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EXTRACTABLE SUBSTANCES FROM NEEDLES OF SIBERIAN FIR *Picea obovata*

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The chemical composition of evergreen needles has been well studied [1-5]. Recently interest in the isolation from needles of polyprenols with several valuable properties has risen. New procedures for isolating low-molecular-weight components from evergreen needles have recently appeared [6, 7]. However, there is little published data on the composition of extractable substances from needles of Siberian fir, one of the forest species of Siberia [8].

We have previously reported on the composition of extractable substances from bark of Siberian fir growing in Altai and Novisibirsk District and monoterpenes of Siberian evergreens [9-11]. Herein we report on the composition of the extract of needles from the Siberian fir *Picea obovata* Ledeb. collected in January in Novosibirsk District.

Air-dried needles (250 g) were ground and extracted with methyl-*t*-butylether. The yield of ether extractable substances was 12.6 g (5.0 mass % of raw material).

Treatment of the extract with aqueous NaOH (2%), reprecipitation from CH_3OH, and chromatography over SiO_2 produced fractions that were analyzed by GC—MS and HPLC. Pure components were identified by physical chemical and spectral methods.

Free acids isolated by extraction with aqueous NaOH (2%) totaled 4.1 g (32.5 mass % of the extract; 1.64 mass % of the air-dried raw material) (henceforth data are given in percent of extract mass if not explicitly stated). This fraction was analyzed as the methyl esters obtained by exhaustive methylation with diazomethane. Identifications were made by comparing spectra with a database. GC—MS spectra were recorded on a Hewlett Packard G 1800 A instrument consisting of a HP 5890 Series II gas chromatograph and HP 5971 mass-selective detector [column 30 m × 0.25 mm × 0.25 μm; HP-5MS sorbent (5% biphenyl, 95% dimethylsiloxane); He carrier gas (1 mL/min); column temperature 2 min at 50°C, 4°C/min to 280°C, 15 min at 280°C; vaporizer temperature 280°C; ion source 170°C].

Free acids were fatty, resin, and "polar" in approximately equal amounts. The fatty acids included components with chain length C_{12}-C_{32}, among which $C_{16:0}$ (4.9%), $C_{18:1}$ (1.7), $C_{18:0}$ (0.7), $C_{22:0}$ (1.0), $C_{24:0}$ (0.8), $C_{26:0}$ (0.4), and $C_{30:0}$ (0.5) dominated. The content of the other fatty acids was insignificant. The resin acids were identified as pimaric, sandaracopimaric, isopimaric, and dehydroabietic (DAB) (90% of total resin acids). Abietic-type acids were absent. Polar acids included alkylferulates, vanillic acid, and the *O*-containing diterpene acids 7-hydroxy-DAB (1.5%), 7-keto-DAB (4.6), and 15-hydroxy-DAB (0.8).

Bound acids obtained upon saponification of the ester fraction that was isolated from total methanol-soluble substances by chromatography over SiO_2 made up 0.47 g (3.73 mass % of extractable substances, 0.19 mass % of raw material) and were analyzed analogously to free acids. Methyl esters of the following acids were identified in the mixture: $C_{16:0}$ (0.5%), 14-methyl-$C_{16:0}$ (0.1), $C_{18:0}$ (0.1), $C_{18:1}$ (1.3), $C_{18:2}$ (0.7), $C_{18:3}$ (0.2), $C_{20:0}$ (0.1), $C_{22:0}$ (0.1), and $C_{20:4}$ (0.1). Minor components, the content of which was less than 0.1 mass % of the extractable substances were also observed: $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:1}$, $C_{17:1}$, $C_{20:1}$, $C_{20:2}$, $C_{20:3}$, $C_{21:0}$, $C_{23:0}$, $C_{24:0}$, $C_{26:0}$ acids. About 10% of the mixture could not be unambiguously identified because of a lack of corresponding spectra in the database.

Components of the unsaponifiable residue of the ester fraction that consisted of a total of 2.4 g (19.0, 0.96%) were a mixture of polyprenols, β-sitosterol, and nonacosan-10-ol. Total hydrocarbons, free polyprenols, free epitorulosol, and β-sitosterol was also isolated during chromatographic purification of neutral components.

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Total high-boiling hydrocarbons (5.0 mass % of neutral components, 3.1 mass % of the extract) were a mixture of aliphatic and sesquiterpene hydrocarbons. Total volatile components have been previously investigated [11].

Hydrocarbons were analyzed after additional purification by GC—MS. They were paraffinic hydrocarbons of composition C_{19}-C_{36}:C_{19}, 0.4%; C_{20}, 0.8; C_{21}, 1.8; C_{22}, 2.9; C_{23}, 4.7; C_{24}, 3.8; C_{25}, 7.4; C_{26}, 2.8; C_{27}, 7.3; C_{28}, 3.3; C_{29}, 10.2; C_{30}, 2.4; C_{31}, 7.3; C_{32}, 1.1; C_{34}, 3.3; C_{35}, 0.6; C_{36}, 0.9 (61%) and sesquiterpenes longipinene (1.2%), longicyclene (0.2), copaene (1.1), β-elemene (0.3), longifolene (2.3), γ-murolene (1.9), β-selinene (1.1), α-murolene (2.3), γ-cadinene (16.6), calamenene (2.2) (mass % of the fraction).

Fractions of free and esterified polyprenols were analyzed using HPLC. Chromatograms were recorded on a Millichrome instrument with a Lichrosorb RP-18 column (6.3 × 0.2 cm), acetone:methanol (3:1) eluent.

Polyprenol acetates made up about 15 mass % of the extractables and consisted of P_{12} (0.1%), P_{13} (0.9), P_{14} (2.9), P_{15} (5.3), P_{16} (3.8), P_{17} (1.4), and P_{18} (0.3). The fraction of free polyprenols consisted of about the same amount and contained P_{12} (0.2%), P_{13} (0.7), P_{14} (3.6), P_{15} (6.2), P_{16} (3.9), P_{17} (1.2), and P_{18} (0.2).

Neutral substances soluble in methanol (1.6 g, 12.7%) contained squalene, phytol, isoabienol, manoyloxide, pimarinol, epitorulosol, and β-sitosterol. They were identified by comparison with authentic samples using TLC, PMR, IR, and UV spectra. The neutral part of the extract also contained free polyprenols, the qualitative and quantitative compositions of which were practically the same as those above, and a mixture of serratene triterpenoids.

Thus, we investigated the composition of ether-extractable substances from needles of Siberian fir *P. obovata* Ledeb. A significant amount of polyprenols both in the free and esterified form was detected in the extract. Their content reached 1.6 mass % of the air-dried needles (>30 mass % of the extract). Neutral triterpenoids, including serratene derivatives that are usual for the genus, made a significant contribution. The free acids included those oxidized at the 7- and 15-position of DAB. Their content was 26.9 mass % of the free acids (0.44 mass % of the raw material).

REFERENCES

1. V. I. Yagodin, *Principles of the Chemistry and Technology of Evergreen Needle Processing* [in Russian], Izd. Leningr. Gos. Univ., Leningrad (1981).
2. *Chemical Composition of Evergreen Needles, Methodological Principles* [in Russian], Zinatne, Riga (1983).
3. E. D. Levin and S. M. Repyakh, *Evergreen Needle Processing* [in Russian], Lesnaya Promyshlennost′, Moscow (1986).
4. V. I. Roshchin, R. A. Baranova, and V. N. Solov′ev, *Khim. Prir. Soedin.*, 168 (1986).
5. V. I. Roshchin, Doctoral Dissertation in Chemical Sciences, Forestry Academy, St. Petersburg (1995).
6. A. A. Koroleva, L. P. Karmanova, and A V. Kuchin, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, **3**, 97 (2005).
7. A. V. Kuchin, L. P. Karmanova, and A. A. Koroleva, *Khim. Interesakh Ustoich. Rasvit.*, **5**, 7 (1997).
8. E. G. Bobrov, *Evergreen Forests of the USSR* [in Russian], Nauka, Leningrad (1978).
9. G. F. Chernenko, E. E. Ivanova, L. I. Demenkova, and E. N. Shmidt, *Khim. Prir. Soedin.*, 760 (1993).
10. G. F. Chernenko, E. E. Ivanova, L. I. Demenkova, and E. N. Shmidt, *Khim. Prir. Soedin.*, 608 (1996).
11. L. M. Pokrovsky and A. V. Tkachev, *ISNPAS-98 Book of Abstracts*, Nov. 30-Dec. 6, Novosibirsk (1998), p. 136.