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# COMPOSITION OF THE PHOSPHOLIPIDS OF THE COTTON PLANT *Gossipium barbadense*


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In recent years, papers have appeared with increasing frequency on the fatty-acid composition and distribution of fatty-acid radicals in phospholipids of plant origin [1-12], the majority of them having been devoted to the study of the lecithins [5-9], and a smaller number to the cephalins [1] and to the phosphatidylinositols [10-12].

Papers on the fatty-acid composition of the lecithins of the cotton plant [7-9] have appeared, and one of them [9] reports the nature of the fatty acids in position 2. There is no such information for the phospholipids of the fine-fibered cotton plant of variety 5904-I in the literature.

We give the results of a determination of the fatty-acid composition of the oil, of the total phospholipids, of the phosphatidylcholines (PChs), of the phosphatidylethanolamines (PEs), and of the phosphatidylinositols (PIs) of the cotton plant *G. barbadense* (variety 5904-I, 1970 crop), and also their position-distribution in the molecules of the PChs, PEs and PIs. The oil and the total phosphatolipids were extracted as described previously. The individual groups of phospholipids were isolated by column chromatography on silica gel and were subfractionated in a thin layer of silica gel in system 1.

The results of a gas chromatographic (GLC) analysis of the fatty acids (Table 1) showed that the fatty-acid compositions of the oil of the total phospholipids are identical qualitatively and similar quantitatively. The individual phospholipids contain the same fatty acids, but their ratios are different, and in order of increasing saturation they form the sequence PChs, PEs, PIs. The amount of unsaturated fatty acids in the PIs is less than that in the PChs and PEs by 26.9-24.1%, respectively, through a decrease in the amount of oleic and linoleic acids, and the amounts of unsaturated acids are greater through an increase in the amounts of palmitic and stearic acids.

The position distribution of the fatty-acid radicals in the phospholipid molecules was determined by enzymatic hydrolysis. For this purpose we used phospholipase $A_2$ from the venom of *Vipera lebetina* obtusa (Azerbaidzhan kufi) [6]. The enzymatic hydrolysis of these phospholipids with 0.1 M tris buffer at pH 10.28 took place completely, but the times of hydrolysis were different and increased in the sequence PChs, PEs, PIs. The completeness of hydrolysis was checked in a thin layer of silica gel in system 1. GLC analysis of


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TABLE 1. Composition and Position Distribution of the Fatty Acids in the Phospholipids of Gossipium barbadense, %

| Acid | Oil | Total phospholipids | PChs | | | PEs | | | PIs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | initial | position | | initial | position | | initial | position | |
| | | | | 1 | 2 | | 1 | 2 | | 1 | 2 |
| 12:0 | 0,2 | 1,1 | 1,2 | 1,1 | 1,4 | 1,2 | 1,2 | 1,2 | 1,0 | 3,0 | 1,7 |
| 14:0 | 0,3 | 0,8 | 0,9 | 0,8 | 0,8 | 0,8 | 0,6 | 0,6 | 1,0 | 2,3 | 1,3 |
| 16:0 | 23,7 | 24,0 | 22,7 | 30,7 | 6,5 | 25,0 | 50,1 | 1,7 | 46,8 | 79,1 | 10,5 |
| 16:1 | 2,7 | 2,2 | 1,2 | 1,2 | 0,8 | 0,8 | 1,3 | 0,6 | 1,5 | 2,5 | 2,0 |
| 18:0 | 2,1 | 1,8 | 1,3 | 2,6 | 0,7 | 1,9 | 2,8 | 0 | 4,2 | 7,5 | 1,2 |
| 18:1 | 19,0 | 18,0 | 28,4 | 28,2 | 32,2 | 14,8 | 7,8 | 18,2 | 9,2 | 2,8 | 16,6 |
| 18:2 | 52,0 | 52,1 | 44,3 | 35,4 | 57,6 | 54,7 | 36,2 | 77,7 | 36,3 | 2,8 | 66,7 |
| Total S* | 26,3 | 27,7 | 26,1 | 35,2 | 9,4 | 28,9 | 54,7 | 3,5 | 53,0 | 91,9 | 14,7 |
| Total U† | 73,7 | 72,3 | 73,9 | 64,8 | 90,6 | 71,1 | 45,3 | 96,5 | 47,0 | 8,1 | 85,3 |
| S:U | 1:2,8 | 1:2,6 | 1:2,8 | 1:1,84 | 1:9,63 | 1:2,4 | 1:0,8 | 1:27,5 | 1:0,9 | 1:0,08 | 1:5,8 |

*S) saturated;
†U) unsaturated.

TABLE 2. Molecular Compositions of the Phospholipids Taking Position Isomerism into Account (%)

| Molecular composition* | PCHs | PEs | PIs |
|---|---|---|---|
| 12:0/12:0 | +† | + | + |
| 14:0/12:0 | + | + | + |
| 16:0/12:0 | 0,5 | 0,7 | 1,5 |
| 16:1/12:0 | + | + | + |
| 18:0/12:0 | + | + | 0,2 |
| 18:1/12:0 | 0,4 | + | + |
| 18:2/12:0 | 0,5 | 0,5 | + |
| 12:0/14:0 | + | + | + |
| 14:0/14:0 | + | + | + |
| 16:0/14:0 | 0,3 | 0,3 | 1,2 |
| 16:1/14:0 | + | + | + |
| 18:0/14:0 | + | + | 0,1 |
| 18:1/14:0 | 0,2 | + | + |
| 18:2/14:0 | 0,3 | 0,3 | + |
| 12:0/16:0 | + | + | 0,3 |
| 14:0/16:0 | + | + | 0,2 |
| 16:0/16:0 | 2,0 | 0,9 | 8,3 |
| 16:1/16:0 | + | + | 0,3 |
| 18:0/16:0 | 0,1 | + | 0,8 |
| 18:1/16:0 | 2,2 | 0,2 | 0,3 |
| 18:2/16:0 | 2,2 | 0,6 | 0,3 |
| 12:0/16:1 | + | + | + |
| 14:0/16:1 | + | + | + |
| 16:0/16:1 | 0,3 | 0,4 | 1,7 |
| 16:1/16:1 | + | + | + |
| 18:0/16:1 | + | + | 0,3 |
| 18:1/16:1 | 0.2 | + | + |
| 18:2/16:1 | 0,3 | 0,2 | + |
| 12:0/18:0 | + | 0 | + |
| 14:0/18:0 | + | 0 | + |
| 16:0/18:0 | 0,2 | 0 | 1,1 |
| 16:1/18:0 | + | 0 | + |
| 18:0/18:0 | + | 0 | 0,1 |
| 18:1/18:0 | 0,2 | 0 | + |
| 18:2/18:0 | 0,3 | 0 | + |
| 12:0/18:1 | 0,3 | 0,3 | 0,5 |
| 14:0/18:1 | 0,3 | 0,2 | 0,4 |
| 16:0/18:1 | 9,8 | 9,2 | 13,1 |
| 16:1/18:1 | 0,4 | 0,3 | 0,4 |
| 18:0/18:1 | 0,8 | 0,5 | 1,2 |
| 18:1/18:1 | 9.2 | 1,5 | 0,5 |
| 18:2/18:1 | 11,4 | 6,2 | 0,5 |
| 12:0/18:2 | 0,6 | 0,9 | 2,0 |
| 14:0/18:2 | 0,5 | 0,7 | 1,5 |
| 16:0/18:2 | 18,0 | 39,0 | 53,0 |
| 16:1/18:2 | 0,7 | 1,0 | 1,4 |
| 18:0/18:2 | 1,5 | 2,1 | 5,0 |
| 18:1/18:2 | 16,2 | 6,0 | 2,0 |
| 18:2/18:2 | 20,1 | 28,0 | 1,8 |
| Disaturated, different acids (S/S) | 1,1 | 1,0 | 5,4 |
| Disaturtaed, same acid (S/S) | 2,0 | 0,9 | 8,4 |
| Diunsaturated, different acids (U/U) | 29,2 | 13,7 | 4,3 |
| Diunsaturated, same acid (U/U) | 29,3 | 29,5 | 2,3 |
| Saturated-unsaturated (S/U) | 32,1 | 53,3 | 78,7 |
| Unsaturated-saturated (U/S) | 6,3 | 1,6 | 0,9 |

*Before the stroke, the acid occupying position 1, and after it the acid occupying position 2

† The symbol + means the presence of the component in an amount of less than 0.1%.

the methyl esters of the acid split off showed that the amounts of unsaturated fatty acids in positions 2 of the molecules of the phospholipids studied were: for the PEs 96.5%, for the PChs 90.6%, and for the PIs 85.4%. The increased amount of unsaturated acids in position 2 is due to oleic and linoleic acids.

The lyso products formed after enzymatic hydrolysis were subjected to alkaline hydrolysis to determine the fatty acids present in position 1. The amounts of saturated acids in this position were: in the PIs 92.0%,

in the PEs 54.7%, and in the PChs 35.2%, this is due to different amounts of the predominating palmitic acid: in the PChs there is only 30.7% of it, in the PEs 50.1%, and in the PIs 79.1%.

In a determination of the possible molecular compositions of the groups of phospholipids investigated, we based ourselves on the experimental results of the positional distribution of the fatty-acid radicals in their molecules, as was done previously [6].

It can be seen from the results obtained (Table 2) that the PChs, PEs, and PIs differ considerably in molecular composition. In the PIs, the amount of diunsaturated species falls considerably and the saturated-unsaturated species increase. Such a sharp change in the molecular composition is connected with the selectivity of the pairing of the fatty acids in the PIs, where the saturated palmitic acid is found predominately in position 1 and the unsaturated linoleic acid in position 2; the 18 :2 acid possesses a lower selectivity of pairing with the 16 :0 acid in the PChs and PEs.

The PEs lack the 18 :0 acid in position 2, and they therefore have fewer molecular species (42) than the PChs and PIs (49 each). The disaturated fractions in the PChs, PEs, and PIs are formed mainly with the 16 :0 acid; diunsaturated same-acid molecules are formed by the 18 :2/18 :2 and 18 :1/18 :1 acids and are characteristic for the PChs and the PEs. It must be noted that in the PChs there is a larger amount of unsaturated-saturated species than in the other phospholipids, and they consist mainly of the 18 :1/16 :0 and 18 :2/16 :0 acids.

Thus, the possible molecular compositions of the individual groups of the phospholipids of the cotton plant depend on the initial composition of the fatty acids and the selectity of their pairing.

## EXPERIMENTAL

The solvents were purified by accepted methods [14]. For chromatographic analysis we used type KSK silica gel of different grain sizes – 100-150 mesh for column chromatography and 150-200 mesh for thin-layer chromatography, the gel being washed with hydrochloric acid and water. As system 1 for TLC we used chloroform–methanol–25% ammonia (65 :35 :5).

The fatty acids of the oil and of the phospholipids were extracted after their cold saponification [15]. The fatty-acid compositions were determined by GLC on a UKh-2 chromatograph at 196-197°C (column 2.5 m long) with 17% of poly(ethylene succinate) on Celite-545 (60-80 mesh) as the stationary phase.

Enzymatic Hydrolysis. A solution of 100 mg of the PChs in 20 ml of moist diethyl ether was placed in a two-necked pear-shaped 30-ml flask, and 3 mg of snake venom dissolved in 0.3 ml of tris buffer (pH 10.28) was added. The flask was connected to a reflux condenser through which a stirrer connected to a motor was passed, and the mixture was carefully stirred at room temperature [6]. After 20 min, hydrolysis had gone to completion.

For the hydrolysis of the PEs and the PIs, the same amounts were taken under the same conditions, but the time of hydrolysis for the PEs was 5 h and for the PIs 47 h.

Separation of the Hydrolysis Products. The fatty acids split off from the PChs were separated from the lyso-PChs by column chromatography. A 50-ml burette was charged with 4 g of silica gel suspended in 20 ml of chloroform. The column was washed with another 40 ml of the solvent, and then the fatty acids were eluted with 120 ml of chloroform–methanol (9 :1) and the lyso-PChs with 80 ml of methanol.

The liberated fatty acids of the PEs and the PIs were separated from the lyso products by preparative TLC in system 1. The free fatty acids were methylated with diazomethane and subjected to GLC analysis.

The fatty acids were liberated from the lyso products by alkaline hydrolysis (10% solution of KOH in methanol, 12 h at room temperature). The fatty acids isolated after the decomposition of the soaps were worked up by the usual methods, methylated with diazomethane, and analyzed by GLC.

## SUMMARY

1. The fatty-acid compositions of the oil, the total phospholipids, and the main groups of phospholipids of the seed kernels of the fine-fibered cotton plant of variety 5904-I have been determined. The complete qualitative identity of the acids present in them and quantitative similarity of the acids in the oil and in the total phospholipids have been shown: in the degree of increasing saturation the phospholipids form the following sequence: phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols.

2. It has been established by enzymatic hydrolysis with phospholipase $A_2$ of kufi venom that the unsaturated fatty acids are present in position of the PCh, PE, and PI molecules – to the extent of 90.6%, 96.5%, and 85.4%, respectively.

3. From the position distribution of the fatty acids in the phospholipids, their possible molecular compositions have been calculated.

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# THE DEPENDENCE OF THE FATTY-ACID COMPOSITION OF COTTONSEED OIL ON THE DEGREE OF UNSATURATION

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The dependence investigated is of considerable interest from the practical point of view. Any strict determination of it would provide fat chemists and merchants with the possibility of quantitatively evaluating the composition of an oil from such a readily available index as the iodine number.

Furthermore, the problem is also of interest for the chemistry of natural compounds and products, particularly from the point of view of Academician S. L. Ivanov's climatic theory of the formation of organic substances [1].

The quality of cottonseed oil is determined primarily, if there are no technological peculiarities in its production, by the ratio of linoleic and oleic acids; this to a certain extent determines the composition and properties of the products of its reduction, and also the conditions of the actual catalytic hydrogenation process. Consequently, in this case it is precisely the dependence of the amount of each of these two unsaturated acids on the degree of unsaturation that is important, while the quantitative differentiation of the acyl radicals has no such importance.

At the present time, various authors have obtained two pairs of equations by which attempts have been made to express the required relationship.

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