# Two New Polyporusterones Isolated from the Sclerotia of *Polyporus umbellatus*

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**In the course of searching for marker components, two new polyporusterones were isolated from the sclerotia of *Polyporus umbellatus*, together with another three known analogs. The structures of the new ones were elucidated as (20*S*,22*R*,24*R*)-16,22-epoxy-3$\beta$,14$\alpha$,23$\beta$,25-tetrahydroxyergost-7-en-6-one (1) and (23*R*,24*R*,25*R*)-23,26-epoxy-3$\beta$,14$\alpha$,21$\alpha$,22$\alpha$-tetrahydroxyergost-7-en-6-one (2) by chemical and spectroscopic means, including HR-FAB-MS, 1D- and 2D-NMR.**

**Key words** *Polyporus umbellatus*; polyporusterone; sclerotia

The crude drug, with Chinese name "Zhuling," prepared from the dried sclerotia of *Polyporus umbellatus* (which belongs to the family of Polyporaceae), is widely distributed in Asia (mainly in China and Japan), Europe and North America. It is used as a folk medicine for diuresis and other kidney diseases for centuries. A series of studies were carried out on the fungal polysaccharides in the earlier days[1—3] and focuses were cast on the minor components in the past decades, resulting in a series of chemical compounds, mainly sterols.[4—7] Nowadays, the market demand for *P. umbellatus* is expanding because of its promising effects, and thereby, it calls for the quality control of the original material. In our research for the marker compounds, we isolated two new polyporusterones, also known as phytoecdysones, (20*S*,22*R*,24*R*)-16,22-epoxy-3$\beta$,14$\alpha$,23$\beta$,25-tetrahydroxyergost-7-en-6-one (**1**) and (23*R*,24*R*,25*R*)-23,26-epoxy-3$\beta$,14$\alpha$,21$\alpha$,22$\alpha$-tetrahydroxyergost-7-en-6-one (**2**), as well as three known polyporusterones (**3**—**5**) from the material of Chinese origin. In this paper, we report on the isolation and structure determination of the two new ones.

Compound **1** was obtained as white amorphous powder, positive in both the Liebermann–Burchard reaction and the Salkowski reaction for steroids. The positive mode HR-FAB-MS showed a $[M+Na]^+$ ion peak at *m/z* 499.3030, corresponding to the molecular formula $C_{28}H_{44}O_6$ (Calcd for $C_{28}H_{44}O_6Na$, 499.3030), possessing seven degrees of unsaturation. The IR spectrum of **1** showed absorption bands for a hydroxyl group (3350 $cm^{-1}$), $\alpha,\beta$-unsaturated cyclic ketone (1650 $cm^{-1}$). The UV spectrum displayed absorption maxima at 242 nm, which is characteristic of $\Delta^7$-6-keto steroidal skeleton.[5,8] In EI-MS the ions at *m/z* 458, 440 were due to the loss of one and two water molecules from the parent molecule, respectively, while the ion at *m/z* 359 was indicative diagnostic of steroids bearing five cycles with a side chain at C-22.[10] The prominent peaks are *m/z* 359 ($[M-C_6H_{13}O_2]^+$; loss of the side chain), *m/z* 341 ($[M-C_6H_{13}O_2-H_2O]^+$; further loss of a water molecule, base peak), *m/z* 300 ($C_{19}H_{24}O_3^+$; fission of the ethereal ring at $C_{17}$–$C_{20}$ and $C_{16}$–O), *m/z* 285 ($C_{18}H_{21}O_3^+$; further loss of a methyl). The $^1$H-NMR spectrum of **1** showed the signal of a trisubsituted double bond at $\delta$ 6.18 as a broad singlet indicating a $\Delta^7$-unsaturation. Four singlets at $\delta$ 0.97, $\delta$ 1.04, $\delta$ 1.47 and $\delta$ 1.56 were accounted by Me-19, Me-18, Me-26 and Me-27, respectively. The doublets at $\delta$ 1.39 ($J$=7.1 Hz) and $\delta$ 1.45 ($J$=7.0 Hz) corresponded to Me-21 and Me-28. The $^{13}$C-NMR spectrum with downfield signals at $\delta$ 202.9, 165.1 and 120.9, is characteristic of phytoecdysones, which have a $\Delta^7$-6-keto steroidal nucleus. Since the molecular formula had seven degrees of unsaturation, with the nucleus accounting for the six ones and no other unsaturated atoms found, it was further convinced of the fifth ring.

The hydroxyl groups in the nucleus were assigned to the usual C-3 and C-14 positions on biogenetic analogy, HMBC correlation (Fig. 2) and comparison with the NMR spectral data (Tables 1, 2) of the known compound **3** (identified as 22,23-epoxy-3$\beta$,14$\alpha$,20$\beta$,24$\beta$-tetrahydroxy-7-en-6-one,[11] whose stereochemistry shown in Fig. 1), deducing their 3$\beta$, 14$\alpha$ configurations respectively. Moreover, the H-3 was observed as a broad singlet, with no a–a *trans* coupling with

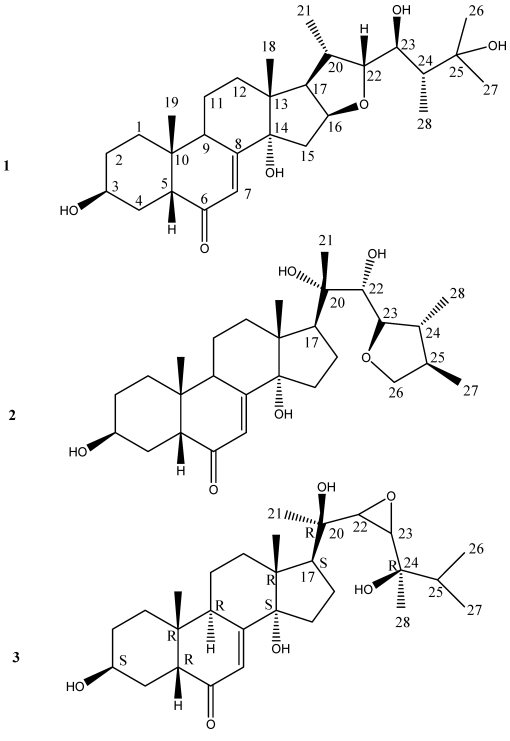

Fig. 1. Structures of Compounds **1**, **2** and **3**

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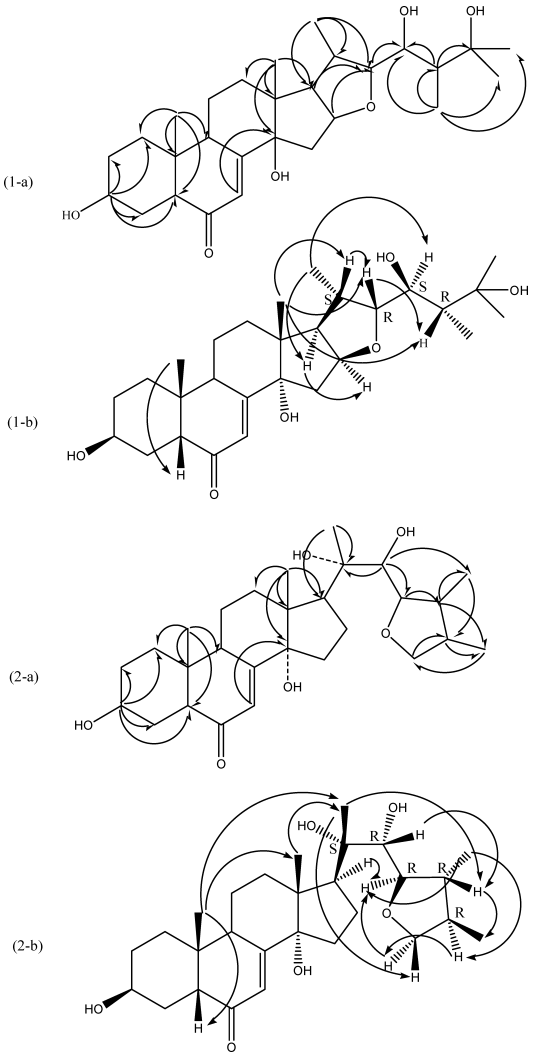


Fig. 2. Important HMBC and NOESY Correlations of Compounds **1** and **2**

(1-a): HMBC correlations of compound **1**; (1-b): NOESY correlations of compound **1**; (2-a): HMBC correlations of compound **2**; (2-b): NOESY correlations of compound **2**.

other protons, which defined its equatorial orientation. Therefore, the C-3 hydroxyl group was further confirmed its $\beta$ orientation.

The NOESY experiment helped to elucidate the stereochemistry of **1** (Fig. 2). On irradiation on 19-Me at $\delta$ 1.04, intense NOE effect was observed with H-5 ($\delta$ 2.97), thus revealing a *cis* A/B junction; when turned to 18-Me at $\delta$ 0.97, H-20 ($\delta$ 2.70), H-22 ($\delta$ 4.44), H-24 ($\delta$ 2.21) were detected, in addition to the 14$\alpha$-OH, indicating a *trans* linked C/D ring with H-20, H-22 and H-24 $\beta$-oriented; the *cis* D/ethereal ring junction was deduced from the strong NOE observed between H-16 and H-17, and corroborated by the NOE between Me-21 and both H-16 and H-17, which coincides with the D/E ring junction and $C_{20}$ configuration of naturally occurring spirostanes,[9] whose data of $C_{16}$ and $C_{17}$ were quite similar to that of **1**; a strong NOE effect was also observed between Me-21 and H-23, which implied a rigid conformation of the side chain with Me-21 close to H-23. The stereochemistry at C-23 was further confirmed by the $H_{22}/H_{23}$ *J* value. According to a combination of molecular dynamics and mechanics calculations in the force field CHARM performing on model compounds with 23(*S*)- and 23(*R*)-hydroxy substituents,[12] the calculated $H_{22}/H_{23}$ *J* value of 23*S*-model was 8.5—8.7 Hz, while the one of 23*R*-model was in the range of 4.5—5.0 Hz. Therefore, the experimental one ($J$=9.1) of **1** helped to determine that its C-23 was of *S* conformation and 23-OH was $\beta$-oriented. Thus, compound **1** was assigned the structure as (20*S*,22*R*,24*R*)-16,22-epoxy-3$\beta$,14$\alpha$,23$\beta$,25-tetrahydroxyergost-7-en-6-one, which is the first reported phytoecdysone with an ethereal ring (ring E) conjunct to the ring D.

Compound **2** was also obtained as white amorphous powder, which gave positive color tests for a sterol. The positive mode HR-FAB-MS showed a $[M+Na]^+$ peak at *m*/*z* 499.3033, in accordance with the molecular formula $C_{28}H_{44}O_6$ (Calcd for $C_{28}H_{44}O_6Na$, 499.3030), also obtaining seven degrees of unsaturation. The IR and UV spectra showed close resemblance to that of **1**, indicating the two of quite the same nucleus. In EI-MS the ions at *m*/*z* 458, 440 were also found, while the ion at *m*/*z* 359 in **1** was turned to be 347 here, indicating the fission at $C_{20}$–$C_{22}$ of the side chain,[10] followed by the ions at *m*/*z* 329 and 311, further loss of one and two water molecules respectively. The base peak at *m*/*z* 99 was due to the fission at $C_{22}$–$C_{23}$ of the side chain. The signal of an olefinic proton at $\delta$ 6.26 (br s) in the $^1$H-NMR spectrum as well as the signals at $\delta$ 203.9, 166.6 and 121.1 in the $^{13}$C-NMR spectrum supported the $\Delta^7$-unsaturation nucleus. And still with no other double bounded atoms found, an independent ring in the side chain would explain the remaining degree of unsaturation. The $^1$H-NMR spectrum also showed five methyls, three of them singlets at $\delta$ 1.05, $\delta$ 1.23, $\delta$ 1.70, assigned as Me-19, Me-18 and Me-21, respectively, the rest two doublets at $\delta$ 0.87 ($J$=6.6 Hz) and $\delta$ 1.27 ($J$=6.5 Hz) corresponding to Me-26 and Me-28. Further analysis of the NMR spectra data of compounds **2** and **3** (see Tables 1, 2) showed both of them sharing quite resemblance in the nucleus, with differences lying in the side chain.

The structure of the side chain was established by the detailed analysis of its HMQC, $^1$H–$^1$H COSY, and HMBC NMR spectroscopic data. The substructure was elucidated from the following HMBC correlations (Fig. 2): the 21-methyl proton signals at $\delta$ 1.70 with C-20, C-17; the signal of H-22 ($\delta$ 3.89) with C-20, C-23 and C-28; the signal of H-24 ($\delta$ 1.94) with C-23, C-28 and C-27; the signals of H-26 ($\delta$ 3.38, 3.89) with C-25, C-27. The NOESY results (Fig. 2) made their stereo configuration clear: on irradiation on 18-Me at $\delta$ 1.23, a strong NOE effect was observed with 21-Me ($\delta$ 1.70), demonstrating its $\beta$ orientation with 21-OH $\alpha$-oriented; a strong NOE effect was also detected between 21-Me and both H-22 and H-24, followed by an effect between H-24 and 27-Me; thus 28-Me and H-25 were both $\alpha$-oriented, a NOE effect between them also occurred; followed by the effect between 28-Me and H-23, the signal between H-22 and H-17. This led us to deduce the structure of compound **2** as (23*R*,24*R*,25*R*)-23,26-epoxy-3$\beta$,14$\alpha$,21$\alpha$,22$\alpha$-tetrahydroxyergost-7-en-6-one, which is rare as a phytoecdysone having an oxygenated $C_{26}$ cycled as a furan ring.

The other two known phytoecdysones isolated were identified as polyporusterone A (**4**) and polyporusteron B (**5**) by comparing their spectral data with literature values.[5]

The biological activity of compound **1** and **2** has not been

Table 1. $^{13}$C-NMR Data of Compounds **1**, **2** and **3** at 125 MHz in Prydine-$d_5$

| Position | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 36.5 | 36.8 | 36.4 |
| 2 | 29.6 | 29.2 | 29.7 |
| 3 | 63.8 | 64.0 | 63.8 |
| 4 | 34.2 | 34.2 | 34.3 |
| 5 | 51.6 | 51.6 | 51.6 |
| 6 | 202.9 | 203.9 | 203.2 |
| 7 | 120.9 | 121.1 | 121.3 |
| 8 | 165.1 | 166.6 | 166.1 |
| 9 | 35.2 | 33.5 | 34.5 |
| 10 | 37.0 | 37.2 | 38.3 |
| 11 | 22.0 | 21.5 | 21.8 |
| 12 | 32.9 | 32.1 | 31.8 |
| 13 | 49.1 | 48.0 | 48.1 |
| 14 | 84.9 | 84.1 | 83.9 |
| 15 | 41.0 | 31.5 | 31.3 |
| 16 | 82.8 | 21.8 | 21.7 |
| 17 | 61.4 | 49.8 | 51.6 |
| 18 | 17.8 | 18.0 | 17.5 |
| 19 | 24.0 | 24.2 | 24.1 |
| 20 | 37.0 | 76.7 | 71.8 |
| 21 | 17.0 | 22.9 | 24.1 |
| 22 | 84.1 | 79.3 | 62.4 |
| 23 | 69.8 | 86.9 | 60.0 |
| 24 | 43.1 | 46.9 | 71.2 |
| 25 | 73.3 | 42.5 | 36.8 |
| 26 | 29.5 | 74.3 | 17.4 |
| 27 | 28.9 | 15.3 | 17.5 |
| 28 | 7.8 | 18.0 | 22.3 |

Table 2. $^{1}$H-NMR Data of Compounds **1**, **2** and **3** at 500 MHz in Prydine-$d_5$

| Position | **1**[a] | **2**[a] | **3**[a] |
|---|---|---|---|
| 3 | 4.13 (1H, br s) | 4.12 (1H, br s) | 4.15 (1H, br s) |
| 5 | 2.97 (1H, m) | 2.98 (1H, m) | 2.97 (1H, m) |
| 7 | 6.18 (1H, br s) | 6.26 (1H, br s) | 6.21 (1H, br s) |
| 9 | 3.54 (1H, br s) | 3.56 (1H, br s) | 3.54 (1H, br s) |
| 16 | 5.29 (1H, m) | 2.47 (1H, m) | 2.43 (1H, m) |
| | | 2.30 (1H, m) | 2.33 (1H, m) |
| 17 | 2.85 (1H, d, $J$=8.0) | 3.46 (1H, t, $J$=9.1) | 3.05 (1H, t, $J$=9.3) |
| 20 | 2.70 (1H, m) | — | — |
| 22 | 4.44 (1H, dd, $J$=5.6, 9.2) | 3.89 (1H, m)[b] | 3.64 (1H, d, $J$=1.9) |
| 23 | 4.69 (1H, br d, $J$=9.1) | 3.89 (1H, m)[b] | 3.31 (1H, d, $J$=1.9) |
| 24 | 2.21 (1H, m) | 1.94 (1H, m) | — |
| 25 | — | 1.70 (1H, m) | 1.92 (1H, m) |
| Me-18 | 0.97 (3H, s) | 1.23 (3H, s) | 1.14 (3H, s) |
| Me-19 | 1.04 (3H, s) | 1.05 (3H, s) | 1.04 (3H, s) |
| Me-21 | 1.39 (3H, d, $J$=7.1) | 1.70 (3H, s) | 1.59 (3H, s) |
| Me-26 | 1.47 (3H, s) | 3.89 (1H, m)[b] | 1.16 (3H, d, $J$=6.8) |
| | | 3.38 (1H, t, $J$=8.4) | |
| H-27 | 1.56 (3H, s) | 0.87 (3H, d, $J$=6.6) | 1.16 (3H, d, $J$=6.8) |
| Me-28 | 1.45 (3H, d, $J$=7.0) | 1.27 (3H, d, $J$=6.5) | 1.38 (3H, s) |

*a*) Numbers of protons, multiplicity and $J$ values in Hz are given in parentheses. *b*) Overlapped.

tested yet, but such activity is expected, since the similar polyporusterones, polyporusterone A—G have been reported the cytotoxic effects on leukemia 1210 cells (L-1210)[5] and polyporusterone A and B, as active substances on hair regrowth.[13]

## Experimental

**General Experimental Methods** UV spectra were obtained from a Hitachi UV-2201 spectrometer and optical rotations on a JASCO J-810 spectropolarimeter. IR spectra were recorded in KBr disks on an Impact 410 FTR spectrophotometer. NMR spectra were acquired in pyridine-$d_5$ on a Bruker Avance-500 FT NMR spectrometer (500 MHz for $^{1}$H and 125 MHz for $^{13}$C) with tetramethylsilane (TMS) as an internal standard. HR-FAB-MS spectra were measured on a Bruker Daltonics. Inc. APEX II. FT-ICRMS spectrometer in positive ion mode, and EI-MS spectra on a Micromass Zabspec spectrometer, respectively. Preparative HPLC were carried on ODS column (Phenomenex 250×10 mm i.d.) with a DAD detector. Silica gel (100—200, 200—300 mesh, Qingdao Mar. Chem. Ind. Co. Ltd.) was used for column chromatography.

**Fungus Material** The sclerotia of *Polyporus umbellatus* were purchased from Luonan County, Gansu province, People's Republic of China, and identified by the corresponding author (Shun-Xing GUO). A voucher specimen (No. ZL20040819) is deposited in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College.

**Extraction and Isolation** The air dried sclerotia (39.2 kg) of *P. umbellatus* were extracted with hot 95% ethanol (3×400 l). After removal of the solvent under reduced pressure at 50 °C, the residue (580 g) was suspended in water and extracted with petroleum ether, dichloromethane ($CH_2Cl_2$), ethyl acetate and *n*-butanol successively. A portion of $CH_2Cl_2$ extract (186.2 g) was subjected to column chromatography over silica gel and eluted with mixtures of $CHCl_3$–MeOH, in increasing order of polarity. The fractions of fr. 148—fr. 162 were combined, rechromatographed over silica gel developed with $CHCl_3$–MeOH (20 : 1), and were purified by HPLC to afford **1** (4 mg), **2** (8 mg), **3** (23 mg), **4** (47 mg), **5** (58 mg). The condition of HPLC was as follows: column: Phenomenex ODS (250×10 mm); mobile phase: 30% $CH_3CN$; flow rate: 2 ml/min; detection: UV 242 nm; each compound and retention time (min): **1** (11.8 min), **2** (42.4 min), **3** (30.8 min), **4** (33.4 min), **5** (26.3 min).

Compound **1**: Obtained as white amorphous powder (MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 242 (4.01). IR (KBr) $\nu_{max}$ cm$^{-1}$: 3350 (OH), 1650 (C=O). HR-FAB-MS (positive) *m/z*: 499.3030 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_6Na$: 499.3030). EI-MS *m/z* (rel. int. %): 458 ($[M-H_2O]^+$, 8), 440 (10), 425 (4), 359 (39), 342 (63), 341 (100), 300 (12), 285 (71), 286 (66), 263 (20), 95 (66), 79 (25). $[\alpha]_D^{20}$ +65.7° ($c$=0.39, MeOH). $^{1}$H- and $^{13}$C-NMR (pyridine-$d_5$) data see Table 1 and 2.

Compound **2**: Obtained as white amorphous powder (MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 242 (3.98). IR (KBr) $\nu_{max}$ cm$^{-1}$: 3350 (OH), 1650 (C=O). HR-FAB-MS (positive) *m/z*: 499.3033 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_6Na$: 499.3030). EI-MS *m/z* (rel. int. %): 476 ($M^+$, 2), 458 (11), 440 (8), 422 (3), 407 (1), 347 (57), 329 (71), 311 (34), 295 (6), 287 (18), 269 (21), 99 (100), 81 (12). $[\alpha]_D^{20}$ +58.9° ($c$=0.41, MeOH). $^{1}$H- and $^{13}$C-NMR (pyridine-$d_5$) data, see Table 1 and 2.

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