12. R. Brandt and C. Djerassi, Helv. Chim. Acta, 51, 1750 (1968).
13. G. G. Abbot and F. D. Gunstone, Chem. Phys. Lipids, 7, 279 (1971); G. G. Abbot and F. D. Gunstone, Chem. Phys. Lipids, 7, 303 (1971).
14. S. A. Barker, J. S. Bromacombe, A. B. Foster, D. H. Whiffen, and G. Zweifel, Tetrahedron, 7, 10 (1959); A. Leboue, Bull. Soc. Chim. Fr., 3037 (1971); J. Muzart and J. P. Pete, Bull. Soc. Chim. Fr., 1373 (1973); D. Gagnaire and M. Leclercq, Bull. Soc. Chim. Fr., 721 (1963).
15. T. Ziminski and E. Borowski, J. Chromatogr., 23, 480 (1966).

PHOSPHOLIPIDS OF THE SEEDS OF KENAF OF VARIETY "UZBEKSKII-1574"

I. Tolibaev, Kh. S. Mukhamedova, and S. T. Akramov

UDC 547.953:665.37

Continuing an investigation of the phospholipids (PL's) of plants of the family Malvaceae, we have studied the seeds of kenaf (*Hibiscus cannabinus*) of the industrial variety "Uzbeksii-1574." The combined PL's were obtained, purified, and fractionated by the method described in previous papers [1, 2]. Two-dimensional chromatography in solvent systems 1 (direction I) and 2 (direction II) showed the presence in the total material of six phosphorus-containing components which were distributed qualitatively and quantitatively in the following way (R in direction II): 0.52, phosphatidylcholines (PC's; 35.6%); 0.70, phosphatidylinositols (PI's; 25.0%); 0.78, phosphatidylethanolamines (PE's; 22.2%); 0.96, N-acylphosphatidylethanolamines (N-acyl-PE's; 9.3%); 0.83, N-acyllysophosphatidylethanolamines (N-acyllyso-PE's; 5.3%); and 0.80, unidentified phospholipids (X; 2.6%).

After the distribution of the total PL's on a column of silica gel, the main and the minor components were finally purified by subfractionation in a thin layer of silica gel in systems 3 and 4. The structures of the fractions mentioned were confirmed by an investigation of the water-soluble products of acid hydrolysis and by IR spectroscopy [3, 4]. The fatty acids of the triglycerides, of the total PL's, and of the individual fractions were determined by saponifying them with alcoholic alkali. The position distributions of the fatty acids in the PC, PE, and PI molecules were determined by enzymatic hydrolysis with the aid of phospholipase A_2 from kufi venom and, after the appropriate working up [1], the fatty acids in the form of their methyl esters were analyzed by GLC (Table 1). The results of the analysis showed that the compositions of the fatty acids of the oils of the total PL's were identical qualitatively and similar quantitatively. The degrees of saturation of the molecules of the individual components of the total PL's rose in the following sequence: PE's → N-acyl-PE's → PC's → PI's → N-acyllyso-PE's. We may note that the phosphatidylcholines isolated from plant materials are almost always more unsaturated than the PE's, but in this case, conversely, the PE's were considerably more unsaturated than the PC's. It was established by means of the results of enzymatic hydrolysis that in the PC's the fatty acids are distributed more selectively between the two positions: 70.5% of saturated acids in position 1 and 91.1% of unsaturated acids in position 2. The results of the position distributions of the fatty acids enabled us to calculate the possible molecular compositions of the PC's, PE's, and PI's:

	PC's	PE's	PI's
Disaturateds	6,4	1,6	10,6
Saturated—unsaturateds	64,0	52,6	55,7
Diunsaturated	27,1	44,5	28,3
Unsaturated—saturateds	2,5	1,3	5,4

The results of the calculations show that the amount of disaturated species in the PE's is considerably smaller than in the PI's and PC's, and the amount of diunsaturated species is greater. This is explained by the comparatively higher degree of unsaturation of the PE molecules than of those of PC's and PI's. The saturated—unsaturated species were formed mainly from the 16:0 and 18:1 or 18:2 acids and the diunsaturated species from the 18.1 and 18.2 acids-

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, N. 6, pp. 776-780, November-December, 1977. Original article submitted June 20, 1977.

 0009-3130/77/1306-0654$07.50

TABLE 1. Compositions and Position Distributions of the Fatty Acids in the Phospholipids

Fatty acid	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Σ S	Σ U
Total phospholipids	0,9	0,7	20,5	1,0	1,6	26,4	47,8	1,1	23,7	76,3
Triglycerides	0,5	0,4	20,9	1,2	3,2	28,8	43,6	1,4	25,0	75,0
Phosphatidylcholines										
total	1,9	1,7	31,6	0,8	2,1	32,1	28,9	0,9	37,3	62,7
position 1	1,8	1,2	63,5	0,7	4,0	16,8	12,0	—	70,5	29,5
position 2	—	2,8	6,1	2,6	—	41,1	45,0	2,4	8,9	91,1
Phosphatidylethanolamines										
total	1,2	1,3	23,3	2,0	1,1	19,3	49,0	2,8	26,9	73,1
position 1	1,1	0,7	50,0	1,8	2,5	11,8	32,1	—	54,3	45,7
position 2	—	0,2	2,7	0,4	—	24,4	69,0	3,3	2,9	97,1
Phosphatidylinositols										
total	—	1,0	40,4	1,0	—	10,9	45,2	1,5	41,4	58,6
position 1	—	2,0	64,1	1,5	—	9,8	22,6	—	66,1	33,9
position 2	—	0,9	15,1	2,5	—	10,1	68,3	3,1	16,0	84,0
N-Acylphosphatidylethanolamines										
total	6,1	5,0	20,0	3,7	4,3	15,2	39,3	6,4	35,4	64,6
O-acyls	1,1	0,9	25,3	0,9	1,2	20,5	49,0	1,1	28,5	71,5
position 1	2,7	2,6	38,5	0,9	3,7	19,1	32,5	—	47,5	52,5
position 2	1,6	1,4	11,0	1,9	1,1	27,2	54,9	0,9	15,1	84,9
N-acyls	0,6	0,5	6,2	1,0	—	9,8	79,4	2,5	7,3	92,7
N-Acyllysophosphatidylethanolamines										
total	7,5	6,6	29,1	4,6	6,5	11,5	32,0	2,2	49,7	50,3
O-acyls	6,2	3,0	8,1	3,0	3,5	9,1	67,1	—	20,8	79,2
N-acyls	11,6	8,0	24,3	8,0	10,2	13,4	24,5	—	54,1	45,9

The analysis of the minor PL's — the N-acyl-PE's and their lyso analogs — was performed as described previously [6, 2] (see Table 1, O- and N-acyls). For a more detailed study of their structure, the N-acyl-PE's were dephosphorylated with a mixture of acetic acid and acetic anhydride. Making use of the specificity of the action of pancreatic lipase on the primary ester groupings in glycerides [7], the diglyceride acetates formed in the dephosphorylation of the N-acyl-PE's were subjected to kipolysis by this enzyme. The lipolysis products were separated by preparative TLC in system 5. The monoglycerides were hydrolyzed with methanolic alkali and the fatty acids from positionsl and 2, in the form of their methyl esters, were subjected to GLC analysis (see Table 1). On the basis of the facts given in Table 1 it may be concluded that the molecules of the N-acyl-PE's are less saturated than those of the N-acyllyso-PE's in relation to the N-acyls (92.7% of unsaturated acids). The predominant amount of unsaturated acids (84.9%) are esterified in position 2 of the N-acyl-PE molecule, as in the case of other phospholipids, and the fatty acids in position 1 have a more saturated nature. The N-acyls of these two minor PL's differ sharply in both qualitative and quantitative composition.

EXPERIMENTAL

For chromatography we used type KSK silica gel: for column chromatography 160-250 μ, and for thin-layer chromatography 125 μ. Thin-layer chromatography (TLC) was performed in the following solvent systems: 1) chloroform—methanol—ammonia (14:6:1); 2) butanol—acetic acid—water (65:20:20); 3) chloroform—ethanol—ammonia (65:35:5); 4) chloroform—methanol—water (65:25:4); and 5) petroleum ether—diethyl ether (85:15).

The GLC of the samples was performed on a UKh-2 instrument under the following conditions: column 2.5 m long, stationary phase 17% of PEGS on Celite 545 (60-80 mesh); carrier gas helium; temperature 197°C.

Partial Deacylation of the N-Acyllyso-PE's. The substance (50 mg) was kept in 0.1 M methanolic NaOH (10 ml) at 37°C for 40 min. Then the mixture was neutralized with 10 ml of

methyl formate and evaporated to dryness, the residue was dissolved in ethanol–water (1:1; 10 ml), and the solution was extracted with petroleum ether (40–60°C, 20 ml). The petroleum ether extract was washed twice with 50% ethanol (2 × 10 ml). The combined aqueous ethanolic extracts were treated three times with chloroform (3 × 15 ml), and the chloroform solution was evaporated. The yield of the petroleum-ether-soluble fraction was 10 mg (fatty acids of the glycerol part of the molecule) and that of the chloroform fraction was 35 mg (traces of FA's and partially deacylated product). The hydrolysis products were separated preparatively in system 3.

The fatty acids were separated and were analyzed by GLC.

The hydrolysis of the partially deacylated product was carried out in 5% KOH/CH_3OH at the boiling point of the mixture. The fatty acids split off were extracted from the acidified solution with ether, dried over Na_2SO_4, methylated, and analyzed by GLC.

Acetolysis of the N-Acyl-PE's. In a sealed tube, 60 mg of the substances and 10 ml of a mixture of acetic acid and acetic anhydride (3:2) was heated at 150°C for 5 h. The reaction product was investigated by TLC in systems 4 and 5. The reaction for phospholipids was negative (Vas'kovskii's reagent). The diglyceride acetates were extracted with a mixture of chloroform, methanol, and water (8:4:3). The chloroform layer was washed several times with water and was dried over Na_2SO_4 and evaporated to dryness. The weight of product obtained was 40 mg. In system 5, the R_f value of the diglyceride acetate was 0.64.

Hydrolysis of the Diglyceride Acetates with Pancreatic Lipase. To 40 mg of the substance in 5 ml of 0.1 M Tris buffer (pH 8.2) were added 40 mg of lipase, 2.5 ml of 0.1 M sodium deoxycholate, and 1.8 ml of 22% $CaCl_2$. The mixture was kept at 37°C for 40 min, and then 2 ml of 20% HCl was added to it. The fatty acids were extracted with ether (3 × 10 ml) and purified by preparative TLC in system 5. The yield of fatty acids from position 1 was 20 mg. The fatty acids were methylated and analyzed by GLC.

The alkaline hydrolysis of the monoglycerides was performed similarly to the hydrolysis of the partially deacylated product.

SUMMARY

Three main and two minor components have been isolated from the total phospholipids of the seeds of kenaf of variety "Uzbeksii-1574" and have been characterized. The fatty-acid compositions of the total phospholipids, of the triglycerides, and of all the components of the phospholipids have been determined. Enzymatic hydrolysis of the main fractions of the phospholipids has been performed, the position distributions of the fatty acids have been determined, and, from this, the possible molecular compositions of the main components have been calculated.

The amide-bound fatty acids and those esterified in the glyceride part of the molecule of the N-acylphosphatidylethanolamines and their lyso analogs have been studied by mild alkaline deacylation. By means of acetolysis followed by lipolysis of the diglyceride acetates obtained with pancreatic lipase the position distributions of the fatty acids in the N-acyl-PE's have been elucidated and it has been shown that position 2 is esterified mainly with unsaturated acids, i.e., the same law is observed as in the main fractions of the phospholipids.

LITERATURE CITED

1. I. Tolibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 289 (1976).
2. I. Tolibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 723 (1976).
3. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Vol. 1, Book 1, Leningrad (1967).
4. G. J. Nelson, Lipids, 3, 104 (1968).
5. E. Stahl, Thin-Layer Chromatography, Allen and Unwin, London/Springer, New York (1969).
6. R. A. Bomstein, Biochim. Biophys. Res. Commun., 21, 49 (1965).
7. M. N. Coleman, J. Am. Oil Chemists' Soc., 38, 685 (1961).