PHOSPHOLIPIDS OF COTTON SEEDS

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The phospholipids of the cotton plant have been little studied chemically [1-4]. We have studied the seeds of Gossypium hirsutum L. (cotton plant) of variety 108-F. The phospholipids were extracted from the acetone-defatted seeds with chloroform–methanol (2:1) [5]. The mixture of solvents was distilled off in a current of nitrogen under reduced pressure. The residue was dissolved in chloroform, and the water-soluble impurities were removed with water. The chloroform solution was distilled to small volume, and the phospholipids were precipitated with an excess of acetone. The flocculent precipitate so produced was separated by centrifuging (3000 rpm). The yield of combined phospholipids was 1.6% of the weight of the seed kernels.

Part of the combined phospholipids was passed through a column of silica gel, and another part was separated into ethanol-soluble and ethanol-insoluble fractions [6, 7]; the latter were also chromatographed on a column. The neutral lipids were eluted with pure chloroform. The phospholipids were eluted with chloroform–methanol with increasing concentrations of methanol. In the separation of the total material, the chloroform–methanol (4:1) fraction gave a pure cephalin fraction and the chloroform–methanol (2:1) eluate gave a very small amount of pure lecithin. The main part of the lecithins is formed in the separation of the ethanol-soluble fraction of the total material on a column of silica gel. The lecithin and cephalin fractions were identified by means of characteristic color reactions for the functional groups (Dragendorff reagent and ninhydrin solution).

To check the purity of the fractions iodine vapor, 50% sulfuric acid, a mixture of a 1% solution of phosphomolybdic acid and 0.4% stannic chloride in 3 N HCl, and also a reagent for phosphorus (solution of ammonium molybdate in a mixture of hydrochloric and perchloric acids) were used [8]. To identify the lecithin, egg lecithin was used as reference material.

To confirm their structures, the cephalins and lecithins were subjected to alkaline [9] and acid [10] hydrolysis, and the fatty acids so split off were analyzed in the form of their methyl esters on a gas-liquid chromatograph. Both the cephalin and the lecithin fractions consisted of eight acids differing qualitatively and quantitatively. The water-soluble hydrolysis products were subjected to paper and thin-layer chromatography. Among the products of the acid and alkaline hydrolyses of the lecithin fraction were identified glycerol, choline, and glycerylphosphorylcholine, respectively (markers – products of the hydrolysis of egg-yolk lecithin). In the hydrolyzates of the cephalin fraction, glycerol, ethanolamine, and glycerylphosphorylethanolamine were detected.

In addition to the phospholipids, the amount of phytin – the main phosphorus-containing compound of cotton seeds – was determined. The material after the extraction of the phospholipids was steeped in 0.5% nitric acid for 15-16 h, and when the filtered acid solution was made alkaline with ammonia to pH 8, an amorphous powder, identified as phytin [11], was obtained. Yield 5.6% of the initial raw material.

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