

Preparation of the Rosavin Solution. About 0.05 g (accurately weighed) of rosavin was dissolved in a 25-ml measuring flask in 60% ethanol, the volume of the solution was made up to the mark with the same ethanol, and the contents were mixed (solution 1).

Solution 1 (1 ml) was pipetted into the column of silica gel and then the same operations were performed with the plant extract.

Preparation of Samples for HPLC. For HPLC analysis, an extract (1:20 in 60% ethanol) was diluted with water in a ratio of 1:4, and 25 μ l was injected into the chromatograph. The eluates from the silica gel column were evaporated to dryness and the residues were dissolved in the HPLC eluent. All the samples were analyzed with the deduction of the peaks of impurities of the corresponding eluates from the control column.

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TERPENOIDS OF THE OLEORESIN OF THE SCHRENK SPRUCE

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UDC 547.595.9

Using the scheme for the group separation of the oleoresins of conifers, the quantitative and qualitative composition of the oleoresin of the Schrenk pine *Picea schrenkiana* Fish. et May has been investigated. Thirty-eight diterpenoids have been identified, one of which (palustradiene) has been detected in conifers for the first time.

The Schrenk spruce *Picea schrenkiana* Fish et May, which belongs to the *Morinda* botanical section is distributed in the Tien-shan mountains [1]. There is little literature information on the chemical composition of its oleoresin — results have been published of an investigation of the volatile components of the neutral fraction, which consists of monoterpenoids and one sesquiterpene (bisabolene [2]) and also of the fractions of the polar components of the total acids, which proved to be the acid succinates of diterpene alcohols [3].

In order to obtain information on the quantitative and qualitative chemical compositions of the whole oleoresin, we have analyzed it by the scheme for the group separation of conifer oleoresins [4]. The results for the neutral fraction, amounting to 41.4% of the oleoresin, are given in Table 1.

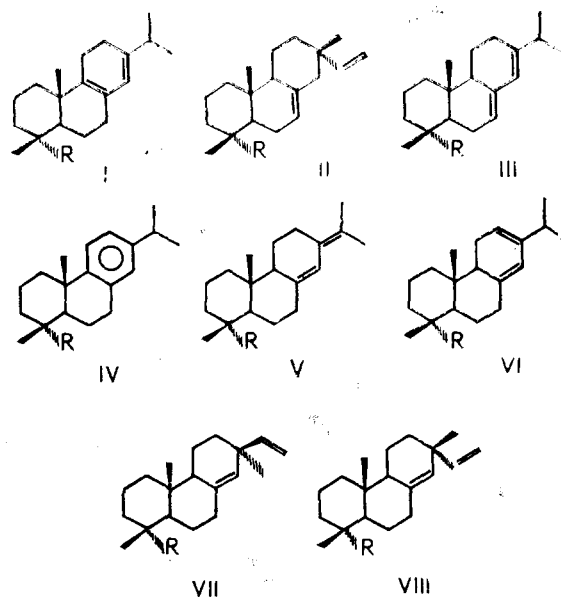
According to GLC, the diterpenes were represented by at least nine compounds, the main ones being palustradiene, isopimeradiene, abietadiene, and dehydroabietane (I-IV: R = CH₃; amounts 1, 7, 0.9, 0.7, and 0.7%, respectively, on the sum of all the hydrocarbons of the oleoresin). After the elimination of the monoterpenes and bisabolene by vacuum distillation, the residue was separated by chromatography on silica gel impregnated with 5% of silver

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TABLE 1. Amounts and Main Components of the Groups of Neutral Components of the Oleoresin of the Schrenk Spruce, %

Group	Amount in the neutral fraction	Main components of the group and their amounts in the group
Hydrocarbons	32,0	Monoterpenes (95.1) Sesquiterpenes (0.6) Diterpenes (4.3)
Aldehydes, methyl esters, oxides	35,4	Diterpene aldehydes (63), methyl esters of diterpene acids (31.5), and oxides (5.5)
Nonpolar tertiary alcohols	9,8	cis-Abienol (83)
Acetyltable monohydric alcohols	13,2	Isocembrol (17) Diterpene alcohols (~95)
Nonacetyltable tertiary alcohols	4,5	Mono- and sesquiterpenoids
Polyfunctional compounds	4,2	Methyl esters of 15-hydroxyabietic and 15-hydroxydehydroabietic

nitrate. This led to the successive isolation of dehydroabietane, a mixture of dehydroabietane and abietadiene, palustradiene, and a mixture of hydrocarbons containing isopimaradiene. Palustradiene has not previously been known as a component of the oleoresins of conifers of the family *Pinaceae* and was identified on the basis of its spectral characteristics and conversion into the known abietadiene under the action of HCl in benzene.



The fractions of aldehydes, acetyltable alcohols, and methyl esters was represented mainly by monotypical compounds with the general formulas (I-VIII) (Table 2). Acids of the same structural types formed the acid fraction of the oleoresin that had been freed from polar acids [3]. The qualitative and quantitative compositions of the mixtures of native methyl esters of diterpene acids and the methylated acid fraction of the oleoresin (without the methyl esters of polar acids, which amounted to 5% of all the acids of the oleoresin [3]) were determined by the GLC method, the identification being confirmed by the use of PMR spectroscopy. The chemical composition of the fraction of acetyltable alcohols (in the form of acetates) and the aldehydes (in the form of the corresponding alcohols) was established from their PMR spectra (400 MHz) using handbook information [5] and the spectra of authentic samples of pimarinol, isopimarinol, and sandaracopimarinol ((VII), (II), and (VIII)), respectively, $R = CH_2OH$.

The aldehydes were represented by only eight compounds of known types, while the primary acetyltable alcohols were more numerous. The mixture of the latter contained about 9% of other compounds including the known [6] labd-8(17), 13E-dien-15-ol (about 8%) and isopimara-8,15-dien-18-ol (traces).

TABLE 2. Chemical Compositions of the Groups of Acetyltable Alcohols, Aldehydes, Free Resin Acids, and Native Methyl Esters of the Oleoresin of the Schrenk Spruce, %

Compound	R			
	CH ₂ OH	CHO	COOH	COOCH ₃
I	25	51	16,1	16,0
II	9	7	29,0	16,0
III	12	6	10,7	4,5
IV	11	24	13,8	45,4
V	22	2	7,1	1,3
VI	5	4	16,1	4,5
VII	5	5	2,3	1,4
VIII	2	1	4,9	—*

*The remaining 10.9% is due to manoyl oxide (2.5%) and 13-epimanoyl oxide (8.4%).

On considering the results obtained (Table 2) it can be observed that on passing from hydrocarbons and alcohols to methyl esters appreciable quantitative changes take place between the groups — the predominance of palustradiene derivatives is replaced by a predominance of methyl dehydroabietate.

The identification of two oxides in the oleoresin — manoyl oxide and 13-epimanoyl oxide — was made by GLC using authentic samples and was confirmed by the presence of their signals in the PMR spectrum of the mixture of methyl esters and oxides.

The fraction of nonpolar tertiary alcohols was represented by two known compounds — cis-abienol and isocembrol (5:1 according to the NMR spectrum), and these were separated by chromatography on silica gel impregnated with 5% of silver nitrate. They have also been found in the oleoresin of the Yeddo spruce [7] but here, as also in the Yeddo spruce, there was no 4-epiisocembrol — a typical component of the oleoresin of pines belonging to the *Haploxylon* subgenus [6, 8]. This permitted the assumption of the stereospecificity of the biosynthesis of isocembrol in some *Picea* species.

The small fraction of nonacetyltable tertiary alcohols contained monoterpenoids described previously [2] and also other compounds, among which there were two so far unidentified tertiary sesquiterpene alcohols. The fraction of polyfunctional compounds consisted to the extent of ~95% of two known abietane hydroxy esters (Table 1) — normal components of conifer oleoresins [9]. They were identified in the mixture by comparison with an authentic sample of the same mixture by TLC and PMR spectroscopy (400 MHz).

On considering the composition of the diterpenoids of the oleoresin of the Schrenk spruce that has been found, it can be observed that it is typical for conifer oleoresins. This spruce differs from close species — Norway spruce and Siberian spruce — by the absence of geranylinalool [10]. Components specific for this species of spruce are possibly present in the microcomponents of the fraction of tertiary nonacetyltable alcohols, the investigation of which is continuing.

EXPERIMENTAL

PMR spectra were recorded on a Bruker AM-400 instrument (400.13 MHz) for solutions in CDCl₃ (with CHCl₃ as internal standard, the signal of which was taken as 7.24 ppm, δ scale). The PMR spectrum of palustradiene was recorded on a Varian A56/60A instrument (60 MHz) for a solution in CCl₄ (TMS). IR and UV spectra were obtained on Specord UV-VIS and UR-20 instruments, respectively. Angles of optical rotation were measured on a Zeiss polarimeter for solutions in CHCl₃.

GLC was conducted on a Chrom-5 instrument (Czechoslovakia) with a 3 mm \times 2 m glass column containing 5% of SE-30 on Chromaton N-Super (0.10-0.16 mm) at temperatures of 85-260°C/6°C for hydrocarbons and 190°C (for methyl esters and oxides) or 9% of DEGS on Chromaton-AW-DMCS (0.20-0.25 mm) at 200°C (for methyl esters). The identification of the compounds from the PMR spectra of the mixtures and their quantitative determination were carried out from the characteristic signals of the olefinic protons in comparison with the spectra of the authentic samples and atlas information [5]. The oleoresin was gathered in 1980 in the Aflatun gorge (Western Tien-Shan).

The separation of the oleoresin into groups of compounds was carried out by a procedure analogous to that described in [4]. From 8.43 g of oleoresin were obtained 3.49 g of neutral and 4.75 g of acid fractions. The latter was methylated with diazomethane, and by chromatography on silica gel 4.52 g of methyl esters (I-VII, R = COOCH₃) and 0.23 g of methyl esters of polar resin acids were isolated.

The neutral fraction yielded 1.12 g of hydrocarbons, 1.23 g of combination of aldehydes, methyl esters, and oxides (later, 0.67 g of alcohols from the aldehydes and 0.52 g of a mixture of native methyl esters and oxides were obtained from it by the method of [4]), 0.35 g of a fraction of nonpolar tertiary alcohols (a mixture of cis-abienol and isocembrol), 0.46 g of acetates of monohydric alcohols, 0.16 g of nonacetyltable tertiary alcohols, and 0.15 g of a fraction of polyfunctional compounds.

Separation of the Hydrocarbons. The monoterpenoids and bisabolene were distilled off under reduced pressure (up to 140°C at 3-5 mm Hg) from the total hydrocarbons (50.0 g) isolated from 376 g of the oleoresin. The still residue (2.35 g) was chromatographed on 70 g of SiO₂ + 5% of AgNO₃. Petroleum ether with the addition of 1, 1.5, and 2% of diethyl ether eluted successively dehydroabietane (0.25 g), a mixture of dehydroabietane and abietadiene (~1:1, PMR spectrum) (0.50 g), and palustradiene (0.80 g). Petroleum ether-diethyl ether (95:5) eluted a fraction (0.90 g) the main component of which was isopimaradiene (TLC, PMR spectrum).

Palustradiene (I, R = CH₃). Colorless oil with n_D^{22} 1.5250 and $[\alpha]_D^{21}$ - 68° (c 5.0); UV spectrum (in heptane): λ_{\max} 267 nm (log ϵ 3.92). PMR spectrum, ppm: 0.85, 0.88, and 0.95 (3 H each, singlets, tertiary Me groups); 0.95 (3 H, d, J = 7 Hz, -CH(CH₃)₂); and 5.32 ppm (1 H, narrow m, W_{1/2} = 3 Hz, H-14). RRTs in GLC (at 180°C): abietadiene - 1.13; dehydroabietadiene - 1.00; palustradiene - 0.90.

Gaseous HCl was passed through a solution of 0.05 g of palustradiene in 10 ml of benzene at 0°C for 20 min after which 20 ml of a saturated solution of sodium bicarbonate was added and the mixture was stirred. The benzene layer was separated off and evaporated to dryness. Chromatography of the residue on 10 g of SiO₂ (with petroleum ether as eluent) yielded 0.04 g of abietadiene having $[\alpha]_D^{20}$ - 78° (c 4.0), identical with an authentic sample according to UV and PMR spectroscopy and TLC.

Isocembrol and cis-Abienol. The chromatography of 0.35 g of the fraction of nonpolar tertiary alcohols on 10 g of SiO₂ + 5% of AgNO₃ yielded successively 0.23 g of cis-abienol and 0.04 g of isocembrol (the eluents being petroleum ether containing 25 and 40% of diethyl ether, respectively); their physicochemical constants ($[\alpha]_D$ for both substances and the melting point for cis-abienol) and their IR, UV, and PMR spectra corresponded to those for authentic specimens.

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