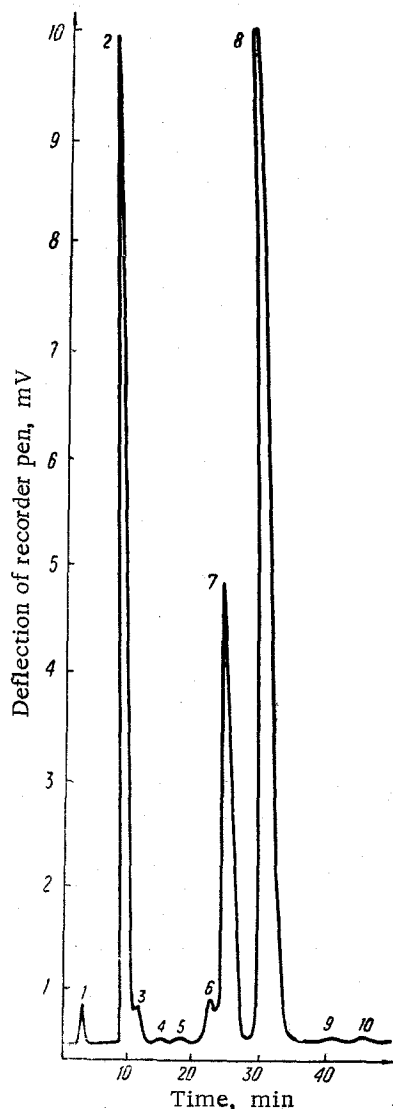


THE FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS OF COTTONSEED OIL

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Khimiya Prirodnykh Soedinenii, Vol. 1, No. 3, pp. 199-201, 1965

In our preceding papers [1-4], data on the composition of the fatty acids and triglycerides of cottonseed oil and the formation and accumulation of the fatty acids in the seeds during maturation have been considered. In addition, the fatty acid components of the lipids (triglycerides, sterol esters, etc.) of the vegetative and generative organs of the cotton plant (in particular, the leaves, flowers, bolls, and bark of the stems and roots) have been given [5]. However, the fatty acid composition of the phospholipids of cottonseed oil has not been studied. There are few investigations on this question in the literature [6], and they are completely lacking for Soviet varieties of cotton.



Chromatogram of the methyl esters of the fatty acids of the phospholipids of cottonseed oil: 1) methyl myristate; 2) methyl palmitate; 3) methyl palmitoleate; 4) methyl palmitlinoleate; 5) methyl palmitlinoleate; 6) methyl stearate; 7) methyl oleate; 8) methyl linoleate; 9) methyl linolenate; 10) methyl eicosadienate.

The present investigation was carried out by using modern analytical methods, particularly chromatographic methods [3, 5, 7]. The paper gives the results obtained in a determination of the fatty acid composition of the phospholipids of cottonseed oil by the methods of adsorption, reverse-phase partition, and gas-liquid chromatography.

Experimental

We investigated cotton seed of the variety 108-F of *Gossypium hirsutum* L., collected in the experimental field plot of the Institute for the Experimental Biology of Industrial and Cereal Crops of the UzSSR Academy of Sciences. The oil was extracted from the seeds with petroleum ether (bp 40-70°) for 24 hr in a Soxhlet apparatus. The solvent was distilled off in vacuum, and the residue was dissolved in petroleum ether to give a 1% solution and was stored in the cold until it was analyzed. This solution was separated on alumina into individual classes of lipids by a method described previously [5]. Then the phospholipids were eluted with a mixture of benzene and acetic acid (9:1). After removal of the solvent, the phospholipids were saponified with 10% alcoholic caustic potash. For this purpose, 300-500 μ g of phospholipids was dissolved in the alcoholic caustic potash solution with heating in a flask with a reflux condenser; after 30 minutes' heating, the alcohol was eliminated by evaporation with the constant addition of water in drops. The potassium salts of the fatty acids were separated from the unsaponifiable ones and were acidified with acetic acid; the fatty acids were extracted with benzene and subjected to chromatographic separation.

By means of reverse-phase paper partition chromatography [1, 7], the presence of the following acids in the phospholipids of cottonseed oil was established: myristic, palmitic, palmitoleic, stearic, oleic, and linoleic; the presence of myristic acid in them had been established previously. Knowing from experience that acids present in small amounts sometimes do not show up in paper chromatography, we subjected the mixture of fatty acids to methylation and then separated the resulting methyl esters by gas chromatography [3]. Under these conditions, we found another four unsaturated fatty acids that had not been detected in paper chromatography. Thus, we have succeeded in showing the presence of ten fatty acids in the phospholipids of cottonseed oil (see figure).

The acids which we isolated were identified from the relationship between the logarithm of the relative retention volume and the number of carbon atoms of the acids concerned [8]. The positions of the double bonds in the four unsaturated acids that we had found for the first time were determined by a method described previously [5]. These acids exhibited the following structures.

<u>No. of carbon atoms:</u> <u>No. of double bonds</u>	<u>Acid</u>
16: 2	Hexadeca-6, 9-dienoic (palmitlinoleic)
16: 3	Hexadeca-6, 9, 12-trienoic (palmitlinolenic)
18: 3	Octadeca-9, 12, 15-trienoic (linolenic)
20: 2	Eicosa-11, 14-dienoic

The quantitative amounts of the acids were calculated from the areas of the peaks found by multiplying the heights of the peaks on the chromatogram by their half-height widths [5].

The Table gives the percentage contents of the fatty acids of the phospholipids of cottonseed oil as compared with the data of Hilditch and Zaky [6].

It is characteristic that fatty acids such as linoleic, oleic, and palmitic are present in large amount not only in the phospholipids but also in the triglycerides of cottonseed oil [1-3]. However, considerable differences are found in the ratios of the other acids: their type and amount are different in the triglycerides and the phospholipids. Thus, the palmitlinoleic, palmitlinolenic, linolenic, and eicosadienic acids that we have found in the phospholipids were not found at all in the triglycerides of cottonseed oil [3]. Palmitlinoleic and palmitlinolenic acids have been identified in

Quantitative Composition of the Fatty Acids of the Phospholipids of Cottonseed Oil (Mole-%)

Fatty Acids	Values found	According to Hilditch and Zaky [6]
Myristic	0.81	—
Palmitic	21.40	17.3
Palmitoleic	0.89	1.5
Palmitlinoleic	Traces	—
Palmitlinolenic	Traces	—
Stearic	2.46	7.3
Oleic	19.87	20.3
Linoleic	54.56	44.4
Linolenic	Traces	—
Arachidic	Not found	2.8
Eicosadienic	Traces	—
Unsaturated C ₂₀ and C ₂₂	—	6.4

small amounts or as traces in the lipids of the vegetative and generative organs of the cotton plant [5]. Linolenic acid, found as traces in the phospholipids of cottonseed oil, is present in large amount (13-56% of the total acids) in the lipids of the leaves and flowers of the cotton plant and in small amounts (1-13%) in the lipids of the bolls, bark of the roots, and bark of the stems. Another unsaturated acid, eicosa-11, 14-dienoic acid, was identified previously in the phospholipids of the bolls and bark of the roots and, in the form of sterol esters, in the flowers, bolls, bark of the stems and bark of the roots of the cotton plant [5]. The other fatty acids that we have found in the phospholipids of cottonseed oil are also present in varying amount in the lipids of the other organs of the cotton plant.

Summary

1. The phospholipids of cottonseed oil contain ten fatty acids.

2. The positions of the double bonds in the aliphatic chain in the newly discovered unsaturated fatty acids — palmitlinoleic, palmitlinolenic, linolenic, and eicosadienic — have been established by the method of gas-liquid chromatography: they are present at the 6, 9; 6, 9, 12; 9, 12, 15; and 11, 14 carbon atoms, respectively, counting from the carboxyl end of the chain. A saturated fatty acid (myristic) has been found in the phospholipids of cottonseed oil for the first time.

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23 December 1964

Scientific Research Institute for the Chemistry and Technology
of Cotton Cellulose, State Committee of the Chemical Indus-
try of Gosplan SSSR, Tashkent