# Four New Triterpenes from Fungus of *Fomes officinalis*

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**In the previous work we reported five lanostane-type triterpenes from the $CHCl_3$ soluble fraction of *Fomes officinalis*. In further study on the isolation of constituents from the $CHCl_3$ soluble fraction, four new triterpenes, fomefficinic acids F (1), G (2) and fomefficinols A (3), B (4), together with seven known compounds officinalic acid (5), fomlactone A (6), fomlactone B (7), fomlactone C (8), laricinolic acid (9), ergosterol (10), ergota-7,22,dien-3$\beta$-ol (11) were isolated. The structures of new compounds were determined by spectroscopic analyses and chemical methods including NMR spectroscopic techniques ($^{13}C$, $^{1}H$, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bonding correlation (HMBC), correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY)).**

**Key words** *Fomes officinalis*; polyporaceae; lanostane-type triterpene; triterpene lactone

*Fomes officinalis* (VILL. ex FR.) is a wood rotting fungus that is found on the trunks of living or dead coniferous trees in the northern regions of China, in the Pacific Northwest United States, Canada and in Europe. It is traditionally used in Chinese Uigur prescription to treat cough and asthma.[1,2] Several triterpenes from this fungus have been characterized since it was first studied in 1804.[3,4] Previously, we have reported five new lanostane-type triterpenes, named fomefficinic acids A—E from the ethanol extract of the dried sclerotium of *F. officinalis*.[5] In our continuing search for bioactive constituents from this fungus, it was found that the EtOH extract of *F. officinalis* possessed marginal cytotoxicity against several human tumor cell lines. By bioassay-guided separation, further chemical investigation on the $CHCl_3$ soluble fraction led to the isolation.

The sclerotium of *F. officinalis*, purchased from Xinjiang, China, were crushed, extracted with 95% EtOH. After removal of excessive EtOH, the extract was partitioned successively with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. After evaporation of the solvent, the $CHCl_3$ soluble residue was subject to column chromatography on silica gel and repeatedly reversed-phase silica gel to afford four new triterpenes, fomefficinic acids F (**1**), G (**2**) and fomefficinols A (**3**), B (**4**), together with 7 known compounds. The known compounds were identified as officinalic acid (**5**),[6] fomlactone A (**6**), fomlactone B (**7**), fomlactone C (**8**),[7] laricinolic acid (**9**),[8] ergosterol (**10**),[9] as well as ergota-7,22,dien-3$\beta$-ol (**11**)[10] (Fig. 1). To our knowledge, known compounds fomlactones A (**6**), B (**7**), C (**8**) and ergosta-7,22,dien-3$\beta$-ol (**11**) were isolated for the first time in *F. officinalis*.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{31}H_{48}O_5$ by HR-EI-MS molecular ion at *m/z* 500.3506 $[M]^+$ and $^{13}C$-NMR spectroscopic analysis. The IR spectrum showed the presence of hydroxyl (3375 $cm^{-1}$) and carboxyl (1697 $cm^{-1}$). The $^{1}H$-NMR spectrum of **1** (Table 1) revealed the presence of five tertiary methyls ($\delta_H$ 1.02, 1.04, 1.11, 1.17, 1.34), a secondary methyl ($\delta_H$ 1.26), a methine ($\delta_H$ 4.62) and an exomethylene group [$\delta_H$ 5.01, 5.05 (each br s)] which were characteristic of 24-methylenelanostane. The $^{13}C$-NMR spectrum of **1** (Table 1) showed 31 resonances, of which 30 were attributed to a triterpene skeleton. The presence of a carboxylic acid ($\delta_C$ 178.8), a ketone function ($\delta_C$ 216.3), two oxygenated carbons [$\delta_C$ 72.3 (C-15) and 66.7 (C-26)] and two olefinic quaternary carbons ($\delta_C$ 133.5, 135.8) indicating the presence of $\Delta^8$ double bond was shown by $^{13}C$-NMR resonances. The $^{1}H$- and $^{13}C$-NMR spectra of **1** were similar to those of fomefficinic acid D,[5] except for the presence of hydroxyl attached to the C-27 and the concomitant the disappearance of the signal of $CH_3$-27, that showed two oxymethyl protons [$\delta_H$ 3.73 (dd, $J$=7.5, 11.5 Hz), 3.95 (dd, $J$=5.5, 11.5 Hz)]. Furthermore, significant cross peaks were observed between H-27 and C-24, 25, 26; H-19 and C-9; H-30 and C-8; H-28, H-29 and C-3; H-15 and C-14, 16, 30 in the heteronuclear multiple bonding correlation (HMBC) spectrum. The 15-OH was assigned to be $\alpha$-orientation due to the nuclear Overhauser effect spectroscopy (NOESY) (Fig. 2) correlation observed between H-15 and $H_3$-18. On the basis of the evidence described above, the structure of **1** was assigned as 15$\alpha$,26-dihydroxy-3-oxo-24-methylenelanosta-8-en-21-oic acid and has been named fomefficinic acid F.

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was determined to be $C_{31}H_{50}O_4$

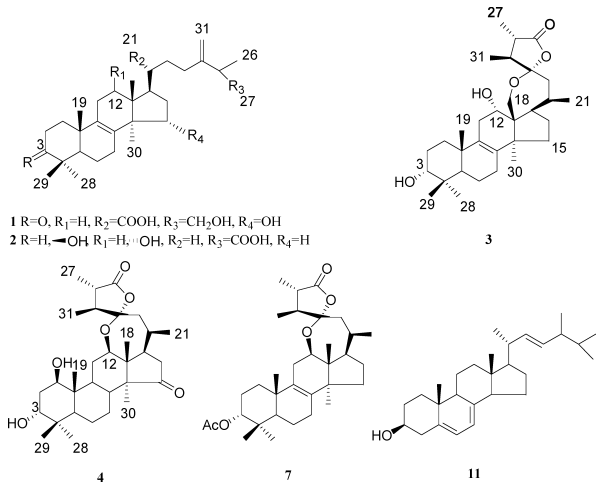


Fig. 1. Structures of **1**—**4**, **7**, **11** from *F. officinalis*

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by HR-EI-MS, which exhibited a molecular ion peak at *m/z* 486.3687 $[M]^+$. The IR spectrum showed the presence of hydroxyl (3433 $cm^{-1}$) and carboxyl (1699 $cm^{-1}$) of COOH. When its $^1H$- and $^{13}C$-NMR data (Table 1) were compared with those of eburicoic acid,[11] the spectra were the same except for two resonances attributed to two hydroxyl groups in **2**. The assignments of the $^1H$- and $^{13}C$-NMR data were facilitated by comparison with those of eburicoic acid[11] and confirmed by HMQC, HMBC and $^1H$–$^1H$ COSY data. The two hydroxyl groups were assigned to C-3 and C-12 based on the HMBC correlations between H-3 and C-4, $CH_3$-28, and $CH_3$-29; H-12 and C-14, 17, 18; $H_2$-11 and C-12, at the same time from the $^1H$–$^1H$ COSY correlations between H-12 and $H_2$-11, H-3 and H-2. On comparing the peak model and coupling constant of H-3 of **2** with that of eburicoic acid,[11] the NOESY associations of H-3 with $H_3$-28 and H-12 with $H_3$-19 suggested $\alpha$-orientation of H-3 and $\beta$-orientations of H-12 (Fig. 2). The information of coupling constant of H-12 (t, $J$=7.5 Hz) implied that the configuration at H-12 to be $\beta$.[12] The absence of isopropyl group in IR and $^1H$-NMR spectra of **2** showed instead a carboxyl group at C-26 by comparing with those of eburicoic acid.[11] Accordingly, compound **2** was determined as 3$\beta$,12$\alpha$-dihydroxy-24-methylene-lanosta-8-en-26-oic acid and has been named fomefficinic acid G.

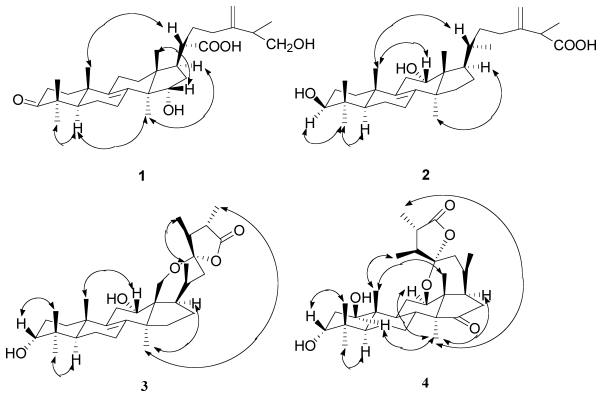


Fig. 2. Key NOESY Correlations of **1**—**4**

Compound **3** was obtained as a colorless needles and showed a molecular ion peak at *m/z* 500.3498 $[M]^+$, consistent with a formula of $C_{31}H_{48}O_5$. The IR spectrum showed the presence of hydroxyl (3543 $cm^{-1}$) and strong absorptions at 1772 and 1759 $cm^{-1}$, suggestive of a strained lactone unit. The $^1H$- and $^{13}C$-NMR data (Table 1) resembled those of fomlactone B (**7**)[7] except for the observation of an oxymethylene [$\delta_H$ 4.01, 4.09 (2H, d); $\delta_C$ 66.4] signal at the same time the absence of the resonance of tertiary methyl in **3**. Significant HMBC correlations of H-18 with C-14, 17, 23; H-17 with C-18 and H-12 with C-18 indicated the conjunction of C-18–C-23 by oxygen functionality. In addition, the correlations of H-12 with C-17, 13; two methyl resonances at $\delta_H$ 1.21 and $\delta_H$ 0.90 with C-3 in the HMBC spectrum indicated

Table 1. NMR Spectroscopic Data (500 MHz, Pyridine-$d_5$) for Compounds **1**—**4**

| Position | **1**[a] | | **2**[a] | | **3**[a] | | **4**[a] | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 36.2 | 1.46, 1.75, m | 36.3 | 1.26, 1.72, m | 32.1 | 1.20, 1.51, m | 79.1 | 4.70, d (4.5) |
| 2 | 33.6 | 2.51, 2.54, m | 31.7 | 1.22, 1.80, m | 20.8 | 1.65, m | 36.7 | 2.21, 2.40, m |
| 3 | 216.3 | | 78.0 | 3.44, dd (7, 9) | 74.8 | 3.59, br s | 74.1 | 3.59, br s |
| 4 | 47.3 | | 39.5 | | 37.3 | | 38.2 | |
| 5 | 51.2 | 1.62, m | 51.0 | 1.45, m | 44.5 | 1.91, m | 44.5 | 2.30, m |
| 6 | 19.8 | 1.10, 1.63, m | 18.7 | 1.08, 1.77, m | 18.3 | 1.63, m | 35.2 | 1.94, 2.38, m |
| 7 | 27.4 | 2.57, m | 28.7 | 1.22, 1.86, m | 25.9 | 0.86, 0.90, m | 27.2 | 1.62, 1.88, m |
| 8 | 135.8 | | 136.7 | | 137.2 | | 51.3 | 2.03, m |
| 9 | 133.5 | | 134.1 | | 133.2 | | 23.0 | 1.03, m |
| 10 | 37.2 | | 37.3 | | 38.1 | | 49.5 | |
| 11 | 21.3 | 1.13, 1.95, m | 35.1 | 2.21, 2.76, m | 35.1 | 2.25, 2.98, m | 27.3 | 2.19, 2.42, m |
| 12 | 30.1 | 1.54, 1.95, m | 72.0 | 4.41, t (7.5) | 74.8 | 4.38, dd (8.5, 17.5) | 75.0 | 4.95, dd (5.5, 13.5) |
| 13 | 45.3 | | 52.3 | | 50.9 | | 60.8 | |
| 14 | 52.2 | | 49.7 | | 50.6 | | 65.1 | |
| 15 | 72.3 | 4.62, dd (5.5, 9.0) | 34.4 | 1.64, 1.96, m | 31.0 | 1.51, 2.01, m | 212.6 | |
| 16 | 39.2 | 2.21, 2.29, m | 25.7 | 1.46, 2.06, m | 26.8 | 1.80, 1.91, m | 31.5 | 1.28, 2.26, m |
| 17 | 46.7 | 2.70, m | 51.4 | 2.08, m | 48.8 | 2.39, m | 43.4 | 2.83, dd (6, 10.5) |
| 18 | 16.9 | 1.17, s | 10.9 | 1.04, s | 66.4 | 4.01, 4.09, d (13.5) | 12.4 | 0.83, s |
| 19 | 18.6 | 1.02, s | 24.3 | 1.02, s | 26.0 | 0.86, s | 18.8 | 2.05, s |
| 20 | 49.0 | 2.65, m | 26.6 | 2.10, m | 30.4 | 2.83, m | 28.1 | 2.14, m |
| 21 | 178.8 | | 21.9 | 1.34, d (6.5) | 22.9 | 0.91, d (7.5) | 19.8 | 0.86, d (7.0) |
| 22 | 31.6 | 1.90, 2.16, m | 34.5 | 1.47, 2.17, m | 39.4 | 1.65, 2.71, m | 40.8 | 1.74, 2.06, m |
| 23 | 34.7 | 2.36, 2.41, m | 33.6 | 2.36, 2.58, m | 110.0 | | 109.7 | |
| 24 | 152.7 | | 150.8 | | 50.3 | 2.02, m | 51.0 | 2.07, m |
| 25 | 43.2 | 2.56, m | 46.5 | 3.55, q (7, 14) | 42.1 | 2.42, m | 41.9 | 2.37, m |
| 26 | 17.2 | 1.26, d (7.0) | 17.1 | 1.52, d (7) | 178.3 | | 176.8 | |
| 27 | 66.7 | 3.73, dd (7.5, 11.5)<br>3.95, dd (5.5, 11.5) | 176.9 | | 12.9 | 1.13, d (6.5) | 13.4 | 1.21, d (6.5) |
| 28 | 18.1 | 1.04, s | 16.4 | 1.07, s | 29.0 | 1.21, s | 29.5 | 1.16, s |
| 29 | 26.4 | 1.11, s | 28.6 | 1.24, s | 22.5 | 0.90, s | 23.0 | 1.06, s |
| 30 | 21.1 | 1.34, s | 19.5 | 1.08, s | 19.4 | 0.98, s | 19.1 | 1.47, s |
| 31 | 109.3 | 5.01, 5.05, s | 110.4 | 5.13, 5.24, s | 12.7 | 1.17, d (7.0) | 12.3 | 0.95, d (7.0) |

*a*) Signals were assigned by HMQC, HMBC.

two hydroxyls group were located at C-3 and C-12. The NOESY associations of H-3 with $H_3$-29 and H-12 with $H_3$-19 suggested $\beta$-orientations of H-12 and H-3 (Fig. 2), similar to fomlactone B (**7**).[7] No NOESY correlative signals of H-18 with $H_3$-30 suggested the $\beta$-orientation of H-18 that might be more stable and reasonable in their stereostructure. Compound **3** is thus named fomefficinol A.

Compound **4** was obtained as a colorless needles and displayed a molecular ion peak at *m/z* 516.3438 $[M]^+$ in the HR-EI-MS spectrum, consistent with a formula of $C_{31}H_{48}O_6$ and the IR absorption bands at 3552 and 1755 $cm^{-1}$ suggested the presence of hydroxyl and carboxyl groups the same as that of **3**. The $^1$H- and $^{13}$C-NMR spectra of **4** showed the presence of a $\gamma$-lactone ring, five tertiary methyl groups and three secondary methyls, for which the features were very similar to those of fomlactone B (**7**)[7] except for the presence of a hydroxyl [$\delta_H$ 4.95 (2H, dd); $\delta_C$ 75.0] signal and carbonyl ($\delta_C$ 212.6) signal and the absence of double bond signal. The location of the carbonyl at C-15 was deduced from the HMBC correlations between H-30 and C-15. The locations of two hydroxyl groups at C-1 and C-3 were deduced from the HMBC of correlations between H-19 and C-1; H-1 and C-2, 5, 10 and other correlation between H-28, H-29 and C-3. Significant NOESY correlations of H-3 with $H_3$-29 indicated $\beta$-orientation, whereas the associations of H-1 and H-12 with $H_3$-30 revealed their $\alpha$-orientation (Fig. 2). Thus, compound **4** has been named fomefficinol B.

## Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on an Impact 400 FTIR spectrometer as KBr pellets, whereas, the optical rotations were acquired on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker AM-500 spectrometer in pyridine-$d_5$, using TMS as internal standard. NMR experiments included the $^1$H–$^1$H COSY, HMQC, HMBC, and NOESY pulse sequences. Mass spectra were recorded on an AutoSpec-Ultima TOF spectrometer. Column chromatography was performed using reversed-phase RP-18 silica (Merck, Germany) and silica gel 60H (400—500 mesh) and TLC was carried out on silica gel $GF_{254}$ plates (0.20—0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, P. R. China).

**Plant Material** Dried sclerotium of *Fomes officinalis* (Vill. ex Fr.) Ames. were purchased from Xinjiang, China in 2003 and identified by Prof. Yong-Ming Liu. Voucher specimens (ALH-03-0918) have been deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Siences and Peking Union Medical College, P. R. China.

**Extraction and Isolation** Dried sclerotium of *F. officinalis* (4.0 kg) were extracted with 95% ethanol two times. The combined extract was evaporated under reduced pressure to give a residue (610 g), which was suspended in water and partitioned with chloromethane. The combined chloromethane-soluble fraction (240 g) was subjected to flash column chromatography on silica gel (100—200 mesh) and eluted with mixtures of $CHCl_3$–MeOH (19 : 1→8 : 2) to obtain fractions F1 (10.0 g). Elution with a 4 : 1 mixture afforded F2 (24.7 g), F3 (13.5 g), F4 (12.2 g), F5 (27.8 g) and F6 (24.5 g), followed by F7 (64.5 g) on elution with MeOH. Purification of fraction F1 (10.0 g) on silica gel (petroleum ether–EtOAc 19 : 1) afforded compound **10** (17 mg) and **11** (16 mg). Fraction F2 (24.7 g) was resolved into 27 subfractions (F2-1—F2-27) by column chromatography on a silica gel 60H (400—500 mesh) [petroleum ether–acetone (19 : 1→1 : 1)]. Compound **6** (12 mg) was purified from fraction F2-3 by column chromatography (silica gel, petroleum ether–acetone 9 : 1). F2-4 was subjected to further column chromatography over reversed-phase silica gel (MeOH–$H_2O$, 80 : 20) to yield compound **4** (15 mg). Compound **5** (20 mg) was obtained from fraction F2-4 by column chromatography over silica gel (petroleum ether–acetone 9 : 1). Compound **7** (12 mg) and **8** (14 mg) were purified from fraction F2-27 by column chromatography over silica gel ($CHCl_3$–MeOH 50 : 1→4 : 1). F5 (27.8 g) was subsequently separated into 15 fractions by silica gel chromatography (49 : 1→4 : 1), of which F5-6 was purified by reversed-phase silica gel (MeOH–$H_2O$, 75 : 25) to furnish compound **9** (75 mg). F5-15 was separately purified by reversed-phase silica gel (MeOH–$H_2O$, 80 : 20) to yield **1** (24 mg), **2** (10 mg) and **3** (14 mg), respectively.

Compound **1**: White amorphous powder. mp 198—201 °C, $[\alpha]_D^{20}$ +16.2 ($c$=0.04, $CHCl_3$ : MeOH, 1 : 1), IR (KBr) $\nu_{max}$: 3375, 2947, 1697, 1645 and 1456 $cm^{-1}$, For $^1$H- and $^{13}$C-NMR spectroscopic data, see Table 1, HR-EI-MS *m/z* 500.3506 $[M]^+$ (Calcd for $C_{31}H_{48}O_5$, 500.3502).

Compound **2**: White amorphous powder. mp 172—175 °C, $[\alpha]_D^{20}$ −54.6 ($c$=0.01, $CHCl_3$ : MeOH, 1 : 1), IR (KBr) $\nu_{max}$: 3433, 2952, 2873, 1699, 1651 and 1456 $cm^{-1}$, For $^1$H- and $^{13}$C-NMR spectroscopic data, see Table 1, HR-EI-MS *m/z* 486.3687 $[M]^+$ (Calcd for $C_{31}H_{50}O_4$, 486.3709).

Compound **3**: Colorless crystalline needles (MeOH). mp 247—249 °C, $[\alpha]_D^{20}$ −25.0 ($c$=0.03, $CHCl_3$ : MeOH, 1 : 1), IR (KBr) $\nu_{max}$: 3543, 2960, 2875, 1772, 1759 and 1454 $cm^{-1}$, For $^1$H- and $^{13}$C-NMR spectroscopic data, see Table 1, HR-EI-MS *m/z* 500.3498 $[M]^-$ (Calcd for $C_{31}H_{48}O_5$, 500.3502).

Compound **4**: Colorless crystalline needles (MeOH). mp 233—235. $[\alpha]_D^{20}$ +30.0 ($c$=0.21, $CHCl_3$ : MeOH, 1 : 1), IR (KBr) $\nu_{max}$: 3552, 3473, 2964, 1755, 1699 and 1454 $cm^{-1}$, For $^1$H- and $^{13}$C-NMR spectroscopic data, see Table 1, HR-EI-MS *m/z* 516.3438 $[M]^+$ (Calcd for $C_{31}H_{48}O_6$, 516.3451).

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