STRUCTURAL INVESTIGATIONS OF THE PHOSPHOLIPIDS OF THE SUNFLOWER

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We have previously [1] reported the isolation and the qualitative and quantitative determination of the composition of the phospholipid complex of the nucleus of the seeds of Helianthus annuus (sunflower), family Compositae, and also the determination of the main components (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidic acids).

There is little information in the literature on the structure of the sunflower phospholipids. Their infrared spectra are given incompletely [2] and without relation to chemical investigations, and it is not known what structure the inositophosphatides of the sunflower possess. The fatty acid compositions of the total [3, 4] and of the free and combined phospholipids [4] have been studied. There is no information on the fatty acid composition of the individual groups of phospholipids.

The present paper gives the results of structural investigations of the phospholipids of the sunflower. By subjecting the combined phospholipids to column chromatography on silica gel, we isolated a pure phosphatidylcholine fraction directly from the column; the other phospholipids, after passage through the column, were purified by rechromatography in a thin layer of silica gel, the phosphatidylethanolamines and phosphatidylinositols in solvent system 1, and the phosphatidic acids in system 2. The indices of the phospholipids obtained in this way are as follows.

Phosphatidylcholines: N/P molar ratio 0.98; $[\alpha]_D^{27}$ +7.45° (c 4; chloroform). The IR spectrum of the compounds has absorption bands at (cm^{-1}): 3380 (OH), 2960, 2935, 2860, 1470, 1382, 1350, 825, and 732 (CH, CH_2, CH_3), 1735, 1180 (ester C=O), 1110, 1070, 1025 (P–O–C), and strong bands at 1250 (P=O) and 975 [$N(CH_3)_3$].

Phosphatidylethanolamines: N/P molar ratio 1.0; $[\alpha]_D^{27}$ +6.1° (c 3.95; chloroform). The IR spectrum shows absorption bands at (cm^{-1}): 2965, 2930, 2860, 1470, 1382, 1320, 810, 720 (CH, CH_2, CH_3), 1735, 1180 (C=O), 1075, 1040 (P–O–C), 1550, 1245 (P=O), 1642, and 1585 due to the deformation vibrations of NH_2 groups. No absorption was observed in the 3500-3100 cm^{-1} region, apparently because of the participation of the NH_2 groups in hydrogen bonds.

Phosphatidylinositols: P 3.35%, no N; $[\alpha]_D^{27}$ +9.7° (c 3.87; chloroform). The IR spectrum exhibited absorption bands at (cm^{-1}): 3400-3200 (OH), 2960, 2932, 2860, 1470, 1380, 725 (CH, CH_2, CH_3); 1732 (C=O, ester). 1117, 1075, 1050, (P–O–C), 1240 (P=O), 955 (P–OH).

Phosphatidic Acids: P 2.0%, no N. IR spectrum of the compounds, cm^{-1}: 3400 (OH), 2965, 2935, 2860, 1465, 1380, 810, 730 (CH, CH_2, CH_3), strong band at 1740, 1175 (ester C=O), 1100, 1080, 1040 (P–O–C), 1240 (P=O), weak band at 960 (P–OH).

The IR spectra of all the phospholipids have absorption bands of the ethylenic bonds of monounsaturated acids in the 1670-1650 cm^{-1} region and bands characteristic for the stretching vibrations of cis-CH = CH bonds (3015 cm^{-1}). The features of the IR spectra of the phospholipids correspond to literature data obtained for glycerophospholipids [2, 5-7].

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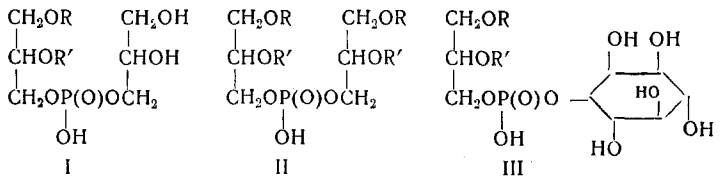
In order to confirm their structure, the individual phospholipids were subjected to acid hydrolysis. Free fatty acids were detected in the hydrolysates of all the phospholipids. To identify the water-soluble fragments of the phospholipid molecules (alcohols, amines), several systems of solvents recommended in the literature [8, 9] were tested in a thin layer of silica gel, but the sharpest separation was achieved in our own system 3 in which reference polyols possess the following R_f values: inositol 0.28, glycerol 0.77, and glycol 0.83; and reference amines the following values: choline 0.07, monoethanolamine 0.20, and serine 0.4. The variations of the values of R_f of the samples and the standards were within ±0.03.

Glycerol and choline were identified in the products of the hydrolysis of the phosphatidylcholines, glycerol and monoethanolamine in those of the phosphatidylethanolamines, and glycerol and inositol in those of the phosphatidylinositols, i.e., the same fragments that we had detected in this fraction under more severe conditions of acid hydrolysis [1]. Glycerol was found in the water-soluble hydrolysate of the phosphatidic acids.

In order to investigate the presence of carbohydrates in the molecules of the phosphatidylinositols, the latter were subjected to additional acid hydrolysis with 0.5 N H_2SO_4, followed by identification of the water-soluble hydrolysis products by paper chromatography in solvent system 4. No carbohydrates were detected.

The structure of each phospholipid was also confirmed by mild alkaline hydrolysis (deacylation). The water-soluble glycerol phosphates formed in the process were identified by paper chromatography in system 5 [10, 11]. The products of the hydrolysis of the phosphatidylcholines were found to contain glycerylphosphorylcholine with R_{GP} (R_f relative to glycerophosphate) 1.50, a reference sample having R_{GP} 1.52, 1.49 [10]; the hydrolysate of the phosphatidylethanolamines contained glycerylphosphorylethanolamine with R_{GP} 0.70, a reference sample having 0.70, 0.64 [10]; and a hydrolysate of the phosphatidylinositols contained glycerylphosphorylinositol with R_{GP} 0.13, 0.10 [10]. The water-soluble products of the deacylation of the phosphatidic acid fractions showed the presence of traces of glycerophosphate with R_{GP} 1.0 (reference sample 1.0) and the spot of a diglycerophosphate with R_{GP} 0.9; 0.83 [11, 12].

The diglycerophosphate may be a product of the mild alkaline hydrolysis both of phosphatidylglycerols (I) and of bis-phosphatidic acids (II). From the IR spectrum of the phosphatidic acid fraction and the result of chemical analysis, it was assumed that the diglycerophosphate was the product of the deacylation of the bis-phosphatidic acids constituting this fraction. On the basis of chemical investigations and IR spectra it may be concluded that the inositophosphatides present in the phospholipid complex of the sunflower are glycerophospholipids and have the structure of monophosphoinositides (III), which were first isolated from commercial soy lecithin and described by Okunara and Nakayama [13].



We have studied the fatty acid compositions of the individual phospholipids and have compared them with the composition of the fatty acids of the initial total phospholipids and with the free and combined lipids (glycerides). The free lipids were extracted in the defatting of the sunflower kernels with petroleum ether, and the bound lipids were extracted with the phospholipids and separated from them by means of acetone [1]. The results of a gas-chromatographic analysis of the fatty acids (Table 1) showed that the fatty acid compositions of the glycerides of the free and bound lipids were similar both with respect to the range of acids and with respect to their proportions. On comparing the fatty acid compositions of the initial phospholipid complex and the glycerides, it can be seen that the former contain a larger amount of saturated acids because of their high palmitic acid content, which is in harmony with the results of Rzhekhin et al. [3].

The fatty acids of the individual phospholipids consist of the same acids as in the phospholipid complex, but in the phosphatidylcholines there are no low-molecular-weight, C_{10-12}, and high-molecular-weight, C_{20-22}, acids, while the bis-phosphatidic acids contain larger amounts of these fatty acids than do the other phospholipids. Furthermore, the latter were found to contain myristoleic acid. In individual phospholipids, as in their initial complex, the same feature of a high content of saturated acids as compared with glycerides due to a large amount of palmitic acid was observed. In respect of the degree of saturation, the sun-

TABLE 1. Fatty Acid Composition of the Lipids of Sunflower Seed Kernels, %

Fatty acid comp.	Lipids						
	glycerides		phospholipids				
	free	combined	initial complex	phosphatidylcholines	phosphatidylethanolamines	monophosphoinositides	bis-phosphatidic acids
$C_{10:0}$	traces	0,3	traces	0	0,6	0,6	traces
$C_{12:0}$	traces	0,3	0,4	0	0,9	1,2	2,6
$C_{14:0}$	traces	0,4	0,3	0,5	0,9	1,2	2,5
$C_{14:1}$	0	0	0	0	0	0	1,9
$C_{16:0}$	6,2	5,7	16,8	10,5	12,6	34,0	21,0
$C_{16:1}$	traces	0,9	0,5	1,3	traces	1,2	2,4
$C_{18:0}$	4,3	4,6	5,0	5,0	5,6	10,4	10,9
$C_{18:1}$	30,7	28,4	14,0	15,0	11,0	5,9	13,2
$C_{18:2}$	56,0	57,2	63,0	67,7	65,8	43,3	42,3
$C_{20:0}$	0,8	1,2	traces	0	1,1	1,1	1,5
$C_{22:0}$	1,0	1,0	traces	0	1,5	1,1	2,7
Sum of saturated acids	13,3	13,5	22,4	16,0	20,6	47,4	36,0
Sum of unsaturated acids	86,7	86,5	77,6	84,0	79,4	52,6	64,0

flower phospholipids form a sequence: phosphatidylcholines, phosphatidylethanolamines, and monophosphoinositides. The amount of oleic acid in all the phospholipids is considerably less than in the glycerides. In the phosphatidylcholines and phosphatidylethanolamines, and also in the initial complex, the amount of linoleic acid is greater than that in the glycerides by 17.7, 9.8, and 7%, respectively. The amount of stearic acid in these phospholipids is close to the amount in the glycerides. A twofold amount of stearic acid as compared with the initial combined phospholipids and a decrease in the amount of linoleic acid by 20.7 and 19.7% is found in the bis-phosphatidic acids and the monophosphoinositides.

EXPERIMENTAL

Chromatography was performed with type KSK silica gel, type S paper of the Leningrad No. 2 paper mill, and the following solvent systems (by volume): 1) chloroform–methanol–water (65:25:4); 2) chloroform–methanol (9:1) [11] or diethyl ether–methanol (9:1); 3) isobutanol–25% ammonia–water (50:7:15), for a thin layer of silica gel; 4) butan-1-ol–pyridine–water (6:4:3), for paper chromatography; and 5) butan-1-ol–acetic acid–water (5:4:1) [10, 11].

The IR spectra of the substances were taken in the form of films on a UR-10 instrument (NaCl prisms for the 680-2000 cm^{-1} region and LiF for the 2000-4000 cm^{-1} region).

Acid Hydrolysis. A sample of phospholipid (20-50 mg) was heated with 3 ml of 3 N HCl [14] in a sealed tube at 100°C for 24 h. After hydrolysis, the free fatty acids were extracted with petroleum ether (40-70°C). Their presence and also the completeness of the hydrolysis were checked by chromatography in a thin layer of silica gel in solvent system 1. The water-soluble hydrolysis products were evaporated in vacuum until the mineral acid had been completely eliminated and were identified by thin-layer chromatography in silica gel in system 3. For this purpose, suitable amounts of the water-soluble hydrolysis products of all the samples and markers of those fragments of the molecules that were to be determined were deposited on each of four plates with dimensions of 13 × 13 cm. The plates were dried first in the air and then in a drying chamber at 105°C for 10 min. After the plates had been cooled to room temperature, the chromatograms were run simultaneously. The markers used were aqueous solutions of the amines choline, monoethanolamine, and serine and of the polyols glycol, glycerol, and inositol. The amines were detected by means of Dragendorff's reagent and ninhydrin [2] and the polyols with a 0.5% aqueous solution of $KMnO_4$ [9] and with potassium periodate–benzidine [15].

Mild Alkaline Hydrolysis. The phospholipids were deacylated by Dawson's method [16]. The completeness of the hydrolysis was checked by the chromatography of the chloroform-soluble hydrolysis products in a thin layer of silica gel in system 1. The water-soluble glycerol phosphates were detected by paper chromatography (system 4) using as markers glycerophosphate and deacylated individual lecithins

and cephalins of egg yolk. The monophosphoinositides and bis-phosphatidic acids were identified by the R_f values of the products of their deacylation and by literature data [11, 12]. To reveal the phosphate esters, Dragendorff's reagent, a 0.2% solution of ninhydrin in a mixture of butan-1-ol and 10% aqueous acetic acid (95 : 5), potassium periodate–benzidine [15], and Dawson's phosphate reagent [16] were used on parallel chromatograms.

Determination of the Fatty Acid Composition. The fatty acids of the lipids were isolated by alkaline hydrolysis and were then methylated with diazomethane and analyzed on a UKh-2 gas-liquid chromatograph at 200°C with a column 2.5 m long, using poly(ethylene succinate) as the stationary phase [17].

SUMMARY

Investigations of the phospholipids of sunflower seeds have been performed which have confirmed the known structures of the phosphatidylcholines and phosphatidylethanolamines. It has been established that the inositophosphatides have the structure of monophosphoinositides. The fatty acid compositions of the individual groups of phospholipids have been studied: they consist mainly of the same selection of acids as the phospholipid complex with the glycerides of the sunflower. The phospholipids form a sequence with respect to increasing saturation: phosphatidylcholines, phosphatidylethanolamines, bis-phosphatidic acids, monophosphoinositides.

A solvent system for the identification of the water-soluble products of the acid hydrolysis of phospholipids by chromatography in a thin layer of silica gel has been proposed.

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