AN INVESTIGATION OF THE STRUCTURE OF THE PHOSPHOLIPIDS OF THE COTTON PLANT OF VARIETY TASHKENT-2

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Continuing a study of the phospholipids (PLs) of wilt-resistant varieties of the cotton plant [1, 2], we have investigated the structure of the main components of the total phospholipids of the seed kernels of the cotton plant of variety Tashkent-2 of the 1972 harvest. The sum of the PLs from the previously acetone-defatted seed kernels obtained by a known method [3] contains a considerable amount of carbohydrates (35-50%). They were freed from carbohydrates by gel filtration through Molselekt G-25 [4]. The yield of purified PLs was 1.3% of the weight of the air-dry kernels, and their phosphorus content was 3.3% [5].

The carbohydrates accompanying the phospholipids were identified qualitatively as disaccharides. After the extraction of the neutral lipids and the PLs, the amount of phytin in the meal was determined (6% of the defatted raw material). The combined PLs were separated into alcohol-soluble and alcohol-insoluble fractions. The group quantitative compositions of the individual components of the combined PLs and their alcohol-soluble and alcohol-insoluble fractions (%) were determined from the phosphorus contents of the spots on a two-dimensional chromatogram:

Phospholipids	Total PLs	Alcohol-soluble fraction	Alcohol-insoluble fraction
Phosphatidylcholines (PCs)	49.6	70.1	6.3
Phosphatidylethanolamines (PEs)	13.0	15.5	27.4
Phosphatidylinositols (PIs)	27.0	6.4	59.6
Lyso-PCs	3.0	3.3	-
Unidentified: X_1	3.3	1.0	4.6
X_2	4.1	3.7	2.1

For separation we used the following systems: 1) chloroform—methanol—ammonia (65:35:5); 2) chloroform—acetone—methanol—acetic acid—water (5:2:1:1:0.5) [6].

Eight spots were detected on the chromatograms, of which six contained phosphorus. Two substances of low polarity were identified by qualitative reactions as steroids [7]. On the basis of often repeated two-dimensional chromatography of the fresh total PLs it may be concluded that the lyso-PCs are a normal product of the metabolism of the cotton plant.

The alcohol-soluble and alcohol-insoluble fractions of the total PLs were chromatographed in two columns of silica gel. The substances were eluted from the columns with mixtures of chloroform and methanol of increasing polarity. Additional purification by preparative TLC yielded chromatographically pure fractions of the PLs, the structures of which were confirmed by their IR spectra, determinations of their N and P contents, and also a study of the products of acid hydrolysis. The IR spectra of the PLs investigated corresponded to those given in the literature for glycerophospholipids [8, 9, 2].

PCs (%): N 1.56, P 3.45; N/P 1.0; $[\alpha]_D$ +2.5° (c 3.0; chloroform). Glycerol and choline were identified among the products of acid hydrolysis.

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TABLE 1

Fatty acid	Triglyceride	Sum of the phospholipids	PCs			PEs			PIs		
			initial	position		initial	position		initial	position	
				α	β		α	β		α	β
$C_{10:0}$	—	2,1	0,8	—	3,3	2,4	3,2	2,8	3,3	2,2	3,1
$C_{12:0}$	—	1,2	0,6	—	1,9	2,1	2,1	2,2	0,9	1,1	2,0
$C_{14:0}$	2,0	1,3	0,6	2,6	1,5	2,7	2,6	2,0	0,8	1,4	2,0
$C_{16:0}$	22,7	23,1	18,0	33,7	1,5	29,3	57,7	5,8	31,3	58,6	2,2
$C_{16:1}$	3,8	2,3	2,2	4,2	1,6	3,1	4,8	1,5	2,4	3,1	—
$C_{18:0}$	3,3	3,0	3,4	5,9	—	2,3	3,6	1,3	6,9	12,0	—
$C_{18:1}$	18,1	17,7	23,4	21,2	24,2	10,5	4,7	14,6	9,8	7,6	7,7
$C_{18:2}$	50,1	49,3	51,0	32,4	66,0	47,6	21,3	69,8	44,6	14,0	83,0
$\Sigma_{sat.fas}$	28,0	30,7	23,4	42,2	8,2	38,8	69,2	14,1	43,2	75,3	9,3
$\Sigma_{unsat.fas}$	72,0	69,3	76,6	57,8	91,8	61,2	30,8	85,9	56,8	24,7	90,7

TABLE 2

Molecular form	PCs	PEs	PIs	Molecular form	PCs	PEs	PIs
	%				%		
10:0/10:0	—	0,1	0,1	14:0/18:1	0,6	0,4	0,1
12:0/10:0	—	0,1	+	16:0/18:1	8,2	8,4	4,5
14:0/10:0	0,1	0,1	+	18:0/18:1	1,4	0,5	0,9
16:0/10:0	1,1	1,6	1,9	10:0/18:2	—	2,2	1,8
18:0/10:0	0,2	0,1	0,4	12:0/18:2	—	1,5	1,0
10:0/12:0	—	0,1	+	14:0/18:2	1,7	1,8	1,2
12:0/12:0	—	+	+	16:0/18:2	22,2	40,3	48,5
14:0/12:0	+	0,1	+	18:0/18:2	4,0	2,5	10,0
16:0/12:0	0,7	1,2	1,2	16:1/10:0	0,1	0,1	0,1
18:0/12:0	0,1	0,1	0,2	18:1/10:0	0,7	0,1	0,2
10:0/14:0	—	0,1	+	18:2/10:0	1,1	0,6	0,4
12:0/14:0	—	+	+	16:1/12:0	0,1	0,1	0,1
14:0/14:0	+	0,1	+	18:1/12:0	0,4	0,1	0,2
16:0/14:0	0,5	1,1	1,2	18:2/12:0	0,6	0,5	0,3
18:0/14:0	0,1	0,1	0,2	16:1/14:0	0,1	0,1	0,1
10:0/16:0	—	0,2	+	18:1/14:0	0,3	0,1	0,2
12:0/16:0	—	0,1	+	18:2/14,0	0,5	0,4	0,3
14:0/16:0	+	0,2	+	16:1/16:0	0,1	0,3	0,1
16:0/16:0	0,5	3,3	1,3	18:1/16:0	0,3	0,3	0,2
18:0/16:0	0,1	0,2	0,3	18:2/16:0	0,5	1,2	0,3
10:0/18:0	—	+	—	10:1/18:0	—	0,1	—
12:0/18:0	—	+	—	18:1/18:0	—	0,1	—
14:0/18:0	—	+	—	18:2/18:0	—	0,3	—
16:0/18:0	—	0,8	—	16:1/16:1	0,1	0,1	—
18:0/18:0	—	+	—	18:1/16:1	0,3	0,1	—
10:0/16:1	—	+	—	18:2/16:1	0,5	0,3	—
12:0/16:1	—	+	—	16:1/18:1	1,0	0,7	0,2
14:0/16:1	+	+	—	18:1/18:1	5,1	0,7	0,6
16:0/16:1	0,5	0,9	—	18:2/18:1	8,0	3,1	1,1
18:0/16:1	0,1	0,1	—	16:1/18:1	2,8	3,3	2,6
10:0/18:1	—	0,5	0,2	18:1/18:2	14,0	3,3	6,3
12:0/18:1	—	0,3	0,1	18:2/18:2	21,3	14,9	11,6

Note. The symbol + shows the presence of the components in an amount of less than 0.1%.

PEs (%): N 1.32; P 3.2; N/P 0.91; $[\alpha]_D$ +2° (c 3.0; chloroform). Glycerol and ethanolamine were the products of acid hydrolysis.

PIs (%): P 3.6, N none; $[\alpha]_D$ +3° (c 3.3; chloroform). On acid hydrolysis, glycerol and inositol were formed.

To study their fatty-acid composition, the total PLs and their main components were subjected to alkaline hydrolysis, and the acids liberated, in the form of their methyl esters, were analyzed by GLC. A chromatogram of the fatty acids of the oil [10], which was obtained by Stahl's method [11], was recorded [10] (Table 1).

The compositions of the fatty acids of the combined material and of the individual fractions were similar qualitatively but differed quantitatively. The degree of saturation of the molecules of the main components rose in the following sequence: PCs → PEs → PIs.

In order to determine the position distribution of the fatty acids, the PLs under investigation were cleaved by phospholipase A in tris buffer (pH 9.4) at 36-38° (bath temperature). The time of enzymatic hydrolysis was considerably shorter than in previous cases [2] (15 min, 4 h, and 6 h for the PCs, PEs, and PIs, respectively). The products of enzymatic hydrolysis were separated and analyzed as described previously [2]. The results on the position distribution of the fatty acids in the PLs (see Table 1) showed that the low-molecular-weight 10:0 and 12:0 acids were absent from the α positions of the PCs, and in the PEs and PIs these acids were distributed uniformly in both positions.

The specificity in the position distribution of the fatty acids (predominant occurrence of unsaturated acids in the β position) in the PE was less pronounced than for the PIs and PCs (85.9, 90.7, and 91.8%, respectively).

On the basis of the results on the position distribution of the fatty acids in the PLs, using a known method [12], we calculated the possible molecular compositions of the PCs, PEs, and PIs (Table 2). As follows from the table, in the PCs, the PEs, and the PIs the saturated–saturated forms amount to 3.4, 9.7, and 6.8%, the saturated–unsaturated forms to 38.7, 59.4, and 68.3%, the unsaturated–saturated forms to 4.8, 4.4, and 2.5%, and the unsaturated-unsaturated forms to 53.1, 26.5, and 22.4%, respectively.

In all the PLs, the saturated–saturated forms contain mainly the 16:0 acid, the saturated–unsaturated forms the 16:0 and 18:2 acids, and the unsaturated–unsaturated forms the 18:1 and 18:2 acids. The number of molecular forms in the PCs is smaller than in the PIs and, particularly, in the PEs, which is due to the absence of low-molecular-weight acids from the α position and of the 18:0 acid from the β position of PC molecule.

EXPERIMENTAL METHOD

The following solvent systems were used for TLC (KSK silica gel): 1) chloroform–methanol–water (65:35:5); 2) chloroform–methanol–25% ammonia (65:35:5). In column chromatography, the fractions were eluted successively with acetone (steroids), chloroform, mixtures of chloroform and methanol (95:5; 9:1; 4:1; 2:1), and methanol.

The IR spectra were taken on a UR-20 spectrometer in the form of films. The gas–liquid chromatography of the mixtures of acids in the form of their methyl esters was performed on a UKh-2 chromatograph in a column 2.5 m long with poly(ethylene succinate) at 197°C. The carrier gas was helium.

The acid hydrolysis of the individual phospholipids was performed in sealed tubes with 3 N HCl at 100°C for 24 h. The amines (choline, ethanolamine) and polyols (glycerol, inositol) that were split off were identified with markers by TLC in the isopropanol–25% ammonia–water systems (5:4:1) [13] and (49:7:14) [14]. Enzymatic hydrolysis was performed by the method described previously [2], but at 37-38°C and without the addition of $CaCl_2$.

The mixture of fatty acids from the seed oil of the cotton plant of the variety Tashkent-2 was isolated by cold saponification with 1 N KOH/CH_3OH. The acids were subsequently treated as described in the literature [11].

Purification of the Combined Phospholipids from Carbohydrates. The gel (1 g) swollen in a mixture of chloroform, methanol, and water (90:10:1) was charged into the column and 0.5 g of the total phospholipids in the form of a 1% solution in the same mixture was added. The eluent was monitored by TLC in the chloroform–methanol–water (65:35:5) system (chromogenic agent 50% sulfuric acid). The elimination of the carbohydrates was complete (the yield of purified total phospholipids was 0.3 g). The carbohydrates were eluted from the column with aqueous methanol.

Acid Hydrolysis of the Carbohydrates. The carbohydrates were boiled under reflux with 1 N sulfuric acid for 24 h. The solution was treated with barium carbonate and filtered, and the filtrate was analyzed by PC (FN7 paper) in the butanol–pyridine–water (6:4:3) system. The chromogenic agent was a solution of aniline phthalate.

Glucose and galactose were found among the hydrolysis products.

SUMMARY

1. The fatty-acid compositions of the total phospholipids of the seed kernels of the cotton plant of variety Tashkent-2, after the elimination of carbohydrates, and of their main components have been studied.

2. It has been found that at 37-38°C the time of enzymatic hydrolysis is considerably shortened.

3. A study of the products of acid and enzymatic hydrolysis has shown the structure of the individual phospholipids.

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COMPOSITION OF THE SEED OILS OF *Origanum tyttanthum* AND *Mentha asiatica*

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Continuing a study of the oils of various species of the family Labiatae [1, 2], we have investigated the compositions of the fatty oils of *Origanum tyttanthum* and *Mentha asiatica*, growing in the Uzbek SSR. These oils have not been studied previously.

The oils isolated from the purified comminuted seeds of the plants mentioned by extraction with petroleum ether were greenish-yellow mobile liquids with a weak sage-like odor which were similar to one another in their main indices (Table 1).

The UV spectra of the oils investigated and the IR spectrum of the *O. tyttanthum* oil showed no specific absorptions or bands. The IR spectrum of the *M. asiatica* oil had weak bands of a hydroxy group at 3450 and 1070 cm^{-1} which were retained in the spectrum of the methyl esters of the fatty acids (MEs). In addition, in the UV spectrum of the MEs of the *M. asiatica* weak absorption appeared at λ_{max}^{hexane} 280 nm.

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