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COMPOSITION OF THE PHOSPHOLIPIDS OF SEEDS OF CERCIS SILIQUASTRUM

M. A. Bitadze, Ts. M. Dalakishvili, and É. P. Kemertelidze

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The phospholipids of the polar lipids of the seeds of the Judas tree and the fatty-acid composition of the N-acylphosphatidylethanolamines, the phosphatidylcholines, and the phosphatidylethanolamines, and also the molecular-species types of the fatty acids of the phosphatidylcholines and the phosphatidylethanolamines have been studied. It has been established that the seeds of the Judas tree contain ten phospholipids. The main fatty acids are palmitic and oleic.

Cercis siliquastrum L. – the Judas tree (family Leguminosae) – is a species from the Mediterranean. It is cultivated as a decorative plant in the Crimean Carpathians, in the Caucasus, and in Central Asia. The Judas tree has scarcely been studied for the presence of biologically active substances. There is only information on the presence in the leaves of the plant of tanning substances of the pyrocatechin and gallotannin series [1, 2].

We have investigated the composition of the phospholipids (PLs) of the seeds of the Judas tree growing in Georgia. The comminuted seeds were extracted with n-hexane, and after the solvent had been distilled off 10-12% of neutral lipids was obtained. The polar lipids were extracted from the defatted raw material by Folch's method [3] and were freed from impurities (systems 1 and 2) [4], as a result of which a total of 2.9% of phospholipids was obtained.

By two-dimensional TLC in a layer of silica gel in systems 3 and 4 [5, 6] the total PLs revealed ten phosphorus-containing spots the quantitative amounts of which were determined spectrophotometrically (%): phosphatidylcholines (PCs) – 48.2; phosphatidylinositols (PIs) – 14.1; phosphatidylethanolamines (PEs) – 13.5; N-acyl-PEs – 7.4; phosphatidylglycerides (PGs) – 5.4; lyso-PCs – 4.5; lyso-PIs – 3.7; phosphatidic acid (PA) – 3.2. Diphenylglycerides (DPGs) and N-acyl-lyso-PEs were detected in trace amounts. As we see, the main components of total PLs of Judas tree seeds are PCs, PIs, and PEs.

To isolate the homogeneous phospholipids, the total material was first fractionated by chromatography on a column of silica gel and was then separated by preparative TLC. As a result, the PCs, PEs, and N-acyl-PEs were isolated, these being identified from their IR spectra [7-9] and the products of acid hydrolysis. The fatty acids split out were analyzed by GLC. To determine the position specificity of the distribution of the fatty acids in the phospholipid molecules we studied the products of their enzymatic hydrolysis.

Fatty acids in the PCs and PEs were represented by five components and those in the N-acyl-PEs by seven (Table 1). In all cases, among the saturated acids palmitic predominated, and among the unsaturated acids oleic. The PEs and PCs differed from one another both qualitatively and quantitatively. The total degree of saturation of the PEs (69.5%) was consid-

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TABLE 1. Composition and Positional Distribution of the Fatty Acids in the PCs, PEs, and N-acyl-PEs of Judas Tree Seeds (wt. %)

Fatty acid	PCs			PEs			N-acyl-PEs		
	total	sn-1	sn-2	total	sn-1	sn-2	total	O-acyls	N-acyls
14:0	1,6	7,1	6,4	Tr.	1,7	—	6,9	1,5	10,5
16:0	25,3	42,9	12,5	55,0	58,5	46,0	25,6	20,2	30,0
17:0	—	—	—	—	—	—	Tr.	Tr.	9,5
18:0	1,5	10,0	—	14,5	10,5	12,8	2,6	2,1	7,0
18:1	53,7	30,5	73,7	18,0	28,8	14,7	34,4	42,6	23,2
18:2	17,9	3,5	7,4	12,5	0,5	27,5	28,3	33,6	15,8
18:3	—	—	—	—	—	—	2,2	Tr.	4,0
Σ_{sat}	28,4	60,0	18,9	69,5	70,7	58,8	35,1	23,8	57,0
Σ_{unsat}	71,6	40,0	81,1	30,5	29,3	42,2	64,9	76,2	43,0

TABLE 2. Molecular Compositions of the PCs and PEs Showing Positional Isomerism (%)

Molecular composition	PCs	PEs	Molecular composition	PCs	PEs
14:0/14:0	0,5	—	18:0/18:0	—	3,7
14:0/16:0	2,7	—	18:0/18:2	—	0,1
14:0/18:0	0,6	—	18:1/14:0	5,2	—
14:0/18:1	2,3	—	18:1/16:0	31,6	8,6
14:0/18:2	0,2	—	18:1/18:0	7,4	1,5
16:0/14:0	0,9	—	18:1/18:1	26,9	4,2
16:0/16:0	5,4	27,4	18:1/18:2	2,6	0,1
16:1/18:0	1,3	4,8	18:2/14:0	0,5	—
16:0/18:1	4,6	13,2	18:2/16:0	3,2	16,5
16:0/18:2	0,4	0,2	18:2/18:0	0,7	2,9
18:0/16:0	—	7,5	18:2/18:1	2,7	7,9
18:0/18:0	—	1,3	18:2/18:2	0,3	0,1
S/S	5,9	28,7	U/S	48,6	29,5
S/S*	5,5	12,3	U/U	27,2	4,3
S/U	7,5	17,2	U/U**	5,3	8,0

*S/S) disaturated mixed fatty acid.
†U/U) diunsaturated mixed fatty acid.

erably higher than that of the PCs (28.4%) and of the N-acyl-PEs (35.1%) which was due to the high level of palmitic acids in the PEs (55.0%).

Among the fatty acids localized on the N atoms in the N-acyl-PEs there were far more saturated acids than in the corresponding O-acyls. The predominating amount of low-molecular mass fatty acids (14:0) was found in the amide-bound form. A large amount (9.5%) of margaric acid was localized on the N atoms. Attention is attracted by the presence of linolenic acid in the N-acyl-PEs although it was absent from the main PLs.

The molecular compositions of the PCs and PEs were determined by using Coleman's mathematical method in Markman's variant [10] (Table 2).

It must be mentioned that a well-known feature was observed: the unsaturated acids predominantly occupied the sn-2 position. A high specificity of the distribution of the FA radicals was noted in the PCs (sn-1 – 60% of saturated; sn-2 – 81.1% of unsaturated).

The main fatty acid radicals in both positions of the glyceride moieties of the molecules were the 16:0, 18:1, and 18:2 types, and combinations of these acids: 16:0/16:0; 16:0/18:1; 18:1/16:0; 18:1/18:0; 18:1/18:1; 18:2/16:0; and 18:2/18:1 formed the bulk of the molecular varieties of the phospholipids: PCs – 45.9%; PEs – 55.3%.

In the PCs, 20 molecular species were detected, and in the PEs 16. The predominating species in the PEs were the 18:1/16:0 and 18:1/18:1 (31.6 and 26.9%), and in the PEs the 16:0/16:0 and 18.2/16:0 species (27.4 and 16.5%).

From the results on the positional species composition of the PLs we calculated their typical compositions. This showed that the typical compositions of the PCs and PEs differed from one another and that this difference depended on the initial FA composition and the positional distribution of the FAs in the PL molecule: in the PCs the unsaturated-saturated (S/U) types predominated – 48.6%; and in the PEs – S/S and U/S (28.7 and 29.5%).

Thus, it has been established that the PCs and PEs differ substantially from one another in their fine structures which is possibly due to a difference in their biosynthesis.

EXPERIMENTAL

The total polar lipids were extracted from the defatted seeds with a 2:1 mixture of chloroform and methanol. Carbohydrate impurities were eliminated by gel filtration through Sephadex G-25 in system 5, and neutral lipids, pigments, and glycolipids by TLC on silica gel in systems 1 and 2 [4]. For chromatography we used silica gel of Chemapol brand (Czechoslovakia).

The quantitative determination of the PLs was based on inorganic phosphorus. The PLs were hydrolyzed with 3 N HCl in sealed tubes.

The enzymatic hydrolysis of the homogeneous PLs was carried out with kufi (Vipera lebetina) venom phospholipase A_2 in borate buffer at pH 7.4. The products of enzymatic hydrolysis were separated by PTLC in system 3; the FAs were methylated with diazomethane and the methyl esters were analyzed by GLC. Lyso compounds were subjected to acid methanolysis, and the FAMEs were studied by the GLC method.

Mild deacylation of the N-acyl-PEs was carried out at 37°C in 0.1 M methanolic NaOH solution [12].

The following solvent systems were used: 1) hexane–ether (7:3); 2) acetone; 3) chloroform–methanol–25% ammonia (65:30:4); 4) chloroform–methanol–acetic acid–water (170:25:25:6); 5) chloroform–methanol–water (90:10:1).

GLC was conducted on a Chrom-41 chromatograph using a stainless steel column filled with 17% of PEGS on Chrom W (60-80 mesh). The length of the column was 2.5 × 4 mm [sic] and the temperature 200°C.

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