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AMOUNTS OF ARACHIDONIC ACID IN THE BUDS OF Populus balzamifera IN THE COURSE OF THE ANNUAL CYCLE

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The paper gives information on the amount of arachidonic acid in the buds of Populus balzamifera. The presence of arachidonic acid in the buds was confirmed by TLC, GLC, coulometric titration, and an iodine number calculation. The dynamics of the amount of arachidonic acid in the buds during the annual cycle are given.

It is known that arachidonic acid, the main precursor of the prostaglandins, is a structural component of the phospholipids of cell membranes, of triacylglycerides, and of esterified cholesterols in animal organisms. There is information on the presence of arachidonic acid in all mammals [1] and also in the lipids of marine organisms [2, 3]. Until very recently, it was considered that vegetable oils did not contain unsaturated acids with five and six double bonds [4]. The presence of arachidonic acid* in some species of lower plants [5] and in clover and buckwheat pollen [6] was later established.

L. Rubchevskaya [7] showed the presence of arachidonic acid in the cambial zone of Larix sibirica. É. Levin, Sh. Alaudinov, and V. Cherepanova were the first to establish the presence of prostaglandins in the living tissues of higher plants [8, 9]. This, in its turn, posed the problem of studying their precursors in these species of plants. Our aim was to establish the presence of arachidonic acid in the buds of Populus balzamifera L., and also to estimate its amount quantitatively in the course of the annual cycle.

The results of determinations of the amounts of arachidonic acid are given in Table 1. The amounts of total lipids differ in the course of the annual cycle. The maximum amount of total lipids is found in December (21.33%) and the minimum in September (13.34%). The amount of neutral lipids ranges from 6 to 48% of the total lipids, depending on the pheno-

*Arachidonic acid contains four double bonds — Publisher.

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TABLE 1. Amounts of Lipids and Arachidonic Acid in the Buds of Populus balzamifera in Various Periods of the Annual Cycle

Month	Total, % on the absolutely dry buds		Arachidonic acid, 10^{-5} g/g of absolutely dry
	lipids	phospholipids	
September	13,34	0,34	0,16
October	17,56	0,18	2,60
November	19,40	0,32	6,74
December	21,33	0,29	4,81
January	16,15	0,57	2,05
February	18,60	0,38	3,30
March	18,80	0,35	3,63
April	18,42	0,47	4,18

logical state of the tree. The amount of phospholipids also changes in the course of the annual cycle, amounting to 1-3% of the total lipids of the buds.

Arachidonic acid was determined in the fractions of neutral lipids and phospholipids. With the aid of TLC in system 1, the neutral lipids were separated into individual classes of substances. Their fatty acid compositions were established by GLC. It was found that arachidonic acid was absent from the mono-, di-, and triacylglycerols and also from the free fatty acids. The main acids were those of the C_{16} and C_{18} series.

The acids isolated from the phospholipids were analyzed by TLC in systems 2 and 3 in the presence of standard arachidonic acid. The R_f values of the standard and one of the zones in the chromatography of the acids coincided, which showed the presence of arachidonic acid in this zone.

To establish the position distribution of the fatty acids in the phospholipid molecules, enzymatic hydrolysis was carried out. Phospholipase A_2, which specifically hydrolyzes the ester bond in the sn-2 position of a phospholipid, was used. The hydrolysis products were separated with the aid of TLC in system 4. The results of the investigation showed that in the phospholipids of the buds of Populus balzamifera the arachidonic acid was present in the sn-2 position.

The arachidonic acid was isolated from the phospholipids by a method similar to that used in working with animal organisms [10]. The R_f value of the arachidonic acid isolated coincided with that of a standard (R_f 0.44, system 6).

According to the results of the GLC under standard conditions of methyl arachidonate and a standard, the retention time of the sample coincided with that of the standard. The refractive index of the sample of arachidonic acid isolated was n_D^{20} 1.4838 (according to the literature, n_D^{20} 1.4824), and the iodine number (mean of the results of three measurements) 333.7, which agrees with the calculated value. According to the results of coulometric titration and from the value of the iodine number, the number of double bonds calculated to one molecule of the arachidonic acid obtained was 4.0.

The quantitative estimation of the amount of arachidonic acid in the sample was carried out by the method of internal normalization, and also by the method of an internal standard, in which a label was used. The dynamics of the amount of arachidonic acid in the buds of Populus balzamifera are shown in Table 1. The amount of arachidonic acid in the buds of Populus balzamifera ranged from 0.2 to 8% of the sum of the acids of the phospholipids in different periods of the annual cycle.

The results obtained indicate that in the period of deep dormancy (from September to December) there is an active formation of arachidonic acid. Its amount reaches a maximum in November, corresponding to $6.74 \cdot 10^{-5}$ g/g of absolutely dry buds. The increase in the amount of arachidonic acid is apparently connected with the fact that in this period there is an active accumulation of reserve substances ensuring various physicochemical processes taking place in the spring-summer months. In the period of forced dormancy some decrease in the amount of arachidonic acid was observed ($2.05 \cdot 10^{-5}$ g/g of absolutely dry buds, January).

With the setting in of vegetative growth (for Populus balzamifera this is the March-April period), the amount of arachidonic acid again rises, reaching $4.18 \cdot 10^{-5}$ g/g of absolutely dry buds (April). This is obviously connected with the beginning of reproductive processes taking place in the tree.

EXPERIMENTAL

Buds of Populus balzamifera collected in the same region of the town of Krasnoyarsk were investigated. The samples of the buds were averaged over the height of the trunk of the tree. The lipids were extracted by the procedure for isolating lipids from plant material that is generally adopted in lipidology [11]. The total lipids were separated into individual classes by column chromatography on silica gel [11].

The TLC of the neutral lipids was carried out on glass plates (20 × 20 cm) coated with KSK silica gel containing 5% of gypsum with solvent system 1) hexane–diethyl ether–acetic acid (85:15:1). The revealing agent was an ethanolic solution of molybdophosphoric acid.

The TLC of the acids isolated from the phospholipids was performed on glass plates coated with KSK silica gel containing 5% of gypsum in solvent systems 2) chloroform–methanol–acetic acid–water (90:6:1:0.75) and 3) hexane–ether–glacial acetic acid (70:30:2).

The enzymatic hydrolysis of the phospholipids was carried out by a standard procedure [11]. The products of enzymatic hydrolysis that had been obtained were separated with the aid of two-dimensional TLC on KSK silica gel containing 5% of gypsum in solvent systems 4) chloroform–methanol–28% ammonia (65:25:5) and 5) chloroform–acetone–methanol–acetic acid–water (6:8:2:2:1). Iodine vapor was used to reveal the spots.

The TLC of the arachidonic acid isolated was performed on Silufol UV-254 plates in solvent system 6) hexane–ether (7:3). The revealing agent was iodine vapor.

The methyl esters of the acids were analyzed with the aid of a Tsvet-100 chromatograph with a flame-ionization detector fitted with an electronic integrator; a 2 mm × 2.0 m glass column filled with Chromaton NAW-DMCS 0.2-0.25 mm impregnated with 5% of SE-30 was used, with helium as the carrier gas and programmed heating of the column from 130 to 280°C at a rate of heating of 8°C/min; the evaporator temperature was 200°C. Standard arachidonic acid in the form of its methyl ester was used as marker.

The mixture of acids was methylated with freshly prepared diazomethane.

CONCLUSION

The phospholipids of the buds of Populus balzamifera L. are a source of arachidonic acid the amount of which depends on the phenological state of the tree and ranges from $0.16 \cdot 10^{-5}$ to $6.74 \cdot 10^{-5}$ g/g of absolutely dry buds.

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