PHOSPHOLIPIDS OF Gossypium barbadense

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The chemical study of the phospholipids of fast-maturing thin-fibered types of cotton has recently begun [1]. In the present communication we give the results of an investigation of the phospholipids of cotton seeds of the fine-fibered variety 5904-I (G. barbadense).

The comminuted seed kernels were defatted with acetone. The phospholipids were extracted by Folch's method [2]. The water-soluble impurities were eliminated by filtering off the precipitate that deposited from a chloroform solution on standing and also by the subsequent treatment of the filtrate with a solution of NaCl [3]. The combined phospholipids purified in this way and reprecipitated by acetone formed a yellow powder readily soluble in chloroform (1.6% of the weight of the undefatted seeds). The amount of phosphorus in this material was 2.4%.

The total phospholipid material and its ethanol-soluble and ethanol-insoluble parts [4] were separated into individual fractions on a column of silica gel. The neutral lipids were eluted with chloroform and the phospholipids with mixtures of chloroform and methanol in various proportions.

In the separation of the combined material, from the $CHCl_3-CH_3OH$ (9 : 1) fraction chromatographically homogeneous phosphatidylethanolamines and phosphatidylinositols containing a small amount of phosphatidylcholines were obtained. From the $CHCl_3-CH_3OH$ (4 : 1) fraction a mixture of lecithins and phosphatidylinositols was eluted. The bulk of this mixture was isolated in the separation of the ethanol-soluble part of the total. By preparative thin-layer chromatography, the mixture was separated into phosphatidylinositols and phosphatidylcholine fractions. The homogeneity was checked in a thin layer of silica gel with the aid of characteristic color reactions.

The absorption bands in the IR spectra of the individual fractions of phospholipids agreed with those given in the literature [5, 6].

For a structural study of the homogeneous fractions of phospholipids, they were subjected to acid hydrolysis. All the fractions yielded mixtures of fatty acids consisting of seven or eight components. A water-soluble product of hydrolysis was subjected to TLC using glycerol, inositol, ethanolamine, and choline as markers.

Glycerol was found in the products of the hydrolysis of all three phospholipids studied. The formation of glycerol, and also the results of IR spectroscopy, confirmed their glycerophospholipid structure.

Ethanolamine was identified from a hydrolyzate of the phosphatidylethanolamines, choline from the phosphatidylcholines, and inositol from the phosphatidylinositols.

EXPERIMENTAL

The solvents were purified and rendered absolute by standard methods [7].

IR spectra were taken on a UR-20 instrument (in the form of films). Chromatography was performed on silica gel (KSK, 100 μ for thin-layer chromatography and 160-250 μ for column chromatography). The solvent system chloroform–methanol–25% ammonia (65 : 35 : 5) was used for TLC.

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Chromogenic agents: for phospholipids containing an amino group – a 0.2% butanolic solution of ninhydrin (with 0.3% of CH_3COOH); for choline-containing phospholipids – Dragendorff's reagent; and for all the phospholipids – the phosphorus reagent [8] and the modified reagent of Vaskovsky and Kostetsky [9] and also 50% H_2SO_4 and iodine vapor. The amount of phosphorus was determined by the method of Taussky and Shorr [10].

For the water-soluble hydrolysis products we used the systems: isopropanol–25% ammonia–water (5:4:1) [11] and 2% ammonia–methanol (2:3) [12]; chromogenic agents: potassium periodate–benzidine; a 1% aqueous solution of $KMnO_4$; a solution of ninhydrin; and Dragendorff's reagent.

Extraction of Phospholipids by Folch's Method. The acetone-defatted seed kernels (168 g) were extracted with a mixture of chloroform and methanol (2:1). The mixture of solvents was distilled off in vacuum in a current of nitrogen to dryness. The residue was dissolved in 25 ml of chloroform and precipitated with 400 ml of acetone. The flocculant precipitated was separated by centrifugation (3000 rpm). Then it was dissolved in chloroform and the solution was left in the refrigerator. On the following day, the crystals of carbohydrates that had deposited were separated off by filtration, and the filtrate was treated with a 0.9% solution of NaCl. Then it was evaporated to dryness and the residue was redissolved in chloroform and reprecipitated with acetone. The yield of total phospholipids was 2.78 g.

Separation of the Total Material into Ethanol-Soluble and Ethanol-Insoluble Parts. A solution of 0.66 g of the total phospholipids in 2 ml of chloroform was treated with 40 ml of ethanol and the mixture was left in the refrigerator. The precipitate that had deposited was separated by centrifugation. The weight of the precipitate was 0.2 g. The solvent was evaporated off to dryness, giving 0.46 g of residue.

Separation of the Combined Material on a Column of Silica Gel. The combined phospholipids (0.42 g) were deposited on a column containing 22 g of silica gel (column diameter 1.8 cm, height 65 cm).

The neutral lipids were eluted with chloroform (5 ml). Then elution was continued with chloroform–methanol (9:1), 75-ml fractions being collected. Fractions 1-4 yielded a mixture of two substances with R_f 0.8 and 0.9. Fractions 4 and 5 yielded by chromatographic elution homogeneous phosphatidylethanolamines with R_f 0.75. A chloroform–methanol (80:20) fraction yielded a mixture of phosphatidylinositols and choline phosphatides (R_f 0.55 and 0.4). The mixture was separated preparatively.

The phosphatidylethanolamines (N 1.58%, P 4.0%; N:P = 1:0.9) consisted of a wax-like substance readily soluble in chloroform. IR spectrum, cm^{-1}: 2940, 2870, 1470, 1385 (CH_2, CH_3, and CH groups), 1745 (C=O), 1080, 1040 (P–O–C), and 1250 (P=O).

The phosphatidylinositols formed a pulverulent colorless substance soluble in chloroform and in mixtures of chloroform and methanol. The IR spectrum showed absorption bands at (cm^{-1}) 3500-3200 (OH), 2940, 2860, 1480, 740 (CH, CH_2, CH_3), 1740 (C=O), 1130, 1080 (P–O–C), 1250 (P=O).

The phosphatidylcholines (N 2.33%, P 3.8%; N:P = 1:1.2) formed a wax-like substance readily soluble in chloroform. IR spectrum: a weak band at (cm^{-1}) 3300-3150 (OH), 2940, 2870, 1470 (CH, CH_2, CH_3), 1740 (C=O), and 1110, a weak band at 1090 (P–O–C), 1260 (P=O), and the band at 980 cm^{-1} that is characteristic for the $\overset{+}{N}(CH_3)_3$ group.

Acid Hydrolysis of the Phospholipids. The phosphatidylethanolamines and phosphatidylcholines (25-mg samples of each) were boiled in a sealed tube with 3 N HCl in the water bath (100°C) for 24 h. Then the tubes were opened, the completeness of hydrolysis was checked by TLC, and the fatty acids split off were extracted with petroleum ether (30-60°C). The hydrolyzate was evaporated in vacuum to dryness, the residue was dissolved in a few drops of water, and the water-soluble hydrolysis products were analyzed by the TLC method. The hydrolysis of the phosphatidylinositols was performed with 6 N HCl.

SUMMARY

From the total phospholipids of the cotton plant of the thin-fibered variety 5904-I (Gossypium barbadense) chromatographically homogeneous fractions of phosphatidylethanolamines, phosphatidylcholines, and phosphatidylinositols have been isolated. The well-known glycerophospholipid structure of these fractions has been established on the basis of their IR spectra and the products of their acid hydrolysis.

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