Triterpenoids from *Abies* species

23.* A lanostane analog of cytotoxic cycloartanolide and other lactones from needle-free shoots of Siberian fir

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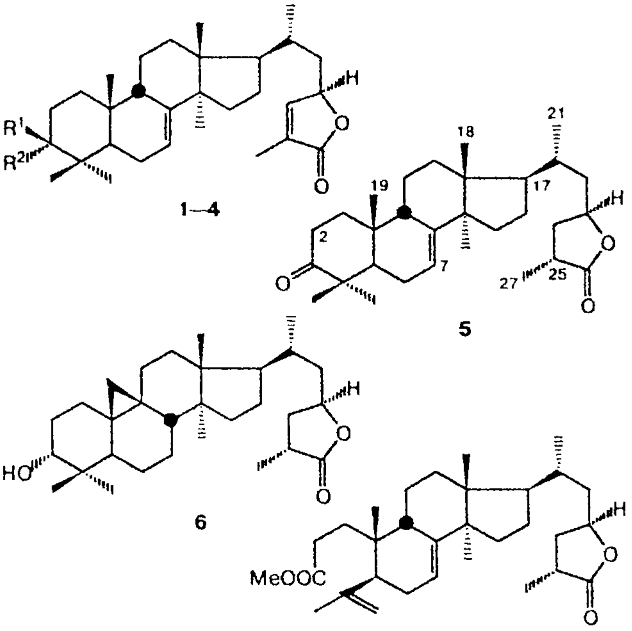
Two new lanostane lactones were isolated from a neutral part of an ethereal extract of dried needle-free shoots of Siberian fir. Their structures were proposed based on comparison of their ^{13}C and ^{1}H NMR spectra with the spectra of known triterpenoid lactones with similar structures.

Key words: triterpenoids; Siberian fir; lactones; NMR spectra.

The bark and coniferous needles of Siberian fir (*Abies sibirica* Ledb.) contain triterpenoid lactones, the best known of which are abieslactone (**1**)[2—4] and its 3-oxo- and 3-hydroxy-analogs **2** and **3**, respectively.[4] For lactone **1** itself,[2] unlike its derivatives,[5] no clear-cut biological activity has been discovered so far.

We studied the chemical composition of an ethereal extract of needle-free 1—3-years old shoots of Siberian fir, which are present in the commercial "fir green." It was found that these shoots contain lactones **1**—**3** and some other hitherto unknown lactones including natural acetate **4**. An oxo lactone with a melting point of 187—188 °C was identified as compound **5** based on the data of ^{13}C NMR (Table 1) and ^{1}H NMR spectra (see Experimental) and on comparison of these spectra with those of closely related analogs, *viz.*, natural oxo lactone **2**[6] and hydroxy lactone **6**[7]. The stereochemistry of this compound corresponds to that of other triterpenoids from Siberian fir, *viz.*, oxolactone **2** and methyl abiesolidate **7**,[8] whose structures were established previously by X-ray diffraction analysis.

The second lactone (m.p. 238—240 °C) has the molecular formula $C_{30}H_{42}O_4$ (determined by high-resolution mass spectrometry). Its ^{13}C NMR spectrum (see Table 1) can be represented as a combination of the corresponding spectra of the steroid part of lactone **2** (see Ref. 6) and the spiropyranolactone fragment of the molecule of pseudolarolide B (**8**),[9] which is a cytotoxic cycloartanolide from the extract of seeds of a Chinese plant, *Pseudolarix kaempferi* Gord. (the *Pinaceae* Lindl. family). Similar correspondence can also be noted for the ^{1}H NMR spectra of lactone **8** and the compound in question (Table 2 and Experimental); hence, structure **9** was proposed for this compound.



1—4

5

6

7

1: R¹ = H; R² = OMe **3**: R¹ = H; R² = OH
2: R¹ + R² = O **4**: R¹ = H; R² = OAc

The ^{1}H NMR spectrum is more sensitive to the appearance of a pyran ring fused with ring *D* of the steroid nucleus in the molecule of lactone **9**. The shape and position of the signal for the H(7) proton correspond to the values expected[10] for 3-oxo-9β-lanost-7-enoids, and the signals for the $H_2C(2)$ protons, which almost coincide in the spectra of oxo lactones **2** and **5**, are substantially different in the ^{1}H NMR spectrum of lactone **9**.

The scarcity of compound **9** at our disposal precluded its X-ray diffraction analysis needed to establish unambiguously its structure.

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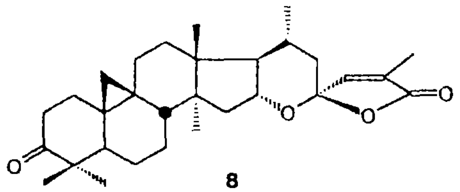
* For Part 22, see Ref. 1.

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 10, pp. 2064—2066, October, 1998.

1066-5285/98/4710-2009 $20.00



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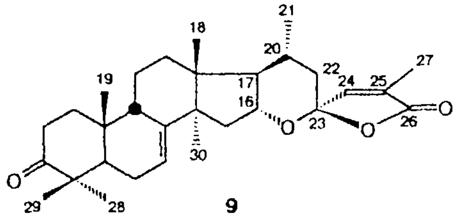

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Table 1. Data of the ^{13}C NMR spectra (δ) of compounds **2**, **5**, **6**, **8**, and **9** ($CDCl_3$)

Atom	2	5	6	8	9
C(1)	34.20 (t)	34.13 (t)			33.30 (t)
C(2)	34.28 (t)	34.29 (t)			33.55 (t)
C(3)	218.92 (s)	218.61 (s)			219.14 (s)
C(4)	47.01 (s)	46.91 (s)			46.76 (s)
C(5)	52.35 (d)	52.27 (d)			53.16 (d)
C(6)	23.01 (t)	22.94 (t)			22.62 (t)
C(7)	121.64 (d)	121.54 (d)			122.16 (d)
C(8)	148.48 (d)	148.43 (s)			146.86 (s)
C(9)	45.67 (d)	45.44 (d)			44.67 (d)
C(10)	35.80 (s)	35.72 (s)			35.91 (s)
C(11)	20.88 (t)	20.82 (t)			19.13 (t)
C(12)	34.39 (t)	34.29 (t)			34.02 (t)
C(13)	44.15 (s)	44.03 (s)		44.6 (s)	43.42 (s)
C(14)	51.93 (s)	51.87 (s)		46.2 (s)	49.31 (s)
C(15)	33.01 (t)	32.98 (t)		43.4 (t)	41.11 (t)
C(16)	28.21 (t)	28.23 (t)		75.5 (d)	75.45 (d)
C(17)	53.47 (d)	53.45 (d)		55.6 (d)	56.30 (d)
C(18)	22.51 (q)	22.44 (q)			22.98 (q)
C(19)	23.12 (q)	23.06 (q)			24.52 (q)
C(20)	33.49 (d)	33.09 (d)	33.06	25.4 (d)	25.36 (d)
C(21)	18.38 (q)	18.14 (q)	18.12	20.2 (q)	20.14 (q)
C(22)		42.53 (t)	42.76	37.8 (t)	37.61 (t)
C(23)		75.77 (d)	76.03	107.2 (s)	107.09 (s)
C(24)		36.37 (d)	36.44	146.3 (d)	146.00 (d)
C(25)		34.07 (d)	34.17	130.7 (s)	130.74 (s)
C(26)		179.91 (s)	180.24	172.3 (s)	172.10 (s)
C(27)		15.84 (q)	15.91	10.4 (q)	10.25 (q)
C(28)	21.30 (q)	21.24 (q)			20.73 (q)
C(29)	28.00 (q)	27.87 (q)			28.39 (q)
C(30)	27.41 (q)	27.34 (q)			26.35 (q)

Table 2. Data of the ^1H NMR spectra of compounds **8**[9] and **9** ($CDCl_3$, tetramethylsilane)

Proton	δ (*J*/Hz)	
	lactone **8**[9]	lactone **9**
HC(15α)	1.42 (dd)	**
HC(15β)	1.96 (dd, *J* = 13, 7.5)	2.04 (dd, *J* = 12.0, 8.0)
HC(16)	4.43 (q, *J* = 7.5)	4.49 (q, *J* = 7.5)
$H_3C(21)$	0.99 (d, *J* = 5.5)	1.01 (d, *J* = 5.5)
HC(24)	6.83 (q, *J* = 1)*	6.82 (q, *J* = 1.5)
$H_3C(27)$	1.87 (d, *J* = 1)	1.87 (d, *J* = 1.5)

* In Ref. 9, this signal was named a doublet.

** The signal was overlapped by other signals.

Experimental

Melting points were determined on a Koffler hot-stage apparatus. IR spectra were recorded on a UR-20 instrument. NMR spectra were measured for $CDCl_3$ solutions on Bruker DRX-500 (operating at 500.13 MHz for ^1H and at 125.76 MHz for ^{13}C) and Bruker AM-400 (operating at 400.13 MHz for ^1H) spectrometers. Mass spectra were run on a Finnigan MAT 8200 instrument (EI, direct inlet, T_{source} = 260 °C, 70 eV). Optical rotation was determined in $CHCl_3$ on a Polamat A polarimeter at 580 nm.

Column chromatography was carried out on KSK SiO_2; the ratio of compound to sorbent was ~1 : 20; hexane—ether (10 to 25%) was used as the eluent. TLC was carried out on Silufol plates; compounds were visualized by spraying with concentrated H_2SO_4.

Fir bough was gathered in winter 1995 at the territory of the Botanical Garden of the Siberian Branch of the RAS (Novosibirsk); shoots were separated from needles, dried at room temperature, and ground to a particle size of less than 1×1×1 mm.

Isolation of the lactone-containing fraction from the extract of shoots. Ground shoots (360 g) were extracted for 113 h at 20 °C with a mixture of ether and ethyl acetate (1 : 1). The extract was decanted, filtered, and treated successively with a 2% aqueous solution of NaOH (2×300 mL) and water (2×200 mL). Drying and removal of the solvent gave 19.6 g of total neutral components of the extract, which was distributed between 100 mL of 80% aqueous ethanol and 150 mL of *n*-pentane. The lower layer (aqueous ethanol) was separated, and the pentane layer was washed with aqueous ethanol (2×100 mL). The aqueous-ethanolic extracts were combined, washed with pentane (2×100 mL), diluted with an equal volume of brine and extracted with a 1 : 1 ether—ethyl acetate mixture (2×100 mL). Drying of the extract (Na_2SO_4) and evaporation of the solvent gave 2.95 g of a lactone-containing fraction as a yellowish oil in a yield of 15% of total of neutral compounds.

Isolation of lactones 4, 5, and 9. Chromatography of the resulting fraction (2.95 g) gave successively a mixture of mono- and diterpenoid nonlactone compounds (0.81 g), a mixture of nonpolar lactones and accompanying compounds (fraction A, 0.57 g), and a fraction of more polar substances (1.24 g). Repeated chromatography of fraction A gave a mixture of lactones (0.11 g) containing lactone **1**, acetate **4** (0.06 g) identical with an authentic sample[4] according to TLC and ^1H NMR spectrum, a mixture (0.07 g) of β-sitosterol and compounds **5** and **9** (fraction B), oxo lactone **2** (0.18 g), and a mixture (0.15 g) of hydroxy lactone **3** and unidentified substances. Fraction B was treated with Ac_2O/Py under known conditions,[4] and subsequent chromatography gave β-sitosterol acetate (0.03 g), lactone **5** (0.01 g), and lactone **9** (0.01 g).

(23*R*,25*R*)-3-Oxolanost-7-en-26,23-olide (5). Crystals with m.p. 187—188 °C (ether—pentane), $[\alpha]_{580}^{20}$ + 17° (*c* 0.59). IR (CCl_4), ν/cm^{-1}: 1770 (γ-lactone), 1703 (C=O), 1380, 1360, 1170. MS, *m/z* (I_{rel} (%)): 454.34540 $[M]^+$ (21), 439 (100), 421 (15), 133 (16), 121 (18), 107 (17), 95 (22). Calculated for $C_{30}H_{46}O_3$: 454.34467. ^1H NMR, δ: 0.78, 0.99, 1.01, 1.07, 1.08 (all s, 5 Me); 0.93 (d, 3 H, C(21)H_3, ${}^3J_{21,20}$ = 6.5 Hz); 1.27 (d, 3 H, C(27)H_3, ${}^3J_{27,25}$ = 7.0 Hz); 2.47 (dd, 2 H, C(2)H_2, J = 6.0 and 8.5 Hz); 2.69 (sextet, 1 H, H(25), ${}^3J_{25,24}$ = ${}^3J_{25,27}$ = 7.5 Hz); 4.63 (m, 1 H, H(23)); 5.62 (dd, 1 H, H(7), J = 7.5 and 3.0 Hz). The data of the ^{13}C NMR spectrum are given in Table 1.

(16*R*,23*S*)-3-Oxo-16,23-epoxylanosta-7,24-dien-26,23-olide (9). Colorless crystals, m.p. 238—240 °C (ether—pentane), $[\alpha]_{580}^{20}$ +26.1° (c 0.65). IR ($CHCl_3$), ν/cm^{-1}: 1770 (γ-lactone), 1705 (C=O), 1390, 1330, 980, 940, 860. MS, *m/z* (I_{rel} (%)): 466.30855 $[M]^+$ (34), 451(78), 433(11), 428(14), 323(53), 313(13), 207(75), 137(100), 119(27). Calculated for $C_{30}H_{42}O_4$: 466.30829. ^{1}H NMR ($CDCl_3$), δ: 0.87, 0.93, 1.00, 1.07, 1.08 (all s, 5 Me); 1.01 (d, 3 H, H_3C(21), ${}^3J_{21,20}$ = 6 Hz); 1.42 (dd, 1 H, HC(5), J = 12.0; 2.5 Hz); 2.22 (m, 1 H, HC(9)); 2.41 (ddd, 1 H, H^aC(2), J = 15.5, 8.5, 6.0 Hz); 2.55 (ddd, 1 H, H^bC(2), J = 15.5, 10.0, 4.0 Hz); 5.66 (dt, 1 H, HC(7), J = 7.5, 3.0 Hz). The signals corresponding to signals of lactone **8** are listed in Table 2.

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Received January 12, 1998;
in revised form March 24, 1998