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GAS-CHROMATOGRAPHIC DETERMINATION OF THE COMPOSITION OF THE VOLATILE COMPONENTS OF THE OLEORESINS OF SOME SPECIES OF CONIFERS

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Using the method of analytical GLC in a glass capillary column, the compositions have been studied of the volatile fractions of the oleoresins of five species of conifers: Larix decidua, Pinus strobus, Pinus mugo, Picea excelsa, and Pinus abies. In the oleoresins 21 monoterpene and 22 sesquiterpene compounds were identified from their relative retention times and with the aid of additives. The main components of the volatile fractions of the oleoresins were α- and β-pinenes and 3-carene among the monoterpenes, and longifolene caryophyllene, δ- and γ-cadinenes and α-murolene among the sesquiterpene compounds. The quantitative analysis of the fractions was carried out by the method of internal normalization from averaged correlation coefficients.

Continuing an investigation of the chemical compositions of the oleoresins of conifers growing in the USSR, we have determined the compositions of the volatile components of the oleoresins of the European larch (Larix decidua Mill.), the Swiss mountain pine (Pinus mugo Turra); the easterm white pine (Pinus strobus L.), and the Norway [Evropeiskaya] spruce (Picea abies Karst.), the areas of which are located in Transcarpathia, and also the Norway [Obyknovennaya] spruce (Picea excelsa Link.) growing in the cis-Urals region. The group compositions and also the compositions of the acid fractions of the oleoresins of these species have been published previously [1].

According to the results obtained, the percentage amounts of the volatile components in the oleoresins studied were approximately the same (14.8-17.4%):

Conifer species	Monoterpenes	High-boiling fraction
Larix decidua	13.4	1.4
Pinus strobus	15.9	0.3
Pinus mugo	16.2	1.2
Picea abies	12.6	3.0
Picea exelsa	15.8	0.6

The high relative content of monoterpene hydrocarbons did not permit the calculation of the quantitative amounts of the other components of the volatile fraction, since the capillary columns used for the analysis possessed a relatively small dynamic range and were very sensitive to overloading. The volatile substances of the oleoresins were therefore first separated into two fractions – monoterpene hydrocarbons, the compositions of which are given below, and high-boiling compounds (Table 1). The quantitative compositions of the fractions of monoterpene hydrocarbons were determined by the method of simple normalization in light of the approximately equal sensitivity of the flame-ionization detector (FID) to the compounds listed below, except for p-cymene (%):

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TABLE 1. Compositions of the High-Boiling Oleoresin Fractions, %

Component	Larix decidua	Pinus mugo	Pinus strobus	Picea abies	Picea exelsa	Correlation coefficient
1. 1,8-Cineole	Tr.	Tr.	Tr.	Tr.	Tr.	0,9
2. Sabinene hydrate	1,4	Tr.	Tr.	Tr.	Tr.	0,9
3. Linalool	1,3	Tr.	Tr.	0,1	Tr.	0,9
4. Borneol	Tr.	Tr.	Tr.	0,1	Tr.	1,0
5. Terpineol-4	Tr.	Tr.	Tr.	Tr.	Tr.	0,9
6. α-Terpineol	Tr.	Tr.	Tr.	0,5	0,2	1,0
7. O-Methylthymol	Tr.	Tr.	Tr.	—	Tr.	1,1
8. Bornyl acetate	Tr.	—	—	0,5	2,1	1,3
9. Linalyl acetate	—	0,2	—	—	—	1,2
10. α-Terpenyl acetate	1,6	0,5	—	2,8	Tr.	1,2
11. α-Longipinene	0,2	—	—	0,6	24,6	1,0
12. Cyclosativene	—	—	—	0,2	0,2	—
13. Copaene	Tr.	6,8	23,2	0,8	0,2	—
14. Longicyclen	Tr.	—	—	0,1	0,4	—
15. Sibirene	—	—	—	8,1	1,6	1,0
16. Longifolene	0,8	—	—	4,1	42,6	1,0
17. Caryophyllen	6,3	22,1	70,2	0,4	2,5	1,0
18. β-Farnesene	0,8	0,2	Tr.	0,9	0,4	—
19. α-Humulene	1,1	0,5	0,6	2,7	0,9	1,0
20. ε-Muurolene	1,3	0,5	—	—	—	—
21. γ-Muurolene	3,2	3,6	0,3	4,3	2,0	1,0
22. α-Muurolene	16,3	37,7	4,2	2,0	0,2	1,0
23. β-Selinene	—	—	—	3,1	0,2	—
24. γ-Cadinene	4,9	8,6	Tr.	20,7	16,7	1,0
25. δ-Cadinene	9,3	16,4	0,6	46,4	4,2	1,0
26. α-Cadinene	22,4	0,3	—	Tr.	—	—
27. Calamenene	2,3	0,5	—	Tr.	—	—
28. Caryophyllene oxide	—	—	Tr.	—	—	—
29. Cubebol	—	Tr.	Tr.	Tr.	Tr.	—
30. δ-Cadinol	—	Tr.	Tr.	Tr.	—	1,0
31. α-Cadinol	—	—	—	Tr.	Tr.	—
Unidentified	23,8	1,9	0,8	1,5	0,9	

Tr) The amount of the component in the fraction did not exceed 0.1%.

Component	Larix decidua	Pinus mugo	Pinus strobus	Picea abies	Picea exelsa
1. Tricylene	Tr.	—	—	Tr.	Tr.
2. α-Pinene	89,1	15,4	70.7	58,8	61,4
3. β-Pinene	4,0	4,1	24,2	34,9	29,1
4. Camphene	1,0	Tr.	1,1	0,9	0,4
5. β-Myrcene	1,0	Tr.	Tr.	0,9	1,0
6. Sabinene	—	Tr.	Tr.	—	0,9
7. 3-Carene	2,1	65,2	3,0	0,8	2,3
8. β-Phellandrene	0,6	2,8	0,2	2,2	2,8
9. Limonene	2,0	8,0	0,7	1,5	1,1
10. Terpinolene	Tr.	Tr.	Tr.	Tr.	—
11. p-Cymene	Tr.	0,6	Tr.	Tr.	Tr.

The amounts of the components of the high-boiling fractions were calculated by the method of internal normalization, since their compositions differed. At a sufficiently high temperature of the evaporator (260°C), it was possible practically to eliminate the fall in sensitivity towards the higher-boiling compounds. However, for different groups of compounds the sensitivity of the detector differed, and for the acetates of terpene alcohols it was substantially lower (Table 1). In order to calculate the quantitative amounts, averaged correlation coefficients were taken; for the monoterpene alcohols 0.9; for the sesquiterpene hydrocarbons, 1.0; and for the acetates of monoterpene alcohols 1.2. A change in the conditions of chromatography (temperature of the analysis, rate of flow of the carrier gas) scarcely changed the correlation coefficients, although it affected the overall sensitivity of the FID and also the order of emergence of individual components. Thus, it was found that at an analytical temperature above 160°C δ-cadinene had a shorter retention time than γ-cadinene, while at 150°C their retention times practically coincided, and with a further lowering of the temperature of analysis the order of emergence of these terpenoids changed. This fact indicates that on the use of literature information [2] for identifying terpene compounds a careful comparison of all the conditions of chromatographic analysis is necessary.

Among the monoterpenes of the oleoresins studied, in all the species apart from the Swiss mountain pine, α-pinene predominated. In the oleoresin of the Swiss mountain pine, as reported previously [3], 3-carene predominated.

The amount of oxygen-containing monoterpenoids was extremely low. Bornyl acetate and α-terpenyl acetate were found in appreciable amounts only in the spruce oleoresins, and sabinene hydrate and linalool only in the larch oleoresin (Table 1), but in these species, as well, the total amount of oxygen-containing monoterpenoids did not exceed 5% of the high-boiling fraction.

Among the sesquiterpene hydrocarbons forming the bulk of these fractions longifolene predominated in the case of Picea excelsa, caryophyllene in the case of Pinus strobus, and compounds of the cadalene series in the cases of Pinus mugo, Larix decidua, and Picea abies. The absence of sesquiterpenes of the longifolane group (longifolene, α-longipinene, and longicyclene) in the pine species studied must be mentioned. The composition of the sesquiterpenes of Pinus strobus, containing practically only two components (caryophyllene and α-copaene) has no analogs among the oleoresins of this genus. From the composition of the sesquiterpene compounds, the oleoresin of the Picea abies was close to that of Far-Eastern species of spruce [4] in which, likewise, δ- and γ-cadinenes predominate, while that of Picea excelsa was similar to Siberian spruce, which has a similar area [5]. For the European larch a great difference was observed between the amount of high-boiling components in the oleoresin (Table 1) and in the essential oil of the shoots [6], where bornyl acetate, caryophyllene, and α-humulene have been found as the predominating compounds.

EXPERIMENTAL

The site and time of collection of the oleoresins have been given in communication [1]. The oleoresins were collected by the method of open blazes from 20-25 trees. A sample (50 g) of each oleoresin was dissolved in 100 ml of diethyl ether and the neutral fraction was separated off in the usual way. From this fraction monoterpenes (60-90°C/10 mm Hg) and high-boiling compounds (90-160°C/3 mm Hg) were obtained by fractional vacuum distillation.

Chromatographic analysis was performed on a Chrom-41 instrument (Czechoslovakia). Glass capillary column 49 m long with an internal diameter of 0.3 mm, the stationary phase (polymethylsiloxane VS-30) being deposited on the internal surface of the column that had previously been silanated with hexamethyldisilazane, by the high-pressure static method from a 0.15% solution in hexane. The efficiency of the column was 3000 theoretical plates/m.

The temperature for the analysis of the monoterpenes was 60°C and the rate of flow of the carrier gas (nitrogen) 30 ml/min.

The analysis of the high-boiling fractions was carried out at 70-170°C with a rate of programming of 4°C per minute. The components were identified from their relative retention times and with the aid of the method of additives. The correlation coefficients for quantitative analysis were determined from model mixtures of individual terpenoids isolated chromatographically from the neutral fractions of the oleoresins of Pinus sylvestris, Larix gmelinii, Picea glehnii, and Abies sibirica.

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