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### A new pregnane steroid from *Poria cum Radix Pini*

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## NOTE

### A new pregnane steroid from *Poria cum Radix Pini*

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A new pregnane steroid and eight known compounds were isolated from *Poria cum Radix Pini*. Their structures were identified by spectroscopic data. All the known compounds were isolated from this species for the first time.

**Keywords:** *Poria cum Radix Pini*; pregnane steroid; *Poria cocos*

#### 1. Introduction

*Poria cum Radix Pini*, known as Fu-Shen in China and with an English name of tuckahoe with pine, is the white sclerotium of *Poria cocos* (Schw.) Wolf with pine roots in the middle, growing in China, Japan, Korea, and North America. As a well-known traditional eastern Asian medicine, *P. cocos* has important applications in diuretic [1], antibacterial, antitumor [2–4], mitogenic, complement activating [5], and immune stimulating activities [6]. Previous phytochemical investigation suggested that *P. cocos* contains 90%  $\beta$ -glucan and 10% various terpenes by dry weight. The predominant triterpenoids in *P. cocos* are pachymic acid and ebuticoic acid [7], which are reported to have antitumor [8,9] and antiemetic activities [10]. The highest proportion of  $\beta$ -glucan extracted from *P. cocos* is called pachyman, which has antitumor [3] and immunomodulatory activities [6].

*P. cum Radix Pini* was reported to possess sedative activity and is frequently used in traditional Chinese medicine

compound prescriptions [11]. In contrast to extensive investigation on *P. cocos*, there exist few studies on the chemical components of *P. cum Radix Pini*. Our chemical investigation on this species led to the isolation of one new pregnane steroid, pregna-7-en-3 $\alpha$ ,11 $\alpha$ ,15 $\alpha$ ,20-quadriol (**1**), together with eight known compounds, namely  $\beta$ -sitosterol (**2**) [12], (2*S*,3*S*)-8 $\alpha$ -(4''-hydroxy-3''-methoxybenzyl)-3 $\beta$ -(4'-hydroxy-3'-methoxybenzyl)- $\gamma$ -butyrolactone (**3**) [13], (+)-nortrachelogenin (**4**) [14], 3-(4-hydroxy-3-methoxyphenyl)-1,2,3-propantriol (**5**) [15], 1,2-bis(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**6**) [16], swertiamarin (**7**) [17], uridine (**8**) [18], and D-arabitol (**9**) [19], as shown in Figure 1. Their structures were determined by spectroscopic analysis and comparing spectral data with those known compounds reported in the literatures. All these compounds were isolated from this species for the first time. In this paper, we report the isolation and structural elucidation of compound **1**.

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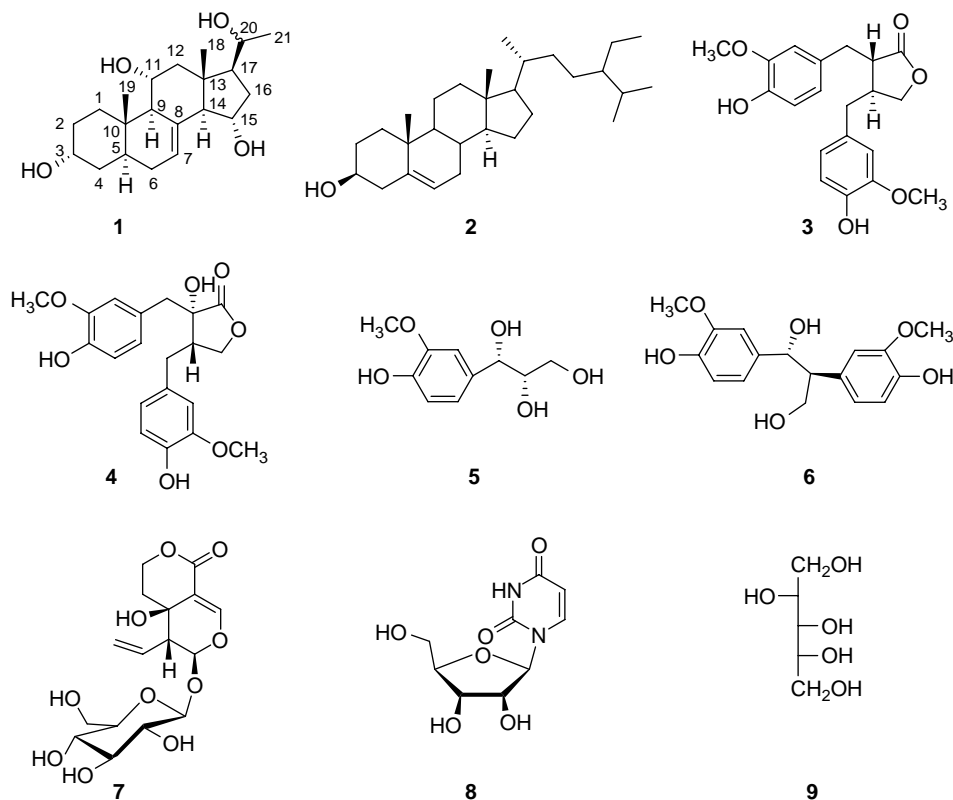


Figure 1. Structures of compounds 1–9.

## 2. Results and discussion

Compound **1** was isolated as a white powder. The molecular formula was determined as  $C_{21}H_{34}O_4$  by HR-ESI-MS at  $m/z$  385.2157  $[M + Cl]^-$ . Its IR spectrum showed absorption bands for hydroxyl ( $3423\text{ cm}^{-1}$ ) and olefinic bonds ( $1629\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum of **1** showed signals for an olefinic proton at  $\delta$  5.58 (1H, dd,  $J = 4.9, 2.5\text{ Hz}$ , H-7), four oxymethine protons at  $\delta$  4.15 (1H, ddd,  $J = 10.1, 7.4, 4.3\text{ Hz}$ , H-15), 3.96 (1H, m, H-3), 3.85 (1H, ddd,  $J = 11.6, 8.2, 5.1\text{ Hz}$ , H-11), 3.54 (1H, m, H-20), and three methyl protons at  $\delta$  1.22 (3H, d,  $J = 6.1\text{ Hz}$ ,  $H_3$ -21), 0.93 (3H, s,  $H_3$ -19), 0.58 (3H, s,  $H_3$ -18). The  $^{13}\text{C}$  NMR and DEPT spectra showed 21 carbon resonances including three methyls, six methylenes, nine methines [one olefinic carbon at  $\delta$  122.1

(C-7)], and three quaternary carbons [one olefinic carbon at  $\delta$  134.6 (C-8)]. These spectroscopic data indicated **1** to be a pregnane-type steroid possessing an olefinic bond. The locations of four hydroxyls and the olefinic bond in **1** were further elucidated by 2D NMR spectra. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Figure 2) showed the following key correlations: H-1/H-2/H-3/H-4/H-5/H-6/H-7; H-9/H-11/H-12; H-14/H-15/H-16/H-17/H-20/H-21. The HMBC spectrum (Figure 2) showed key correlations of H-3/C-1, C-4; H-11/C-10, C-12, C-9; H-15/C-17, C-14, C-8; H-20/C-17; H-6/C-7, C-8; H-9/C-7. From these data, in combination with their chemical shifts, we can safely conclude that compound **1** was a pregnane-type steroid with four hydroxyls located at C-3, C-11, C-15, C-20, and an olefinic bond located at C-7.

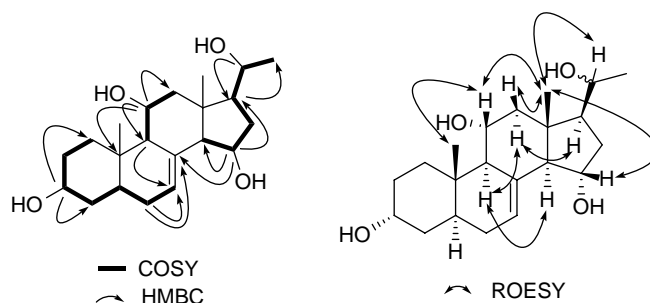


Figure 2. Key 2D NMR correlations of compound **1**.

The relative configuration of **1** was established by ROESY experiments. The ROESY spectrum (Figure 2) showed correlations of H-15/H<sub>3</sub>-18; H-11/H<sub>3</sub>-18, H<sub>3</sub>-19; H-20/H<sub>3</sub>-18; H-12a/H<sub>3</sub>-18; H-9/H-12b, H-14; H-12b/H-17, suggesting that these pairs of protons are spatially vicinal. The hydroxyl group at C-3 was assigned to be  $\alpha$  by a comparison of its NMR spectral data with those of (*E*)-agawone [20]. The above evidences indicated the relative configurations for each fused ring and some chiral centers as shown in Figure 2. The absolute configuration at C-20 still remained unknown due to scarcity of the material. On the basis of these findings, the structure of **1** was established as pregna-7-en-3 $\alpha$ ,11 $\alpha$ ,15 $\alpha$ ,20-quadiol.

### 3. Experimental

#### 3.1 General experimental procedures

Optical rotation was measured using a Horiba SEPA-300 polarimeter. UV spectrum was recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectrum was obtained on a Tensor 27 with KBr pellet. 1D and 2D NMR spectra were recorded on a Bruker AV-400 or a DRX-500 spectrometer with TMS as an internal standard. EI-MS and FAB-MS were measured on a Finnegan-4510 spectrometer and a VG Autospec-3000 spectrometer, respectively. HR-ESI-MS were determined with an API QSTAR Pulsar 1

spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), RP-18 gel (40–63  $\mu$ m; Daiso Co., Osaka, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography.

#### 3.2 Plant material

The material of *P. cum Radix Pini* was purchased from Yunnan Corporation of Materia Medica, Yunnan Province, China, and identified by Mr Y.H. Deng, at Yunnan Corporation of Materia Medica. A voucher specimen (CHYX0004) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

#### 3.3 Extraction and isolation

The air-dried powder of *P. cum Radix Pini* (10 kg) was extracted two times, each with 50 liters of boiling water for 3 h to give a crude extract (327 g), which was suspended in water and successively partitioned with EtOAc and *n*-BuOH (each 3  $\times$  2 liters). The EtOAc and *n*-BuOH extracts were combined (48 g) and loaded onto silica gel column chromatography (200–300 mesh) eluted with a stepwise gradient of CHCl<sub>3</sub>–MeOH (99:1 to 70:30) to obtain five fractions on the basis of their TLC characteristics. Fraction 1 (11 g) was

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of compound **1** in  $\text{CD}_3\text{OD}$ .

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1a	34.9	1.62 (1H, m)
1b		2.20 (1H, m)
2a	29.5	1.58 (1H, m)
2b		1.65 (1H, m)
3	66.9	3.96 (1H, m)
4	36.5	1.54 (2H, m)
5	35.9	1.84 (overlapped)
6a	30.6	1.29 (1H, m)
6b		1.73 (1H, m)
7	122.1	5.58 (1H, dd, 4.9, 2.5)
8	134.6	
9	57.9	1.78 (1H, m)
10	36.9	
11	70.0	3.85 (1H, ddd, 11.6, 8.2, 5.1)
12a	51.4	1.36 (1H, t, 11.6)
12b		2.10 (1H, dd, 11.6, 5.1)
13	43.4	
14	62.9	1.94 (1H, m)
15	70.5	4.15 (1H, ddd, 10.1, 7.4, 4.3)
16a	38.4	1.84 (overlapped)
16b		2.16 (1H, m)
17	56.9	1.69 (1H, m)
18	14.7	0.58 (3H, s)
19	12.2	0.93 (3H, s)
20	70.4	3.54 (1H, m)
21	23.9	1.22 (3H, d, 6.1)

chromatographed on a silica gel column eluted with petroleum ether– $\text{Me}_2\text{CO}$  (9:1 to 7:3) and then subjected to Sephadex LH-20 ( $\text{CHCl}_3$ – $\text{MeOH}$  6:4) to yield **2** (8 mg) and **3** (3 mg). Fraction 2 (4 g) was separated on a silica gel column eluting with  $\text{CHCl}_3$ – $\text{MeOH}$  (20:1 to 5:1), and then purified by preparative TLC ( $\text{CHCl}_3$ – $\text{Me}_2\text{CO}$ , 6:1) to afford **1** (3 mg) and **4** (6 mg). Fraction 3 (5 g) was applied to Sephadex LH-20 ( $\text{MeOH}$ ) followed by a RP-18 column chromatography ( $\text{MeOH}$ – $\text{H}_2\text{O}$ , 20:80 to 100:0) to yield **5** (4 mg) and **6** (11 mg). Likewise, fraction 4 (9 g) was fractionated by a silica gel column eluted with  $\text{CHCl}_3$ – $\text{MeOH}$  (15:1 to 5:1), in combination with an RP-18 column

( $\text{MeOH}$ – $\text{H}_2\text{O}$ , 40:60 to 100%  $\text{MeOH}$ ) and Sephadex LH-20 ( $\text{MeOH}$ ) to give **7** (10 mg), **8** (14), and **9** (33 mg).

### 3.3.1 *Pregna-7-en-3 $\alpha$ ,11 $\alpha$ ,15 $\alpha$ ,20-quadriol (1)*

A white powder;  $[\alpha]_{\text{D}}^{27.6} + 16.54$  ( $c = 0.11$ ,  $\text{MeOH}$ ). UV ( $\text{MeOH}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ): 390 (1.92), 204 (3.59) nm. IR ( $\text{KBr}$ )  $\nu_{\text{max}}$ : 3423, 2964, 2925, 1629, 1447, 1376, 1071, 1033, 1017  $\text{cm}^{-1}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data, see Table 1. FAB-MS (negative ion):  $m/z$  349  $[\text{M} - \text{H}]^-$ . HR-ESI-MS (negative ion):  $m/z$  385.2157  $[\text{M} + \text{Cl}]^-$  (calcd for  $\text{C}_{21}\text{H}_{34}\text{O}_4\text{Cl}$ , 385.2145).

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