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GLYCOLIPIDS OF THE WOODY VERDURE OF *Larix sibirica*

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The compositions of the glycolipids of the main fractions of the woody verdure of the Siberian larch — needles and shoots — have been studied. The dynamics of the amounts of individual glycolipids in the course of vegetation have been established. The qualitative and quantitativae compositions of the fatty acids of the total glycolipids of the needles and shoots have been determined.

Improvement in the technology of the complex processing of woody verdure (WV) must be based on a careful investigation of the chemical composition and a differentiation of the trees treated with respect to species identification. The Siberian larch *Larix sibirica* is the most widespread timber-forming species of Siberia, the Altai, and the Far East [1]. Its needles and shoots contains a whole series of compounds possessing high biological activity. Literature information on the polar lipids of various tissues of Siberian larch relates primarily to the phospholipids [2-4], and there is no information on the glycolipids (GLs) of the needles and shoots.

We have previously reported the composition of the total lipids of the WV of the Siberian larch [5]. The task of the present investigation included a study of the qualitative compositions and dynamics of the levels of individual components of the GLs in the course of vegetation in the needles and shoots of the Siberian larch in order to determine the possibility of its use as an additional source of biologically active compounds. The GLs were isolated from the total lipids with the aid of column chromatography (CC) on silica gel [5]. The best separation of the total GLs and their more complete freeing from accompanying impurities was achieved by two-dimensional chromatography in a thin layer of silica gel. As eluents we used solvent systems 1 (first direction), and 2 (second direction) [6]. The substances were identified by comparing the chromatographic mobilities of the compounds under investigation and "markers", together with literature information on R_f values, and also from quantitative reactions and spectral characteristics.

Resolution of the zones of five substances was achieved: sulfoquinovosyldiglycerides (SQVDGs), digalactosyldiglycerides (DGDGs), ceramide-oligoheterosides (COGs), sterol glycosides (SG)s, and monogalactosyldiglycerides (MDGDGs). The R_f values of individual GLs agreed with those given in the literature [7, 8].

A comparison of the qualitative and quantitative indices of the GLs of the needles and shoots (Table 1) shows that the group composition of the GLs was more diverse in the needles. During the whole vegetation period the MGDG and DGDG fractions predominated in the GLs of the needles and of the shoots.

The amount of GLs in the tissues of the WV of the Siberian larch depended on the state of development of the tree in the periods investigated. The following law was found with respect to the levels of MGDGs, GDGDs, SQVDGs, and COGS of the needles and also for the MGDVs and SQVDGs of the shoots: at the beginning of vegetation (May) their amount was small; in the period of the greatest physiological activity their maximum amount was observed in July for the needles and in June for the shoots; and at the end of vegetation (September) the amount of GLs gradually fell. The dynamics of the levels of DGDGs in the shoots and also of the GSs in the needles of the Siberian larch differed from the groups of GLs considered above. The amount of DGDGs in the shoots was a maximum at the beginning of vegetation (May), and a minimum in June. It must be mentioned that in the shoots the level of DGDGs scarcely changed during the whole vegetation period: 0.24-0.27% on the absolutely dry weight. Characteristic for the needles was a gradual decrease in the amount of SGs from May to September. The opinion that sterols and their derivatives stabilize some properties of membranes [9], and, in particular,

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TABLE 1. Composition of the Glycolipids of the Woody Verdure of *Larix sibirica*

Glycolipid	Amounts over the months, %, on the									
	May		June		July		August		September	
	absolutely dry mass	sum of the glycolipids	absolutely dry mass	sum of the glycolipids	absolutely dry mass	sum of the glycolipids	absolutely dry mass	sum of the glycolipids	absolutely dry mass	sum of the glycolipids
Needles										
SQVDGs	0.02	4.25	0.06	6.91	0.13	7.26	0.05	5.67	0.01	4.72
DGDGs	0.09	26.53	0.22	22.87	0.29	16.07	0.16	19.46	0.07	24.18
COGs	0.02	5.45	0.08	8.72	0.17	9.16	0.10	11.27	0.04	12.84
SGs	0.06	15.83	0.05	5.21	0.04	2.39	0.04	4.42	0.03	10.13
MGDGs	0.17	47.94	0.54	56.29	1.17	65.12	0.51	59.18	0.14	48.13
Shoots										
SQVDGs	0.11	15.14	0.21	18.77	0.13	16.02	0.09	13.07	0.08	12.35
DGDGs	0.27	36.08	0.24	21.14	0.25	29.18	0.26	38.11	0.26	41.40
MGDGs	0.36	48.78	0.69	60.09	0.47	54.80	0.33	48.82	0.30	46.25

decrease their mobility, permits the assumption that the maximum level of GSs in the early stage of development of the needles is connected with precisely this function.

The fatty acid compositions of the total GLs were determined by GLC analysis (Table 2). The qualitative compositions of the fatty acids of the total GLs of the needles and the shoots were identical, but the amounts of fatty acids differed, The main fatty acids of the GLs were unsaturated species, the amount of which throughout the vegetation period exceeded 50% of the total weight of acids. At the beginning of vegetation (May-June) the sum of the unsaturated acids of the GLs of the shoots was higher than that of the needles.

The total degree of unsaturation of the fatty acids increased for the GLs of the needles from May to July, and for those of the shoots from May to June. At the end of vegetation, the amount of unsaturated acids fell. The dynamics of the amount of saturated acids had the opposite nature.

The sharpest changes in the levels of fatty acids of the GLs of the needles took place in the period of activation of growth processes and the formation of young needles — in May-July. At the beginning of vegetation (May) a maximum level was observed of the main fatty acids, palmitic, oleic, and linolenic. The smallest amount of linolenic acid was found in the May samples of needles. The intensive synthesis of linolenic acid began in June, and its level was a maximum in July. At the same time, the amount of saturated acids and also those of oleic and linoleic acids decreased, and in July a minimum was observed. At the end of vegetation, the amount of linolenic acid gradually fell and the proportion of the less unsaturated acids - oleic and linoleic — and also of all the saturated acids of the total GLs of the needles rose. It must be mentioned that the dynamics of the level of palmitoleic acid were similar to those of linolenic acid.

In the total GLs of the shoots, as in the GLs of the needles, the bulk of the saturated acids consisted of palmitic acid. Its minimum (13.09%) was found in July. Then the amount of palmitic acid increased and reached a maximum (29.90%) in September. Among the unsaturated acids of the GLs of the shoots linolenic acid predominated: its amount ranged between 32.98 and 47.13%, the maximum being in June and the minimum in September. A considerable proportion of the unsaturated acids of the GLs of the shoots also consisted of linolenic acid. Throughout the vegetation period its level decreased from 26.68% (May) to 16.00% (September).

In the total GLs of the shoots the level of myristic acid was higher than in the GLs of the needles while the amounts of palmitoleic, stearic, and oleic acids were considerably lower. The amounts of arachidic and behenic acids in the total GLs of the needles and of the shoots were approximately the same.

With the aid of paper chromatography (PC) in system 4 it was established that the main carbohydrate components isolated after severe acid hydrolysis of the GLs were galactose and glucose, while traces of mannose and arabinose were also detected,

A comparison of the results obtained with literature information on the amounts of GLs in plants permits us to consider that the needles and shoots of the Siberian larch may serve as a promising source for obtaining these substances.

TABLE 2. Fatty Acid Compositions of the Total Glycolipids of the Woody Verdure of *Larix sibirica* (%, GLC)

Acid	Needles					Shoots				
	May	June	July	August	September	May	June	July	August	September
$C_{12:0}$	1.15	0.98	0.85	1.12	1.38	1.68	1.28	1.72	1.98	2.23
$C_{14:0}$	2.23	1.52	0.82	0.96	1.05	3.11	2.53	2.86	3.04	3.48
$C_{16:0}$	24.00	18.32	13.22	15.28	18.47	19.23	13.09	20.33	23.88	29.90
$C_{16:1}$	7.23	8.12	10.33	8.16	7.94	4.15	5.81	5.14	4.63	4.02
$C_{18:0}$	5.3	2.43	2.03	4.37	6.33	2.05	1.46	1.65	1.84	2.16
$C_{18:1}$	15.46	13.24	7.61	9.98	10.58	5.34	8.29	6.03	5.73	5.25
$C_{18:2}$	26.13	21.34	17.87	18.25	18.85	26.68	18.32	17.28	16.13	16.00
$C_{18:3}$	15.12	31.52	44.96	37.85	30.79	34.94	47.13	42.16	39.19	32.98
$C_{20:0}$	2.02	1.33	1.25	2.19	2.24	1.28	0.94	1.35	1.78	2.02
$C_{22:0}$	1.53	1.20	1.06	1.24	2,37	1.54	1.15	1.48	1.80	1.96
Total saturateds	36.06	25.78	19.23	25.76	31.84	28.89	20.45	29.39	34.32	41.75
Total unsaturateds	63.94	74.22	80.77	74.24	68.16	71.11	79.55	70.61	65.68	58.25

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument (chloroform). GLC was conducted on a Tsvet-100 instrument with a flame-ionization detector and programmed heating of the column. The stationary phase used was SE-30 deposited on Chromaton NAW-DMCS with a particle size of 0.20-0.25 mm (5%). The diameter of the glass column was 4 mm and its length 2 m. The carrier gas was helium at the rate of 90 ml/min. The column was heated from 100 to 280° at 8°C/min. The temperature of the detector was 320°C, and that of the evaporator 250°C.

TLC was conducted on Silufol plates and 5/40 silica gel (Czechoslovakia) containing 5% of gypsum. The following solvent systems were used; 1) chloroform–methanol–water (65:25:4); 2) acetone–toluene–acetic acid–water (60:60:2:1); 3) hexane–diethyl ether–acetic acid (85:15:1); and 4) ethyl acetate–pyridine–water (5:1:5).

Samples of the WV of the Siberian larch were taken throughout the vegetation period (May-September) in the second decade of each month from 40-year-old trees growing in the Emel'yanovo experimental leskhoz [forestry farm], Krasnoyarsk region. An average sample of the WV was separated manually into needles and shoots. The number of trees from which the samples of WV were taken ensured the necessary representativity of the samples [10].

The total lipids were isolated by the Bligh–Dyer method [11]. The CC of the lipids was conducted on KSK silica gel (100-150 mesh). The GLs were eluted with acetone, and the solvent was driven off in a rotary evaporator at 35-40°C under a reduced of nitrogen. The amounts of the individual GLs were determined by Dubois' method from the amounts of galactose in the spots of the substances obtained after two-dimensional TLC [12].

Alkaline Hydrolysis of the GLs. Samples of the GLs (5-10 mg) were subjected to alkaline deacylation under the conditions of [13]. The fatty acids isolated were methylated with a freshly prepared solution of diazomethane. The resulting fatty acid methyl esters were additionally purified by TLC in solvent system 3 and were analyzed by GLC. Acid hydrolysis was carried out by the procedure described in [13]. For PC we used solvent system 4. The spots were revealed with aniline phthalate.

The IR spectra of the GL fractions corresponded to the results given in the literature [14]. The following absorption bands were present in the IR spectrum (cm^{-1}): 1380, 1470, 2870, 2930, 2960 (CH_3, CH_2, CH), 1750 (C=O), 3450 (OH), 1740 (R–CO–OR), 1650–1550 (NH), 670, 800, 1260, 1420, 1350 (R–O–SO_2–R).

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