then washed with aqueous sodium bisulfate and with water and was dried with anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed on a column of silica gel in system 4a. This led to the isolation of 30 mg of the amorphous completely methylated product, the IR spectrum of which showed no absorption in the region of hydroxy groups. Mass spectrum of the permethylate, m/z: M^+ 848, 788, 716, 703, 539, 483, 451, 349 (disaccharide fragment), 317, 262, 220, 203, and 189 (terminal trimethylrhamnose). The permethylate was heated in a 5% aqueous methanolic (1:1) solution of sulfuric acid (80°C, 6 h). The mixture was diluted with water, the methanol was distilled off, and the solution was heated for another 2 h. After neutralization with barium carbonate, 2,3,4-tri-O-methyl-L-rhamnose and 2,3-di-O-methyl-L-arabinose were detected in the hydrolysate with the aid of TLC in systems 4a and 1c with markers. The methylated sugars were not revealed with the Bonner reagent [6].

The genin was identified by TLC in system 1c with the methyl ester of 23-O-methylhederagenin.

SUMMARY

A new triterpene glycoside – dipsacobioside, having the structure of hederagenin 3-O-[O-α-L-rhamnopyranosyl-(1→4)-α-L-arabinopyranoside) – has been isolated from the roots of Dipsacus azureus Schrenk.

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TRITERPENOIDS FROM Abies SPECIES

VII. NEW LANOSTANE LACTONES FROM SIBERIAN FIR NEEDLES

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UDC 547.595.9

Two new lanostane lactones have been isolated from the neutral fraction of an ethereal extract of Siberian fir needles by chromatography, and their structures have been established on the basis of their spectral characteristics and chemical transformations.

In preceding communications, it was shown that (24Z)-3,23-dioxo-9β-lanosta-7,24-dien-26-oic acid (I) is one of the main components of the acidic fraction of an ethereal extract of the needles of the Siberian fir Abies sibirica Ledeb. [1, 2]. It is impossible not to observe the similarity of the structure of side chain of this substance with that of the molecule of β-acetylacrylic acid (II), which has been the object of special investigations

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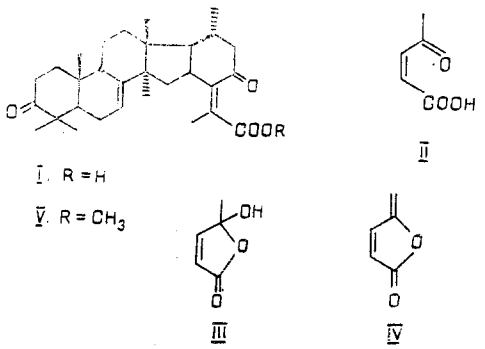
 0009-3130/89/2502-0176 $12.50

[3, 4] in connection with its capacity for giving the tautomeric form (III) (angelica lactone). The anhydro form of the latter (protoanemonin) (IV) is known as a natural compound that has been isolated from plants of the family Ranunculaceae [5].

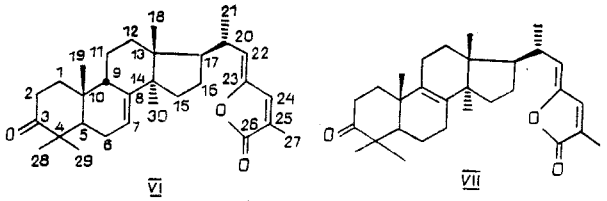


I. R = H

V. R = CH_3

II III IV

In views of the facts given, it is natural to put the question of the possibility of the existence of acyclic tautomeric form for the acid (I) (it has been described only in the form of the methyl ester (V) [1, 6]) and the presence of the anhydro form of the latter in Siberian fir needles. Since all the compounds under consideration are neutral, to search for them we used the neutral fraction of an ethereal extract of the needles. By column chromatography on silica gel, a fraction, eluted before β-sitosterol was isolated which contained, according to its IR spectrum, γ-lactones (ν_{max} 1770 cm^{-1}). By treatment with acetic anhydride in pyridine followed by chromatography, it was possible to remove from it acetylatable compounds of nonlactone nature (triterpenoids, fatty alcohols) and to obtain the total lactones with a yield of 3.8% on the neutral fraction of the extract or 0.2% on the initial needles. According to GLC, they were represented by only two compounds, which it was possible to separate chromatographically. The structures and stereochemistries of the molecules of the lactones isolated, which are shown in formulas (VI) and (VII) were established on the basis of the following results:



VI VII

Lactone (VI) (the main one, making up about 80% of the mixture) had the empirical formula $C_{30}H_{42}O_3$ (high-resolution mass spectrometry). In its UV spectrum, an intense maximum was observed at 280 nm (ε 25,000) analogous to that for protoanemonin (IV) [3], while the PMR spectrum differed from that for the diekto ester (V) only by the signals of protons present in the side chain of the molecules (see Table 1; the assignments were confirmed by double-resonance experiments). When lactone (VI) was treated with an ethanolic solution of alkali, followed by acidification, a product not identical, according to TLC, with the initial compound was formed that gave, on methylation, by diazomethane, an ester identical, according to TLC, PMR spectroscopy, and its circular dichroism (CD) curve, with the known methyl ester (V). In the light of the UV and PMR spectra, this transformation is in fact a proof of the structure and stereochemistry (apart from the configuration of the Δ^{22} double bond) of the molecule of the lactone (VI).

The structure of the second compound isolated (VII) was established on the basis of its spectral characteristics alone. In its mass spectrum it was possible to observe, in addition to the peak of the molecular ion (m/z 450; 31%) the peaks of the ions $(M - 15)^+$ (16%), $(M -$ side chain$)^+$ (m/z 313, 10%), and ions with m/z 137 (100%) formed through cleavage of the (VII) molecule at the C-17–C-20 bond. All the other peaks were weak (less than 8%). On the whole, the mass spectrum did not differ substantially from that for lactone (VI).

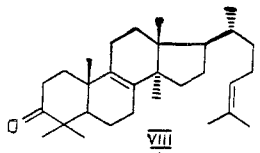
In the UV spectrum of the compound under investigation a maximum was observed at 280 nm (ε 22,000) while the PMR spectrum (see Table 1 and the Experimental part) could be

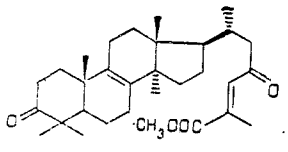
TABLE 1. Chemical Shifts (ppm) and Observed Spin-Spin Coupling Constants (Hz, shown in parentheses) of the Signals in the PMR Spectra of Compounds (VI), (VII), (VIII), and (X) *

Compound	2H-2	H-7	H-20	H-22	H-24	Me-20	Me-25
VI	2,47**	5,61 dt (7,0; 3,0; 3,0)	2,86 tq (10; 10; 6,7)	4,95 d (10)	6,95 q (1,5)	1,02 d (6,7)	1,96 d (1,5)
VII	2,39 ddd (15,5; 6,5; 3,5) 2,56 ddd (15,5; 11,0; 5,0)	—	2,96 tq (10; 10; 6,7)	4,95 d (10)	6,95 q (1,5)	1.04 d (6,7)	1,97 d (1,5)
VIII	2,39 ddd (15,5; 6,5; 3,5) 2.56 ddd (15,5; 11,0; 5,0)	—	—	—	—	0,89 d (6,0)	—
X	2,52**	5,67 dt (7,0; 3,0; 3.0)	—	—	6,88 br.s	1,01 d (6,5)	1,96 br.s

*Arbitrary designations, d – doublet; t – triplet; q – quartet; br.s – broadened singlet.
**The signal has the form of a doublet of doublets with splittings of 8.0 and 6.5 Hz and an integral intensity of 2H; proof of its assignment to 2H-2 has been given in [6].

represented as a combination of two groups of signals one of which coincided with that for lactone (VI) (protons of the side chain) and the other (the signals of the methyl groups and of the 2H-2 protons) with the signals of the cyclic part of the molecule of lanosta-8,24-dien-3-one (VIII) [7]. We have given a special discussion of the characteristic nature of the form of the signals for the 2H-2 protons in a preceding communication [8] in determining the structure of the analogous lanost-8-enoic ester (IX) isolated from the methylated acid fraction of the needles under investigation.


VIII



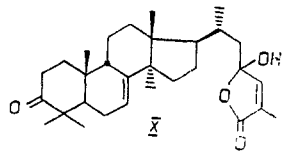
IX

In the CD curve for the lactone (VII) an extremely intense negative Cotton effect ($\Delta\varepsilon_{280} \approx -11$) is observed which corresponds to the absorption maximum in the UV spectrum and to an electronic transition in the conjugated system of the side chain of the molecule. The same effect appears on the CD curve of lactone (VI), the absolute configuration of which has been shown by correlation with the ester (V). Thus, lactone (VII) belongs to the same stereochemical series, and formula (VII) reflects its absolute configuration. It must be mentioned that the contribution of the 3-keto group to the observed Cotton effect for both compounds is not determining, as can be deduced from a consideration of model compounds. For the ester (V), the dichroic absorption due to the 3-keto group amounts to +0.84, and for ester (IX) it is +1.48 [8].

The molecule of lactone (VII) has the 22Z-configuration (as shown in its formula), as was established on the basis of an observation of the Overhauser effect (5.0%) on the signal of the H-22 proton when the H-24 proton was irradiated. The signal of the H-20 proton did not change in this situation. The molecule of lactone (VI) has the same configuration of this bond, as was established by a similar experiment.

Thus, the first part of the problem had been solved – an anhydro derivative of a tautomeric form of the acid (I) [the lactone (VI)] had been found in the needles. In addition to this, a similar derivative of its as yet unknown $\Delta^{8(9)}$-isomer [lactone (VII)] was isolated. We obtained the actual tautomeric form of the acid (I) (formula X) in the chromatographic separation of the acid fraction of the extract of the needles under investigation. The search for it was carried out in the light of Shaw's results [3] showing that the cyclic form (III) passes in an aqueous alkaline solution into the open form (β-acetylacrylic acid).

Consequently, the hydroxylactone (X), if it is present in the needles, must be converted into the sodium salt of the acid (I) on treatment of the ethereal extract. Then, on the chromatography of the total acids on silica gel [2], the acid (I), as may be assumed, is converted partially into the tautomer (X) because of the acidic properties of the sorbent. In



X

consideration of these hypotheses, we turned to the fractions obtained on the chromatography of the sum of the "strong" acids described in communication (II). When the crystalline part of fraction 4 enriched with the acid (I) was methylated with diazomethane, the mixture of the ester (V) and an unknown compound with the same R_f value as the product before methylation was obtained. It was possible to isolate the latter by two crystallizations, but on an attempt to extract it from the mother liquors by chromatography on silica gel it was found that this substance decomposed totally and irreversibly on the sorbent used without forming products eluted by diethyl ether.

The compound obtained proved to be the desired tautomeric form of the acid (I), and its structure, expressed by the formula (X), was confirmed by the results of IR spectroscopy (absorption bands of γ-lactone, keto, and hydroxy groups), UV spectra (no strong maxima in the 230-300 nm region), and mass and PMR spectra (see Table 1).

On mild dehydration with phosphorous oxychloride in pyridine, compound (X) gave the expected lactone (VI), and this is a proof of its structure. Formula (X) also reflects the absolute configuration of the molecule, since the ORD curve of this substance showed a positive Cotton effect corresponding to the 9β-lanost-7-en-3-oic fragment of the molecule [6].

In view of its structure, it could be assumed that the main direction of the fragmentation of the molecule of the hydroxylactone (X) (m/z 468) on electron impact would be dehydration followed by the formation of stable ions with m/z 137 the peak of which is the main one in the mass spectra of the lactones (VI) and (VII). However, it was found that the intensity of the latter in the spectrum was only 33%, and the main peak was that of ions with m/z 325, as also in the mass spectra of the 3,23-diketolanostenoids [6, 8]. This interesting fact can be considered as proof of the occurrence of the tautomeric transformation (X) → (I), that the molecular ion of the hydroxylactone X undergoes before fragmentation.

In the present work we did not set ourselves the aim of finding the optimum conditions for the tautomeric transformation of the acid (I) into the hydroxylactone (X). It may merely be mentioned that in the eluates obtained on the chromatography of the sum of the acids from the needles under investigation the latter was not detected by TLC and was most probably formed on the evaporation of the solvent and the drying of the product in vacuum (in our case – fraction 4 [2]). In a qualitative experiment it was established that the hydroxylactone (X) does not change on storage in the pure form or in chloroform solution (0°C, one week), while acid (I) in chloroform solution isomerizes partially into the hydroxylactone (X).

The chain of transformations (I) → (X) → (VI) can be regarded as modeling the formation of compound (VI) in the plant. However, the possibility of its occurrence even without the participation of enzymes cannot be excluded.

EXPERIMENTAL

PMR spectra were recorded for $CDCl_3$ solutions on a Bruker WP-200 SY instrument (200.13 MHz) (δ scale) internal standard $CHCl_3$, the signal of which was taken as 7.24 ppm). High-resolution mass spectra were obtained on a Finnigan MAT 8200 instrument. UV spectra (of solutions in ethanol) and IR spectra were recorded on Specord UV-Vis and UR-20 instruments, respectively. Angles of optical rotation and CD and ORD curves were obtained on a Spectropol-1 spectropolarimeter for solutions in chloroform. The GLC of the sum of the lactones was recorded on a Chrom-5 instrument (5% of SE-30 on Chromaton N-Super, 0.160-0.200 mm) with

a column 1 m long, a thermostat temperature of 270°C, an evaporator temperature of 300°C, and a rate of flow of nitrogen of 40 ml/min.

Air-dry type KSK silica gel with a grain size of 0.07-0.16 mm was used for chromatography at a ratio of substance and sorbent of ~1:20, the eluent in all cases being petroleum ether with increasing concentrations (from 0 to 100%) of diethyl ether.

The Siberian fir needles were gathered in the Novosibirsk province and were dried in the air at room temperature.

Isolation of the Lactones (VI) and (VII). By extraction with diethyl ether in a Soxhlet apparatus (50 h), 74.00 g of the air-dry needles yielded an extract which was treated as described in [2]. The yield of the neutral fraction of the extract was 3.78 g (5.1% on the needles). Its chromatography led to the isolation of 2.72 g of combined nonlactonic compounds (the eluent being petroleum ether with an increasing concentration, from 0 to 20%, of diethyl ether), 0.37 g of a lactone-containing fraction (the eluent being petroleum ether with additions of 20 and 25% of diethyl ether), and 0.29 g of a mixture of more polar compounds which have not yet been studied.

A solution of 0.37 g of the lactone-containing fraction in 5 ml of pyridine was treated with 1.5 ml of acetic anhydride, and the mixture was left at room temperature for 7 h. After the usual working up and chromatography, 0.20 g of unidentified acetates of fatty and triterpene alcohols (the eluent being petroleum ether with 10% of diethyl ether) and 0.15 g of the sum of the lactones (VI) and (VII), in a ratio of ~4:1 (TLC) were isolated. Crystallization of the latter from petroleum ether gave 0.05 g of the lactone (VI), and chromatography of the mother liquor on silica gel impregnated with silver nitrate (5%) led to the successive isolation of 0.02 g of lactone (VII), 0.02 g of a mixture of compounds (VI) and (VII) (~1:1, TLC), and 0.05 g of lactone (VI).

3-Oxo-9β-lanosta-7,22Z,24-trien-26,23-olids (VI). Crystals with mp 218-220°C (from petroleum ether), $[\alpha]_D^{20}$ −51.4° (c 0.078), $C_{30}H_{42}O_3$ (found m/z 450.3121; calculated, 450, 3134). Mass spectrum (m/z %): 450 (36) − M^+, 435 (36) − $(M - 15)^+$, 313 (12) − (M − side chain)$^+$, 137 (100) − $(M - C_{22}H_{33}O)^+$. UV spectrum: λ_{max} 280 nm (25,000). IR spectrum (in $CHCl_3$), cm^{-1}: 1700 (C=O), 1755 (γ-lactone). CD spectrum: $\Delta\varepsilon_{280} \approx -11$ ($c = 1.7 \cdot 10^{-3}$ M). PMR spectrum, ppm: 0.82, 0.97, 1.00, 1.07, 1.08 (singlets, each 3 H, angular methyl groups); the other signals are described in Table 1.

Conversion of Lactone (VI) into the Ester (V). A solution of 0.05 g of lactone (VI) in 10 ml of ethanol was treated with 5 ml of a 10% ethanolic solution of sodium hydroxide, and the mixture was heated in the water bath at 60-70°C until the initial substance had disappeared (about 15 min; monitoring by TLC). The solution was cooled to room temperature, diluted with 30 ml of water, and acidified with 5% hydrochloric acid (10 ml), after which 20 ml of a saturated aqueous solution of sodium chloride was added and it was extracted with diethyl ether (3 × 30 ml). The ethereal extract was treated with an excess of an ethereal solution of diazomethane, and the solvent and the excess of diazomethane were rapidly eliminated. This gave 0.03 g of a product identical with the ester (V) according to TLC and its PMR spectrum. The CD spectrum of a solution in methanol coincided with that described [2], and for a solution in chloroform the same Cotton effects were observed at 290 ($\Delta\varepsilon$ = +1.87) and 341 nm ($\Delta\varepsilon$ = −0.84) ($c = 2.5 \times 10^{-4}$ M).

3-Oxo-lanosta-8,22Z,24-trien-26,23-olide (VII). Crystals with mp 247-248°C (from petroleum ether) $[\alpha]_D^{20}$ −62.3° (c 0.032). UV spectrum: λ_{max} 280 nm (ε 22,000); IR spectrum (in CCl_4), cm^{-1}: 1710 (C=O), 1775 (γ-lactone). CD spectrum: $\Delta\varepsilon_{280} \approx -11$ ($c = 0.7 \times 10^{-3}$ M). PMR spectrum, ppm: 0.76, 0.86, 1.06, 1.08, and 1.10 (singlets, 3 H each, angular methyl groups); the other signals are described in Table 1.

Isolation of the Hydroxylactone (X). An ethereal solution of the crystals (1.00 g) that had deposited from fraction 4 (4.20 g) [2] was treated with an ethereal solution of diazomethane. The product was crystallized from ethanol, to give 0.63 g of a mixture of substances (X) and (V) (~1.1, TLC). The mother liquor was evaporated to dryness and the residue was crystallized from a mixture of petroleum ether and diethyl ether (~1:5). This gave 0.11 g of the hydroxylactone (X) in the form of a microcrystalline powder.

23-Hydroxy-3-oxo-9β-lanosta-7,24-dien-26,23-olide (X). Crystals, with mp 177-181°C, $[\alpha]_D^{20}$ + 34.9° (c 0.10). Mass spectrum (m/z, %): 468 (22) − M^+, 453 (30) − $(M - 15)^+$, 435 (13) − $(M - H_2O - 15)^+$, 137 (33) − $(M - C_{22}H_{35}O_2)^+$. IR spectrum (in $CHCl_3$), cm^{-1}: 1710 (C=O); 1775

(γ-lactone); 3590 (OH). PMR spectrum, ppm: 0.82, 1.00, 1.02, 1.12, 1.13 (singlets, 3 H each, angular methyl groups); the other signals are described in Table 1. ORD: $[\alpha]_{311}$ + 500° (peak), $[\alpha]_{285}$ 0°, $[\alpha]_{274}$ −105° (valley) (c 0.10).

Dehydration of the Hydroxylactone (X). A solution of 0.030 g of compound (X) in 2 ml of pyridine cooled to 0°C was treated dropwise with 0.5 ml of phosphorus oxychloride. The reaction mixture was kept at −5°C for 15 h. After the usual working up, a product was obtained which, according to TLC, consisted of a mixture of the lactone (VI) and the initial compound. Its chromatography on silica gel gave 0.008 g of the lactone (VI), identical, according to TLC and its PMR spectrum, with an authentic sample.

SUMMARY

1. Two new lanostanoids have been isolated from the neutral fraction of an ethereal extract of Siberian fir needles, and their structures have been established as 3-oxo-9β-lanosta-7,22Z,24-trien-26,23-olide and 3-oxolanosta-8,22Z,24-trien-26,23-olide.

2. The tautomeric form of 3,23-dioxo-9β-lanosta-7,24Z-dien-26-oic acid — 23-hydroxy-3-oxo-9β-lanosta-7,24-dien-26,23-olide — has been obtained and characterized by its constants and spectral characteristics.

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