

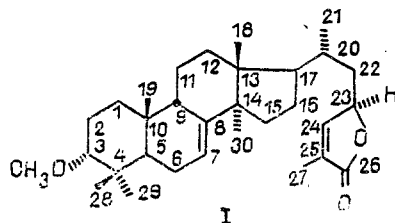
Four lanostane lactones have been isolated from an ethereal extract of Siberian fir bark — abieslactone and its hydroxy and keto analogs, and also (25R)-24,25-dihydroabieslactone, the structure of which was established on the basis of a chemical and spectral correlation with known compounds.

One of the features of *Abies* (fir) species is the high degree of expression of the synthesis of triterpenoids of the lanostane and related types in them. In the literature, in addition to a series of acids, a number of neutral triterpenoids have been described all the representatives of which are γ -lactones — abieslactone [1, 2], cyclograndisolide, and epicyclograndisolide [3], 3 α -hydroxylanost-9(11)-en-26,23-olide [4], abietospiran [5], and firmanolide and epifirmanolide [6].

The aim of the present work was to investigate the neutral triterpenoids of the bark of the Siberian fir (*Abies sibirica* Ledeb. Although the chemical compositions of ethereal and benzene extracts of this bark have recently been studied in detail [7], the authors concerned give no information whatever about the presence of triterpenoids in them. Nevertheless, the high content of triterpene acids in the oleoresin of the Siberian fir [8] shows the possibility of the presence of neutral compounds of a similar type in the bark.

The triterpenoids sought were in fact present in the bark investigated and, as was shown, they precipitated almost completely (TLC monitoring) from the neutral fraction of the ethereal extract of the bark when it was treated with petroleum ether. Their total yield was 2.2% on the neutral fraction taken, 0.8% on the initial extract, and 0.03% on the air-dry bark. In TLC on Silufol, the product obtained gave three spots. The substances corresponding to them were isolated by column chromatography on silica gel with yields of 41, 24, and 28%, respectively, on the total.

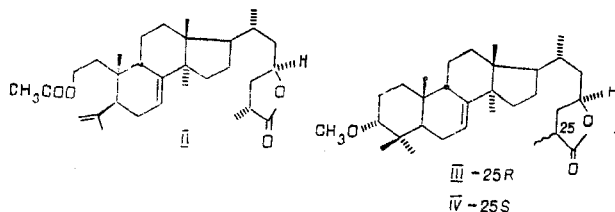
According to its PMR spectrum, the first substance was a mixture of two compounds (20:1) the main one of which was isolated by crystallization and was identified as abieslactone (I).



The second substance, which proved to be a new triterpenoid, was obtained from the mother liquor by additional chromatography and by crystallization from hexane. Its molecule contained two hydrogen atoms more than the abieslactone molecule and had the empirical formula $C_{31}H_{50}O_3$ (high-resolution mass spectrometry). The IR spectrum of the compound under investigation contained no absorption maxima in the 210-400 nm region, while in the IR spectrum a band was observed from a saturated γ -lactone ring (1770 cm^{-1}). The PMR spectrum of the compound could be represented as a combination of two groups of signals, one of which coincided with precisely the same signals in the spectrum of abieslactone (H-3, H-7, OCH_3 ,

singlet signals of five tertiary methyl groups), and the other with the signals of the protons of the side chain of the molecule of a known compound [methyl abiesolidate (II)] [9]. From these facts (in the light of the elementary composition found), it was possible to suggest the structure of (25R)-24,25-dihydroabieslactone (III) for the lactone under investigation, and this was then confirmed by the following facts.

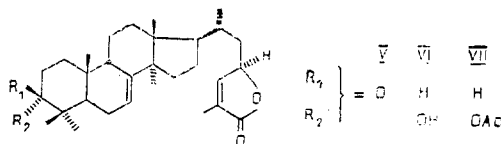
24,25-Dihydroabieslactone, which was obtained by Uyeo [1] by the catalytic hydrogenation of abieslactone, differed with respect to its PMR spectrum from the compound which we had isolated and, in the light of the results given in [10] it can be ascribed the (25S)-configuration [formula (IV)].



When this experiment was repeated, we obtained the same dihydro product the spectrum of which was identical with the spectrum of the lactone derivative (III), while the signals for the H-23 proton and for the CH₃-25 methyl group in the PMR spectrum coincided with those of known 25-epiabiesolidate derivatives [9]. Epimerization of the lactone (IV) under alkaline conditions gave a chromatographically irresolvable mixture of the lactones (IV) and (III) (2.6:1), in the PMR spectrum of which signals characteristic for each compound (H-23 and CH₃-25) were observed separately. As described in [9], the epimerization of methyl abiesolidate (II) took place analogously with the formation of a similar irresolvable pair of epimers at C-25.

Formula (III) corresponds to the absolute configuration of the molecule of the methoxylactone isolated, since its circular dichroism curve (in methanol) was a smooth negative curve at wavelengths below 215 nm, as also for the synthetic 24,25-dihydroabieslactone (IV) [11].

A substance corresponding to the second spot obtained in the TLC of the initial mixture of lactones was identified from its constants and spectral characteristics as (23R)-3-oxo-9 β -lanosta-7,24-dien-26,23-olide (V), obtained previously by partial synthesis [10].



The last, most polar, component of the mixture of lactones from the extract of the bark under investigation differed, according to its PMR spectrum, from abieslactone only by the presence of a hydroxy group in place of a methoxy group [structure (VI)]. This was confirmed by its IR spectrum (ν_{\max} 3620 cm⁻¹), by the formation of the corresponding acetate (VII) on acetylation with acetic anhydride in pyridine, and by the formation of the known ketolactone (V) on oxidation with pyridinium chlorochromate in methylene chloride.

Lactone (VI) was mentioned by Kutney [2] as a minor component of an extract of the bark of *Abies amabilis* (Dougl.) Forbes, but he gave no experimental information about this compound.

The ¹³C NMR spectra of all the lactones obtained were recorded, and their details are given in Table 1. The assignment of the signals for compounds (III-VI) was made on the basis of a comparison with the spectra of known triterpenoids [6, 12-15] and γ -lactones [16]. Details of the ¹³C NMR spectrum of abieslactone were published in [6], but there is some doubt as to the unambiguity of the assignment by the authors of the signals for the C-4 and C-10 atoms (37.6 and 35.7 ppm, respectively). Their places should probably be interchanged (as has been done in Table 1) and the signals of these atoms in the spectra of the four other lactones considered be interpreted in accordance with this. The basis for

TABLE 1. Chemical Shifts (ppm) and Multiplicities of the Signals in the ^{13}C Spectra of Compounds (I, III, and IV-VI)

No. of the atom	I a,d	III b,c	IV b,g	V a,c	VI a,e
1	29,88 t	30,52 t	30,39t	34,21t	29,70 t
2	20,25 t	20,79 t	20,71t	34,05t	25,63 t
3	85,73 d	85,82 d	85,95d	218,64s	76,65 d
4	35,51 s	36,09 s	35,99s	46,87s	36,60 s
5	42,73 d	43,48 d	43,27d	45,37d	42,50 d
6	22,90 t	23,52 t	23,42t	22,88t	23,00 t
7	121,44 d	122,26 d	122,01d	121,49d	121,52 d
8	148,31 s	148,89 s	148,78s	148,33s	148,50 s
9	48,44 d	49,19 d	48,98d	52,21d	48,43 d
10	37,47 s	38,00 s	37,94s	35,66s	37,28 s
11	22,60 t	23,88 t	28,24t	20,74t	22,77 t
12	33,09 t	33,73 t	33,63t	32,90t	33,19 t
13	43,50 s	43,91 s	43,91s	44,02s	43,65 s
14	52,66 s	53,24 s	53,17s	51,78s	52,78 s
15	35,19 t	35,75 t	35,69t	34,21t	35,25 t
16	28,43 t	28,96 t	28,92t	28,08t	28,46 t
17	53,80 d	54,40 d	54,28d	53,34d	53,89 d
18	23,68 q	23,61 q	23,96q	22,37q	23,62 q
19	24,33 q	24,81 q	24,80q	22,99q	24,26 q
20	33,28 d	33,48 d	33,63d	33,36d	33,37 d
21	18,21 q	18,36 q	18,54q	18,24q	18,30 q
22	40,26 t	42,69 t	42,84t	40,34t	40,38 t
23	78,84 d	74,82 d	75,99d	78,81d	78,91 d
24	149,54 d	36,22 t	38,27t	149,49d	149,52 d
25	129,20 s	33,96 d	36,01d	129,35 s	129,36 s
26	174,20 s	174,41 s	179,23s	174,24 s	174,25 s
27	10,43 q	15,83 q	15,19q	10,49 q	10,49 q
28	28,55 q	29,14 q	29,07q	27,82 q	28,52 q
29	23,65 q	23,91 q	24,03q	21,17 q	23,30 q
30	30,71 q	31,05 q	31,11q	27,26 q	30,59 q
OCH ₃	56,67 q	56,68 q	56,87q	—	—

- a) internal standard - the signal of CDCl_3 at 76.90 ppm;
b) internal standard - the signal of C_6D_6 at 128.0 ppm;
c) for a 0.075 M solution in CDCl_3 ; d) for a 0.240 M solution in CDCl_3 ; e) for a 0.048 M solution in CDCl_3 ;
f) for a 0.027 M solution in C_6D_6 ; g) for a 0.370 M solution in a mixture of CDCl_3 and C_6D_6 (1:1).

this change was a displacement of the singlet of the chemical shift of 35.51 ppm into the 36.60 ppm position on the passage from abieslactone to its hydroxy analog (VI), which shows that this signal relates to the C-4 atom [17]. The signal at 37.47 ppm, which, in this interpretation of the spectrum of abieslactone, is assigned to the C-10 atom, changed only slightly [37.28 ppm for (VI)], which corresponds to its assignment to C-10. However, the appreciable downfield shift of the signal for the C-10 atom in the passage from the 3-keto derivative (V) (35.66 ppm) to the 3-methoxy and 3-hydroxy derivatives (I), (III), (IV), and (VI) (see Table 1) remains not fully understood. This is probably due to the specific nature of ring A in the molecules of the 3-oxo- Δ^7 -9 β -lanostene derivatives reported previously [10] in a study of their optical properties.

EXPERIMENTAL

The PMR spectra were recorded on a Bruker WP-200 SY instrument (200.13 MHz) for solutions in CDCl_3 (δ scale, internal standard chloroform, the signal of which was taken as 7.24 ppm). ^{13}C NMR spectra were recorded on Bruker WP-200 SY (50.32 MHz) and Bruker AC 200 (50.323 MHz) instruments. Arbitrary designations in the descriptions of the NMR spectra are: s - singlet; d - doublet; t - triplet; q - quartet; m - multiplet. High-resolution mass spectra were obtained on a Finnigan MAT 8200 instrument. UV spectra (for solutions in ethanol) and IR spectra were recorded on Specord UV-Vis and UR-20 instruments, respectively. Angles of optical rotation were determined for solutions in chloroform on a Zeiss polarimeter. CD curves were recorded on a Spectropol 1 spectropolarimeter for solutions in methanol. Melting points were determined on a Kofler stage.

Type KSK silica gel with a grain size of 0-0.14 mm was used for chromatography at a ratio of substance to sorbent of ~1:30.

The Siberian fir bark was collected in July, 1987, in the Khakass Autonomous Oblast and was ground and dried in the air.

Isolation of Lactones (I, III, V, and VI). The extraction of 1.2 kg of the Siberian fir bark with diethyl ether in a Soxhlet apparatus for 50 h yielded 150 g (12.5%) of extract, a 52.7 g portion of which was separated into acid (33.9 g) and neutral (18.4 g) fractions by the usual method. The neutral fraction of the extract was mixed with 200 ml of hot petroleum ether (bp 40-70°C) and the mixture was allowed to cool to room temperature. The resulting precipitate of the total mixture of lactones (0.41 g) was filtered off. The total amount of lactones was treated in this way, and a portion of it (1.00 g) was chromatographed with methylene chloride containing increasing amounts (from 0 to 10%) of diethyl ether as eluent. This yielded successively 0.41 g of a mixture of abieslactone and lactone (III), 0.24 g of the ketolactone (V), and 0.28 g of the hydroxylactone (VI).

Crystallization of the 0.41-g fraction from ethyl acetate yielded 0.32 g of abieslactone, and the subsequent chromatography of the mother liquor using petroleum ether-diethyl ether (3:1) as eluent and crystallization of the product from hexane gave 0.02 g of the (23R,25R)-24,25-dihydroabieslactone (III).

Abieslactone (I). Crystals with mp 238-242°C (ethyl acetate), $[\alpha]_D^{21} -96.9^\circ$ (c 4.85) (according to the literature [1]: mp 252-253°C (ethyl acetate: $[\alpha]_D^{22} -113^\circ$ (c 1.00)). PMR spectrum, ppm: 0.89, 0.92, 0.93, 0.97, 0.99 (each 3 H, singlets, tertiary methyl groups); 0.97 (3 H, d, $J \approx 6$ Hz, CH_3 -20), 1.89 (3 H, t, $J = 1.6$ Hz, CH_3 -25); 2.79 (1 H, narrow m, H-3); 3.25 (3 H, s, OCH_3); 4.94 (1 H, dddq, $J = 10.5, 3.0, 1.6$, and 1.6 Hz, H-23); 5.50 (1 H, dt, $J = 7.0, 3.0$ Hz, H-7); 6.97 (1 H, quintet, $J = 1.6$ Hz, H-24).

When 0.05 g of abieslactone was hydrogenated by the procedure described in [1], 0.05 g of lactone (IV) was obtained with mp 212.5-213.5°C (hexane), its PMR and mass spectra coinciding with those in the literature [1].

(23R,25R)-3 α -Methoxy-5 α ,9 β -lanost-7-en-26,23-olide (III). Crystals with mp 195-197°C (hexane) $[\alpha]_D^{21} -66.6^\circ$ (c 0.6). IR spectrum (KBr), cm^{-1} : 1770 (γ -lactone). Empirical formulas $\text{C}_{31}\text{H}_{50}\text{O}_3$ (found, m/z 470.3758; calculated, 470.3760). Mass spectrum (m/z , %): 470 (32) - M^+ , 455 (31) - $(\text{M} - 15)^+$, 423 (100) - $(\text{M} - 15 - \text{CH}_3\text{OH})^+$, 316 (25), 301 (12), 175 (20). PMR spectrum, ppm: 0.89, 0.91, 0.93, 0.97, 1.00 (each 3 H, singlets, tertiary methyl groups); 0.92 (3 H, d, $J \approx 6$ Hz, CH_3 -20); 1.26 (3 H, d, $J = 7.0$ Hz, CH_3 -25); 3.26 (3 H, s, OCH_3); 5.50 (1 H, dt, $J = 6.0$ and 3.0 Hz, H-7); 2.79 (1 H, narrow m H-3); 2.69 (1 H, sextet, $J = 7.5$ Hz, H-25; on double resonance with the suppression of this signal, the doublet signal of the CH_3 -25 proton was converted into a singlet); 4.62 (1 H, m, H-23).

23R-3-Oxo-5 α ,9 β -lanosta-7,24-dien-26,23-olide (V). Crystals with mp 224-228°C (ethanol), $[\alpha]_D^{21} +22.9^\circ$ (c 2.62). The IR, mass, CD, and PMR spectra coincided with those for an authentic sample obtained as described in [10].

(23R)-3 α -Hydroxy-5 α ,9 β -lanosta-7,24-dien-26,23-olide (VI). Crystals with mp 243-245°C (ethyl acetate-diethyl ether), $[\alpha]_D^{21} -70.9^\circ$ (c 2.96). IR spectrum (in CCl_4), cm^{-1} : 1770 (γ -lactone), 3620 (OH). CD spectrum: positive CE at 250 nm, $\Delta\epsilon = 0.14$ (c = $1.9 \cdot 10^{-3}$ M). Empirical formula $\text{C}_{30}\text{H}_{46}\text{O}_3$ (found, m/z 454.3436; calculated, 454.3447). Mass spectrum, m/z , %): 454 (36) - M^+ , 439 (29) - $(\text{M} - 15)^+$, 421 (100) - $(\text{M} - 15 - \text{H}_2\text{O})^+$. PMR spectrum, ppm: 0.90 and 0.92, 0.96, 0.99, 1.00 (each 3 H, singlets, tertiary methyl groups); 0.98 (3 H, d, $J \approx 6$ Hz, CH_3 -20); 1.89 (3 H, t, $J = 1.6$ Hz, CH_3 -25); 3.41 (1 H, m, H-3); 4.95 (1 H, dddq, $J = 11.0, 3.0, 1.6$, and 1.6 Hz, H-23); 5.53 (1 H, dt, $J = 6.0$ and 3.0 Hz, H-7); 6.97 (1 H, quintet, $J = 1.6$ Hz, H-24).

Oxidation of the Hydroxylactone (VI) to the Ketolactone (V). A solution of 0.045 g of compound (VI) in 5 ml of methylene chloride was treated with 0.02 g of sodium acetate and 0.05 g of pyridinium chlorochromate. After the mixture had been stirred at room temperature for 1 h and had been worked up in the usual way, chromatography yielded 0.04 g of a product the crystallization of which in methanol gave 0.02 g of the ketolactone (V), identical with the natural material in terms of TLC and PMR and CD spectra.

Epimerization of the Lactone (IV). A solution of 0.03 g of lactone (IV) in 15 ml of methanol was treated with 3 ml of a 20% solution of potassium hydroxide in 20% aqueous methanol. The reaction mixture was kept at 50-60°C for 1 h, after which it was acidified

with hydrochloric acid to pH 2, diluted with water, and extracted with diethyl ether. After the solution had been dried and the ether had been driven off, a product (0.03 g) was obtained which on TLC coincided with the initial compound. PMR spectrum 0.89, 0.91, 0.93, 0.97, 1.00 (each 3 H, singlets, tertiary methyl groups); 1.25 and 1.26 (totaling 3 H, doublets with $J = 7.0$ Hz each, protons of the $\text{CH}_3\text{-25}$ groups of the molecules of (III) and (IV), respectively); 3.26 (3 H, s, OCH_3); 4.43 and 4.63 (totaling 1 H, H-23 of compounds (III) and (IV), respectively; ratio of the integral intensities 2.6:1; 5.50 (1 H, dt, $J = 6.0$ and 3.0 Hz, H-7).

Preparation of the Acetate (VII). A solution of 0.10 g of lactone (VI) in 5 ml of pyridine was treated with 2 ml of acetic anhydride and the mixture was left at room temperature for 12 h. After the usual working up, 0.08 g of the acetate (VII) was obtained with mp 200-203°C (diethyl ether-methanol), $[\alpha]_D^{21} -63.5^\circ$ (c 0.63). IR spectrum (in KBr), cm^{-1} : 1240, 1740 (OAc); 1760 (α -butenolide). PMR spectrum, ppm: 2.03 (3 H, s, OOCCH_3); 4.62 (1 H, narrow m, H-3).

SUMMARY

Four lanostane lactones have been isolated from an ethereal extract of Siberian fir bark, and for that one of them which proved to be a new triterpenoid the structure of (23R,-25R)-3 α -methoxy-5 α ,9 β -lanost-7-en-26,23-olide has been established.

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