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COMPOSITION OF THE PHOSPHOLIPIDS OF Gossypium barbadense

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We have previously [1] reported the isolation of the total phospholipids from the seed kernels of the fine-fibered cotton plant of variety 5904-I by Folch's method [2] and their purification by precipitation with acetone. The phospholipids obtained in this way amounted to 1.6% on the absolutely dry kernels, and the amount of phosphorus in them was 2.4%.

Continuing an investigation of these phospholipids, we have determined their qualitative composition by one-dimensional chromatography in a thin layer of silica gel in system 1 [3]. The chromatogram showed 10 spots of substances which were identified by chemical tests [4-5] and by comparison with markers; the R_f values of the substances were: 1 – 0.96; 2 – 0.92; 3 – 0.80; 4 – 0.78; 5 – 0.75; 6 – 0.55; 7 – 0.40; 8 – 0.20; 9 – 0.10; and 10 – 0.05. Six spots (1, 3, 5, 6, 7, and 8) gave a positive reaction for phosphorus [5]. We identified these substances as X_1-polyglycerophosphatides (1), X_2-polyglycerophosphatides (3),* phosphatidylethanolamines (5), phosphatidylcholines (6), phosphatidylinositols (7), and lysophosphatidylcholines (8).

After the chromatograms had been sprayed with sulfuric acid and slowly heated, the substances with R_f 0.92 and 0.78 gave a violet color, and then carbonized to black. These substances were isolated preparatively by chromatography on a column of silica gel and were characterized qualitatively as steroids [6]. The substances with R_f 0.10 and 0.05 were identified as carbohydrates, and visually they amounted to a considerable part of the sample studied.

The results of the investigation performed showed the complexity of the composition of the total material isolated, which contained as accompanying components, in addition to the phospholipids, two other groups in each case – steroids and carbohydrates. The presence of carbohydrates made further experiments difficult, since they interfered with the separation of the total phospholipids into individual components by column chromatography on silica gel.

The most widely used method of purifying lipid extracts from nonlipid impurities is that given in our first paper [1]. In this method, water and aqueous solutions of salts are used for washing, but this is most effective for lipid extracts of animal tissues containing only slight amounts of water-soluble impurities. Nonlipid components can also be separated by gel filtration on Sephadexes G-25 [7-10] and LH-20 [11, 12].

To free the total phospholipids from carbohydrates we used Molselekt G-25. For 1 g of dry gel we used 100-125 mg of total phospholipids. After the gel had been swollen in a mixture of chloroform, ethanol, and water (90 : 10 : 1) [10], it was charged into a column, and then the sample was deposited in the same mixture of solvents and it was eluted. The completeness of the filtration of the phospholipids was checked in a thin layer of silica gel in system 1. Purification from carbohydrates was complete, and the yield of phospholipids was 1.0% on the absolutely dry kernels and 68.8% on the impure material applied to the column; its phosphorus content had risen to 4.15%. The Molselekt used was discharged from the column, and the carbohydrates were re-

*For X_1- and X_2-polyglycerophosphatides, see the following paper.

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generated by washing with water. The aqueous solutions were evaporated to dryness in a rotary evaporator. The dry residue of carbohydrates amounted to 31.2% of the initial phospholipid fraction and contained no phospholipids, as was shown by chromatography in a thin layer of silica gel in system 1. Then the carbohydrates were subjected to acid hydrolysis and the water-soluble hydrolysis products were analyzed by descending paper chromatography in solvent system 3 [13]. Glucose and galactose were found; no monosaccharides were found in intact carbohydrates. Consequently, the carbohydrates accompanying the phospholipids were disaccharides.

For the complete quantitative characteristics of the individual groups of phospholipids of the purified total material, they were subjected to two-dimensional chromatography in the thin layer of silica gel in system 1 for the first direction and in system 2 for the second direction. The phosphorus content was determined by D. T. Tevekelov's method [14] in each spot, and it was referred to the total amount of phosphorus in all the spots. The mean results of three determinations were as follows (%): X_1-polyglycerophosphatides 2.4; X_2-polyglycerophosphatides 7.1; phophatidylethanolamines 14.1; phosphatidylcholines 50.4; phosphatidylinositols 20.4; and lysophosphatidylcholines 5.6.

Thus, we have established that the phospholipids of the seed kernels of the thin-fibered cotton plant of variety 5904-I contain six groups of phospholipids, and the substances accompanying them consist of steroids and disaccharides.

EXPERIMENTAL

Chromatography. Thin-layer chromatography was performed in silica gel (type KSK, less than 150 mesh) washed with nitric acid, water, acetone, and chloroform, and paper chromatography was performed on type M ["slow"] paper of Leningrad mill No. 2.

The following solvent systems were used as the mobile phases: 1) chloroform–methanol–25% ammonia (65 : 35 : 5) [3]; 2) chloroform–methanol–water (65 : 25 : 4) [15]; and 3) butan-1-ol–pyridine–water (6 : 4 : 1) [13].

Purification of the Phospholipids from Carbohydrates. For swelling 26 g of Molselekt G-25 (100-320 μm, Reanal, Budapest)was left in 10 ml of chloroform–methanol–water (90 : 10 : 1) for 18-20 h [10]. The swollen gel together with the solvent was charged into a column (2 × 12 cm, working dimensions), the cone of which had previously been packed with medical absorbent cotton. A solution of 3.2 g of phospholipids in 10 ml of the same mixture of solvents was deposited on the column and subjected to gel filtration, being eluted with 100 ml of the same mixture. The filtrate was evaporated in a rotary evaporator at 30-40°C, and the residue was dried to constant weight. Yield 2.2 g. The gel was discharged from the column, and the carbohydrates were washed out on a Büchner funnel with 700-800 ml of distilled water. The aqueous filtrate was evaporated in a rotary evaporator at 70-80°C (towards the end with the addition of methanol). The yield of carbohydrate residue was 1 g. After the carbohydrates had been eluted, the gel was dehydrated with 300 ml of acetone and 150 ml of methanol and was stored at +4°C in a mixture of chloroform and methanol (4 : 1). After being used four times, the Molselekt had not lost its activity.

Acid Hydrolysis of the Carbohydrates. A mixture of 50 mg of the dry carbohydrate residue and 2 ml of 7% HCl was heated in a sealed tube in the water bath for 4 h. The hydrolyzate was evaporated in a porcelain dish until all the acid had been driven off. The residue was dissolved in a few drops of water and was subjected to qualitative paper chromatography in system 3. The spots of the carbohydrates were revealed with an alcoholic solution of o-toluidine and salicylic acid. Glucose, mannose, galactose, and arabinose were used as markers.

Quantitative Determination of the Group Composition of the Phospholipids. A solution of the phospholipids (30-40 μg of lipid phosphorus) was deposited on one plate (13 × 13 cm) coated with a thin layer of silica gel G and the components were separated by two-dimensional chromatography. After the chromatogram had been run and the solvents had been evaporated off, the plates were sprayed with 50% sulfuric acid and heated at 140-150°C for 35-40 min; the carbonized spots were scraped into centrifuge tubes and treated as described by Dyatlovitskaya et. al., [16], and the mineralization of the sample and the phosphorus was determined by Tevekelov's method [14].

SUMMARY

1. The combined phospholipids of the seed kernels of the cotton plant have been completely freed from accompanying carbohydrates by gel filtration on Molselekt G-25.

2. It has been established by two-dimensional chromatography in a thin layer of silica gel that the phospholipids of the seed kernels of the cotton plant of thin-fibered variety 5904-I consist of X_1- and X_2-polyglycerophosphatides (2.4 and 7.1%, respectively), phosphatidylethanolamines (14.1%), phosphatidylcholines (50.4%), phosphatidylinositols (20.4%), and lysophosphatidylcholines (5.6%).

3. The accompanying substances of the phospholipids of the cotton plant form two groups each of steroids and disaccharides.

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THE STRUCTURE OF THE PHOSPHOLIPIDS OF THE COTTON PLANT AND THE POSITION SPECIFICITY OF THEIR FATTY ACIDS

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We have previously described the isolation of the phospholipids of the seed kernels of the thin-fibered cotton plant of variety S-6029 and the determination of their qualitative and quantitative compositions [1]. The combined phospholipids isolated contained a large amount of carbohydrates (29%), which interfered with subsequent fractionation. The carbohydrates were eliminated by gel filtration through Molselekt G-25 in the chloroform–methanol–water (90 : 10 : 1) system [2]. The phospholipids purified in this way amounted to 1.2% on the absolutely dry kernels, and their phosphorus content had risen from 2.57 to 3.3%.

The combined phospholipids were separated into ethanol-soluble and ethanol-insoluble fractions and these were then chromatographed on columns of silica gel. As eluents we used acetone, chloroform, and chloroform–methanol with gradually increasing concentrations of methanol up to the pure alcohol. The fractions isolated were analyzed qualitatively by thin-layer chromatography in systems 1 and 2 [3, 4]. The use of acetone permitted, in the first place, the separation of practically all the substances still remaining in the phospholipids (mainly steroid-containing substances), which amounted to 8.8% of the material transferred to the column. It also freed the column from loads and improved the fractionation of the phospholipids. Chloroform eluted neutral

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