18-*nor*-Abietatrienes from the Cones of *Larix kaempferi*

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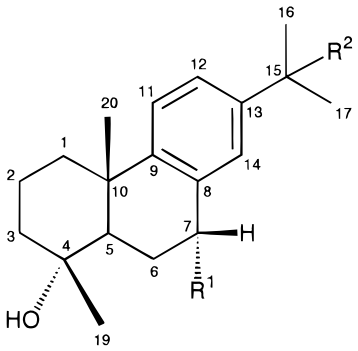
Two novel norditerpenediols, 18-*nor*-abieta-8,11,13-triene-4,15-diol (**1**) and 18-*nor*-abieta-8,-11,13-triene-4,7α-diol (**2**), were isolated from the cones of *Larix kaempferi*, together with two known diterpenes, abieta-8,11,13-triene-15,18-diol and abieta-8,11,13-triene-7α,18-diol. The structures of **1** and **2** were determined on the basis of chemical and spectral evidence.

Previously, we reported the isolation of abieta-8,11,-13,15-tetraen-18-oic acid, 16-*nor*-15-oxodehydroabietic acid, and 12,15-dihydroxydehydroabietic acid from the leaves of *Larix kaempferi* (Lamb.) Carr. (Pinaceae), together with 12 known compounds, including (−)-α-cadinol, oplopanone, larixol, two dehydroabietinols, and seven dehydroabitic acids including a pair of 9α,13α-epidioxy- and 9*β*,13*β*-epidioxyabiet-8(14)-en-18-oic acids.[1] No detailed study, however, has so far been reported on extracts of the cones of *Larix* species. Careful fractionation of a $CHCl_3$ extract of fresh cones of *L. kaempferi*, by Si gel column chromatography and reversed-phase HPLC, furnished two new compounds, **1** and **2**, and several known compounds. Two of the known compounds were identified as abieta-8,11,13-triene-15,18-diol[2,3] and abieta-8,11,13-triene-7α,18-diol[4] by comparison of their IR, ^{1}H NMR, and ^{13}C NMR, and EIMS data with those already published. We now report the characterization of **1** and **2**.



	R^1	R^2
1	H	OH
2	OH	H
2a	OAc	H

Compound **1** was assigned the molecular formula $C_{19}H_{28}O_2$ by HREIMS. Its IR spectrum contained absorption bands, indicating a hydroxyl group and a benzene ring. The ^{1}H and ^{13}C NMR spectra showed signals for a tertiary methyl group, a tertiary methyl group geminal to an oxygen function, two equivalent methyls of a hydroxyisopropyl group,[5] and an aromatic ring characteristic of abieta-8,11,13-trienes. No signal was observed for a methine proton geminal to a hydroxyl group. Except for the presence of signals of a hydroxyisopropyl group and the absence of those for an isopropyl group, the above ^{1}H and ^{13}C NMR spectral data were closely similar to those of the known compounds, 18-*nor*-abieta-8,11,13-trien-4-ol[6−8] and 19-*nor*-abieta-8,11,-13-trien-4-ol.[7,8] The HMBC spectrum of **1** provided cross correlations (Figure 1), indicating that the two hydroxyl groups of **1** should be placed at C-4 and C-15. The chemical shift values of signals for Me-20, C-5, C-19, and C-20 were closely similar to those of 18-*nor*-abieta-8,11,13-trien-4-ol rather than those of 19-*nor*-abieta-8,-11,13-trien-4-ol. In the NOESY experiment (Figure 1), a significant correlation was observed between signals at *δ* 1.16 and 1.24, indicating Me-20 and Me-19 (geminal to a tertiary hydroxyl group) have a 1,3-diaxial relationship. Thus, the structure of **1** was proved to be 18-*nor*-abieta-8,11,13-triene-4,15-diol.

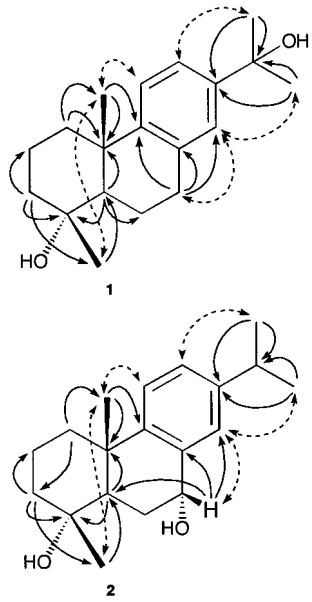


Figure 1. HMBC (solid arrow) and selected NOESY (dashed arrow) correlation of **1** and **2**.

Compound **2** was also assigned the molecular formula $C_{19}H_{28}O_2$ by HREIMS. Its IR spectrum contained absorptions characteristic for hydroxyl and aromatic groups. The ^{1}H and ^{13}C NMR spectra revealed signals for a tertiary methyl group, a tertiary methyl group geminal to a hydroxyl group, an isopropyl group, a benzene ring corresponding to the C-ring of **1**, and a hydroxymethine group. Acetylation of **2** afforded a

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hydroxymonoacetate (**2a**). The presence of an isopropyl group and a secondary hydroxyl group and the absence of a hydroxyisopropyl group suggested that **2** was a positional isomer of **1**. This assumption was supported by the $^1H-^1H$ COSY, HMQC, and HMBC experiments. The HMBC data exhibited correlations as shown in Figure 1, indicating **2** to be either 18-*nor*- or 19-*nor*-abieta-8,11,13-triene-4,7ξ-diol. The C-7 signal of **2** observed at δ_C 68.1 was compatible with those reported for 7α-hydroxyabieta-8,11,13-trienes,[9,10] which was shifted ca. 2 ppm to higher field than those of the known 7*β*-hydroxyabieta-8,11,13-trienes.[9,11] Furthermore, the C-5 signal was shifted upfield 6.1 ppm from that of **1**, whereas those of C-6, C-12, and C-14 were shifted 9.1, 4.6, and 3.1 ppm downfield, respectively, from those of **1** due to the addition of a 7α-hydroxyl group in the molecule. The NOESY data provided cross correlations as shown in Figure 1, indicating that the two hydroxyl groups of **2** have 4α- and 7α-configurations. Hence, compound **2** was proved to be 18-*nor*-abieta-8,11,13-triene-4,7α-diol.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. IR spectra were recorded as KBr disks using a Perkin−Elmer 1720X FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Varian XL-300 and INOVA 500 spectrometer with standard pulse sequences operating at 300 and 500 MHz and 74.5 and 125 MHz, respectively. $CDCl_3$ was used as solvent and TMS as internal standard. EIMS and HREIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over Si gel (70∼230 mesh, Merck) and Cosmosil 75C_{18}-OPN (Nacarai Tesque), and MPLC was carried out with Cosmosil 40C_{18}-PREP (Nacarai Tesque). Preparative HPLC was carried out using a TOSOH system equipped with a CCPM-prep Pump, a SC-8020 system controller, and a TSK-GEL ODS-80Ts (21.5 × 300 mm). Fractions obtained from column chromatography were monitored by TLC (Merck Si gel 60 HF_{254}). Preparative TLC was carried out on Merck Si gel PF_{254} plates (0.5 mm).

Plant Material. The fresh cones of *L. kaempferi* were collected on the grounds of National Forest Tree Breeding Station, Komoro City, Nagano Prefecture, Japan, on July 30, 1995. Voucher specimens are deposited in the Herbarium of the Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences, Takatsuki, Japan (voucher no. LK-957K).

Extraction and Isolation of Compounds. The fresh cones of *L. kaempferi* (1.45 kg) were chopped and extracted with $CHCl_3$ (4L × 5). Evaporation of the extract in vacuo yielded a dark yellow residue (123 g), which was divided into 10 fractions by preliminary Si gel (2.0 kg) column chromatography using $CHCl_3$, $CHCl_3$−EtOAc (gradiently), EtOAc, and EtOAc−MeOH (1:1). Rechromatography of residue 9 collected from the EtOAc eluates (12.10 g) over a Si gel (200 g) column with $CHCl_3$−MeOH (100: 1) successively afforded residues **A** (484 mg) and **B** (859 mg) from the fraction nos. 29−37 and 38−44 (each fraction: 100 mL). Residue **A** was purified by reversed-phase MPLC using Cosmosil 40C_{18}-PREP (ODS) column and 60% MeOH−H_2O to give compound **2** (7 mg) as a crystalline solid. Residue **B** was subjected to ODS open column chromatography. Elution with 70% MeOH−H_2O afforded residues **C** (208 mg), **D** (42 mg), and **E** (68 mg) from the fraction nos. 1−12, 13−26, and 39−44 (each fraction: 30 mL). Residue **C**, purified by HPLC (ODS) using 65% MeOH−H_2O, furnished compound **1** (6 mg). Recrystallization of residue **D** from *n*-hexanes−EtOAc afforded abieta-8,-11,13-triene-15,18-diol, 30 mg, mp 100−101 °C, $[\alpha]^{26}_D$ +2° (*c* 0.50, EtOH). Residue **E** was purified by preparative TLC (*n*-hexane−EtOAc, 2:1) to afford abieta-8,11,13-triene-7α,18-diol, 26 mg (Me_2CO), mp 87−88 °C, $[\alpha]^{26}_D$ −1° (*c* 0.43, EtOH). Physical and spectral data of these latter two compounds were compatible with those already reported in the literature.[2−4]

18-*nor*-Abieta-8,11,13-triene-4,15-diol (1): colorless needles (*n*-hexanes−EtOAc); mp 140−141 °C; $[\alpha]^{26}_D$ −13° (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3279 (OH), 2970, 2930, 1610, 1498, and 1458 (aromatic ring), 1175, 986, 876 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.24 (1H, dd, J = 8.0, 2.0 Hz, H-12), 7.22 (1H, d, J = 8.0 Hz, H-11), 7.17 (1H, d, J = 2.0 Hz, H-14), 2.93 (2H, m, H-7α, H-7*β*), 2.26 (1H, ddd, J = 13.0, 4.0, 2.8 Hz, H-1*β*), 2.12 (1H, dddd, J = 13.0, 7.0, 1.5, 1.5 Hz, H-6α), 1.88 (1H, dddd, J = 13.0, 3.5, 3.3, 1.5 Hz, H-3*β*), 1.77 (1H, m, H-2α), 1.70 (1H, m, H-6*β*), 1.66 (1H, m, H-2*β*), 1.60 (1H, m, H-5α), 1.57 (6H, s, Me-16, Me-17), 1.44 (1H, ddd, J = 13.0, 13.0, 4.0 Hz, H-1α), 1.39 (1H, ddd, J = 13.0, 13.0, 4.5 Hz, H-3α), 1.24 (3H, s, Me-19), 1.16 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.4 (s, C-9), 146.0 (s, C-13), 134.8 (s, C-8), 125.0 (d, C-14), 124.6 (d, C-11), 122.0 (d, C-12), 72.4 (s, C-4), 72.3 (s, C-15), 52.4 (d, C-5), 42.7 (t, C-3), 38.3 (s, C-10), 38.0 (t, C-1), 31.6 (q × 2, C-16, C-17), 30.5 (t, C-7), 24.5 (q, C-20), 23.0 (q, C-19), 20.5 (t, C-2), 18.0 (t, C-6); EIMS (70 eV) *m/z* 288 $[M]^+$ (29), 273 $[M - Me]^+$ (100), 255 $[273 - H_2O]^+$ (70), 227 (6), 197 (8), 185 (17), 157 (10), 131 (11), 43 (39); HREIMS *m/z* 288.2077 (calcd for $C_{19}H_{28}O_2$, 288.2088).

18-*nor*-Abieta-8,11,13-triene-4,7α-diol (2): colorless needles (*n*-hexane−EtOAc); mp 85−86 °C; $[\alpha]^{26}_D$ −15° (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max} 3371 (OH), 2965, 2931, 2867, 1568, 1497, and 1457 (aromatic ring), 1149, 983, 858 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.19 (1H, d, J = 8.2 Hz, H-11), 7.16 (1H, d, J = 2.0 Hz, H-14), 7.12 (1H, dd, J = 8.2, 2.0 Hz, H-12), 4.81 (1H, dd, J = 4.0, 1.7 Hz, H-7*β*), 2.87 (1H, septet, J = 6.9 Hz, H-15), 2.23 (1H, br dd, J = 13.0, 1.7 Hz, H-6α), 2.21 (1H, ddd, J = 13.1, 3.8, 2.3 Hz, H-1*β*), 1.94 (1H, br d, J = 13.0 Hz, H-5α), 1.89 (1H, ddd, J = 13.0, 13.0, 4.0 Hz, H-6*β*), 1.83 (1H, dddd, J = 13.3, 3.3, 3.3, 1.2 Hz, H-3*β*), 1.73 (1H, m, H-2α), 1.63 (1H, m, H-2*β*), 1.37 (1H, ddd, J = 13.1, 13.1, 3.8 Hz, H-1α), 1.32 (1H, ddd, J = 13.3, 13.3, 4.4 Hz, H-3α), 1.24 (6H, d, J = 6.9 Hz, Me-16, Me-17), 1.18 (3H, s, Me-19), 1.08 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 146.5 (s, C-13), 146.4 (s, C-9), 135.9 (s, C-8), 128.1 (d, C-14), 126.6 (d, C-12), 124.7 (d, C-11), 71.8 (s, C-4), 68.1 (d, C-7), 46.3 (d, C-5), 42.6 (t, C-3), 38.7 (s, C-10), 37.7 (t, C-1), 33.5 (d, C-15), 27.1 (t, C-6), 24.0 (q, C-17), 23.9 (q, C-16), 23.5 (q, C-20), 22.5 (q, C-19), 20.5 (t, C-2); EIMS (70 eV) *m/z* 288 $[M]^+$ (100), 255 $[M - Me - H_2O]^+$ (41), 237 $[255 - H_2O]^+$ (51), 195 (67), 185 (17),

143 (89), 109 (41), 85 (83), 43 (62); HREIMS *m/z* 288.2098 (calcd for $C_{19}H_{28}O_2$, 288.2088).

Compound 2 Acetate (2a). A mixture of compound **2** (2.4 mg) in dried pyridine−Ac_2O (1:1, 1 mL) was left at room temperature overnight. Workup as usual yielded a solid (2.8 mg) that was recrystallized to furnish monoacetate (**2a**), 2.0 mg (*n*-hexane−EtOAc), as colorless needles: mp 156−157 °C, $[\alpha]^{26}_D$ +4° (*c* 0.21, $CHCl_3$); IR (KBr) ν_{max} 3553 (OH), 2932, 2867, 1726, and 1249 (OAc), 1497 and 1457 (aromatic ring) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.24 (1H, d, J = 8.2 Hz, H-11), 7.17 (1H, dd, J = 8.2, 2.0 Hz, H-12), 7.07 (1H, d, J = 2.0 Hz, H-14), 6.06 (1H, br d, J = 4.0 Hz, H-7β), 2.86 (1H, septet, J = 7.0 Hz, H-15), 2.26 (1H, ddd, J = 13.0, 4.0, 2.3 Hz, H-1β), 2.24 (1H, m, H-6α), 2.07 (3H, s, OCOCH_3), 1.95 (2H, m, H-5α, H-6β), 1.90 (1H, ddd, J = 13.5, 3.0, 3.0 Hz, H-3β), 1.79 (1H, m, H-2α), 1.66 (1H, m, H-2β), 1.48 (1H, ddd, J = 13.0, 13.0, 4.0 Hz, H-1α), 1.42 (1H, ddd, J = 13.5, 13.5, 4.5 Hz, H-3α), 1.22 (6H, d, J = 7.0 Hz, Me-16, Me-17), 1.22 (3H, s, Me-19), 1.11 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8 (s, O*C*OCH$_3$), 147.2 (s, C-9), 146.6 (s, C-13), 132.1 (s, C-8), 128.4 (d, C-14), 127.1 (d, C-12), 124.6 (d, C-11), 71.7 (s, C-4), 70.3 (d, C-7), 47.3 (d, C-5), 42.6 (t, C-3), 38.4 (s, C-10), 37.5 (t, C-1), 33.5 (d, C-15), 25.0 (t, C-6), 24.0 (q, C-17), 23.8 (q, C-16), 23.5 (q, C-20), 22.7 (q, C-19), 21.7 (q, OCO*C*H$_3$), 20.4 (t, C-2); EIMS (70 eV) *m/z* 330 $[M]^+$ (1), 312 $[M - H_2O]^+$ (22), 270 $[M - AcOH]^+$ (94), 255 $[270 - Me]^+$ (25), 237 $[255 - H_2O]^+$ (33), 195 (30), 185 (21), 169 (34), 155 (24), 143 (57), 85 (34), 43 (100); HREIMS *m/z* 330.2187 (calcd for $C_{21}H_{30}O_3$, 330.2193).

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