Novel A-Seco-Rearranged Lanostane Triterpenoids from Abies sachalinensis

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From the needles of Abies sachalinensis, novel rearranged lanostane type triterpenes, 1—4, were isolated along with a known triterpene (5). The structures of the new compounds, 1—4, were elucidated to be 3,4-seco-8-(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14(30),22Z,24-pentaen-26,23-olide-3-oic acid, methyl 3,4-seco-8-(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14(30),22Z,24-pentaen-26,23-olide-3-oate, 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14,22Z,24-pentaen-26,23-olide-3-oic acid and methyl 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14,22Z,24-pentaen-26,23-olide-3-oate, respectively, by means of spectral experiments, especially two dimensional NMR spectroscopy, such as 1H-detected multiple quantum coherence (HMOC), 1H-detected heteronuclear multiple bond connectivity (HMBC) and 1H–1H-correlation spectroscopy (COSY) experiments. These new compounds have novel structures containing A-seco, rearranged spiro structure and a γ-lactone conjugated with a diene. Some of these compounds showed potent antibacterial activity against gram positive bacteria.

Key words Abies sachalinensis; Pinaceae; rearranged lanostane; A-seco-lanostane; antibacterial activity

Gymnosperm trees are very important in forestry and paper manufacturing, but large amounts of the trees are discarded in the manufacturing process for wooden products and papers. There have been fewer reports on bioactive constituents from gymnosperm plants compared with other plants. In the course of our program on researching biologically active constituents from the abandoned parts of forestry trees, we found that several coniferous plants including Abies sachalinensis (Pinaceae, Japanese name “Todomatsu”) showed potent anti-bacterial activity against gram positive bacteria, Staphylococcus aureus and Bacillus subtilis. A. sachalinensis trees are distributed in Hokkaido, Japan and Sakhalin, Russia. From Abies spp. plants, many kinds of lanostane triterpenes, including A-seco and rearranged derivatives, were isolated.1—4) From A. sibirica, an abiesonic acid having an A-seco and rearranged structure, was isolated5) and from A. mariesii, a mariesic acid C having a rearranged structure, was isolated.5) From A. sachalinensis, epijuvabione type sesquiterpenes were isolated,5) but there have been few reports about constituents from A. sachalinensis.5) From the antibacterially active AcOEt soluble fraction of needles of A. sachalinensis, four new A-seco-rearranged lanostane derivatives (1—4) were isolated along with a known triterpene (5).5) This paper deals with the isolation and structural determination of these novel lanostane derivatives and their antibacterial activity against gram positive bacteria.

Results and Discussion

A methanol extract of the needles of A. sachalinensis showed potent antibacterial activity against gram positive bacteria. The activity partitioned into the AcOEt soluble fraction of the methanol extract, so that fraction was chromatographed on silica gel. The resultant fractions were further separated by HPLC using an octadesyl silica (ODS) column as described in the Experimental to give four new rearranged lanostane triterpenes, named abiesanolide A (1), B (2), C (3) and D (4) along with a known rearranged lanostane (5).

Abiesanolide A (1) gave pseudo molecular ion peak, m/z 465.2983 [MH]+, consistent with the formula, C30H40O4 from high resolution (HR)-FAB-MS. The UV spectrum of 1 showed an absorption band at 281 nm (ε 18357). The IR spectrum of 1 showed an absorption band at 1754 cm⁻¹ (strained lactone carbonyl) and 1715 cm⁻¹ (carboxyl carbonyl). The 1H-NMR spectrum of 1 showed the characteristic signals for two exomethylene [δ 4.82 (1H, s), 4.86 (1H, s), 4.73 (1H, s), 4.69 (1H, s)], three olefinic protons [δ 5.39 (1H, dd, J=6.8, 3.6 Hz), 5.15 (1H, d, J=10.4 Hz), 6.93 (1H, q, J=1.6 Hz)] two singlet methyl groups [δ 0.86 (3H, s), 0.94 (3H, s)], two vinylmethyl groups [δ 2.02 (3H, s), 1.78 (3H, s)] and a doublet methyl group [δ 1.02 (3H, d, J=7.6 Hz)]. The 13C-NMR spectrum of 1 showed thirty carbon signals and showed the presence of a carboxyl group (δ 180.8), a lactone group (δ 171.2), two exomethylene carbons (δ 112.3, 105.7), three trisubstituted olefine groups (δ 121.1, 144.8, 118.8, 147.0, 128.4) and a quaternary carbon (δ 63.8). The 1H-NMR and 13C-NMR data in the A—D ring part of 1 showed a similar signal pattern and chemical shifts with those of dimethyl abiesonate having a 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14(30)-trien structure, whose stereo structure was determined by X-ray analysis.5) These data indicated that 1 was an A-seco-rearranged lanostane triterpene having a γ-lactone conjugated with a diene moiety. The H—H-correlation spectroscopy (COSY) spectrum of 1 was utilized for an alignment of the side chain and 1H-detected multiple quantum coherence (HMOC) spectrum of 1 was utilized for the assignment of 1H-NMR and 13C-NMR signals. The 1H-detected heterocyclic multiple bond correlation (HMBC) experiment of 1 showed many C–H long range correlations as shown in Fig. 1. The Me-29 protons at δ 1.78 showed a correlation with an exomethylene carbon (δ 112.3) and C-5 (δ 30.8). The exomethylene protons at C-30 showed a correlation with a spiro carbon (δ 63.8) at C-13 and C-15 (δ 28.9). The doublet
methyl protons (δ 1.02) showed a correlation with a methine carbon (δ 118.8) at C-22 and a quaternary carbon (δ 50.5) at C-17. The Me-18 protons showed a correlation with a methine (δ 37.9) at C-20 and C-13. The Me-19 protons showed a correlation with C-1 (δ 30.2), C-5 (δ 44.2) and C-9 (δ 48.8). The C-1 methylene protons (δ 1.5—1.7) showed a correlation with a carboxyl carbon (δ 180.0). The olefinic proton (δ 5.39) at C-7 showed a correlation with C-5 (δ 44.2) and C-9 (δ 48.8). The olefinic proton (δ 5.15) showed a correlation between Me-18 and H-7. This fact indicated that a δ 1.53 (3H, br s)].

This was supported from the 13C-NMR spectrum data as shown in Fig. 1. Thus the structure of 1 was determined to be 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14(30),22,24-pentaen-26,23-olide-3-oic acid.

Abiesanolide B (2) gave the molecular formula, C34H42O6, from a pseudomolecular ion [MH]+ at m/z 479.3170 in HR-FAB-MS. IR spectrum of 2 showed an absorption band at 1767 cm⁻¹ (strained lactone carbonyl), 1740 cm⁻¹ (ester carboxyl) and 1198 cm⁻¹ (ester C–O). The 1H-NMR and 13C-NMR spectra of 2 showed almost the same signal patterns with those of 1 (See Table 1 and Experimental section) except for the presence of the carbomethoxy group [δ 3.66 (s) in 1H-NMR and δ 51.6, 174.8 in 13C-NMR]. These indicated that 2 was the methyl ester of 1. This was confirmed by HMBC experiment. Thus the structure of 2 was determined to be methyl 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14(30),22,24-pentaen-26,23-olide-3-oate.

Abiesanolide C (3) gave the molecular formula, C34H42O6 from HR-FAB-MS, m/z 465.2983. The UV spectrum of 3 showed a similar absorption band (ε 24615 at 279 nm) to that of 1. The IR spectrum of 3 showed an absorption band at 1771 cm⁻¹ (strained lactone) and 1711 cm⁻¹ (carboxyl). The 1H-NMR spectrum of 3 showed almost the same signal pattern with that of 1 except for the absence of the exomethylene at C-30 and a newly arisen trisubstituted olefin group [δ 5.25 (1H, br s)] and a vinylmethyl group [δ 1.53 (3H, br s)].

This was supported from the 13C-NMR spectrum data as shown in Table 1. These facts indicated that 3 was transformed from 1 by double bond migration from C-14,30 to C-14,15. This was confirmed by the HMBC experiments as shown in Fig. 1. Thus the structure of 3 was determined to be 3,4-seco-8(14→3R)abeo-17,13-friedo-9β-lanosta-4(28),7,14,22,24-pentaen-26,23-olide-3-oic acid.

Abiesanolide D (4) gave the molecular formula, C34H42O6 from pseudomolecular ion [MH]+ at m/z 479.3177 in HR-FAB-MS. The UV spectrum of 4 showed an absorption band at 280 nm (δ 15512). The IR spectrum of 4 showed absorption bands at 1768 cm⁻¹ (strained lactone carbonyl), at 1740 cm⁻¹ (ester carboxyl) and at 1193 cm⁻¹ (ester C–O). The 1H-NMR and 13C-NMR spectra showed almost the same signal patterns with those of 3 except for the presence of car-
Homothexy group [δ 3.66 (3H, s) in 1H-NMR and δ 51.6, 174.9 in 13C-NMR] instead of a free carboxyl group in 3. Thus the structure of 4 was determined to be methyl 3,4-seco-8(14→13R)abeo-17,13-friedo-9β-lanosta-4(28),7,14,22Z,24-penta-26,23-olide-3-oate.

Compound 5 was identified as 3,4-seco-4(28),6,8(14),22Z,24-mariesapentaen-26,23-olide-3-oic acid by means of spectral data, which was isolated from A. sibirica.\(^9\) The 13C-NMR data for 5 is reported for the first time.

The five A-seco-rearranged lanostane derivatives, 1—5, were tested for their antibacterial activity against gram positive bacteria, Staphylococcus aureus and Bacillus subtilis, in the same way as described in the previous paper.\(^10\) Of these, 1 and 5 showed a minimum inhibitory concentration (MIC) at 12.5 μg/ml, but 2, 3 and 4 showed no activity up to a concentration of 200 μg/ml.

Compounds 1—5 have novel structures transformed from lanostane triterpenes. A leaving group at C-17 of the hypothesized intermediate A migrates to give a carbocation at C-1 and different pathways (a, b, c) to give hypothetical intermediate B was oxidized to give the Z form followed by enolization and lactonization to give the γ-lactone derivatives such as 1—5. Oxidation of Me-26 of B gave E form, which gave enone carboxylic acid, such as mariesic acid as shown in Fig. 2.

**Experimental**

Optical rotations were determined with JASCO P-1010 polarimeter in MeOH at 25°C. UV spectra were obtained with a Hitachi U3410 spectrometer. IR spectra were obtained with a Perkin Elmer GX FT-IR spectrometer. 1H-NMR and 13C-NMR spectra were recorded on a JEOL α-500 spectrometer in CDC\(_3\), using tetramethylsilane (TMS) as an internal standard (1H-NMR at 500 MHz, 13C-NMR at 125 MHz). H-H COSY, HMOC and HMBC and difference NOE experiments were carried out on the same spectrometer. HR-FAB-MS were obtained with a JEOL HX110 mass spectrometer. Analytical TLC were performed by using precoated Silica gel 60 F\(_{254}\) (Merck) plates, and detection for the spots was accomplished by UV absorption and spraying with 50% H\(_2\)SO\(_4\) followed by heating. HPLC was carried out using an ODS column (YMC R-ODS7 packed column).

**Extraction and Isolation** Air dried needles of A. sachalinensis (1.5 kg), collected in Assabu, Hokkaido, Japan, in September 1997, were extracted with MeOH under reflux to give the MeOH extract, which was suspended in water and extracted with AcOEt to give the AcOEt soluble fraction (100 g). The residual water layer was extracted with n-ButOH to give the n-ButOH soluble fraction (60 g) and the aqueous fraction. Of these three fractions, the AcOEt soluble fraction showed most potent antibacterial activity against gram positive bacteria. S. aureus and B. subtilis. The active AcOEt fraction (50 g) was separated by silica gel (SiO\(_2\)) chromatography using gradient CHCl\(_3\)--MeOH (50:1→3:1) solvent system to give 10 fractions. Fraction 1 (30 g) was chromatographed on a SiO\(_2\) column using a hexane--AcOEt solvent system to give eight fractions (1'—8'), of which fraction 2' (5 g) was further chromatographed on a SiO\(_2\) column using a hexane--AcOEt solvent system to give eleven fractions (1''—11''). Of these, fraction 11'' (200 mg) was repeatedly purified by HPLC using an ODS column and 85% CH\(_3\)CN to give 2 (10 mg) and 4 (12 mg). Fraction 3 (8 g) was chromatographed on a SiO\(_2\) column using a gradient hexane--AcOEt solvent system to give seven fractions (1''—7''), of which fraction 2'' (500 mg) was repeatedly purified by HPLC using an ODS column and 85% CH\(_3\)CN to give 1 (40 mg), 3 (25 mg) and 5 (70 mg).

Abiesanolide A (1): White amorphous solid, [α]\(_D\) +310° (c=0.016, MeOH), UV \(λ_{max}\) nm (ε) (MeOH): 203.5 (11252), 281 (18537), IR \(ν_{max}\) cm\(^{-1}\) (KBr): 3070, 2960, 2870, 1768, 1740, 1193, HR-FAB-MS; m/z 465.2983 [MH]\(^+\) (Caled for C\(_{31}\)H\(_{41}\)O\(_{2}\); 465.2983) 1H-NMR (CDCl\(_3\)) δ: 0.86 (3H, s, Me-19), 1.02 (3H, d, J=7.6 Hz, Me-21), 1.42 (2H, overlap, H-11, 12), 1.5—1.7 (2H, overlap, H-1), 1.6 (2H, overlap, H-16), 1.7 (1H, overlap, H-11), 1.78 (3H, s, Me-29), 1.82 (1H, m, H-20), 2.02 (3H, s, Me-27), 2.03 (1H, overlap, H-5), 2.11 (1H, br d, J=18.8 Hz, H-6), 2.30 (2H, t, J=8.4 Hz, H-2), 2.33 (1H, br d, J=18.8 Hz, H-6), 2.46 (2H, m, H-15), 3.07 (1H, dq, J=10.4, 7.6 Hz, H-20), 4.73 (1H, br s, H-30), 4.69 (1H, br s, H-30), 4.82 (1H, br s, H-28), 4.86 (1H, br s, H-28), 5.15 (1H, d, J=10.4 Hz, H-22), 5.39 (1H, dd, J=6.8, 3.6 Hz, H-7), 6.93 (1H, q, J=1.6 Hz, H-24).

**Abiesanolide B (2):** White amorphous powder, [α]\(_D\) +297° (c=0.5, MeOH), IR \(ν_{max}\) cm\(^{-1}\) (KBr): 3080, 1768, 1740, 1193, HR-FAB-MS; m/z 479.3170 [MH]\(^+\) (Caled for C\(_{31}\)H\(_{41}\)O\(_{2}\); 479.3161), 1H-NMR (CDCl\(_3\)) δ: 0.85 (3H, s, Me-19), 0.94 (3H, s, Me-18), 1.02 (3H, d, J=7.2 Hz, Me-21), 1.58 (2H, overlap, H-1), 1.6 (2H, overlap, H-16), 1.78 (3H, s, Me-29), 1.99 (3H, J=10.4, 7.2 Hz, H-20), 3.66 (3H, s, COOMe), 4.70 (1H, br s, H-30), 4.73 (1H, br s, H-30), 4.82 (1H, br s, H-28), 4.85 (1H, br s, H-28), 5.14 (1H, d, J=10.4 Hz, H-22), 5.39 (1H, dd, J=6.4, 3.6 Hz, H-7), 6.93 (1H, q, J=1.6 Hz, H-24).

**Abiesanolide C (3):** A white amorphous powder, [α]\(_D\) -370° (c=0.034, MeOH), UV \(λ_{max}\) nm (ε) (MeOH): 278 (24615), IR \(ν_{max}\) cm\(^{-1}\) (KBr): 3090, 2970, 2920, 2850, 1740, 1640, \(ν_{max}\) cm\(^{-1}\) (KBr): 3070, 2960, 2870, 1768, 1740, 1193, HR-FAB-MS; m/z 465.2983 [MH]\(^+\) (Caled for C\(_{31}\)H\(_{41}\)O\(_{2}\); 465.2983).
1771, 1711, HR-FAB-MS: m/z 465.2983 [MH]+ (Caled for C_{30}H_{41}O_{4}; 465.2304), 1H-NMR (CDCl₃) δ: 0.88 (3H, d, J = 6.8 Hz, Me-21), 0.91 (3H, s, Me-18), 0.92 (3H, s, Me-19), 1.53 (3H, br s, Me-27), 1.76 (3H, s, Me-29), 1.99 (3H, s, Me-27), 3.02 (1H, dq, J = 10.8, 6.8 Hz, H-20), 4.79 (1H, br s, H-28), 4.82 (1H, br s, H-28), 5.14 (1H, d, J = 10.8 Hz, H-22), 5.25 (1H, br s, H-15), 5.30 (1H, dd, J = 6.4, 4.0, H-7), 6.96 (1H, q, J = 1.2 Hz, H-24), 13C-NMR data is shown in Table 1.

Abiesanolide D (4): White amorphous powder, [α]_{D}^{23} = 314° (c = 1.2, MeOH), UV λ_{max} nm (e) (MeOH): 280 (15512), IR ν_{max} cm⁻¹ (KBr); 3080, 1768, 1740, 1193. HR-FAB-MS; m/z 479.3177 [MH]+ (Caled for C_{31}H_{43}O_{4}; 479.3161), 1H-NMR (CDCl₃) δ: 0.88 (3H, d, J = 6.8 Hz, Me-21), 0.90 (3H, s, Me-18), 0.92 (3H, s, Me-19), 1.54 (3H, br s, Me-30), 1.76 (3H, s, Me-29), 1.99 (3H, s, Me-27), 3.03 (1H, dq, J = 10.8, 6.8 Hz, H-20), 3.66 (3H, s, COOMe), 4.79 (1H, br s, H-28), 4.81 (1H, br s, H-28), 5.14 (1H, d, J = 10.8 Hz, H-22), 5.24 (1H, br s, H-15), 5.29 (1H, dd, J = 6.4, 4.3 Hz, H-7), 6.96 (1H, q, J = 1.6 Hz, H-24), 13C-NMR data is shown in Table 1.

3,4-Secoo-4(28),6,8(14),22-Z,24-mariesapentaen-26,23-olide-3-oic Acid (5): White amorphous powder, [α]_{D}^{23} = 343° (c = 0.036, MeOH), UV λ_{max} nm (e) (MeOH): 254 (29249), IR ν_{max} cm⁻¹ (KBr); 3400(br), 1754, 1715, HR-FAB-MS; m/z 464.2925 [M]+ (Caled for C_{30}H_{40}O_{4}; 464.2926), 1H-NMR (CDCl₃) δ: 0.78 (3H, s, Me-30), 0.83 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.01 (3H, d, J = 7.6 Hz, Me-21), 1.77 (3H, s, Me-29), 2.00 (3H, s, Me-27), 2.61 (1H, d, J = 5.6 Hz, H-5), 3.15 (1H, dq, J = 10.4, 7.6 Hz, H-20), 4.75 (1H, br s, H-28), 4.95 (1H, br s, H-28), 5.18 (1H, d, J = 10.4 Hz, H-22), 5.36 (1H, dd, J = 10.0, 5.6 Hz, H-6), 6.20 (1H, d, J = 10.0 Hz, H-7), 6.97 (1H, q, J = 1.6 Hz, H-24), 13C-NMR data is shown in Table 1.

**Antibacterial Activity** The antibacterial activity test was carried out by the agar plate dilution method against gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* as previously reported.¹⁰

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**References**


