TERPENOIDS OF *Pinus funebris*

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The complete chemical composition of the oleoresin of the Chinese pine growing in the Far East has been studied. It has been shown that the main components of the monoterpenes are α-pinene, β-phellandrene, and α-terpineol. The sesquiterpenes contain longifolene and compounds related to it. The oleoresin of the Chinese pine is distinguished by a high content of diterpene cembrane compounds. Among the resin acids, dehydroabietic acid predominates (42.4%).

The Chinese pine *Pinus funebris* Kom. [*P. tabulaeformis*] is widespread on the territory of the USSR in the low mountain areas of the southern Maritime Territory and has been described as a hybrid of the Scotch pine (*Pinus sylvestris*) and the Japanese red pine (*Pinus densiflora*) [1]. The composition of the oleoresin of the Scotch pine has been studied fairly completely [2-4]. There is no detailed information on the chemical composition of the resin of the Japanese red pine in the literature. Information has been found on the sesquiterpenes of the oleoresin [5] and on the composition of the terpentine of the essential oil (*Pinus densiflora*) [6]. It was of interest to compare the set of terpenoids of the oleoresin of the Chinese pine with that of the oleoresin of the "parent" species. In the present paper we consider the results of a study of the total chemical composition of the oleoresin of the Chinese pine.

After treatment of the oleoresin with an aqueous solution of caustic soda, neutral and acid components were obtained. The subsequent chromatography of the neutral fraction on alumina yielded hydrocarbons and oxygen-containing compounds, and mono-, sesqui-, and diterpenoids were then obtained by fractional distillation. Each fraction was investigated separately.

The composition of the monoterpene hydrocarbons was studied by the GLC method. We identified camphene, α- and β-pinenes, 3-carene, myrcene, limonene, β-phellandrene, and γ-terpinene, the main components of the fraction being α-pinene (50%) and β-phellandrene (25%).

From the fraction of oxygen-containing monoterpenoids we isolated by chromatography and identified from their PMR spectra α-terpenyl acetate, bornyl acetate, α-terpineol, terpineol-4, sabinene hydrate, and methyleugenol and its isomer 1-allyl-2,4-dimethoxybenzene. The structure of the last-mentioned compound was established by comparing its IR and ^{1}H and ^{13}C NMR spectra with those of methyleugenol and 1,2,3-substituted benzenes. The main components of this fraction of the oleoresin were methyleugenol and α-terpineol.

The sesquiterpene hydrocarbons of the oleoresin of the Chinese pine consisted mainly of compounds of the longifolane series — longifolene, α-longipinene, and longicyclene, the total amount of which made up 83% of this fraction. In addition to these, caryophyllene and α-humulene were identified.

From the oxygen-containing sesquiterpenoids we isolated cubebol, epicubebol, caryophyllene α-oxide, and the main component of this fraction — longiborneol.

The neutral diterpenoids amounted to 10% of the initial oleoresin and consisted of hydrocarbons, aldehydes, alcohols, and esters.

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The main diterpene hydrocarbons were pimaradiene, isopimaradiene, and dehydrobietane. The oxygen-containing diterpenoid fraction included 20% of aldehydes and methyl esters of resin acids in a ratio of 5:1 according to PMR. The main components of this fraction were pimarinal, isopimarinal, and methyl dehydroabietate, which were identified from their spectral characteristics and by direct comparison (TLC) with authentic samples.

Diterpene alcohols were represented by isocembrol and 4-epi-isocembrol, the amount of which made up 50% of all the oxygen-containing diterpene compounds. In addition to these alcohols, pimarinol and isopimarinol (∿20%) were found.

The most polar compounds contained hydroxy derivatives of methyl esters of resin acids (∿1%) — methyl 15-hydroxydehydroabietate and methyl 15-hydroxyabietate, the latter being the predominating component in this fraction.

The composition of the acid fraction of the oleoresin (65% of the oleoresin) was investigated in the form of methyl esters of resin acids by the GLC method. In the usual set of acids for conifer oleoresins (abietic, dehydroabietic, neoabietic, levopimaric/palustric, pimaric, and isopimaric), dehydroabietic acid (42%) and abietic acid (25%) predominated.

The information that we obtained on the composition of the terpenoids of the oleoresin of the Chinese pine showed that it differed markedly from the other *Pinus* species of the *Diploxylon* subgenus in its content of the macrocyclic cembrane diterpenoids that are characteristic for the oleoresin of the pines of the *Haploxylon* subgenus [7].

Considerable amounts of the monoterpene β-phellandrene also form a distinguishing feature of this species of pine; β-phellandrene is more frequently found as the main component in the oleoresin of firs and spruces [8].

The main components found in the oleoresin of the Scotch pine are α-terpineol, methyleugenol, pimarinal, pimarinol, isopimarinol, longifolene, and sesquiterpenoids of the cadalene series [3, 4]. No bicyclic cadalene sesquiterpenes were found in the oleoresins of the Chinese and the Japanese red pines [5], which again shows the closeness of these species.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in CCl_4 solution and in KBr tablets, and NMR spectra on Varian A-56/60A instruments for solutions of the substances in CCl_4 and $CDCl_3$, with HMDS as the internal standard, its chemical shift being taken as $\delta = 0.05$ ppm. Specific rotations were determined for solutions in chloroform. The oleoresin of the Chinese pine was collected in the Arsen'ev region of the Maritime Territory in the summer of 1979.

Treatment of the Oleoresin. The oleoresin (600 g) was treated with 2% aqueous caustic soda, and the neutral compounds were separated by extraction with diethyl ether. After the solvent had been distilled off, 166 g of neutral substances were obtained. The aqueous solution of sodium salts yielded 392 g of resin acids. The neutral compounds were chromatographed on Al_2O_3 (activity grade II, 2 kg). Petroleum ether eluted 112 g of hydrocarbons, and ethanol eluted 53 g of oxygen-containing terpenoids. Fractional vacuum distillation of the hydrocarbons gave 73 g of monoterpenes (bp 70-100°C/10 mm Hg), 30 g of sesquiterpenes (bp 80-120°C/1 mm Hg), and 9 g of diterpenes (still residue). When the oxygen-containing compounds (96 g) were distilled, a fraction of mono- and sesquiterpenes was obtained (5.3 g, bp 100-140°C/0.1 mm Hg), and the still residue contained diterpenoids.

The Monoterpene Hydrocarbons. The qualitative and quantitative analysis of the monoterpenes was carried out by the GLC method on a Chrom-41 instrument. For analysis we used a 3.75 m × 3 mm column with the stationary phase TCEP (10%) on Chromosorb W at a column temperature of 75°C with a rate of flow of carrier gas (nitrogen) of 30 ml/min. The following were identified by means of their relative retention times: α-pinene (50.1%), camphene (0.9%), β-pinene (17.5%), 3-carene (traces), myrcene (2.3%), limonene (2.9%), β-phellandrene (25%), and γ-terpinene (0.8%).

The Sesquiterpene Hydrocarbons. For the analysis of the sesquiterpene hydrocarbons we used a capillary column, 50 m × 0.2 mm with the stationary phase OV-225, the temperature of the column being programmed from 120 to 170°C at the rate of 1°C per minute, with a rate of flow of the carrier gas (nitrogen) of 5 ml/min. The following were identified with the aid of the method of additives: longifolene (78.6%), α-longipinene (2.5%), longicyclene (2.2%), caryophyllene (11.5%), and α-humulene (1.5%).

The Oxygen-Containing Mono- and Sesquiterpenoids. The oxidized mono- and sesquiterpenoids (5 g) were chromatographed on silica gel (70 g, 0.07-0.10 mm). Petroleum ether with increasing amounts of diethyl ether eluted 8 fractions (I-VIII).

The rechromatography of fraction I (0.2 g) on SiO_2 + 20% of $AgNO_3$ gave 1-allyl-2,4-dimethoxybenzene (0.12 g). IR spectrum, cm^{-1}: 913, 1042, 1160, 1210, 1265, 1290, 1366, 1510, 1614, 1640, 1727, 3080. ^1H PMR spectrum, ppm: 3.20 (2 H, doublet, J = 5.5 Hz); 3.58 (3 H, singlet); 3.62 (3 H, singlet); 4.79 (1 H, multiplet); 5.00 (1 H, multiplet); 5.54-6.20 (1 H, multiplet); 6.25 (1 H, doublet of doublets, J = 8.8 Hz, J = 2.5 Hz); 6.87 (1 H, doublet, J = 8.8 Hz); 6.28 (1 H, doublet, J = 2.5 Hz). ^{13}C NMR, ppm: singlets at 159.2, 157.9, and 120.7 ppm, doublets at 137.2, 129.7, 103.8, and 98.3; and triplets at 114.7 and 33.7, with a double-intensity quartet at 55.0 ppm.

According to PMR spectroscopy, fraction II (0.04 g) consisted of a mixture of α-terpenyl acetate and bornyl acetate in a ratio 2:1; fraction (III) (0.06 g) of α-caryophyllene oxide; fraction V (0.3 g) of a mixture of cubebol and epicubebol in a ratio of 1:1; fraction VI (0.6 g) of α-terpineol; and fraction VII (0.50 g) of sabinene hydrate.

The chromatography of fraction IV (2.3 g) on SiO_2 + 20% of $AgNO_3$ (60 g) led to the isolation of longiborneol (0.2 g), terpineol-4 (0.07 g), methyleugenol (1.1 g), and nerolidol (0.05 g). The longiborneol had mp 110-110.5°C (petroleum ether—diethyl ether (1:1)); ^1H NMR (ppm) 0.82 (6 H, singlet); 0.92 (6 H, singlet); 3.66 (1 H, doublet of doublets, J = 5 Hz, J = 2 Hz); ^{13}C NMR (ppm): singlets at 51.2, 50.1, and 33.4; doublets at 79.4, 64.6 and 34.0; triplets at 41.0, 35.1, 30.3, 26.4, and 22.5; and quartets at 29.2, 28.9, 22.5, and 13.0.

Fraction VIII (0.12 g) consisted of a mixture of polar compounds and we did not investigate it.

The Diterpene Hydrocarbons. The still residue obtained on the vacuum distillation of the diterpene hydrocarbons was analyzed by the GLC method on a 2.5 m × 3 mm column with the stationary phase 5% of SE-30 on Chromaton NAW at a column temperature of 162°C and an evaporator temprature of 200°C with a rate of flow of carrier gas (nitrogen) of 30 ml/min. By the method of adding authentic samples, pimaradiene, (40.6%), isopimaradiene (14.0%), dehydroabietane (11.8%), and cembrene (8.5%) were identified.

Chromatography of the hydrocarbon fraction (1 g) on SiO_2 + 5% of $AgNO_3$ led to the isolation of pimaradiene (0.09 g) with n_D^{20} 1.5235, $[\alpha]_D^{20}$ +50.7° (c 2.9), isopimaradiene (0.05 g) with n_D^{20} 1.5217, $[\alpha]_D^{20}$ −35.3° (c 5.0) and 0.06 g of a fraction containing dehydroabietane (according to PMR and GLC).

The Oxygen-Containing Diterpenoids. The mixture of oxygen-containing diterpenoids (10 g) was chromatographed by the method of rapid chromatography [9] on SiO_2. Petroleum ether with the addition of 5% of diethyl ether eluted a fraction (2 g) containing aldehydes and methyl esters. Rechromatography on SiO_2 + 5% $AgNO_3$ (30 g) yielded pimarinal (1 g) with mp 62-64°C (according to the literature [10]: mp 52-54°C), isopimarinal (0.35 g) with $[\alpha]_D^{20}$ −29° (c 5.8), and methyl dehydroabietate (0.1 g), which was identical with an authentic sample according to TLC, GLC, and spectral characteristics. Petroleum ether—diethyl ether (95:15) yielded a fraction (5 g) containing two components, according to TLC (SiO_2 + $AgNO_3$). Additional chromatography on SiO_2 + 10% of $AgNO_3$ yielded isocembrol (4 g) with n_D^{20} 1.5025, $[\alpha]_D^{20}$ +60.7° (c 2.8) [11] and 4-epi-isocembrol (0.5 g) with n_D^{20} 1.5007, $[\alpha]_D^{20}$ +94° (c 3.2), which was identified by a comparison of spectral characteristics with an authentic sample [12].

Petroleum ether—diethyl ether (75:25) yielded a fraction (2 g) of primary alcohols. Rechromatography (SiO_2) led to the elution of pure pimarinol (1.4 g with n_D^{20} 1.5259, $[\alpha]_D^{20}$ +60.5° (c 4.5) and isopimarinol (0.5 g) with mp 86-87°C, n_D^{20} 1.5250, $[\alpha]_D^{20}$ −40.1° (c 4.2) (according to the literature [10]: mp 85-86°C, $[\alpha]_D^{20}$ −24.6° (c 1.8)).

Diethyl ether eluted a fraction (1 g) of hydroxy esters. Additional purification of the mixture of SiO_2 (25 g) yielded methyl 15-hydroxyabietate with n_D^{20} 1.5189, $[\alpha]_D^{20}$ −44°, (c 3.6) and methyl 15-hydroxydehydroabietate with mp 82-84°C (according to the literature [13]: mp 82-83°C).

The Acids of the Oleoresin of the Chinese Pine. The resin acids of the oleoresin (5 g) were treated by a method described previously [14] and gave 4.6 g of methyl esters. Analysis of the combined methyl esters was carried out by the GLC method on 5% of XE-60 on Chromaton (column 2.5 m × 3 mm; column temperature 214°C; rate of flow of carrier gas (nitrogen) 25 ml/min). Identification was carried out from retention times and by the method of adding individual compounds. The acid fraction of the oleoresin contained abietic (24.7), levopimaric/palustric (13.8%), isopimaric (11.3%), dehydrobietic (42.4%), pimaric (5.7%), and neoabietic (2%) acids.

SUMMARY

1. The complete chemical composition of the oleoresin of *Pinus funebris* Kom., growing in the Far East has been studied.

2. It has been shown that the main components of the monoterpenes are α-pinene, β-phellandrene, α-terpineol, and methyleugenol. The sesquiterpenes are represented by longifolene and compounds related to it.

3. The composition of the diterpenoids differs from those of the oleoresins of species of pine of the *Diploxylon* subgenus studied previously by their high content of cembrane compounds.

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