Abietane Diterpenoids from the Cones of *Larix kaempferi*

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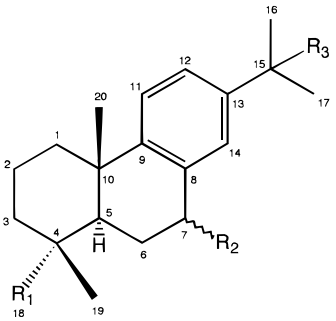
Three new abietane-type diterpenes, 7α,15-dihydroxyabieta-8,11,13-trien-18-al (**1**); 15,18-dihydroxyabieta-8,11,13-trien-7-one (**2**); and 18-*nor*-4,15-dihydroxyabieta-8,11,13-trien-7-one (**3**), were isolated from the cones of *Larix kaempferi*, together with three known diterpenes, abieta-8,11,13-trien-18-yl succinate, 16-*nor*-15-oxoabieta-8,11,13-trien-18-oic acid, and 7*β*-hydroxyabieta-8,11,13-trien-18-oic acid. The structures of **1**−**3** were determined on the basis of chemical and spectral evidence.

Recently, we reported the isolation of 18-*nor*-abieta-8,-11,13-triene-4,15-diol and 18-*nor*-abieta-8,11,13-triene-4,7α-diol from the cones of *Larix kaempferi* (Lamb.) Carr. (Pinaceae), together with two known diterpenes, abieta-8,11,13-triene-15,18-diol and abieta-8,11,13-triene-7α,18-diol.[1]

Further investigation of a $CHCl_3$ extract of the fresh cones of *L. kaempferi* furnished three new compounds (**1**−**3**), together with three known compounds. The known compounds were identified as abieta-8,11,13-triene-18-yl succinate,[2] 16-*nor*-15-oxoabieta-8,11,13-trien-18-oic acid,[3] and 7*β*-hydroxyabieta-8,11,13-trien-18-oic acid[4] by comparison of their physical, IR, ^{1}H and ^{13}C NMR, and EIMS data with those already published. Compound **5** was previously isolated from the leaves of *L. kaempferi*.[3] We now report the characterization of **1**−**3**.



	R_1	R_2	R_3
1	CHO	α–OH	OH
1a	CHO	α–OAc	OH
2	CH_2OH	= O	OH
2a	CH_2OAc	= O	OH
2b	CH_2OAc	= O	OAc
3	OH	= O	OH

Compound **1** was assigned the molecular formula $C_{20}H_{28}O_3$, by HREIMS. Its IR spectrum indicated absorption bands for hydroxyl groups, an aldehyde group, and a benzene ring. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed signals for two tertiary methyl groups, two equivalent methyls of a hydroxyisopropyl group,[1] an aromatic ring characteristic for an abieta-8,11,13-triene, and an aldehyde group [δ_H 9.30 (1H, s); δ_C 206.2 (d)]. Acetylation of **1** afforded a monoacetate (**1a**). Except for the absence of a carboxyl group at C-18 and the presence of an aldehyde group, close resemblances were observed in the ^{1}H and ^{13}C NMR spectra with analogous data of the known compound 7α,15-dihydroxyabieta-8,11,13-trien-18-oic acid.[5] The HMBC spectrum of **1** provided cross correlations shown in Figure 1, indicating that an aldehyde group should be placed at C-4. The configuration of the C-7 hydroxyl group of **1** was determined as pseudoaxial 7α based on the ^{13}C NMR chemical shift values at C-5, C-6, C-7, and C-14 by comparison with those of 7*β*-hydroxyabieta-8,11,13-trien-18-oic acid.[4] The unambiguous structure of **1** was determined from NOESY correlations between H-19 with H-20, and the aldehyde proton with the H-3α and H-5α protons (Figure 1). Therefore, compound **1** was determined to be 7α,15-dihydroxyabieta-8,11,13-trien-18-al.

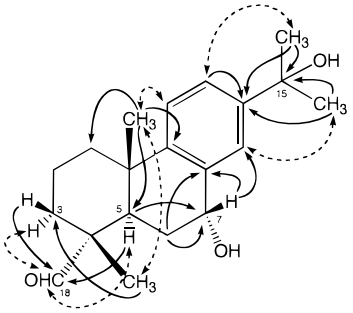


Figure 1. HMBC (plain arrow) and key NOESY (dashed arrow) interactions of compound **1**.

Compound **2** was also established with the molecular formula $C_{20}H_{28}O_3$, by HREIMS. Its UV and IR spectra showed absorptions for hydroxyl groups, an α,*β*-unsaturated ketone, and a conjugated aromatic ring. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed signals for two tertiary methyl groups, a hydroxyisopropyl group, a primary hydroxyl group, a ketone group, and an aromatic ring characteristic of an abieta-8,11,13-triene. Acetylation of **2** afforded a monoacetate (**2a**) and a less polar product, diacetate (**2b**), in the ratio 2:1. The ^{1}H and ^{13}C NMR spectral data of **2** resembled those of abieta-8,11,13-triene-15,18-diol,[1] except for the presence of a ketone group instead of a methylene group at C-7. This inference was supported by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments. The HMBC and NOESY data are shown in Figure 2. Therefore, compound **2** could be represented as 15,18-dihydroxyabieta-8,11,13-trien-7-one.

Compound **3** was assigned the molecular formula $C_{19}H_{26}O_3$, by HREIMS. The UV and IR spectra indicated absorptions for hydroxyl groups, an α,*β*-unsaturated ke-

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**, **1a**, **2**, **2a**, **2b**, and **3** in $CDCl_3$[a]

proton	1	1a	2	2a	2b	3
H-1α	1.49, m	1.50, m	1.53, m	1.57, ddd (13.3, 13.3, 4.0)	1.56, ddd (13.0, 13.0, 4.0)	1.57, m
H-1*β*	2.35, ddd (13.0, 3.5, 3.5)	2.37. ddd (13.0, 3.5, 3.5)	2.33, ddd (13.0, 3.5, 3.5)	2.37, ddd (13.0, 3.5, 3.5)	2.34, ddd (13.0, 3.5, 3.5)	2.33, ddd (12.5, 2.5, 2.5)
H-2α	1.84, m	1.84, m	1.76, m	1.78, m	1.77, m	1.85, d quintet (13.5, 3.0)
H-2*β*	1.84, m	1.84, m	1.81, m	1.83, m	1.82, m	1.69, dddt (13.5, 13.5, 13.5, 3.0)
H-3α	1.51, ddd (13.5, 13.5, 5.0)	1.48, m	1.59, ddd (13.3, 13.3, 3.8)	1.47, m	1.46, m	1.45, ddd (13.5, 13.5, 3.0)
H-3*β*	1.38, dt (13.5, 3.0, 3.0)	1.40, ddd (13.5, 3.0, 3.0)	1.38, ddd (13.3, 3.8, 3.8)	1.47, m	1.46, m	1.93, ddd (13.5, 3.0, 3.0)
H-5α	2.34, dd (13.0, 2.0)	2.34, dd (13.0, 2.0)	2.26, dd (12.7, 5.3)	2.21, dd (10.8, 7.0)	2.23, dd (10.8, 7.5)	2.13, dd (14.5, 4.0)
H-6α	1.46, m	1.52, m	2.65, m	2.68, m	2.66, m	3.00, dd (18.0, 4.0)
H-6*β*	2.06, m	2.08, ddd (14.0, 13.0, 4.3)	2.65, m	2.68, m	2.66, m	2.63, dd (18.0, 14.5)
H-7*β*	4.79, dd (4.5, 1.5)	5.97, dd (4.3, 1.5)				
H-11	7.26, d (8.5)	7.30, d (8.5)	7.34, d (8.5)	7.38, d (8.5)	7.35, d (8.5)	7.36, d (8.5)
H-12	7.38, dd (8.5, 2.0)	7.44, dd (8.5, 2.0)	7.68, dd (8.5, 2.0)	7.75, dd (8.5, 2.0)	7.52, dd (8.5, 2.0)	7.73, dd (8.5, 2.0)
H-14	7.46, d (2.0)	7.34, d (2.0)	7.99, d (2.0)	8.08, d (2.0)	7.98, d (2.0)	8.08, d (2.0)
H-15						
H-16	1.57, s	1.56, s	1.54, s	1.59, s	1.76, s	1.58, s
H-17	1.58, s	1.56, s	1.55, s	1.66, s	1.77, s	1.59, s
H-18	9.30, s	9.27, s	3.13, d (11.5) 3.46, d (11.5)	3.74, d (11.5) 3.84, d (11.5)	3.73, d (11.5) 3.83, d (11.5)	
H-19	1.17, s	1.17, s	0.93, s	1.03, s	1.02, s	1.30, s
H-20	1.19, s	1.21, s	1.25, s	1.28, s	1.26, s	1.21, s
C(15)OCOMe					2.05, s	
C(18)OCOMe		2.08, s		2.02, s	2.04, s	

[a] Values were recorded at 500 MHz, δ in ppm, J (in parentheses) in Hz; assignments from ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY data.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**, **1a**, **2**, **2a**, **2b**, **3** ($CDCl_3$)[a]

carbon	1	1a	2	2a	2b	3
1	37.5 t	37.4 t	37.5 t	37.3 t	37.3 t	37.2 t
2	17.8 t	17.7 t	18.2 t	18.0 t	18.0 t	20.1 t
3	31.9 t	32.0 t	34.7 t	35.2 t	35.2 t	42.5 t
4	49.2 s	49.1 s	37.7 s	37.7 s	37.7 s	71.5 s
5	37.3 d	38.1 d	42.2 d	43.2 d	42.8 d	50.9 d
6	30.9 t	28.3 t	35.9 t	36.0 t	35.9 t	35.0 t
7	67.8 d	70.1 d	199.8 s	198.8 s	198.6 s	198.9 s
8	135.7 s	131.8 s	130.4 s	130.4 s	130.6 s	130.4 s
9	147.1 s	147.9 s	154.5 s	154.2 s	154.2 s	153.5 s
10	36.7 s	36.6 s	37.6 s	36.6 s	36.5 s	38.6 s
11	124.3 d	124.4 d	123.7 d	123.7 d	123.7 d	124.0 d
12	124.9 d	125.4 d	130.6 d	130.6 d	130.1 d	130.6 d
13	147.0 s	147.2 s	147.1 s	147.2 s	144.0 s	147.2 s
14	126.0 d	126.4 d	122.9 d	123.1 d	123.1 d	123.1 d
15	72.3 s	72.2 s	72.2 s	72.3 s	81.0 s	72.2 s
16	31.6 q	31.6 q	31.5 q	31.6 q	28.5 q	31.6 q
17	31.7 q	31.7 q	31.5 q	31.7 q	28.7 q	31.6 q
18	206.2 d	205.6 d	70.6 t	71.6 t	71.4 t	
19	14.0 q	14.1 q	17.3 q	17.3 q	17.3 q	22.7 q
20	24.3 q	24.3 q	23.8 q	23.9 q	23.9 q	22.7 q
C(15)OCO*Me*					22.3 q	
C(15)O*C*OMe					169.8 s	
C(18)OCO*Me*		21.5 q		20.9 q	21.0 q	
C(18)O*C*OMe		170.5 s		171.0 s	171.1 s	

[a] Values were recorded at 125 MHz, δ in ppm; assignments from DEPT, HMQC, and HMBC experiments.

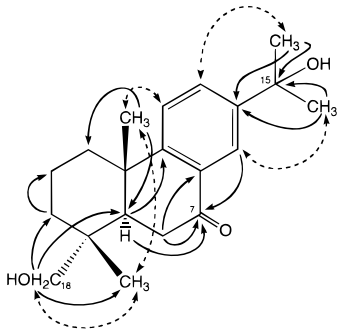

Figure 2. HMBC (plain arrow) and key NOESY (dashed arrow) interactions of compound **2**.

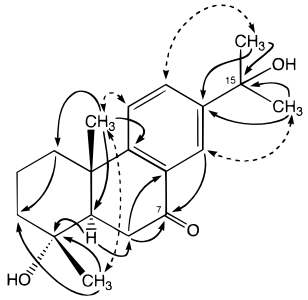

Figure 3. HMBC (plain arrow) and key NOESY (dashed arrow) interactions of compound **3**.

tone, and a conjugated aromatic ring. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed signals for two tertiary methyl groups, a hydroxyisopropyl group, a tertiary hydroxyl group, and an aromatic ring characteristic of an abieta-8,11,13-triene. Comparing the ^{13}C NMR data of **3** with that of **2**, compound **3** shows a signal attributed to a quaternary oxygenated carbon [δ_C 71.5 (s)], while the C-18 signal of **2** [δ_C 70.6 (t)] was absent. Together with the molecular ion at *m*/*z* 302.1889 in EIMS, these data suggested that compound **3** was a new norabietatriene. The HMBC spectrum of **3** exhibited the cross correlations shown in Figure 3, indicating that two hydroxyl groups should be placed at C-4 and C-15, and a ketone group at C-7. In the NOESY spectrum (Figure 3), a significant correlation was observed between the signals of H-20 and H-19 geminal to a hydroxyl group, indicative of a 1,3-diaxial relationship. Thus, compound **3** was characterized as 18-*nor*-4,15-dihydroxyabieta-8,11,13-trien-7-one.

Compounds **1**−**3** have not yet been reported in the literature. Although the ^{1}H and ^{13}C NMR data of compound **2a** have been reported,[6] some differences in the ^{13}C NMR assignments were observed in the present study (Table 2).

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on a Hitachi 150−20 spectrophotometer, and IR spectra were recorded using a Perkin−Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on Varian XL-300 and INOVA 500 spectrometers 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 Cosmocil 75 C_{18}−OPN (ODS, Nacarai Tesque), and MPLC was carried out with Si gel (230−400 mesh, Merck) and Cosmocil 40 C_{18}−PREP (ODS, 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) column. Fractions obtained from column chromatography were monitored by TLC (Si gel 60 HF_{254}). Preparative TLC was carried out on Merck Si gel PF_{254} plates (20 × 20 cm, 0.5 mm thick).

Isolation of Compounds. Preliminary Si gel column chromatography of the $CHCl_3$ extract of the fresh cones of *L. kaempferi* has been reported previously, with separation into 10 fractions.[1] Rechromatography of fraction 8 (9.87 g), eluted with $CHCl_3$−EtOAc (2:1) from the preliminary Si gel column chromatography, over Si gel (200 g) with a solvent gradient from *n*-hexane−EtOAc (4:1) to 100% EtOAc afforded fractions **a**−**k**. Rechromatography of fraction **f** (169 mg), eluted from *n*-hexane−EtOAc (2:1), over ODS column with MeOH−H_2O (3:1) furnished 7β-hydroxyabieta-8,11,13-trien-18-oic acid, 39 mg, $[\alpha]^{23}_D$ +21° (*c* 0.41, EtOH).[4] Fraction **g** (1.65 g), obtained from *n*-hexane−EtOAc (1:1), was repeatedly purified by ODS column chromatography with MeOH−H_2O (4:1) to give successively compound **2** (120 mg) and compound **1** (8 mg). Rechromatography of fraction **h** (241 mg), obtained from *n*-hexane−EtOAc (1:1), was purified using MPLC (ODS) with MeOH−H_2O (4:1), and HPLC with MeCN−H_2O (7:3) furnished compound **3** (20 mg).

Fraction **F** (2.17 g), collected from the early fractions in the rechromatography of fraction 9, was subjected to MPLC (Si gel). Elution with *n*-hexane−EtOAc (3:1) successively afforded two gummy residues from fractions 13−28 (**F-1**, 338 mg) and 29−48 (**F-2**, 195 mg), respectively. Rechromatography of **F-1** over an ODS column with MeOH−H_2O (4:1) furnished crude 16-*nor*-15-oxoabieta-8,11,13-trien-18-oic acid (12 mg), which was methylated by diazomethane etherate to afford a methyl ester (6 mg), identical in all respects with an authentic sample.[3] Fraction **F-2** was also purified using an ODS column. Elution with MeOH−H_2O (4:1) of the column furnished abieta-8,11,13-trien-18-yl succinate, 20 mg, $[\alpha]^{23}_D$ +32° (*c* 1.0, $CHCl_3$).[2]

7α,15-Dihydroxyabieta-8,11,13-trien-18-al (1): colorless oil; $[\alpha]^{23}_D$ −17° (*c* 0.21, $CHCl_3$); IR (film) ν_{max} 3380 (OH), 2971, 2931, 1717 (−CHO), 1498 and 1456 (aromatic ring) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 316 $[M]^+$ (12), 301 $[M - Me]^+$ (100), 298 $[M - H_2O]^+$ (36), 280 $[M - 2H_2O]^+$ (9), 269 (16), 265 (15), 195 (20), 155 (14), 59 (12); HREIMS *m*/*z* 316.2044 (calcd for $C_{20}H_{28}O_3$, 316.2037).

Acetylation of Compound 1. A mixture of compound **1** (2 mg) in dried pyridine−Ac_2O (1:1, 1 mL) was left at room temperature overnight. Workup as usual yielded a residue (3 mg), which was purified by preparative TLC (*n*-hexane−EtOAc, 3:1) to furnish a monoacetate (**1a**), 1.8 mg, as a colorless oil: $[\alpha]^{23}_D$ +21° (*c* 0.20, $CHCl_3$); IR (film) ν_{max} 3445 (OH), 2920, 2850, 1731 and 1238 (OAc), 1718 (−CHO), 1504 and 1463 (aromatic ring) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 358 $[M]^+$ (0.5), 340 (3), 316 (5), 298 (100), 286 (13), 255 (24), 173 (23), 155 (27), 141 (17), 59 (27), 43 (49); HREIMS *m*/*z* 358.2142 (calcd for $C_{22}H_{30}O_4$, 358.2142).

15,18-Dihydroxyabieta-8,11,13-trien-7-one (2): viscous oil; $[\alpha]^{23}_D$ −11° (*c* 1.38, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 253 (3.96) and 299 (3.26) nm; IR (film) ν_{max} 3408 (OH), 2972, 2932, 1668 (aryl C=O), 1497 and 1457 (aromatic ring), 1149, 983, 858 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 316 $[M]^+$ (6), 301 $[M - Me]^+$ (100), 283 $[M - Me - H_2O]^+$ (7), 203 (19), 187 (6), 115 (4), 43 (15); HREIMS *m*/*z* 316.2044 (calcd for $C_{20}H_{28}O_3$, 316.2037).

Acetylation of Compound 2. A mixture of compound **2** (20 mg) and dried pyridine-Ac_2O (1:1, 2 mL) was left at room temperature overnight. The reaction mixture was evaporated under reduced pressure to give a residue (23 mg), which showed two spots on TLC (*n*-hexane−EtOAc, 3:1). Si gel column chromatography of the residue yielded a monoacetate (**2a**), 12 mg, as a viscous oil: $[\alpha]^{23}_D$ −12° (*c* 1.06, $CHCl_3$); IR (film) ν_{max} 3463 (OH), 2971, 2934, 1739 and 1238 (OAc), 1681 (aryl C=O), 1607, 1491 and 1459 (aromatic ring) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 358 $[M]^+$ (10), 343 $[M - Me]^+$ (100), 298 (15), 283 (45), 265 (24), 203 (24), 187 (78); HREIMS *m*/*z* 358.2150 (calcd for $C_{22}H_{30}O_4$, 358.2142), and a diacetate (**2b**), 6 mg, as a viscous oil: $[\alpha]^{23}_D$ −40° (*c* 0.46, $CHCl_3$); IR (film) ν_{max} 2935, 1737 and 1241 (OAc), 1683 (aryl C=O), 1610, 1492 and 1466 (aromatic ring) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 400 $[M]^+$ (10), 357 (41), 341 (97), 325 (39), 280 (44), 265 (72), 245 (24), 185 (29), 43 (100); HREIMS *m*/*z* 400.2250 (calcd for $C_{24}H_{32}O_5$, 400.2248).

18-*nor*-4,15-Dihydroxyabieta-8,11,13-trien-7-one (3): viscous oil; $[\alpha]^{23}_D$ +6° (*c* 1.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 253 (3.94) and 297 (3.31) nm; IR (film) ν_{max} 3417 (OH), 2973, 2934, 1673 (aryl C=O), 1607, 1490 and 1457 (aromatic ring) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; EIMS (70 eV) *m*/*z* 302 $[M]^+$ (5), 287 $[M - Me]^+$ (100), 269 $[M - Me - H_2O]^+$ (6), 241 (5), 199 (12), 171 (5), 43 (14); HREIMS *m*/*z* 302.1889 (calcd for $C_{19}H_{26}O_3$, 302.1881).

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References and Notes

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