SUMMARY

The carbohydrate and amino acid compositions of red clover growing in the Northern Caucasus have been studied qualitatively by paper chromatography.

The amounts of macroelements potassium, sodium, calcium, and magnesium in the total ash content have been determined quantitatively.

Phytin, a mixture of Ca and Mg salts of inositol phosphate, has been isolated and identified.

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A STUDY OF THE PHOSPHOLIPIDS OF THE COTTON PLANT OF VARIETY 159-F IN THE PROCESS OF ITS DEVELOPMENT

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The distribution of phosphorus with the secretion of phospholipids (PLs) in six stages of development of the cotton plant has been studied. It has been shown that additional extraction with methanol leads to the isolation of further PLs. Both the seeds and cotton-plant bushes during growth contain phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, X_1, and X_2, and also unidentified PLs, one of which, Y_2, is phosphatidic acid according to qualitative reactions, chromatographic mobility, and literature information.

The completing stage of the development of the cotton plant is represented by the ripe seeds, the phospholipids (PLs) of which have been relatively well studied [1, 2].

Ganieva and Rakhmanova [3], who investigated the lipids of cotton-plant leaves, showed that the phases of development and the positions of the leaves on the plant are not reflected

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TABLE 1. Yields of Extracts of the Cotton Plant of Variety 159-F and the Amounts and Distribution of Phosphorus in Them According to Vegetation Periods

Vegetation period	Fraction	Yield of extractable substances, %		Phosphorus, %			
		on the cotton plant	on their total	in the cotton plant and fraction	in the fractions, on the total amount of phosphorus	in the fractions, on the total extractable phosphorus	lipid phosphorus as a fraction of the total phosphorus
Beginning of vegetation — four true leaves (I)	Cotton plant	100.0	—	0.018	100.0	—	
	Chloroform-methanolic + methanolic	2.1	58.6	0.45	53.5	92.8	21.0
	Aqueous	1.5	41.4	0.05	4.1	7.2	
	Meal	9.7	—	0.08	42.4	—	
Vegetation — about 20 true leaves (II)	Cotton plant	100.0	—	0.039	100.0	—	
	Chloroform-methanolic	0.9	20.5	0.78	17.5	41.9	
	Methanolic	1.6	38.7	0.38	16.1	38.6	15.2
	Aqueous	1.7	40.8	0.18	8.1	19.5	
	Meal	13.3	—	0.17	58.3	—	
Budding (III)	Cotton plant	100.0	—	0.024	100.0	—	
	Chloroform-methanolic	1.0	24.1	0.57	23.6	49.9	
	Methanolic	0.8	18.7	0.34	10.9	23.1	22.2
	Aqueous	2.4	57.2	0.13	12.8	27.0	
	Meal	14.2	—	0.09	52.7	—	
Flowering (IV)	Cotton plant	100.0	—	0.038	100.0	—	
	Chloroform-methanolic	1.1	22.6	0.90	24.9	47.5	
	Methanolic	1.6	32.8	0.48	19.3	36.9	34.2
	Aqueous	2.1	44.6	0.15	8.2	15.6	
	Meal	18.9	—	0.10	47.6	—	
Massive fruit-bearing (V)	Cotton plant	100.0	—	0.024	100.0	—	
	Chloroform-methanolic	0.8	28.2	0.47	14.5	46.7	
	Methanolic	1.5	17.8	0.22	4.4	14.1	11.4
	Aqueous	0.5	54.0	0.20	2.2	39.2	
	Meal	17.6	—	0.0	68.9	—	
Guza-paya (cotton stems and bolls) (VI)	Cotton plant	100.0	—	0.053	100.0	—	
	Chloroform-methanolic	0.6	19.0	0.46	5.5	20.6	
	Methanolic	1.4	41.6	0.34	8.9	33.2	5.4
	Aqueous	1.3	39.4	0.50	12.3	46.2	
	Meal	71.6	—	0.06	73.3	—	

in the qualitative composition of the fatty acids in the lipids but greatly affect their quantitative composition. This also applies to the PLs [4].

We have studied the changes taking place in the qualitative and quantitative compositions of the PLs of the cotton plant according to vegetation periods. The PLs were exhaustively extracted with chloroform–methanol (2:1) [5] and with methanol. An aqueous re-extract obtained from the chloroform–methanol extract was separated off and treated with chloroform. All the extracts were evaporated, the meal was dried and weighed, and the amount of phosphorus was determined (Table 1).

Phospholipids are present in methanolic extracts of the cotton plant at all stages of its development.

The chloroform–methanolic and methanolic extracts of the cotton plant at the stage of four true leaves contained the same PLs, and therefore the two extracts were combined, but in the other stages the extracts differed from one another and were not combined. The aqueous extracts and the meal contained no lipid phosphorus, which shows the presence of other types of phosphorus-containing substances. The total amount of phosphorus in the aqueous extracts and the meal rose from 46.5% (I) to 85.6% (VI), except for the flowering stage when it was 55.8%. The amount of lipid phosphorus rose from 15.2% (II) to 34.2% (IV) and then fell to 5.4% (VI), with the exception of stage I, when it was 21.0%.

The qualitative and quantitative compositions of the PLs in the extracts of the cotton plant of variety of 159-F were determined by two-dimensional TLC in systems 1 and 2. The results, which are given below, show the chromatographic mobility, R_f, and the percentage amounts of the individual PLs expressed as phosphorus (PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine):

	Y_1	PI	PC	PE	X_1	X_3	Y_2	Y_3	Y_4
R_f in system 1	0,28	0,25	0,44	0,46	0,50	0,55	0,22	0,71	0,12
R_f in system 2	0	0,22	0,77	0,53	0,44	0,74	0,45	0,71	0,27
Stage of vegetation (Table 1)									
I	—	11,0	39,0	10,3	16,9	5,9	5,9	11,0	—
II	2,7	6,0	54,9	6,4	14,5	8,6	1,7	5,2	—
III	2,8	6,1	40 3	9,8	13,6	13,9	7,2	6,3	—
IV	3,4	10,5	29,0	8,2	11,7	4 8	22,1	10,3	—
V	5,3	9,6	25,6	13,0	11,8	10,4	14,8	9,5	—
VI	6,6	10,3	9,2	—	—	—	62,4	—	11,5

As compared with ripe seeds [1], the cotton bushes in stages (I)-(V) also contain PC, PI, PE, and X_1 and X_3, but there are unidentified PLs — Y_1, Y_2, and Y_3. In the guza-paya (cotton stems and bolls) stage some PLs (PE, X_1, X_3, and Y_3) has disappeared and a new PL (Y_4) has appeared, while the amount of Y_2 has increased.

On comparing all the stages, we can see that the amount of PCs first increases and then gradually falls, the amount of X_1 gradually decreases, and, conversely, the amount of Y_1 increases.

On the basis of qualitative reactions, chromatographic mobility, and literature information [7, 8], it may be concluded that Y_2 is phosphatidic acid.

Thus, during the development of the cotton plant great changes take place both in the qualitative and in the quantitative composition of its PLs.

EXPERIMENTAL

Cotton plants were grown to the stage of four true leaves under artificial illumination in room conditions on washed and calcined sand, and to the other stages by the use of ordinary agrotechnology in the G. S. Zaitsev All-Union Scientific-Research Institute for the Breeding and Production of Seeds.

The PLs were isolated in the following way. The cotton bushes were frozen with liquid nitrogen, comminuted, and extracted with a mixture of chloroform and methanol (2:1) and with methanol. The water present in the plants, on passing into the chloroform-methanolic solution, formed a separate layer. The aqueous layer was separated off and was washed with chloroform, the washings being combined with the chloroform-methanolic extract. The extracts were evaporated at 40°C in a rotary evaporator. For two-dimensional TLC, aliquots of the chloroform-methanolic and methanolic extracts were combined.

For TLC we used type KSK silica gel with a size of less than 150 mesh.

The following solvent systems were used for two-dimensional TLC: direction I — 1) chloroform—methanol—25% ammonia (10:5:2); direction II — 2) chloroform—methanol—acetic acid—water (14:5:1:1).

To identify the PLs we employed the usual reagents and methods [6].

The quantitative compositions of the individual groups of PLs were determined by the method of Dyatlovskaya et al. [9] after their separation by two-dimensional TLC.

SUMMARY

1. It has been established that in the process of development of the cotton plant changes take place in the qualitative and quantitative compositions of the total PLs. In almost all stages of development the PCs predominate, their amount first increasing to 54.9% (stage II) and then gradually falling to 9.2% (stage VI). The amounts of PIs and PEs range from 6 to 10%. Unidentified PLs have been detected that were not found in cotton seed kernels.

2. The distribution of phosphorus in the extracts according to the vegetation periods of the cotton plant of variety 159-F has been studied.

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MODIFICATION OF NATURAL COUMARINS REACTION OF KHELLACTONE ESTERS WITH AMINES

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The reaction of diesters of khellactone with primary and secondary amines under mild conditions has given derivatives of 4'-aminodihydroseselin. Under more severe conditions, not only the replacement of a 4'-acyloxy group by an amino group but also the opening of the lactone ring with the formation of the corresponding cinnamamide takes place. The ease of hydrolysis of the 3'-acyloxy group and subsequent esterification of the alcohols formed and also the use of various amines makes it possible to obtain very diverse acyloxy and amino derivatives.

Derivatives of khellactone (I) are widely distributed in plants of the family *Umbelliferae* [1], and a number of these compounds are found in plants of the genus *Seseli* L. [2]. Interest in substances of this type is due to their considerable biological activity and, in particular, their marked spasmolytic action [3] — visnadin, a compound of this class, is used abroad as a spasmolytic agent for the treatment of diseases of the cardiovascular system [4].

The modification of the structure of khellactone esters, for example, by the introduction of an amino group, could considerably modify the activity of these substances and also lead to the formation of compounds, the solubility of salts of which in water would be considerably higher than for the initial lipophilic esters. In order to obtain new biologically

TABLE 1. Synthesis of 4'-Aminocoumarins

Compound	4'-Aminocoumarin		mp, °C	Yield, %	Initial khellactone diester	Method
	R_2	NR_2^3				
IIIa	$COCH_2CH(CH_3)_2$		84	68 9	Ia	A
IIIb	"		142	24,5	Ia	B
IIIc	"	$-\overset{+}{N}H_2-(CH_2)_3CH_3Cl^-$	249 (decomp.)	49.6	Ia	B
IIId	"	$-\overset{+}{N}H_2CH_2CH_2(CH_3)_2Cl$	237 (decomp.)	62.0	Ia	B
IIIe	$COCH_3$		157	53.0	Ib	B
IIIf	"		180	25.0	Ib	B
IIIg	"	$-N(C_2H_5)_2$	111	39,5	Ib	B

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