PHOSPHOLIPIDS OF Crambe amabilis

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We have investigated the phospholipid (PL) complex of the seeds of Crambe amabilis (family Cruciferae) growing in the Bostanlykskii region of Tashkent oblast in the environs of Burchmulla. The yield of combined phospholipids obtained by Folch's method and freed from carbohydrates by gel filtration on Sephadex G-25 was 0.85% of the weight of the air-dried seeds. On a two-dimensional chromatogram (direction I – system 1; direction II – system 2) seven phosphorus-containing spots were revealed, and their quantitative distribution was as follows:

Phosphatidylcholines (PCs)	56.1
Phosphatidylethanolamines (PEs)	20.6
Phosphatidylinositols (PIs)	13.2
Phosphhatidylserines (PSs)	3.0
N-Acylphosphatidylethanolamines (N-acyl-PEs)	2.8
N-Acyllysophosphatidylethanolamines (N-acyllyso-PEs)	2.3
Lysophosphatidylcholines (lyso-PCs)	2.0

It can be seen from this that the total PLs of this species of Crambe differ from those of C. schugnana [1] both qualitatively and quantitatively. It is interesting to note that in the seeds of the plants that we studied previously [1, 2] the amount of PIs was greater than of PEs. In this case, conversely, there are more PEs (20.6%) than PIs (13.2%). The total PLs were separated into their components by column chromatography on silica gel and, in contrast to the total PLs of other seeds [1, 2], the individual PL fractions of the combined material were eluted from the column in homogeneous form.

In the individual PL fractions, the nitrogen and phosphorus contents were determined, the IR spectra were recorded, and the water-soluble products of acid hydrolysis were analyzed. On the basis of the results obtained, the PLs under study were assigned to the glycerophospholipid group. The fatty acids of the triglycerides, of the total PLs, and of the individual homogeneous fractions of it were isolated under mild conditions and, in the form of methyl esters, were analyzed by GLC (Table 1).

The compositions of the fatty acids of the oil and of the total phospholipids were the same so far as concerns the acids present, but their ratios were different: in both cases the FAs consisted of a set of 11 acids among the saturated representatives of which palmitic acid predominated, but its amount in the triglycerides was one third of its amount in the total PLs. Among the unsaturated acids in the oil, erucic predominated (30.1%), while in the total PLs it was linoleic (24.9%).

The individual PLs contained the same main acids, but the minor acids were distributed nonuniformly: the PCs contained no low-molecular acids – $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ –, while the PCs contained the $C_{10:0}$ acid. Arachidic acid ($C_{20:0}$) was absent from the PCs, the N-acyl PCs, and their lyso analogs. According to increasing degree of saturation, the PLs formed the following sequence: PCs → PEs → PIs → N-acyllysoPEs → PSs → N-acyl-PEs. The most saturated fraction of this combined material consisted of the N-acyl-PEs, in which more than half (50.7%) of the acids were saturated.

The position distribution of the FAs in the glyceride part of the PC, PE, PI, and PS molecules was determined by enzymatic hydrolysis using as the source of phospholipase A_2 the venom of the Azerbaidzhan kufi in 0.1 M Tris buffer with pH 8.5. From the results of GLC analysis of the FAs (see Table 1) it can be seen that,

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TABLE 1. Fatty Acids of the Triglycerides, of the Total Phospholipids, and of the Individual Fractions of the Phospholipids of the Seeds of Crambe amabilis

Fraction	Fatty acid											ΣS	ΣU
	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:1		
Triglycerides	2,0	1,8	0,8	4,5	2,0	2,0	18,5	14,0	21,1	3,2	30,1	14,3	85,7
Phospholipids	1,6	1,4	0,5	13,0	3,7	3,3	23,3	24,9	13,7	8,3	6,3	28,1	71,9
Phosphatidylcholines													
total	—	—	—	7,2	2,0	1,9	42,4	26,2	16,3	1,7	2,3	10,8	89,2
position 1	—	—	—	14,2	1,7	1,0	50,0	17,0	8,0	3,6	4,5	18.8	81,2
position 2	-	—	—	1,6	1,0	—	35,8	35,8	25,8	—	—	1,6	98,4
Phosphatidylethanolamines													
total	1,2	1,0	1,0	12,0	2,1	1,4	20,0	35,6	18,0	4.8	2,9	21,4	78,6
position 1	3	2,5	2,5	21,5	4,0	2,1	17.1	19,5	13,6	8,6	5,6	40,2	59,8
position 2	—	—	—	1,5	1,0	0,9	23,8	50,0	22,8	—	—	2,4	97,6
Phosphatidylinositols													
total	0,4	0,8	0,4	24,0	1,4	0,4	20,0	30,0	16,3	3,3	3,0	29,3	70,7
position 1	1,6	2,1	1,0	48,5	1,3	1,4	20,5	12,3	2,0	5,0	4,3	59,6	40,4
position 2	—	—	—	1,0	0,6	—	20,4	48,0	30,0	—	—	1,0	99,0
Phosphatidylserines													
total	—	6,1	0,5	30,4	1,5	4,2	16,0	25,0	13,2	—	3,1	41,2	58,8
position 1	-	8,7	1,0	60,4	—	—	9,2	16,4	4,3	—	—	70,1	29,9
position 2	—	3,3	—	5,1	1,2	4,1	21,1	36,1	23,1	—	6	12,5	87,5
N-Acylphosphatidylethanolamines													
total	20,0	2,8	3,9	20,0	4,4	4,0	13,5	17,5	6,5	—	7,4	50,7	49,3
O-acyl	10.2	2,6	3,6	25,3	4,5	5,3	17,4	19,0	9,5	—	2,6	47,0	53,0
N-acyl	30,0	3,1	4,6	15,5	4,0	2,5	8,6	16.0	7,1	—	8,6	55,7	44,3
Lysophosphatidylcholines	6,0	8,0	2,2	16,6	3,7	1,7	30,0	23,0	10,0	1,7	2,1	31,2	68,8
N-Acyllysophosphatidylethanolamines	3,6	13,7	1,0	16,1	2,1	2,0	20,0	25,6	11,5	—	4,4	36,4	63,6

as was to be expected, the unsaturated acids occupied position 2 in the molecules of the PLs studied: 99% in the PIs, 98.4% in the PCs, 97.6% in the PEs, and 87.5% in the PSs. The PIs were characterized by a high specificity of the distribution of the acids: 59.6% of the saturated acids were esterified in position 1. Positions 2 of the PC and PI molecules contained no stearic acid, while in the phosphatidyl serines this acid was present completely in position 2. In the PCs, PEs, and PIs, the erucic acid was mainly esterified in position 1, and in the PCs exclusively in position 2. Arachidic acid is esterified only in position 1 in the molecules of the main PL fractions.

In the N-acyl-PE molecules, the fatty acids localized on the N atom are more saturated (55.7% of saturated acids) than the acids in the glycerol moiety of the molecule (O-acyl groups). The degree of saturation of the N-acyl groups is due to an unusually high content of $C_{10:0}$ (30%). The overwhelming amount of erucic acid is present in the nitrogen-bound form.

Information on the position distribution of the FAs in the PC, PE, PI, and PS molecules enabled their possible molecular compositions to be calculated.

Type	PCs	PEs	PIs	PSs
Disaturated	0.2	0.5	0.5	8.1
Diunsaturated	77.8	58.5	39.6	26.9
Saturated–unsaturated	20.6	39.9	59.6	62.0
Unsaturated–saturated	1.4	1.1	0.3	3.0

The number of molecular forms calculated for the PCs was 32, for the PEs 45, for the PIs 38, and for the PSs 34. In the PCs, the amounts of disaturated and saturated–unsaturated types decreases considerably. This change in the molecular composition of the PCs is connected with the selectivity of the pairing of the fatty acids of the PCs, where the saturated $C_{10:0}$, $C_{12:0}$, and $C_{14:0}$ acids are completely absent, and the stearic acid is present in position 2. This situation is also explained by the fact that the amount of the 16 : 0 acid in position 1 (14.2%) is smaller than in the other PL fractions and, consequently, so is the total degree of unsaturation of the molecule. In all cases, disaturated types are formed with palmitic acid. The diunsaturated types are predominant for the PCs and PEs, and the saturated-unsaturated types for the PIs and PSs.

EXPERIMENTAL

As absorbent for chromatographic analyses we used type KSK silica gel with particle sizes of up to 125 μm which had been washed with hydrochloric acid, water, and acetone, and ground in a ball mill together with 5% of gypsum for thin-layer chromatography and the same material with a particle size of 160-250 μm for column

chromatography. The following solvent systems were used for chromatography: 1) chloroform–methanol–water (65 : 35 : 5), and 2) chloroform–methanol–25% ammonia (65 : 35 : 5). The fatty acids of the oil and of the phospholipids were isolated by alkaline saponification (5% KOH in methanol, room temperature. 15-18 h). The mixtures of fatty acid methyl esters were separated on a UKh-2 chromatograph using a 2500 × 4 mm column containing 18% of polyethyleneglycol succinate on Celite-545 (60-80 mesh) at 197°C with helium as the carrier gas. The pressure of helium at the outlet was 2.5 atm. The IR spectrum was recorded on a UR-20 instrument with the substances in the form of films. The phosphorus was determined quantitatively by a known method. The acid, alkaline, and enzymatic hydrolyses of the PLs were performed by procedures described previously [2]. The N-acyl-PEs and N-acyllyso-PEs were also analyzed as described previously [3].

SUMMARY

The phospholipid complex of seeds of the plant Crambe amabilis has been studied for the first time. From the combined phospholipids freed from carbohydrates three main components (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) and four minor components (lysophosphatidylcholine, N-acylphosphatidylethanolamine, phosphatidylserine, and N-acyllysophosphatidylethanolamine) have been isolated and characterized.

The position distributions of the fatty acids in the phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine molecules have been determined by enzymatic hydrolysis with phospholipase A_2. On the basis of these results, their possible molecular compositions have been calculated. The compositions of the fatty acids esterified in the glyceride moiety and acylating the nitrogen atom in the N-acylphosphatidylethanolamine molecule have been established.

LITERAUTRE CITED

1. Yu. A. Tadshibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 109 (1977).
2. Yu. A. Tadshibaev, Kh. S. Mukhamedova, and S. T. Akramov, Khim. Prirodn. Soedin., 435 (1976).
3. Kh. S. Mukhamedova and S. T. Akramov, Khim. Prirodn. Soedin., 580 (1976).

AMOUNTS OF PHOSPHOLIPIDS AND PHYTIN IN THE SEEDS OF VARIOUS PLANTS

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Over a number of years, we have systematically studied the phospholipid complexes of the seeds of a number of plants of the family Malvaceae: various types of cotton plant [1-5] and kenaf [6].

In order to determine the materials of the greatest interest in the theoretical or practical respect, we have begun to study the seeds of plants belonging to different families for their phospholipid and phytin contents.

The phospholipids were extracted by chloroform–methanol (2 : 1) from the comminuted and acetone-defatted seeds. For the preliminary purification of the combined phospholipids from accompanying substances (oil, pigments, carbohydrates, steriods, etc.), the dry extract was treated with acetone. The acetone-insoluble residue was dissolved in chloroform–methanol–water (90 : 10 : 1), and for final purification from carbohydrates this mixture was passed through a column with preswollen Molselekt G-25. As compared with the combined phospholipids from other sources, the combined phospholipids from the seeds of Onopordon acanthium L. and Rhaponticum integrifolium (family Compositae) possess some unusual properties: they do not precipitate from a concentrated chloroform solution on the addition of acetone, and when a dry residue is treated with acetone a

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