18-Succinyloxyabieta-8,11,13-triene as a new component from green shoots of the Siberian fir

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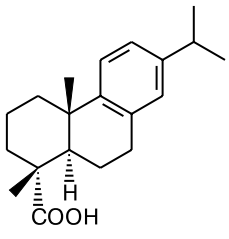
A new diterpene derivative, 18-succinyloxyabieta-8,11,13-triene, was isolated from the acidic fraction of the extract from wood greens of a Siberian fir. The chemical structure of the molecule was established on the basis of spectroscopy and confirmed by partial synthesis from abieta-8,11,13-trien-18-ol.

Key words: diterpenoids, abieta-8,11,13-trien-3-ol, succinates, 2D NMR spectroscopy.

The wood greens (fresh shoots with needles) of the Siberian fir (*Abies sibirica* L.) are known as a raw material for the production of a plant growth regulator used in Russia,[1,2] whose biological activity is attributed to triterpenic acids mainly concentrated in the needles.[3] The deneedled shoots, which are a component of wood greens, contain some triterpenic acids that were identified in the needles and some other acidic components, *e.g.*, diterpenic acids and *n*-alkyl ferulates.[4]

A TLC analysis has shown that the acidic fraction of the ethereal extract of the wood greens contains a component that is absent in the extract of Siberan fir needles but present in the extract of deneedled shoots. This component is manifested in the chromatogram between the bands of the references, well-known dehydroabietic acid[5] (**1**) and the least polar triterpenoic ketoacid of the Siberian fir, firmanic acid.[6,7]

Methylation of the acidic fraction of the extract from the wood greens of Siberian fir with diazomethane followed by column chromatography gave the methyl ester of the new component as a colorless oil. Using high-resolution mass spectrometry, the molecular formula of this compound was found to be ($C_{25}H_{36}O_4$), and using



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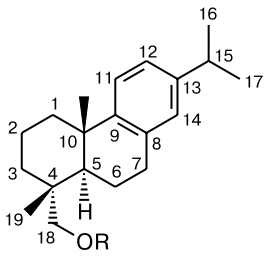

2—4

R = MeO(O)C(CH_2)$_2$C(O) (**2**), H (**3**), HO(O)C(CH_2)$_2$C(O) (**4**)

^{1}H and ^{13}C NMR spectra, it was identified as the methyl ester of 18-succinyloxyabieta-8,11,13-triene (**2**). The structure of compound **2** was confirmed by its partial synthesis, namely, succinylation of the known[8] abieta-8,11,13-trien-18-ol (dehydroabietinol, (**3**) on treatment with succinic anhydride in DMF in the presence of 4-dimethylaminopyridine followed by methylation of the resulting acid **4**.

The ^{13}C NMR spectrum of compound **2** was interpreted using 2D NMR procedures: ^{1}H—^{1}H (COSY), ^{13}C—^{1}H NMR (COLOC), and the ^{13}C NMR data for the known labdane succinate.[9]

In the reverse-phase HPLC using 85% aqueous MeOH containing 0.05 *M* H_3PO_4 as the eluent, the peaks for acids **1** and **4** coincided, the capacity factor *k´* being 2.5 for the common peak. When the proportion of MeOH in the eluent decreased to 82%, these peaks were partially resolved, the *k´* factor increased to 3.5 for acid **1** and to 3.9 for acid **4**.

Experimental

IR spectra were measured on a Vector 22 instrument. NMR spectra were recorded for solutions in $CDCl_3$ on a Bruker DRX-500 spectrometer (operating at 500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C); for COSY and COLOC 2D experiments (7 Hz), the standard Bruker software was used.

High-resolution mass spectra (EI, 70 eV) were run on a Finnigan MAT 8200 mass spectrometer. The optical rotation was measured (580 nm) on a Polamat A polarimeter. Column chromatography was carried out on SiO_2 (KSK) with a grain size of 0.05—0.14 mm using a mixture of petroleum and diethyl ethers with increasing (0 to 60%) content of the latter as the eluent. TLC was performed on Silufol plates, visualization was accomplished by spraying with H_2SO_4. HPLC was carried out

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on a Milikhrom microcolumn liquid chromatograph (Ob'-4), UV detector, 200 nm, a 64×2 mm column, the Lichrosorb RP-18 sorbent (Merck), 5 μm grain size. The eluent was prepared by mixing MeOH and 0.05 *M* aqueous H_3PO_4 (85 : 15 or 82 : 18, v/v, respectively). The elution rate was 100 μL min^{-1}. The concentration of the solution under analysis was about 12 mg mL^{-1} in MeOH, the sample volume was 1.4 μL. The column temperature was 30 °C. The column was heated by passing an ac voltage of 0.5 V in a thermostat designed at the Engineerung Department of the Novosibirsk Institute of Organic Chemistry, Siberian Branch of the RAS.

The starting mixture of acids from the extract of wood greens of the Siberian fir was isolated in 6% yield by a previously described procedure[1] from the raw material collected in the Novosobirsk region. Before treatment with aqueous alkali, the ethereal extract was filtered through a paper filter.

Acid **1** (see Ref. 5) for HPLC and alcohol **3** (see Ref. 8a) for the synthesis of ester **2** were prepared by known procedures.

18-Succinyloxyabieta-8,11,13-triene methyl ester (2). ***A.*** An ethereal solution of CH_2N_2 was added in small portions to a stirred solution containing a mixture (3.0 g) of the acids from the wood greens of Siberian fir in Et_2O (30 mL) until the yellow color persisted (excess CH_2N_2). The solvent was immediately evaporated *in vacuo* and the residue was chromatographed on SiO_2 (100 g) to give successively 0.31 g of a mixture of the methyl esters of gum acids (elution with a mixture of petroleum ether with 2% Et_2O); 0.15 g of a mixture of esters with compound **2** as the major component (petroleum ether with 8% Et_2O), and 2.50 g of the methyl esters of triterpenoic acids (elution with Et_2O). Repeated chromatography of a 0.15 g fraction on SiO_2 (8 g) gave 0.08 g of ester **2** as a colorless oil with $[\alpha]_{580}^{23}$ + 36.1° (*c* 1.66; $CHCl_3$). IR (CCl_4), ν/cm^{-1}: 1740 (C=O); 1498, 1216, 1159, 1001. MS, *m/z* (I_{rel} (%)): 400 $[M]^+$, (7), 268 (24), 254 (21), 253 (100), 225 (6), 211 (14), 115 (21), 43 (15). Found, *m/z*: 400.26181 $[M]^+$. Calculated for $C_{25}H_{36}O_4$: 400.26134. ^{1}H NMR, δ: 0.93 (s, 3 H, $H_3C(19)$); 1.20 (s, 3 H, $H_3C(20)$); 1.21 (d, 6 H, 2 $H_3C(16)$ and $H_3C(17)$, J = 7.0 Hz); 1.35—1.45 (m, 3 H, 2 H(1), $H(3)_{ax}$); 1.61 (dd, 1 H, H(5), J = 11.5 and 3.0 Hz); 1.63—1.79 (m, 4 H, 2 H(2), 2 H(6)); 2.27 (dm, 1 H, $H(3)_{eq}$, J_{gem} = 13 Hz); 2.59 (nar.m, 4 H, $-(C\underline{H}_2)_2-$COOMe); 2.80 (m, 1 H, H(7A)); 2.81 (sept, 1 H, H(15), J = 7.0 Hz); 2.87 (br. ddd, 1 H, H(7B), J = 17, 7, 2 Hz); 3.59 (s, 3 H, COOMe); 3.75 (d, 1 H, H(18A), J_{AB} = 10.7 Hz); 3.97 (d, 1 H, H(18B), J = 10.7 Hz); 6.87 (d, 1 H, H(14), $J_{12,14}$ = 1.5 Hz); 6.97 (dd, 1 H, H(12), $J_{12,14}$ = 1.5 Hz, $J_{11,12}$ = 8.0 Hz); 7.15 (d, 1 H, H(11), $J_{11,12}$ = 8.0 Hz). ^{13}C NMR, δ: 38.19 (t, C(1)); 18.42 (t, C(2)); 35.46 (t, C(3)); 36.75 (s, C(4)); 44.24 (s, C(5)); 18.91 (t, C(6)); 30.05 (t, C(7)); 134.62 (s, C(8)); 146.98 (s, C(9)); 37.30 (s, C(10)); 124.11 (d, C(11)); 123.73 (d, C(12)); 145.41 (s, C(13)); 126.73 (s, C(14)); 33.30 (d, C(15)); 23.83 (q, C(16)); 23.84 (q, C(17)); 72.62 (t, C(18)); 17.62 (q, C(18)); 17.29 (q, C(19)); 25.20 (q, C(20)); 172.14 (s, C(1′)); 28.81 (t, C(2′)); 28.76 (t, C(3′)); 172.48 (s, C(4′)); 51.57 (q, O$\underline{C}H_3$).

B. An ethereal solution of CH_2N_2 was added to a solution of acid **4** (0.10 g) in Et_2O (5 mL) until the yellow color persisted. The removal of the solvent gave 0.1 g of ester **2** as a colorless oil with $[\alpha]_{580}^{23}$ +35° (*c* 2.50; $CHCl_3$) identical to the sample obtained from the wood greens extract according to TLC and NMR spectra.

18-Succinoyloxyabieta-8,11,13-triene (4). Succinic anhydride (0.35 g) and 4-dimethylaminopyridine (0.05 g) were added to a solution of alcohol **3** (0.80 g)[8a] in DMF (10 mL). The mixture was stirred for 5 h at 40—45 °C, cooled to room temperature, 2% aqueous NaOH (40 mL) was added, and the unreacted alcohol **3** (0.60 g) was extracted with Et_2O (2×50 mL). The target acid **4** (0.15 g, 53% based on the consumed reactant) was isolated from the alkaline aqueous solution after acidification with hydrochloric acid to pH = 2 and extraction into Et_2O (2×50 mL). Colorless oil. IR (CCl_4), ν/cm^{-1}: 1740 ($\underline{CO}_2$Me); 1716 ($\underline{CO}$OH); 1482, 1238, 1167, 997. The ^{1}H NMR (500 MHz, $CDCl_3$, δ) spectrum differs markedly from the ^{1}H NMR spectrum of ester **2**, in particular, it contains a broad multiplet at 2.58—2.67 ppm ($-(C\underline{H}_2)_2-CO_2H$), instead of the signal at 2.59 ppm, and no signal for the protons of the CO_2Me group.

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