WAXY SUBSTANCES FROM THE NEEDLES OF THE SIBERIAN LARCH

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Continuing investigations begun earlier on waxy substances of Siberian conifers [1, 2], we have studied the dynamics of the amount of unsaponifiable and saponifiable components and the fatty acid compositions of the waxy substances from the needles of the Siberian larch.

The needles were gathered in the period May–September, 1989, in the second decade of each month from 40-year-old Siberian larch trees growing in the Emel'yanovo region of Krasnoyarsk krai. The samples of needles were averaged over the whole crown of a tree. The number of trees from which the samples of needles were taken ensured the necessary representativeness of the samples. The needles were ground and were exhaustively extracted with gasoline and then with ethanol at the boiling point of the solvent. The extracts obtained were kept in the refrigerator at −10°C for 24 h. The waxy substances that precipitated were separated from the extracts, and those extracted by gasoline and by ethanol were combined. The amount of waxes obtained was found gravimetrically. The amounts of saponifiable and unsaponifiable substances in the waxes were determined [3]. The composition of the fatty acids from the waxy substances was determined by GLC on a Biokhrom 1 chromatograph as described in [4]. The results obtained are given in Tables 1 and 2.

It can be seen from Table 1 that in the period from May to September the amount of waxy substances in Siberian larch needles varies within the range of 3.4-7.9% on the absolutely dry substance of the needles. With the growth and aging of the needles the level of waxy substances in them more than doubles. The accumulation of wax in the needles takes place through an increase in the amount of saponifiable substances. Sixteen individual acids from C_6 to C_{30} were detected in the acids of the waxy substances.

TABLE 1. Dynamics of the Amount of Waxy Substances in Siberian Larch Needles

Month	Amount, % on the absolutely dry substance	Saponifiable substances	Unsaponifiable substances
		% on the weight of the waxy substances	
May	3,4	80,4	19,5
June	3,6	87,7	12,2
July	4,0	88,8	11,1
August	4,5	88,8	11,1
September	7,9	88,8	11,0

TABLE 2. Fatty Acid Composition of the Waxy Substances of Siberian Larch Needles, % on the Sum of the Acids

Acid	May	June	July	August	September
$C_{6:0}$	1,5	1,3	1,0	1,2	1,0
$C_{12:0}$	2,5	2,0	1,8	1,5	1,2
$C_{14:0}$	3,2	2,0	1,5	1,0	1,1
$C_{16:0}$	30,0	29,9	30,7	28,0	23,3
$C_{16:1}$	3,8	2,0	1,5	1,1	1,2
$C_{18:0}$	1,8	1,4	2,0	1,6	1,1
$C_{18:1}$	8,8	9,0	9,1	9,0	8,3
$C_{18:2}$	11,2	10,0	10,9	8,3	6,8
$C_{18:3}$	5,4	3,0	3,2	2,7	3,5
$C_{20:0}$	2,2	2,0	1,8	1,9	1,5
$C_{20:1}$	1,5	0,5	0,6	0,9	1,0
$C_{22:0}$	5,1	10,5	10,4	13,1	15,2
$C_{24:0}$	9,6	10,4	11,0	12,3	14,1
$C_{26:0}$	1,5	1,4	1,0	2,0	2,2
$C_{28:0}$	1,9	1,6	1,5	2,1	3,5
$C_{30:0}$	10,0	13,0	12,0	13,3	15,0

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The bulk of the acids throughout the whole vegetation period of May to September consists of the $C_{16:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{22:0}$, $C_{24:0}$, and $C_{30:0}$ species, which make up more than 80% of the total acids. The end of the vegetation period (August–September) is characterized by the accumulation of the long-chain acids $C_{22:0}$, $C_{24:0}$, and $C_{30:0}$ in the waxy substances.

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FLAVONOIDS OF Lathyrus sativus

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The epigeal part of Lathyrus sativus L. (grass pea) gathered in the flowering period in Khar'kov province was exhaustively extracted with 70% ethanol. The alcoholic extract was concentrated in vacuum to an aqueous residue, and the flavonoids were extracted with ethyl acetate. Paper chromatography (two-dimensional ascending PC in the butanol–acetic acid–water, 4:1:2, and 15% acetic acid systems) revealed no less than 20 substances of flavonoid nature. To isolate individual compounds, the ethyl acetate fraction from 3 kg of raw material was deposited on a column of polyamide sorbent and was eluted successively with chloroform and mixtures of ethanol and chloroform. As a result, flavonoid substances (I-VI) were isolated and identified.

Substance (I) – $C_{16}H_{12}O_4$, mp 256-258°, λ_{max} 249, 302 nm (ethanol), identified as formononetin.

Substance (II) – $C_{15}H_{10}O_6$, mp 330-331°, λ_{max} 256, 268, 350 nm (ethanol), identified as luteolin.

Substance (III) – $C_{15}H_{10}O_6$, mp 274-276°, λ_{max} 266, 367 nm (ethanol), identified as kaempferol.

Substance (IV) – $C_{15}H_{10}O_7$, mp 310-312°, λ_{max} 256, 370 nm (ethanol), identified as quercetin [2].

Substance (V) – $C_{21}H_{19}O_{10}$, mp 233-235°, λ_{max} 255, 367 nm (ethanol), and Substance (VI) – $C_{21}H_{20}O_{11}$, mp 283-285°, λ_{max} 260, 375 nm (ethanol). In the products of acid and enzymatic hydrolysis we detected kaempferol (V), quercetin (VI), and L-rhamnose. UV spectroscopy with ionizing and complex-forming reagents showed the presence of free hydroxy groups in the C-3, C-5, and C-4' positions, and in (VI) also at C-3'. In the PMR spectra (DMSO), in addition to the protons of the aromatic moieties H-2' (d, 7.72 ppm, J = 1.95 Hz), H-6' (d, 7.58 ppm, J = 8.8 Hz), H-5' (d, 6.88 ppm, J = 8.8 Hz), H-8 (d, 6.74 ppm, J = 1.95 Hz), H-6 (d, 6.41 ppm, J = 1.95 Hz, and, in (V) H-3' (d, 6.92 ppm, J = 8.8 Hz), the protons of the carbohydrate moieties were observed (3.60-5.14 ppm). The anomeric proton of rhamnose was strongly shifted into the weak-field region (d, 5.54 ppm) and appeared in the form of a well-defined doublet with a splitting constant J = 1.5 Hz; the signal of the methyl group of rhamnose was found at 1.12 ppm with J = 5.87 Hz, which confirmed the α configuration of the glycosidic bond. The results obtained enabled substance (V) to be characterized as kaempferol 7-O-α-L-rhamnofuranoside and substance (VI) as quercetin 7-O-α-L-rhamnofuranoside [1-4].

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