PHOSPHOLIPIDS OF THE SEED KERNEL OF SUNFLOWERS

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Until recently, the phospholipid complex of crops with a high yield of oil, especially Helianthus annuus (sunflower), family Compositae, has been studied inadequately. Only the sum of the bound and free phosphatides and their fatty acid compositions have been investigated; the group composition of the phospholipids of oils extracted from the high-oil-content seeds of sunflowers has been characterized [1–5]. In this paper we give the results of a study of the qualitative and quantitative composition of the native phospholipid complex of the kernel of the high-oil-content sunflower of the variety "peredovik" [leader].

The phospholipids were extracted from the petroleum-ether-defatted kernel with a mixture of chloroform and methanol (2 : 1), and they were separated from carbohydrates and other water-soluble impurities, and then purified with acetone. The phospholipid fraction obtained in this way consisted of an almost colorless, fairly hard mass. The mean yield based on the absolutely dry weight of the kernel was 1.1%, and the phosphorus content 3.06%.

The qualitative composition of the mixture of phospholipids was determined by two-dimensional chromatography. Eight spots were obtained on the chromatogram and they were identified by chemical tests and by comparison with markers which had the following R_f values in solvent system 1: X_1-cerebrosides 0.82, phosphatidic acids 0.76, X_2 cerebrosides 0.72, phosphatidylethanolamines 0.65, phosphatidylcholines 0.41, phosphatidylinositol 0.41, X_1-glycolipids 0.11, and X_2-glycolipids 0.04. The use of two-dimensional chromatography permitted the phosphatidylcholines and phosphatidylinositols to be separated.

Individual groups of phospholipids and the components corresponding to them were isolated by preparative chromatography in a thin layer of silica gel in solvent system 1. Their amount relative to the total substance (wt. %) and their phosphorus content were also determined (table).

Characteristics of the Individual Fractions of the Phospholipids Isolated by Preparative Chromatography

Substance	Content			External form of the fractions
	wt. %	P in the fractions, %	phospholipids from the P, %	
X_1-Cerebrosides Phosphatidic acids	7.8	1.0	Not determined	Light-brown rubberlike substance with solid white inclusions
X_2-Cerebrosides Phosphatidylethanolamines	19.6	3.52	25.5	Light-yellow waxy substance
Phosphatidylcholines*	39.8	3.80	51.5	Light-yellow rubberlike substance
Phosphatidylinositols*	17.8	3.82	23.0	Almost colorless pulverulent substance
X_1-Glycolipids	10.0	0.24	—	Colorless brittle substance
X_2-Glycolipids	5.0	0.07	—	

*Separated and determined quantitatively by rechromatography in solvent system 2.

We see from the table, that the X_1- and X_2-cerebrosides were characterized together with the phosphatidic acids, since their similar R_f values and small quantity made it difficult to separate them under the experimental conditions. The substances provisionally identified as glycolipids contained only small amounts of phosphorus and therefore, cannot, be described as phospholipids. The figures in column 4 of the table show that half of the total content of the phospholipids is represented by phosphatidylcholines and the other half by phosphatidylethanolamines and phosphatidylinositols, in nearly equal quantities, together with a small amount of phosphatidic acids. The content of true phospholipids amounts to 0.9% of the weight of the absolutely dry kernel.

To characterize the individual phospholipids more completely, the total starting material was subjected to two-dimensional chromatography and the content of phosphorus in each spot was determined and related to the total amount

of phosphorus in all the spots. The mean results of three determinations were as follows:

Substance	Content, % of P of phospholipids in nature	
X_1 Cerebrosides	0	—
Phosphatidic acids	4,0	4,3
X_2-Cerebrosides	0	—
Phosphatidylethanolamines	21,3	22,6
Phosphatidylcholines	48,0	51,3
Phosphatidylinositols	20,3	21,8
X_1-Glycolipids	4,6	—
X_2-Glycolipids	1,8	—

Consequently, the content of the main components of the phospholipid complex determined in this way approximates to the content determined by the gravimetric method (see table). Two-dimensional chromatography enabled the amount of phosphatidic acids to be determined.

Thus, the phospholipids of the sunflower kernel consist of phosphatidocholines (51.3%), phosphatidylethanolamines (22.6%), phosphatidylinositols (21.8%), and phosphatidic acids (4.3%). Associated components in the phospholipid complex are cerebrosides and glycolipids.

EXPERIMENTAL

The solvents used were purified and made absolute by generally known methods [7].

Isolation of the phospholipids. The phospholipids were extracted by three treatments with chloroform–methanol (2 : 1) from comminuted sunflower kernels that had been defatted with petroleum ether (40–50° C). For 50 g of kernels we used 300 ml of solvent. Defatting and extraction were carried out at room temperature. The miscella obtained was freed from the bulk of the carbohydrates by filtration after 14–16 hr standing at +4° C. The filtrate was concentrated, and the residue was treated with petroleum ether, and then chloroform for further purification from sugars. The petroleum ether–chloroform solution was washed with water (20% by volume) to eliminate water-soluble impurities and was evaporated to dryness. The residue was dissolved in chloroform to form a 25% solution, and the phospholipids were precipitated with a twenty-fold volume of acetone. The precipitate was kept at -12° C for 14–16 hr, then filtered off on a No. 4 porous glass filter, washed with chilled acetone, and dissolved in chloroform. The phospholipid fraction purified in this way contained no neutral lipids. The yield of lipid phosphorus with a single acetone purification was 97%.

Chromatography. The work was carried out by chromatography in a thin layer of type KSK silica gel (smaller than 150 mesh) which had been washed with HCl, water, acetone, methanol, and chloroform. After treatment, the silica gel had pH 4.5, as determined by Jantzen and Andreas's method [8]. Silica gel, 30 mg, with 5% of gypsum mixed with double the amount of water was deposited per cm^2 of the plates. Two solvent systems were used as the mobile phase: 1) chloroform–methanol–water (65 : 25 : 4) [6, 9], and 2) chloroform–methanol–25% ammonia (14 : 6 : 1) [6].

One-dimensional chromatography was carried out in system 1 and two-dimensional chromatography in system 1 (first direction) and system 2 (second direction).

One-dimensional preparative chromatography was carried out on 18 × 24 cm plates (80–100 mg of the initial phospholipid complex in the form of a 20% solution in chloroform was separated on one plate) and two-dimensional chromatography on 13 × 13 cm plates.

Identification of the substances. The cerebrosides were identified by three chemical tests. 1) The initial mixture of phospholipids was separated by the two-dimensional method, and the plates were dried for 1 min and sprayed with a 0.04% solution of Bromothymol Blue in 0.01 N NaOH. The cerebrosides appeared in the form of green spots on a blue background [10].

2) The mixture was separated by one-dimensional chromatography, the solvent was driven off, and the plates were sprayed with a reagent consisting of 20 ml of 10% ethanolic diphenylamine, 100 ml of conc HCl, and 80 ml of glacial acetic acid. Then they were heated at 105° C; gray-blue spots appeared on a light-gray background [9].

3) Voskovsky and Kostetsky's modified reagent [11] for phospholipids did not reveal phosphorus in this class of substances.

We observed that after the separation of the initial phospholipids by one-dimensional chromatography with subsequent showing up of the spots of the substances with iodine vapor and then with 50% H_2SO_4 and heating at 150° C, the spot corresponding to the cerebrosides changed color from gray to dirty violet to black. The color of the spots corresponding to the phospholipids changed from light brown to black, and that of the glycolipids from gray to black. This change in the color of the cerebrosides may serve as an additional qualitative characteristic for them.

Phosphatidic acids. These acids were identified preparatively from the presence of phosphorus and the lilac fluorescence on observation in UV light after the chromatograms had been sprayed with a 0.0004% solution of Rhodamine 6 G [3].

Phosphatidylcholines and phosphatidylethanolamines. The substances were identified by specific chemical tests [3] and also by the coincidence of their R_f values with markers, lecithin and cephalin (isolated from egg yolk).

Phosphatidylinositols. The substances were identified from the products of their acid hydrolysis with 6 N HCl at 120° C for 24 hr. Fatty acids, glycerol, and inositol were detected. The polyols were compared with reference samples, pure glycerol and inositol, after chromatography of the water-soluble hydrolysis products in a thin layer of silica gel as described by Kaufman et al. [12].

Glycolipids. These compounds gave a positive reaction with an ethanolic solution of diphenylamine [3, 9], and are colored by an alkaline solution of silver nitrate [3]. The products of hydrolysis with 6 N HCl were found to contain fatty acids, inositol and carbohydrates, while the glycolipids gave a weak positive reaction for phosphorus with the reagent used by Voskovsky and Kostetsky [11].

Methods of quantitative determination. The initial phospholipids were separated into individual fractions by preparative chromatography. The zones corresponding to these fractions were removed and were treated first with chloroform–methanol (1 : 1) and then with methanol. The silica gel was separated by filtration, the filtrate was evaporated, and the residue was dried to constant weight. The yields were determined in wt. %. Aliquots of the solutions of the individual groups of phospholipids and the components corresponding to them were analyzed for their phosphorus content by the modified micro method described by Tevekelov [13].

The group composition of the phospholipids was determined from the phosphorus content in the spots of the substances obtained by two-dimensional chromatographic separation. After the chromatogram had been run and the solvents had been evaporated off, the plates were sprayed with 50% H_2SO_4 and heated at 150° C for 40–50 min. The charred spots were transferred to test tubes and treated as described by Dyatlovitskaya et al. [6]. The phosphorus was determined by Tevekelov's method [13].

CONCLUSIONS

The qualitative and quantitative composition of the phospholipid complex of the kernels of seeds of the oil-rich Helianthus annuus and of the substances associated with them have been investigated by chromatography in a thin layer of silica gel.

The phospholipids consist of four groups of substances: phosphatidylcholines (51.3%), phosphatidylethanolamines (22.6%), phosphatidylinositols (21.8%), and phosphatidic acids (4.3%).

The substances associated with the phospholipid complex are cerebrosides and glycolipids, forming two groups in each case.

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