Fomitellic Acids, Triterpenoid Inhibitors of Eukaryotic DNA Polymerases from a Basidiomycete, *Fomitella fraxinea*

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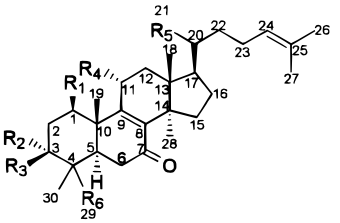
Four new triterpenoid compounds, **1**−**4**, were isolated from the mycelium of a basidiomycete, *Fomitella fraxinea*, and their structures determined by spectroscopic analyses. Compounds **1**−**5** inhibited calf DNA polymerase α and rat DNA polymerase *β*, with respective minimum inhibitory concentration (MIC) ranges of 35−75 and 90−130 *μ*M.

Eukaryotic DNA polymerases are designated as α, *β*, *γ*, *δ*, and *ϵ*, each responsible for different DNA syntheses.[1] The current intense interest to understand the precise in vivo role of the polymerases and of the factors controlling their activity has prompted us to undertake a major search for inhibitors of these enzymes.[2−8] The search for the *β*-type polymerase inhibitors is especially urgent, because the role of the *β*-type polymerase in vivo is still obscure. Although several DNA *β*-polymerase inhibitors have been reported, including the nucleotide dideoxy TTP,[9] flavonoids,[10] sulfate- or sialic acid-containing glycolipids,[11] phospholipids,[12,13] and fatty acids,[14−16] more potent agents are still needed. In this paper, we report the isolation and structure determination of four new triterpenoids (**1**, **2**, **3**, and **4**) and a known compound (**5**) from a mushroom, *Fomitella fraxinea*.

We have previously reported the establishment of an assay[14,15] to detect DNA polymerase inhibitors and have used it to screen microbial secondary metabolites.[14] In the present study, colonies of actinomycetes, fungi, and mycelia of basidiomycetes were collected from field soil near our laboratory. Each mycelia from the actinomycetes (ca. 1500 strains), fungi (200 strains), and basidiomycetes (200 strains) were homogenized in a Waring blender and the Me_2CO-soluble extract evaluated in the DNA polymerase assay system. A basidiomycete, *Fomitella fraxinea*, produced four new triterpenes, which were found to inhibit the activity of mammalian DNA polymerases α and *β* in vitro and to prevent the growth of mammalian cells. The compounds, given the trivial the names of fomitellic acids A (**1**), B (**2**), C (**3**), and D (**4**), were purified by HPLC. In addition, a known compound (**5**) was isolated and identified as 3*β*-hydroxylanosta-8,24-dien-21-oic acid based on its molecular formula, $C_{30}H_{48}O_3$, and its physicochemical and spectrometric data.[17,18]

Results and Discussion

Fomitellic acid A (**1**) was crystallized from EtOAc; its molecular formula of $C_{30}H_{46}O_6$ was determined by HREIMS (*m*/*z* 502.3289), which was consistent with the



	R_1	R_2	R_3	R_4	R_5	R_6
1	OH	H	OH	OH	Me	COOH
2	OH	H	OH	H	Me	COOH
3	OH	H	OH	OMe	Me	COOH
4	OH	OH	H	H	Me	COOH
5	H	H	OH	H	COOH	Me

^{1}H- (Table 1) and ^{13}C-NMR spectra (Table 2). Methylation with CH_2N_2 afforded a methyl ester showing a methyl proton at 4.00 ppm. The ^{1}H-NMR spectrum of **1** showed 42 nonexchangeable protons, including an olefinic proton and seven methyl groups. Analyses of the ^{13}C-NMR, DEPT, and HSQC spectra of **1** further identified the presence of seven methyl carbons; seven methylene carbons; six methine carbons, three of which had oxygen substituents; four sp^2-hybridized carbons, one of which was protonated; four sp^3-hybridized quaternary carbons; a carbonyl carbon; and a carboxyl carbon. The IR spectrum showed hydroxyl (3390 cm^{-1}) and carbonyl (1706 and 1663 cm^{-1}) absorptions. The homonuclear ^{1}H−^{1}H connectivity of **1** was determined using COSY and TOCSY experiments, and the results are presented in Figure 1. The skeleton of **1** was constructed from the HMBC experiment. The observed two- and three-bond correlations are listed in Table 3, and some of the correlations are illustrated in Figure 1. An important aspect of the analyses was the observed HMBC correlations of the carboxyl carbon signal at *δ* 180.2 (s, C-29) with the protons at *δ* 4.09 (CH, H-3), 2.33 (CH, H-5), and 1.18 (CH_3, H-30). These correlations identified the position of the carboxylic acid as C-29. In the case of **5**, the carboxyl carbon signal at *δ* 180.6 (s, C-21) was correlated to three protons at *δ* 2.03 (CH, H-17) and 1.50 (CH_2, H-22). Other important correlations in the HMBC spectrum of **1** observed at *δ* 201.8 (C=O, C-7) with *δ* 2.33 (CH, H-5), 2.80, and 1.98 (CH_2, H-6); *δ* 142.8 (C=, C-8) with *δ* 4.81 (CH, H-11), 2.04 and 1.54 (CH_2, H-15), and 1.11 (CH_3, H-28); and *δ* 160.9 (C=, C-9) with *δ* 4.16 (CH, H-1), 4.81 (CH, H-11), 2.40 and 1.86 (CH_2, H-12), and 1.27 (CH_3, H-19) sug-

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Table 1. ^{1}H NMR Assignments of Fomitellic Acids A (**1**), B (**2**), C (**3**), and D (**4**) in CD_3OD Solutions (500 MHz)

position	A (**1**)	B (**2**)	C (**3**)	D (**4**)
1	4.16 (1 H, dd, J = 11.0, 4.4 Hz)	3.82 (1 H, dd, J = 11.5, 4.4 Hz)	4.38 (1 H, dd, J = 11.7, 4.6 Hz)	4.49 (1 H, dd, J = 11.5, 4.9 Hz)
2	Ha 1.90 (1 H, m)	Ha 1.95 (1 H, m)	Ha 1.81 (1 H, m)	Ha 1.85 (1 H, m)
	Hb 1.95 (1 H, m)	Hb 1.85 (1 H, m)	Hb 1.95 (1 H, m)	Hb 1.95 (1 H, m)
3	4.09 (1 H, dd, J = 12.0, 4.4 Hz)	4.05 (1 H, dd, J = 11.5, 4.7 Hz)	4.04 (1 H, dd, J = 12.3, 4.1 Hz)	3.95 (1 H, dd, J = 2.3, 2.2 Hz)
4				
5	2.33 (1 H, dd, J = 14.8, 2.5 Hz)	2.24 (1 H, dd, J = 12.5, 1.4 Hz)	2.38 (1 H, dd, J = 14.4, 3.2 Hz)	2.78 (1 H, m)
6	Ha 1.98 (1 H, m)	Ha 2.72 (1 H, dd, J = 15.1, 13.7 Hz)	Ha 2.03 (1 H, m)	Ha 1.88 (1 H, m)
	Hb 2.80 (1 H, dd, J = 14.8, 14.8 Hz)	Hb 1.95 (1 H, m)	Hb 2.79 (1 H, dd, J = 15.4, 14.8 Hz)	Hb 2.56 (1 H, dd, J = 15.9, 14.3 Hz)
7				
8				
9				
10				
11	4.81 (1 H, dd, J = 8.8, 7.7 Hz)	Ha 2.56 (1 H, dd, J = 22.0, 4.4 Hz)	5.00 (1 H, dd, J = 9.0, 4.8 Hz)	Ha 2.45 (1 H, ddd, J = 20.9, 9.3, 8.8 Hz)
		Hb 2.91 (1 H, m)		Hb 2.78 (1 H, dd, J = 14.3, 2.2 Hz)
12	Ha 2.40 (1 H, dd, J = 13.7, 9.0 Hz)	1.82 (2 H, m)	Ha 1.89 (1 H, m)	1.74 (2 H, m)
	Hb 1.86 (1 H, m)		Hb 2.29 (1 H, dd, J = 13.1, 9.0 Hz)	
13				
14				
15	Ha 2.04 (1 H, m)	Ha 1.71 (1 H, m)	Ha 1.90 (1 H, m)	Ha 1.52 (1 H, m)
	Hb 1.54 (1 H, m)	Hb 2.01 (1 H, m)	Hb 2.03 (1H, m)	Hb 1.84 (1 H, m)
16	Ha 1.29 (1 H, m)	Ha 1.35 (1 H, m)	Ha 1.28 (1 H, m)	1.30 (2H, m)
	Hb 1.94 (1 H, m)	Hb 1.94 (1 H, m)	Hb 1.96 (1 H, m)	
17	1.55 (1 H, m)	1.45 (1 H, m)	1.56 (1 H, m)	1.38 (1 H, m)
18	0.70 (3 H, s)	0.69 (3 H, s)	0.69 (3 H, s)	0.60 (3 H, s)
19	1.27 (3 H, s)	1.18 (3 H, s)	1.23 (3 H, s)	1.11 (3 H, s)
20	1.38 (1 H, m)	1.07 (1 H, m)	1.09 (1 H, m)	0.97 (1 H, m)
21	0.98 (3 H, d, J = 6.6 Hz)	0.96 (3 H, d, J = 5.5 Hz)	0.97 (3 H, dd, J = 6.3 Hz)	0.86 (3 H, d, J = 6.0 Hz)
22	Ha 1.42 (1 H, m)	1.43 (2 H, m)	Ha 1.07 (1 H, m)	1.20 (2 H, m)
	Hb 1.38 (1 H, m)		Hb 1.47 (1 H, m)	
23	Ha 1.95 (1 H, m)	Ha 1.92 (1 H, m)	Ha 1.65 (1 H, m)	Ha 1.81 (1 H, m)
	Hb 2.02 (1 H, m)	Hb 2.05 (1 H, m)	Hb 1.97 (1 H, m)	Hb 1.92 (1 H, m)
24	5.09 (1 H, dd, J = 7.7, 7.1 Hz)	5.09 (1 H, dd, J = 7.2, 7.1 Hz)	5.09 (1 H, dd, J = 7.7, 7.1 Hz)	5.00 (1 H, dd, J = 7.2, 7.1 Hz)
25				
26	1.67 (3 H, s)	1.67 (3 H, s)	1.60 (3 H, s)	1.57 (3 H, s)
27	1.60 (3 H, s)	1.60 (3 H, s)	1.66 (3 H, s)	1.50 (3 H, s)
28	1.11 (3 H, s)	0.92 (3 H, s)	1.07 (3 H, s)	0.87 (3 H, s)
29				
30	1.18 (3 H, s)	1.17 (3 H, s)	1.17 (3 H, s)	1.08 (3 H, s)
OCH_3			3.38 (3 H, s)	

Table 2. ^{13}C NMR Assignments of Fomitellic Acids A (**1**), B (**2**), C (**3**), and D (**4**) in CD_3OD Solutions (125 MHz)

position	A (**1**)	B (**2**)	C (**3**)	D (**4**)
1	73.0 d	73.1 d	71.7 d	70.4 d
2	36.9 t	38.4 t	38.2 t	35.2 t
3	72.9 d	72.7 d	72.7 d	72.1 d
4	54.9 s	54.5 s	54.4 s	54.5 s
5	45.2 d	45.1 d	45.4 d	41.0 d
6	38.9 t	38.6 t	38.9 t	38.8 t
7	201.8 s	200.1 s	201.5 s	201.0 s
8	142.8 s	140.0 s	143.2 s	141.2 s
9	160.9 s	169.3 s	162.6 s	166.5 s
10	47.4 s	46.3 s	46.5 s	46.2 s
11	66.3 d	27.4 t	74.6 d	23.8 t
12	43.0 t	31.5 t	40.4 t	31.4 t
13	49.5 s	45.6 s	48.2 s	45.4 s
14	47.6 s	49.2 s	49.4 s	49.2 s
15	33.9 t	33.4 t	34.2 t	33.5 t
16	28.7 t	29.8 t	29.0 t	29.7 t
17	50.7 d	50.2 d	51.4 d	50.5 d
18	16.7 q	16.2 q	17.5 q	16.5 q
19	15.0 q	14.4 q	16.5 q	18.9 q
20	37.1 d	37.4 d	37.2 d	37.4 d
21	19.1 q	19.3 q	19.0 q	19.2 q
22	37.2 t	37.3 t	37.3 t	37.4 t
23	25.7 t	25.8 t	25.8 t	25.8 t
24	126.0 d	126.1 d	126.1 d	126.1 d
25	131.8 s	131.8 s	131.8 s	131.8 s
26	25.9 q	25.9 q	25.9 q	25.9 q
27	17.7 q	17.7 q	17.7 q	17.7 q
28	25.8 q	25.5 q	25.0 q	25.3 q
29	180.2 s	180.5 s	179.7 s	180.5 s
30	11.5 q	10.8 q	10.8 q	11.2 q
OCH3			56.1 q	

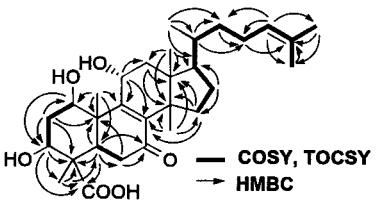

Figure 1. COSY, TOCSY, and selected HMBC correlations of fomitellic acid A (**1**).

gested that the α,β unsaturated ketone was located on the B-ring of lanostane skeleton. The positions of the three hydroxyl groups (C-1, C-3, and C-11) were also determined from the ^1H spectrum of **1**. The stereochemistry of these hydroxyl groups were identified as equatorial based on coupling constant analysis. Because the weak cross peaks of the carboxyl carbon (C-29) with H-3 (ax) and H-5 (ax) and the strong cross peaks of the methyl carbon (C-30) with those protons in the HMBC spectrum were observed, it is suggested that the carboxylic acid could be equatorial.

Fomitellic acid B (**2**) was crystallized from EtOAc and shown to have a molecular formula of $C_{30}H_{46}O_5$ determined by HRFABMS (*m/z* 487.3395 for $C_{30}H_{47}O_5$). This molecular formula implied loss of one oxygen from fomitellic acid A (**1**). The ^1H-NMR, ^{13}C-NMR, DEPT, HSQC, and HMBC spectra of **2** closely resembled those of fomitellic acid A (**1**). When the chemical shifts of **2** were compared with those of **1**, the chemical shift differences of both C-11 and H-11 were Δ38.9 for ^{13}C and Δ1.90 or Δ2.25 for ^1H. These upfield shifts implied that the methine of **1** at C-11 was changed to methylene. An olefinic carbon at δ 169.3 (C-9) has the correlations with two methylene protons at δ 2.91 and 2.56 (H-11) and 1.82 (H-12), which were connected with the carbons at δ 27.4 (C-11) and 31.5 (C-12) in the HSQC apectrum, respectively. Thus, fomitellic acid B was determined as the 11-deoxy derivative of fomitellic acid A (**1**) and has the structure of **2**.

Table 3. HMBC Assignments of Fomitellic Acid A (**1**)

position	A (**1**)	B (**2**)
1	H-2, 3, 19	H-2, 3, 19
2	H-1, 3	H-1, 3
3	H-1, 2, 5, 30	H-1, 2, 30
4	H-2, 3, 5, 30	H-2, 3, 5, 30
5	H-6, 19, 30	H-6, 19, 30
6	H-5	H-5
7	H-5, 6	H-5, 6
8	H-11, 15, 28	H-11, 15, 28
9	H-1, 11, 12, 19	H-1, 5, 11, 12, 19
10	H-1, 2, 5, 6, 11, 19	H-1, 2, 5, 19
11	H-12	H-12
12	H-11, 18	H-11, 18
13	H-12, 15, 18, 28	H-11, 12, 16, 18, 28
14	H-12, 15, 17, 18, 28	H-12, 15, 16, 18, 28
15	H-16, 28	H-16, 17, 28
16	H-15, 17	H-15, 17
17	H-12, 16, 18, 21, 22	H-12, 15, 16, 18, 20, 21, 22
18	H-12, 17	H-12, 17
19	H-1, 5	H-1, 5
20	H-17, 21	H-16, 21, 23
21	H-17, 20	H-17
22	H-20, 21, 24	H-17, 20, 21, 23
23	H-20, 22, 24	H-24
24	H-23, 26, 27	H-23, 26, 27
25	H-23, 26, 27	H-23, 24, 26, 27
26	H-24, 27	H-24, 27
27	H-24, 26	H-24, 26, 27
28	H-15	H-15
29	H-3, 5, 30	H-3, 5, 30
30	H-3, 5,	H-3, 5

Fomitellic acid C (**3**) was obtained as a white powder. A molecular formula was determined by HRFABMS as $C_{31}H_{48}O_6$ (*m/z* 517.3506 for $C_{31}H_{49}O_6$), which implied the addition of a methyl group to fomitellic acid A (**1**). In the HMBC spectrum a carbon at δ 74.6 (C-11) was correlated to a methoxy proton at δ 3.38 (OCH_3). The olefinic carbons at δ 162.6 (C-9) and 143.2 (C-8) correlated with a proton at δ 5.00 (CH, H-11). Thus, the structure of fomitellic acid C was determined to be **3**, an 11-*O*-methyl derivative of fomitellic acid A.

Fomitellic acid D (**4**) was obtained as a white powder. A molecular formula was determined by HRFABMS as $C_{30}H_{46}O_5$(*m/z* 487.3423 for $C_{30}H_{47}O_5$). This molecular formula was the same as that for fomitellic acid A (**1**). The ^1H-NMR, ^{13}C-NMR, DEPT, HSQC, and HMBC spectra of **4** were similar to those of fomitellic acid B (**2**) with an exception of the coupling constants $J = 2.2$, 2.3 Hz for H-3 at δ 3.95 in contrast to $J = 4.7$, 11.5 Hz at δ 4.05 for fomitellic acid A (**1**). This implied that the structure of fomitellic acid D is a 3α-isomer of fomitellic acid A as presented in **4**.

Compounds **1**−**5** effectively inhibited calf DNA polymerase α and rat DNA polymerase β (Table 4).

Experimental Section

General Procedures. ^1H-, ^{13}C-, and 2D NMR measurements were performed on a UNTIY 500 at 500 MHz for ^1H and 125 MHz for ^{13}C. DEPT data were collected on a GEMINI 300. All NMR spectra were recorded in $CDCl_3$ solutions, and the spectra are referenced to the residual CD_3OD peak at 3.30 ppm for ^1H or 49.8 ppm for ^{13}C. Both HREIMS and LREIMS were

Table 4. Minimum Inhibitory Concentration (MIC) Values of Fomitellic Acids Against DNA Polymerases[a]

compound	MIC (μM) DNA polymerase α	DNA polymerase β
fomitellic acid A (**1**)	75	125
fomitellic acid B (**2**)	30	90
fomitellic acid C (**3**)	75	130
fomitellic acid D (**4**)	35	95
3β-hydroxylanosta-8,24-dien-21-oic acid (**5**)	70	100
linoleic acid[b]	60	50

[a] Activity criteria: MIC ≤ 150 μM is considered active. [b] Positive control.

measured on a JEOL DX300 mass spectrometer. HR-FABMS (high-resolution fast atom bombardment mass-spectra) were measured on a JMS HX-110 mass spectrometer. The IR absorption spectra were measured using a Nicolet Impact 400 instrument on KBr pressed-disk samples. Specific rotations were recorded on a Digital Polar meter, JASCO DIP-370.

All reagents and fermentation media components were purchased from Wako Chemical Industries (Tokyo, Japan). TLC plates were purchased from Merck (Darmstadt, Germany) Si gel 60F_{254}, with a layer thickness of 0.5 mm, and the R_f values were determined by using plates 20 cm in length. $CHCl_3$−MeOH−H_2O (v/v/v 10:1:0.5) was used as the solvent system, and after being developed, the compounds were visualized with UV at 254 nm.

DNA Polymerase Inhibition Assay. Assays were performed as previously described.[14,15] Briefly, neutralized crude samples were dissolved in MeOH and sonicated for 30 s. Then 4 μL of the sonicated sample was mixed with 16 μL of calf DNA polymerase α (0.05 units) or rat DNA polymerase β (0.05 units) and held at 0 °C for 10 min. Then 8 μL of the inhibitor−enzyme mixture was added to 16 μL of the standard reaction mixture for DNA polymerase α containing 50 mM Tris−HCl (pH 7.5), 1 mM dithiothreitol, 1 mM $MgCl_2$, 10 μM poly(dA), 5 μM (dT), 10 μM [^{3}H]-dTTP (100 cpm/pmol), 15% (v/v) glycerol. The standard reaction mixture for DNA polymerase β was the same reaction mixture as used above but also containing 150 mM KCl. After incubation at 37 °C for 60 min, the radioactive DNA product was collected on a DEAE-cellulose paper (DE81) disk as described by Lindahl et al. (1970).[19] The radioactivity was measured using a scintillation counter.

Biological Material. More than 200 species of fruiting bodies of basidiomycetes were collected from fields in the vicinity of Noda City of Chiba Prefecture, Japan. The inter-piece (1 cm^3) of each of the fruiting bodies was transferred to an agar slant containing 2% glucose, 0.5% dry yeast, and 2% agar at pH 4.8 and cultured for 14 days at 30 °C. The strain producing a DNA polymerase inhibitor was identified as *Fomitella fraxinea* by the authors. The strain, which will be deposited at the National Insitute of Bioscience and Human-Technology of Japan, is kept in our laboratory.

Extraction and Isolation. The mycelia grown on agar slants were transferred to 50 flasks of liquid medium containing 2% glucose and 0.5% dry yeast at pH 4.8 and cultured for 14 days at 30 °C. The culture (0.1 L × 50) was filtered to remove the mycelia (150 g, dry wt) and, after homogenization by Waring blender, was extracted with Me_2CO (5 L) for 3 days. Evaporation of the solvent yielded 2 g of a yellow waxy material. The extract was partitioned between *n*-butanol (1 L) and H_2O (1 L), adjusted to pH 2, and the organic layer evaporated. The fraction was purified by *n*-hexane (0.5 L)−80% MeOH (0.5 L) partition. The 80% MeOH layer portion (0.87 g) was subjected to Sephadex LH-20 column (2.0 × 40 cm) eluted with MeOH. The active fractions (0.25 g) were purified through a Si gel column chromatography (Wakogel C-200, 200 mesh, 2.0 × 30 cm) using $CHCl_3$−MeOH−H_2O (v/v/v 10:1:0.5). Finally, compounds **1** (4.1 mg), **2** (3.4 mg), **3** (1.1 mg), **4** (0.7 mg), and **5** (5.2 mg) were purified by preparative HPLC [on YMC A-323 column (C_{18}−ODS) 250 × 10 mm in 5% AcOH in 80% MeOH with flow rate at 3 mL/min detected UV at 254 nm] to give their yields of 28.3%, 23.4%, 7.6%, 4.8%, and 35.9%, respectively.

Fomitellic acid A (1): crystal (EtOAc); $[\alpha]_D$ (23 °C) +13 (*c* 0.6, MeOH); mp 218−219 °C; R_f 0.38 (EtOAc−MeOH−H_2O 10:1:0.5); EIMS *m/z* 502 (M^+, 100); HRE-IMS calcd for $C_{30}H_{46}O_6$ 502.3295, found 502.3289; IR λ_{max} (KBr) 3390, 1706, 1663 cm^{-1}.

Fomitellic acid B (2): crystal (EtOAc); $[\alpha]_D$ (23 °C) +8 (*c* 0.57, MeOH); mp 258 °C (dec); R_f 0.38 (EtOAc−MeOH−H_2O 10:1:0.5); FABMS *m/z* 487.3 (M^+ + H, 100); HRFABMS calcd for $C_{30}H_{47}O_5$ 487.3425, found 487.3395; IR λ_{max} (KBr) 3385, 1717, 1637 cm^{-1}.

Fomitellic acid C (3): amorphous solid; R_f 0.38 (EtOAc−MeOH−H_2O 10:1:0.5); EIMS *m/z* 516 (M^+, 58); HRFABMS calcd for $C_{31}H_{49}O_6$ 517.3529, found 517.3506; IR λ_{max} (KBr) 1702, 1663 cm^{-1}.

Fomitellic acid D (4): R_f 0.38 (EtOAc−MeOH−H_2O 10:1:0.5); EIMS *m/z* 486 (M^+, 100); HRFABMS calcd for $C_{30}H_{47}O_5$ 487.3382, found 487.3423.

3β-Hydroxylanosta-8,24-dien-21-oic acid (5): R_f 0.38 (EtOAc−MeOH−H_2O 10:1:0.5); EIMS *m/z* 456 (M^+, 69); IR λ_{max} (KBr) 3431, 2942, 1702 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.77 (3H, s, H-18), 0.79 (3H, s, H-30), 0.97 (3H, s, H-29), 0.98 (3H, s, H-19), 0.99 (3H, s, H-28), 1.04 (1H, dd, J = 2.0, 10.6 Hz, H-5), 1.21 (1H, m, H-1a), 1.22 (1H, m, H-15a), 1.35 (1H, m, H-16a), 1.46 (1H, m, H-12a), 1.50 (2H, m, H-22), 1.55 (1H, m, H-7a), 1.58 (3H, s, H-26), 1.61 (2H, m, H-2), 1.64 (1H, m, H-15b), 1.66 (3H, s, H-27), 1.68 (1H, m, H-12b), 1.70 (1H, m, H-7b), 1.73 (1H, m, H-1b), 1.94 (2H, m, H-23), 1.95 (1H, m, H-16b), 2.01 (2H, m, H-11), 2.03 (1H, m, H-17), 2.06 (2H, m, H-6), 2.11 (1H, ddd, J = 3.4, 11.5 Hz, H-20), 3.16 (1H, dd, J = 4.7, 11.3 Hz, H-3), 5.08 (1H, dd, J = 8.1, 13.5 Hz, C-24); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.1 (q, C-30), 16.4 (q, C-18), 17.7 (q, C-26), 19.4 (t, C-7), 19.6 (q, C-19), 21.9 (t, C-11), 24.6 (q, C-28), 25.8 (q, C-27), 27.0 (t, C-23), 27.6, (t, C-6), 28.1 (t, C-16), 28.4 (t, C-2), 28.6 (q, C-29), 30.0 (t, C-12), 31.5 (t, C-15), 33.7 (t, C-22), 37.0 (t, C-1), 38.2 (s, C-10), 39.9 (s, C-4), 45.5 (s, C-14), 48.4 (d, C-17), 49.5 (d, C-20), 50.6 (s, C-13), 51.9 (d, C-5), 79.6 (d, C-3), 124.9 (d, C-24), 132.8 (s, C-25), 135.5 (s, C-8), 136.0 (s, C-9), 180. 6 (s, C-21).

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