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AGE CHANGES IN THE COMPOSITION OF THE EXTRACTIVE SUBSTANCES OF THE BARK OF *Picea obovata*

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The chemical composition of the bark of the Siberian spruce Picea obovata *Ledb. has been studied. The bark of young trees contains esters of linoleic acid and of polyprenols and polyalcohols, and also isocembrol. In addition to these, components have also been identified which are characteristic for an extract of spruce bark of mature age:* cis-*abienol, epimanool oxide, dehydroabietinal, triterpene alcohols of the serratene type, and fatty (C_{16}-C_{26}) and resin acids.*

We have previously reported the composition of the extractive substances of the bark of the Siberian spruce *Picea obovata* Ledb. growing in Altai and obtained from trees more than 100 years old [1]. In the present paper we give the results of the chemical composition of the bark of young Siberian spruce trees(about 40 years old) growing in the same region. A feature of the raw material studied is the fact that it contains 36% of outer bark (rhytidome) and 64% of inner bark (phloem). We extracted the components of the raw materials separately with diethyl ether (DE) and found the phloem contained about 2% of extractive substances and the rhytidome 7.3%.

The phloem extract contained a considerable amount of free acids (50%) and neutral substances (28%), together with a residue insoluble in DE (10%) and compounds soluble in water (10%). In the residue we found pinosylvin and its monomethyl ether. In the neutral fraction of the extract, 40% consisted of esters, 35% of fatty and triterpene alcohols, and 20% of β-sitosterol, campesterol, and polyfunctional polar compounds. The acid fraction of the phloem extract contained saturated and unsaturated fatty and resin acids, and also phenolic acids, the presence of which is characteristic for extracts of spruce phloem [2].

Analysis of the bark extract showed that the free acids amounted to 64%, and of them 47% were resin acids. The neutral fraction of the extract (28%) consisted of a mixture of hydrocarbons (6%) including monoterpenes (14%), sesquiterpenes (35%), and diterpenes (45%); the ester fraction amounted to 29%, triterpenoids and β-sitosterol to 40%, and polar compounds to 4%.

In view of the fact that DE extracted a considerable amount of acids, polyphenols, and water-soluble substances from the bark and phloem, we investigated a hexane extract of the bark (not separated from the phloem), the yield of which amounted to 3.6%. The extract was treated with a 10% solution of NaOH in order to separate it into acid and neutral substances. The acid fraction of the extract amounted to 56% and contained 20 components, among which we identified saturated and unsaturated fatty acids and resin acids (66% of the total acids), the bulk of which consisted of dehydroabietic acid (28%). The neutral fraction of the extract was chromatographed, using solvents with increasing polarity. The hydrocarbons (6% of the total neutral substances) included aliphatic hydrocarbons and mono-, sesqui-, and diterpenes.

The ester fraction was eluted and was saponified with alcoholic alkali. The neutral unsaponifiable substances were separated from the reaction mixture. By crystallization of the neutral substances and subsequent chromatography of the mother solution we isolated and identified mixtures of β-sitosterol with campesterol and of polyprenols with dolichols. By chromato-mass spectrometry, among the neutral components we identified nona-2,6-dien-1-ol, dehydroabietinal, and epimanool oxide. The two latter components coincided in polarity with esters and were eluted together from the neutral part of the extract. No polyprenols and dolichols were detected in a petroleum ether extract of spruce bark [1].

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The acid fraction of the reaction mixture consisted of saturated and unsaturated fatty acids, among which the main one was linoleic acid (43%). The presence of linoleic acid in spruce bark is probably a taxonomic characteristic of this species [3].

From the neutral part of the extract we extracted isocembrol containing none of the 4- epimer (according to its NMR spectrum), *cis*-abienol, fatty alcohols — docosanol and tetracosanol —, β-sitosterol, and campesterol. The presence of triterpenoids of the serratene type is characteristic for spruce bark extracts [1, 3, 5]. In the extracts studied we found 3α-methoxy- and 3β-methoxy-21β-hydroxy-Δ^{14}-serratenes and 3α-methoxy-21β-hydroxy-Δ^{13}-serratene, but we did not detect triterpene ketones and diols. The polar fractions of the neutral substances of the extract were not investigated.

On comparing these results with those obtained previously [1], it may be noted that the total extract of the bark and phloem of young trees growing in Altai was distinguished by a high content of linoleic acid esters and by the presence of polyprenol and dolichol esters and of cembrene and isocembrol, which is probably due to a high phloem content. The extract contained no esters of vicinal aliphatic diols and triterpene diols.

EXPERIMENTAL

The bark of the Siberian spruce was collected in Altai in July, 1992, the age of the trees being 35-40 years.

IR spectra were recorded on a UR-20 instrument in CCl_4 solutions and in KBr tablets, and KBr spectra on a Bruker AC 200 instrument in $CDCl_3$ (200.13 MHz), δ-scale, internal standard $CHCl_3$, the signal of which was taken as 7.24 ppm. The GLC of the neutral substances was conducted on a Chrom-5 instrument with the phase SE-30 on Chromaton Super (0.16-0.20), column temperature 260°C, carrier gas nitrogen, 30 ml/min. The GLC of the methyl esters of the acids was conducted on a Chrom-5 instrument with a glass column and the phase 9% of DEGS on Chromaton N-AW, DMCS (0.20-0.25), with the column temperature rising from 140 to 220°C, and with the phase SE-30 on Chromaton Super (0.16-0.20) at a column temperature of 260°C with the carrier gas nitrogen, 30 ml/min.

The analysis of the total methyl esters and the neutral substances obtained after the saponification of the esters was performed on a Hewlett-Packard 5890 "A" chromatograph with a MSD 5971 mass-selective detector. For chromatography we used KSK silica gel (0.063-0.16 mm) and the freshly distilled solvents DE and petroleum ether (PE) with bp 40-60°C. The melting points of the substances were determined on a Kofler stage.

Preparation of the Phloem Extract. The air-dry comminuted phloem separated from the bark (187 g) was extracted with diethyl ether in a Soxhlet apparatus for 6 h. The yield of extract was 3.6 g (1.9%).

Treatment of the Extract. The extract was dissolved in DE and the solution was treated with 2% aqueous NaOH to eliminate free acids, which gave 0.86 g (28%) of neutral substances. After the solution of salts had been treated with 5% HCl, in addition to 1.7 g of acids we obtained 0.3 g of a residue in which pinosylvin and its methyl ether were determined (by TLC), and 0.35 g of water-soluble substances.

Neutral Substances. The neutral fraction of the phloen extract was chromatographed on a column. By successive elution we obtained hydrocarbons (1%), esters (40%), and fatty alcohols, β-sitosterol, and triterpenoids (20%).

The Acids of the Extract. The free acids of the phloem consisted of a mixture of resin acids and fatty (saturated and unsaturated) acids. The percentage composition of the fraction (analysis in the form of methyl esters): $C_{16:0}$ (1.8); $C_{17:0}$ (0.6); $C_{18:1}$ (4.0); $C_{18:2}$ (4.1); $C_{18:3}$ (0.3); X_1 (4.0); sandaracopimaric (3.7); isopimaric (11.7); levopimaric (7.5); dehydroabietic (21.6); abietic (13.3), neoabietic (9.7); $C_{22:0}$ (3.7); $C_{24:0}$ (1.0); X_2 (4.5); 15-hydroxydehydroabietic (5.1); and 15-hydroxyabietic (4.6).

Alkaline Hydrolysis of the Ester Fraction of the Phloem. A solution of 0.4 g of the fraction in 5% alcoholic KOH was heated at 80°C for 3 h. After the usual working up, 0.12 g of a mixture of neutral substances and 0.2 g of acids were obtained. In the acid fraction we identified even straight-chain saturated and unsaturated aliphatic acids (%): $C_{12:0}$ (0.5); $C_{14:0}$ (0.3); $C_{16:0}$ (11.0); $C_{18:1}$ (16.9); $C_{18:2}$ (1.5); $C_{18:3}$ (5.2); $C_{20:0}$ (5.0); $C_{22:0}$ (5.4); $C_{24:0}$ (10.4). Among the neutral unsaponifiable substances a mixture of β-sitosterol and campesterol (75%) predominated. A multicomponent mixture of alcohols present in small amounts was not identified.

Preparation of an Extract of the Rhytidome (Bark). Bark containing no phloem (85 g) was extracted with DE; the total yield of extract was 6 g (7.3%).

Treatment of the Extract. The extract was treated with 1% aqueous NaOH in order to separate it into neutral and acid components. This gave 3.8 g (64%) of acids and 1.67 g (28%) of neutral substances.

The isolation of the acids from the extract was achieved by succesive treatment with 1% aqueous NaOH (fraction 1) and with a saturated solution of $NaHCO_3$ (fraction 2). Composition (%): methyl isopimarate/methyl levopimarate (35), methyl dehydroabietate (35), methyl abietate (13), methyl neoabietate (5), and methyl 15-hydroxyabietate/15-hydroxydehydroabietate (8). Fraction 2 contained free phenolic acids (ferulic, *p*-coumaric, vanillic), and 15-hydroxydehydroabietic acid (TLC results and PMR spectra).

The neutral part of the extract (1.67 g) contained hydrocarbons (0.1 g, 6%; here and below, on the total neutral substances), which were represented by monoterpenes (10%), sesquiterpenes (35%), and diterpenes (45%); esters (0.49 g, 29%); fatty alcohols and β-sitosterol (0.32 g, 20%); triterpene alcohols (0.32 g, 20%); and polar compounds (0.07 g, 4%).

Isolation of a Total Extract of Phloem and Bark. The air-dry raw material (bark not separated from the phloem) (512 g) was ground and extracted with hexane in a Soxhlet apparatus for 6 h. The yield of extract was 19 g (3.7%). The total yield of extract from 2607 g of bark amounted to 93 g (3.6%).

Treatment of the Extract. A solution of 50 g of the extract in DE was treated with 1% aqueous NaOH. This gave 19.6 g (39.2%) of neutral substances and 27.8 g (55.6%) of free acids.

The Free Acids of the Extract. The free acids were treated with an ethereal solution of diazomethane and the resulting methyl esters were analyzed by GLC. They consisted of 20 components, among which we identified the following (%): $C_{16:0}$ (2.3); $C_{18:1}$ (2.9); $C_{18:2}$ (3.9); $C_{18:3}$ (1.1); sandaracopimaric (2.4); isopimaric (17.1); levopimaric (10.4); dehydroabietic (31.5); abietic (8.6); neoabietic (4.2); X (3.3); $C_{22:0}$ (2.3), $C_{24:0}$ (1.8). The other components, the amounts of which in the mixture ranged from 0.1 to 1.5%, were not identified.

The Neutral Substances of the Extract. The total neutral substances (10.0 g) were chromatographed on silica gel (160 μm, ratio 1:15) with, as eluent, PE containing increasing amounts of DE. PE eluted the total hydrocarbons (0.6 g) which included monoterpenes (15%), sesquiterpenes (47.5%), and diterpenes (37%) (among the latter 9% of cembrene was found), and also the C_{16}-C_{18} *n*-alkanes. Then a mixture of PE with 5% of DE eluted 1.6 g of total esters, a mixture with 10% of DE eluted 2.1 g of a tertiary alcohol fraction, mixtures with 20-30% of DE eluted 2.1 g of triterpene alcohol, a mixture with 40% of DE eluted 1 g of a fraction of fatty alcohols and alkyl ferulates; a mixture with 50% DE eluted 1.6 g of esters of phenolic acids and β-sitosterol; and, finally, DE alone eluted 1.1 g of a fraction of polar substances which was not investigated further.

Alkaline Hydrolysis of the Esters. A solution of 0.67 g of the ester fraction in 10 ml of 5% alcoholic KOH was heated at 100°C for 3 h. After the usal working up of the reaction mixture, 0.34 g of neutral substances and 0.18 g of acids were obtained. Crystallization of the neutral substances from methanol gave 0.12 g of a mixture of β-sitosterol and campesterol (90:10, GLC).

The mother solution after the crystallization of the sterol (0.2 g) was chromatographed on silica gel (0.71 mm, ratio 1:15). A mixture of PE with 2% of DE eluted 0.08 g of total nonpolar substances, which were analyzed by chromato-mass spectrometry. On the basis of an analysis of the mass spectra, we identified 3,4-dimethylbenzaldehyde (veratraldehyde) (12.6%), dehydroabietinal (20%), and epimanool oxide (60%).

A mixture of PE with 5% of DE led to the isolation of nona-2,6-dien-1-ol (5%, chromato-mass spectrometry) and the total oligoisoprenoid alcohols (0.02 g) which was analyzed by HPLC (conditions: Milikhrom instrument, 2 × 60 mm column, sorbent LiChrosorb RP-18, eluent: acetone–methanol (75:25), UV detector at a wavelength of 210 nm, rate of elution 100 μl/min). Composition of the mixture (%): polyprenols — C_{65} (2), C_{70} (12), C_{75} (25), C_{80} (18), C_{85} (3); dolichols — C_{65} (2), C_{70} (10.5), C_{75} (19), C_{80} (8), C_{85} (0.6). The PMR spectrum of the fraction coincided with that of an authentic sample of combined polyprenols and dolichols. Then PE containing 20% of DE eluted 0.1 g of a mixture of β-sitosterol and campesterol (83:17, GLC).

The acids (0.18 g) were treated with diazomethane, and the resulting methyl esters were chromatographed. This gave 0.15 g of total methyl esters, which were analyzed by chromato-mass spectrometry. The following acids were identified (%): $C_{16:0}$ (7.5), $C_{18:3}$ (19.4), $C_{18:2}$ (43.5), $C_{18:0}$ (traces), $C_{18.2}$ (iso) (2.4), $C_{20:0}$ (6.9), $C_{22:0}$ (8.5), $C_{24:1}$ (4.0), $C_{24:0}$ (5.5).

Tertiary Alcohols. The alcohol fraction (2.1 g) was acetylated, and after the usual working up, followed by chromatography, we isolated *cis*-abienol (1.0 g), isocembrol (0.66 g), 21β-acetoxy-3α-methoxy-Δ^{14}-serratene (0.1 g) (mp 199-201°C), and a mixture of the latter and its Δ^{13}- isomer (ratio 1:2 according to its ^{13}C NMR spectrum) [4]. The individual compounds isolated had IR and PMR spectra and constants identical with those of authentic samples.

Triterpene Alcohols. The triterpene alcohol fraction (2.1 g) was rechromatographed, with the isolation of 21β-hydroxy-3α-methoxy-Δ^{14}-serratene (1.1 g), mp 304-306°C (lit. [5]: 307.5-308°C); 21β-hydroxy-3β-methoxy-Δ^{14}-serratene (0.82 g), mp 273-276°C (lit. [5]: 276-277.5°C]. The IR and PMR spectra of the alcohols isolated were identical with those of authentic samples.

Saturated Fatty Alcohols. The primary alcohol fraction (1.0 g) was analyzed by GLC. The composition of the fraction (%): X_1 (1.1); X_2 (7.2); $C_{16:0}$ (1.6); $C_{20:0}$ (13); $C_{22:0}$ (18.6); $C_{24:0}$ (46.3); campesterol (1.1); β-sitosterol (5.5); X_3 (1.1).

β-Sitosterol. The fraction (1.6 g) was recrystallized from PE, to give 0.8 g of a substance with 130-132°C consisting, according to GLC, of a mixture of β-sitosterol (83%) and campesterol (17%). The mother solution was chromatographed, with the isolation of 0.2 g of a mixture of esters of phenolic acids (alkyl ferulates and alkyl coumarates from their PMR spectra) and 0.1 g of Δ^4-stigmasten-3-one, identical with an authentic sample [1].

REFERENCES

1. G. F. Chernenko, E. E. Ivanova, Yu. V. Gatilov, I. Yu. Bagryanskaya, and É. N. Shmidt, Khim. Prir. Soedin., 654 (1992).
2. A. S. Gromova, V. I. Lutskii, and N. A. Tyukavkina, Khim. Prir. Soedin., 798 (1974).
3. T. Norin and B. Winell, Acta Chem. Scand., **26**, 2286 (1972).
4. G. F. Chernenko, I. Yu. Bagryanskaya, Yu. V. Gatilov, G. E. Sal'nikov, É. N. Shmidt, and V. I. Mamatyuk, Khim. Prir. Soedin., 662 (1992).
5. J. P. Kutney, I. H. Rogers, and I. W. Rowe, Tetrahedron, **25**, 3731 (1969).