TABLE 1. Fatty-Acid Compositions of the Minor Fractions of the Phospholipids, %

Fatty acid	Lysophosphatidylcholine	N-Acyllysophosphatidylethanolamine (X_2-phospholipid)	N-Acylphosphatidylethanolamine (X_1-phospholipid)		
			total	O-acyl	N-acyl
$C_{10:0}$	1,6	—	4,0	3,9	—
$C_{12:0}$	1,4	6,9	5,5	2,1	6,7
$C_{14:0}$	1,2	5,2	6,6	2,3	4,7
$C_{16:0}$	19,2	18,8	32,0	29,3	42,6
$C_{16:1}$	2,3	4,6	6,3	2,9	6,5
$C_{18:0}$	3,6	4,3	4,6	4,8	1,4
$C_{18:1}$	20,0	13,8	13,6	20,0	9,7
$C_{18:2}$	50,7	46,4	27,4	34,7	28,4
Σ_S	27,0	35,2	52,7	42,4	55,4
Σ_{US}	73,0	64,8	47,3	57,6	44,6

The behavior of X_1-PL on mild alkaline hydrolysis confirmed the possibility of a N-acylphosphatidylethanolamine structure of this fraction and the nonidentity of glyceryl-phosphoryl-N-acylethanolamine with X_2-PL confirmed the structure of the latter as an N-acyllysophosphatidylethanolamine.

In its chromatographic behavior (systems 1 and 2), X_1-PL did not differ from the product of the reaction of acetyl chloride with PE. The IR spectra of the latter also had absorption bands of an amide carbonyl at 1650 and 1530 cm^{-1}.

N-Acyl-PEs (phosphatidylethanolamides) and their lyso analogs were first isolated from wheat flour [6] and were then detected in soybeans, haricot beans, and the seeds of oats, rape, peas [7], etc. We are the first to have found such phospholipids in the seeds of the cotton plant.

As can be seen from Table 1, among the saturated acids in all the samples palmitic acid predominates, and linoleic predominates among the unsaturated acids; the molecule of the N-acyl-PE is more saturated than that of the N-acyllyso-PE.

The lysophosphatidylcholine was eluted from the column with a mixture of chloroform and methanol (2 : 1) (together with the PC) and with methanol (pure) and had R_f 0.1-0.15 (systems 1 and 2). The structure of the lyso-PC was determined on the basis of an analysis of its IR spectrum [3] by determining its N and P contents (2.4 and 4.9%, respectively), and also by a study of the products of its acid hydrolysis. Among the hydrolysis products were found FAs, glycerol, and choline (TLC with glycerol and choline as markers).

For analysis, the fatty acids were isolated by cold saponification and were analyzed by GLC (see Table 1). More than 50% of the acids consisted of linoleic acid.

From its fatty-acid composition, the structure of a 1-acyl-3-glyceryl-phosphorylcholine may be put forward for the lyso-PC.

On the basis of repeated chromatographic results on the fresh total PLs, it may be considered that the lyso-PC is not an artefact but is present in the total material in the native form [1].

EXPERIMENTAL

For column chromatography we used type KSK silica gel (160-250 μ) and DEAE-cellulose in microgranular form, which was prepared by a known method and converted into the acetate form [8].

The following solvent systems were used: 1) chloroform–methanol–water (65 : 35 : 5); 2) chloroform–methanol–ammonia (65 : 35 : 5); 3) isopropanol–ammonia–water (7 : 1 : 2) [9]; and 4) 2% NH_3–CH_3OH [10].

The acid hydrolysis of the PLs was performed in sealed tubes with 3 N HCl at 100°C for 24 h. The IR spectra were taken on a UR-20 instrument in the form of films. Gas–liquid chromatography was performed on a UKh-2 instrument at 197°C with poly(ethylene succinate) (17%) on Celite-545 (60-80 mesh) as the stationary phase.

Separation of the Total Phospholipids on DEAE-Cellulose. A column of cellulose (50 g) was washed with chloroform–methanol (2 : 1) and with methanol, and the combined phospholipids (1 g) in chloroform solution

were added and were eluted with: chloroform, neutral lipids; chloroform–methanol (9:1), PC, lyso-PC, and traces of PE; chloroform–methanol (4:1), PE, traces of PC, and lyso-PC; chloroform–methanol (2:1) containing 0.4% of ammonium acetate, X_1- and X_2-phospholipids and traces of pigment (40 mg); the same mixture with 1% of ammonium acetate, phosphatidylinositols.

Hydrogenation of N-Acylphosphatidylethanolamine. The hydrogenation of this material (40 mg) was performed in the presence of a palladium catalyst (supported on aluminum powder) in a current of hydrogen with vigorous stirring for 1 h. The hydrogenation product consisted of a white powder.

IR spectrum: ν_{max} 1650, 1540 cm^{-1}.

Production of Glycerophosphoryl-N-acylethanolamine. N-Acylphosphatidylethanolamine (X_1-PL, 40 mg) was incubated at 37°C in 10 ml of 0.1 M methanolic alkali for 80 min. Then the mixture was neutralized with methyl formate and evaporated to dryness. The dry residue was treated with 5 ml of a mixture of ethanol and water (1:1) and 10 ml of petroleum ether (40-60°C). The mixture was shaken and the lower layer was separated off. The petroleum ether solution was washed with the ethanol–water (1:1) mixture (2×5 ml). The combined aqueous ethanolic extracts were reextracted with chloroform. The yield of the petroleum ether fraction was 12 mg – fatty acids of the glycerol part of the molecule; and that of the chloroform fraction was 24 mg – traces of FAs and glyceryl-phosphoryl-N-acylethanolamine. The hydrolysis products were separated preparatively in system 2.

Preparation of N-Acetylphosphatidylethanolamine. A solution of 20 mg of PE [1] in 5 ml of chloroform (without ethanol) was treated with 0.5 ml of triethylamine and 0.3 ml of acetyl chloride. The mixture was left with protection by a calcium chloride tube for 1.5-2 h (the solution acquired a reddish color). TLC of the mixture showed the presence of only one phosphorus-containing compound, with R_f 0.9 (the R_f value of the initial PE was 0.5; system 2), which was not revealed by ninhydrin.

Then the solution was shaken with 10% sodium bicarbonate solution (2×10 ml), the upper layer was separated off, and the lower, chloroform, layer was evaporated to small volume and purified by preparative TLC in system 2, and its IR spectrum was recorded.

SUMMARY

The minor components of the total PLs of seeds of the cotton plant of variety 108-F have been studied.

N-Acylphosphatidylethanolamine and N-acyllysophosphatidylethanolamine have been isolated from cotton seed for the first time and some of their physicochemical properties and their fatty-acid compositions have been studied.

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