New Lanostane-Type Triterpenes from *Fomes officinalis*

Xia WU, JunShan YANG,* Liang ZHOU, and YueSheng DONG

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing 100094, People's Republic of China. Received June 25, 2004; accepted August 6, 2004

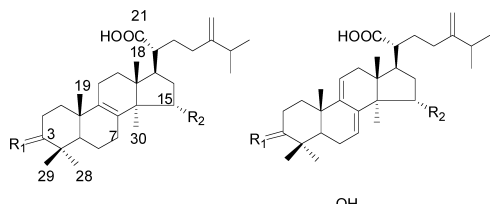
Five new lanostane-type triterpenes, named fomefficinic acid A—E (1—5), were isolated from the dried sclerotium of *Fomes officinalis*, respectively. Their structures were established as 24-methylene-3-oxo-lanost-8-en-21-oic acid (1), 3α,15α-dihydroxy-24-methylene-lanosta-7,9(11)-dien-21-oic acid (2), 3α,15α-dihydroxy-24-methylene-lanost-8-en-21-oic acid (3), 15α-hydroxy-3-oxo-24-methylenelanost-8-en-21-oic acid (4), 15α-acetoxy-3-oxo-24-methylenelanosta-7,9(11)-dien-21-oic acid (5), by spectral analysis and chemical methods as well as comparison with known compounds.

Key words *Fomes officinalis*; polyporaceae; triterpene; lanostane

Fomes officinalis (VILL. ex FR.) AMES is a wood rotting fungus which is found on the trunks of living or dead coniferous trees in the northern regions of China and in the Pacific Northwest United States, Canada and Europe. It is traditionally used in Chinese Uigur prescription to treat cough and asthma.[1,2] A variety of triterpenes from this fungus have been characterized since it was first studied in 1804.[3,4] In this paper, we report the isolation and characterization of five new lanostane-type triterpenes **1**—**5** by extensive NMR experiment and chemical methods.

Results and Discussion

Compound **1** was obtained as white needles and exhibited a positive Libermann–Burchard reaction. Its molecular formula was determined to be $C_{31}H_{48}O_3$ by HR-EI-MS ([M^+], *m/z* 468.3603). Its IR spectrum showed hydroxyl (3430 cm^{-1}) and carboxyl (1705 cm^{-1}). The ^{1}H-NMR data of **1** (Table 1) exhibited two secondary methyl [δ 1.01, 1.02 (each 3H, d, J=7.0 Hz)], five tertiary methyl groups [δ 0.79, 0.91, 1.06, 1.09, 1.11 (each 3H, s)], two coupled broad signals at δ 4.69 and 4.76 (each 1H, br s) which were characteristic of 24-methylenelanostane. The ^{13}C-NMR spectrum of **1** (Table 1) provided evidence of a lanostane skeleton. It showed the appearance of two olefinic quaternary carbons (δ 135.0, 133.1) indicating the presence of Δ^8 one double bond, one carboxyl moiety (δ 181.8) and a ketone function at δ 217.6. Therefore, the ^{13}C-NMR spectrum of **1** was similar to that of versisponic acid C (**6**),[5] except for the disappearance of the signal of α-acetoxy at C-15 in **1**. Furthermore, significant cross peaks were observed between the H-17/C-21, H-19/C-9, H-30/C-8, H-28/C-3 and H-29/C-3 in the HMBC experiment. Thus, the structure of compound **1** was determined to be 24-methylene-3-oxo-lanost-8-en-21-oic acid and has been named fomefficinic acid A.



1 R_1=O, R_2=H

2 R_1=OH/H, R_2=OH

3 R_1=OH/H, R_2=OH

4 R_1=O, R_2=OH

5 R_1=O, R_2=OAc

6 R_1=O, R_2=OAc

Compound **2** was obtained as white amorphous powder and exhibited a positive Libermann–Burchard reaction. The IR spectrum showed hydroxyl (3400 cm^{-1}) and carboxyl (1700 cm^{-1}), and a molecular formula of $C_{31}H_{48}O_4$ was deduced from its HR-EI-MS ([M^+], *m/z* 484.3545). The ^{1}H-NMR spectrum of **2** indicated the presence of seven methyl protons (δ 0.95, 0.98, 1.00, 1.13, 1.15, 1.16, 1.36), an exomethylene group (δ 4.84, 4.88) and two oxymethines protons [δ 3.62 (br s), 4.77 (dd, J=6, 9.5 Hz)]. The UV spectrum showed an absorption maximum at 236 nm, 244 nm and 252 nm, suggesting the presence of a $\Delta^{7,9(11)}$ conjugated diene system. This was fully supported by ^{13}C-NMR data of **2** (Table 1), which showed the signals of C-7 (δ 122.3), C-8 (δ 142.0) and C-9 (δ 147.3), C-11 (δ 115.9) revealing the presence of $\Delta^{7,9(11)}$. In addition, there are one carboxyl resonance at δ 178.6 (C-21) and two oxymethine resonances at δ 75.1 (C-3) and δ 73.8 (C-15) in the ^{13}C-NMR of **2**. Furthermore, compared with those of **1**, a significant difference in the ^{1}H-NMR of **2** was that the CH_3-30 appeared at δ 1.36 instead of δ 0.91, the signal due to C-30 exhibited an upfield shift from δ 24.3 to δ 18.3. These shifts could be explained with γ-effect caused by the hydroxyl attached to the C-15. This result was also supported by correlations between the H-15/C-30 and H-15/C-8 in the HMBC spectrum. In the NOESY spectrum, important correlations were observed between the H-29/H-3 and H-18/H-15, the hydroxyl configurations at C-3 and C-15 should be α-configuration. Thus, the structure of compound **2** was established as 3α,15α-dihydroxy-24-methylene-lanosta-7,9(11)-dien-21-oic acid and has been named fomefficinic acid B.

Compound **3** was obtained as white amorphous powder and exhibited a positive Libermann–Burchard reaction. It was assigned the molecular formula $C_{31}H_{50}O_4$ by HR-EI-MS ([M^+], *m/z* 486.3687), which showed 2 mass units more than **2**. The IR spectrum showed hydroxyl (3440 cm^{-1}) and carboxyl (1700 cm^{-1}). In the ^{1}H-NMR data of **3** (Table 1), there are seven methyl protons at δ 0.92, 0.97, 1.00, 1.07, 1.20, 1.21, 1.28, an exomethylene group (δ 4.85, 4.88), and two oxymethines protons [δ 3.61 (br s), 4.65 (dd, J=6, 9.5 Hz)]. Comparison of the ^{13}C-NMR data of **3** (Table 1) with those of **2** indicated that the major differences were the

* To whom correspondence should be addressed. e-mail: JunShanYang@hotmail.com

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data of **1**—**3**

Position	**1** ($CDCl_3$)[a]		**2** (pyridine-d_5)[b]		**3** (pyridine-d_5)[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.60 m, 2.10 m	36.1	1.72 m, 2.25 m	30.7	1.80 m, 1.94 m	31.9
2	2.39 m, 2.55 m	34.5	2.12 m, 2.14 m	23.5	2.03 m, 2.08 m	27.4
3		217.6	3.62 br s	75.2	3.61 br s	75.1
4		47.1		37.9		38.2
5	2.09 m	47.3	2.04 m	43.6	2.04 m	44.6
6	1.63 m, 1.66 m	19.3	1.92 m, 2.02 m	31.9	1.62 m, 1.77 m	18.8
7	2.03 m, 2.05 m	26.3	6.50 br s	122.3	1.78 m, 2.04 m	26.9
8		135.0		142.0		135.3
9		133.1		147.3		134.7
10		36.9		38.0		37.6
11	1.06 m, 2.03 m	20.9	5.47 d (5.5)	115.9	1.03 m, 2.02 m	21.2
12	1.26 t, 1.66 m	30.4	1.84 m, 2.06 m	26.8	1.94 m, 2.01 m	30.8
13		44.2		48.9		49.1
14		49.6		52.6		52.2
15	1.41 m, 1.72 m	28.8	4.77 dd (6, 9.5)	73.8	4.65 dd (6, 9.5)	72.5
16	1.38 m, 1.96 m	27.0	2.20 m, 2.29 m	39.6	2.23 m, 2.27 m	39.4
17	1.59 m	51.2	2.64 m	45.0	2.69 m	45.5
18	0.79 s	16.0	1.15 s	16.9	1.21 s	16.9
19	1.11 s	18.6	1.13 s	23.1	1.07 s	19.4
20	2.32 m	47.6	2.74 m	46.4	2.64 m	46.7
21		181.8		178.6		178.7
22	1.69 m, 1.72 m	30.9	2.38 m, 2.70 m	36.8	1.97 m, 2.14 m	30.2
23	1.95 m, 2.05 m	31.9	2.31 m, 2.42 m	32.7	2.29 m, 2.43 m	32.7
24		155.1		155.8		155.9
25	2.22 m	33.7	2.24 m	34.2	2.24 m	34.2
26	1.02 d (7.0)	21.8	1.00 d (7)	22.0	1.00 d (7)	22.0
27	1.01 d (7.0)	21.7	0.98 d (7)	21.9	0.97 d (7)	21.9
28	1.06 s	21.2	1.16 s	29.1	1.20 s	29.0
29	1.09 s	26.1	0.95 s	23.2	0.92 s	22.7
30	0.91 s	24.3	1.36 s	18.3	1.28 s	18.1
31	4.69 s, 4.76 s	106.8	4.84 s, 4.88 s	107.1	4.85 s, 4.88 s	107.1

a) Signals were assigned by HMQC, HMBC. *b*) Signals were assigned by HMQC, HMBC and NOESY.

numbers and positions of double bond. The signals of C-8 (δ 135.3) and C-9 (δ 134.7) of **3** showed the presence of Δ^8 one double bond. There are two oxymethine resonances at δ 75.1 (C-3) and δ 72.5 (C-15). Close similarity of these spectral data with these of **2** described above suggested that **3** is a higher homologue of **2**. Thus, the structure of **3** was established as 3α,15α-dihydroxy-24-methylene-lanost-8-en-21-oic acid and has been named fomefficinic acid C.

Compound **4** was obtained as white amorphous powder and exhibited a positive Libermann–Burchard reaction. It showed [M^+] at m/z 484.3557 ($C_{31}H_{48}O_4$) in the HR-EI-MS. The IR spectrum showed hydroxyl (3440 cm^{-1}) and carboxyl (1710 cm^{-1}). The ^{1}H-NMR spectrum showed seven methyl protons at δ 0.98, 1.00, 1.01, 1.03, 1.10, 1.18, 1.35, an exomethylene group (δ 4.85, 4.89) and an oxymethines protons [δ 4.63 (dd, J=6, 9.5 Hz)]. The ^{1}H-, ^{13}C-NMR spectra of **4** were similar to those of **3**, except for the presence of a ketone function at δ 216.3 (C-3) and meanwhile the disappearance of the H-3 signal. In conclusion, compound **4** was determined as 15α-hydroxy-3-oxo-24-methylenelanosta-8-en-21-oic acid and has been named fomefficinic acid D.

Compound **5** was obtained as white amorphous powder and exhibited a positive Libermann–Burchard reaction. It showed [M^+] at m/z 524.3502 ($C_{33}H_{48}O_5$) in the HR-EI-MS. The IR spectrum showed hydroxyl (3440 cm^{-1}) and carboxyl (1735 cm^{-1}). The UV spectrum showed an absorption maximum at 235 nm, 243 nm and 251 nm, suggesting the presence of a $\Delta^{7,9(11)}$ conjugated diene system. Two coupled broad signals at δ 4.86 and 4.88 in the ^{1}H-NMR data of **5** (Table 2) are characteristic of 24-methylenelanostane. The ^{13}C-NMR spectrum of **5** was similar to that of **6**, except for the signals of C-7 (δ 121.5), C-8 (δ 142.1) and C-9 (δ 144.6), C-11 (δ 116.6) revealing the presence of $\Delta^{7,9(11)}$ conjugated diene system. Thus, the structure of compound **5** was determined to be 15α-acetoxy-3-oxo-24-methylenelanosta-7,9(11)-dien-21-oic acid and has been named fomefficinic acid E.

Experimental

General Experimental Procedures Melting points were determined on an X4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. UV spectra were measured with a Hitachi UV-2201 spectrophotometer and IR spectra with an Impact 400 FTIR spectrometer. NMR spectra were recorded in pyridine-d_5 or $CDCl_3$ with an INOVA 500 NMR spectrometer, using TMS as internal standard. NMR experiments included the HMQC, HMBC and NOESY pulse sequences. Coupling constants (J value) are given in Hz. Mass spectra were recorded on an AutoSpec Ultima-TOF spectrometer. Silica gel (300—400 mesh), Silica gel 60H (500—600 mesh) and silica gel GF_{254} sheets (0.20—0.25 mm) (both from Qingdao Haiyang Chemical Co., Qingdao, P. R. China) were used for column chromatography and TLC, respectively.

Plant Material Dried sclerotium of *Fomes officinalis* (Vill. ex Fr.) Ames was purchased from Xinjiang P. R. China and identified by Prof. Yong-Min Liu. A voucher specimen (ALH-03-0918) is deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, P. R. China.

Extraction and Isolation Dried sclerotium of *Fomes officinalis* (4.0 kg) were extracted with 95% ethanol two times. The concentrated extract was

Table 2. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data of **4**, **5**

Position	**4** (pyridine-d_5)[a]		**5** (pyridine-d_5)[a]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.45 m, 2.60 m	36.2	1.65 m, 2.17 m	36.7
2	1.52 m, 1.88 m	34.7	2.35 m, 2.74 m	34.2
3		216.3		216.4
4		46.7		47.4
5	1.70 m,	51.2	1.59 dd (5, 11)	50.8
6	1.67 m, 1.69 m	19.8	1.91 m, 2.05 m	23.6
7	2.64 m, 2.66 m	27.4	5.81 d (6)	121.5
8		135.3		142.1
9		133.5		147.6
10		37.1		37.3
11	2.00 br s	21.3	5.50 d (5.5)	116.6
12	2.02 m, 2.24 m	30.1	2.37 m, 2.58 m	35.3
13		45.3		45.6
14		52.2		51.6
15	4.63 dd (6, 9.5)	72.3	5.40 dd (5.5, 9.5)	77.6
16	2.28 m, 2.38 m	39.3	2.22 m, 2.25 m	36.4
17	2.78 m	47.3	2.53 m	46.8
18	1.18 s	16.9	1.11 s	16.5
19	1.03 s	18.7	1.03 s	18.4
20	2.71 m	49.1	2.70 m	47.7
21		178.8		178.5
22	2.05 m, 2.22 m	31.8	2.36 m, 2.72 m	35.3
23	2.38 m, 2.51 m	32.7	2.31 m, 2.46 m	33.7
24		155.8		154.8
25	2.32 m	34.2	2.25 m	34.5
26	1.00 d (6.5)	22.0	1.10 d (6.5)	21.3
27	0.98 d (6.5)	21.9	1.08 d (6.5)	21.8
28	1.10 s	26.4	1.00 s	26.7
29	1.01 s	21.1	1.13 s	21.6
30	1.35 s	18.2	1.20 s	19.8
31	4.85 s, 4.89 s	107.1	4.86 s, 4.88 s	107.3
–$COCH_3$			2.17 s	170.8, 21.3

a) Signals were assigned by HMQC, HMBC.

suspended in water and extracted with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The chloroform-soluble fraction (240 g) was subjected to low-pressure column chromatography (LPLC) on silica gel (300—400 mesh), eluting with $CHCl_3$–MeOH (19 : 1—8 : 2) mixtures to afford Fr. 1—7. Fr. 1 (40 g) was chromatographed on a silica gel (300—400 mesh) column eluting with petroleum ether–ethyl acetate (10 : 0—8 : 2) to afford **1** (10 mg). Fr. 6 (24.5 g) was chromatographed on a silica gel (300—400 mesh) column eluting with $CHCl_3$–MeOH (10 : 1—8 : 2) to give four fractions [A (4.6 g), B (6 g), C (3 g) and D (1.4 g)]; Fraction B was subjected to repeated column chromatography, eluting with $CHCl_3$–MeOH (97 : 3), to yield **2** (12 mg) and **3** (23 mg); Fraction C was subjected to repeated column chromatography, eluting with $CHCl_3$–MeOH (95 : 5), to yield **4** (10 mg); Fraction D was further purified by MPLC over silica gel 60H (400—500 mesh) and eluted with $CHCl_3$–MeOH (96 : 4—90 : 10) gave **5** (7 mg).

Fomefficinic Acid A (**1**): White needles, mp 201—203 °C, $[\alpha]_D^{20}$ +4.6° (c=0.06, $CHCl_3$: CH_3OH, 1 : 1), UV (CH_3OH) λ_{max} (log ε): 204 (4.70) nm, IR (KBr) ν_{max}: 3430, 2960, 2875, 1705, 1640, 1460, 1450, 1375, 1298, 1280, 1200, 1100, 890 cm^{-1}, ^{1}H-, ^{13}C-NMR data see Table 1, EI-MS *m/z* (%): 468 ([M]$^+$, 38), 453 (100), 435 (15), 425 (5), 407 (24), 339 (8), 311 (20), 309 (23), 297 (43), 271 (16), 245 (11), 229 (8), 203 (10), 185 (14), 173 (18), 157 (25), 145 (27), 119 (35), 97 (40), 81 (30), 69 (38), HR-EI-MS *m/z* 468.3603 [M]$^+$ (Calcd 468.3603).

Fomefficinic Acid B (**2**): White amorphous powder, mp 194—196 °C, $[\alpha]_D^{20}$ +25.8° (c=0.05, $CHCl_3$: CH_3OH, 1 : 1), UV (CH_3OH) λ_{max} (log ε): 236 (3.90), 244 (4.03), 252 (4.01) nm, IR (KBr) ν_{max}: 3400, 2962, 2932, 2640, 1700, 1640, 1456, 1380, 1280, 1235, 1170, 1050, 990, 895 cm^{-1}, ^{1}H-, ^{13}C-NMR data see Table 1, EI-MS *m/z* (%): 484 ([M]$^+$, 100), 466 (13), 451 (32), 423 (10), 311(9), 255 (8), 227 (19), 197 (6), 185 (10), 171 (13), 145 (12), 119 (15), 109 (10), 95 (11), 83 (19), 69 (15), 55 (21), HR-EI-MS *m/z* 484.3545 [M]$^+$ (Calcd 484.3553).

Fomefficinic Acid C (**3**): White amorphous powder, mp 203—205 °C, $[\alpha]_D^{20}$ +26.7° (c=0.04, $CHCl_3$: CH_3OH, 1 : 1), UV (CH_3OH) λ_{max} (log ε): 205 (4.70) nm, IR (KBr) ν_{max} 3440, 2960, 2950, 2840, 1700, 1680, 1450, 1379, 1280, 1165, 1050, 980, 880 cm^{-1}, ^{1}H-, ^{13}C-NMR data see Table 1, EI-MS *m/z* (%): 486 ([M]$^+$, 100), 471 (47), 453 (75), 435 (25), 357 (8), 297 (7), 289 (9), 257 (8), 241 (17), 215 (11), 187 (52), 173 (13), 145 (10), 135 (17), 121 (16), 109 (13), 105 (11), HR-EI-MS *m/z* 486.3687 [M]$^+$ (Calcd 486.3709).

Fomefficinic Acid D (**4**): White amorphous powder, mp 205—207 °C, $[\alpha]_D^{20}$ +37.5° (c=0.04, $CHCl_3$: CH_3OH, 1 : 1), UV (CH_3OH) λ_{max} (log ε): 204 (4.60) nm, IR (KBr) ν_{max}: 3440, 2960, 1710, 1638, 1450, 1375, 1270, 1170, 1050, 990, 880 cm^{-1}, ^{1}H-, ^{13}C-NMR data see Table 2, EI-MS *m/z* (%): 484 ([M]$^+$, 100), 482 (55), 469 (25), 451 (49), 449 (9), 405 (5), 367 (12), 325 (7), 295 (13), 287 (28), 273 (14), 257 (14), 245 (10), 203 (9), 185 (13), 145 (18), 119 (25), 97 (22), 81 (19), 69 (31), 55 (43), HR-EI-MS *m/z* 484.3557 [M]$^+$ (Calcd 484.3553).

Fomefficinic Acid E (**5**): White amorphous powder, mp 207—209 °C, $[\alpha]_D^{20}$ +46.7° (c=0.03, $CHCl_3$: CH_3OH, 1 : 1), UV (CH_3OH) λ_{max} (log ε): 235 (4.04), 243 (4.10), 251 (4.00) nm, IR (KBr) ν_{max}: 3440, 2960, 2940, 1735, 1700, 1630, 1380, 1250, 1040, 880 cm^{-1}, ^{1}H-, ^{13}C-NMR data see Table 2, EI-MS *m/z* (%): 524 ([M]$^+$, 68), 469 (100), 449 (21), 421 (16), 368 (8), 307 (20), 257 (27), 241 (6), 183 (7), 97 (19), 83 (7), 69 (10), 55 (16), HR-EI-MS *m/z* 524.3502 [M]$^+$ (Calcd 524.3502).

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