PHOSPHOLIPIDS OF THE COTTON PLANT OF VARIETY TASHKENT-3.
II. STRUCTURE OF THE MAIN PHOSPHOLIPID FRACTIONS

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Continuing an investigation of the phospholipids of the cotton plant of variety Tashkent-3 [1], we have studied the composition of the combined and the individual components. The combined phospholipids were obtained by a known method.

After the extraction of the phospholipids (PLs), the meal yielded 6% of phytin. Fractionation of the combined PLs into their components was performed on columns of silica gel after they had been separated into alcohol-soluble and alcohol-insoluble fractions. The columns were eluted with $CHCl_3$–CH_3OH mixtures with a gradually increasing concentration of methanol. The fractions were additionally purified by preparative thin-layer chromatography, and chromatographically pure phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), and the minor fractions PL–X_2 and PL–X_3 were isolated.

The main phospholipids obtained in this way had the following indices: PCs–P/N = 0.93; PEs–P/N = 1.14; PIs–N, none, P 3.6%.

The IR spectra of these main fractions and also of the two minor fractions agreed with those in the literature for glycerophospholipids [2, 3]. These substances were identified by means of characteristic color reactions in a thin layer of silica gel. In system 2, substances X_2 and X_3 had R_f 0.6 and 0.9, respectively. Both substances were revealed only by the Vas'kovskii reagent.

For identification, acid hydrolysis of the homogeneous fractions was performed, with the subsequent comparison of the hydrolysis products with markers by thin-layer chromatography in system 3. The chromogenic agents were the Dragendorff reagent, ninhydrin solution, a 0.5% solution of $KMnO_4$, and potassium metaperiodate–benzidine. In all the fractions, glycerol and fatty acids were detected after hydrolysis. In addition, in the case of the PCs choline was found, in the case of the PEs ethanolamine, in the case of the PIs inositol, and for substance X_3 a ninhydrin-positive spot which, after comparison with markers by TLC in system 4, proved to be ethanolamine.

The fatty-acid compositions of the total phospholipids and of the homogeneous fractions were determined by alkaline hydrolysis. For comparison, the fatty acids of the triglycerides of the oil were also isolated and analyzed [4].

The results of the GLC analysis showed that the compositions of the fatty acids of the oils and of the combined phospholipids were identical qualitatively and similar quantitatively. The fatty-acid compositions of the individual phospholipid fractions had some differences: the PEs lacked the low-molecular-weight $C_{10:0}$ and $C_{12:0}$ acids, and the PIs, X_2, and X_3 lacked the $C_{10:0}$ acid.

With respect to increasing saturation, the individual phospholipid fractions formed the following sequence: $X_3 \rightarrow PCs \rightarrow PEs \rightarrow PIs \rightarrow X_2$.

On the basis of the specific action of phospholipase A [5], we performed the enzymatic hydrolysis of the PCs, PEs, and PIs to determine the position distribution of the fatty acids.

This gave the fatty acids in the β position. The products of enzymatic hydrolysis were separated by preparative thin-layer chromatography (TLC), and the lysophospholipids were

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TABLE 1. Fatty-Acid Compositions of the Phospholipids and the Position Distributions of the Fatty Acids, %

Fatty acid	Tryglyceride	Combined phospholipids	Phosphatidylcholines			Phosphatidylethanolamines			Phosphatidylinositols			X_2	X_3
			initial	position		initial	position		initial	position			
				α	β		α	β		α	β		
10:0	3,4	0,8	3,6	7,4	6,1	—	—	—	—	—	—	—	—
12:0	0,9	0,7	0,5	0,6	1,1	—	—	—	1,3	1,7	4,4	9,6	1,6
14:0	0,8	0,5	0,4	0,4	0,6	0,1	0,4	0,6	0,6	1,1	2,1	7,4	1,3
16:0	22,5	24,8	13,4	23,7	1,5	20,9	42,7	1,5	33,1	55,7	5,4	29,7	8,3
16:1	0,9	2,0	0,8	0,9	0,4	1,3	1,6	0,6	1,5	3,3	—	7,5	1,5
18:0	1,3	0,6	2,1	4,4	—	1,2	2,9	—	5,3	10,8	2,1	10,2	3,1
18:1	15,0	13,5	26,5	24,7	26,0	12,7	8,0	14,0	9,6	9,7	9,7	13,2	11,5
18:2	55,2	57,1	52,7	37,9	64,3	63,8	44,4	83,3	48,6	17,7	76,3	22,4	72,7
Sum of the saturated acids	28,9	27,4	20,0	36,5	9,3	22,2	46,0	2,1	40,3	69,3	14,0	56,9	14,3
Sum of the unsaturated acids	71,1	72,6	80,0	63,5	90,7	77,8	54,0	97,9	59,7	30,7	86,0	43,1	85,7

hydrolyzed with methanolic alkali. The fatty acids from the α and β positions were methylated with diazomethane, and their methyl esters were analyzed by gas–liquid chromatography. The results are given in Table 1.

On the basis of the results of the position distribution of the fatty acids the possible diglyceride compositions of the phospholipids were calculated by Coleman's method [6] as modified by Markman et al. [7, 8]. It was calculated that the number of molecular species in the PEs was smaller than in the PIs and PCs, which is due to the absence of the $C_{10:0}$ and $C_{12:0}$ acids in the initial molecule. In the PCs, the disaturated PCs are formed mainly from the low-molecular-weight $C_{10:0}$ and $C_{12:0}$ acids and the $C_{16:0}$ acid; in the PEs and PIs they are formed mainly from the $C_{16:0}$ acid.

In the PIs the amount of diunsaturated species is considerably smaller than in the PEs and PCs, and saturated–unsaturated species predominate. In all the individual fractions, the diunsaturated species are formed mainly from the $C_{18:1}$ and $C_{18:2}$ acids. On summarizing the results of calculation, the following possible group diglyceride compositions of the phospholipids are obtained (%):

Molecular Species	PCs	PEs	PIs
Disaturated	3.3	1.0	9.6
including monoacid	0.9	0.7	3.3
Saturated–unsaturated	33.1	45.1	59.6
Unsaturated–saturated	6.0	1.1	4.4
Diunsaturated	57.6	52.8	26.4
including monoacid	30.8	38.2	14.5

EXPERIMENTAL METHOD

We used type KSK silica gel with a particle size of 160-250 μ for column chromatography and with a particle size of about 125 μ for TLC. Solvent systems for TLC: 1) $CHCl_3$–CH_3OH–H_2O (65:35:5); 2) $CHCl_3$–CH_3OH–25% NH_3 (65:35:5); 3) isopropanol–25% NH_3–H_2O (7:1:2) [9]; and 4) 2% NH_3–CH_3OH (2:3) [10]. Chromatograms of the methyl esters of the fatty acids were obtained on a UKh-2 gas–liquid chromatograph with a column 3 m long containing as the solid phase Celite-545 (80-100 mesh) impregnated with 17% of poly(ethylene succinate); temperature 196-198°C; carrier gas helium. The IR spectra were recorded on a UR-20 instrument in the form of films. The quantitative determinations of phosphorus were performed by a known method.

Isolation and Separation of the Combined Phospholipids. Seed kernels of the cotton plant of variety Tashkent-3 that had been comminuted and dried over sulfuric acid were defatted with acetone, and the combined phospholipids were extracted with $CHCl_3$–CH_3OH (2:1). The resulting extract was dissolved in chloroform and the solution was filtered. The phospholipids were precipitated from the chloroform solution with acetone. The precipitate was separated off by centrifuging.

To free them from carbohydrates, the combined phospholipids were passed in $CHCl_3$–CH_3OH–H_2O (90:10:1) through a column of Molselekt G-25 previously swollen in the same mixture. The eluate was monitored by TLC in system 1. The elimination of the carbohydrates was complete. The yield of purified combined phospholipids was 1.3% of the weight of the air-dry kernels. The combined phospholipids obtained were separated into alcohol-soluble and alcohol-insoluble fractions, and each, separately, was chromatographed on columns of silica gel. Elution was performed with acetone, chloroform, chloroform–methanol mixtures in various ratios, and with methanol. In all cases, mixtures of two or three phospholipids were obtained which were then separated preparatively in systems 1 and 2.

The acid and alkaline hydrolyses of the individual phospholipids were performed as described previously [11, 12].

Enzymatic Hydrolysis. To 87 mg of the PCs dissolved in 15 ml of ether was added 3 mg of the venom of the Azerbaidzhan kufi [*Vipera lebetina* L.] in 0.4 ml of 0.1 M tris buffer (pH 9.7). The reaction mixture was stirred mechanically (20°C) and hydrolysis was completed in 1 h.

The β fatty acids were separated from the lysophosphatidylcholines (LPCs) by preparative TLC in system 1. The LPCs were hydrolyzed with methanolic alkali, and the liberated fatty acids (α position) were extracted with ether. Both the α and the β fatty acids were investigated by GLC.

The enzymatic hydrolysis of the PEs and PIs was performed similarly, but at 36-38°C for 6 h. The separation and analysis of the hydrolysis products were performed as described for the PCs.

SUMMARY

1. Individual fractions of phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and the X_2 and X_3 phospholipids have been obtained from the combined phospholipids, freed from carbohydrates, of the seed kernels of the cotton plant of variety Tashkent-3.

2. The compositions and position distributions of the fatty acids in the main fractions of phospholipids have been studied.

3. The possible molecular composition of the phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols have been calculated.

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