Triterpenoids from *Abies* species
19.* (22*Z*)-3α-Hydroxy-17,14-fridolanosta-7,14,22,24-tetraen-26,23-olide, a new lactone from the needles of the Siberian fir

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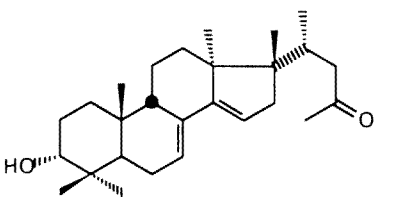
A new triterpenoid lactone with a reported rearranged lanostane skeleton was isolated from the neutral fraction of the extract from the needles of Siberian fir. Its structure was established on the basis of chemical transformations and NMR spectra using two-dimensional (COSY, COLOC) NMR spectroscopy.

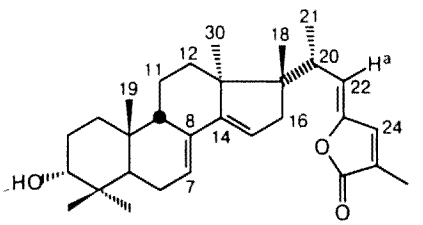
Key words: triterpenoids, lactones, two-dimensional NMR spectroscopy, HPLC.

The neutral fraction of the ether extract from the needles of the Siberian fir (*Abies sibirica* Ledb.) was recently found[2] to contain two isomeric lanostane lactones with similar (*Z*)-γ-alkylidene-α,β-butenolide chromophore groups in their molecules. To continue the study of the neutral components of the polar fraction of the extract from the needles of this ligneous plant abundant in Siberia, we have isolated a new triterpene lactone with the composition $C_{30}H_{42}O_3$ (high resolution mass spectroscopy) in 0.018% yield from the air-dried raw material. According to the IR spectrum, the molecule of this compound has a secondary hydroxyl group and a γ-lactone ring, which is a part of the γ-alkylidene-α,β-butenolide fragment according to the UV spectrum.[2] Treatment with an alkaline ethanolic solution is known to result first in the ordinary opening of the lactone ring[2] with the formation of β-acylacrylic acid and then, after an extended reaction time, in fragmentation to methyl ketone.[3] Under these conditions the lactone under study resulted in the known[3] trinor-17,14-fridolanostane hydroxyketone **1**. Thus, the starting lactone has structure **2** (with undefined configuration of the Δ^{22} double bond).

The interpretation of all of the signals in the NMR spectra of lactone **2** (Table 1), thus confirming its structure, was performed by $^1H-^1H$ (COSY) and $^{13}C-^1H$ (COSY, COLOC) two-dimensional NMR spectroscopy. It should be mentioned that the assignments of the signals of the C(11), C(12), C(21), and C(30) atoms in the ^{13}C NMR spectra of the three other neutral derivatives of 17,14-fridolanostane given in Ref. 4 were made without the use of the minimum necessary number of two-dimensional spectra, which do not need a large amount of a sample ($^1H-^1H$ (COSY) and $^{13}C-^1H$ (COSY, COLOC)), and are invalid. Their positions should therefore be changed according to the data in Table 1.



1



2

The assignment of the signals of C(11) and C(12) in the ^{13}C NMR spectrum of the compound under study is based on the presence of cross peaks in the COLOC spectrum resulting from the interaction of C(8) with H(11a) and H(11b) and of C(14) with H(12α). The assignment of the signal of C(8) is based on the presence of C(8)/H(6a) and C(8)/H(6b) cross peaks in the aforementioned spectrum. The assignment of the signal of C(14) is made on the basis of the cross peaks resulting from the interaction of this atom with the protons at C(16). The H(30)/H(12β) cross peak in the two-dimensional $^1H-^1H$ (COSY) NMR spectrum allowed unambiguous identification of the singlet of $C(30)H_3$ in the ^1H NMR spectrum and detection of the signal of C(30)

* For Part 18 see Ref. 1.

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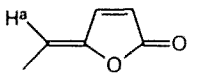
Table 1. ^{13}C and ^{1}H NMR spectral data for compound **2** ($CDCl_3$, $SiMe_4$, δ, ppm, *J*/Hz, c = 12 mg·mL^{-1})

Atom number (*i*)	δC^i	δH^i (*J*)
1	28.64 t	0.90; 1.98
2	25.10 t	1.57; 1.93
3	76.47 d	3.42 sh.m
4	37.04 s	—
5	37.85 d	1.50 (dd, 11.5; 5.0)
6	23.00 t	1.86; 1.96
7	120.97 d	5.56 m
8	136.33 s	—
9	53.00 d	1.35
10	34.67 s	—
11	24.93 t	1.29; 1.72
12	32.01 t	1.20 (α-H(C-12); 1.67 (β-H(C-12)
13	51.71 s	—
14	152.95 s	—
15	114.34 d	5.17 (dd, 3.0; 2.0)
16	44.54 t	1.96; 2.22 (br.d, 15.0)
17	50.50 s	—
18	17.00 q	0.97 s
19	22.23 q	0.94 s
20	36.26 d	3.21 (dq, 10.0; 7.0)
21	17.21 q	0.99 (d, 7.0)
22	118.12 d	5.17 (br.d, 10.0)
23	146.73 s	—
24	137.87 d	6.95 (qd, 1.5; 0.4)
25	128.67 s	—
26	171.07 s	—
27	10.37 q	1.97 (d, 1.5)
28	28.07 q	0.95 s
29	22.88 q	0.90 s
30	19.10 q	0.78 s

in the ^{13}C NMR spectrum using the spectrum of $^{13}C-^{1}H$ correlation (COSY). The presence of the H(21)/C(21) cross peak in the two-dimensional $^{13}C-^{1}H$ (COSY) NMR spectrum made it possible to assign the signal for C(21) in the ^{13}C NMR spectrum.

To determine the configuration of the Δ^{22} double bond in the molecule of lactone **2**, we carried out its photoisomerization. The resulting product (~20%) differed from the starting compound only by the positions of the signals of H(20), H(22), and H(24) in the ^{1}H NMR spectrum (see Experimental). The changes in the positions of only these signals indicate that the product is an isomer of the initial lactone in the geometry of the Δ^{22} double bond. A comparison of the chemical shifts of H^a in the ^{1}H NMR spectra of the two pairs of *Z*,*E*-isomers, namely, lactones **2** and **3** [5] (δ 5.17 and 5.25 ppm, respectively) and the photoisomer of lactone **2** and lactone **4** [5] (δ 5.67 and 5.80 ppm, respectively), allowed one to conclude that the initial lactone **2** has the (22*Z*) configuration, and its photoisomer has the (22*E*) configuration.



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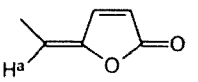

4

The (22*E*)-isomer of lactone **2** is absent in the extract from the needles of the Siberian fir. It is easily separated from lactone **2** by TLC on Silica gel, but has the same retention time in reverse phase HPLC (elution with aqueous MeOH or MeCN).

Experimental

The melting point was determined on a Kofler unit. The IR spectrum was recorded on a UR-20 instrument in CCl_4. NMR spectra were taken on a Bruker AM-300 spectrometer using the Bruker standard programs for registration of the two-dimensional $^{1}H-^{1}H$ (COSY) and $^{13}C-^{1}H$ (COSY, COLOC) NMR spectra. The mass spectrum (EI, 70 eV) was registered on a Finnigan MAT-8200 mass spectrometer. Column chromatography was performed on Silica gel KSK with a compound—sorbent ratio of 1 : 20 (elution with pentane with 10—40 % diethyl ether). TLC was carried out on Silufol plates. The spots were visualized by spraying with conc. H_2SO_4. The optical rotation was determined on Polamat A ($[\alpha]_{580}$) and Zeiss ($[\alpha]_D$) polarimeters.

(22*Z*)-3α-Hydroxy-17,14-fridolanosta-7,14,22,24-tetraen-26,23-olide (2). Isolation of the neutral fraction from the ether extract of the needles of the Siberian fir (yield 4.4 % from air-dried needles) and its chromatography on SiO_2 were performed as described in Ref. 2. The eluate containing the compound eluted just after β-sitosterol (the internal standard), which was visualized as a reddish brown spot by TLC, was evaporated to dryness. The residue was crystallized from pentane—ether to give pure lactone **2** (yield 0.018 % from the starting material), m.p. 217—220 °C; $[\alpha]^{15}_{580}$ −41° (*c* 0.44). UV (EtOH), λ_{max}/nm: 231 (ε 11000), 280 (ε 20000). IR (CCl_4), ν/cm^{-1}: 3620 (OH), 1780 (γ-lactone). ^{13}C and ^{1}H NMR spectra are given in Table 1. MS, *m*/*z*: 450.31260 $[M]^+$. Calculated for $C_{30}H_{42}O_3$: 450.31338.

Photoisomerization of lactone 2. A solution of lactone **2** (15 mg) in a hexane—ether mixture (60 mL) was irradiated in a quartz cuvette with a DRSh-1000 high-pressure mercury vapor lamp with cooling by a ventilator. Then the solution was evaporated to dryness to yield 15 mg of a crystalline residue. Its ^{1}H NMR spectrum contained all the signals of lactone **2** and also the signals of its (22*E*)-isomer, which did not correspond to those of the former and had an integral intensity of 29 % of that of the corresponding signals of the (22*Z*)-isomer. ($CDCl_3$, δ, *J*/Hz): 0.75 (s, 3 H, H(30)), 1.01 (d, 3 H, H(21), $^{3}J_{21,20}$ = 7.0), 2.00 (d, 3 H, H(27), $^{4}J_{27,24}$ = 1.5), 2.81 (dq, 1 H, H(20), $^{3}J_{20,22}$ = 11.0, $^{3}J_{20,21}$ = 7.0), 5.67 (d, 1 H, H(22), $^{3}J_{22,20}$ = 11.0), 7.26 (dq, 1 H, H(24), $^{4}J_{24,27}$ = 1.5, $^{4}J_{24,22}$ = 0.8).

Synthesis of trinor-17,14-fridolanostane hydroxyketone 1 from lactone 2. A solution of lactone **2** (40 mg) in an ethanolic

solution of NaOH (15 mL) was heated for 4 h at 70 °C, cooled to 20 °C, and diluted with a saturated aqueous solution of NaCl (30 mL) and Et_2O (30 mL). The mixture was shaken vigorously, and the ether extract was washed once again with an aqueous solution of NaCl. The solvent was removed, and the residue was chromatographed to give 27 mg of hydroxy ketone **1**, m.p. 84—86 °C (hexane), $[\alpha]_D^{20}$ +52.4° (*c* 2.2; $CHCl_3$) (*cf.* Ref. 3: m.p. 84—86 °C, $[\alpha]_D^{20}$ +54.3° ($CHCl_3$)). The ^1H NMR spectrum of the compound obtained corresponds to that of the standard hydroxy ketone **1**.

HPLC. The purity of compounds **1** and **2** was determined on a Milikhrom microcolumn liquid chromatograph[6] (64×2 mm column, LiChrosorb RP-18 (5 mm) (Merck), 30 °C).

Elution with MeOH—H_2O (85 : 15 vol.): the capacity coefficients k' are 5.4 for ketone **1**, and 5.9 for lactone **2**. Elution with MeOH—H_2O (75 : 25 vol.): k' = 8.1 for **1**, and k' = 9.8 for **2**. Detection: 200/230 and 200/280 nm, respectively. The mixture of isomers **2** is detected as a single peak in both eluents.

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