Lanost-9(11)-ene-3,24,27-triol (III). The mixture obtained on the chromatography of the sterol fraction and containing β-sitosterol, compound (I) and epitorulosol acetate (90 mg) was dissolved in 3 ml of THF (dry), and 0.09 g of lithium tetrahydroaluminate and then another 3 ml of THF were added and the mixture was boiled on the water bath at 80°C for 2 h. After the usual working up, the reaction mixture was chromatographed on silica gel. PE–DE (3:1) eluted a fraction (24 mg) containing β-sitosterol and unchanged triterpene (I); PE–DE (1.5:1) eluted 10 mg of epitorulosol; and PE–DE (1:1) eluted a fraction (12 mg) the recrystallization of which from acetonitrile with the addition DE gave 6 mg of compound (III) with mp 155-157°C.

PMR spectrum (400 MHz): 0.63, 0.73, 0.86, 0.94, 1.04 (3 H each, s, tertiary methyl groups), 0.89 (3 H, d, J = 6.5 Hz, CH_3-21), 1.25 (3 H, d, J = 6.5 Hz, CH_3-26), 3.0 (1 H, m, H-24), 3.41 (1 H, m, H-3), 3.60 (2 H, dd, J = 4, 5, and 12 Hz, dd, J = 8.5 and 12 Hz 2H-27), 5.25 (1 H, m, H-11). For the ^{13}C NMR spectrum (400 MHz), see Table 1. Mass spectrum, m/z (%): 458(11) – M^+, 440 (20) – $(M - 18)^+$, 425(45), 407(32), 400(53), 385(78), 367(100), 313(90), 295(10), 246(15), 229(14), 213(15), 201(15), 187(20), 175(30), 161(25), 147(20), 135(28), 119(35), 81(27), 69(32), 55(40), 43(45).

Compound (I) (8 mg) was reduced with lithium tetrahydroaluminate, as described above. After working up, 6 mg of the initial compound (I) and traces of compound (III) (according to TLC) were obtained.

When 9 mg of compound (II) was reduced, again only traces of compound (III) (according to TLC) were detected, and the acetate (II) was isolated in unchanged form.

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EXTRACTIVE SUBSTANCES OF THE BARK OF Picea ajanensis

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A study of the chemical composition of a petroleum ether extract of the bark of Yeddo spruce growing in Khabarovsk Territory has shown that the neutral fraction of the extract contained waxes, glycerides, diterpenoids – epimanool, cis- and trans-abienols, and phyllocladenol, and triterpenoids – the epimeric 3α- and 3β-methoxy-21-hydroxy-Δ^{14}-serratenes and a ketone which has been ascribed the structure of 21-keto-3β-methoxy-Δ^{14}-serratene.

There is information in the literature on the study of the chemical composition of the terpenoid components of extracts of the bark of various species of spruce [1-3]. The phenolic substances of the bark of the Yeddo, Korean, and Siberian spruces have been investigated [4]. However, the lipid and terpene substances of extracts of domestic species have been studied inadequately.

In the present paper we give information on a study of the group and component compositions of a petroleum ether extract of the bark of the Yeddo spruce Picea ajanensis Fisch. (Picea jezoensis) growing in Khabarovsk Territory. The Yeddo spruce is the main forest-forming species of the Far East and is widely involved in forestry operations, but the bark finds

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no use and is stored in the form of wastes, although it may be a source of a whole series of valuable substances.

The yield of the petroleum ether extract of Yeddo spruce bark depends greatly on the times of collecting and storing of the bark and ranges from 2 to 4% of the weight of the air-dry bark. The extract was treated successively with sodium bicarbonate and caustic soda to separate the acidic components which make up about half the extract (~48%). Analysis of the methyl esters of the free acids of the extract was carried out by gas-liquid chromatography (GLC). It was established that the acid fraction of the extract consisted of fatty and resin acids, among which behenic, lignoceric, isopimaric, and dehydroabietic acids predominated.

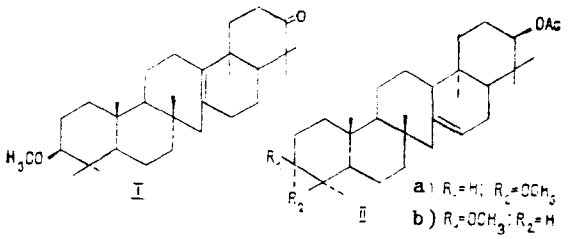
The neutral substances of the extract were separated by chromatography into hydrocarbons and oxygen-containing compounds. The hydrocarbons amounted to 4.5% of the neutral substances and were represented mainly by sesquiterpenes, among which δ-cadinene predominated.

The oxygen-containing compounds were separated by adsorption chromatography with increasing polarity of the eluting system.

The neutral part of the extract contained esters and glycerides (30%), fatty alcohols, triterpene ketones and alcohols (30%), tertiary alcohols (15%), sterols (10%), and polyfunctional compounds. The ester fraction consisted of groups of compounds with different polarities. The nonpolar esters were treated with alcoholic alkali, which led to the formation of β-sitosterol (86%), campesterol (12%), and an unidentified compound (1.7%; GLC results). On alkaline hydrolysis, the secondary group, of polar esters, gave predominantly acids: oleic (16.4%), linoleic (41.4%), and linolenic (28.1%), while stearic, behenic, and lignoceric acids were detected in trace amounts.

The tertiary diterpene alcohols were represented by cis- and trans-abienols, epimanool, and phyllocladenol. Among the fatty alcohols the main ones were docosanol and tetracosanol (74%). The presence of considerable amounts of fatty alcohols and acids is probably characteristic for extracts of spruce bark [3].

The amount of triterpenoids in the extract was fairly high, and they were represented by compounds of the serratane type, which are frequently found among the extractive substances of the bark of various species of conifers [3, 5, 6]. We isolated a triterepene ketone to which, on the basis of its PMR, IR, and mass spectra and a comparison of them with the spectra of known serratane ketones, and also an analysis of literature information, we ascribed the structure (I) -3β- methoxy-Δ^{13}-seraten-21-one. Δ^{13}-Isomers of ketones have previously been obtained under the action of mineral acids [7].



Triterpene alcohols were represented by 3α- and 3β-methoxy-21-hydroxy-Δ^{14}-serratenes, which were isolated and characterized in the form of their acetates (IIa and IIb). The amount of free β-sitosterol and free campesterol, which are characteristic of the extractive substances of conifers, was small, the bulk of the sterols being present in the extract in the form of esters. The polar fractions of the extract contained, in addition to polyfunctional compounds, products of the oxidation and polymerization of the labile diterpenoids (epimanool, cis- and trans-abienols) (checked by TLC).

EXPERIMENTAL

The bark of the Yeddo spruce was collected in Kabarovsk Territory in March-April, 1988. The IR spectra of the compounds were recorded on a UR-20 instrument in CCl_4 solutions and in KBr tablets. ^1H and ^{13}C NMR spectra were recorded on a Bruker WP 200SY instrument (^1H – 200.13 MHz; ^{13}C – 50.32 MHz) and a Bruker AC-200 MHz instrument in chloroform solutions, δ scale. The following abbreviations have been adopted in indicating the multiplicities of the

signals in the NMR spectra: s) singlet; d) doublet; t) triplet; q) quartet; m) multiplet. Melting points were determined on a Kofler stage. Mass spectra were taken on a Finnigan MAT 8200, pW, instrument.

Adsorption chromatography was conducted on type KSK silica gel with a grain size of 0.063-0.1 mm. The eluent used was petroleum ether (40-70°C) (PE) with the addition of from 0 to 100% of diethyl ether (DE). Extraction of the air-dried bark was carried out in a Soxhlet apparatus with petroleum ether (70- 100°C) as the solvent at an extraction time of 8 h. From 345 g of Yeddo spruce bark, after elimination of the solvent, 11.8 g of extract was obtained in the form of a dark green viscous mass with a resinous odor. The total amount of bark extracted was 1.15 kg, and the yield of extract was 29.8 g.

Working Up of the Extract. The extract (6 g) was dissolved in DE and the solution was washed successively with saturated aqueous sodium bicarbonate and 1% aqueous caustic soda. The ethereal solution of the extract was washed with water and dried over sodium sulfate, the yield of neutral substances amounting to 3.7 g. The solution of sodium salts of acids was acidified with 5% HCl solution to pH 2-3 and was extracted with DE, and, after the usual working up, 1.7 g of "weak" acids, 0.09 g of "strong" acids and 0.5 g of a precipitate (which was not investigated) were obtained.

Composition of the Acidic Part of the Extract. The free acids (0.5 g) were dissolved in DE and this solution was treated with an ethereal solution of diazomethane. After the solvent had been driven off, 0.52 g of methyl esters was obtained, and they were analyzed by GLC. The analysis was performed on a Chrom-5 chromatograph with the phase 9% of DEGS/Chromaton N-NW DMCS (0.20-0.25 mm), the length of the column being 2 m and its temperature 200°C and the carrier gas being nitrogen at a rate of 30 ml/min. Results of the analysis (%): methyl stearate) 2.0; methyl oleate) 3.3; methyl linoleate) 1.3; methyl arachidate) 1.3; unidentified) 2.2 and 0.4; methyl behenate) 6.3; methyl pimarate) 0.8; unidentified) 2.2; methyl levopimarate/methyl palustrate) 12.8; methyl isopimarate/methyl lignocerate) 32.8 (ratio 1:1); methyl abietate) 12.0; methyl dehydroabietate) 19.3; methyl neoabietate) 5.2.

Neutral Substances of the Extract. The neutral part of the extract (10 g) was chromatographed on silica gel (0.1 mm) at a ratio of substance and sorbent of 1:15. The results of the separation are given below:

Fraction No.	Solvent	Yield	Group of Compounds	Remarks
1	PE	0.45	Hydrocarbons	Mono- and sesquiterpenes
2	PE:DE (5%)	1.7	Esters	Waxes
3	PE:DE (10%)	1.3	"	Glycerides
4	PE:DE (20%)	1.0	Tertiary alcohols, ketones	Diterpene alcohols, triterpenoids
5	"	0.65	Fatty alcohols	C_{20}–C_{22}–C_{24}
6	PE:DE (30%)	0.6	Alcohols	Triterpenoids
7	PE:DE (40%)	1.2	Sterols	Sterols, alcohols
8	PE:DE (50%)	1.1	Polar	Polyfunctional compounds
9	DE	1.0	"	Polymers

Saponification of the Esters. A. Fraction 2 was dissolved in 20 ml of alcoholic KOH (5%) and the solution was heated at 60°C for 3 h. After cooling, the reaction mixture was worked up in the usual way. The neutral part of the reaction mixture amounted to 1.02 g and the acidic part to 0.51 g. Analysis of the component composition was carried out by the GLC method. Composition of the neutral part (%): β-sitosterol) 86.1; campesterol) 12.1; unidentified) 1.7. Recording conditions: Chrom-4 instrument, phase S-30 on Chromaton Super 0.16-0.20; column temperature 260°C; carrier gas nitrogen at the rate of 30 ml/min.

Analysis of the composition of the acidic part of the reaction mixture was performed under the conditions given for the methyl esters. The following acids were identified (%): palmitic) 16.4; stearic) 0.9; arachidonic) 12.2; behenic) 10.3; and lignoceric) 7.5; the remaining 14 components of the mixture in amounts of from 0.6 to 4.6% were not identified.

B. Fraction 3 was worked up by the procedure described above. After the usual treatment 0.2 g of neutral substances and 1.0 g of acidic substances were obtained. Composition of the acid part (%): linoleic) 16.4; oleic) 41.1; linolenic) 28.1; C_{20}) 1.6; C_{22}) 1.2; C_{24}) 1.0. Recording conditions: Chrom-5; phase) 9% DEGS/Chromaton N-NW, DMCS (0.20-0.25); column

3 mm × 2 m, column temperature 129-228°C/min [sic]; evaporator temperature 280°C; carrier gas) nitrogen at the rate of 20 ml/min. The neutral compounds were represented by C_{18}–C_{24} alcohols.

Tertiary Alcohols. From fraction 4 by repeated chromatography, cis- and trans-abienols and epimanool were isolated in a ratio of 2:6.9:1 (according to GLC) and were identified by comparison with authentic samples in relation to spectral characteristics, TLC, and GLC.

Phyllocladenol with mp 182-183°C was isolated when fraction 6 was chromatographed; it gave no depression of the melting point with an authentic sample of phyllocladenol, and the PMR spectra of the two compounds were identical.

Triterpene Ketones. 3β-Methoxy-21-keto-Δ^{13}-serratene (I). After the elimination of the tertiary alcohols, fraction 4 yielded a crystalline product which, after repeated recrystallization from ethyl acetate, had mp 206-210°C, $[\alpha]_{594}^{22}$ + 77.24° (c 0.725), $\nu_{max}^{CCl_4}$, cm^{-1}: 1110, 1380, 1460, 1705. PMR spectrum (200 MHz), ppm: 0.73, 0.79, 0.81, 0.95, 0.98, 1.0, 1.03 (3 H each, s, tertiary methyl groups), 2.7 (1 H, m, H-3), 3.45 (3 H, s, $-OCH_3$). ^{13}C NMR spectrum, ppm: 16.20 (q), 18.50 (q), 19.0 (t), 19.18 (q), 20.01 (t), 20.69 (q), 22.03 (t), 22.45 (t), 26.83 (q), 27.98 (q), 28.11 (t), 33.82 (t), 34.37 (t), 35.60 (s), 36.43 (t), 37.68 (s), 38.32 (s), 38.53 (s), 39.16 (t), 43.73 (t), 47.09 (s), 51.82 (d), 52.27 (t), 54.78 (d), 57.40 (q), 63.94 (d), 88.45 (d), 129.58 (s), 142.87 (s), 217.99 (s). Empirical formula – $C_{31}H_{50}O_2$ (found, m/z 454.3821; calculated, 454.3811). Mass spectrum, m/z (%); 454(100) – M^+, 439(35) – $(M - 15)^+$, 422(27), 407(65), 221(28), 203(25), 189(25), 175(10), 161(15), 147(15), 135(25),121(25), 107(23), 95(27), 81(25), 69(28), 55(27).

3β-Methoxy-21-acetoxy-Δ^{14}-serratene (IIb). A solution of fraction 6 in pyridine was treated with acetic anhydride and left overnight at room temperature. After the usual working up, the reaction mixture was chromatographed, and the solvent system PE–DE (20%) led to the isolation of a crystalline substance with mp 198-200°C, $[\alpha]_{594}^{24}$ + 6.3° (c 0.112) (according to the literature [1], mp 199-201°C, $[\alpha]_D$ +4.5°), $C_{33}H_{54}O_3$. M^+ – 498.4073 (high-resolution mass spectrometry). $\nu_{max}^{CCl_4}$, cm^{-1}: 1110, 1250, 1740. PMR spectrum (200 MHz), ppm: 0.67, 0.73, 0.79, 0.81, 0.84, 0.91 (3 H each, s, tertiary methyl groups), 2.06 (3 H, s, Ac), 2.52 (1 H, m, H-3), 3.33 (3 H, s, $-OCH_3$), 4.54 (1 H, m, H-21), 5.4 (1 H, s, H-15).

3α-Methoxy-21-acetoxy-Δ^{14}-serratene (IIa). The repeated chromatography of the combined acetates with PE–DE (30%) led to the isolation of a crystalline substance with mp 199-203°C, identical according to its IR, PMR, and mass spectra with an authentic sample of 3α-methoxy-21-acetoxy-Δ^{14}-serratene (according to the literature [1]: mp 205-207°C, $[\alpha]_D$ – 64.6°), PMR spectrum (200 MHz), ppm: 0.67 (3H), 0.81 (9H), 0.90 (6H), 0.92 (3H), (s, tertiary methyl groups), 2.05 (3 H, s, Ac), 2.55 (1 H, t, J = 2.5 Hz, H-3), 3.28 (3 H, s, $-OCH_3$), 4.42 (1 H, m, H-21), 5.4 (1 H, s, H-15).

β-Sitosterol. When fraction 7 was recrystallized from ethanol, β-sitosterol, identical in relation to its melting point (138-139°C) and its IR and PMR spectrum with an authentic sample, was obtained.

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