

Two Novel Lanostane-Type Triterpenes from the Stem Bark of *Abies mariesii*

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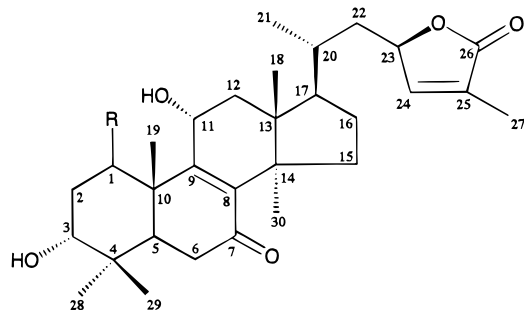
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Two novel lanostane-type triterpene lactones, 3 α ,11 α -dihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide (**1**) and 1 α ,3 α ,11 α -trihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide (**2**), were isolated from the stem bark of *Abies mariesii*, together with a known triterpene, 3-oxo-9 β *H*-lanosta-7,24-dien-26,23(*R*)-olide. The structures of **1** and **2** were determined on the basis of 2D NMR techniques.

Abies mariesii Mast. (Pinaceae) is a tall evergreen tree growing deep in the mountains from central to northern Japan. Previously, we reported the isolation of abieslactone (3 α -methoxy-9 β *H*-lanosta-7,24-dien-26,23(*R*)-olide)¹ and three gammaceran-type triterpenes, gammaceran-3,21-dione, gammaceran-3 α ,21 β -diol, and 3 α -hydroxygammaceran-21-one from the stem bark of *A. mariesii*.² Further investigation of the CHCl₃ extract of the stem bark of *A. mariesii* led to the isolation of two minor lanostane-type triterpene lactones together with a known compound. The known compound was identified as 3-oxo-9 β *H*-lanosta-7,24-dien-26,23(*R*)-olide by direct comparison with a sample that had been isolated from *Abies firma* and *Abies veitchii*.^{3,4}

Compound **1** was assigned the molecular formula C₃₀H₄₄O₅ by HREIMS. Its UV and IR spectra indicated absorption bands for hydroxyl groups (ν_{\max} 3455 cm⁻¹), a γ -hydroxy- α,β -unsaturated six-membered ring ketone (λ_{\max} 244 nm, ν_{\max} 1660 cm⁻¹), and an α,β -unsaturated- γ -lactone (ν_{\max} 1741 cm⁻¹). Its ¹H and ¹³C NMR spectra (Table 1)



1 R = H₂

2 R = α -OH

showed five tertiary methyl groups, a secondary methyl group, two secondary hydroxyl groups [δ_{H} 3.52 (t), δ_{C} 75.0 (d); δ_{H} 4.53 (dd), δ_{C} 65.4 (d)], a tetrasubstituted double bond [δ_{C} 141.5 (s), 160.4 (s)], a carbonyl group [δ_{C} 200.3 (s)], and a 4-substituted 2-methyl-2-butenolide ring [δ_{H} 1.92 (t), 4.98 (ddd), 6.97 (quint); δ_{C} 10.6 (q), 78.7 (d), 129.5 (s), 149.6 (d), 174.4 (s)], which were very similar to the functionalities of abieslactone and its analogues.⁵ The gross structure of **1** was proved by extensive 2D NMR experiments involving the determination of its ¹H–¹H COSY, HOHAHA, HMQC, and HMBC spectra. The homonuclear ¹H–¹H connectivity of **1** was determined using ¹H–¹H COSY and HOHAHA spectra, and the observed two- and three-bond correlations

Table 1. NMR Data for Compound **1** (125 and 500 MHz, CDCl₃)^{a,b}

position	δ_{C}	δ_{H}	COSY (H → H)	HMBC (C → H)
1 α	28.6 t	2.32 m	1 β , 2 β	Me-19
1 β		1.60 m	1 α , 2 α , 2 β	
2 α	25.3 t	1.74 m	1 β , 2 β , 3 β	
2 β		2.04 m	1 α , 1 β , 2 α , 3 β	
3 β	75.0 d	3.52 t (2.5)	2 α , 2 β	Me-28; Me-29
4	37.7 s			H-5 α ; Me-28; Me-29
5	44.7 d	2.30 dd (14.5, 3.0)	6 α , 6 β	H ₂ -6; Me-19; Me-28; Me-29
6 α	36.8 t	2.36 dd (15.8, 3.0)	5, 6 β	
6 β		2.49 dd (15.8, 14.5)	5, 6 α	
7	200.3 s			H ₂ -6
8	141.5 s			Me-30
9	160.4 s			Me-19
10	40.5 s			H-5 α ; H ₂ -6; Me-19
11 β	65.4 d	4.53 dd (9.0, 4.6)	12 α , 12 β	H ₂ -12
12 α	44.0 t	1.88 dd (14.0, 4.6)	11 β , 12 β	Me-18
12 β		2.48 dd (14.0, 9.0)	11 β , 12 α	
13	47.7 s			Me-18; Me-30
14	48.1 s			Me-18; Me-30
15 α	32.7 t	2.07 ddd (12.8, 9.5, 2.0)	16 α , 16 β	Me-30
15 β		1.63 m	16 α , 16 β	
16 α	27.9 t	1.87 m	15 α , 15 β , 16 β , 17	
16 β		1.28 m	15 α , 15 β , 16 α , 17	
17	50.3 d	1.55 m	16 α , 16 β , 20	Me-18; Me-21
18	17.1 q	0.68 s	12 β	H ₂ -12; H-17 α
19	19.6 q	1.24 s	1 α , 1 β	H-5 α
20	33.4 d	1.78 m	17, 21, 22	H-17 α ; Me-21
21	18.4 q	1.04 d (6.5)	20	
22	40.3 t	1.37 ddd (14.0, 10.5, 2.5)	20, 23	Me-21
		1.52 m	20, 23	
23	78.7 d	4.98 ddd (11.0, 2.5, 1.5)	22, 24, 27	H-24
24	149.6 d	6.97 quintet (1.5)	23, 27	Me-27
25	129.5 s			Me-27
26	174.4 s			H-24; Me-27
27	10.6 q	1.92 t (1.5)	23, 24	
28	27.4 q	0.98 s		Me-29
29	21.8 q	0.96 s		H-5 α ; Me-28
30	25.1 q	1.12 s	15 β	H ₂ -15

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^a Assignments confirmed by decoupling, H/H COSY, NOESY, HMQC, and HMBC spectra. ^b *J* values are given in Hz.

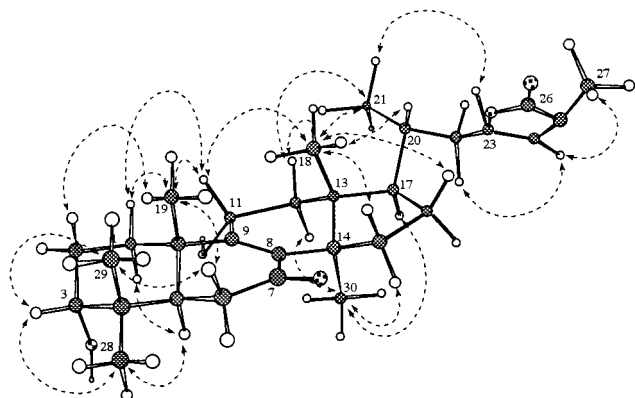


Figure 1. NOESY correlations observed for compound 1.

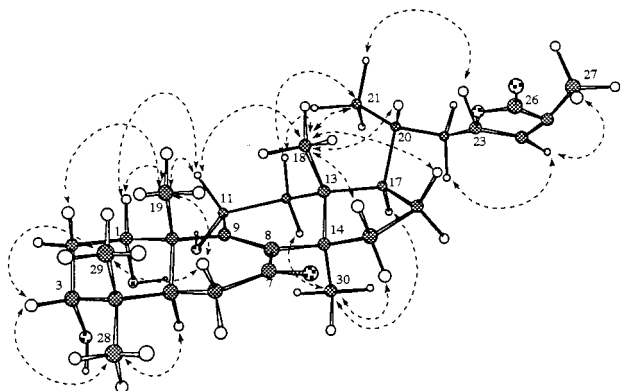


Figure 2. NOESY correlations observed for compound 2.

in the HMBC spectrum are listed in Table 1. One of the two hydroxyl groups in **1** could be placed at the usual C-3 position and the second one at an allylic position because cross-peaks between H-3 (δ_{H} 3.52, t) and H-2 (δ_{H} 1.74, 2.04, each m) and H-11 (δ_{H} 4.53, dd) and H-12 (δ_{H} 1.88, 2.48, each dd) were observed in the ^1H - ^1H COSY spectrum. Thus, the planar structure of **1** was assigned as 3,11-dihydroxy-7-oxo-8,24-dien-26,23-olide. The configurations of the two hydroxyl groups were established by 2D-NOESY spectroscopy and coupling constants (Figure 1). Since cross-peaks were observed between H-3 [δ_{H} 3.52 (t, $J = 2.5$ Hz)] and Me-28 (δ_{H} 0.98, s) and Me-29 (δ_{H} 0.96, s), and H-11 [δ_{H} 4.53 (dd, $J = 9.0, 4.6$ Hz)] and Me-18 (δ_{H} 0.68, s) and Me-19 (δ_{H} 1.24, s), the configuration of the hydroxyl group at C-3 is α -axial and that at C-11 is α -equatorial. The stereochemistry of C-23 was determined as *R* by comparison with the ^1H and ^{13}C NMR chemical shift values of those of abieslactone and its analogues.²⁻⁵ Accordingly, the structure of **1** was elucidated as 3 α ,11 α -dihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide, which has not yet been reported in the literature.

Compound **2** was assigned the molecular formula $\text{C}_{30}\text{H}_{44}\text{O}_6$ by HREIMS. The UV and IR spectra indicated absorption bands for hydroxyl groups (ν_{max} 3345 cm^{-1}), a γ -hydroxy- α,β -unsaturated six-membered ring ketone (λ_{max} 244 nm, ν_{max} 1659 cm^{-1}), and an α,β -unsaturated- γ -lactone (ν_{max} 1738 cm^{-1}). Its ^1H and ^{13}C NMR spectra (Table 2) showed five tertiary methyl groups, a secondary methyl group, three secondary hydroxyl groups [δ_{H} 3.62 (brs), δ_{C} 77.0 (d); δ_{H} 4.19 (dd), δ_{C} 72.1 (d); δ_{H} 4.65 (dd), δ_{C} 64.3 (d)], a tetrasubstituted double bond [δ_{C} 144.8 (s), 157.8 (s)], a carbonyl group [δ_{C} 199.7 (s)], and a 4-substituted 2-methyl-2-butenolide ring [δ_{H} 1.92 (t), 4.98 (ddd), 7.00 (quint); δ_{C} 10.6 (q), 78.8 (d), 129.5 (s), 149.7 (s), 174.5 (s)]. The above spectral data of **2** including the lactone moiety resembled

Table 2. NMR Data for Compound **2** (125 and 500 MHz, CDCl_3)^{a,b}

position	δ_{C}	δ_{H}	COSY (H \rightarrow H)	HMBC (C \rightarrow H)
1 α	72.1 d			Me-19
1 β		4.19, dd (2.8, 3.0)	2 α , 2 β	
2 α	29.2 t	2.13 ddd (15.4, 3.3, 3.0)	1 β , 3 β	
2 β		2.22 ddd (15.4, 2.8, 2.5)	1 β , 3 β	
3 β	77.0 d	3.62 brs	2 α , 2 β	Me-28; Me-29 H-5 α ; Me-28; Me-29
4	38.2 s			Me-28; Me-29
5	39.1 d	2.70 dd (15.0, 3.0)	6 α , 6 β	H ₂ -6; Me-19; Me-28; Me-29
6 α	36.3 t	2.44 dd (15.3, 3.0)	5	H-5 α
6 β		2.56 dd (15.3, 15.0)	5	
7	199.7 s			H-5 α ; H ₂ -6
8	144.8 s			H-11 β ; Me-30
9	157.8 s			Me-19
10	47.2 s			H ₂ -6; Me-19
11 β	64.3 d	4.65 dd (9.2, 4.1)	12 α , 12 β	H ₂ -12
12 α	44.1 t	1.95 dd (13.8, 4.1)	11, 12 β	Me-18
12 β		2.41 dd (13.8, 9.2)	11, 12 β	
13	47.8 s			H ₂ -12; Me-18; Me-30
14	48.6 s			Me-18; Me-30
15 α	32.8 t	2.05 ddd (12.8, 9.5, 2.0)	15 β	Me-30
15 β		1.58 m	15 α	
16 α	28.0 t	1.90 m	17	
16 β		1.28 m	16 α , 17	
17	50.6 d	1.59 m	20	Me-18; Me-21
18	17.7 q	0.66 s	12 α	H ₂ -12; H-17 α
19	19.0 q	1.21 s		H-5 α
20	33.4 d	1.77 m	17, 21, 22	Me-21
21	18.4 q	1.04 d (6.4)	20	
22	40.3 t	1.52 ddd (14.1, 11.0, 3.0)	20, 23	Me-21
23	78.8 d	4.98 ddd (11.0, 2.5, 1.6)	22, 24, 27	H-24
24	149.7 d	7.00 quintet (1.6)	23, 27	Me-27
25	129.5 s			Me-27
26	174.5 s			Me-27
27	10.6 q	1.92 t (1.6)	23, 24	
28	27.7 q	1.06 s		Me-29
29	21.4 q	0.96 s		H-5 α ; Me-28
30	24.9 q	1.21 s		

^a Assignments confirmed by decoupling, H/H COSY, NOESY, HMQC, and HMBC spectra. ^b J values are given in Hz.

those of **1**, except for the presence of a hydroxyl group instead of a methylene group at C-1. This assumption was supported by its ^1H - ^1H COSY, HOHAHA, HMQC, and HMBC experiments (Table 2). In the HOHAHA spectrum of **2**, long-range correlations between H-1 (δ_{H} 4.19, dd) and H-3 (δ_{H} 3.62, brs) were observed. The configuration of the C-1 hydroxyl group was found to be α -axial, since cross-peaks between H-1 β and H-11 β (δ_{H} 4.65, dd) and Me-19 (δ_{H} 1.21, s) were clearly observed in the NOESY spectrum (Figure 2). Therefore, compound **2** was determined to be the novel compound 1 α ,3 α ,11 α -trihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide.

Although lanostane triterpenes having a 1 α ,3 α -diaxial hydroxyl group such as **2** have not yet been isolated from natural sources, lanostan-1 α ,3 α -diol has been obtained from lanost-2-ene as a synthetic product.⁶ Recently, 1 β ,3 β -

dihydroxy-7-oxolanosta-8,24-dien-29-oic acid, 1 β ,3 β ,11 α -trihydroxy-7-oxolanosta-8,24-dien-29-oic acid, 1 β ,3 β -dihydroxy-11 α -methoxy-7-oxolanosta-8,24-dien-29-oic acid, and 1 β ,3 α -dihydroxy-7-oxolanosta-8,24-dien-29-oic acid were obtained from the mycelium of a Basidiomycete, *Fomitella flaxinea*.⁷

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. UV spectra were recorded using a Perkin-Elmer 1720X FTIR spectrophotometer. ¹H and ¹³C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. CDCl₃ was used as the solvent and TMS as the internal standard. EIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over silica gel (70–230 mesh), and medium-pressure liquid chromatography (MPLC) was carried out with silica gel (230–400 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 HF₂₅₄). Preparative TLC was carried out on Merck silica gel PF₂₅₄ plates (20 × 20 cm, 0.5 mm thick).

Plant Material. The stem bark of *A. mariesii* Mast. was collected in the mountainous terrain under the control of National Yamaguchi Forestry Office, Fukushima Prefecture, Japan, in July 1994. A voucher specimen (AM-9407-1) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences. The extraction was carried out in April 1997.

Extraction and Isolation. The chopped stem bark (21.85 kg) of *A. mariesii* was extracted with CHCl₃ in an automatic percolator at 60 °C for 1 week. The CHCl₃ extract (1.36 kg) was chromatographed on silica gel using *n*-hexane–CHCl₃ (1:1), CHCl₃, CHCl₃–EtOAc (5:1–1:1), EtOAc, and EtOAc–MeOH (1:1) as eluents and divided into eight fractions (I–VIII). Fraction II (52.24 g) was rechromatographed by silica gel column chromatography using CHCl₃ to afford a crystalline solid (201.6 mg) (fractions 101–110), which was recrystallized from MeOH–CHCl₃ to give 3-oxo-9 β H-lanosta-7,24-dien-26,23-(*R*)-olide (152.3 mg). This compound was identified by direct comparison with an authentic sample obtained from *A. firma* and *A. veitchii*. Subsequent column chromatography with CHCl₃–EtOAc (10:1) gave a gummy product (516.8 mg)

(fractions 206–232), which was repeatedly subjected to silica gel MPLC with CHCl₃–EtOAc (10:1) and preparative TLC [CHCl₃–MeOH (9:1)] to give compounds **1** (8.7 mg) and **2** (3.2 mg).

3 α ,11 α -Dihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide (1): mp 82–85 °C (MeOH–CHCl₃); [α]_D –16° (*c* 0.47); UV λ_{\max} (EtOH) 244 nm (ϵ 5000); IR ν_{\max} (KBr) 3455 (OH), 2961, 2928, 2873, 1741 ($\alpha\beta$ -unsaturated γ -lactone), 1660 (C=C–O), 1457, 1384 (*gem*-dimethyl) 1275, 1028, 1004, 987, 944 cm^{–1}; ¹H and ¹³C NMR, see Table 1; EIMS (70 eV) *m/z* 484 [M]⁺ (100), 469 [M – Me]⁺ (5), 466 [M – H₂O]⁺ (28), 451 [M – Me – H₂O]⁺ (19), 433 [M – Me – 2H₂O]⁺ (8), 423 [M – Me – H₂O – CO]⁺ (10), 330 [C₂₀H₂₆O₄] (72), 263 (7), 121 [C₉H₁₃] (7), 97 [C₅H₅O₂] (5); HREIMS *m/z* 484.3188 (C₃₀H₄₄O₅, requires 484.3186).

1 α ,3 α ,11 α -Trihydroxy-7-oxolanosta-8,24-dien-26,23(*R*)-olide (2): amorphous solid; [α]_D +20° (*c* 0.29); UV λ_{\max} (EtOH) 244 nm (ϵ 7000); IR ν_{\max} (KBr) 3345 (OH), 2959, 2923, 2852, 1738 ($\alpha\beta$ -unsaturated γ -lactone), 1659 (C=C–O), 1457, 1384 (*gem*-dimethyl), 1162, 1061, 1035 cm^{–1}; ¹H and ¹³C NMR, see Table 2; EIMS (70 eV) *m/z* 500 [M]⁺ (37), 482 [M – H₂O]⁺ (24), 467 [M – H₂O – Me]⁺ (28), 464 [M – 2H₂O]⁺ (26), 449 [M – 2H₂O – Me]⁺ (29), 421 [M – 2H₂O – Me – CO]⁺ (26), 367 (20), 356 (100), 330 [C₂₀H₂₆O₄] (33), 276 (22), 121 [C₉H₁₃] (32), 97 [C₅H₅O₂] (45); HREIMS *m/z* 500.3134 (C₃₀H₄₄O₆, requires 500.3135).

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