Fomlactones A−C, Novel Triterpene Lactones from *Fomes cajanderi*

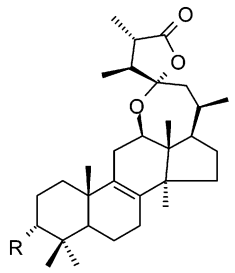
Jian He,*,† Xiao-Zhang Feng, Yang Lu, and Bin Zhao

Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Xian Nong Tan 1, 100050 Beijing, People's Republic of China

Received April 30, 2003

Investigation of the neutral ether extracts of the fungus *Fomes cajanderi* led to the isolation of three novel ketal lactones named fomlactones A (**1**), B (**2**), and C (**3**). These compounds were identified by analysis of IR, HRMS, CD, and 1D and 2D NMR data. The structure of fomlactone C (**3**) was confirmed by X-ray diffraction analysis.

In the course of our screening of fungi for novel compounds, we systematically studied the chemical constituents of *Fomes cajanderi* P. Karst, which is a wood-rotting fungus belonging to the polyporaceous family (Basidiomycetes) that grows on broad-leaved trees of China. The genus *Fomes* is known as a rich source of lanostane-type triterpenes.[1] Some fatty acids and ergostane and lanostane derivatives have been isolated from this genus,[2,3] but for *Fomes cajanderi* Karst, no reports have appeared to date. In the present paper we report the isolation and structure elucidation of three novel ketal triterpene lactones, fomlactones A (**1**), B (**2**), and C (**3**), from this fungus.



1 R = OAc **2** R = OH **3** R = =O

The powdered fruit bodies of *Fomes cajanderi* were successively extracted with petroleum ether and diethyl ether. The neutral ether extracts were separated by column chromatography on silica gel to give three pure crystalline compounds, named as fomlactones A (**1**), B (**2**), and C (**3**). The structures of **1**−**3** were determined by analysis of HRMS, 2D NMR, and CD spectra, and **3** was confirmed by X-ray crystallographic evidence.

The molecular formula of fomlactone A ($C_{33}H_{50}O_5$) was established by HREIMS combined with ^{13}C, DEPT data, which, together with a positive Liebermann−Burchard test, indicated a triterpenoid structure. The IR spectrum showed strong absorptions at 1780 and 1248 cm^{-1}, suggestive of a strained lactone unit, and at 1734 cm^{-1}, which combined with a methyl signal at 2.07 in the ^1H NMR indicated an acetate group. The presence of five tertiary methyl groups, three secondary methyl groups, and two oxygen-bearing methine units [δ 4.65 (br s, w/2 = 3 Hz) and 4.35 (t, J = 8.0 Hz)] was observed in ^1H NMR spectrum of fomlactone A. The ^{13}C and DEPT spectra contained two ester carbonyl signals (δ 177.6 and 170.9), two nonprotonated olefinic carbons (δ 135.0 and 133.6), one ketal carbon (δ 110.0), two oxygen-bearing methines (δ 77.9 and 75.8), and nine methyl groups. Analysis of these data, as well as comparison with those of lanostane,[4,5] suggested that fomlactone A possesses a lanostane triterpene skeleton with the C-3 position substituted by an acetoxy group.[1,3] Because of shifts observed for carbon signals of the A-ring[3] and the appearance of the H-3 signal as a broad singlet (w/2 = 3.0 Hz), the stereochemistry of H-3 was proposed to be β-equatorial. On comparing the carbon chemical shifts of **1** with those of lanostane,[4] the lower carbon signal at δ 52.6 of C-13 indicated C-13 neighbored with an electroattracting group, which was further confirmed by HMBC correlations of C-12 (δ 75.8) with H-18 (δ 0.77), and of C-18 (δ 11.7) with H-12 (δ 4.35). These results led to the assumption that C-12 was substituted by an oxygen atom as shown.

Furthermore, the IR band at 1780 cm^{-1} and the ^{13}C NMR signal at δ 177.6 indicated the presence of a lactone group in the molecule. However, the NMR data showed only two oxygenated sp^3 carbon signals, and these two signals were attributed to C-3 and C-12 as noted above. Analysis of the ^{13}C NMR, DEPT, and HMBC data revealed a ketal carbon signal at δ 110.0 (C-23), which correlated with 4.35 (H-12), indicating that the lactone group was linked to C-12 and that C-12 and C-23 were connected by an ether bridge.

The configuration at C-17 was proposed as shown by analogy with that of lanostane triterpenes.[4] This information, together with the coupling constant of H-12 (t, J = 8.0 Hz), implied that H-12 has an α-axial orientation. Moreover, the configuration at C-23 was determined by the CD spectrum, which showed a positive Cotton effect at 239 nm. With the rule of W. Klyne,[6,7] we determined the *S* configuration of C-23. This result was supported by X-ray crystallographic analysis of fomlactone C.

The IR, NMR, and EI mass spectral data of fomlactones B (**2**) and C (**3**) were very similar to those of **1** except for the data of the A-ring. Fomlactone B showed the α-OH substitution at C-3 by the IR absorption band at 3543 cm^{-1}, a new ^1H NMR signal at δ 3.43 (brs, w/2 = 6.0 Hz), and ions in the EI mass spectrum at m/z 484 $(M)^+$ and 466 $(M^+ - 18)$.

The conclusion that C-3 of **3** was a ketone carbon was supported by the EIMS molecular ion at m/z 482, additional IR absorption at 1705 cm^{-1}, and the apparently additional signals for H-2 in the ^1H NMR spectrum at δ 2.45−2.55. These data enabled assignment of the structures of fomlacones B and C as shown.

To confirm the structures and stereochemistry of these compounds, fomlactone C (**3**) was subjected to a single-

* To whom correspondence should be addressed. Tel: (520) 741-1691. Fax: (520) 741-1468. E-mail: jianhe@email.arizona.edu.
† Current address: Southwest Center for Natural Products Research and Commercialization, 250E Valencia Rd., Tucson, AZ 85706-6800.

10.1021/np0301994 CCC: $25.00
Published on Web 08/20/2003

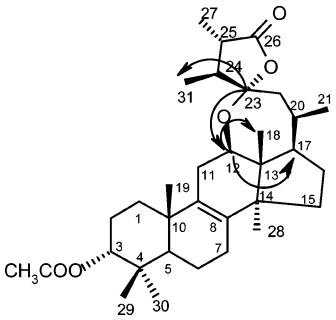


Figure 1. Selected HMBC correlations (C−H) observed for **1**.

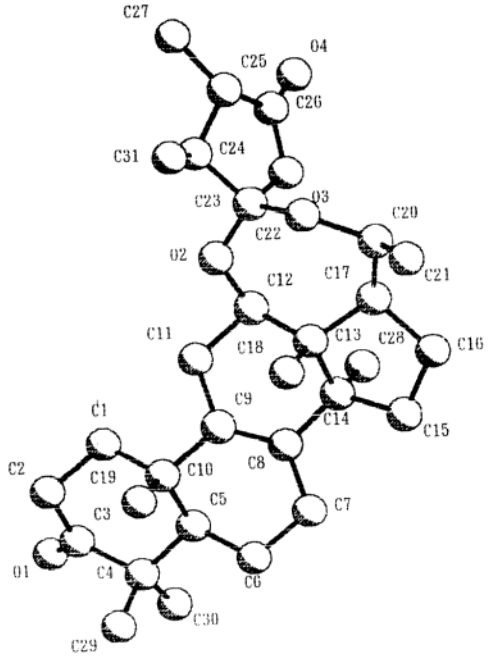


Figure 2. X-ray structure of **3**.

crystal X-ray diffraction study. The crystal data are given in the Experimental Section, and the structure and stereochemistry of the molecule are represented in Figure 2.

The results indicated that fomlactone C is a lanostane-type triterpenoid with novel structural features. In the crystal structures, ring A adopted a twist-chair conformation, rings B and C adopted semichair conformation, and ring D was in an envelope conformation. The rings A/B, C/D, E/C, and E/D were all *trans*-fused, and E and F were spiro-fused. Combined with the known absolute configuration of the lanostane skeleton, the configurations at each chiral position were assigned as 12*R*, 17*R*, 20*R*, 23*S*, 24*S*, and 25*S*.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotations were measured in $CDCl_3$ solution at 15 °C. IR spectra were taken with a Perkin-Elmer 683 spectrophotometer. EIMS and HREIMS data were obtained with a VG ZAB-2F spectrometer. NMR spectra were obtained with a Varian Mercury-300 NMR spectrometer using TMS as internal standard. The CD spectrum was run on a Jasco-J 500C spectrometer. TLC used silica gel F_{254}. Separation and purification were performed by column chromatography on silica gel (200−300 mesh).

Fungal Material. The fungi were collected from Jiling Province, China, in 1997 and identified by Professor Bai, Wang (Chang Bai Mountain Protection Institute, Jiling Province, China). A voucher specimen (970803) was deposited in the herbarium of the Department of Botany, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Table 1. ^{13}C NMR Data (δ) for Fomlactone A (**1**) and B (**2**)[a] ($CDCl_3$)

position	**1**	**2**
1	30.9	30.3
2	23.6	23.7
3	77.9	75.9
4	36.7	36.9
5	45.2	44.1
6	17.8	18.0
7	25.6	25.7
8	135.0	135.0
9	133.6	133.5
10	36.8	37.6
11	42.3	40.1
12	75.8	75.8
13	52.6	52.6
14	48.1	49.2
15	29.4	29.5
16	23.2	25.7
17	42.9	42.3
18	11.7	11.8
19	18.9	18.9
20	32.9	29.5
21	19.7	19.7
22	32.9	31.8
23	110.0	109.9
24	28.7	28.1
25	50.6	50.6
26	177.6	177.4
27	13.8	13.6
28	24.7	24.7
29	27.6	28.1
30	21.8	22.2
31	13.1	13.0
CH_3COO	170.9	
CH_3COO	21.4	

[a] Assignment were established by DEPT and HMBC spectra and reference to literature.[3,4]

Table 2. ^1H NMR Data (δ mult, w/2 and J in Hz) for Fomlactones A (**1**), B (**2**), and C (**3**) ($CDCl_3$)

position	**1**	**2**	**3**
2			2.45−2.55 m
3	4.65 (br s, w/2 = 3.0)	3.43 (br s, w/2 = 6.0)	
12	4.35 (t, J = 8.0)	4.33 (t, J = 8.7)	4.35 (t, J = 8.0)
18	0.77 s	0.76 s	0.79 s
19	0.99 s	0.97 s	0.97 s
21	0.98 (d, J = 7.0)	0.99 (d, J = 6.0)	0.99 (d, J = 8.0)
27	1.26 (d, J = 7.0)	1.25 (d, J = 6.0)	1.23 (d, J = 6.0)
28	1.01 s	1.00 s	1.06 s
29	0.92 s	0.95 s	1.12 s
30	0.85 s	0.87 s	1.09 s
31	1.11 (d, J = 7.0)	1.10 (d, J = 7.0)	1.12 (d, J = 7.0)
CH_3COO	2.07 s		

Extraction and Separation. Air-dried fungal material (740 g) was thoroughly extracted with petroleum ether in a Soxhlet apparatus under reflux. After removal of the solvent, the residue was further extracted with MeOH and the solvent was removed under reduced pressure. The MeOH extract (ca. 200 g) was suspended in H_2O and partitioned with Et_2O. The combined Et_2O layers (ca. 180 g) were extracted again with 0.5 M aqueous NaOH to obtain neutral and acidic fractions. The neutral Et_2O extract (ca. 11 g) was subjected to column chromatography by gradient elution with petroleum ether and EtOAc to yield fomlactones A (**1**, 13 mg), B (**2**, 15 mg), and C (**3**, 0.9 mg).

Fomlactone A (1): white crystalline needles (MeOH); mp 184−186 °C; $[\alpha]^{15}_D$ +30° (c 0.02, $CHCl_3$); IR (KBr) ν_{max} 1780, 1734, 1375, 1248 cm^{-1}; CD ($CHCl_3$) $\Delta\epsilon$ (nm) +9.8 (239); ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 1; EIMS *m/z*

526 $[M]^+$ (10), 511 (39), 493 (9), 451 (52), 433 (27), 153 (100); HREIMS *m/z* 526.3686 (calcd for $C_{33}H_{50}O_5$, 526.3658).

Fomlactone B (2): white crystalline needles (MeOH); mp 278−280 °C; $[\alpha]^{15}_D$ +37° (*c* 0.06, $CHCl_3$); IR (KBr) ν_{max} 3543, 1759, 1221 cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR data, see Table 1; EIMS: *m/z* 484 $[M]^+$ (10), 469 (100), 466 (1), 451 (20), 153 (25); HREIMS *m/z* 484.3550 (calcd for $C_{31}H_{48}O_4$, 484.3553).

Fomlactone C (3): white crystals (MeOH); mp 246−250 °C; $[\alpha]^{15}_D$ +40° (*c* 0.03, $CHCl_3$); IR (KBr) ν_{max} 1778, 1705 cm^{-1}; ^1H NMR data, see Table 2; EIMS *m/z* 482 (10), 467 (68), 449 (20), 153 (100); HREIMS *m/z* 482.3415 (calcd for $C_{31}H_{46}O_4$, 482.3396).

X-ray Crystal Data of 3.[8] Fomula $C_{31}H_{46}O_4$, D_{cal} = 1.169 g/cm^3. Single-crystal X-ray diffraction data were collected by using a MAC Science DIP 2030k image plate with graphite monochrome Mo Kα radiation. The crystal (0.03 × 0.20 × 0.50 mm) belongs to the orthorhombic system, space group $P2_12_12_1$. Accurate cell parameters are a = 7.572(1) Å, b = 9.924(1) Å, c = 36.434(1) Å, V = 2737.8 $Å^3$, Z = 4. There were 2196 reflections, of which 2054 ($|F|^2 \geq 8\sigma|F|^2$) were observed. The position of 22 nonhydrogen atoms was obtained directly from an E-map. The structure was solved by direct methods using SHELXS-86 for the refinement. Positions of the other nonhydrogen atoms were obtained, and the kind of atoms was determined by using least-squares calculations and difference Fourier methods in turn. Geometric calculations and difference Fourier methods proved positions of all hydrogen atoms. The structure was finally refined to R_f = 0.073 and R_w = 0.068.

Acknowledgment. The authors thank Professor Ke Di, Cheng of the Department of Biosynthesis, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, for the donation of fungal material.

References and Notes

(1) Batta, A. K.; Rangaswami, S. *J. Chem. Soc., Perkin Trans. 1* **1975**, *5*, 451−455.
(2) Anderson, C. G.; Epstein, W. W.; Van Lear G. *Phytochemistry* **1972**, *11*, 2847−2852.
(3) Keller, A. C.; Maillard, M. P.; Hostettmann, K. *Phytochemistry* **1996**, *41*, 1041−1046.
(4) Sharma, M. C.; Ohira, T.; Yatagai, M. *Phytochemistry* **1994**, *37*, 201−203.
(5) Lakshmi, V.; Raj, K.; Kapil, R. S. *Phytochemistry* **1989**, *28*, 943−945.
(6) De Grazia, C. G.; Klyne, W.;Scopes, P. M.; Sparrow, D. R.; Whalley, W. B. *J. Chem. Soc. (C)* **1966**, 896−899.
(7) Jennings, J. P.; Klyne, W.; Scopes, P. M. *J. Chem. Soc. (C)* **1965**, 7211−7229.
(8) Crystallographic data for the structure reported in this paper have been deposited with the Combridge Crystallographic Data Center (deposition number CCDC 177785). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

NP0301994