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N-ACYLATED PHOSPHOLIPIDS AND LYSOPHOSPHOLIPIDS OF WILT-RESISTANT VARIETIES OF THE COTTON PLANT

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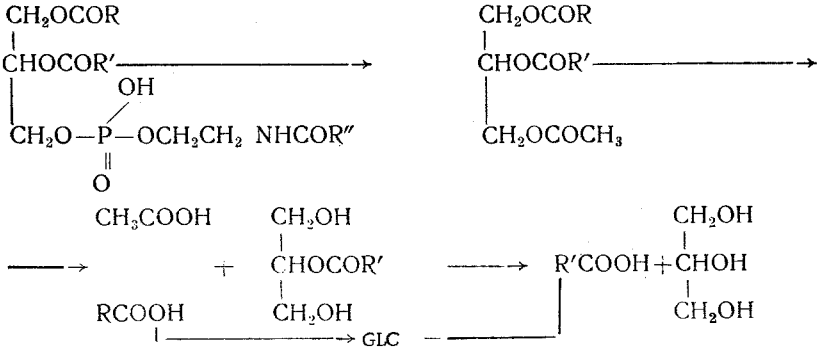
As part of the continuing investigations of the minor phospholipids of the seeds of wilt-resistant varieties of the cotton plant, we have studied the N-acylated phospholipids (N-acylphosphatidylethanolamines and N-acyllysophosphatidylethanolamines) of the varieties Tashkent-1 and Tashkent-3, and also the lysophosphatidylcholines of the varieties Tashkent-1, Tashkent-2, and Tashkent-3. The N-acylated phosphatidylethanolamines were isolated, identified, and analyzed similarly to those from the variety Tashkent-2 [1] by Scheme 1.

The analysis showed the position distributed of the fatty acids in the N-acylphosphatidylethanolamines (Table 1). It follows from Table 1 that the low-molecular-weight $C_{10:0}$ acid, the amount of which in the varieties Tashkent-2 [1] and Tashkent-3 was fairly considerable, was absent from the N-acylphosphatidylethanolamines of the variety Tashkent-1. It is mainly because of this acid that the percentage of low-molecular-weight acids was high in the N-acylphosphatidylethanolamines of the varieties Tashkent-2- and Tashkent-3. The total degree of unsaturation of the molecules of the N-acylphosphatidylethanolamines decreased in the sequence: Tashkent-1 → Tashkent-2 → Tashkent-3. In the N-acylphosphatidylethanolamines of the three varieties of cotton plant, the saturated acids were attached predominantly to the N atom (Table 1).

In the N-acyllysophosphatidylethanolamines, by the methods described previously [1], we established the positions esterified by the fatty acids: as in the case of N-acyllysophosphatidylethanolamines from the variety Tashkent-2, it was position 2 that was substituted in the molecules studied.

In the NMR spectrum there is a multiplet at 5.2 ppm (δ scale) which is characteristic for β-substituted lysophospholipids [2].

The N-acyllysophosphatidylethanolamines of the three varieties had fairly large total unsaturation due to a high content of unsaturated fatty acids in the glycerol moiety of the molecule (O-acyls). Among the fatty acids localized on the N atom there were far more saturated acids than in the corresponding O-acyls. The predominating amount of low-molecular-weight fatty acids ($C_{10:0}$, $C_{12:0}$, $C_{14:0}$) in the N-acylated phospholipids was present in the amide-bound form.



Scheme 1

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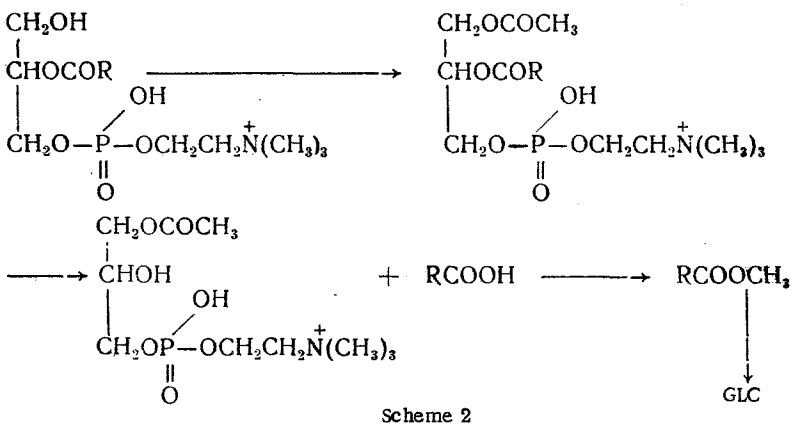
TABLE 1. Fatty-Acid Compositions of the N-Acylated Phosphatidylethanolamines

Acid	Tashkent-1								Tashkent-3							
	N-Acyl-PEs					N-Acyllyso-PEs			N-Acyl-PEs					N-Acyllyso-PEs		
	total	O-acyls			N-acyls	total	O-acyls	N-acyls	total	O-acyls			N-acyls	total	O-acyls	N-acyls
		total	position							total	position					
			1	2							1	2				
10:0	—	—	—	—	—	—	—	—	16,4	2,2	4,4	—	26,7	—	—	—
12:0	4,1	1,5	—	1,1	5,2	1,5	1,6	9,6	6,6	2,2	0,9	3,0	10,2	1,5	1,9	9,0
14:0	3,9	1,5	7,5	1,1	5,0	1,3	3,0	7,0	5,8	3,2	0,8	2,3	8,6	1,3	2,3	6,5
16:0	25,2	28,2	23,8	25,5	39,8	23,6	22,8	23,0	20,9	25,6	30,6	28,3	12,2	23,6	25,4	24,7
16:1	5,2	2 2	4,6	1,1	4,3	1,3	2,5	5,2	3,3	2,5	0,8	2,4	5,5	1,3	3,3	7,6
18:0	4,6	2,0	11,3	—	3,5	3,9	1,9	5,5	3,3	4,0	4,0	—	4,6	3,9	4,0	4,2
18:1	12,9	12,1	22,5	10,1	17,6	21,1	13,3	17,9	11,0	13,8	11,8	16,8	15,1	21,1	12,9	12,7
18:2	44,1	52,5	30,3	61,1	24,6	47,3	54,9	31,8	32,7	46,5	46,7	47,2	17,1	47,3	50,2	35,3
ΣS	37,8	33,2	42,6	27,7	53,5	30,3	29,3	45,1	53,0	37,2	40,7	33,6	62,3	30,3	33,6	44,4
ΣU	62,2	66,8	57,4	72,3	46,5	69,7	70,7	54,9	47,0	62,8	59,3	66,4	37,7	69,7	66,4	55,6

In the study of the lysophosphatidylcholines, we set ourselves the aim of determining what positions were occupied by the acyl residues in this molecule. Under the conditions of our experiment, which are favorable for the enzymatic hydrolysis of phosphatidylcholine [3], phospholipase A_2 does not act on the lysophosphatidylcholines. There is information [4] that phospholipase A_2 can hydrolyze lysophospholipids, but the rate of this reaction is only 0.1% (in relation to that for the phosphatidylcholines). Consequently, the lysophosphatidylcholines were converted into phosphatidylcholines by acylation with acetyl chloride.

We subjected the synthetic phosphatidylcholine to enzymatic hydrolysis with phospholipase A_2, which acts specifically on position 2 (Scheme 2). The fatty acids obtained from position 2 in the form of the methyl esters were analyzed by GLC: they were identical with the fatty acids of the initial lysophosphatidylcholine.



Scheme 2

Thus, β-substitution in the lysophosphatidylcholine molecule has been established. The NMR spectra of the latter also confirmed that position 2 was esterified: in the 5.2 ppm region (δ scale) a multiplet corresponding to a >CHOCOR grouping was observed [2].

The results of the study of the fatty acid composition of the lysophosphatidylcholines of the three varieties of cotton plant showed that degree of unsaturation of these molecules rose from the variety Tashkent-1 to Tashkent-3:

Variety of cotton plant	Fatty acid									
	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	ΣS	ΣU
Tashkent-1	—	3,4	2,0	27,6	2,0	3,4	21,2	40,4	36,4	63,6
Tashkent-2	3,1	1,6	2,3	22,6	2,7	4,2	20,1	43,4	33,8	66,2
Tashkent-3	3,6	2,8	2,0	21,7	1,5	—	25,8	42,6	30,1	69,9

The amount of low-molecular-weight fatty acids rose in the same sequence, the $C_{10:0}$ acid being absent from the variety Tashkent-1.

EXPERIMENTAL

For **chromatography** we used KSK silica gel with a particle size of 160-250 μm (column chromatography) and about 125 μm (TLC). GLC was performed on a UKh-2 instrument at 198°C in a column 2.5 m long filled with poly(ethylene succinate) on Celite-545.

The hydrolysis and mild deacylation of the N-acylated phospholipids was performed as described previously [1].

The NMR spectra were taken on a JNM-4H 100/100 MHz instrument in $CDCl_3$ solution.

Acylation of the Lysophosphatidylcholines. A solution of 50 mg of lysophosphatidylcholines in 12.5 ml of ethanol-free $CHCl_3$ (the chloroform was twice washed with an equal volume of distilled water and was dried over Na_2SO_4) was treated with 1.2 ml of triethylamine and 0.8 ml of acetyl chloride. The mixture was stirred and it was left at room temperature for two hours. Then the phosphatidylcholine synthesized was isolated by preparative TLC. Enzymatic hydrolysis of the synthetic phosphatidylcholine was performed with the aid of phospholipase A_2 as described previously [3].

SUMMARY

The minor phospholipids of the seeds of cotton plants of varieties Tashkent-1, Tashkent-2, and Tashkent-3 have been isolated and studied. The position distribution of the fatty acids in the N-acylphosphatidylethanol-

amines has been established.

On the basis of the results of a chemical study and also of NMR spectroscopy, it is suggested that the N-acyllysophosphatidylethanolamines are 2-acylglycerophosphoryl-N-acylethanolamines, and that the lysophosphatidylcholines are 2-acylglycerophosphorylcholines.

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STUDY OF THE FRACTIONAL AND FATTY-ACID COMPOSITIONS OF THE PHOSPHOLIPIDS OF *Psoralea* SEEDS

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Psoralea drupacea Bge (drupe scurf pea) is a wild-growing medicinal and fodder plant of the family Leguminosae (Fabaceae) that is widespread in Central Asia [1].

We have studied the phospholipids (PLs) of the seeds of this plant collected in the environs of the village of Tobolino, Saragachskii region, Chimkent oblast. The purified and comminuted seeds were defatted with acetone and extracted with chloroform–methanol (2:1). According to TLC, the total PLs obtained were contaminated with carbohydrates, neutral lipids, and substances of steroid nature. They were freed completely from carbohydrates by gel filtration in chloroform–methanol–water (19:10:1) through Molselekt G-25. The neutral lipid and steroid impurities were eliminated by column chromatography on silica gel (eluents: chloroform and acetone). The mean yield of total PLs freed from impurities was 1.1% of the weight of the air-dry seeds, and the amount of phosphorus in them was 3.1%.

To determine the quantitative composition of the total PLs we used two-dimensional TLC on silica gel in solvent systems 1 and 2. Nine phosphorus-containing spots were detected, six of which were identified from their R_f values, comparison with markers, and characteristic color reactions. The R_f values of the components of the total material in the second direction were: lysophosphatidylcholines (lyso-PCs) 0.1; phosphatidylinositols (PIs) 0.3; x_1-PL 0.4; x_2-PL 0.65; phosphatidylcholines (PCs) 0.7; x_2-PL 0.75; N-acylphosphatidylethanolamines (N-acyl-PEs) 0.95; and N-acyllysophosphatidylethanolamines (N-acyllyso-PEs) 0.98.

The amounts of the individual components in the total PLs were determined from the phosphorus contents of each spot on the two-dimensional chromatogram, and their ratio to the total. The fractions of the total PLs were distributed in the following sequence (%): PCs 35.3; PEs 23.4; PIs 21.8; N-acyl-PEs 7.2; N-acyllyso-PEs 3.2; lyso-PCs 3.0; x_1-PL 2.6; x_2-PL 1.8; x_3-PL 1.7.

It can be seen from the figures given that the main components of the total PLs are PCs, PEs, and PIs, i.e., the same situation is observed as for other plants.

It is known that in the defatting of the seeds a very small amount of PLs (traces of PIs or even PEs and their N-acylated analogs) passes into the acetone. In the case of *Psoralea* seeds a considerable amount of N-acyl-PEs and traces of PEs was detected by TLC in the neutral lipid fraction (acetone extract). The lyso analogs of the acylated PEs were completely absent. In order to determine the amount of N-acyl-PEs in the oil we used column chromatography on silica gel. The neutral lipids were eluted with chloroform and acetone and the PLs with chloroform–methanol (95:5). The N-acyl-PEs were freed from pigments and traces of PEs by preparative TLC in system 3. Thus, from the oil of 100 g of seeds we obtained 70 mg of chromatographically pure N-acyl-PEs. Consequently, the true amount of this type of phospholipid in the total is far higher.

The total PLS were separated into homogeneous fractions by column chromatography on silica gel followed

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