NEUTRAL DITERPENOIDS OF OLEORESINS OF FIVE SPECIES OF CONIFERS OF TRANSCARPATHIA

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UDC 547.914

The compositions of the neutral diterpenoids of the oleoresins of five species of conifers growing in the Transcarpathia have been studied. It has been found that the oleoresins of Abies alba M., Larix decidua M., and Picea excelsa L. contain more than 50% of neutral diterpenoids. The group and qualitative compositions of the oxygen-containing diterpenoids have been determined. In the oleoresins of Abies alba, Picea excelsa, and Picea abies tertiary alcohols – cis-abienol and isocembrol – predominate, while in Larix decidua the main component is the hydroxy ester larixyl acetate. Primary alcohols related to the resin acids have been found in all the oleoresins investigated. Features of the distribution of diterpenoids according to the species of conifers have been revealed. The results obtained are necessary for the chemotaxonomy of conifers of the family Pinceae.

We have previously reported the composition of the resin acids of the oleoresins of conifers growing in Transcarpathia [1]. In the present paper we give the results of an investigation of the neutral diterpenoids of the oleoresins of the Swiss mountain pine (Pinus mugo T.), Eastern white pine (Pinus strobus L.), silver fir (Abies alba M.), Norway [europeiskaya] spruce (Picea abises K.), European larch (Larix decidua M.), and Norway [obyknovennaya] spruce (Picea excelsa L.).*

The separation of the oleoresins into neutral and acid components was carried out by a known procedure [1]. The neutral compounds were separated into hydrocarbons and oxygen-containing substances, and each group was investigated separately. The group compositions of the neutral diterpenoids from the oleoresins are given in Table 1.

The diterpene hydrocarbons in the oleoresins were present in insignificant amounts (see Table 1) and were represented mainly by tricyclic diterpenes, among which abietadiene, dehydroabietane, and isopimaradiene were identified. In the oleoresins of the Swiss mountain pine and the P. excelsa a monocyclic hydrocarbon – cembrene – was detected.

The oxygen-containing diterpenoids formed the bulk of the neutral high-boiling compounds. The quantitative and qualitative compositions of the aldehydes and primary alcohols of the oleoresins are given in Tables 1 and 2.

In addition to the compounds mentioned above, epimanoyl oxide and methyl dehydroabietate were found in the oleoresin of Pinus mugo. The dimethyl ether of pinosylvin and an aromatic compound with empirical formula $C_{12}H_{16}O_3$ [M^+ 208.1 (100%)] were isolated in insignificant amount from the same oleoresin. According to its IR spectrum the latter compound contained a disubstituted double bond (910, 1620 cm^{-1}), a methoxy group (1110, 1210, 1300 cm^{-1}), and a benzene ring (1490, 1510 cm^{-1}). The UV spectrum showed absorption maxima at 220 nm (ε 4000) and 260 nm (ε 2500) corresponding to a conjugated double bond and benzene ring.

In the PMR spectrum of the compound, a doublet at 1.83 ppm (3H, J = 6.5 Hz) was assigned to the signal of a methyl group present at a double bond. Singlets at 3.83 ppm (6H) and 3.84 ppm were assigned to the signals of three methoxy groups in an aromatic ring. Sig-

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*The oleoresin of the Picea excelsa was collected in Chelyabinsk province.

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 812-816, November-December, 1988. Original article submitted December 9, 1988.

TABLE 1. Compositions of the Neutral Diterpenoids of Oleoresins

Species	Amount of neutral components, %	Hydrocarbons	Oxygen-containing	Group compositions of the oxygen-containing diterpenoids, %				
		% on the neutral components		aldehydes and methyl esters, % of the oxygen-containing compounds	primary alcohols	teritary alcohols	hydroxy esters	diols
Pinus mugo	34,0	0,58*	10.7	41,0**	13,8	—	6,1	4,7
Pinus strobus	26,6	0,3	36,8	39,0	22,5	4,2	2,0	—
Abies alba	50,6	0,48	38,5	1,5***	14,0	80,0	—	—
Picea excelsa	48,5	1,5	48,8	3,5**	25,0	45,0	4,8	—
Picea abies	34,5	1.4	49,2	17,0**	7,0	50,0	10,0	—
Larix decidua	54,6	2,5*	63,5	18,0**	7,6	10,0	53,0	—

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*Sum of sesqui- and diterpene hydrocarbons.

**No methyl esters of resin acids.

***No aldehydes.

TABLE 2. Compositions of the Primary Alcohols and Aldehydes of the Oleoresins, %*

Species	Alcohols					
	primarinol	isopimarinal	dehydroabietinal	abietinal	neoabietinal	palustrol and levopimarinal
Pinus mugo	+**	—	10	15	+	5
Pinus strobus	20,3	24,6	13,0	15,0	6,7	23,1
Abies alba	31,8	16,7	21,1	3,0	—	7,3
Picea excelsa	2,9	7.6	44,0	16,4	8.4	19,5
Picea abies	11,3	—	29.5	11.6	9,9	15,2
Larix decidua	—	28.8	11.5	25,4	6,2	29,0

Species	Aldehydes						
	primarinol	isopimarinal	dehydroabietinal	abietinal	neoabietinal	palustral + levopimarinal	agathol
Pinus mugo	+	—	10.5	20,0	2,5	16.0	10,0
Pinus strobus	—	17.9	24,4	21,5	—	36 2	—
Abies alba	+	+	+	+	—	—	—
Picea excelsa	—	—	—	—	—	—	—
Picea abies	—	38,6	28,1	11,4	—	14.0	—
Larix decidua	—	30,1	17,9	17,9	6,9	27,2	—

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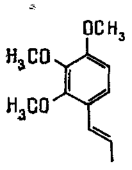
*Determined by GLC in the corresponding fractions (Table 1).

**Trace amounts of the components.

nals at 6.14 ppm (1H, m) and 6.52 ppm (1H, d, J = 16 Hz) corresponded to the olefinic protons of a disubstituted double bond. In the weak-field region of the spectrum, doublets at 7.09 ppm (1H, J = 8.7 Hz) and 6.61 ppm (1H, J = 8.7 Hz) were assigned to ortho-protons of a benzene ring.

On the basis of the facts presented, the structure of 1,2,3-trimethoxy-4-propenylbenzene (I) is proposed for the substance isolated.



I

Aromatic compounds have been found previously in the high-boiling neutral fraction of the oleoresin of the Scotch pine [2].

Together with primary alcohols, all the oleoresins contained tertiary alcohols, the amount of which varied greatly from species to species (Table 1), and in the Swiss mountain pine they were completely absent. In the oleoresin of the silver fir, practically only one alcohol — cis-abienol — was found, while in Picea excelsa and Picea abies it was detected in admixture with isocembrol in ratios of 1:2 and 3.5:1, respectively. cis-Abienol was present in insignificant amounts in the oleoresin of Pinus strobus. Only one tertiary alcohol was found in Larix decidua — epimanool.

In all the oleoresins studied apart from those of Abies alba and Larix decidua there were polyfunctional tricyclic hydroxy esters: methyl 15-hydroxydehydroabietate and methyl 15-hydroxyabietate. In the oleoresin of Pinus mugo they were found in a ratio of 3:1; in Pinus strobus, 2:3; and in Picea excelsa, 2:1; while in Picea abies methyl 15-hydroxydehydroabietate was the main component. The bicyclic hydroxyester larixyl acetate and the diterpene diol larixol were present in considerable amounts in the oleoresin of the European larch (Table 1).

The study of the chemical composition of the oleoresins of the conifers of Transcarpathia has shown that individual species of conifers retain characteristic genus features regardless of their growth site.

EXPERIMENTAL

The time and place of collecting the oleoresins investigated have been given in [1]. The UV spectra of the compounds isolated were recorded on a UR-20 instrument for solutions in carbon tetrachloride, and the NM spectra for solutions in CCl_4 on a Varian A 56-60 A instrument (δ-scale) and for solutions in $CDCl_3$ on a Bruker WP-200 SY H^1-200.13 MHz instrument.

Gas-liquid chromatography (GLC) of the diterpene hydrocarbons was performed on a Chrom-41 instrument using a 0.3 mm × 20 m capillary column at 180°C with PMS as the liquid phase and nitrogen as the carrier gas. The acetates of the diterpene alcohols were analyzed by GLC on a Chrom-5 instrument with a glass capillary column, the temperature of the column being raised from 180 to 230°C at the rate of 2°C/min.

Adsorption chromatography was performed on silica gel with a grain size of 0.8-0.63 mm and on silica gel impregnated with silver nitrate. The components were eluted with petroleum ether and mixtures of petroleum ether with diethyl ether. The course of chromatography was monitored and the individuality of the components was checked by thin-layer chromatography on Silufol plates (Czechoslovakia).

All the oleoresins investigated were separated into neutral and acid components by treatment with a 1% solution of caustic soda, the neutral substances being isolated by extraction with diethyl ether.

The neutral fraction of the oleoresin was then separated into hydrocarbons and oxygen-containing compounds by percolation through Al_2O_3, activity grade (I-II), at a rate of substance to sorbent of 1:10 (see Table 1).

Vacuum distillation of the hydrocarbon fraction yielded mono- (60-100°C/10 mm Hg), sesqui- (120-150°C, 3 mm Hg), and diterpene compounds (still residue). In those cases where the amount of diterpene hydrocarbons was insignificant, the combined sesqui- and diterpenes were investigated. Analysis of the mixtures of diterpene hydrocarbons was carried out by adsorption chromatography on silica gel impregnated with silver nitrate and by GLC.

The oxygen-containing compounds were analyzed by adsorption chromatography.

The procedure for separating neutral substances of the oleoresins is given for the case of the oleoresins of the European larch as an example.

Neutral Substances of the Oleoresin of the European Larch. The combined neutral compounds (22.5 g) were separated by chromatography on alumina (activity grade II-III) into hydrocrbons (6.5 g) and oxygen-containing compounds (14.5 g). Vacuum distillation of the hydrocarbons gave monoterpenes (5.5 g) and combined sesqui- and diterpenes (0.6 g) in which the main diterpene hydrocarbon — dehydroabietane — was identified by GLC.

Separation of the Oxygen-Containing Diterpenoids. The oxygen-containing compounds (3 g) were chromatographed on silica gel (0.8-0.63 mm) by the method of flash chromatography [3].

Petroleum ether—diethyl ether (97:3) eluted the combined aldehydes (0.54 g), which were reduced to alcohols [4], and the latter, in the form of their acetates, were analyzed by GLC (Table 2).

Petroleum ether—diethyl ether (95:5) eluted epimanool (0.29 g) and then the total primary alcohols (0.23 g), which were acetylated and identified by GLC (Table 2).

Petroleum ether—diethyl ether (85:15) led to the isolation of larixyl acetate (1.59 g) with bp 80°C, identical in physical and spectral characteristics with an authentic sample.

Diethyl ether eluted larixol (0.32 g) with mp 97-98°C, giving no depression of the melting point with an authentic sample.

CONCLUSIONS

1. The composition of the neutral diterpenoids of the oleoresins of five species of conifers growing in Transcarpthia has been studied. Features of the distribution of the diterpenoids according to species have been revealed.

2. It has been established that for each type of oleoresin the predominance of one or a few diterpenoids is characteristic. In Pinus mugo the main compounds are diterpene aldehydes, in A. alba the main components is cis-abienal, in Picea abies and P. excelsa it is isocembrol, and in the oleoresin of Larix decidua it is larixyl acetate.

LITERATURE CITED

1. V. I. Bol'shakova, É. N. Shmidt, L. I. Demenkova, and V. A. Pentegova, Khim. Prir. Soedin., 212 (1987).
2. V. Yu. Puzanova, B. A. Radbil', V. A. Raldugin, and V. A. Pentegova, Khim. Prir. Soedin., No. 4, 12 (1983).
3. W. C. Still and M. J. Kahn, J. Org. Chem., 43, 2923 (1978).
4. G. Hilgetag and A. Martini, Weygand—Hilgetag's Preparative Organic Chemistry, Wiley, New York (1972) [Russian translation from the German: Khimiya, Moscow (1968), p. 65].

TRITERPENOIDS FROM Abies SPP.

VI. "ISOFIRMANIC" AND (24E)-LANOSTA-8,24-DIENE-3,23-DION-26-OIC ACIDS FROM SIBERIAN FIR NEEDLES

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UDC 547.595,9:547.914.4

Two triterpene acids have been isolated in the form of methyl esters from an extract of Siberian fir needles, and their structures have been established as 9β-lanosta-7,25-diene-3,23-dion-26-oic and (24E)-lanosta-8,24-dien-3,23-dion-26-oic acids, respectively.

It has been shown previously that the main lanostane acids in an extract of needles of the Siberian fir Abies sibirica Ledb. are the diketo acids (I) and (II), the structures of the corresponding methyl esters of which (III) and (IV) have been established [1, 2]. Acid (I) was recently described by Hasegawa et al. under the name of "firmanoic" [3]. They isolated it in the form of the methyl ester from an extract of the seeds of the fir Abies firma Sieb. et Zucc. A second acid ("isofirmanoic") was isolated from the same extract, and the structure expressed by formula (V) was established for its methyl ester on the basis of spectral characteristics.

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. S. M. Kirov Leningrad Academy of Wood Technology. Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 816-820, November-December, 1988. Original article submitted January 4, 1988.