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POLYPRENOLS AND DOLICHOLS FROM THE NEEDLES, NEEDLE-FREE SHOOTS, AND BARK OF *Pinus sibirica*

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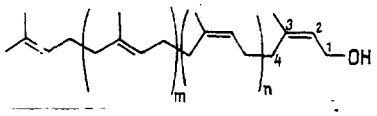
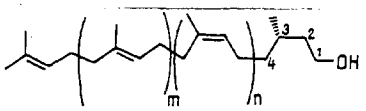
The presence of bioregulators of animal organisms — dolichols — has been established in the needles, needle-free shoots, and bark of the Siberian stone pine. The qualitative and quantitative compositions of the mixtures of the isoprenologues and polyprenols have been established by the HPLC method. In addition, known diterpene alcohols ((E,E,E)-geranylgeraniol and labd-8(17),13E-dien-15-ol) esterified with higher fatty acids have been identified in the bark.

Polyprenols (**1**) and their 2,3-dihydro analogues — dolichols (**2**) — are well-known oligoisoprenoid alcohols fulfilling important biological functions in living organisms and usually present in the form of mixtures of isoprenologues, free or esterified with acetic or phosphoric acid or with higher fatty acids [1, 2].



1 (m=2 or 3, n = from 4 upwards)



2 (m=2, n=10-20)

The polyprenols are contained predominantly in the photosynthesizing tissues of plants, and some of the richest sources of them are species of the family Pinaceae Lindl. [3, 4]. The polyprenols from plants of these species are stereochemically analogous to dolichols from animal organisms — their molecules contain only two double bonds with the (E)-configuration.

In the needles of conifers, polyprenols are usually present in the form of acetates [3]. In the bark of *Larix decidua* Mill. [5] and the inner bark of the fir *Abies sibirica* Ledb. [6] they are esterified with higher fatty acids. In conifers, dolichols have previously been found in the form of esters only in an extract of the inner bark of the Siberian fir [6].

In the present paper we give for the first time the results of an investigation of the oligoisoprenoids of the needles, shoots, and bark of the Siberian stone pine *Pinus sibirica* R. Mayr. We have previously shown the presence of polyprenols and dolichols in the bark of this tree [7].

The initial raw material was treated by the usual method (see the Experimental part), and for the investigation we used neutral fractions of ethereal extracts. By chromatographing the neutral fraction of an extract of the needles we obtained successively the total hydrocarbons, a mixture of esters, and a fraction of hydroxyl-containing compounds from which, by using an authentic sample of fir polyprenols as a marker for TLC we isolated the total free polyprenols of the stone pine needles (yield 0.09% on the initial raw material).

According to TLC using markers, the ester fraction contained a mixture of acetates of polyprenols and esters of sterols with higher fatty acids, and also a diterpene ester — methyl lambertianate. The alkaline hydrolysis of the fraction, followed

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TABLE 1. Qualitative and Quantitative Compositions of the Isoprenologues of the Stone Pine Polyprenols*

C_i	Needles		Bound	Free
	Shoots	Bark		
C_{55}	0.1	0.1	-**	0.6
C_{60}	0.2	0.2	-**	1.1
C_{65}	0.5	0.7	-**	1.7
C_{70}	2.3	2.5	10.9	3.9
C_{75}	11.4	12.2	22.6	15.2
C_{80}	28.3	27.1	33.2	32.4
C_{85}	32.5	32.2	27.5	29.5
C_{90}	16.4	18.0	4.7	12.7
C_{95}	5.0	5.1	1.0	2.8
C_{100}	2.1	1.8	0.0	0.1

*As percentages of the sum of the isoprenologues shown.
**Not determined.

TABLE 2. Qualitative and Quantitative Compositions of the Isoprenologues of the Stone Pine Dolichols

C_i	Needles	Shoots	Bark
C_{70}	5.5	7.0	4.2
C_{75}	16.5	26.5	13.8
C_{80}	33.6	41.4	47.8
C_{85}	31.5	21.3	30.0
C_{90}	9.5	3.7	4.1

*As percentages of the total isoprenologues shown.

by column chromatography of the unsaponifiable part, led to the isolation of unchanged methyl lambertianate, a mixture of β-sitosterol and campesterol (GLC), and the total bound polyprenols of the needles (0.22% of the initial raw material). The PMR spectrum of the latter was typical for polyprenols [8] but showed, in addition, a weak signal (at 3.65 ppm) characteristic for the protons of the (—CH_2—O—) group of dolichol molecules [8]. The integral intensity of this signal amounted to 5% of the intensity of the signal at 4.07 ppm (—CH_2—O—) of polyprenol molecules [8]. Consequently, the molar ratio of polyprenols and dolichols could be estimated as 95:5. We treated this sample by Ravi's method [9] and isolated the total dolichols of the needles. The PMR spectrum of the sample of dolichols obtained corresponded to that given in the literature [8]. The polyprenals formed in the separation of the dolichols [9], were reduced to polyprenols with lithium tetrahydroaluminate in diethyl ether. The results of the qualitative and quantitative analysis of the dolichols and polyprenols of the stone pine needles by the HPLC method are given in Tables 1 and 2 (determination with the use of known samples of fir polyprenols and the sea buckthorn dolichols).

No dolichols were detected in the total free polyprenols. In a typical chromatogram of the bound polyprenols of the Siberian stone pine needles, because of their small amount the dolichols appeared only as small diffuse shoulders on the right-hand sides of the peaks of the polyprenols. On an HPLC chromatogram of the free polyprenols only the peaks of the polyprenols were observed.

The polyprenols of Siberian stone pine needles belong to the series with $m = 2$ (formula **1**) that is typical for coniferous species, since the relative integral intensities of the signals of the PMR spectrum at 1.73 ppm ($C\underline{H}_3C(R)=CH—CH_2OH$) and 1.58 ppm ($C\underline{H}_3$ at double bonds with the (E)-configuration) were 1 and 3, respectively [6].

We have reported the presence of nonpolar esters in the neutral fraction of extracts of the shoots and bark of the stone pine previously [7, 10]. In them, as was found, the polyprenols are, like the sterols, bound with higher fatty acids (TLC with markers). These fractions, isolated by chromatography, were subjected to alkaline hydrolysis. The unsaponifiables were chromatographed on column of silica gel, using the total polyprenols from the needles as a marker. The total oligoisoprenoid alcohols isolated (yields 0.019% and 0.005% from the shoots and bark, respectively), coinciding according to TLC with the polyprenols of the stone pine needles, proved to be mixtures of polyprenols and dolichols with a high relative content of the latter (38 and 41% for the shoots and bark, respectively; HPLC, PMR spectra).

The dolichols were isolated by Ravi's method [9]. The qualitative and quantitative compositions of the isoprenologues of the polyprenols and dolichols from the shoots and bark of the stone pine are given in Tables 1 and 2. Their PMR spectra showed that the polyprenols of the shoots and bark belonged to the same series as the polyprenols of the needles.

The total acids obtained on the hydrolysis of the ester fractions of the shoots and bark contained the usual saturated and unsaturated fatty acids, among which oleic, linoleic, and stearic predominated (GLC).

The mixture of esters from the bark proved to be complex in composition and when its unsaponifiable part was chromatographed we isolated, in addition to the total methyl esters of resin acids, oligoisoprenoids and sterols [7], a mixture of diterpene alcohols (composition according to GLC as percentages of their total: 30.6% of (E,E,E)-geranylgeraniol, 23.2% of isopimarinol, 11.8% of abietinol, 10.1% of dehydroabietinol, 8.9% of labd-8(17),13E-dien-15-ol, and 8.8% of sandaracopimarinol). These alcohols were present in the bark extract in the form of esters of higher fatty acids, and the acetates obtained from them gave on TLC a single diffuse spot with a R_f value substantially smaller than for the initial ester fraction of the bark before saponification. These diterpene alcohols are common components of the wood or oleoresin of the Siberian stone pine [11, 12], but have not been known previously in the form of natural esters with higher fatty acids.

This is the first time that dolichols have been detected in the needles of conifers. The increase in their proportion in the total oligoisoprenoids of the shoots and bark (relative to the amount of polyprenols) is apparently typical for coniferous plants.

EXPERIMENTAL

PMR spectra were recorded on Bruker AC-200 (200.13 MHz) and Bruker AM-400 (400.13 MHz) instruments for solution in $CDCl_3$, δ-scale. Gas-liquid chromatography was conducted on a Chrom-5 instrument (Czechoslovakia) using a 3 mm × 2 m glass column with 5% of SE-30 on Chromaton N-Super (0.16-0.20), t_{col} = 120-270°C/4°C. Analyses by the HPLC method were conducted on a Milikhrom instrument with a 0.2 × 6.3 cm column containing the sorbent LiChrosorb RP-18 with methanol—acetone (1:3) as eluent. For adsorption chromatography we used air-dry silica gel of the "Armsorb" brand (from the firm Akunk, Erevan) with a grain size of 0.100-0.140 mm.

The bark and litter of the stone pine were gathered in 1992 in the region of Lake Teletskoe. The bark was separated from the cork, ground, and dried in the air. The litter was separated by hand into the needles and needle-free shoots. Of the latter only those aged 0.5-1.5 years were taken and these were comminuted and, like the needles, dried in the air to the air-dry state (brittleness). The dried material was comminuted further in a knife grinder to a particle size of 0.2-1.5 mm and was extracted with diethyl ether (DE) in a Soxhlet apparatus for a day. Each ethereal extract (volume of about 1.5 liters) was treated with a 1% solution of NaOH (3 × 0.3 liter) to eliminate acids, and the ethereal layer after the treatment with the alkali solution was washed with water and dried over anhydrous sodium sulfate. After the solvent had been driven off, the neutral fractions of extracts of the needles (12.13 g), shoots (12.91 g), and bark (12.60 g) were obtained from 354, 309, and 780 g of needles, shoots, and bark, respectively (acids — 15.63, 27.57, and 30.2 g, respectively).

Isolation of the the Oligoisoprenoid Fractions. The neutral fraction of the needle extract (12.13 g) was chromatographed on 200 g of silica gel. Hexane eluted a mixture of hydrocarbons (5.78 g), hexane—DE (97:3) an ester fraction (2.5 g), and hexane—DE (96:4-90:10) a fraction containing the polyprenols (0.71 g), the rechromatography of which on 6 g of silica gel with hexane containing 10% of DE yielded a mixture of polyprenols (0.33 g). We did not investigate the polar fractions (2.16 g).

The fraction containing the oligoisoprenoid esters of the shoots (2.16 g) was obtained as described in [10], and those of the bark (2.5 g) as described in [7].

The alkaline hydrolysis of the ester fractions was carried out by the following general procedure: each fraction was dissolved in 30 ml of 10% of alcoholic NaOH, and the solution was heated in the boiling water bath for 1 h. After the usual working up, the acids were methylated with diazomethane and were recorded by GLC, while the unsponifiable residues (1.91, 1.01, and 1.52 g for the needles, shoots, and bark, respectively), were chromatographed on silica gel (ratio of substances and sorbent 1:20).

The Separation of the Unsaponifiable Residue. The following were obtained successively by elution with mixtures of hexane and DE (3, 8, 10, and 20%): methyl lambertianate from the needles and shoots (0.9 and 0.32 g, respectively) and a mixture of methyl esters of resin acids from the bark (0.11 g; eluent with 3% of DE), the total oligoisoprenoid alcohols (from the needles 0.76 g, from the shoots 0.006 g, and from the bark 0.04 g; eluent with 8% of DE), the total diterpene alcohols (0.27 g; 10% of DE in the case of the bark), and a mixture of β-sitosterol with campesterol (0.17, 0.56, and 1.09 g for the needles, shoots, and bark, respectively; eluent with 20% of DE).

The separation of the mixture of isoprenols and dolichols was carried out by the method of [9] followed by the reduction of the polyprenals with lithium tetrahydroaluminate in DE at room temperature. After the reaction with MnO_2 and chromatography on silica gel, 0.76 g of the total oligoisoprenoids of the needles yielded 0.03 g of dolichols and isoprenals the reduction of which gave 0.70 g of polyprenols. For the extract of the shoots, the yields of products amounted to 0.02 g of dolichols and 0.03 g of polyprenols, and for the bark the corresponding amounts were 0.01 and 0.02 g, respectively.

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