MINOR PHOSPHOLIPIDS OF THE SEEDS OF THE COTTON PLANT OF VARIETY "TASHKENT-2"

STRUCTURE OF THE N-ACYLLYSOPHOSPHATIDYLETHANOLAMINE

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N-Acylated phospholipids have been found in many plant materials [1-3], including the cotton plant [4, 5]. From the seeds of the cotton plant of the wilt-resistant variety "Tashkent-2" we have isolated two minor components the amount of which were 4.1 and 3.3%, respectively, of the total phospholipids (PL's). On separating the total PL's on a column of silica gel, the minor components were eluted with mixtures of $CHCl_3$ and CH_3OH (95:5 and 9:1), and they were freed from the pigments present in them by passing these fractions through Al_2O_3 [6] and by their additional separation by preparative TLC.

To identify the minor phospholipids isolated we used various physicochemical methods of investigation. Both substances, which in the initial form have negative reactions with ninhydrin, after acid hydrolysis showed the presence in the reaction products of ethanolamine, glycerol, and fatty acids (FA's). Consequently, it may be assumed that the amino group of the ethanolamine in the initial compound is not present in the free form but is bound by an amide bond to a fatty acid. This was confirmed by IR spectroscopy: there were absorption bands at 1650 and 1540 cm^{-1} which did not disappear on hydrogenation (amide carbonyl). In addition, when thin-layer chromatograms of the minor substance N-acyl-PE with a synthetic standard obtained by acylating phosphatidylethanolamine were compared the coincidence of their R_f values (0.9 in systems 1 and 2) showed their identity [4]. On acylation, the lyso product also gave N-acyl-PE.

Since phospholipase A_2 does not act on N-acylated phospholipids [1], to investigate the position distribution of the FA's we selected the following method. By means of the acetolysis reaction, the N-acyl-PE's were converted into diglyceride acetates [9], which were subjected to lipolysis with pancreatic lipase isolated from porcine pancreas [10]. The monoglycerides (MG's) and FA's obtained were separated preparatively in system 3. Then the MG's were subjected to alkaline deacylation and the FA's from position 2 were obtained. The FA's were methylated with diazomethane and analyzed by GLC (Table 1).

It was established that the FA compositions of the two minor components were identical qualitatively. In the quantitative respect, however, there were substantial differences. In its degree of saturation (43.0%), the molecule of the N-acyl-PE was close to the most saturated of the main components of the total PL's – phosphatidylinositol (43.2%) [11] – while, on the other hand, the N-acyllyso-PE was one of the most unsaturated fractions (after the phosphatidylcholine). In both molecules, the N-acyls were more saturated than the corresponding O-acyls. In the N-acyl-PE, as for the main components of the total PL's, position 1 was esterified by more saturated FA's than position 2, the saturation here being due to a considerable extent to a contribution of the low-molecular-weight acids ($C_{10:0}$, $C_{12:0}$, and $C_{14:0}$), which were absent from position 2.

In order to determine the positions of the aryl radicals in the N-acyllyso-PE (R_f 0.9 in system 1 and 0.65 in system 2) we performed dephosphorylation by acetolysis followed by the action of pancreatic lipase on the monoglycerides obtained. No lipolysis took place (TLC and GLC). Pancreatic lipase acts specifically on the ester bond in position 1, and therefore in this case it may be assumed that there were no FA's in this position and, consequently, position 2 of the N-acyllyso-PE molecule is esterified. Thus, it has been established that the lyso analog of acylated phosphatidylethanolamine has the structure of 2-acylglycerophosphoryl-N-acetylethanolamine. Such a high degree of unsaturation of the glycerol moiety of the N-acyllyso-PE molecule is also evidence in favor of the structure that we have proposed, since it is known that unsaturated FA's in glycerophospholipids predominantly occupy position 2.

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TABLE 1. Fatty-Acid Compositions of the Minor Phospholipids

Fatty acid	N-acylphosphatidylethanolamine					N-acyllysophosphatidylethanolamine		
	total	O-acyls			N-acyls	total	O-acyls	N-acyls
		total	position					
			1	2				
10:0	7,0	12,7	8,6	—	9,7	2 5	—	6,0
12:0	3,5	2,4	7,9	—	7,0	1,4	0,7	6,7
14:0	3,5	1,6	7,0	—	6,8	1,5	0,6	6,6
16:0	24,6	16,7	23,3	24,0	24,1	16,1	26,6	20,8
16:1	4,1	2,4	5,4	9,0	6,0	1.4	2,8	6 0
18:0	4,4	4,3	5,8	8,4	8,5	9,3	—	12,7
18:1	15,4	14,6	9,7	22,8	15,7	24,9	10,0	18,0
18:2	37,5	45,3	32,3	35,8	22,2	42,9	59,3	23,2
Σ_s	43,0	37,7	52,6	32,4	56,1	30,8	27,9	52,8
Σ_u	57,0	62,3	47,4	67,6	43,9	69,2	72,1	47.2

EXPERIMENTAL

For TLC (KSK silica gel, up to 125 μ) we used the following solvent systems: 1) $CHCl_3-CH_3OH-NH_4OH$ (25%) (65:35:5); 2) $CHCl_3-CH_3OH-H_2O$ (65:35:5); and 3) petroleum ether–diethyl ether (85:15).

The N-acyl-PE and the N-acyllyso-PE were hydrogenated in ether over a palladium catalyst [4]. Gas–liquid chromatography was carried out in a UKh-2 instrument at 198°C in a column 2.5 m long filled with polyethylene glycol succinate (17%) on Celite-545

The total fatty acids of the phospholipids investigated were determined by deacetylation according to Stahl [7]. The FA's of the glycerol moiety of the molecule (O-acyls) were split off under conditions of mild alkaline hydrolysis (0.1 M $NaOH/CH_3OH$) [8], and the amide-bound FA's (N-acyls) were obtained by saponification according to Stahl [7].

Preparation of Diglyceride Acetates. In a sealed tube, a solution of 90 mg of N-acyl- PE in 10 ml of acetic acid–acetic anhydride (3:2) was heated at 150°C for 5 h. The acetolysis products were investigated by TLC in system 3 – no phospholipids were detected.

The reaction mixture was separated in the $CHCl_3-CH_3OH-H_2O$ (8:4:3) system. The diglyceride acetates passed into the chloroform layer, which was washed several times with distilled water and was dried over Na_2SO_4. After the distillation of the solvent, 46 mg of diglyceride acetates (R_f 0.55 in system 3) was obtained.

Lipolysis. To 46 mg of diglyceride acetates was added 46 ng of crude pancreatic lipase dissolved in a mixture of 9 ml of 0.1 M Tris buffer (pH 8.4), 2.5 ml of 0.1% Na deoxycholate solution, and 1.8 ml of 22% $CaCl_2$ solution. The mixture was kept at 37-40°C for 30 min with mechanical stirring. At the end of the reaction, 2 ml of 20% HCl was added and the mixture was extracted three times with ether. The ethereal layer was dried over Na_2SO_4. On chromatography in a thin layer, the ethereal solution was found to contain FA's (position 1) and monoglyceride acetates (R_f 0.65 in system 3), and these were separated by preparative chromatography.

The acetolysis and lipolysis of the N-acyllyso-PE's were performed in the same way as for the N-acyl-PE's.

SUMMARY

The minor phospholipids from the seeds of the cotton plant of variety "Tashkent-2" have been isolated and studied. It has been established that the FA's attached to the N atom are more saturated than the corresponding O-acyls. For the N-acyllsyo-PE the structure of a 2-acylglycerophosphoryl-N-acylethanolamine has been proposed. As in the main fractions, in the minor components of the phospholipids of the seeds of the cotton plant of variety "Tashkent-2" the saturated fatty acids mainly occupy position 2.

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NEUTRAL LIPIDS OF THE SEEDS OF *Eremostachys moluccelloides*

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We have previously reported on individual components of the fatty oil of the seeds of *Eremostachys moluccelloides*, family Labiatae [1-3]. In the present paper we give the results of a further study of the total neutral lipids isolated from the seeds of plants collected in 1976 (in the Tashkent oblast).

The neutral lipids (NL's) of the ripe seeds of *E. moluccelloides* were chromatographed on a column of silica gel. Elution with hexane yielded the total hydrocarbons, high-molecular-weight alcohols, pigments, esters, and sterols (fraction I, 1% of the weight of the initial extract) [1]; hexane–diethyl ether (95 : 5) eluted the triglycerides (TG's) (II, 89.6%); hexane–diethyl ether (92 : 8) eluted the total free fatty acids (FFA's) and the sterols and diglycerides (DG's) (III, 7.2%); diethyl ether eluted the monoglycerides (MG's) (IV, 0.8%); and methanol eluted the phospholipids (V, 1.4%).

Assignments to the appropriate classes were made on the basis of the results of TLC [4] and spectral (IR and NMR) characteristics. The fact that fraction (V) consisted of phospholipids was confirmed by the qualitative reaction with the Vas'kovskii reagent. The phospholipid fraction was not studied.

Since halogen-containing acids have previously been isolated from the oil of *E. moluccelloides* [3], each of the fractions obtained was subjected to the Beilstein test for the presence of halogen. Fractions II, III, and V gave a positive result. The presence of a helogen in fractions II and III was confirmed by the results of neutron-activation analysis (performed by R. Khamidova of the Institute of Nuclear Physics of the Academy of Sciences of the Uzbek SSR). This showed that fraction II contained 4 ppm of Br and 160 ppm of Cl, and fraction III contained 90 ppm of Br and 1100 ppm of Cl.

The composition of fraction I has been described previously [1]. The fatty-acid composition of the TG fraction (GLC) is given in Table 1. The composition of the acids of the TG's differs from that of the TG's of the seeds of *E. moluccelloides* collected in 1974 by a lower content of the $C_{20:1}$ acid and by the appearance of small amounts of the $C_{16:1}$ and $C_{18:3}$ acids [1]. The glyceride composition of the TG's calculated from the results of enzymatic hydrolysis is represented by the following types (%):

GlS_3 – none	GlS_2U – 0.2	GlSUS – 0.3
GlUSU – 1.6	GlSUU – 10.0	GlU_3 – 87.9 [5].

By chromatographing fraction III again on a column of silica gel, we isolated di-2-ethylhexyl phthalate identified by its NMR spectrum [2] and FFA's [hexane–diethyl ether (92 : 8)], free sterols and DG's [hexane–diethyl ether (1 : 1)]. The Beilstein test was positive for the FAA fraction and the DG fraction (weak).

In all cases the separation was monitored by TLC on Silufol plates in system 1. According to TLC, the DG fraction represented the sum of the α, β-(R_f 0.2) and of the α,α'-(R_f 0.26) isomers with a predominance of the latter. The compositions of the fatty acids of the total DG's (GLC) are given in Table 1. The IR spectrum

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