Rearranged Lanostane Triterpenoids from *Abies sachalinensis* (III)

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To isolate more rearranged lanostane-type triterpenes from *Abies sachalinensis*, continuous chemical investigation of the ethyl acetate soluble fraction of the methanol extract of *A. sachalinensis* afforded two new rearranged lanostane-type triterpenes (1, 2). Their structures were elucidated to be 3,4-*seco*-4(28),7,12,24-mariesatetraen-26,23-olide-23-hydroxy-3-oic acid (1) and ethyl 3,4-*seco*-8(14→13*R*)*abeo*-17,13-*friedo*-4(28),7,14,24-lanostatetraen-26,23-olide-23-hydroxy-3-oate (2), respectively. The structure of these compounds was determined by spectral studies, especially by two-dimensional (2D)-NMR and high-resolution (HR)-MS. Compounds 1 and 2 have a tautomeric lactone structure in the side chain.

Key words *Abies sachalinensis*; Pinaceae; rearranged lanostane-type triterpene; tautomerism

Many interesting rearranged lanostane-type triterpenes have been isolated from *Abies* sp. plants (Pinaceae).[1—3] As a member of this genus, *A. sachalinenis*, (Todomatsu in Japanese), is usually used as an important raw material in paper manufacture. In our previous work, many rearranged lanostane-type derivatives were isolated from the ethyl acetate (EtOAc) soluble fraction of methanol (MeOH) extract,[4,5] some of which had tautomeric lactone structure in their side chains.[5] Further chemical investigation of this fraction for the purpose of identifying many more novel constituents led to the isolation of two new triterpenoids (**1**, **2**). Structural determinations of these compounds were carried out mainly by spectral analysis. This paper reports the isolation and structural elucidation of the new compounds.

The molecular formula of compound **1** was determined to be $C_{30}H_{42}O_5$ based on the $[M-H]^-$ ion peak at *m/z* 481.2839 in its high-resolution (HR)-ESI-MS (negative ion mode). The UV spectrum of **1** showed maximum absorption at 245 nm and the IR spectrum of **1** showed absorption bands at 1744 cm^{-1} (strained lactone carbonyl) and 1710 cm^{-1} (carboxyl carbonyl). The ^{1}H-NMR spectrum of **1** showed the presence of six methyl groups [δ_H 0.92 (3H, s), 0.94 (3H, s), 0.97 (3H, d, J=6.3 Hz), 1.04 (3H, s), 1.74 (3H, s), 1.94 (3H, s)], an exomethylene group [δ_H 4.74 (1H, s), 4.79 (1H, s)], three olefinic protons [δ_H 5.57 (1H, dd, J=6.9, 2.7 Hz), 5.50 (1H, br s), 6.82 (1H, br s)] together with other alkyl proton signals. The ^{13}C-NMR spectrum of **1** showed the presence of a carboxyl group (δ_C 179.9), an exomethylene group (δ_C 112.4, 148.8), four olefinic carbons (δ_C 118.3, 118.6, 147.2, 155.8), five methyls (δ_C17.6, 23.9, 25.2, 27.9, 28.0), three methines (δ_C 36.3, 43.8, 45.1), three quaternary carbons (δ_C 36.0, 48.0, 48.6) and so on. The ^{13}C-NMR spectrum of **1** also showed characteristic signals supposed to belong to the side chain part, in which some signals appeared as broad-weak signals such as C-20, 21, 22 and 26 (δ_C 36.3, 17.6, 40.6, 171.7, respectively), and some signals were not observed such as C-23, 24, 25 and 27. (Table 1) This phenomenon is based on the tautomeric hemiacetal structure of γ-lactone, the same as that of abiesanolides E and F.[5] In abiesanolides E and F, some carbons belong to side chain appeared as broad-weak signals, suggesting that **1** was also a rearranged lanostane-type derivative having the same tautomeric γ-lactone side chain as that of abiesanolides E and F.[5] Direct correlations between proton and carbon signals were determined by the HMQC spectrum of **1**. The HMBC spectrum of **1** showed the correlations in Fig. 2; H-7 (δ_H 5.50, 1H, br s) to C-5, 9 and 14 (δ_C 45.1, 43.8, 48.0); H-12 (δ_H 5.57, 1H, dd, J=6.9, 2.7 Hz) to C-9, 14 and 17 (δ_C 43.8, 48.0, 48.6); H-28 (δ_H 4.74, 1H, br s, 4.79, br s) to C-4, 5 and 29 (δ_C 148.8, 45.1, 25.2); H-19 (δ_H 0.92, 3H, s) to C-1, 5 and 9 (δ_C 29.4, 45.1, 43.8); H-30 (δ_H 1.04, 3H, s) to C-8, 13 and 15 (δ_C 147.2, 155.8, 38.1); H-1 (δ_H 1.58, 1H, m, 1.78, 1H, over lap) to C-3 (δ_C 179.9). These HMBC correlations showed the presence of the 3,4-*seco*-4(28),7,12-mariesatriene structure of the A—D ring part of **1**. Thus, the structure of **1** was determined as 3,4-*seco*-4(28),7,12,24-mariesatetraen-26,23-olide-23-hydroxy-3-oic acid and was named abiesanolide I.

Compound **2** was obtained as a colorless amorphous solid. The molecular formula of **2** was determined to be $C_{32}H_{46}O_5$ based on the $[M-H]^-$ at *m/z* 509.3241 in its HR-ESI-MS (negative ion mode) and $[M+Na]^+$ at *m/z* 533.3237 in its HR-ESI-MS (positive ion mode). Absorption bands at 3373, 1764, 1707 cm^{-1} in IR spectrum **2** suggested the presence of hydroxyl, lactone and carboxyl ester groups in the structure. The ^{1}H-NMR spectrum of **2** showed the presence of an ethoxy group [δ_H 4.11 (2H, q, J=7.1 Hz), 1.24 (3H, t, J=7.1 Hz)], an exomethylene group [δ_H 4.82 (1H, br s), 4.79 (1H, br s)], two olefinic methyl groups [δ_H 1.78 (3H, s), 1.58 (3H, s)], two singlet methyl groups [δ_H 0.91 (3H, s), 0.95 (3H, s)], a doublet methyl group [δ_H 0.87 (3H, br d, J=6.8 Hz)], two olefinic protons [δ_H 5.32 (1H, q-like), 5.20

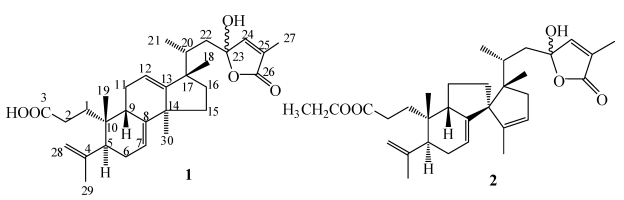

Fig. 1. Structures of Compounds **1** and **2**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2** (in $CDCl_3$)

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	1.58 (m)	29.4	1.56 (overlap)	30.0
	1.78 (overlap)		1.60 (m)	
2	2.31 (t, 8.3 Hz)	28.9	2.26 (t, 8.2 Hz)	29.5
3		179.9		174.4
4		148.8		149.2
5	2.08 (overlap)	45.1	2.08 (overlap)	44.4
6	1.75 (overlap)	29.4	2.02 (overlap)	30.2
	1.89 (overlap)		2.32 (m)	
7	5.50 (br s)	118.6	5.32 (t-like)	120.5
8		147.2		144.1
9	2.08 (overlap)	43.8	2.16 (overlap)	47.7
10		36.0		36.5
11	2.00 (overlap)	22.2	1.44 (m)	24.9
	1.89 (ddd, 14.4, 11.7, 2.7 Hz)		1.68 (overlap)	
12	5.57 (dd, 6.9, 2.7 Hz)	118.3	1.63 (m)	29.3
			1.69 (overlap)	
13		155.8		66.0
14		48.0		148.7
15	1.63 (m)	38.1	5.20 (br s)	121.9
	1.77 (overlap)			
16	1.23 (m)	32.9	1.95 (overlap)	40.5
	1.75 (overlap)			
17		48.6		52.7
18	0.94 (s)	28.0[b]	0.95 (s)	21.1
19	0.92 (s)	23.9	0.91 (s)	24.2
20		36.3[b]		35.1[b]
21	0.97 (d, 6.3 Hz)	17.6[b]	0.87 (d, 6.8 Hz)	20.2[b]
22		40.6[b]		40.5[b]
23		[a]		[a]
24	6.82 (br s)	[a]	6.58 (br s)	[a]
25		[a]		[a]
26		171.7[b]		171.8[b]
27	1.94 (br s)	[a]	1.95 (br s)	[a]
28	4.74 (br s)	112.4	4.79 (br s)	112.2
	4.79 (br s)		4.82 (br s)	
29	1.74 (s)	25.2	1.78 (s)	25.8
30	1.04 (s)	27.9	1.58 (s)	14.9
O–$\underline{CH_2}$–CH_3			4.11 (q, 7.1 Hz)	60.3
O–CH_2–$\underline{CH_3}$			1.24 (t, 7.1 Hz)	14.2

a) Not detected. *b*) Broad-weak signal.

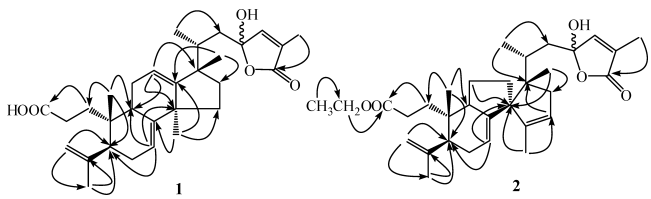
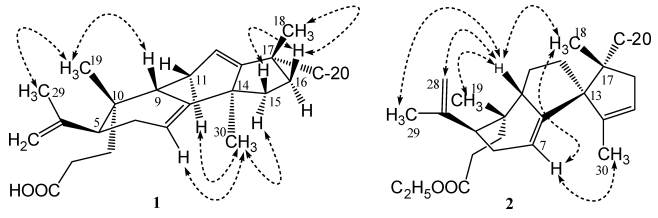


Fig. 2. Selective HMBC Correlations of Compounds **1** and **2**

(1H, br s)], a broad olefinic methyl group [δ_H 1.94 (3H, br s)], and a broad olefinic proton [δ_H 6.58 (1H, br s)]. The ^{13}C-NMR spectrum of **2** showed the presence of an ethoxycarbonyl group (δ_C 14.2, 60.3, 174.4), an exomethylene group (δ_C 112.2, 149.2), the other four olefinic carbons (δ_C 120.5, 121.9, 144.1, 148.7), and several broad-weak carbon signals, the same as those of abiesanolides E and F.[5] Broad Me-21 and Me-27 signals in the ^{1}H-NMR spectrum and broad ^{13}C-NMR signals belonging to the side chain part indicated that **2** had the same tautomeric hemiacetal γ-lactone structure in the side chain as that of abiesanolides E and F.[5] The HMBC experiment of **2** showed the correlations in Fig. 2; H-28 (δ_H 4.82, 1H, br s, 4.79, 1H, br s) to C-4, 5 and 29 (δ_C 149.2, 44.4, 25.8); H-19 (δ_H 0.91, 3H, s) to C-1, 5, 9 (δ_C 30.0, 44.4, 47.7); H-7 (δ_H 5,32, q-like) to C-5, 9 and 13 (δ_C 44.4, 47.7, 66.0); H-30 (δ_H 1.58, 3H, br s) to C-13 and 15 (δ_C 66.0, 121.9); H-15 (δ_H 5.20, 1H, br s) to C-13, 30 and 17 (δ_C 66.0, 14.9, 52.7); H-18 (δ_H 0.95, 3H, s) to C-13, 16 and 20 (δ_C 66.0, 40.5, 35.1); the methylene protons of ethyl group (δ_H 4.11, q, J=7.1 Hz) to C-3 (δ_C 174.4). These results showed that **2** had a rearranged-carbon skeleton having a 3-*O*-ethyl-3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*—4(28),7,14-lanostatrien-3-oic acid part. ^{1}H- and ^{13}C-NMR spectral data of the ring part of **2** were similar to those of 3,4-*seco*-8(14→13*R*)*abeo*-17,13-*friedo*-4(28)7,14,22*Z*,24-lanostapentaen-26,23-olide-3-oic acid (abiesanolide C).[4] Thus, the structure of **2** was determined to be ethyl 3,4-*seco*-8(14→13*R*)*abeo*-17,13-*friedo*-9β-lanosta-4(28),7,14,24-tetraen-26,23-olide-23-hydroxy-3-oate and was named abiesanolide J.

Compounds **1** and **2** should be derived from a lanostane derivative by migration of a methyl group and a C–C bond in the D-ring. Thus the configurations at C-5, 9, 10, 14 and 20



1 2

Fig. 3. Key NOE Correlations of **1** and **2**

of the lanostane derivative should be conserved in **1** and **2**. Me-18 of **1** and **2** were migrated from 13β-position of the lanostane derivative, so the configuration of Me-18 should be β. These expectations were confirmed by ROESY experiments. In compound **1**, Me-19 showed NOE correlations with H-9 and Me-29; H-16β with Me-18 and H-15β; Me-30 with H-7, H-11α and H-15α. In compound **2**, H-9 showed correlations with Me-18, Me-19, Me-29 and H-28; Me-18 with H-7 and H-9. These data indicated that the stereochemistry of **1** and **2** were identical with those of reported rearranged lanostane derivatives[3—5] as shown in Fig. 3.

Compounds **1** and **2** showed broad weak signals or no signals in the ^{13}C-NMR spectrum of the side chain part. This phenomenon should be based on tautomeric γ-lactone having a hemiasetal structure. Compounds **1** and **2** also have a novel rearranged lanostane-type structure characteristic of the triterpenes of *Abies* plants.

Experimental

Melting points were recorded on Yanaco MP-3 micro-melting point apparatus and the temperatures were not corrected. UV and IR spectra were obtained by a U-2001 spectrophotometer (Hitachi) and FT-IR spectroscopy (Perkin Elmer), respectively. NMR spectra were recorded on a JEOL-α-500 (^{1}H-NMR; 500 MHz, ^{13}C-NMR; 125 MHz) spectrometer using $CDCl_3$ as the solvent and TMS as an internal standard. Two-dimensional (2D)-NMR was performed under the usual conditions. Optical rotations were measured with a JASCO P-1010 polarimeter at room temperature. HR-MS and EI-MS experiments were carried out on a JEOL-HX110 mass spectrometer. Preparative and analytical HPLC was carried out on reverse phase columns (Mighty sil RP-18 and 8, Kantho Chemical Co., Ltd.) with the CH_3CN–H_2O solvent system. Silica gel 60 (Merck) was used for column chromatography. Analytical and preparative thin layer chromatography (PLC) was carried out on precoated Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) and spots were visualized by spraying the plates with 50% H_2SO_4 solution, followed by heating.

Plant Material Needles of *A. sachalinensis* were collected in Assabu, Hokkaido, Japan in September 1997, and a voucher specimen was deposited in the herbarium of The Faculty of Life and Enviromental Sciences, Prefectural University of Hiroshima.

Extraction and Isolation The extraction process was the same as to the reported in our previous articles.[1,2] Air-dried needles of *A. sachalinensis* (1.5 kg) were refluxed with MeOH and the filtrate was evaporated under reduced pressure to give MeOH extracts. The extract was then suspended in water and partitioned successively with EtOAc and *n*-BuOH to afford an ethyl acetate soluble fraction (150 g), *n*-BuOH extract (60 g) and aqueous residues. EtOAc layer showed the most potent antibacterial activity against gram-positive bacteria *S. aureus* and *B. subtilis*. The EtOAc extract was chromatographed on a silica gel column with the gradient solvent system $CHCl_3$–MeOH to afford ten fractions (Fr. 1—10), and Fr. 3 (16.0 g) was further purified by preparative HPLC with reverse phase columns and preparative layer chromatography (PLC) to afford compounds **1** (15.0 mg) and **2** (25 mg).

Compound **1**: Colorless amorphous solid. mp 142—144 °C (MeOH), $[\alpha]_D$ −63.4° (c=0.031, $CHCl_3$), HR-ESI-MS (negative ion mode): m/z 481.2839 $[M-H]^-$ (Calcd for $C_{30}H_{41}O_5$; 481.2801). UV λ_{max} nm (ε) ($CHCl_3$): 245 (3213), 278 (sh). IR ν_{max} cm^{-1} (KBr): 3075, 2960, 1744, 1710, 1639, 1452, 1377. ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

Compound **2**: Colorless amorphous solid. mp 112—114 °C (MeOH), $[\alpha]_D$ −55.7° (c=0.055, $CHCl_3$), HR-ESI-MS (positive ion mode): m/z 533.3237 $[M+Na]^+$ (Calcd for $C_{32}H_{46}O_5Na$, 533.3243). HR-EI-MS (negative ion mode): m/z 509.3241 $[M-H]^-$ (Calcd for $C_{32}H_{45}O_5$, 509.3267). UV: λ_{max} nm (ε) (MeOH): 213 (9470), 242sh (3870). IR: ν_{max} cm^{-1} (KBr): 3373, 2956, 1764, 1707, 1439, 1283. ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

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