098, 1033, 897; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data are shown in table 1; negative ESITOF–MS *m/z* 497.2775 $[M - H]^-$; negative HRESI–MS *m/z* 497.3278 $[M - H]^-$ (calcd for $C_{31}H_{45}O_5$, 497.3272).

3.3.5 25-Hydroxypachymic acid (*5*)

White amorphous powder; $C_{33}H_{52}O_6$; $[\alpha]_D^{20}$ + 20.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε): 200 (2.42); IR (KBr) ν_{max} cm^{-1}: 3733, 3417, 2955, 2926, 2855, 1734, 1707, 1680, 1642, 1458, 1376, 1250, 1171, 1096, 1032, 902, 858; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data are shown in table 1; negative ESITOF–MS *m/z* 543.3360 $[M - H]^-$; negative HRESI–MS *m/z* 543.3686 $[M - H]^-$ (calcd for $C_{33}H_{51}O_6$, 543.3691).

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