8. T. M. Khomenko, D. V. Korchagina, Yu. V. Gatilov, I. Yu. Bagryanskaya, and V. A. Barkhash, Zh. Org. Khim., 27, No. 3, 599-609 (1991).

EXTRACTIVE SUBSTANCES OF THE BARK OF Picea obovata

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The chemical composition of a petroleum ether extract of the bark of the Siberian spruce has been studied. Extracts included saturated aliphatic alcohols and C_{16}–C_{24} acids – abietic, dehydroabietic, isopimaric, oleic, lineolic, and linolenic – alkyl ferulates, ketones, and alcohols of the serratene type, and also Δ^4-stigmasten-3-one. Onoceradienedione and onoceradienediol – precursors of the serratene triterpenoids – and also a saturated vicinal diol – triacontane-10,11-diol – have been isolated from the extractive substances of conifer in the native form for the first time.

Pices obovata Lebd. (Siberian spruce) is the most widespread species of all forest-forming dark-needled conifers of the taiga zone in the mountain-taiga belt, the area of which stretches from the north of Scandanavia to the shores of the Sea of Okhotsk [1]. The extractive substances of the bark of the Siberian spruce have scarcely been studied, although there is information in the literature on the composition of the bark of other species of spruce [2-4].

The present work was devoted to a study of the chemical comosition of an extract of the bark of the Siberian spruce growing in the Altai. The extract was obtained by treating the bark with petroleum ether (PE) (bp 70-100°C), and its yield amounted to 1-1.8%. The extract, consisting of a grease-like light brown product, was separated into acidic and neutral components by the usual method.

The acidic part of the extract was treated with diazomethane and the result ethyl esters were then analyzed by the GLC method. The main components of the acidic fraction were the C_{18}–C_{24} saturated fatty acids, among which lignoceric acid (14.6%) predomianted, unsaturated fatty acids – oleic (15%) and linoleic (8%), and resin acids – isopimaric (11%) and dehydropimaric and abietic (32.6%), and also alkyl ferulates, which are typical components of bark extracts from coniferous plants [2, 5].

The neutral part of the extract was separated into groups of substances and individual compounds by adsorption chromatography. A nonpolar fraction was deluted by PE (bp 40-70°C) and was analyzed by the GLC method. It was found to contain 29 components and consisted of n-alkanes (C_{16}–C_{28} and C_{17}–C_{31} [sic]) the amounts of the individual components of which ranged from 0.3 to 9.2%. With an increase in the polarity of the eluting system, fractions of esters, ketones, tertiary alcohols, primary alcohols, triterpene alcohols, β-sitosterol, and diols were isolated successively.

The ester fraction was saponified with alcoholic alkali, as a result of which neutral unsaponifiable substances and the sum of the "bound" acids were obtained, and the latter were analyzed by the GLC method in the form of their methyl esters. It was established that the "bound" acids consisted mainly of saturated fatty acids (C_{14}–$_{24}$) with a predominance of behenic (22%) and lignoceric (28%), and also unsaturated acids – oleic (6.6%), lineolic (11.5%), and linolenic (14%).

The composition of the neutral unsaponifiable substances obtained on the saponification of the esters was unusual. In addition to β-sitosterol and the total fatty alcohols – the usual components of extractive substances, in this fraction the main component (64%) was a

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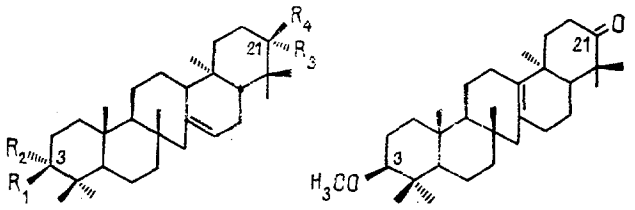
saturated diol – triacontane-10,11-diol. Aliphatic diols are usually present in various natural materials in the bound form [6]. Diols have not previously been isolated from the extractive substances of conifer bark.

The ketone fraction was rechromatographed, and the epimeric 3β-and 3α-methoxy-21-oxo-Δ^{14}-serraten4es (Ia and Ib) and 3β-methoxy-21-oxo-Δ^{13}-serratene (II) were isolated, these being identified by comaprison with authentic samples in terms of melting points and spectral characteristics.

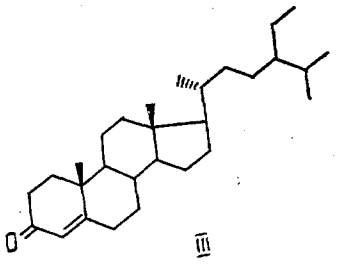


I II

a) R_1=OCH_3; R_2=H; R_3+R_4=O
b) R_1=H; R_2=OCH_3; R_3+R_4=O
c) R_1=OCH_3; R_2=H; R_3=H; R_4=OH
d) R_1=H; R_2=OCH_3; R_3=H; R_4=OH

From the fraction of tertiary alcohols we isolated cis-abienol, the presence of which is characteristic for spruce oleoresin [7]. Among the primary alcohols behenyl and lignoceryl (C_{22} and C_{24}) predominated. From the fraction of triterphenols we isolated 3β- and 3α-methoxy-21β-hydroxy-Δ^{14}-serratenes (Ic and Id) which were identified in the form of acetates from their physical constants and by comparison with authentic samples in terms of melting poitns and PMR spectra.

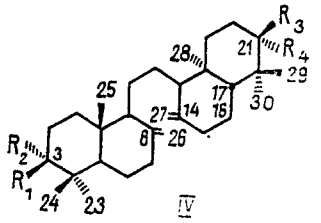
In order to isolate the accompanying compounds, the triterpene alcohol fraction was acetylated, and the serratene derivatives were separated by chromatography in the form of acetates. From the unacetylated fraction two compounds were isolated by rechromatography. The first consisted of an unsaturated ketone (III) the spectral characteristics of which coincided with those described previously for stigmast-4-en-3-one (Δ^4-sitosten-3-one) [8, 9]. It is possible that this enol is either a product of the autooxidation of β-sitosterol, which is always present in the extracts, or a product of the microbiological degradation of sterols [9]. It must be mentioned that interest has recently arisen in the synthesis of unsaturated ketones from β-sitosterol [10].



III

The second crystalline compound had intense bands in its IR spectrum at 890, 1650, and 1700 cm^{-1}, while its UV spectrum lacked absorption in the 210-260 nm region. In the PMR spectrum there were the signals of three methyl groups at 0.79, 0.96, and 1.06 ppm and, in the weak-field in the spectrum two singlets at 4.6 and 4.88 ppm, each with an intensity of 1 H, which were assigned to the protons of an exomethylene group. In the ^{13}C NMR spectrum the signals of 15 carbon atoms were observed, and in the mass spectrum the molecular ion had m/z 438. On the basis of its spectral characteristics, the compound isolated was ascribed the structure of α-onoceradienedione (4a) which has been obtained previously by partial synthesis [11] but has not been detected in the extractive substances of conifers in the native form. (Formula, top, following page.)

The polar fraction of the extract was acetylated. As a result of repeated chromatography of the acetylation products, a substance with mp 215-219°C was isolated with a PMR spectrum close to that of α-onoceradienedione (IVa). Its ^{13}C NMR spectrum contained the signals of 17



IV

a) $R_1+R_2=O$; $R_3+R_4=O$
b) $R_1=OAc$; $R_2=H$; $R_3=H$; $R_4=OAc$
c) $R_1+R_2=O$; $R_3=H$; $R_4=OAc$
d) $R_1=OH$; $R_2=H$; $R_3=H$; $R_4=OH$

carbon atoms, and the molecular ion, with m/z 526, corresponded to the empirical formula C_{34}-$H_{54}O_4$. To determine the structure of the substance isolated and to refine the structure of compound (IVa), we made an x-ray structural analysis (XSA), with the aid of which the structure of ketone (IVa) was confirmed and the structure of the new compound (Vb) was established. Both substances, (IVa) and (IVb), are unique by virtue of the fact that they possess a symmetry which is retained even in the crystal; the structures, relative configurations, and spatial arrangements of the molecules are shown in Figs. 1 and 2, respectively. The molecule of the acetate (IVb) was located on a second-order axis of rotation passing through the middle of the C11–C12 bond. The geometry of the molecule was the usual one, the rings having the chair form. The molecule of the ketone (IVa) was present on a C_2 axis of symmetry, analogously to the molecule of the acetate (IVb). The bond lengths in the ketone (IVa) were the normal ones. We may note a flattening of the chair conformation of ring A in the region of the ketone group (the torsional angles C1–C2–C3–C4, C2–C3–C4–C5, and C3–C4–C5–C10 are, respectively, 40.5°, −33.0°, and 41.5°). On comparing the (IVb) and (IVa) molecules, on the whole the difference can be seen in the C9 C11–C12–C13 torsional angles – −163.9° in the acetate (IVb) and +161.3° in the ketone (IVa).

The second substance, with mp 191-193°C, isolated on the chromatography of the acetate of the polar fraction, was, according to its IR and PMR spectra, a ketoacetate (1250, 1710, 1720 cm^{-1}; 2.02 ppm), and it had a molecular mass of 482 and the empirical formula $C_{32}H_{50}O_3$. The PMR spectrum showed the signals of six methyl groups and the signals of the olefinic protons of two exomethylene double bonds (4.54, 4.60, 4.82, and 4.87 ppm, with an intensity of 1 H each(). A multiplet with a chemical shift of 4.5 ppm (1 H) was assigned to the signal of a proton geminal to an acetate group. The ^{13}C NMR spectrum showed the signals of 32 atoms. To the compound isolated we ascribed the structure of 21-acetoxy-3-oxoonoceradiene (IVc). The reduction of the ketoacetate (IVc) led to α-onoceradienediol (onocerin) (IVd). From the fraction containing polyfunctional compounds we isolated native onocerin (IVd), coinciding with a synthetic sample on TLC. It must be mentioned that the presence of various functions in onoceradiene destroys the symmetry of the molecule that we observed in the case of compound (IVc), and this has not been described previously.

It has been shown that onoceradienes are biogenetic precursors of the serratene triterpenoids [12] which have been found in consdierable amounts in the extractive substances of conifer barks [13]. It is an interesting fact that onoceradiene has been obtained from abienol [14], and this triterpenoid may probably be a product of the transformation of cis-abienol which is present in Siberian spruce bark.

Among the polyfunctional polar compounds of the extract we found a saturated diol, which was identified as triacontane-10,11-diol, identical with the alcohol obtained on the saponification of the ester fraction.

The study of the chemical composition of a petroleum ether extract of Siberian spruce bark has shown that its main components are saturated fatty alcohol and acids, and also serratene triterpenoids. The presence of onoceradiene derivatives and of an oxidation product of β-sitosterol – stigmast-4-en-3-one – proved unexpected.

EXPERIMENTAL

The bark of the Siberian spruce was collected in the Altai in July, 1990.

IR spectra were recorded on a UR-20 instrument in CCl_4 solution and in KBr tablets. PMR spectra were recorded in $CDCl_3$ on a Bruker-200 instrument (200.13 MHz) (δ scale, internal standard chloroform, the signal of which is 7.24 ppm). ^{13}C NMR spectra were recorded on Bruker AC-200 (50.32 MHz) and Bruker AM-400 (10.02 MHz) instruments. Mass spectra were taken on a Finnigan MAT 8200, pW instrument.

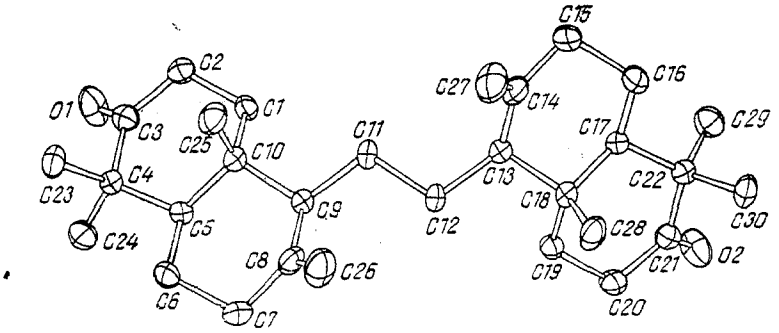


Fig. 1. Structure, relative configuration, and spatial arrangement of onoceradienedione (IVa).

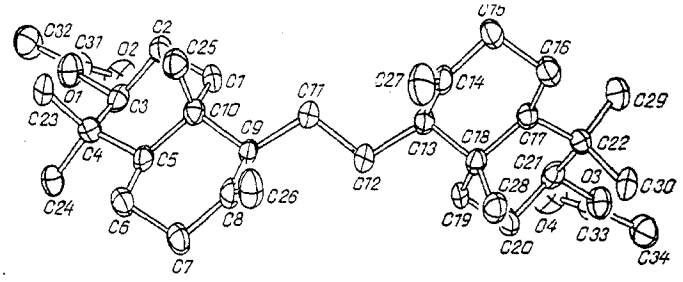


Fig. 2. Structure, relative configuration, and spatial arrangement of onoceradienediol diacetate (IVb).

The GLC of the neutral substances was conducted on a Chrom-4 instrument with the phase SE-30 on Chromaton-Super 0.16-1.20 at a column temperature of 260°C with the carrier gas nitrogen at the rate of 30 ml/min. The conditions for recording the methyl esters are given below.

For chromatography we used type KSK silica gel with a grain size of 0.063-0.16 mm and freshly distilled solvents: PE (40-70°C) and dimethyl ether (DE). The melting points of the substances were determined on a Kofler stage. Angles of optical rotation were obtained on a Zeiss polarimeter for solutions in chloroform.

Preparation of the Extract. The air-dry spruce bark (540 g) was extracted with PE (70-100°C) in a Soxhlet apparatus for 5 h. The ether was distilled off, giving 7 g of extract. A total of 3.12 kg of bark was treated and the total yield of extract was 38 g (1.2%).

The extract was treated by two methods [3, 15]. A. When a solution of 18 g of the extract in 200 ml of methanol was cooled, a precipitate (10 g) deposited, which was separated off and was dissolved in DE; the resulting solution was heated with aqueous NaOH (during this procedure, a voluminous flocculent precipitate difficult to separate formed); after working up, 5.3 g of neutral and 4 g of acidic substances were obtained. The filtrate was evaporated and dissolved in DE, and the solution was treated successively with sodium bicarbonate solution (yield of acid 0.17 g) and with 4% aqueous caustic soda solution (yield of acid 4.0 g); the ethereal solution of the unsaponifiable substances was washed with water and dried, the ether was distilled off to give 3 g of neutral substances.

Method B. The extract (10 g) was dissolved in hot acetone and the solution was left at room temperature for a day. The resulting precipitate was filtered off and the acetone was evaporated off. The product (9.5 g) was dissolved in DE and the solution was worked up as described above. This gave 4.0 g of neutral substances and 4.7 g of acid substances. The neutral and acidic fractions of the extract were then investigated separately.

Analysis of the Free Acids of Extracts. The acids obtained by method A (filtrate) (4 g) were treated with diazomethane and the resulting methyl esters were analyzed by GLC. The analysis was conducted on a Chrom-5 instrument with a glass column 3 mm × 2 m containing 9% of the phase DEGS on Chromaton N-AW, DMCS (0.20-0.25), using nitrogen as the carrier gas.

The mixture of methyl esters consisted of 23 components, among which the following were present in substantial amounts (%): C_{16} (4.8), C_{17} (3.0), C_{18} (0.7), $C_{18:1}$ (6.0), $C_{18:2}$ (9.2),

X_1 (7.0), C_{20} (4.1), C_{21} (2.0), C_{22} (8.5), X_2 (8.6), C_{24} (9.9). The total methyl esters from the precipitate (method A) were analyzed by GLC, and had the following composition (%): C_{16} (2.9), $C_{16:1}$ (0.9), C_{18} (0.3), $C_{18:1}$ (15.6), $C_{18:2}$ (8.7), $C_{18:3}$ (0.4), C_{20} (2.6), C_{22} (8.8), C_{24} (14.6), methyl pimarate (3.2), methyl isopimarate (11.0), and methyl dehydrobietate (32.6).

Adsorption chromatography of the methyl esters from the precipitate showed that this fraction contained serratene triterpenoids, which are sparingly soluble in methanol. In order to separate the triterpenoids, the extract was treated with acetone (method B). The component composition of the acid part of the extract (by method B) corresponded to that given above. It was established that the acidic part of the extract consisted of saturated and unsaturated faty acids (50%), resin acids (40%), and alkyl ferulates (2%).

Neutral Substances of the Extract. The neutral parts of the extracts obtained by methods A and B were combined, and a weighed sample (2.7 g) was chromatographed on silica gel (at a ratio of 1:15) in mixtures of PE and DE with increasing amounts of the latter. PE–DE (5%) eluted 0.94 g of an ester fraction; PE–DE (10%) 0.71 g of a ketone fraction; PE–DE (15%) 0.52 g of an alcohol fraction; PE–DE (25%), 1 g of triterpene alcohol; PE–DE (40%), 0.4 g of β-sitero-sterol; and PE–DE (50%) 0.3 g of a diol fraction.

Saponification of the Esters. The ester fraction (0.94 g) was dissolved in 5% methanolic OH and the solution was boiled for 4 h. After the usual working up, 0.64 g of unsaponifiable neutral substances and 0.16 g of acids were obtained, and the latter were treated with diazomethane and the resulting methyl esters were analyzed by GLC. The composition of the methyl esters of the "bound" acids was (%): $C_{14:0}$ (0.2), $C_{16:0}$ (3), $C_{16:1}$ (2), $C_{18:0}$ (0.5), $C_{18:1}$ (6.6), $C_{18:2}$ (11.5), X_1 (9), $C_{18:3}$ (3), C_{20} (14), C_{22} (22), C_{24} (28), X_2 (0.4), X_3 (2.3). The neutral unsaponifiable substances were analyzed by GLC. By comparison with authentic samples it was establishedthat the neutral fraction consisted of all the C_{16}–C_{24} fatty alcohols, β-sitosterol (13.9%), and triacontane-10-11-diol (64.6%).

Triterpene Ketones. The rechromatography of the ketone fraction (0.3%) led to the successive isolation of:

0.03 g of 3β-methoxy-21-oxo-Δ^{14}-serratene (Ia) with mp 265-270°C (methanol), $[\alpha]_D^{22}$ −63.5° (c 1.01); lit [3]: mp: 240-243° $[\alpha]_D$ − 71.5°;

0.05 g of 3α-methoxy-21-oxo-Δ^{14}-seratene (Ib) with mp 265-270°C (methanol), $[\alpha]_D^{22}$ −15° (c 1.12); lit. [3]: mp: 270-271°, $[\alpha]_D$ −16°; and

0.05 g of 3β-methoxy-21-oxo-Δ^{13}-serratene (II) with mp 204-209°C (ethyl acetate), $[\alpha]_D^{22}$ +65.5° (c 0.5); lit. [16]: mp: 206-210°, $[\alpha]_D$ + 77.2°. The IR and ^1H and ^{13}C NMR spectra were identical with those of authentic samples.

cis-Abienol. cis-Abienol (0.1 g), with mp 40-41°C was isolated from the fraction containing triterpene ketones. Its IR and PMR spectra coincided with those of an authentic sample.

Saturated Fatty Alcohols. According to GLC results, the alcohol fraction contained eicosanol (12%), docosanol (40%), and tetracosanol (47%).

Triterpene Alcohols. The triterpene alcohol fraction (1.0 g) was acetylated and, after the usual working up and chromatography of the product with PE–DE (10%) the following were obtained successively: 0.1 g of 21β-acetoxy-3β-methoxy-Δ^{14}-serratene, mp 198-199°C; lit. [3]; mp 199-201°C; and 0.15 g of 21β-acetoxy-3α-methoxy-Δ^{14}-serratene, mp 202-203°C, identical in terms of its IR and ^1H and ^{13}C NMR spectra with an authentic sample.

Stigmast-4-en-3-one (III). After the separation of the alcohol acetates, with increasing polarity of the eluent (15%) a crystalline substance was isolated with mp 86-87°C (ethanol), $[\alpha]_D^{22}$ + 84° (c 2.01), identical according to its UV, IR, and PMR spectra with stigmast-4-en-3-one [9]. UV spectrum (λ_{max}, nm: 240, ε 16,000). IR spectrum: ν_{max}^{KBr}, cm^{-1}: 1650, 1700. PMR spectrum (ppm): 0.85 (s, 18-CH_3), 0.87 (d, J = 7 Hz, 26-CH_3), 0.90 (d, J = 7 Hz, 27-CH_3), 0.93 (t, J = 7 Hz, 29-CH_3), 1.00 (d, J = 7.5 Hz, 21-CH_3), 1.06 (s, 19-CH_3), 2.3-2.34 (m, 2H-2), 5.69 (br.s, 1H-4).

Onaceradienedione (IVa). On further chromatography with PE–De(20%), a diketone (IVa) was isolated with mp 179-181°C (methanol), $[\alpha]_D^{24}$ + 11.5° (c 0.87), lit. [11]: 183-185°C (chlf–methanol). IR spectrum, ν_{max}^{KBr}, (cm^{-1}): 890, 1390, 1460, 1640, 1700. PMR spectrum (ppm): 0.80, 0.99, 1.07 (each 6H, s, tertiary methyl groups), 2.30-2.67 (m, 2H-2 and 2H-20), 4.61 (br.s,

1H-26 and 1H-27) and 4.89 (br.s, 1H-26 and 1H-27). Its ^{13}C NMR spectrum is given in Table 1. Mass spectrum, m/z: 438 (M^+).

Onaceradiene-3,21-diol Diacetate (IVb). On rechromatography of the fraction obtained after the separation of the ketones with PE–DE(50%), 0.2% of a polar fraction was eluted, and this was rechromatographed to give 50 mg of a substance with mp 215-219°C (ethanol), $[\alpha]_D^{24}$ + 26.3° (c 1.14). IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1720. PMR spectrum, (ppm): 0.64, 0.81, 0.86 (s, each 6H, tertiary methyl groups), 2.03 (s, 6H, CH_3–OCO), 450 (m, 1H-3 and 1H-21), 4.53 (br.s, 1H-26 and 1H-27) and 4.80 (br.s, 1H-26 and 1H-27). Its ^{13}C NMR spectrum is given in Table 1. Mass spectrum, m/z: 526 (M^+). Empirical formula $C_{31}H_{52}O_4$ (found, m/z 526.2022; calculated, 526.4022).

The x-ray structural analysis of compounds (IVa) and (IVb) was conducted on a Syntex $P2_1$ diffractometer (Cu-K_α radiation, graphite monochromator). The crystals of the acetate (IVb) were rhombic a = 15.143 (2), b = 7.4307 (8), c = 13.780 (2) Å, V = 1550.6 (3) $Å^3$, space group $P2_12_12$, $C_{34}H_{54}O_4$, z = 2, M = 526.80, d_{calc} = 1.13 g/cm^3. The intensities of 1201 independent reflections with $2\theta < 114°C$ were measured by the $2\theta/\theta$ scanning method, and 744 of the observed reflections ($F_0 > 4\sigma$) were used in the calculations. The crystals of ketone (IVa) were also rhombic: a = 6.269 (3), b = 7.887 (4), c = 26.285 (9) Å, V = 1300 (1) $Å^3$, space group $P22_12_1$, $C_{30}H_{46}O_2$, z = 2, M = 438.69, d_{calc} = 1.12 g/cm^3. The intensities of 1050 independent reflections with $2\theta < 116°C$ were measured by the ω-scanning method. In view of the large dimensions (0.2 × 1.0 × 2.0 mm^3) and poor quality of the crystal with 125 strongest ($I > 5 \cdot 10^5$ pulses/s) reflections were not used in the calculations.

TABLE 1. Chemical Shifts (ppm) and Multiplicities of the Signals in the ^{13}C NMR Spectra of Compounds (IVa, b, c, and d)*

C atom	IVa	IVb	IVc	IVd
1,19	34,60 t	36,58 t	34,61 t, 36,62 t	37,02 t
2,20	37,62[a] t	22,16 t	37,44[a] t, 22,25 t	22,61 t
3,21	216,72 s	80,73 d	216,70 s, 80,68 d	78,86 d
4,22	47,64 s	37,92 s	47,62 s, 37,83 s	39,09[a] s
5,17	55,15 d	54,67 d	54,76 d, 55,12 d	54,73 d
6,16	22,61 t	23,72 t	22,61 t, 23,72 t	24,01 t
7,15	37,48[a] s	37,99 t	37,83[a] t, 37,91 t	38,24 t
8,14	147,39 s	148,11 s	147,98 s, 147,51 s	148,36 s
9,13	56,44 d	56,98 d	56,41 d, 57,08 d	57,57 d
10,18	39,05 s	38,98 s	38,99 s, 38,99 s	39,25[a] s
11,12	24,98 t	24,18 t	24,17 t, 25,03 t	27,96 t
23,29	25,04 i	28,11 q	25,94 q, 28,09 q	28,23 q
24,30	21,52 q	16,40 q	21,53 q, 16,36 q	15,26 q
25,28	13,96 q	14,48 q	13,95 q, 14,49 q	14,44 q
26,27	104,47 t	106,70 t	106,79 t, 107,37 t	106,64 t
$OCOCH_3$	—	170,85 s	170,82 s	—
$\bar{O}C\bar{O}CH_3$	—	21,20 q	21,16 q	—

[a]The chemical shifts may change places within a given column.
*In the assignment of the chemical shifts of the signals of the carbon atoms we made use of information in [18].

TABLE 2. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms of Ketone (IVa)

Atom	x/a	y/b	z/c	Ueq, $Å^2 \times 10^3$
Cl	3714(8)	1303(4)	649(1)	52
C2	4205(8)	−414(4)	889(1)	60
C3	4703(7)	−318(5)	1449(1)	53
C4	3396(6)	870(4)	1781(1)	44
C5	2691(6)	2490(4)	1485(1)	41
C6	1086(7)	3548(5)	1794(1)	55
C7	739(9)	5293(5)	1546(1)	64
C8	64(7)	5042(5)	998(1)	54
C9	1710(6)	4078(4)	698(1)	42
C10	1916(6)	2247(4)	925(1)	38
C11	1339(8)	4089(4)	114(1)	53
C23	1554(8)	−210(5)	1995(1)	59
C24	4821(8)	1408(6)	2236(1)	69
C25	−186(7)	1294(5)	867(1)	56
C26	−1850(8)	5513(6)	841(2)	75
O	6113(6)	−1186(5)	1626(1)	84

The coordinates of the independent atoms are given; the numbering corresponds to the complete molecule (see Fig. 1).

TABLE 3. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms of the Acetate (IVb)*

Atom	x/a	y/b	z/c	$U_{eq.}$ Å$^2\times10^3$
C1	7917(4)	9650(10)	1980(5)	55
C2	6996(5)	9605(10)	1533(5)	58
C3	6740(4)	7656(10)	1311(5)	58
C4	6718(4)	6448(10)	2207(5)	53
C5	7648(4)	6598(10)	2686(5)	49
C6	7765(4)	5320(11)	3553(5)	64
C7	8730(4)	5247(11)	3882(6)	65
C8	9072(4)	7106(9)	4081(5)	53
C9	8992(4)	8328(9)	3208(4)	43
C10	7990(4)	8538(9)	2915(5)	45
C11	9496(4)	10123(10)	3307(5)	54
C23	5935(4)	6924(11)	2885(5)	63
C24	6560(5)	4521(11)	1859(6)	76
C25	7502(4)	9466(11)	3740(5)	61
C26	9322(4)	7597(14)	4964(6)	76
C31	5785(6)	7816(12)	−57(7)	73
C32	4842(5)	7641(13)	−410(6)	96
O1	5856(3)	7639(7)	886(4)	70
O2	6399(4)	8122(10)	−596(4)	105

*The coordinates of the independent atoms are given; the numbering corresponds to the complete molecule (see Fig. 2).

The structures were interpreted by the direct method using the SHELX-86 program and was refined by means of SHELX-76 program in the full-matrix anisotropic-isotropic (for the H atoms) approximation. The coordinates of the hydrogen atoms were calculated geometrically after each cycle of refinement. The final values of the R factors were: R = 0.0497, R_W = 0.0556, S = 1.03, $W^{-1} = \sigma^2 + 0.0027\ F^2$ for IVb. R = 0.0677, R_W = 0.0767, S = 1.31, $W^{-1} = \sigma^2 + 0.007\ F^2$ (868 $F_0 > 10\sigma$) for IVa. The coordinates of the crystallographically independent atoms of the ketone IVa) and the acetate (IVb) are given in Tables 2 and 3, respectively.

β-Sitosterol. The fractions (0.4 g) obtained by chromatography of the neutral substances was recrystallized from PE, giving β-sitosterol with mp 139-140°C the IR and PMR spectra of which coincided completely with those of an authentic sample.

21-Acetoxy-3-oxoonoceradiene (IVc). We acetylated 3 g of the mixture of polar compounds obtained from the neutral fraction of the extract. The reaction product (0.28 g) was chromatographed from PE–DE(5%), and 14 mg of sitosterol acetate was isolated, while PE–DE (10% led to 80 mg of compound IVb, with mp 191-193°C (acetone), $[\alpha]_D^{24}$ + 24.6° (c 2.03) IR spectrum, ν_{max}^{KBr}, cm^{-1}: 905, 1250, 1705, 1730. PMR spectrum, ppm: 0.64, 0.79, 0.81, 0.84, 0.99, 1.07 (s, each 3H, tertiary methyl groups), 2.02 (s, CH_3–OCO), 4.50 (m, 1H-21), 4.54, 4.60, 4.82 and 4.87(br.s, each 1H, 2H-26 and 2H-27). The ^{13}C NMR spectrum is given in Table 1. Mass spectrum m/z: 482 (M^+). Empirical formula $C_{32}H_{50}O_3$ (found, m/z 482.3769; calculated 482.3760).

Onoceradiene Diol (IVd). Lithium tetrahydroaluminate* was added to 20 mg of compound (IVc) in 2 ml of THF, and the mixture was boiled under reflux for 2 h. After the usual working up, the product was chromatographed, and PE–DE(50%) yielded 9 mg of the diol (IVd) with mp 232-237°C (chlf), $[\alpha]_D^{24}$ + 37.5° (c 0.4), lit. [11]: 202-203° (chlf–methanol). PMR spectrum, ppm: 0.63, 0.75, 0.98 (s, each 6H, tertiary methyl groups), 3.23 (dd, J = 4.5 and 11 Hz, 1H-3 and 1H-21), 4.54 (br.s, 1H-26 and 1H-27) and 4.81 (br.s, 1H-26 and 1H-27). The ^{13}C NMR spectrum is given in Table 1. Mass spectrum, m/z: 422 (M^+). Empirical formula $C_{30}H_{50}O_2$ (found, m/z 442.3801; calculated, 442.3811).

The reduction of 32 mg of onaceradienedione (IVa) under similar conditions gave 18 mg of the diol (IVd), identical with that described above.

Triacontane-10,11-diol. From the polar fractions of the extract by repeated chromatography we isolated a product (0.1 g) with mp 115-118°C (methanol), which was identified as triacontane-10,11-diol; lit. [17]: 115-116°C. IR spectrum, $\nu_{max}^{CCl_4}$, cm^{-1}: 1110, 1390, 1470, 3600; the diacetate of the triacontane-10,11-diol had mp 60-61°C.

*No amount given – Translator.

LITERATURE CITED

1. E. G. Bobrov, Forest-Froming Conifers of the USSR [in Russian], Nauka, Leningrad (1978).
2. T. Norlin and B. Winell, Acta Chem. Scand., 26, 2289 (1972).
3. J. P. Kuthey, I. H. Rogers, and J. W. Rowe, Tetrahedron, 25, 3731 (1969).
4. N. A. Vasil'eva and I. S. Geles, The Chemistry and Use of the Extractive Substances of Trees: Abstracts of Lectures at the IIIrd All-Union Scientific and Technical Conference [in Russian], Gor'kii (1990), p. 98.
5. M. L. Laver and H. H. L. Fang, J. Agric. Food Chem., 37, 114 (1989).
6. . . Kolattukudy, Chemistry and Biochemistry of Natural Waxes, Elsevier, Amsterdam (1976), p. 247.
7. V. A. Pentegova, Zh. V. Dubovenko, V. A. Raldugin, and É. N. Schmidt, The Terpenoids of Doniferous Plants [in Russian], Nauka, Novosibirsk (1987), p. 71.
8. J. W. Rowe, Phytochemistry, 4, 1 (1965).
9. T. W. Norin and B. Winell, Acta, Chem. Scand., 26, 2297 (1972).
10. N. V. Kovganko and Zh. N. Kashkan, Khim. Prir. Soedin., No. 6, 771 (1990).
11. D. H. R. Barton and K. H. Overton, J. Chem. Soc., 2639 (1955).
12. Y. Tsuda, T. Sano, K. Kawaguchi, and Y. Inubushi, Tetrahedron Lett., 1279 (1964).
13. T. Norin, Phytochemistry, 11, 1231 (1972).
14. R. M. Carman and H. C. Deeth, Aust. J. Chem., 24, 1099 (1971).
15. J. de Pascual Teresa, A. F. Barrero, L. Muriel, A. San Feliciano, and M. Grande, Phytochemistry, 19, 1153 (1980).
16. G. F. Chernenko, E. E. Ivanova, L. I. Demenkova, and É. N. Shmidt, Khim. Prir. Soedin., No. 5, 645 (1990).
17. X. A. Dominguez and V. Barragan, J. Org. Chem., 30, 2049 (1965).
18. K. L. Dhar, O. P. Suri, and K. A. Suri, Indian J. Chem., 29B, 911 (1990).

POLYFUNCTIONAL TRITERPENOIDS FROM THE BARK OF YEDDO SPRUCE

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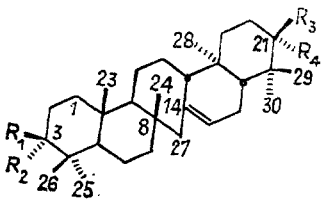
The composition of the polyfunctional triterpenoids of an extract of the Yeddo spruce has been studied. Five serratene triterpenoids have been isolated: 21β-hydroxyserrat-14-en-3-one (I), 3β-hydroxyserrat-14-en-21-one (II), serratenediol (III), episerratenediol (XII), and diepiserratenediol (V) in the form of its acetate. The structures of the compounds were confirmed by ^{13}C NMR spectra and XSA.

We have previously reported the chemical composition of a petroleum ether extract of Yeddo spruce but the polar compounds in it were not investigated [1]. The present work was devoted to their study.

By rechromatography of the total polar compounds we isolated two ketoalcohols (I and II) and three isomeric triterpenediols (III-V) which had been found earlier in bark extracts from various pine species [2-5]. The contradictory statements about the melting points of these compounds found in the literature compelled us to carry out an analysis of the PMR and ^{13}C NMR spectra of compounds (I-V).



I. $R_1+R_2=O$; $R_3=OH$; $R_4=H$
Ia. $R_1+R_2=O$; $R_3=OAc$; $R_4=H$
II. $R_1=OH$; $R_2=H$; $R_3+R_4=O$
III. $R_1=OH$; $R_2=H$; $R_3=H$; $R_4=OH$
IV. $R_1=OH$; $R_2=H$; $R_3=OH$; $R_4=H$
IVa. $R_1=OAc$; $R_2=H$; $R_3=OAc$; $R_4=H$
V. $R_1=H$; $R_2=OAc$; $R_3=OAc$; $R_4=H$
VI. $R_1=H$; $R_2=OCH_3$; $R_3=OAc$; $R_4=H$

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