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PHOSPHOLIPIDS OF RIPE *Sophora japonica* SEEDS

Kh. S. Mukhamedova and A. I. Glushenkova

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Nine classes of lipids have been isolated in homogenous form from ripe seeds of the Japanese pagoda tree, and their fatty acid compositions have been established. The positional distributions of the fatty acids in the phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols have been determined.

We have reported previously on the change in the phospholipid (PL) complex in ripening seeds of the Japanese pagoda tree (*Sophora japonica*, fam. Fabaceae) [1]. Continuing our study of this plant, we have investigated the fractional and fatty acid compositions of the PLs of its seeds.

The total lipids were extracted from the ground seeds with a 2:1 mixture of chloroform and methanol. According to TLC, the total extractive substances consisted of neutral lipids (NLs), polar lipids (PoLs), carbohydrates, amino acids, pigments, etc.

In order to free the PLs from carbohydrates, a solution of the total lipids in chloroform–methanol–water (90:10:1) was passed through a column of Molselekt G-25 [2]. The PLs were freed from NLs, glycolipids, and pigments by column chromatography (CC) on silica gel, and from amino acids as described in [3]. The total yield of NLs from the *Sophora* seeds was 7%, and that of PLs 1.1% on the weight of the seeds, the phosphorus content of the total NLs being 3.3%.

On two-dimensional chromatography in solvent systems 1 (first direction) and 2 (second direction), 9 classes of PLs were detected, with the following R_f values: 0.05 (0.1) — lysophophatidylcholines (lyso-PCs); 0.15 (0.4) — phosphatidylinositols (PIs); 0.35 (0.3) — PCs; 0.5 (0.6) — phosphatidylethanolamines (PEs); 0.8 (0.9) — N-acyl-PEs; 0.6 (0.85) — N-acyl-lyso-PEs; 0.1 (0.75) — phosphatidic acids (PAs); 0.4 (0.7) — phosphatidylglycerols (PGs); and 0.5 (0.9) — DPGs.

The total PLs were separated by CC and PTLC into the nine classes. The classes isolated were identified by comparing their chromatographic mobilities and qualitative reactions for functional groups.

The fatty acids of the total PLs and of the homogeneous classes were split off by alkaline hydrolysis, and their compositions were investigated by GLC (Table 1). A high level of the 16:0 and 18:2 acids was found in all the samples; in the N-acyl-lyso-PEs the 18:2 species made up 51.7% of the weight of the acids. In the total PLs, trace amounts were found of the 21:0 acid, localized mainly in the N-acyl-PEs. The presence of the $C_{20}-C_{24}$ acids in small amounts is a characteristic biological feature of the oils of many plants of the Leguminosae family [4, 5], but in the PLs these acids are either totally absent [5, 6] or have been detected in trace amounts [7]. The PGs contained a higher level of saturated FAs (64.5%) than the other PLs, while the N-acyl-lyso-PEs contained the smallest amount of them (15.1%). In order of decreasing unsaturation, the individual classes of PLs of the *Sophora* seeds can be arranged in the following way: N-acyl-lyso-PEs → PCs → PEs → N-acyl-PEs → lyso-PCs → PIs → DPGs → PAs → PGs.

The positional distributions of the fatty acid radicals in the PCs, PEs, and PIs were determined by enzymatic hydrolysis using phospholipase A_2 from kufi venom. The fatty acids split out from the sn-2 position of the glycerol part of the molecule were methylated with diazomethane and the methyl esters were subjected to GLC analysis. The FAs from the sn-1 position were determined after the alkaline hydrolysis of the lyso-products, methylation, and GLC analysis. It can be seen from Table 1 that the bulk of the unsaturated (U) acids was localized in the sn-2 position, and the saturated (S) acids in the sn-1 position. The higher content of U acids in the sn-2 position is due mainly to the high levels of the 18:2 and 18:1 acids, while that of the S acids in the sn-1 position is due to the 16:0 acid. These distinctive features, characteristic of plant PLs, appeared in all the classes of *Sophora* seed PLs. However, with respect to the positional distribution of the FAs in the molecule, the classes

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 578-582, July-August, 1997. Original article submitted January 21, 1997.

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TABLE 1. Fatty Acid Compositions of the Total PLs of Ripe Seeds of the Japanese Pagoda Tree and of Individual Classes of Them

FAs	Total PLs	PCs			PEs			PIs			N-acyl-PEs			lyso-PCs	N-acyl-lyso-PEs	PGs	PAs	DPGs
		tot.	sn-1	sn-2	tot.	sn-1	sn-2	tot.	sn-1	sn-2	tot.	N-ac.	O-ac.					
10:0	0.6	0.7	1.4	1.2	6.0	0.3	7.7	2.5	2.7	2.2	Tr.	Tr.	Tr.	Tr.	0.3	1.1	1.0	2.5
12:0	0.7	0.5	1.0	0.5	3.7	0.6	3.0	0.8	1.3	2.2	Tr.	Tr.	Tr.	2.7	0.3	4.9	2.3	6.5
14:0	1.0	0.6	1.0	0.6	0.6	0.5	1.5	0.7	1.7	1.2	1.5	1.1	2.6	3.0	0.9	1.6	3.2	3.3
15:0	1.0	-	-	-	0.3	0.5	1.4	-	-	-	1.0	1.2	1.2	2.0	Tr.	2.0	2.5	4.9
16:0	16.9	11.9	22.3	5.2	9.4	20.7	5.0	35.1	63.8	6.2	17.1	15.3	13.5	20.2	8.6	37.3	30.8	32.5
16:1	1.5	1.1	2.0	1.0	0.8	0.8	2.2	-	-	-	2.3	2.1	2.1	Tr.	3.3	Tr.	3.0	1.1
17:0	1.2	1.0	1.8	1.1	0.9	0.7	2.4	2.6	3.2	2.2	1.2	0.9	1.1	Tr.	0.9	2.3	4.0	2.8
17:1	0.7	-	-	-	0.7	0.6	2.2	-	-	-	1.0	1.9	1.1	-	Tr.	1.5	2.3	3.6
18:0	4.1	5.8	9.3	1.9	4.9	9.6	3.5	4.3	8.6	2.9	6.1	6.4	5.5	9.6	4.1	15.3	19.3	6.7
18:1	19.6	25.0	23.1	25.9	14.6	17.2	12.1	8.6	7.2	12.1	16.1	14.6	16.1	23.2	24.5	12.7	10.2	10.8
18:2	49.9	50.9	38.1	60.6	56.2	46.1	55.6	42.7	11.5	65.8	48.5	44.5	53.6	37.2	57.1	20.2	19.4	26.3
18:3	2.8	2.5	Tr.	2.0	1.9	2.4	3.1	2.4	Tr.	5.2	4.0	1.0	3.2	2.1	Tr.	1.1	2.0	-
21:0	Tr.	Tr.	Tr.	-	Tr.	Tr.	Tr.	-	-	-	1.2	2.0	Tr.	Tr.	Tr.	Tr.	-	-
ΣS	25.4	20.5	36.8	10.5	25.8	32.9	24.5	46.3	81.3	16.9	28.1	26.9	23.9	37.5	15.1	64.5	63.1	59.2
ΣU	74.6	79.5	63.2	89.5	74.2	67.1	75.5	53.7	18.7	83.1	71.9	63.1	76.1	62.5	84.9	35.5	36.9	41.8

TABLE 2. Position-Species Compositions of the PCs, PEs, and PIs of the *Sophora* Seeds (not including species with levels of less than 0.1%)

Species sn-2–sn-1	PCs	PEs	PIs	Species sn-2–sn-1	PCs	PEs	PIs
10:0–16:0	0.3	1.7	1.4	18:0–16:0	0.5	0.8	2.1
10:0–18:0	0.1	0.8	0.3	18:0–18:0	0.2	0.4	0.3
10:0–18:1	0.3	1.4	0.2	18:0–18:1	0.5	0.6	0.2
10:0–18:2	0.5	3.6	0.3	18:0–18:2	0.7	1.6	0.3
10:0–18:3	–	0.2	–	18:0–18:3	–	0.1	–
12:0–16:0	0.1	0.6	1.4	18:1–10:0	0.3	–	0.3
12:0–18:0	–	0.3	0.3	18:1–12:0	0.3	0.1	0.1
12:0–18:1	0.2	0.6	0.2	18:1–14:0	0.3	–	0.2
12:0–18:2	0.2	1.4	0.3	18:1–16:0	5.8	2.5	7.7
12:0–18:3	–	0.1	–	18:1–16:1	0.6	0.1	–
14:0–16:0	0.2	0.3	0.8	18:1–17:0	0.6	0.2	0.4
14:0–18:0	–	0.2	0.1	18:1–18:0	2.4	1.2	1.1
14:0–18:1	0.1	0.3	0.1	18:1–18:1	5.9	2.1	0.9
14:0–18:2	0.3	0.7	0.2	18:1–18:2	9.8	5.6	1.4
15:0–16:0	–	0.3	–	18:1–18:3	–	0.3	–
15:0–18:0	–	0.1	–	18:2–10:0	0.8	0.2	1.8
15:0–18:1	–	0.3	–	18:2–12:0	0.6	0.4	0.9
15:0–18:2	–	0.7	–	18:2–14:0	0.6	0.3	1.1
16:0–16:0	1.3	1.1	4.1	18:2–15:0	–	0.3	–
16:0–17:0	0.2	–	0.2	18:2–16:0	13.5	11.4	42.0
16:0–18:0	0.5	0.5	0.6	18:2–16:1	1.2	0.5	–
16:0–18:1	1.2	0.9	0.5	18:2–17:0	1.1	0.4	2.1
16:0–18:2	2.0	2.3	0.7	18:2–17:1	–	0.4	–
16:0–18:3	–	0.2	–	18:2–18:0	5.7	5.3	5.6
16:1–16:0	0.2	0.5	–	18:2–18:1	14.0	9.6	4.7
16:1–18:0	0.2	0.2	–	18:2–18:2	23.1	25.5	7.6
16:1–18:1	0.2	0.4	–	18:2–18:3	–	1.3	–
16:1–18:2	0.4	1.0	–	18:3–10:0	–	–	0.1
16:1–18:3	–	0.1	–	18:3–12:0	–	–	0.1
17:0–16:0	0.2	0.5	1.4	18:3–14:0	–	–	0.1
17:0–18:0	0.2	0.2	0.3	18:3–16:0	0.5	0.7	3.3
17:0–18:1	0.2	0.5	0.2	18:3–17:0	–	–	0.1
17:0–18:2	0.5	1.1	0.3	18:3–18:0	0.2	0.4	0.5
17:0–18:3	–	0.1	–	18:3–18:1	0.5	0.6	0.4
17:1–16:0	–	0.5	–	18:3–18:2	0.8	1.7	0.6
17:1–18:0	–	0.2	–				
17:1–18:1	–	0.4	–				
17:1–18:2	–	1.0	–				
17:1–18:3	–	0.1	–				

of PLs studied have their distinguishing features. Thus, the greatest positional specificity is observed in the PIs, where 81.3% of the sn-1 positions are esterified by S acids and 83.1% of the sn-2 positions by U acids. The least specific distribution of the FAs is in the PEs.

To determine possible molecular compositions of the PCs, PEs, and PIs we based ourselves on the experimental results for the positional distribution of the FAs in their molecules and the method of calculation given in [8]. For the PC and PIs we calculated 49 species each, and for the PE 67, of which 36, 33, and 47, respectively, were present in amounts of less than 1% (Table 2). In all cases the predominant species were formed by combinations of the 16:0, 18:1, and 18:2 acids. Of course there were appreciable differences in the quantitative ratios of the individual species. These differences naturally affect the type composition of the PLs, which is calculated by summing the saturated and unsaturated species separately:

		PCs	PEs	PIs
Disaturated	(SS)	3.8	7.9	13.2
Unsaturated-saturated	(US)	33.0	24.9	67.5
Saturated-unsaturated	(SU)	6.7	16.4	3.6
Diunsaturated	(UU)	56.5	50.8	15.7

In the PIs the US species predominated, and in the PCs and PEs the UU species, which is explained by the greater unsaturation of the PE and, particularly, the PC molecules.

Thus, the main components (PCs, PEs, and PIs) of the PLs of the *Sophora* seeds differed substantially in their fine structure, which is probably due to a specificity of their acylation in biosynthesis according to the different roles of the individual species in the occurrence of cell processes.

In order to study the distribution of FAs in the N-acyl-PE molecule, the acids were split off from the glycerol part of the molecule (O-acyls) by deacylation under mild conditions, as in [9]. The resulting glycerylphosphoryl-N-acylethanolamine was subjected to alkaline deacylation, and the N-acyls were split out (Table 1). As can be seen from Table 1, the N-acyls were more saturated than the O-acyls, which agrees with the literature [6, 7, 9, 10]. It was also found that the 21:0 acid was present only in the N-acyl-PE fraction, and this exclusively in the amide-bound form, as in the case of [7].

EXPERIMENTAL

The separation of the PLs into individual classes and the monitoring of the individual fractions were conducted by CC and TLC. For TLC we used silica gel L 5/40, and for CC silica gel 100/160. The solvent systems were: 1) chloroform–methanol–ammonia (65:35:5); 2) chloroform–acetone–methanol–acetic acid–water (40:20:10:10:3). The alkaline hydrolysis of the PLs was conducted in a 10% methanolic solution of KOH at room temperature [11]. The enzymatic analysis of the main fractions of the total PLs was achieved with the aid of phospholipase A_2 in Tris buffer, pH 9.8, at 37°C.

The FAMEs were analyzed on a Chrom-4 GLC with a flame-ionization detector in a steel column (4 mm × 2.5 m), with the stationary phase PEGS (17%) on Celite-545 (80-100 mesh), the carrier gas helium, evaporator temperature 250°C, thermostat temperature 198°C.

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