COMPOSITION OF THE PHOSPHOLIPIDS OF SEEDS OF *Dipthychocarpus strictus*

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The majority of plants of the family Cruciferae contain erucic acid, but some of them do not contain this acid in the oil [1]. These include *Dipthychocarpus strictus*. We have studied the phospholipids (PL) of its seeds. The combined phospholipids obtained by a known method showed the qualitative reaction for sulfur [2]. However, after appropriate treatment they no longer showed the reaction for sulfur.

The comminuted seeds were defatted with acetone and the phospholipids were extracted by two methods: by Folch's method [3] and by hot ethanolic extraction. The extract obtained by the first method consisted of a red resinous mass containing a considerable amount of pigments, carbohydrates, and amino acids, and on treatment with acetone the bulk of it precipitated with the phospholipids. The bulk of the carbohydrates and amino acids was eliminated by the dialysis of a chloroform solution of the phospholipids against water. Final purification from carbohydrates was achieved by gel filtration of the combined phospholipids in chloroform–methanol–water (90:10:1) through Sephadex G-25.

The yield of purified total phospholipids was 0.45% of the weight of the air-dry seeds, and their phosphorus content was 3.3%.

The ethanolic extract contained less pigments, amino acids, and carbohydrates. The dry residue after the distillation of the ethanol was treated several times with petroleum ether (40-60°C). The bulk of the phospholipids passed into solution and the residue consisted of a pink resin containing traces of phospholipids (by TLC).

The petroleum ether solution was evaporated to dryness, and the residue was dissolved in a small volume of chloroform and was precipitated with acetone. The resin was treated with chloroform and was likewise precipitated with acetone. The two precipitates were separated by centrifuging and combined and were passed in chloroform–methanol–water (90:10:1) through Sephadex G-25. The yield of total phospholipids was 0.4%, and the phosphorus content 3.4%. The qualitative and quantitative compositions of the phospholipids were determined by two-dimensional TLC in a fixed layer of silica gel in solvent systems 1 and 2. Six phosphorus-containing spots were found.

The amounts of the individual substances were determined from the amounts of phosphorus [4] in the corresponding spots on the chromatogram [5] (%):

Phospholipids Fraction	Amount, %
Phosphatidylcholines (PC)	50.2
Phosphatidylinositol (PI)	22.9
Phosphatidylethanolamines (PE)	12.2
Lysophosphatidylcholines (lyso-PC)	10.4
Minor phospholipid fractions	4.3

The IR spectra of these homogeneous fractions agree with literature information for glycerophospholipids [6, 7]. The molar N:P ratio for the phosphatidylcholines, phosphatidylethanolamines, and lysophosphatidylcholines was ~1.0.

The structures of these homogeneous fractions were confirmed by studying the products of their acid hydrolysis. The hydrolyzates of all the phospholipids were found to contain fatty acids and glycerol. Among the water-soluble hydrolysis products we identified choline

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TABLE 1. Fatty-Acid Compositions of the Triglycerides of the Total and Separate Fractions of the Phospholipids

Fatty acid	Tryglycerides	Total phospholipids	Individual fractions			
			phosphatidylcholines	phosphatidylethanolam.	phosphatidylinositols	lysophosphatidylcholines
$C_{14:0}$	2,0	0,6	1,6	1,2	1,2	5,0
$C_{16:0}$	10,0	23,3	11,6	17,3	36,8	21,4
$C_{16:1}$	2,5	2,2	3,1	3,0	3,6	3,6
$C_{18:0}$	0,9	2,0	1,4	2,3	2,5	6,2
$C_{18:1}$	9,1	20,1	26,8	23,7	10,7	37,4
$C_{18:2}$	11,3	19,2	24,5	25,5	14,1	15,2
$C_{18:3}$	64,2	32,6	31,0	27,0	31,1	11,2
$\Sigma_{sat.\ acids}$	12,9	25,9	14,6	20,8	40,5	32,6
$\Sigma_{unsat.\ acids}$	87,1	74,1	85,4	79,2	59,5	67,4

from the PC, ethanolamine from the PE, and inositol from the PI (in systems 3 and 4).

The sum and the individual homogeous fractions of the phosphatidylcholines, phosphatidylinositols, phosphatidylethanolamines, and lysophosphatidylcholines were deacylated by alkaline saponification, and the fatty acids split off, in the form of their methyl esters, were analyzed by GLC. For comparison we also recorded a chromatogram of the fatty acids of the glycerides of the oil [1] (Table 1).

It can be seen from Table 1 that the triglycerides of the oil are more unsaturated than the combined phospholipids; in all the fractions, among the saturated acids palmitic predominates. The degree of saturation of the individual fractions of the phospholipids increases in the following sequence:

$$PC \rightarrow PE \rightarrow \text{lyso-PC} \rightarrow PI$$

The position specificity of the fatty acids in the molecules of the phospholipids investigated was determined by studying the products of their enzymatic hydrolysis by phospholipase A. The enzymatic hydrolysis of the homogeneous fractions of the PC, PI, and PE was performed in a tris buffer medium at pH 8.4.

The products of enzymatic hydrolysis were separated by preparative TLC. The corresponding lyso compounds that were isolated were subjected to alkaline hydrolysis. The acids from positions 1 and 2 were analyzed, after methylation with diazomethane, by GLC (Table 2).

In all the fractions studied, as was to be expected, a specific distribution of the saturated and unsaturated acids was observed: The percentages of unsaturated acids esterifying position 2 of the molecules were 96.6 for the PC, 94 for the PE, and 96.3 for the PI.

A determination of the position distribution of the fatty acids using Coleman's method of calculation in the modification due to A. L. Markman et al. [8] enabled us to calculate the diglyceride compositions of the phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols (Table 3).

The meal after the extraction of the neutral lipids and phospholipids were found to contain 3.6% of phytin, calculated on the initial seeds.

EXPERIMENTAL

The solvents used were purified and rendered absolute by known methods. For column chromatography we used type KSK silica gel and Al_2O_3 (activity grade II). The solvent systems used were: 1) chloroform–methanol–water (65:35:5); 2) chloroform–methanol–25% ammonia (65:35:5); 3) isopropanol–25% ammonia–water (7:1:2) [9]; and 4) 2% NH_3–methanol (3:2) [10].

The IR spectra of samples in the form of films were taken on a UR-20 instrument. Gas–liquid chromatography was performed on a UKh-2 instrument at 197°C with poly(ethylene succinate) as the stationary phase.

Purification of the Total Phospholipids from Carbohydrates. The total phospholipids (2.3 g) in 300 ml of chloroform–methanol–water (90:10:1) was passed through a column containing 5 g of Sephadex G-25. The elimination of carbohydrates was complete (TLC in system 1). The yield of combined phospholipids was 2 g.

TABLE 2. Position Distribution of the Fatty Acids in the Main Fractions of the Phospholipids of *Dipthychocarpus* Seeds

Fatty acids	Phosphatidylcholine			Phosphatidylethanolamine			Phosphatidylinositol		
	initial	position		initial	position		initial	position	
		1	2		1	2		1	2
$C_{14:0}$	1,6	1,9	1,8	1,2	0,7	0,6	1,2	1,0	1,1
$C_{16:0}$	11,6	21,0	1,6	17,3	27,1	4,6	36,8	66,7	2,6
$C_{16:1}$	3,1	—	2,9	3,0	1,5	2,9	3,6	3,1	1,3
$C_{18:0}$	1,4	4,1	—	2,3	2,4	0,8	2,5	4,0	—
$C_{18:1}$	26,8	31,8	23,6	23,7	33,0	18,0	10,7	10,2	9,4
$C_{18:2}$	24,5	19,7	28,8	25,5	21,7	30,1	14,1	6,0	21,8
$C_{18:3}$	31,0	21,5	41,3	27,0	13.5	43,0	31,1	9,0	63,8
$\Sigma_{sat.\ acids}$	14,6	27,0	3,4	20,8	30,3	6,0	40,5	71,7	3,7
$\Sigma_{unsat.\ acids}$	85,4	73,0	96,6	79,2	69,7	94,0	59,5	28,3	96,3

TABLE 3. Possible Molecular Compositions of the Phosphatidylcholines (PC), Phosphatidylethanolamines (PE), and Phosphatidylinositols (PI) (%)

Fatty acid		Calculated for			Fatty acid		Calculated for		
		PC	PE	PI			PC	PE	PI
16:0	14:0	0,4	0,1	0,7	18:2	18:1	5	3,6	0,5
18:0	14:0	0,1	+	+	18:3	18:1	5,5	2,3	0,7
16:0	16:0	0,3	1,1	1,6	18:2	14:0	0,4	0,1	0,1
18:0	16:0	0,1	0,1	+	18:2	16:0	0,3	0,9	0,2
14:0	16:0	—	0,3	0,3	18:2	16:1	0,6	0,6	+
14:0	18:0	—	+	—	18:2	18:0	—	0,2	—
16:0	18:0	—	0,2	—	14:0	18:2	0,7	2,0	1,4
18:0	18:0	—	+	—	16:0	18:2	6,1	8,0	14
16:1	14:0	—	+	+	16:1	18:2	—	0,4	0,4
18:1	14:0	0,6	0,1	+	18:0	18:2	1,2	0,7	0,7
16:1	16:0	—	+	+	18:1	18:2	8,3	9,0	2,0
18:1	16:0	0,6	1,5	0,2	18:2	18:2	5	6,5	1,2
14:0	16:1	0,1	0,2	0,1	18:3	18:2	6,2	4,0	1,3
16:0	16:0	+	0,8	0,8	18:3	14:0	0,4	+	0,2
16:1	16:1	—	+	+	18:3	16:0	0,3	0.6	0,2
18:0	16:1	0,1	+	+	18:3	16:1	0,7	0,4	+
18:1	16:1	1	0,9	0,2	18:3	18:0	—	0,1	—
16:1	18:0	—	+	—	14:0	18:3	1	3,0	3
18:1	18:0	—	0,2	—	16:0	18:3	9	10,0	40
16:1	18:1	—	0,3	0,2	16:1	18:3	—	0,6	1,9
16:0	18:1	5	4,5	5,0	18:0	18:3	1,7	1,0	1,0
14:0	18:1	0,5	1,3	0,8	18:1	18:3	13,2	16,0	6,3
18:0	18:1	1	0,4	0,3	18:2	18:3	8,1	9,0	3,3
18:1	18:1	7,5	5,6	1,0	18:3	18:3	9	6,4	4,2
Total					SS		0,9	1,7	2,6
					UU		2,6	3,1	8,0
					SU		26,4	29,6	67,0
					US		70,1	65,6	22,4

Separation of the Combined Phospholipids on Silica Gel. The combined phospholipids in chloroform were deposited on a column of silica gel and eluted with chloroform, mixtures of chloroform and methanol and various ratios, and then with methanol. The chloroform extracted the neutral lipids; chloroform–methanol (9:1) extracted the pigments, traces of PE, and unidentified phospholipids; chloroform–methanol (4:1) extracted PE and PI; chloroform–methanol (3:1) extracted PI and PC; chloroform–methanol (2:1) extracted PC and lyso-PC; and methanol extracted lyso-PC.

Separation of the Combined Phospholipids by Means of Al_2O_3. A solution of 0.7 g of the combined phospholipids in 5 ml of chloroform was deposited on a column containing 20 g of alumina.

Chloroform eluted 47 mg of neutral lipids; methanol eluted 350 mg of PC and traces of lyso-PC; chloroform–methanol (2:1) containing 0.1% of ammonium acetate eluted 20 mg of PC, and traces of lyso-PC and PE; the same mixture with 0.25% of ammonium acetate eluted 30 mg of PE and traces of unidentified phospholipids; and the same mixture with 1% of ammonium acetate eluted 210 mg of PI, traces of PE, and unidentified phospholipids. The individual fractions were further purified by preparative TLC.

Acid Hydrolysis of the Individual Phospholipids. Mixtures of 35-50 mg of the individual fractions of the phospholipids and 2-3 ml of 10% HCl in sealed tubes were heated in the boiling water bath for 24 h. Then the tubes were opened, the fatty acids were extracted from the acid solution with petroleum ether (40-60°C), and the residue was evaporated in vacuum and dissolved in water and the hydrolysis products were analyzed by TLC in systems 3 and 4. Glycerol, choline, ethanolamine, and inositol were used as markers.

Enzymatic Hydrolysis of the Phospholipids. A) Phosphatidylcholines. A solution of 93 mg of the sample in 15 ml of ether was treated with 2.7 mg of snake venom dissolved in 0.4 ml of 0.1 M Tris buffer. The mixture was stirred at room temperature.

After 2 h, the solvent was evaporated off, the residue was dissolved in chloroform—methanol (2:1), and the hydrolysis products were separated preparatively in system 1: R_f 0.9 (iodine vapor) — fatty acids from position 1: R_f 0.15 — lyso-PC (iodine vapor and the Dragendorff and Vas'kovskii reagents).

B) Phosphatidylethanolamines. A sample (70 mg) was dissolved in 10 ml of ether and 3.4 mg of snake venom in 0.8 ml of tris buffer (pH 9.4) was added, and the mixture was stirred mechanically at room temperature for 18 h. Then the hydrolysis products were worked up as in the case of the phosphatidylcholines.

C) Phosphatidylinositols. A solution of 80 mg of the sample in 10 ml of ether was mixed with 3.4 mg of snake venom in 0.9 ml of 0.1 M Tris buffer. The reaction was complete after 12 h.

SUMMARY

The qualitative and quantitative compositions of the total phospholipids of the seeds of *Dipthychocarpus* have been determined. The fatty-acid compositions and position specificities of the distribution of the fatty acids in the main phospholipids of *Dipthychocarpus* seeds have been determined and, on this basis, their possible molecular composition has been calculated.

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