Triterpenoids from *Abies* species

22.* Isolation of the cyclic tautomer of (24*Z*)-3,23-dioxolanosta-8,24-dien-26-oic acid, a new component of the acidic fraction of the extract from Siberian fir needle

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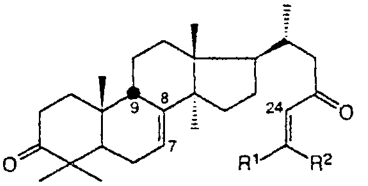
A new component, namely, the cyclic tautomer of previously unknown (24*Z*)-3,23-dioxolanosta-8,24-dien-26-oic acid, was isolated from the acidic fraction of the extract from Siberian fir needle. The structure of the title compound was established based on the ^{1}H NMR and circular dichroism spectra.

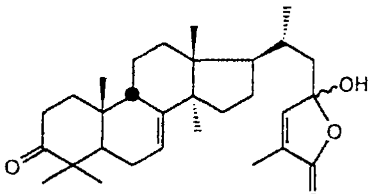
Key words: triterpenoids; Siberian fir; tautomerism; circular dichroism; HPLC.

Siberian fir needle (*Abies sibirica* Ledb.) is a rich source of triterpenoids of the 9β-lanostane series and biogenetically related structural types most of which are carboxylic acids.[2,3] Isomeric acids **1** and **2** are the first compounds of this type reported in the literature. Acid **2** (24*Z*-isomer) was characterized only as its methyl ester **3**.[4,5] Like other representatives of β-acylacrylic acids,[6] acid **2** is, apparently, prone to undergo conversion into the corresponding cyclic tautomer **5**. More recently, compound **5** was isolated from a mixture of free acids extracted from fir needle and was characterized by the physicochemical constants and spectral data.[7]



1: R¹ = COOH; R² = Me
2: R¹ = Me; R² = COOH
3: R¹ = Me; R² = CO$_2$Me
4: R¹ = CO$_2$Me; R² = Me

Methyl ester of acid **6**, which differs from compound **4** by the position of the endocyclic double bond, was identified as the minor component of the methylated mixture of acids from the fir needle under study.[8] It is reasonable to suggest that the (24*Z*)-isomer of this acid is also present in the natural mixture of triterpenic acids from the fir needle. Most likely, this isomer exists as the cyclic tautomer, lactol **7**. Actually, a crystalline precipitate was gradually formed upon storage (in a sealed tube) of an ethereal solution of the multicomponent (TLC) fraction, which has been eluted before the fraction from which lactol **5**[3] was isolated during silica gel



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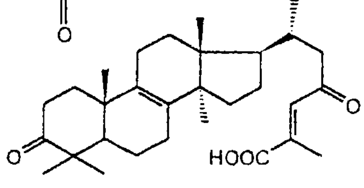

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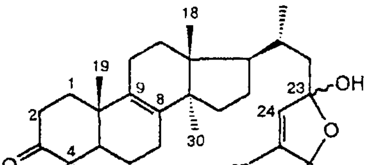

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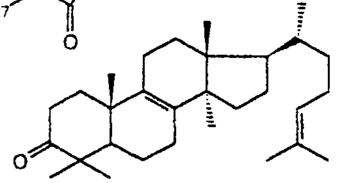

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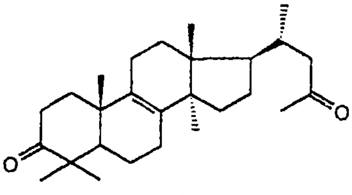

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* For Part 21, see Ref. 1.

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chromatography of a mixture of acids of the ethereal extract. According to the data of HPLC, the precipitate is a mixture of lactol **5** and a new (previously unidentified) compound. Rerystallization of this precipitate from MeOH gave a new compound of composition $C_{30}H_{44}O_4$ (high-resolution mass spectrometry), which (according to TLC and HPLC) is homogeneous but melts in a wide temperature range (183—193 °C). The IR spectrum of this compound has the characteristic absorption bands identical to those observed in the IR spectrum of lactol **5**, which is indicative of the presence of the γ-lactone ring, the hydroxy group, and the nonconjugated ketone group. The data obtained, taking into account the data of ^1H NMR spectroscopy, suggest that structure **7** can be assigned to the compound under study. Actually, the ^1H NMR spectrum of this compound (Table 1) can be considered as a superposition of the ^1H NMR spectrum of the cyclic portion of the molecule of the known (20*R*)-lanosta-8,24-dien-3-one **8** [8] and the ^1H NMR spectrum of the lactone fragment of molecule **5** [7] (the HC(24) and $H_3C(27)$ protons). It should be noted that this spectrum (the operating frequency was 500 MHz) shows several signals of both expected 23-epimers of lactol **7** (Table 1). According to the relative integral intensities of these signals, the ratio of the above-mentioned epimers **A** and **B** was 6 : 5. Previously, the C(23)-epimers of lactol **5** have not been detected because of the low operating frequency of the NMR spectrometer used (200 MHz for ^1H),[7] whereas in the spectrum obtained on an instrument operating at 500 MHz, analogous double signals for the $C(18)H_3$ and HC(24) atoms are observed.

The ^{13}C NMR spectrum of compound **7** is very informative (Table 2). The assignment of the signals of this spectrum was made based on the published data on related compounds, which were obtained with the use of two-dimensional NMR spectroscopy.[9,10]

Table 1. Chemical shifts (δ) and spin-spin coupling constants (*J*/Hz) of selected signals in the ^1H NMR spectra of ketone **8** and lactol **7** ($CDCl_3$, c = 5.7 mg mL^{-1}, T = 30 °C)

Atom	**7**	**8** [8]
$H_3C(18)$	0.716 (epimer **A**), 0.706 (epimer **B**)	0.69 s
CH_3*	0.870 (d, 3 H, ^4J = 0.6)	0.86 s
CH_3*	1.046 (s, 3 H)	1.05 s
CH_3*	1.072 (s, 3 H)	1.07 s
CH_3*	1.099 (d, 3 H, ^4J = 0.4)	1.10 s
$C(21)H_3$	1.037 (d, 3 H, $^3J_{21,20}$ = 6.1)	0.89 d
$C(27)H_3$	1.920 (d, 3 H, $^4J_{27,24}$ = 1.6)	—
$H_aC(2)$	2.390 (ddd, 1 H, J = 15.7, 6.7, 3.5)	2.39 (ddd, J = 15.5, 5.6, 3.0)
$H_bC(2)$	2.560 (ddd, 1 H, J = 15.7, 11.0, 7.0)	2.56 (ddd, J = 15.5, 11.0,5.0)
HC(24)	6.822 (q, $^4J_{24,27}$ = 1.6, epimer **B**), 6.836 (q, $^4J_{24,27}$ = 1.6, epimer **A**)	

* The angular Me groups at the C(4), C(10), and C(14) atoms.

The circular dichroism (CD) spectrum of tautomer **7** has the positive Cotton effect at 286 nm (the $n \to \pi^*$ transition in the C(3)=O group), which indicates[11] that structural formula **7** describes also the absolute configuration of the molecule of this compound.

When dissolved in an ethanolic solution of alkali followed by heating under conditions reported for 23-oxolanost-24-en-26-oic acids,[12] lactol **7** was converted into crystalline diketone **9** as a result of fragmentation.[12,13] This process was readily monitored by HPLC. Initially, acid **6** and its (24*Z*)-isomer, which was detected by HPLC as tautomer **7** because of the acidic nature of the eluent, were formed. These compounds were gradually converted into diketone **9**. Isomeric lactol **5** and methyl ester **4** behave in a similar manner. The presence of the C(23)-epimers of lactol **7** does not lead to broadening of the peak of compound **7** on the chro-

Table 2. ^{13}C NMR spectrum of lactol **7** (a mixture of the C(23)-epimers) ($CDCl_3$, δ, the signal of the residual $CHCl_3$ was taken as the internal standard, δ 76.924, c = 5.7 mg mL^{-1}, T = 30 °C)

Atom C	Signal of the major epimer	Signals that coincide for both epimers	Signal of the minor epimer
1		35.962 t	
2		34.520 t	
3		217.774 s	
4		47.318 s	
5		51.125 d	
6		20.951 t	
7		26.217 t	
8	133.136 s		133.186 s
9	135.040 s		135.023 s
10		36.824 s	
11		30.699 t*	
12	30.833 t		30.816 t
13	44.426 s		44.376 s
14	50.021 s		50.034 s
15		19.324 t*	
16	28.529 t		28.591 t
17	50.770 d		50.810 d
18	20.804 q		21.381 q
19		18.610 q	
20	32.748 d		32.930 d
21	15.651 q		15.613 q
22	44.085 t		43.900 t
23	105.904 s		106.269 s
24	147.400 d		147.256 d
25	131.906 s		131.783 s
26	171.400 s		171.373 s
27	10.388 q		10.368 q
28		26.100 q	
29		21.212 q	
30		24.133 q	

* The values may be reversed.

matogram, whereas the peak of lactol **5** is slightly distorted.

The concentration of lactol **7** in a mixture of acids obtained from a freshly prepared sample of *Abies sibirica* needle was 2.7% (determined with the use of the external standard).

Experimental

The melting temperatures were determined on a Kofler stage. The IR spectra were obtained on an UR-20 instrument. The NMR spectra were recorded on Bruker DRX-500 (the operating frequency for ^{1}H and ^{13}C were 500.13 and 125.76 MHz, respectively) and Bruker AM-400 (the operating frequency for ^{1}H was 400 MHz) spectrometers in $CDCl_3$ solutions. The mass spectra (EI, 70 eV) were obtained on a Finnigan MAT 8200 instrument. The CD spectrum* was recorded on a JACSO J-600 dichrograph in MeOH. The optical rotation was measured on a Polamat A polarimeter in $CHCl_3$. Fir needle was collected in the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk).

HPLC was carried out on a Milikhrom microcolumn liquid chromatograph (64×2-mm column; LiChrosorb RP-18 (5 mm) (Merck) as the sorbent; detection: 200/240 nm).

23-Hydroxy-3-oxolanosta-8,24-dien-26,23-olide (7). Crystals, m.p. 183—193 °C (MeOH). MS, *m/z* (I_{rel} (%)): 468.32508 $[M]^+$ (11) (calculated for $C_{30}H_{44}O_4$: 468.32394), 453 $[M-CH_3]^+$ (21), 450 $[M-H_2O]^+$ (7), 435 $[M-H_2O-CH_3]^+$ (11), 325.25309 (100) $[C_{23}H_{33}O]^+$ (calculated for $C_{23}H_{33}O$: 325.25313), 137 (29). IR ($CHCl_3$), ν/cm^{-1}: 1700 (C=O), 1765 (γ-lactone), 3030 (C=C), 3590 (OH). ^{1}H NMR spectrum is given in Table 1. CD spectrum (MeOH): $\Delta\varepsilon_{286}$ = +1.96 (c 5.98 · 10^{-3} mol L^{-1}).

25,26,27-Trinorlanost-8-ene-3,23-dione (9) was prepared from compound **7** according to the procedure reported in Ref. 12 in a yield of 60%. Crystals, m.p. 125.5—126.5 °C (hexane—diethyl ether), $[\alpha]_{580}$ +82° (c 1.78). MS, *m/z* (I_{rel} (%)): 398.31852 $[M]^+$ (55) (calculated for $C_{27}H_{42}O_2$: 398.31846), 383 $[M-15]^+$ (100), 364 (25), 340 (8), 325 (86), 121 (25), 43 (41). IR (CCl_4), ν/cm^{-1}: 1711 (C=O), 1705 (sh, C=O), 1175, 1115.

^{1}H NMR, δ: 0.73, 0.86, 1.03, 1.05, 1.08 (all s, 5 Me), 0.88 (d, 3 H, J = 7 Hz, $H_3C(21)$), 2.09 (s, 3 H, $H_3C(24)$).

The products, which were obtained by treatment of compound **7** with alkali, were analyzed by HPLC at a column temperature of 35 °C; a mixture of MeOH and a 0.05 *M* H_3PO_4 aqueous solution (90 : 10 v/v) was used as the eluent. The capacity coefficients k' were 1.99 (**5**), 3.58 (**1**), 2.38 (**7**), 4.29 (**6**), and 4.74 (**9**).

Samples of the acidic fraction of the extract from the fir needle under study were analyzed at a column temperature of 24 °C; a mixture of MeOH and a 0.05 *M* H_3PO_4 aqueous solution (85:15 v/v) was used as the eluent. For lactols **5** and **7**, the values of k' were 2.76 and 3.30, respectively.

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