PHOSPHOLIPIDS OF THE RIPENING SEEDS OF *Sophora japonica*

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The change in the class and fatty acid compositions of the phospholipids during ripening of the seeds of the Japanese Pagoda tree (buds, flowers, unripe and ripe seeds) have been studied. It has been found that the ripening of the seeds is accompanied by complex transformations in the phospholipid complex indicating the important role of phospholipids in the processes of biosynthesis.

The change in the phospholipids (PLs) of leguminous crops over the phases of the development of the plants is considered in [1, 2], and as the seeds ripen in [3-5]. There is no information in the literature on the PLs of *Sophora*.

The tree *Sophora japonica* L. (Japanese pagoda tree), family leguminosae, belongs to an oil-poor medicinal species, and it is grown in gardens and is used for providing greenery for streets.

The medicinal action of a tincture from *Sophora* buds is due to the combined flavonoids present in it [6]. The *Sophora* buds are used as a raw material for the production of a medicinal preparation of rutin [7].

We have studied the class and fatty-acid compositions of the PLs of the developing seeds of the Japanese pagoda tree growing in Tashkent. The mass flowering of this tree is observed in the middle of July, and complete ripeness at the end of October.

The samples for analysis were taken from the same tree, the buds in the period of mass budding, the flowers in the period of mass flowering, two samples of unripe seeds with an interval of 30 days, and ripe seeds.

The phytosynthesizing tissues contain relatively stable enzymes causing the degradation of lipids [8]. These enzymes can be inactivated with water (80°C) [9], with hot isopropanol [8], with 0.25% acetic acid (85°C) [10], etc.

Using fresh buds as an example, we found that the class and fatty-acid compositions of the total PLs obtained after the destruction of the enzymes both with isopropanol and with water were identical. Although this treatment of the fresh raw material prevents the formation of artifacts, under these conditions a considerable amount of nonlipid impurities passes into the extract, and this makes it necessary to perform careful additional treatment of the extracts in order to obtain the pure combined PLs.

The PLs freed from accompanying impurities were obtained in the following amounts (as percentages of the weight of the sample): for the buds 0.28; for the flowers 0.6; for the unripe seeds 0.5 (sample I) and 0.7 (sample II); and for the ripe seeds 1.1. These results indicate that during ripening the amount of PLs in the seeds increases. The same tendency has been observed in a study of the seeds of other plants [11, 12].

It is known that the role of the PLs in metabolic processes is exceptionally great [13, 14], even though their amount in various organs is relatively small.

The sum of the PLs was analyzed by two-dimensional chromatography. The results of the investigation and the class composition of the PLs of the *Sophora* seeds showed that in different stages of ripening they consisted of 8-11 components (Table 1). Quantitative analysis showed that ripening was accompanied by complex transformations in the phospholipid complex of the seeds.

As is known, the PGs and, particulary, the PAs are important intermediate products in the synthesis of the PLs, the DAGs, and the TAGs [15], being readily converted into other compounds.

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TABLE 1. Change in the Composition of the Phospholipids during the Ripening of *Sophora* Seeds

Class of PLs	Amounts of individual components, % on wt. of PLs				
	buds	flowers	unripe seeds		ripe seeds
			sample 1	sample 2	
Phosphatidylcholines	20,3	22,0	26,7	30,5	38,5
Phospatidylethanolamines (PEs)	14,0	19,2	17,0	16,2	15,1
Phosphatidylinositols (PIs)	17,0	19,2	19,0	19,3	20,1
Phosphatidic acids (PAs)	16,0	15,1	12,0	10,0	1,2
N-Acyl-PEs	9,1	7,3	7,0	6,5	6,8
N-Acyl-lyso-PEs	6,8	3,0	2,5	2,2	2,1
Phosphatidylglycerols (PGs)	13,9	8,2	7,8	7,5	5,9
Lyso-PCs	2,9	4,0	3,1	2,8	2,3
Phosphatidylserines (PSs)	—	—	1,2	Tr.	—
X_1-PLs	—	2,0	3,7	5,0	5,5
X_2-PLs	—	—	—	Tr.	2,5

TABLE 2. Change in the Fatty-Acid Composition of the Total Phospholipids during the Ripening of *Sophora* Seeds (wt.%)

Fatty acid	Buds	Flowers	Unripe seeds		Ripe seeds
			sample 1	sample 2	
10:0	1,5	1,0	0 9	1,0	0,5
12:0	1,0	0,5	0,8	1 7	0,7
14:0	0,8	0 9	1,0	1,0	1 0
15:0	1,7	0,7	1,0	1,0	Tr.
16:0	18 5	24,2	30,4	25,6	17,6
16:1	Tr.	1,0	1,0	Tr.	1,8
17:0	1,2	1,4	1,0	Tr.	Tr.
18:0	3,0	3 3	5,4	5,2	6,3
18:1	6,3	2,8	10,3	15,0	20,8
18:2	44,7	36,7	34,7	38,8	48,7
18:3	21 3	27,5	12,6	10,7	2,6
Sum of the saturated (S) acids	27,7	32,0	41,4	35,5	27,1
Sum of the unsaturated (U) acids	72,3	68 0	58 6	64,5	72,9
U/S	2,6	2,1	1,4	1,8	2,7

It is precisely these components that underwent the greatest changes. Thus, the amount of PAs fell from 16% in the buds to 1.2% in the ripe seeds and that of PGs from 13.9 to 5.9%, respectively, while PSs were present in the stage of the unripe seeds and were absent in the ripe seeds. The uniform increase in the amounts of PCs and PIs (from 20.3 and 17.0% in the buds to 38.5 and 20.1% in the ripe seeds, respectively) indicates that these components are the key products of the biosynthesis of PLs in the plant.

The considerable decrease in the proportion of PAs and PGs and the increase in the amount of PCs during the ripening of *Sophora* seeds is in harmony with literature information on the change in the group composition of the PLs in the developing seeds of the soybean [4] and of other plants [16].

Each sum of the PLs was deacylated by alkaline saponification, and the fatty acids split out were analyzed by GLC (Table 2). During the development of the *Sophora* seeds an intensive metabolism of the fatty acids of the phospholipids takes place. The greatest quantitative changes are undergone by the 16:0, 18:1, 18:2, and 18:3 acids. A uniform decrease in the ratio of the unsaturated and saturated acids up to the stage of the unripe seeds (including sample I) takes place mainly through an increase in the amount of the 16:0 and 18:0 acids and a decrease in the amount of 18:2 and 18:3 acids. The amount of the 18:1 acid rises gradually during the seed-ripening process. By the stage of complete ripeness of the seeds the amount of the 16:0 acid has fallen sharply and that of the 18:2 acid has risen. These changes in the quantitative composition of the individual fatty acids also leads to an appreciable increase in the ratio of unsaturated and saturated acids in the ripe seeds.

In the dynamics of the change in the fatty acid composition of the PLs of *Sophora*, as the seeds ripen a high metabolic activity of the 18:3 acid is observed. A considerable fall in the amount of this acid may be connected with its participation in the formation of the ethylene that is necessary for the ripening of the seeds [16, 17].

The results obtained indicate a high metabolic activity of the free acids and the importance of the functional role of the phospholipids for maintaining the stability of the biological membranes during the ripening of *Sophora* seeds.

EXPERIMENTAL

To determine the composition of the total PLs we used two-dimensional TLC in a layer of silica gel in the following solvent systems: chloroform–methanol–25% ammonia (65:35:5) (direction I), and cloroform–methanol–acetone–acetic acid–water (10:5:4:2:1) (direction II). The PLs were identified by comparison with markers and by means of characteristic reactions. The quantitative compositions of the individual classes of PLs were determined on the basis of an analysis of the phosphorus content of each spot and its relationship to the total [18].

The fatty acids were isolated after alkaline hydrolysis by a known method [19]. The fatty acid methyl esters were analyzed on a Chrom-4 instrument with a flame-ionization detector using a 4 mm × 2.5 m column filled with Chromaton N-AW impregnated with 15% of Reoplex-400, at 210°C and with a rate of flow of the carrier gas (helium) of 115 ml/min.

Extraction of the Lipids from *Sophora* Buds. The freshly gathered buds were covered with boiling isopropanol. After 2-3 min, the solvent was poured off and the buds were ground in a mortar in the presence of hot isopropanol. The hot solution was filtered with suction and the residue on the filter was washed with boiling isopropanol. This operation was repeated twice. Then the residue was ground in a mortar in a mixture of chloroform and methanol (2:1), the mixture was filtered, the filtrate was transferred to separatory funnel, and extraction with the mixture given above by the steeping method was carried out six times. All the filtrates were combined and were evaporated in vacuum to dryness. The residue was a green mass sparingly soluble in chloroform. The further treatment of the combined lipids was performed by known methods [20].

SUMMARY

The change in the class and fatty-acid compositions of the phospholipids during the ripening of the seeds of the Japanese pagoda tree has been studied.

It has been found that the ripening of the seeds is accompanied by profound transformations in the phospholipid complex showing the important role of phospholipids in the processes of biosynthesis.

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LIPIDS FROM EXTRACTS OF *Chlorella vulgaris*

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The composition of ethanolic (I) and gasoline (II) extracts of the cultivated microalga *Chlorella vulgaris* has been studied. With the aid of CC, TLC, qualitative reactions, GLC, and UVS the following classes of compounds have been detected in them. From the neutral lipids: hydrocarbons, carotenoids, traces of sterols and their esters, fatty acid esters, tri- and diacylglycerols, free fatty acids, and chlorophylls; and from the polar lipids: di- and monogalactosylglycerols, phosphatidylethanolamine, lecithin, phosphatidylinositol, phosphatidylserine, and three sphingosine bases. The polar lipids I and II made up 52.4 and 50.2% of the total, respectively. As compared with extract I, extract II was somewhat enriched with neutral lipids, including provitamins of the A group and vitamins of the F group. In the fatty acids of chlorella, 19 components were detected, the main ones being the 16:0 acid and 18:2 and 18:3 acids.

Green and blue-green algae are rich sources of proteins, carbohydrates, vitamins, and lipids [1]. A technology has been developed for the industrial cultivation of some species of these algae, including chlorella, the biomass of which is used as a protein-vitamin additive in animal husbandry, sericulture, and plant growing [2].

An intensive study of the lipids of green and blue-green algae is being performed mainly for scientific purposes: The role of the composition and structure of the lipids in the process of photosynthesis, in the adaptation of the algea to varying conditions of growth, and the value of this class of compounds in chemotaxomony are being investigated [3].

At the same time, the production by some species of algae of a considerable amount of lipids and the pronounced physiological activity of a number of lipid extracts [4] shows the value of the lipids as independent components and the desirability of the complex processing of the algal biomass.

The composition of algal lipids is very labile and is determined largely by the conditions of growth [3, 5].

In the present paper we give information on the composition of the lipids of two samples of extracts of the cultivated alga *Chlorella vulgaris*: an ethanol extract (sample 1) and a gasoline extract (sample 2).

The extracts were separated by column chromatography on silica gel. This led to the isolation of a number of individual classes of lipids and also of some mixed fractions which were then separated by preparative TLC.

The assignment of the lipids to definite classes was made from their chromatographic mobility on TLC in systems 1-5 in comparison with model samples, and also by comparison with literature information [6].

The compositions of the lipids of the two chlorella extracts are given below (% on the weight of the extracts):

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