# AN INVESTIGATION OF THE PHOSPHOLIPIDS OF SEEDS OF THE COTTON PLANT OF VARIETY 108-F

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In a preceding communication [1] we gave preliminary results of an investigation of the phospholipids of the seeds of the cotton plant of variety 108-F (Gossypium hirsutum). In the present paper we describe the results of a study of the fractional composition of the combined phospholipids, the characteristics of individual homogeneous fractions, and the fatty-acid compositions both of the total phospholipids of seeds of the cotton plant of variety 108-F and the individual fractions of them.

The combined phospholipids were obtained from the acetone-defatted seed kernels by Folch's method [2]. The purified total material appeared on two-dimensional TLC as five phosphorus-containing components the amounts of which were determined from the amounts of phosphorus in the corresponding spots on the chromatogram [3]:

| Phospholipid fraction | Amount, % |
|---|---|
| Phosphatidylcholines | 52,63 |
| Phosphatidylinositols | 22,80 |
| Phosphatidylethanolamines | 17,52 |
| Polyglycerophosphatides | 3,50 |
| Lysophosphatidylcholines | 3,55 |

The quantitative yield of phospholipids on their separation by two-dimensional TLC was 89%. The mineralization of the phosphorus was performed at 170-180°C for 3-4 h. The phosphorus content was determined by the method of Taussky and Shorr [4].

The polyglycerophosphatides were identified from the position of the spots and their positive reaction for phosphorus.

The presence of lysophosphatidylcholines is possibly due to a degradation of the phosphatidylcholines in the process of the extraction and purification of the combined phospholipids. The main components of the total material were isolated by separating the ethanol-soluble and ethanol-insoluble fractions on a column of silica gel. The subsequent preparative fractionation was performed in a thin layer of silica gel. For the separation of the phosphatidylethanolamines from the phosphatidylcholines we used the chloroform–methanol–water (65 : 35 : 5) system and for the separation of a mixture of phosphatidylcholines and phosphatidylinositols we used the chloroform–methanol–25% ammonia (65 : 35 : 5) system. The homogeneous fractions of phospholipids obtained in this way had the following indices:

Phosphatidylcholines. N 2.30, P. 3.86%; molar ratio N:P = 1 : 0.8, $[\alpha]_D^{20}+8°$ (c 1.0; $CHCl_3$–$CH_3OH$, 2 : 1).

Phosphatidylinositols. P. 3.0%; N none.

Phosphatidylethanolamines. N 1.87, P. 3.5%; molar ratio N:P = 1 : 0.9.

The IR spectra of these fractions agreed with those given in the literature for glycerophospholipids [5, 6].

To confirm the structure of the phosphatidylinositols, they were subjected to acid hydrolysis. Among the water-soluble hydrolysis products on TLC we found glycerol and inositol [isopropanol–25% ammonia–water (50 : 7 : 15) system [7], where "isopropanol" should be read for "isobutanol"; chromogenic agents: potassium periodate–benzidine; $I_2$ vapor; 0.5% aqueous solution of $KMnO_4$]. The literature does not contain sufficiently complete information on the fatty-acid composition of the total and individual fractions of

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TABLE 1

| Fatty acids | Triglycerides | Total phospholipids | Individual fractions | | |
|---|---|---|---|---|---|
| | | | phosphatidylcholines | phosphatidylethanolamines | phosphatidylinositols |
| $C_{10:0}$ | — | 1,1 | — | 4,10 | — |
| $C_{12:0}$ | — | 2,04 | 1,17 | 2,40 | 2,98 |
| $C_{14:0}$ | 1,81 | 1,36 | 1,14 | 2,05 | 2,11 |
| $C_{16:0}$ | 22,00 | 24.85 | 17,50 | 25,03 | 31,91 |
| $C_{16:1}$ | 1,75 | 1,26 | 1,30 | 1,93 | 1,19 |
| $C_{18:0}$ | 2,90 | 1,75 | 1,00 | 1,78 | 4,82 |
| $C_{18:1}$ | 16,33 | 15,33 | 26,88 | 12,66 | 9,99 |
| $C_{18:2}$ | 55,20 | 52,30 | 51,00 | 50,04 | 47,00 |
| Sum of the saturated acids | 26,71 | 31,10 | 20,81 | 35,36 | 41,82 |
| Sum of the unsaturated acids | 73,28 | 68,89 | 79,18 | 64,63 | 58,18 |

the phospholipids of the seeds of the variety of cotton plant that we have studied [8]. The fatty acids were isolated from the total and the individual fractions of the phospholipids by alkaline saponification. The mixture of fatty acids obtained was methylated with a freshly prepared solution of diazomethane, and the methyl esters were analyzed on a gas–liquid chromatograph. In order to compare the results, a chromatogram of the triglycerides of the oil was obtained [9]. The results of the analysis of the fatty-acid composition of the phospholipids of seeds of the cotton plant of variety 108-F are given in Table 1.

It can be seen from Table 1 that the fatty-acid compositions of the triglycerides and of the combined phospholipids are very similar with respect to the acids involved, with the exception of the low-molecular-weight $C_{10}$ and $C_{12}$ acids, which are absent from the triglycerides. The fatty acids of the combined phospholipids have a more saturated nature than those of the triglycerides. The amounts of the main acids in the triglycerides and in the combined phospholipids agree approximately with those given in the literature [8, 7].

Among the saturated acids palmitic predominates, and among the unsaturated acids linoleic. The main phospholipids of the seed kernels of the cotton plant of variety 108-F form the following sequence with respect to increasing degree of saturation: phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols.

## EXPERIMENTAL METHOD

For two-dimensional chromatography we used the following systems: direction I, chloroform–methanol–ammonia (65 : 35 : 5); direction II, chloroform–methanol–acetone–acetic acid–water (63 : 21 : 10 : 21 : 11). The methyl esters of the fatty acids were analyzed on a UKh-2 gas–liquid chromatograph at 196° with poly(ethylene succinate) as the stationary phase.

Separation of the Total Material according to Solubilities in Ethanol. The combined phospholipids (0.65 g) obtained from the acetone-defatted seed kernels were extracted with a mixture of chloroform and methanol (2 : 1) and dissolved in 2 ml of chloroform, and this solution was treated with 20 ml of ethanol. The flocculant precipitate that had formed was left in the refrigerator for several hours. Then the ethanol was separated off by centrifuging (3000 rpm), and the precipitate was washed twice with ethanol. The weight of the ethanol-soluble fraction was 0.45 g and the ethanol-insoluble fraction 0.188 g.

Fractionation of the Ethanol-Soluble Fraction of the Combined Material on a Column. The ethanol-soluble fraction (158 mg) of the combined phospholipids was transferred to a column (d 1.6 cm, h 25 cm) containing 8.5 g of silica gel and eluted in the following sequence:

I) chloroform (neutral lipids);

II) chloroform–methanol (9 : 1) 26 mg (mixture of phosphatidylinositols and phosphatidylethanolamines);

III) chloroform–methanol (8 : 1) 30 mg (phosphatidylethanolamines and traces of phosphatidylcholines); and

IV) chloroform–methanol (7 : 3) 90 mg (phosphatidylcholines).

Column Chromatography of the Ethanol-Insoluble Fraction of the Combined Phospholipids. Part of the combined material (178 mg) was separated on a column (d 1.6 cm, h 25 cm) containing 9.0 g of silica gel and was eluted with chloroform–methanol. The chloroform–methanol (9 : 1) fraction yielded 90 mg of a mixture of phosphatidylethanolamines and phosphatidylinositols, while the (8 : 1) fraction gave 65 mg of a mixture of phosphatidylethanolamines (very small amount) and phosphatidylinositols with traces of phosphatidylcholines.

The mixture obtained was subfractionated by preparative TLC.

Acid Hydrolysis of the Phosphatidylinositols. A mixture of 47 mg of the phosphatidylinositols and 3 ml of 6 N HCl in a sealed tube was heated in the boiling water bath for 24 h. Then the tube was opened, the acid solution was extracted with petroleum ether (40-70°C) and evaporated in vacuum, the residue was dissolved in water, and the water-soluble hydrolysis products were analyzed by TLC. Inositol and glycerol were identified.

Alkaline Hydrolysis of the Phospholipids. A sample weighing 25-35 mg of the phosphatidylcholines or the phosphatidylethanolamines in 1 ml of 0.1 N KOH (in methanol) was heated on the water bath under reflux for 20 min. Then the solvent was evaporated off, the residue was dissolved in water, the solution was acidified with 10% HCl, and the free fatty acids were extracted with diethyl ether. After the ether had been distilled off, the fatty acids were methylated with diazomethane.

A mixture of 30 mg of the phosphatidylinositols in 1 ml of 10% methanolic KOH was left at room temperature for 20 h. The subsequent working up was performed as described above.

## SUMMARY

1. It has been found that the main components of the combined phospholipids of the seed kernels of the cotton plant of variety 108-F are phosphatidylcholines, phosphatidylinositols, and phosphatidylethanolamines.

2. The fatty-acid composition of the combined phospholipids and of individual homogeneous fractions of them have been studied.

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