1β-hydroxylfriedelin, a new natural pentacylic triterpene from the sclerotia of *Polyporus umbellatus*

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A new pentacylic triterpene, 1β-hydroxylfriedelin, has been isolated from the sclerotia of *Polyporus umbellatus* (Pers.) Fries (Polyporaceae) together with 18 known compounds (**2**–**19**). The structures of these compounds were elucidated on the basis of spectral data.

Keywords: 1β-hydroxylfriedelin, *Polyporus umbellatus*, Polyporaceae

The Chinese traditional medicine "Zhuling", is prepared from the dried sclerotia of *Polyporus umbellatus* (Pers.) Fries (Polyporaceae). It is distributed and used in Asia (mainly in China and Japan), Europe and North America. *P. umbellatus* is used as a diuretic and anti-tumour drug in China.[1-3] As reported previously, the chemical constituents of *P. umbellatus* are mainly polysaccharides and steroids.[4-8] We have recently examined the diuretic components from the sclerotia of *P. umbellatus* by a bioactivity-directed approach.[9] In continuation of our chemical studies of *P. umbellatus*, we describe here the isolation and structural elucidation of further metabolites, including a new pentacylic triterpene, 1β-hydroxylfriedelin (1), together with 18 known metabolites (2–19), of which two (9 and 19) and nine (10–18) are reported for the first time from the Polyporaceae and *P. umbellatus*, respectively. The structures of these metabolites were elucidated on the basis of spectral data.

Compound **1** was obtained as colourless needles. Its IR spectrum indicated the presence of a carbonyl group (1709 cm^{-1}) and a hydroxyl group (3418 cm^{-1}). Its positive APCI-MS, negative APCI-MS and positive TOF-MS spectrum exhibited quasi-molecular ion peaks at *m/z* 443.5 $[M + H]^+$, 477.5 $[M + Cl]^-$ and 465.3712 $[M + Na]^+$, respectively, which combined with the ^1H and ^{13}C NMR data (Table 1), showed that the molecular formula was $C_{30}H_{50}O_2$. This was further confirmed by the ^{13}C NMR and ^{13}C NMR (DEPT) spectra (Table 1), which displayed 30 carbon signals including eight methyls [involving one up-field shift methyl, C-23 (δ_C 7.02)], nine methylenes, five methines, seven sp^3 quaternary carbon atoms, and one carbonyl. The above information indicated that **1** might be a pentacylic triterpenoid bearing a 4-methyl substituent in A-ring. The deduction was confirmed by examination of 2D NMR data. The ^{13}C NMR data of **1** was similar to those of friedelin,[10] and the major difference was that a methylene at C-1 (δ_C 21.7) in friedelin was replaced by an oxymethine at C-1 (δ_C 74.1) in **1**. Two proton spin systems involving H-1/H_2-2 and H-1/H-10 in the ^1H–^1H COSY spectrum of **1** indicated that the oxymethine was an hydroxyl substituent at C-1. NOESY cross peaks of H-2α with H-1, and of H-10α with H-1 of **1** revealed that the 1-OH in **1** was β-oriented. The correlations observed in the HSQC and ^1H-^1H COSY spectra, in combination with the HMBC data, allowed the assignments of all the resonances in the ^1H and ^{13}C NMR spectrum (Table 1). Hence the structure of **1** was established as 1β-hydroxylfriedelin (Fig.1).

Table 1 ^1H and ^{13}C NMR assignments of compounds **1**[a]

No.	dH J (Hz)	dC	No.	dH J (Hz)	dC
1a	4.09 (brs)	74.1 (d)	15b	1.28 (m)	
2a	1.96 (m)	30.2 (t)	16a	1.42 [b]	36.1 (t)
2b	1.87 [b]		16b	1.56 [b]	
3		213.3 (s)	17		30.1 (s)
4a	2.81 (dd, 7.0)	53.5 (d)	18b	1.57 [b]	42.9 (d)
5		42.8 (s)	19a	1.37 [b]	35.6 (t)
6a	1.34 (m)	41.4 (t)	19b	1.56 [b]	
6b	1.74 (m)		20		28.3 (s)
7a	1.41(m)	18.4 (t)	21a	0.93 (m)	32.9 (t)
7b	1.51(m)		21b	1.48 (m)	
8a	1.87 (m)	52.6 (d)	22a	1.96 (m)	39.4 (d)
9		37.1 (s)	22b	1.96 (m)	
10a	1.56 (m)	53.3 (d)	23	0.89 (d, 7.0)	7.0 (q)
11a	1.28 (m)	35.5 (t)	24	0.72 (s)	14.4 (q)
11b	1.37 (m)		25	0.86 (s)	17.9 (q)
12a	1.96 (m)	30.6 (t)	26	0.99 (s)	20.4 (q)
12b	1.87 (m)		27	1.05 (s)	18.9 (q)
13		38.4 (s)	28	1.18 (s)	32.2 (q)
14		39.8 (s)	29	0.95 (s)	35.2 (d)
15a	1.51 (m)	32.6 (t)	30	1.00 (s)	31.9 (q)

[a]Data were determined at 500 MHz in $CDCl_3$ with d in ppm and *J* in Hz; [b]overlapped.

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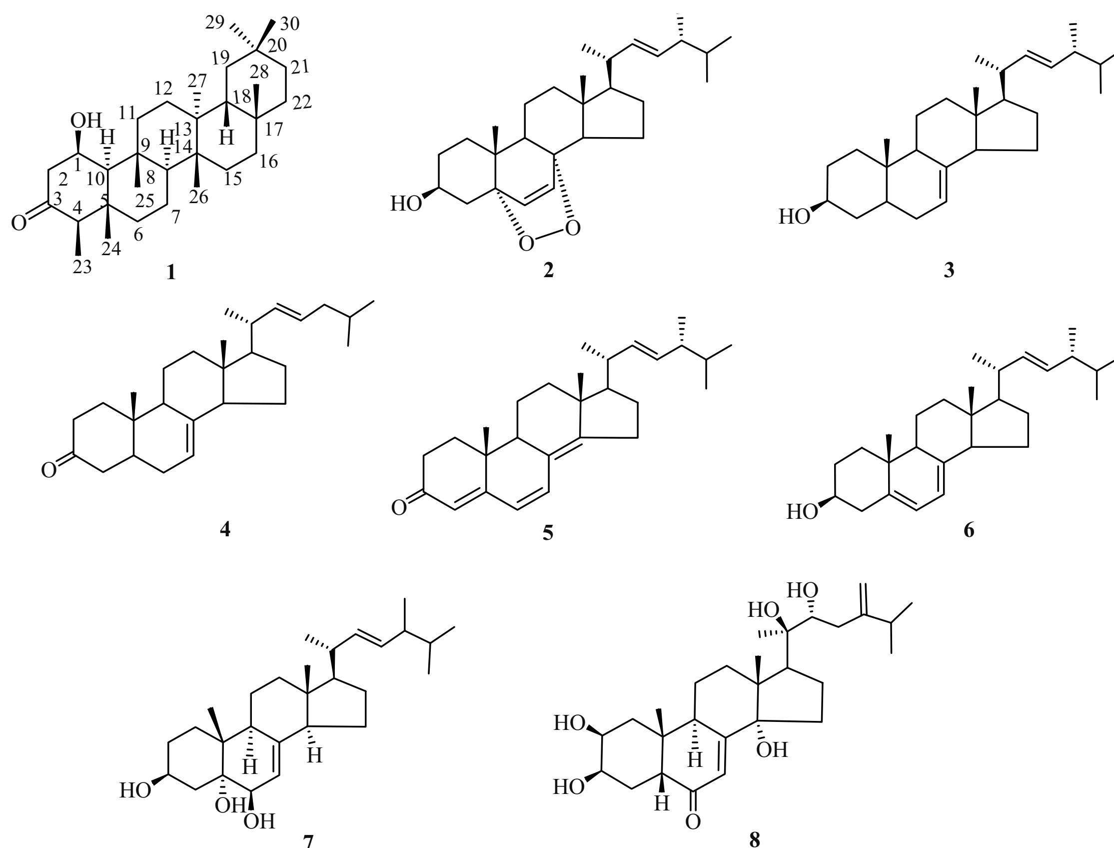

Fig. 1 Structure of compounds **1–8** from *P. umbellatus*.

The structures of the known compounds were identified based on spectral analysis (^{1}H NMR, ^{13}C NMR, 2D NMR and MS) and by comparison of their spectral data with those reported previously. Their structures are shown in Fig. 1.

Experimental

UV and IR spectra were recorded on a HITACHI J-2000 spectrometer and a Thermo IR100 spectrometer, respectively. Optical rotations were measured on a JASCO-20 polarimeter. APCI-MS and TOF-MS spectra were obtained on Waters micromass ZQ and AXIMA-CFR plus instruments. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer with TMS as the internal standard. Separation and purification were performed by column chromatography on silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd.).

Plant material

The dried sclerotia of *P. umbellatus* were collected from Shaanxi Province of China in October 2007, and identified by Prof. Ya-Zhou Wang (College of Life Sciences, Northwest University). A voucher specimen (No. 071013) is deposited at Biomedicine Key Laboratory of Shaanxi Province, Northwest University, China.

Extraction and isolation

The dried and powdered the sclerotia of *Polyporus umbellatus* (5.0 kg) were exhaustively extracted with methanol to yield a crude brown extract after evaporation of the solvent *in vacuo*. The residue was suspended in 80% methanol and partitioned with *n*-hexane, ethyl acetate and *n*-butanol successively. The *n*-hexane extract (19.0 g) was subjected to silica gel chromatography using mixtures of *n*-hexane–EtOAc (20 : 1, 10 : 1 and 5 : 1 each eluent) to yield eight fractions (Fr. A1–Fr. A8). Fr. A1 (*n*-hexane–EtOAc 20 : 1) was purified by column chromatography on silica gel monitored by TLC and recrystallisation yielded 1-hydroxyl-friedelin (**1**, 15.6 mg). Fr. A2 (*n*-hexane–EtOAc 20:1) was washed with petroleum ether to afford crude crystals. Recrystallisation yielded ergosta-7, 22-dien-3-one (**4**, 25 mg).[11] Fr. A3 (*n*-hexane–EtOAc 10 : 1) was repeatedly chromatographed by silica gel and Sephadex LH-20 to obtain ergosterol (**6**, 100 mg),[12] ergosta-4,6,8(14),22-tetraen-3-one (**5**, 13.5 mg)[13] and 4-hydroxybenzaldehyde (**18**, 8 mg).[14] Fr. A6 (*n*-hexane: EtOAc 5 : 1) was then purified by successive column chromatography on silica gel and monitored by TLC to afford (22E, 24R)-ergosta-7, 22-dien-3β-ol (**3**, 43.2 mg) [15] and 5α, 8α-epidioxy-(22E, 24R)-ergosta-6, 22-dien-3β-ol (**2**, 311.7 mg).[16] The EtOAc extract (9 g) was submitted to chromatography on silica gel, eluting with mixtures of $CHCl_3$-MeOH to yield five fractions (Fr. B1–Fr. B5). Fr. B1 ($CHCl_3$–MeOH 20 : 1) was further purified by repeated silica gel and Sephadex LH-20 chromatography to yield emodin (**9**, 51.4 mg).[17] Fr. B3 ($CHCl_3$–MeOH 5 : 1) was submitted to chromatography on silica gel. Elution with mixtures of petroleum ether-acetone (2 : 1) yielded (22E, 24R)-ergosta-7, 22-dien-3β,5α,6β-triol (**7**, 51.6 mg).[18] Fr. B5 ($CHCl_3$-MeOH 5 : 1) was subjected to chromatography on silica gel and an ODS column eluted with (MeOH–H_2O, 20:80→50:50) to give polyporusterone B (**8**, 127 mg)[19] and cerebroside B (**19**, 55.6 mg).[20] The *n*-butanol extract (20 g) was subjected to further silica gel chromatography. Elution with $CHCl_3$–MeOH, gradually increasing the polarity with MeOH affording six fractions (Fr. C1–Fr. C6). Further purification was carried out with silica gel, Sephadex LH–20 chromatography and preparative HPLC RP-C18 column. Fraction C1 ($CHCl_3$–MeOH, 10 : 1) yielded 5-hydroxymethylfurfuraldehyde (**15**, 6 mg)[21] Fraction C2 ($CHCl_3$–MeOH, 10 : 1) gave nicotinic acid (**16**, 8 mg)[22] and ferulic acid (**10**, 2.3 mg)[23] Fraction C5 ($CHCl_3$–MeOH, 5 : 1) gave uracil (**14**, 12 mg).[17] Fraction C5 ($CHCl_3$–MeOH, 5 : 1) gave uridine (**12**, 25 mg),[23] adenosine (**13**, 20 mg).[23] Fraction C6 (CHCl3–MeOH, 5 : 1) gave D-mannitol (**11**, 18 mg) [24] and succinic acid (**17**, 5 mg).[23]

1β-hydroxylfriedelin (**1**), colourless needles; $[\alpha]^{20}_D$ – 65°(*c* 0.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (1.81), 267 (1.75) nm; IR (KBr): 3418 (OH), 1709 (CO) cm^{-1}; APCIMS (positive mode) *m/z*: 443.5 $[M + H]^+$; APCIMS (negative mode) *m/z*: 477.5 $[M + Cl]^-$; TOF-MS (positive mode) *m/z*: 465.3712 $[M + Na]^+$ (Calcd for $C_{30}H_{50}O_2Na$: 465.3718); ^{1}H and ^{13}C NMR data are shown in Table 1.

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