ISOLATION OF THE PHOSPHOLIPIDS OF THE COTTON PLANT AND THEIR COMPOSITION

L. A. Shustanova, A. Sh. Isamukhamedov, and S. T. Akramov

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We have investigated the phospholipids of the seed kernels of cotton plants of the medium-fiber variety S-4727 and the thin-fiber variety S-6029. The indices of the seed kernels of the cotton plants were as follows (%):

Variety	Kernel content	Oil content	Total P	Lipid P	Lipid P on the total
S-4727	56.3	38.8	1.12	0.057	5.09
S-6029	58.1	35.4	1.12	0.058	5.17

The amounts of lipid phosphorus were found from the figures of Table 1.

The presence of gossypol in cotton seed kernels was responsible for a specific feature of the isolation of the free and bound lipids. In contrast to other authors, who used acetone for defatting the kernels [1, 2] and extracted the free and bound lipids together [3], we extracted the free lipids by the successive treatment of the comminuted kernels with petroleum ether and acetone and the bound lipids with a mixture of chloroform and methanol (2: 1) [4]. This change of solvents enabled us to obtain a large fraction of free lipids practically free from gossypol, the latter being concentrated in the acetone fraction.

The yields of extractive substances and of lipid phosphorus in the extraction of the free and bound lipids are shown in Table 1. As can be seen from Table 1, 7.7% of the lipid phosphorous in the variety S-4727 is present in the bound state, and in S-6029 the corresponding figure is 5.1%. Qualitative chromatography in a thin layer of silica gel showed that this lipid phosphorus is in the form of phosphatidylcholines.

The bulk of the phospholipids was extracted together with the other bound lipids by the mixture of chloroform and methanol. The completeness of the extraction of the phospholipids was checked by treating the extracted meal with water-saturated butan-1-ol [5]; this isolated mainly the inorganic phosphorus (about 1% of the total), since no appreciable amounts of phospholipids were found on a qualitative chromatogram. The results obtained show that in the kernels of the seeds of variety S-4727 the lipid phosphorus amounted to 5.09% of the total and in variety S-6029 to 5.17%.

In the extraction of the bound lipids, carbohydrates were extracted with them, and these were separated partially in the form of crystalline precipitates when the crude phospholipids were treated with petroleum ether and then with chloroform [6]. The precipitates of the carbohydrates of the kernels of the seeds of variety S-4727 isolated in this way amounted to 2.1% of the total extracted substances and contained 2.3% of the lipid phosphorus. For the variety S-6029 these figures were 0.9 and 0.6%, respectively.

Petroleum ether—chloroform solution of the bound lipids were washed with water to eliminate water-solubrle impurities and, after concentration, the phospholipids were precipitated with acetone. In the acetonic purification, the yield of lipid phosphorus was 95-97%. The yield of the combined phospholipids of the kernels of seeds of variety S-4727 was 1.34% with a phosphorus content of 2.70%; for S-6029 the corresponding figures were 1.4 and 2.57%.

The qualitative and quantitative compositions of the phospholipids were determined by

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TABLE 1

Extractant and extractive substances	Variety of cotton plant	Yield of the fraction		Content of p in the fraction	Distribution of the lipid P	
		on the kernels	on all the fractions		on the kernels	on the total P
		%				
Free lipids						
Petroleum ether (glycerides, fatty acids, traces of gossypol, etc.)	S-4727	31,3	72,4	0,0045	0,0014	2,5
	S-6029	26,3	72,4	0,0038	0,001	1,7
Acetone (gossypol, glycerides, fatty acids, phospholipids, etc.)	S-4727	6,1	14,2	0,05	0,003	5,2
	S-6029	5,0	13,5	0,046	0,002	3,4
Bound lipids						
Chloroform–methanol (2:1) (phospholipids, carbohydrates, glycerides, etc.)	S-4727	6,1	13,4	1,20	0,0525	92,3
	S-6029	5,3	14,4	1,12	0,055	94,9

TABLE 2

Fatty acids*	Petroleum ether extract		Acetone extract		Glycerides		Total phospholipids	
	%							
	S-4727	S-6029	S-4727	S-6029	S-4727	S-6029	S-4727	S-6029
	Free lipids				Bound lipids			
10:0	0,5	2,2	0,4	1,2	1,0	2,2	0,6	3,5
12:0	0,4	0,9	0,3	0,6	1,0	1,8	0,5	0,7
14:0	0,5	0,7	0,5	0,5	1,1	1,9	0,5	0,5
16:0	24,0	23,4	22,8	23,5	21,7	21,0	25,4	27,9
16:1	Tr	1,3	0,5	1,2	1,0	2,1	0,7	0,9
18:0	0,7	0,8	1,4	1,1	1,2	0,2	2,3	0,9
18:1	18,1	13,6	25,4	21,6	16,9	14,4	17,3	13,2
18:2	54,3	57,1	48,1	50,3	55,4	56,4	52,0	52,4
18:3	1,5	0	0,7	0	0,7	0	0,7	0
Total saturated	26,1	28,0	25,3	26,9	26,0	27,1	29,3	33,5
Total unsaturated	73,9	72,0	74,7	73,1	74,0	72,9	70,7	66,5

*The figure before the colon denotes the number of carbon atoms in the chain of the acid, and the figure after the colon the number of double bonds.

two-dimensional chromatography in a thin layer of silica gel. In the case of variety S-4727 a chromatogram showed eleven spots of substances, seven of which proved to contain phosphorus. The spots were identified by specific reagents for the determination of the functional groups of phospholipids [7] and by comparison with markers. In solvent system I the spots had the following R_f values: 1 — 0.81; 2 — 0.78; 3 — 0.68; 4 — 0.63; 5 — 0.57; 6 and 7 — 0.40; 8 — 0.28; 9 — 0.20; 10 — 0.08; 11 — 0.02. Substances 6 and 7 were separated by two-dimensional chromatography.

The following phospholipids were identified: 2 — x_1-polyglycerophosphatides (2.8%); 4 — x_2-polyglycerophosphatides (5.8%); 5 — phosphatidylethanolamines (11.4%); 6 — phosphatidylcholines (52.0%); 7 — phosphatidylinositols (21.8%); 8 — lysophosphatidylcholines (1.7%); and 9 — an unidentified phospholipid (4.5%). By qualitative reactions [8], substances 1 and 3 were identified as sterol-containing, and 10 and 11 were identified as carbohydrates [7].

We have published information on the phospholipid composition of the kernels of seeds of variety S-6029 previously [9]. In this variety, on the whole, the same phospholipids and

substances composing them were found as in variety S-4727; only the x_1-polyglycerophosphatides were not found. In both varieties, the main component, involving half the lipid phosphorus, consisted of phosphatidylcholines and the bulk of the other half consisted of phosphatidylinositosl, and then phosphatidylethanolamines; polyglycerophosphatides, lysophosphatidycholines, and an unidentified polar lipid were present in small amounts. We determined and compared with one another the fatty-acid compositions of all the lipid fractions obtained in the extraction of the phospholipids (Table 2).

The fatty-acid compositions of the free and the bound lipids (glycerides) and the phospholipids were qualitatively identical, but the lipids of variety S-4727 contained small amount of linoleic acid. On the whole, the fatty-acid composition of this variety has a somewhat more unsaturated nature than for variety S-6029, and this was particularly noticeable for the combined phospholipids. The fatty-acid compositions of the combined phospholipids of both varieties were more saturated than the other lipid fractions because of a higher content of palmitic acid, which is in harmony with literature information for phospholipids of other oil crops [10-12]. Of the unsaturated fatty acids, linoleic and oleic predominated.

In both varieties of the cotton plant, the freee lipids extracted by acetone contained more oleic acid and less linoleic acid than the bulk of the glycerides, which was extracted by petroleum ether; the amount of stearic acid in the free lipids was not much more than one third of that in variety 108-F and the new "Tashkent" breeding varieties [13].

EXPERIMENTAL METHOD

The solvents were prepared by generally used methods [14]. For chromatography in a thin leyer (TLC) we used type KSK silica gel (less than 150 mesh) fixed with 5% of gypsum. Solvent systems for two-dimensional chromatography: I) chloroform–methanol–water (65:25:4); II) chloroform–methanol–ammonia (14:6:1) [15].

For qualitative TLC we used one-dimensional chromatography and the solvent systems mentioned.

Extraction of Phospholipids. All the extraction operations were performed at room temperature and the extracts were evaporated in a rotary evaporator at 35-40°C under vacumm in an atmosphere of nitrogen and were then brought to constant weight in a vacuum-drying chest at the same temperature. They were filtered through a No. 4 porous glass filter under vacuum.

The seed kernels (25 g) were ground and extracted with petroleum ether by steeping for 30 min (3 × 75 ml) and were filtered off and were then treated with acetone (10 × 75 ml). The combined lipids (including the phospholipids) were extracted by steeping the defatted kernels with 75 ml of a mixture of chloroform and methanol (2:1) for 16-18 h and filtering and treating the residue with the same mixture of solvents (9 × 75 ml, without steeping). The combined chloroform–methanol extracts were evaporated to dryness and treated successively with petroleum ether (3 × 15 ml) and chloroform (3 × 20 ml). The precipitates of hydrocarbons were filtered off or were separated by centrifuging (4000 rpm). The combined filtrates were washed with water (25% by volume) twice to eliminate water-soluble impurities and were evaporated. The residue was dissolved in chloroform (double volume), precipitated with a twelvefold amount of acetone, left at −15 to −20°C for 16-18 h, and filtered off, and the residue was washed with cooled acetone (2 × 10 ml) and dissolved in chloroform. The chloroform solution of phospholipids was evaporated and dried to constant weight, and their yield was determined and analyzed.

Determination of Phosphorus. In the free lipids, the phosphorus was determined by the gravimetric method [7], and in the other lipid fractions in aliquots by Tevkelov's colorimetric micromethod [16].

The group compositions of the phospholipids were determined from the amounts of phosphorus in the spots of the substances obtained by separating the phospholipid fraction by two-dimensional thin-layer chromatography. After the chromatograms had been run and the solvent had been evaporated off, the plates were sprayed with 50% sulfuric acid and were heated at 150°C for 50 min. The carbonized spots corresponding to the individual phospholipids were scraped into test tubes and treated as recommended by Dyatlovitskaya et al. [15]. The mineralization of the samples and the determination of the phosphorus were performed as described in Stahl's Handbook [16].

Determination of the Fatty Acids. A 20-30-mg sample of lipids was subjected to cold saponification [17], and the fatty acids were isolated, methylated with diazomethane, and analyzed in a UKh-2 gas–liquid chromatograph at 197-198°C; column 2.25 m × 4 mm with 17% of PÉGS on Celite-545 (80-100 mesh); carrier gas helium.

SUMMARY

The yields of the free and bound lipids of the seed kernels of cotton plants of varieties S-4727 and S-6029 have been determined by their successive treatment with selective solvent, and the total and fractional proportions of lipid phosphorus have been found.

The phospholipids have been solated and their group composition has been determined; it has been established that the main components are phosphatidylcholines, phosphatidylinositols, and phosphatidylethanolamines, while polyglycerophosphatides, lysophosphatidylcholines, and unidentified polar phospholipid are present in small amounts.

The fatty-acid compositions of the free and bound lipids and phospholipids have been studied.

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