USE OF THE VASKOVSKY–SVETASHEV REAGENT FOR COMPLEX DETECTION OF PHOSPHOLIPIDS AND IN THEIR PREPARATIVE SEPARATION BY THE TLC METHOD

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It has been established that the Vaskovsky–Svetashev reagent has no influence on the general and positional composition of the fatty acids of phospholipids. For the qualitative identification of PLs on one plate it is possible to use successively iodine, ninhdrin, the Dragendorff reagent, the Vaskovsky–Svetashev reagent, 50% H_2SO_4, and the Malachite Green reagent.

A procedure for investigating lipids by TLC has been described in detail in [1, 2]. The phospholipids (PLs) are one of the most representative classes of polar lipids, and a number of reagents are used for their qualitative detection [2, 3]. A specific reagent for detecting phospholipids which contains an acid in extremely low concentration is the reagent proposed by Vaskovsky and Svetashev [3].

The aim of our investigation was to determine the influence of the Vaskovsky–Svetashev reagent on the change in the fatty acid composition of the PLs isolated from cotton seed and also on the identification of the PLs on TLC.

We prepared an artificial mixture of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), N-acyl-PE, and lyso-PC (20% each). After the chromatography of the combined PLs, the plates were sprayed with the Vaskovsky–Svetashev reagent, the top of the colored layer of silica gel, equal to 1/10-1/5 of the layer of adsorbent, was removed, and so was the lower layer of adsorbent containing none of the reagent, and they were transferred to Schott filters and eluted first with a mixture of chloroform, methanol, and 25% ammonia, and then with a mixture of chloroform and methanol. Elution and the

TABLE 1

Phosphatidylcholine		Acid								
		12:0	16:0	16:1	18:0	18:1	18:2	18:3	Σs	Σu
Not treated with the reagent										
Total		1,2	19.1	2 1	3,7	24,7	46,3	2,3	24,0	76,0
Position	1	1,9	33 9	2,1	6,2	24,2	30,2	1,5	42,0	58,0
	2	0.5	4.3	2,1	1.2	25.2	62,4	4,3	6,0	94,0
Treated with the reagent										
Total		0,8	20,0	1,1	3,7	23 8	47,7	2.9	24,5	75,5
Position	1	1,0	36,6	1,3	6,4	22.7	30,0	2,0	44 0	56,0
	2	0,6	3,4	0,9	1,0	24,9	65,4	3,8	5.0	95.0
Top fraction										
Total		0,8	13,9	1,1	2,8	25,4	54,6	1 4	17,5	82,5
Position	1	0,9	25,5	1,2	4,9	26,6	38,1	2,8	31,3	68,7
	2	0,7	2,3	1,0	0,7	24,2	71,1	—	3,7	96.3
Middle fraction										
Total		1,1	21,6	1,5	4,4	26,2	43,1	2,1	27,1	72 9
Position	1	1,5	39,9	1,7	7,1	21,9	24,7	3,2	48,5	51,5
	2	0,7	3,3	1,3	1,7	30,5	61,5	1,0	5,7	94,3
Bottom fraction										
Total		1,5	24,2	2 4	6,1	22,6	41,7	1,5	31 8	68,2
Position	1	0,7	3 .9	0,2	9,0	20.6	30 9	1.7	46,6	53,4
	2	2,3	11.5	4,6	3,2	24,6	52,5	1 3	17,0	83,0

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native state of the PLs were monitored by one- and two-dimensional TLC in systems 1 and 2. In all cases, a single spot was observed which showed the absence of hydrolytic processes.

The influence of this reagent on the change in the fatty acid moiety of a PL was studied with PC as an example. Chromatography was performed in system 1, and the plates were sprayed with the Vaskovsky–Svetashev reagent and treated as described above. The fatty-acid compositions and positional distribution of the acyl radicals in the PC molecules, both those treated with the reagent and the initial ones, were identical (Table 1) and, consequently, the reagent had no effect on the structure of the PCs.

The results of the investigation performed led to the following conclusions:

1. After spraying with the Vaskovsky–Svetashev reagent, the colored layer can be used to determine the total fatty-acid composition and the uncolored layer for enzymatic hydrolysis and other purposes. In the use of the reagent, accuracy in the determination of the PL zones over the width of the chromatographic plate is achieved.

2. On TLC (see Table 1) distribution of the PLs with respect to molecular species is observed over the width of the chromatographic plate.

A disadvantage of I_2 (the usual reagent for PLs and other lipids) is that on the detection of PL with incomplete elimination of the I_2 there is a loss of unsaturated fatty acids, while the complete elimination of I_2 requires from 12 to 36 h and more. The use of the Vaskovsky–Svetashev reagent for PLs, however, permits them to be detected rapidly with no change in their fatty-acid compositions.

The following order of visualization of TLC plates by reagents in the qualitative identification of PLs is proposed: I_2 vapor; after the elimination of the I_2, ninhydrin; then the Dragendorff reagent, the Vaskovsky–Svetashev reagent, carbonization with 50% H_2SO_4, decolorization with perchloric acid at 200-250°C, and spraying with the Malachite Green reagent [4]. This procedure permits a more reliable identification of the various PLs on one plate.

EXPERIMENTAL

Individual PLs were obtained as described in [5]. The plates were prepared with the use of KSK silica gel containing 5% of gypsum, the thickness of the layer of silica gel being ~1 mm and the size of the plates 20 cm^2.

On each plate 100-150 mg of PLs in chloroform was deposited. Solvent systems: 1) chloroform–methanol–25% ammonia (70:30:5); 2) chloroform–methanol–acetic acid–water (14:5:1:1). The silica gel was removed from the plates into a Schott No. 3 filter immediately after spraying with the Vaskovsky–Svetashev reagent and was eluted with 10 ml of chloroform–methanol (1:1). The GLC analysis of the fatty acid methyl esters was performed as described in [6].

CONCLUSIONS

It has been established that the Vaskovsky–Svetashev reagent has no influence on the fatty-acid compositions of phospholipids.

The quantitative identification of phospholipids on one plate can be carried out by the stagewise use of iodine, ninhdyrin, the Dragendorff reagent, the Vaskovsky–Svetashev reagent, 50% H_2SO_4, and the Malachite Green reagent.

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