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A STUDY OF THE PHOSPHOLIPIDS OF KENAF

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In the present paper we give the results of an investigation of the structure of the main components of the total phospholipids of kenaf of variety Kuban'-333, grown in the Uzbek experimental station for bast crops [1]. The previously ground seed kernels were defatted with acetone. The total phospholipids were extracted by Folch's method [2] and were freed from carbohydrates by filtration through Molselekt G-25 [3]. The combined phospholipids were separated into ethanol-soluble and ethanol-insoluble fractions and each separately was passed through a column of silica gel. The neutral lipids were eluted with chloroform, and the phospholipids with mixtures of chloroform and methanol of increasing polarity. The final purification of the main fractions (phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols) and the two minor fractions (X_1 and X_2) of phospholipids was performed by preparative chromatography in a thin layer of silica gel in solvents systems 1 and 2. This gave homogeneous fractions with the following constants:

Phosphatidylcholines (PCs): molar ratio N/P = 0.9; $[\alpha]_D^{20} + 6.3^0$ (c 2.0; $CHCl_3$);

Phosphatidylinositols (PIs): no N; P 3.3%;

Phosphatidylethanolamines (PEs): molar ratio N/P = 1.1.

The IR spectra of the fractions obtained coincided with those of glycerophospholipids [4, 5]. To confirm the structure of the main components, we carried out acid hydrolysis. The following products were found in the hydrolyzate: from the PCs, choline; from the PIs, inositol; from the PEs, ethanolamine; and, in the hydrolyzates of all the fractions, glycerol. The amines and polyols were identified from their Rf values with markers in a thin layer of silica gel in systems 3 and 4. The revealing agents were a solution of ninhydrin, Dragendorff's reagent, a 1% solution of potassium metaperiodate, and benzidine solution. To determine their fatty-acid compositions, the phospholipids were subjected to alkaline hydrolysis. The fatty acids were analyzed in the form of methyl esters by GLC. The results of the analysis of the fatty acid compositions of the total phospholipids and of the individual fractions are given in Table 1. The fatty acids of the total phospholipid and its components are similar qualitatively, but the ratios of the individual acids differ. In the individual fractions of the phospholipids of kenaf seeds, from 21.4 to 37% of saturated fatty acids containing mainly palmitic acid and from 63 to 78.6% of unsaturated acids with a predominance of linoleic acid are found. The degree of unsaturation of the phospholipid molecules rises in the following sequence: PIs → X_1-PL → PEs → PCs → X_2-PL.

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TABLE 1. Composition of Position Distribution of the Fatty Acids in the Phospholipids

Fatty acids	Total phospholipids	Phosphatidylcholine			Phosphatidylethanolamine			Phosphatidylinositol			Minor components	
		initial	position		initial	position		initial	position			
			1	2		1	2		1	2	X_1-PL	X_2-PL
$C_{12:0}$	0,6	0,7	1,2	1,1	1,6	1,6	1,0	1,1	1,3	0,6	1,5	1,9
$C_{14:0}$	0,4	0,6	1,3	—	1,8	1,3	1,3	1,0	0,9	0,5	1,4	1,4
$C_{16:0}$	29,7	19,5	33,5	3,4	20,2	40,6	4,1	32,7	70,4	2,3	26,7	15,2
$C_{16:1}$	1,5	2,6	2,1	1,8	2,7	2,3	2,0	2,0	3,8	0,9	2,7	2,1
$C_{18:0}$	2,1	2,0	2,3	—	3,6	2,7	—	2,2	5,3	—	4,3	2,9
$C_{18:1}$	21,3	32,0	29,4	35,6	17,1	12,9	27,9	15,5	8,7	19,2	19,1	11,4
$C_{18:2}$	43,3	41,9	30,2	55,2	49,1	38,6	62,8	44,7	9,6	75,1	42,6	64,3
$C_{18:3}$	1,1	0,7	—	1,9	3,9	—	0,9	0,8	—	1,4	1,7	0,8
Σ_S	32,7	22,2	38,3	4,5	27,2	46,2	6,4	37,0	22,1	3,4	33,9	21,4
Σ_U	67,3	77,8	61,7	95,5	72,8	53,8	93,6	63,0	77,9	96,6	66,1	78,6

TABLE 2. Possible Molecular Compositions of the Phosphatidylcholines (PCs), Phosphatidylinositols (PIs), and Phosphatidylethanolamines (PEs)

Molecular composition	PCs	PIs	PEs	Molecular composition	PCs	PIs	PEs
12:0/12:0	+	+	+	16:1/16:1	+	+	+
14:0/12:0	+	+	+	18:0/16:1	—	+	0,1
16:0/12:0	0,4	0,4	0,4	18:1/16:1	0,5	0,1	0,3
16:1/12:0	+	+	—	18:2/16:1	0,6	0,1	0,8
18:0/12:0	+	+	+	12:0/18:1	0,4	0,2	0,4
18:1/12:0	0,3	0,1	0,2	14:0/18:1	0,4	0,1	0,3
18:2/12:0	0,4	0,1	0,4	16:0/18:1	12,5	13,5	11,4
12:0/14:0	—	+	+	16:1/18:1	0,7	0,7	0,6
14:0/14:0	—	+	+	18:0/18:1	0,8	1,2	0,8
16:0/14:0	—	0,4	0,6	18:1/18:1	10,3	1,7	3,6
16:1/14:0	—	+	+	18:2/18:1	10,5	1,8	10,8
18:0/14:0	—	+	+	12:0/18:2	0,6	1,0	1,0
18:1/14:0	—	+	0,2	14:0/18:2	0,7	0,7	0,8
18:2/14:0	—	0,1	0,6	16:0/18:2	19,2	52,8	25,5
12:0/16:0	—	+	0,1	16:1/18:2	1,2	2,9	1,5
14:0/16:0	—	+	0,1	18:0/18:2	1,2	4,0	1,7
16:0/16:0	1,2	1,7	1,6	18:1/18:2	16,4	6,5	8,1
16:1/16:0	0,1	0,1	0,1	18:2/18:2	16,9	7,2	24,1
18:0/16:0	0,1	0,1	0,1	12:0/18:3	+	+	+
18:1/16:0	1,0	0,2	0,5	14:0/18:3	+	+	+
18:2/16:0	1,0	0,2	1,6	16:0/18:3	0,7	1,0	0,4
12:0/16:1	+	+	+	16:1/18:3	+	0,1	+
14:0/16:1	+	+	+	18:0/18:3	—	0,1	—
16:0/16:1	0,7	0,7	0,8	18:1/18:3	0,6	0,1	0,1
				18:2/18:3	0,6	0,1	0,4

Note. The symbol + means that the component was present in an amount of less than 0.1%.

In order to study the position distribution of the fatty acids, the main fractions of the phospholipids were subjected to enzymatic hydrolysis with phospholipase A, which splits off the acyl radical in position 2 of phospholipids. The source of the enzyme was the venom of the Azerbaidzhan kufi in 0.1 M tris buffer with pH 10.0. The hydrolysis reaction was monitored by TLC in solvent systems 1 and 2. The products of enzymatic hydrolysis were separated by preparative TLC in system 2. The free fatty acids from position 2 were desorbed from the chromatograms and were methylated and analyzed by GLC (see Table 1), while the lyso products were subjected to alkaline hydrolysis and the fatty acids split off from position 1 were extracted from ether and analyzed as described PIs, and PEs, respectively).

As follows from Table 2, the ratios of the types of fatty acids (saturated and unsaturated) were as follows: S/S–1.7, 2.6, 2.84%; S/U–37.15, 75.3, 43.2%; U/S–2.8, 0.8, 3.56%; and U/U–58.35, 21.3, 50.4% (in the PCs, PIs, and PEs, respectively).

The results obtained permit the statement that the unsaturated acids occupy position 2 in the molecules of the main fractions of the phospholipids.

On the basis of the results of the position distribution of the fatty-acid radicals in the PC, PE, and PI molecules, using a modified Coleman calculation [6-8], we determined their possible molecular compositions (Table 2). The results of the calculations show that in the PCs and PEs the U/U molecular types predominate and in the PIs the S/U types. In all the phospholipids investigated, the S/U types were formed mainly from the 16 : 0 and the 18 : 1 and 18 : 2 acids, and the U/U types from the 18 : 1 and 18 : 2 acids.

EXPERIMENTAL

Chromatography was performed with KSK silica gel; for thin-layer chromatography the fraction up to 125 μm and for column chromatography the 160-250 μm fraction. The solvent systems were: 1) chloroform–methanol–25% ammonia (65 : 35 : 5), 2) chloroform– methanol–water (65 : 25 : 4); 3) isopropanol–25% ammonia–water (5 : 4 : 1) [9], 4) isopropanol–25% ammonia–water (49 : 7 : 14) [10], and 5) butan-1-ol–pyridine–water (6 : 4 : 3) [11].

The UV spectra were taken on a UR-20 instrument with the substances in the form of films. Gas–liquid chromatography was performed on a UKh-2 chromatograph with a column 2.5 m long filled with poly(ethylene succinate) at 198°C. The carrier gas was helium [12].

Extraction of the Total Phospholipids. The ground kenaf seeds (100 g) were extracted with acetone at room temperature. From the dried meal the total phospholipids were extracted with chloroform–methanol (2:1). The solvents were distilled off under a current of nitrogen, the residue was dissolved in chloroform, and the solution was filtered; the filtrate was concentrated to small volume, and the phospholipids were precipitated with acetone. The precipitate was separated off by centrifuging (1.6 g) and was dissolved in chloroform–ethanol–water (90 : 10 : 1) and passed through a column containing Molselekt G-25 swollen in the same mixture. The completeness of freeing from carbohydrates was checked by the TLC of the eluate in systems 1 and 2. The yield of purified total phospholipids was 1.0 g. The carbohydrates were desorbed with aqueous methanol (0.55 g).

Acid Hydrolysis of the Carbohydrates. The carbohydrates were heated with 1 N H_2SO_4 for 12 h. Then the solution was treated with barium carbonate and filtered, and the filtrate was analyzed by PC (FN-17 paper, Czechoslovakia) in systems 5. The revealing agent was aniline phthalate. Glucose and galactose were found.

Fractionation of the Ethanol-Soluble Fraction of the Combined Material in a Column. The combined phospholipids (690 mg) in chloroform were deposited on a column containing 35 g of silica gel. Elution was performed with acetone and chloroform and then with mixtures of chloroform and methanol of increasing polarity:

I. Acetone–substances of steroid nature (30 mg).

II. Chloroform–neutral lipids (20 mg).

III. Chloroform–methanol (9:1)–mixture of X_1 and X_2 phospholipids, traces of PEs and PIs (100 mg).

IV. Chloroform–methanol (4:1)–mixture of PEs and PCs (240 mg).

V. Chloroform–methanol (2:1)–PCs and traces of PIs and lyso-PCs (200 mg).

VI. Methanol–lysophosphatidylcholines, traces of phosphatidylcholines (80 mg).

The distribution of the ethanol-insoluble fraction of the combined material was performed similarly. In this case, eluates enriched with phosphatidylinositols were obtained.

Acid Hydrolysis of the Main Components of the Total Phospholipids. The PCs, PE, and PIs (30-40 mg each) in 3 ml of 3 N HCl (or 6 N HCl in the case of the PIs) were boiled in sealed tubes in the water bath for 24 h. Then the tubes were opened and the fatty acids were extracted from the acid solutions with petroleum ether (40-60°C). The residue was evaporated to dryness and dissolved in water, and the hydrolysis products were analyzed by TLC. Glycerol, choline, ethanolamine, and inositol were used as markers.

Alkaline Hydrolysis of the Phospholipids. The PCs, PEs, and PIs, and X_1- and X_2-PLs (30-40 mg each) were hydrolyzed with 5% KOH in CH_3OH (1.5-2 ml) on the water bath under reflux for 30 min. The solvents were evaporated off, the residue was dissolved in water, the solution was made alkaline with 10% HCl, and the fatty acids were extracted with petroleum ether (3 × 10 ml). The combined petroleum ether solutions were washed with distilled water and dried over Na_2SO_4, and the solvent was distilled off. The fatty acids obtained were methylated with diazomethane and analyzed by GLC.

Enzymatic Hydrolysis. Phosphatidylcholines. A solution of 200 mg of the sample in 25 ml of ether was treated with 4 mg of snake venom dissolved in 0.8 ml of 0.1 M tris buffer (pH 10.10). The mixture was stirred mechanically at room temperature. Hydrolysis was performed for 30 min. Then the solvent was evaporated to dryness and the residue was dissolved in a mixture of chloroform and methanol (2:1). The hydrolysis products were separated preparatively in system 2. The yield of fatty acids from position 2 was 87 mg, and the yield of lysophosphatidylcholine 110 mg.

Phosphatidylethanolamines. The phosphatidylethanolamines (200 mg) were dissolved in 25 ml of ether, and 4.1 mg of snake venom dissolved in 0.8 ml of 0.1 M tris buffer with pH 10.0 was added. The mixture was stirred mechanically, the temperature of the water bath being kept at 37°C. The time of enzymatic hydrolysis was 5 h. The hydrolysis products were worked up as in the case of the PCs. The yield of fatty acids was 90 mg and the yield of lysophosphatidylethanolamine 100 mg.

Phosphatidylinositols. The phosphatidylinositols (120 mg) were hydrolyzed under conditions similar to those for the PEs. The time of hydrolysis was 12 h. The yield of fatty acids was 50 mg and the yield of lysophosphatidylinositol 67 mg. The alkaline hydrolysis of the lyso compounds was performed in a similar way to the hydrolysis of the initial phospholipids.

SUMMARY

The fatty-acid composition of the combined phospholipids and also of the main and two minor components of the phospholipids have been studied. On the basis of the results of acid hydrolysis and also IR spectroscopy, the glycerophospholipid structure of the main components of the total material has been confirmed. These main components have been subjected to enzymatic hydrolysis: the position distribution of the fatty acids have been determined and, from these, the possible diglyceride compositions of the phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols have been calculated. It has been shown that the phospholipids are accompanied by 35-40% of carbohydrates which have been characterized qualitatively as disaccharides.

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