Lanostane Triterpenoids and Triterpene Glycosides from the Fruit Body of *Fomitopsis pinicola* and Their Inhibitory Activity against COX-1 and COX-2

Kazuko Yoshikawa,* Megumi Inoue, Yuki Matsumoto, Chika Sakakibara, Hideki Miyataka, Hitoshi Matsumoto, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-Cho, Tokushima 770-8514, Japan

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Two new lanostane triterpenoids (**1**, **2**) and **10** new lanostane triterpene glycosides (**3**−**12**) have been isolated from the fruit bodies of *Fomitopsis pinicola*. Their structures were established primarily by NMR experiments and chemical methods, and their biological activity against COX-1 and COX-2 was investigated.

In the course of our program aimed at the discovery of biologically active compounds from fungi,[1] we have initiated the chemical study of *Fomitopsis pinicola* (Pers.) Pat. (Polyporaceae), which grows on trees in needle-leaved forests throughout Japan.[2,3] An earlier study of this fungus resulted in the isolation of lanostane triterpenes, and no biological activity was reported.[4−6] In our investigation, two new lanostane triterpenes, named fomitopinic acids A (**1**) and B (**2**), and **10** new lanostanoid glycosides named fomitosides A (**3**), B (**4**), C (**5**), D (**6**), E (**7**), F (**8**), G (**9**), H (**10**), I (**11**), and J (**12**) were isolated from the 70% EtOH extract of the fruit bodies of *F. pinicola*. We describe here the isolation and structure elucidation of compounds **1**−**12**, primarily by extensive NMR experiments and chemical degradation. The configuration of C-24 in **1** was verified by Mosher's method. The antiinflammatory activity against cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) was examined.

Results and Discussion

Fomitopinic acid A (**1**) gave an $[M + Na]^+$ peak at *m/z* 511.3385 in its HRFABMS. It corresponds to a molecular formula of $C_{30}H_{48}O_5$, requiring seven unsaturation equivalents. Absorptions at 3400, 1710, 1070, and 1040 cm^{-1} in the IR spectrum suggested hydroxy and carbonyl groups in **1**. The ^1H NMR spectrum of **1** exhibited seven methyl singlets at δ 0.96, 1.00, 1.04, 1.04, 1.13, 1.46, and 1.51 and one methine proton at δ 3.83 (dd, $J = 10.5$, 1.9 Hz). The 30 carbon signals were sorted into seven methyl, ten methylene, four methine, one of which had an oxygen substituent (δ 79.4), five sp^3 quaternary, one of which had an oxygen substituent (δ 72.6), two sp^2 [δ 135.0 (s), and 133.6 (s)], and two carbonyl carbons (δ 216.3 and 179.1) by DEPT experiment. These data suggest that **1** is a tetracyclic triterpene. The structure of **1** was deduced from detailed analysis of ^1H and ^{13}C NMR data aided by 2D NMR including COSY, HMQC, HMBC, and ROESY experiments. The COSY spectrum revealed connectivities of C-1 to C-2, C-5 to C-7, C-11 to C-12, C-15 to C-17 and C-20, and C-22 to C-24. HMBC correlations completed the definition of all the functional groups in the lanostane framework. Long-range correlations of Me-18 (δ 1.04) to C-12, C-13, C-14, and C-17, Me-19 (δ 1.00) to C-1, C-5, C-9, and C-10, Me-26 (δ 1.46) and Me-27 (δ 1.51) to C-24 and C-25, Me-28 (δ 1.13) and Me-29 (δ 1.04) to C-3, C-4, and C-5, Me-30 (δ 0.96) to C-8, C-13, C-14, and C-15, and H-20 [δ 2.67 (dt, $J = 11.0$, 3.3 Hz)] to C-21 revealed one double bond at C-8, two hydroxy groups attached at C-24 and C-25, and two carbonyl groups attached at C-3 and C-21. The relative configuration of **1** was established by a ROESY experiment. Significant NOE correlations between H-20 (δ 2.67) and Me-18 (δ 1.04) and between Me-30 (δ 0.96) and H-17 [δ 2.48 (m)] indicated an α-orientation of H-17 and *R*-configuration of C-20. For the determination of the absolute configuration at C-24 in the side chain, the advanced Mosher's method was applied.[7] The (*S*)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) ester (**1b**) and the (*R*)-MTPA ester (**1c**) were prepared from the methyl ester of **1** (**1a**). The ^1H NMR chemical shifts of the (*R*)-MTPA ester (**1c**) were subtracted from the values of the (*S*)-MTPA ester (**1b**) [$\Delta\delta = \delta$ (*S*)-MTPA − δ (*R*)-MTPA] and are shown in Figure 1. The negative $\Delta\delta$ values for H_2-16, H-17, H-20, H_2-22, and H_2-23 and positive values of H-24, H_3-26, and H_3-27 unambiguously indicated that C-24 had an *S*-configuration. Hence, the assumed structure of fomitopinic acid A was assigned as 24*S*,25-dihydroxy-3-oxolanost-8-en-21-oic acid.

Fomitopinic acid B (**2**) gave a molecular formula of $C_{32}H_{52}O_6$ from an $[M + Na]^+$ peak at *m/z* 555.3658 in its HRFABMS. The IR spectrum of **2** showed absorptions for hydroxy (3395 cm^{-1}) and acetyl (1730 and 1235 cm^{-1}) functions. Detailed comparison of the ^{13}C NMR data of **2** and **1** revealed that **2** differed from **1** in the A-ring (C-1-C-5, C-28), indicating the presence of an acetoxy group [δ 170.0 (s), 21.1(q) and 78.0 (d)] in **2**. The secondary hydroxy carbon at δ 78.0 was correlated with the downfield shifted signal at δ 4.83 (brs) in the HMQC experiment, which was assigned at H-3 by the COSY experiment. H-3 showed HMBC long-range correlation with the carbonyl carbon at δ 170.0 of the acetyl group. The α-orientation of 3-*O*Ac could be confirmed from its coupling pattern and ROESY correlation between H-3 (δ 4.83) and H_3-28 (δ 0.95) and H_3-29 (δ 0.87). The lanostane relative configuration of **2** was confirmed by ROESY analysis as was described for **1**. However, the absolute configuration of C-24 in **2** could not be elucidated because of lack of material. Thus, the structure of fomitopinic acid B was assigned as 24,25-dihydroxy-3α-acetoxylanost-8-en-21-oic acid.

Fomitoside A (**3**) had a quasi-molecular ion peak at *m/z* 625.3711 $[M + Na]^+$ in the HRFABMS, which matched a formula of $C_{35}H_{54}O_8$. The NMR spectra of **3** indicated a lanostane glycoside. The ^1H NMR spectrum of **3** exhibited one anomeric proton as a doublet ($J = 8.0$ Hz) at δ 6.25.

* To whom correspondence should be addressed. Tel: +81-88-622-9611. Fax: +81-88-655-3051. E-mail: yosikawa@ph.bunri-u.ac.jp.

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Chart 1

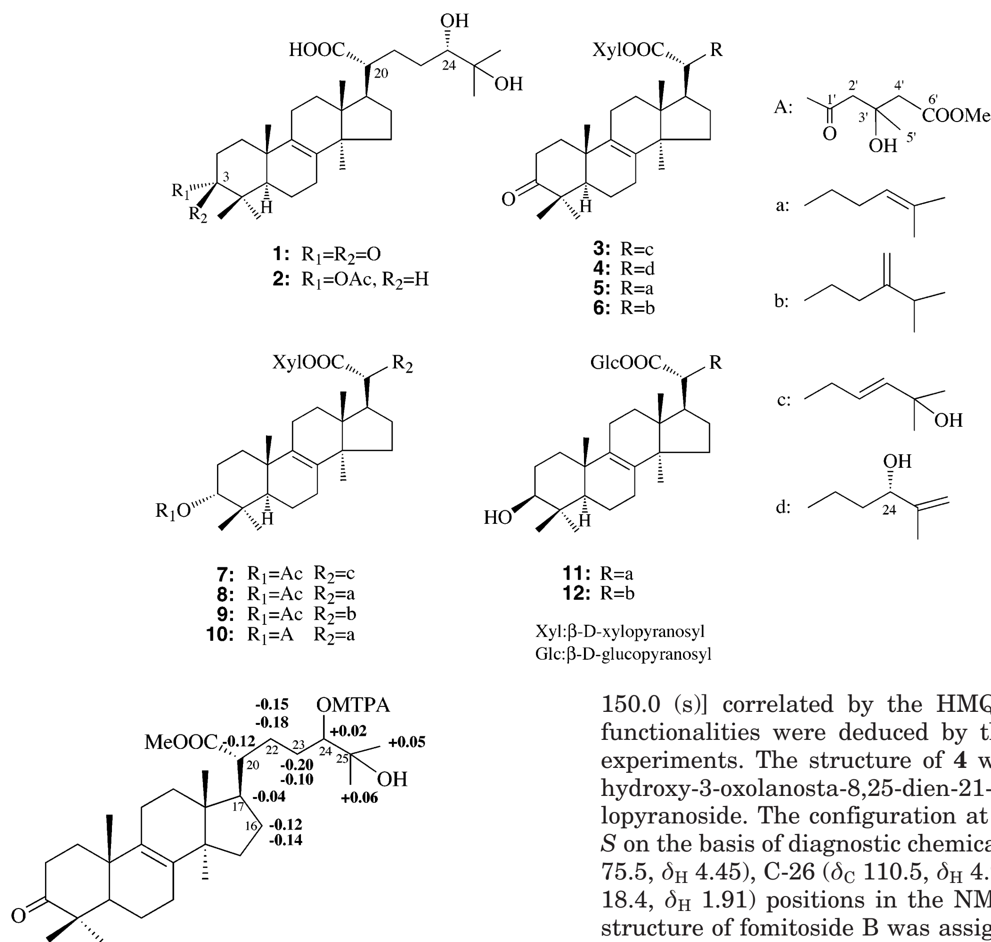

Figure 1. ^{1}H NMR chemical shift differences for MTPA esters of **1a**.

Analysis of the COSY spectrum suggested the presence of a β-D-xylopyranosyl unit in **3**. On acid hydrolysis with 3% H_2SO_4, **3** liberated D-xylose, identified by HPLC analysis using an optical rotation detector.[8] Detailed comparison of the ^{13}C NMR data obtained for **3** and **1** revealed that **3** differed from **1** in the side chain (C-21-C-27). It is composed of two tertiary methyls [δ 30.7 (q) $\times$ 2], one methylene [δ 36.4 (t)], one disubstituted double bond [δ 123.1 (d), 142.5 (d)], one tertiary carbinol [δ 70.0 (s)], and one carbonyl carbon [δ 175.0 (s)] by the DEPT experiment. The analysis of 2D NMR (COSY, HMQC, HMBC, and ROESY) data revealed 25-hydroxy-3-oxolanosta-8,23-dien-21-oic acid as the aglycone of **3**. The xylose moiety was attached at C-21 through an ester linkage by HMBC long-range correlation from H-1 (δ 6.25) of xylose to C-21 (δ 175.0). The coupling constants for H-23 [δ 6.25 (ddd, J = 15.4, 7.1, 7.1 Hz)] and H-24 [δ 6.16 (d, J = 15.4 Hz)] indicated the E-substituted double bond. Therefore, the structure of fomitoside A was assigned as **3**.

Fomitoside B (**4**) gave the same molecular formula of $C_{35}H_{54}O_8$ from an [M + Na]$^+$ peak at m/z 625.3720 in the HRFABMS as **3**. The ^{13}C NMR spectrum for **4** was similar to that obtained for **3**, suggesting the presences of the carbonyl at C-3 (δ 216.2) and of a β-D-xylopyranosyl moiety at C-21 (δ 176.0) through an ester linkage. However, the carbon signals due to the side chain (C-22–C-27) were different from those of **3**. It is composed of one tertiary methyl (δ_C 18.4, δ_H 1.91), two methylenes [δ_C 33.8, δ_H 2.08 (m), 2.14 (m), and δ_C 29.7, δ_H 1.95 (m), 2.14 (m)], one secondary carbinol [δ_C 75.5, δ_H 4.45 (t, J = 5.7 Hz)], and exo-methylene [δ_C 110.5, δ_H 4.93, 5.26 (each brs), and δ_C 150.0 (s)] correlated by the HMQC experiment. These functionalities were deduced by the COSY and HMBC experiments. The structure of **4** was determined as 24-hydroxy-3-oxolanosta-8,25-dien-21-oic acid 21-O-β-D-xylopyranoside. The configuration at C-24 was assigned as S on the basis of diagnostic chemical shifts of the C-24 (δ_C 75.5, δ_H 4.45), C-26 (δ_C 110.5, δ_H 4.93, 5.26), and C-27 (δ_C 18.4, δ_H 1.91) positions in the NMR spectra.[9] Thus, the structure of fomitoside B was assigned as **4**.

Fomitoside C (**5**) had the molecular formula $C_{35}H_{54}O_7$ as established by HRFABMS, which differs from **4** by one oxygen atom. The ^{13}C NMR spectrum for **5** was also similar to that of **4**, suggesting the presence of the carbonyl group at C-3 (δ 216.2) and of a β-D-xylopyranosyl linkage at C-21 (δ 175.8). The signals due to C-22–C-27 of **5** were in good agreement with those of trametenolic acid (**13**), which has a 24-ene functionality in the side chain.[10] Thus, the structure of fomitoside C was assigned as 3-oxolanosta-8,24-dien-21-oic acid 21-O-β-D-xylopyranoside.

Fomitoside D (**6**) had the molecular formula $C_{36}H_{56}O_7$ by HRFABMS, 14 mass units (CH_2) higher than that of **5**. The ^{1}H NMR spectrum of **6** exhibited the presence of the characteristic exo-methylene protons at δ 4.89 (brs) and 4.95 (brs), which accounted for the CH_2 function and the β-D-xylopyranosyl group. The ^{13}C NMR spectrum for **6** was similar to that of **5**, except for the side chain. The ^{13}C NMR data due to C-22–C-27 and C-31 of **6** were analogous to those of eburicoic acid (**14**),[10] which suggested a methylene at C-24 instead of a hydrogen in **5**. The structure of fomitoside D was thus assigned as 3-oxolanosta-8,24(31)-dien-21-oic acid 21-O-β-D-xylopyranoside.

The molecular formula, $C_{37}H_{58}O_9$, of fomitoside E (**7**) was deduced from HRFABMS. The ^{1}H NMR and ^{13}C NMR spectra of **7** exhibited the presence of the acetyl group (δ_H 1.94, δ_C 170.0, 21.1), in addition to the β-D-xylopyranosyl group. Comparison of the ^{13}C NMR data of **7** with those of **3** revealed that **7** had an acetyl function at C-3 [δ 78.0 (d)] instead of a carbonyl function. Hβ-3 was observed at δ 4.84 as a singlet, and the carbon signals in the A-ring were similar to those of **2**. Hence, the structure of fomitoside E was assigned as 25-hydroxy-3α-acetoxylanost-8-en-21-oic acid 21-O-β-D-xylopyranoside.

Table 1. ^{13}C NMR Spectoscopic Data (δ) for **1**−**13** (in pyridine-d_5, 150 MHz)

C no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	36.1	31.4	36.1	36.4	36.4	36.1	31.1	31.4	31.4	31.5	36.3	36.3	36.1
2	34.6	23.9	35.0	35.0	35.0	34.7	23.9	23.9	23.9	23.9	28.9	29.0	28.7
3	216.3	78.0	216.1	216.2	216.2	216.4	78.0	78.0	78.0	78.1	78.2	78.2	78.0
4	47.3	37.2	47.6	47.6	47.6	47.3	37.2	37.3	37.3	37.2	39.8	39.8	39.5
5	51.2	46.0	51.5	51.5	51.5	51.2	46.0	46.0	46.0	46.1	51.5	51.1	50.9
6	19.5	18.6	19.9	19.0	19.9	19.6	18.6	18.6	18.6	18.6	19.0	19.1	18.7
7	26.4	26.5	26.8	26.8	26.8	27.2	26.5	26.7	26.6	26.5	27.1	27.1	26.6
8	133.6	134.4	135.1	135.2	133.9	135.0	134.2	134.2	134.3	134.1	135.2	135.4	134.0
9	135.0	135.0	134.3	133.9	135.0	133.8	134.2	135.2	135.6	135.2	134.1	134.6	134.6
10	37.0	37.5	37.3	37.4	37.4	37.1	37.5	37.5	37.5	37.5	37.6	37.7	37.4
11	21.2	21.6	21.7	21.7	21.7	21.3	21.7	21.8	21.8	21.7	21.7	21.8	21.3
12	29.3	29.6	29.2	29.3	29.3	29.0	29.2	29.3	29.3	29.3	29.3	29.4	29.4
13	44.8	45.1	45.2	45.2	45.2	44.9	45.2	45.2	45.2	45.2	45.2	45.3	44.9
14	49.8	50.1	50.1	50.1	50.1	49.8	50.0	50.5	50.0	50.0	50.0	50.0	49.9
15	30.8	31.1	31.2	31.2	31.2	30.9	31.4	31.2	31.2	31.1	31.2	31.2	30.9
16	27.4	27.7	27.3	27.5	27.6	27.2	28.1	27.6	27.5	27.6	27.6	27.6	27.5
17	47.8	48.0	47.6	48.0	47.9	47.6	47.5	47.8	47.9	47.8	47.9	47.9	47.7
18	16.4	16.7	16.9	16.9	16.9	16.6	16.8	16.8	16.9	16.8	16.7	16.7	16.3
19	18.6	19.4	19.0	19.9	19.0	18.7	19.4	19.4	19.4	19.4	19.7	19.8	19.4
20	49.8	50.1	49.4	49.0	48.6	48.4	49.3	48.6	48.6	48.6	48.8	48.8	49.0
21	179.1	179.1	175.0	176.0	175.8	175.9	175.4	175.9	175.9	175.9	175.8	176.0	178.6
22	31.0	31.1	36.4	29.7	33.8	32.0	36.2	33.8	32.4	33.8	33.8	32.2	33.3
23	30.5	30.9	123.1	33.8	26.7	32.2	123.1	26.5	32.5	26.7	26.8	32.6	26.7
24	79.4	79.7	142.5	75.5	124.8	155.8	142.4	124.8	155.8	124.7	124.8	155.8	124.9
25	72.6	72.9	70.0	150.0	132.0	34.2	70.0	132.0	34.5	131.9	131.9	34.5	131.6
26	25.8	26.2	30.7	110.5	26.1	22.1	30.7	26.1	22.2	26.1	26.1	22.4	25.8
27	25.9	26.3	30.7	18.4	18.2	21.9	30.7	18.2	22.3	18.2	18.1	22.3	17.7
28	26.3	28.1	26.7	26.7	26.7	26.4	22.2	24.7	24.7	28.3	28.9	29.0	28.6
29	21.1	22.2	21.7	21.7	21.7	21.3	28.1	28.1	28.1	22.2	16.8	16.8	16.4
30	24.4	24.7	24.8	24.8	24.8	24.5	24.7	22.2	22.4	24.7	24.7	24.8	24.5
31						107.3			107.3			107.2	

The molecular formula, $C_{37}H_{58}O_8$, of fomitoside F (**8**) was deduced from HRFABMS. The ^{1}H NMR and ^{13}C NMR spectra of **8** were similar to those of 3α-acetoxylanosta-8,24-dien-21-oic acid.[6] The only difference was the presence of the β-D-xylopyranosyl (δ 6.26) group at C-21 (δ 175.4), confirmed by the HMBC experiment. Consequently, the structure of fomitoside F was assigned as **8**.

Fomitoside G (**9**) gave an [M ($C_{38}H_{60}O_8$) + Na]$^+$ peak at *m/z* 667.4181 in its HRFABMS. The ^{1}H NMR spectrum for **9** exhibited the presence of the β-D-xylopyranosyl group, and the ^{13}C NMR spectra of **9** were similar to those of **6**. The only difference was the presence of the acetoxy group [δ 21.1, 170.0, 78.0 (C-3)] instead of the carbonyl group at C-3 (δ 216.4) in **6**. Thus, the structure of fomitoside G was assigned as 3α-acetoxylanosta-8,24(31)-dien-21-oic acid 21-*O*-β-D-xylopyranoside.

Fomitoside H (**10**) gave an [M ($C_{42}H_{66}O_{11}$) + Na]$^+$ peak at *m/z* 769.4495 in its HRFABMS. Detailed comparison of the ^{13}C NMR data of **10** with those of **8** revealed that they differed in the ester group attached at α-OH of C-3. The carbon signals due to the acyl moiety, that is, the tertiary methyl (δ_C 28.4, δ_H 1.69) attached to the carbon atom [δ 69.9 (s)] bearing a hydroxy group, and signals of two methylene groups [δ_C 46.4, δ_H 2.96, 3.00 (each d, J = 14.7 Hz), and δ_C 46.0, δ_H 3.01, 3.07 (each d, J = 14.7 Hz)], one carbonyl (δ 171.3), and methoxycarbonyl signal [δ_H 3.60 (s), δ_C 171.9, 51.5] indicated the presence of a methyl ester of a 3-hydroxy-3-methylglutaryl (HMG) moiety.[6] H-3 [δ 4.84 (brs)] was correlated with the carbonyl carbon at δ 171.3 (C-1′) of the HMG moiety in the HMBC experiment. Thus, the structure of fomitoside H was assigned as **10**.

Fomitoside I (**11**) has a molecular formula of $C_{36}H_{58}O_8$ as established by HRFABMS. The ^{1}H NMR and ^{13}C NMR spectra for **11** exhibited the lanostane β-glucopyranosyl group. Acid hydrolysis with 3% H_2SO_4 of **11** liberated D-glucose, which was identified to be similar to D-xylose in **3**. The ^{13}C NMR spectrum for **11** was similar to that obtained for trametenolic acid (**13**),[10] which had β-OH at C-3. The anomeric proton (δ 6.40) showed a long-range correlation with C-21 (δ 175.8) by the HMBC experiment. Thus, the structure of fomitoside I was assigned as trametenolic acid 21-*O*-β-D-glucopyranoside.

The molecular formula, $C_{37}H_{60}O_8$, of fomitoside J (**12**) was established by HRFABMS, 14 mass units (CH_2) more than **11**. The ^{13}C NMR spectrum for **12** was similar to that obtained for **11**, in addition to the β-D-glucopyranosyl group. However, the side chain (C-22−C-27, C-31) was the same as that of eburicoic acid (**14**),[10] having the exo-methylene at C-24. Thus, the structure of fomitoside J was determined as eburicoic acid 21-*O*-β-D-glucopyranoside.

The COX-1 and COX-2 inhibitor activities of the constituents isolated from this fungus were studied.[11,12] Aspirin was used as positive control. The results are listed in Table 3. Relatively, test compounds effectively inhibited COX-2. In particular, compounds **1**, **7**, and **8** showed significant activities (IC_{50} 0.15−1.15 μM) corresponding to indomethacin (IC_{50} 0.60 μM)[13] against COX-2. The search for selective inhibitors of COX-2 is considered important, on the basis of the theory that the side effects, such as gastric lesions, that occurred from inhibition of COX-1 activity were observed with aspirin and indomethacin.[14] Until now, very few compounds of natural origin have been reported to possess COX-2 inhibitory effects.[15] This is the first report of the potent selective inhibitory of COX-2 by lanostane triterpenoids and their glycosides.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO FT/IR-5300, and NMR spectra on a Varian UNITY 600 spectrometer in C_5D_5N and $CDCl_3$ using TMS as internal standard. NMR experiments included COSY, DEPT, HMQC, HMBC, and ROESY. Coupling constants (J values) are given in Hz. The FABMS were measured on a JEOL JMS-PX303 mass spectrometer.

Material. The fruit bodies of *Fomitopsis pinicola* were collected at Tokushima, Japan, in autumn 1999. A voucher

Table 2. ^{13}C NMR Spectroscopic Data (δ) for **2**−**12** (in pyridine-d_5, 150 MHz)

	2	3	4	5	6	7	8	9	10	11	12
acyl moiety											
1′	21.1					21.1	21.1	21.1	171.3		
2′	170.0					170.0	170.0	170.0	46.4		
3′									69.9		
5′									171.9		
6′									28.4		
OMe									51.5		
sugar moiety		xyl	xyl	xyl	xyl	xyl	xyl	xyl	xyl	glc	glc
1		96.5	96.6	96.6	96.4	96.4	96.5	96.6	96.4	95.9	95.9
2		73.9	74.0	73.9	73.7	73.9	73.9	73.9	73.7	74.2	74.2
3		78.9	78.9	79.0	78.8	78.9	79.0	79.0	78.8	79.4	79.4
4		71.1	71.1	71.1	70.9	71.1	71.1	71.1	70.9	71.5	71.5
5		68.2	68.3	68.3	68.1	68.2	68.3	68.3	68.1	79.2	79.2
6										62.7	62.8

Table 3. IC_{50} Values (M)[a] of the Isolated Compounds

compound	COX-1	COX-2
1	(18.1%)	1.15×10^{-6}
5	1.91×10^{-3}	5.11×10^{-3}
6	3.33×10^{-3}	2.39×10^{-3}
7	(57.2%)	0.15×10^{-6}
8	(27.5%)	1.13×10^{-6}
9	(21.7%)	1.85×10^{-5}
10	7.39×10^{-5}	(70.1%)
aspirin	1.88×10^{-2}	4.97×10^{-2}
indomethacin[b]	0.10×10^{-6}	0.60×10^{-6}

[a] IC_{50} based on duplicate four-point titration. Values in parentheses are percent inhibition at 10^{-5} g/mL where IC_{50} values were not determined. [b] Indometacin was used as the reference compound.

specimen (TB 3015) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. The fresh fruit bodies (550 g) of *F. pinicola* were extracted with 70% EtOH at room temparature for 6 weeks. The EtOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble portion (23.0 g) was subjected to silica gel column chromatography with $[(Me)_2CH]_2O$−MeOH (50:1−25:6) to afford fractions 1−6. Fraction 4 (4.9 g) was passed through silica gel with $[(Me)_2CH]_2O$−MeOH−H_2O (25:3:0.1−25:8:0.1) to afford fractions 4-1−4-5. Fraction 4-1 was passed through silica gel with $[(Me)_2CH]_2O$−MeOH (50:1−25:1) and preparative HPLC (ODS, 93% MeOH) to afford fomitopsic acid (93.5 mg).[6] Fraction 4-2 was passed through silica gel with $[(Me)_2CH]_2O$−MeOH (25:1−25:4) to afford fractions 4-2-1−4-2-4. Fractions 4-2-2, 4-2-3, and 4-2-4 were successively purified by preparative HPLC (ODS, 65−90% MeOH) to afford fomitopinic acids A (**1**, 19.8 mg) and B (**2**, 3.2 mg) and fomitosides F (**8**, 47.0 mg) and G (**9**, 6.1 mg) from fraction 4-2-2, fomitosides D (**6**, 10 mg) and F (**8**, 10.6 mg) from fraction 4-2-3, and fomitosides D (**5**, 19.8 mg), G (**9**, 4.0 mg), and H (**10**, 13.5 mg) from fraction 4-2-4. Similar purification by silica gel column chromatography and preparative HPLC (ODS, 70−90% MeOH) afforded fomitosides A (**3**, 3 mg), B (**4**, 5.0 mg), I (**11**, 3 mg), and J (**12**, 3.5 mg) from fraction 4-3 and fomitosides C (**5**, 24.0 mg) and E (**7**, 10.0 mg) from fraction 4-4.

Fomitopinic acid A (1): colorless needles ($CHCl_3$); mp 182−184 °C; $[\alpha]^{25}_D$ +33.8° (*c* 1.1, MeOH); FT-IR (dry film) ν_{max} 3400, 1710, 1070, 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.96 (3H, s, Me-30), 1.00 (3H, s, Me-19), 1.04 (6H, s, Me-18, 29), 1.13 (3H, s, Me-28), 1.46 (3H, s, Me-26), 1.51 (3H, s, Me-27), 2.48 (1H, m, H-17), 2.67 (1H, dt, J = 11.0, 3.3 Hz, H-20), 3.83 (1H, dd, J = 10.5, 1.9 Hz, H-24); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 487; HRFABMS *m/z* [M + Na]$^+$ 511.3385 (calcd for $C_{30}H_{48}O_5$ + Na, 511.3399).

Fomitopinic acid B (2): amorphous powder; $[\alpha]^{25}_D$ +16.7° (*c* 0.3, MeOH); FT-IR (dry film) ν_{max} 3395, 1730, 1235, 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.85 (3H, s, Me-30), 0.87 (3H, s, Me-29), 0.92 (3H, s, Me-19), 0.95 (3H, s, Me-28), 1.04 (3H, s, Me-18), 1.46 (3H, s, Me-26), 1.51 (3H, s, Me-27), 1.92 (3H, s, Ac), 2.44 (1H, m, H-17), 2.67 (1H, dt, J = 11.0, 3.3 Hz, H-20), 3.82 (1H, dd, J = 9.7, 1.9 Hz, H-24), 4.83 (1H, brs, H-3); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 531; HRFABMS *m/z* [M + Na]$^+$ 555.3658 (calcd for $C_{32}H_{52}O_6$ + Na, 555.3662).

Fomitoside A (3): amorphous powder; $[\alpha]^{25}_D$ +1.1° (*c* 0.2, MeOH); FT-IR (film) ν_{max} 3400, 1720, 1700, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.93 (3H, s, Me-30), 0.96 (3H, s, Me-19), 1.03 (3H, s, Me-29), 1.04 (3H, s, Me-18), 1.14 (3H, s, Me-28), 1.55 (3H, s, Me-26), 1.56 (3H, s, Me-27), 2.45 (1H, m, H-17), 2.75 (1H, dt, J = 9.4, 3.3 Hz, H-20), 6.16 (1H, d, J = 15.4 Hz, H-24), 6.25 (1H, ddd, J = 15.4, 7.1, 7.1 Hz, H-23), 6.25 (1H, d, J = 8.0 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 601; HRFABMS *m/z* [M + Na]$^+$ 625.3711 (calcd for $C_{35}H_{54}O_8$ + Na, 625.3716).

Fomitoside B (4): amorphous powder; $[\alpha]^{25}_D$ −18.3° (*c* 0.2, MeOH); FT-IR (film) ν_{max} 3400, 1720, 1700, 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.92 (3H, s, Me-30), 0.96 (3H, s, Me-19), 1.02 (3H, s, Me-29), 1.05 (3H, s, Me-18), 1.21 (3H, s, Me-28), 1.91 (3H, s, Me-27), 2.39 (1H, m, H-17), 2.72 (1H, dt, J = 11.3, 3.3 Hz, H-20), 4.45 (1H, t, J = 5.7 Hz, H-24), 4.93, 5.26 (each 1H, brs, H_2-26), 6.27 (1H, d, J = 8.0 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 601; HRFABMS *m/z* [M + Na]$^+$ 625.3720 (calcd for $C_{35}H_{54}O_8$ + Na, 625.3716).

Fomitoside C (5): amorphous powder; $[\alpha]^{25}_D$ +31.4° (*c* 2.4, MeOH); FT-IR (film) ν_{max} 3400, 1700, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.94 (3H, s, Me-30), 0.96 (3H, s, Me-19), 1.03 (3H, s, Me-29), 1.06 (3H, s, Me-18), 1.13 (3H, s, Me-28), 1.64 (6H, s, Me-26, 27), 2.41 (1H, m, H-17), 2.72 (1H, dt, J = 9.4, 3.3 Hz, H-20), 5.28 (1H, m, H-24), 6.27 (1H, d, J = 8.0 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 585; HRFABMS *m/z* [M + Na]$^+$ 609.3777 (calcd for $C_{35}H_{54}O_7$ + Na, 609.3767).

Fomitoside D (6): amorphous powder; $[\alpha]^{25}_D$ +36.6° (*c* 0.6, MeOH); FT-IR (film) ν_{max} 3410, 1720, 1700, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.97 (6H, s, Me-19, 30), 1.03 (6H, d, J = 6.3 Hz, H-26, 27), 1.04 (3H, s, Me-29), 1.08 (3H, s, Me-18), 1.13 (3H, s, Me-28), 2.74 (1H, dt, J = 9.4, 3.3 Hz, H-20), 4.89, 4.95 (each 1H, brs, H_2-31), 6.23 (1H, d, J = 8.3 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 599; HRFABMS *m/z* [M + Na]$^+$ 623.3920 (calcd for $C_{36}H_{56}O_7$ + Na, 623.3924).

Fomitoside E (7): amorphous powder; $[\alpha]^{25}_D$ +1.6° (*c* 0.7, MeOH); FT-IR (film) ν_{max} 3400, 1730, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.84 (6H, s, Me-29, 30), 0.92 (6H, s, Me-19, 28), 1.05 (3H, s, Me-18), 1.54 (3H, s, Me-26), 1.55 (3H, s, Me-27), 1.94 (3H, s, Ac), 2.74 (1H, dt, J = 9.4, 3.3 Hz, H-20), 4.84 (1H, brs, H-3), 6.14 (1H, d, J = 15.2 Hz, H-24), 6.25 (1H, ddd, J = 15.4, 7.1, 7.1 Hz, H-23), 6.24 (1H, d, J = 8.0 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* [M − H]$^-$ 645; HRFABMS *m/z* [M + Na]$^+$ 669.3976 (calcd for $C_{37}H_{58}O_9$ + Na, 669.3979).

Fomitoside F (8): colorless needles (MeOH); mp 185−186.6 °C; $[\alpha]^{25}_D$ −3.4° (*c* 2.6, MeOH); FT-IR (film) ν_{max} 3400, 1735, 1030 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.83 (3H, s, Me-30), 0.86 (3H, s, Me-29), 0.92 (3H, s, Me-28), 1.07 (3H, s, Me-18), 1.63 (6H, s, Me-26, 27), 1.94 (3H, s, Ac), 2.48 (1H, m, H-17), 2.72 (1H, dt, J = 9.4, 3.3 Hz, H-20), 4.84 (1H, brs, H-3), 5.27 (1H,

m, H-24), 6.26 (1H, d, J = 8.0 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS m/z $[M - H]^-$ 629; HRFABMS m/z $[M + Na]^+$ 653.4015 (calcd for $C_{37}H_{58}O_8$ + Na, 653.4029).

Fomitoside G (9): amorphous powder; $[\alpha]^{25}_D$ +5.0° (c 0.7, MeOH); FT-IR (film) ν_{max} 3400, 1730, 1030 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.83 (3H, s, Me-30), 0.87 (3H, s, Me-29), 0.92 (6H, s, Me-19, 28), 1.02 (6H, d, J = 6.3 Hz, Me-26, 27), 1.06 (3H, s, Me-18), 1.93 (3H, s, Ac), 2.48 (1H, m, H-17), 2.72 (1H, dt, J = 9.4, 3.3 Hz, H-20), 4.84 (1H, brs, H-3), 4.87, 4.93 (each 1H, br s, H_2-31), 6.27 (1H, d, J = 8.3 Hz, H-1 of xyl); ^{13}C NMR (C_5D_5N), see Table 1; FABMS m/z $[M - H]^-$ 643; HRFABMS m/z $[M + Na]^+$ 667.4181 (calcd for $C_{38}H_{60}O_8$ + Na, 667.4186).

Fomitoside H (10): amorphous powder; $[\alpha]^{25}_D$ −67.4° (c 0.2, MeOH); FT-IR (film) ν_{max} 3400, 1740, 1700 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.84 (3H, s, Me-30), 0.86 (3H, s, Me-29), 0.91 (3H, s, Me-19), 0.98 (3H, s, Me-28), 1.06 (3H, s, Me-18), 1.62 (6H, s, Me-26, 27), 2.39 (1H, m, H-17), 2.72 (1H, dt, J = 11.3, 3.3 Hz, H-20), 4.84 (1H, brs, H-3), 5.27 (1H, m, H-24), 6.27 (1H, d, J = 8.0 Hz, H-1 of xyl), acyl moiety 1.66 (3H, s, Me-3′), 2.96, 3.00 (each, 1H, d, J = 14.7 Hz, H-2′), 3.01, 3.07 (each, 1H, d, J = 14.7 Hz, H-4′), 3.60 (3H, s, OMe); ^{13}C NMR (C_5D_5N), see Table 1; FABMS m/z $[M - H]^-$ 745; HRFABMS m/z $[M + Na]^+$ 769.4495 (calcd for $C_{42}H_{66}O_{11}$ + Na, 769.4503).

Fomitoside I (11): amorphous powder; $[\alpha]^{25}_D$ +1.77° (c 0.1, MeOH); FT-IR (film) ν_{max} 3400, 1720, 1050 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.94 (3H, s, Me-29), 0.98 (3H, s, Me-19), 1.06 (3H, s, Me-18), 1.09 (3H, s, Me-28), 1.24 (3H, s, Me-30), 1.62 (3H, s, Me-26), 1.64 (3H, s, Me-27), 2.75 (1H, dt, J = 9.4, 3.3 Hz, H-20), 3.42 (1H, dd, J = 11.2, 4.4 Hz, H-3), 5.20 (1H, m, H-24), 6.40 (1H, d, J = 8.0 Hz, H-1 of glc); ^{13}C NMR (C_5D_5N), see Table 1; FABMS m/z $[M - H]^-$ 617; HRFABMS m/z $[M + Na]^+$ 641.4035 (calcd for $C_{36}H_{58}O_8$ + Na, 641.4029).

Fomitoside J (12): amorphous powder; $[\alpha]^{25}_D$ +22.1° (c 0.3, MeOH); FT-IR (film) ν_{max} 3400, 1710 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.97 (3H, s, Me-29), 0.98 (3H, s, Me-19), 1.02 (6H, d, J = 6.3 Hz, Me-26, 27), 1.06 (3H, s, Me-18), 1.09 (3H, s, Me-28), 1.24 (3H, s, Me- 30), 2.75 (1H, dt, J = 11.4, 3.3 Hz, H-20), 3.43 (1H, dd, J = 11.2, 4.4 Hz, H-3), 4.85, 4.92 (each 1H, brs, H_2-31), 6.42 (1H, d, J = 8.0 Hz, H-1 of glc); ^{13}C NMR (C_5D_5N), see Table 1; FABMS m/z $[M - H]^-$ 631; HRFABMS m/z $[M + Na]^+$ 655.4190 (calcd for $C_{37}H_{60}O_8$ + Na, 655.4186).

Methylation of Fomitopinic Acid A (1). A solution of **1** (3.8 mg) in MeOH (0.5 mL) and CH_2Cl_2 (1.5 mL) was treated with $(Me)_3SiCHN_2$ (1.0 mL) for 40 min at room temperature. Workup gave the methyl ester of **1** (**1a**, 4.0 mg). **1a**: amorphous solid; ^{1}H NMR (C_5D_5N) δ 0.88 (3H, s, H_3-30), 0.90 (3H, s, H_3-19), 1.02 (3H, s, H_3-18), 1.05 (3H, s, H_3-29), 1.14 (3H, s, H_3-28), 1.49 (3H, s, H_3-26), 1.52 (3H, s, H_3-27), 2.31 (1H, q, J = 7.5 Hz, H-17), 2.55 (1H, m, H-20), 3.74 (3H, s, COO*Me*), 3.76 (1H, dd, J = 7.5 Hz, H-24).

Preparation of (*S*)- and (*R*)-MTPA Esters of 1a. To a solution of **1a** (1 mg) in pyridine (40 μL) was added (+)-(*S*)-MTPA-Cl (10 μL), and the mixture was stirred for 30 min at room temperature. To the reaction mixture was then added 0.1 M HCl. The mixture was extracted with EtOAc (× 3), the organic layer was washed with 5% $NaHCO_3$ and then saturated NaCl, and the solvent was evaporated to give the (*S*)-MTPA ester (**1b**, 0.7 mg). By the same procedure, the (*R*)-MTPA ester (**1c**, 0.7 mg) was prepared.

(*S*)-MTPA ester of 1a (1b): ^{1}H NMR ($CDCl_3$) δ 0.90 (6H, s, Me-19 and Me-30), 1.03 (6H, s, Me-18 and Me-29), 1.15 (3H, s, Me-28), 1.40 (1H, m, H_2-16), 1.93 (1H, m, H_2-22), 1.96 (1H, m, H_2-23), 1.98 (2H, m, H_2-16), 2.00 (1H, m, H_2-22), 2.26 (1H, dt, J = 9.3, 3.3 Hz, H-17), 2.53 (1H, dt, J = 9.3, 3.3 Hz, H-20), 5.51 (1H, dd, J = 9.3, 3.3 Hz, H-24), 7.68−7.36 (15H, m, Ph-H × 3).

(*R*)-MTPA ester of 1a (1c): ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, s, Me-30), 0.94 (3H, s, Me-19), 1.03 (6H, s, Me-18), 1.06 (6H, s, Me-29), 1.15 (3H, s, Me-28), 1.26 (1H, m, H_2-16), 1.78 (1H, m, H_2-22), 1.82 (1H, m, H_2-22), 1.86 (2H, m, H_2-16, 23), 1.99 (1H, m, H_2-23), 2.22 (1H, dt, H-17), 2.41 (1H, dt, H-20), 5.53 (1H, dd, J = 9.3, 3.3 Hz, H-24), 7.68−7.36 (15H, m, Ph-H × 3).

Acid Hydrolysis of Fomitoside A (3). A solution of **3** (5 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100 °C for 2 h. The reaction mixture was diluted with H_2O, neutralized with Amberlite IRA-35, and evaporated in vacuo to dryness. The identification and the configuration of the sugar were determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC {Shodex RSpak NH_2P-50 4E, MeCN−H_2O −H_3PO_4 (95:5:1), 1 mL/min, 47 °C} by comparison with an authentic sugar (10 mmol each of D-xylose and L-xylose). The sugar portion gave the peak of D-(+)-xylose at 9.10 min.

Acid Hydrolysis of Fomitosides B−J (4−12). A solution of each compound **4**−**12** (each 2 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100 °C for 2.0 h. The reaction mixture was diluted with H_2O and then neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification and the configuration of the sugar were carried out in the same manner as described for **3** to give D-(+)-xylose at 9.10 min from compounds **4**−**10** and D-(+)-glucose at 20.7 min from compounds **11** and **12**.

COX-1- and COX-2-Catalyzed Prostaglandin Biosynthesis Assay in Vitro. Experiments were performed according to Futaki et al.,[16] with modification. In brief, 2 units of COX-1/COX-2 enzyme were suspended in 0.1 M Tris-HCl buffer (pH 7.5) containing hematin (1 mM) and phenol (2 mM), as cofactors. The reaction medium was preincubated with sample for 2 min at 37 °C, and 51.4 μM [1-^{14}C]arachidonic acid (Sigma, St. Louis, MO) was added and incubated for 2 min at 37 °C. To terminate the reaction and extract PGE_2, 400 μL of n-hexane−EtOAc (2:1, v/v) was added to the reaction mixture and the preparation was centrifuged at 2000 rpm for 1 min. The organic solvent phase was discarded. The extraction procedure was repeated twice, then 50 μL of EtOH was added to the aqueous phase, and the preparation centrifuged at 2000 rpm for 1 min. The amount of PGE_2 was measured by radioimmunoassay using a liquid scintillation counter. COX-1 (EC1.14.99.1, isolated from ram seminal vesicles, Cayman Chemical Company, Ann Arbor, MI) and COX-2 (isolated from sheep placenta, purity 70%, Cayman Chemical Company, Ann Arbor, MI) were used.

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