DETERMINATION OF THE MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINES

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Numerous facts indicate that the structure and a number of the most important properties of biological membranes depend on the structure of the phospholipids. In recent years, considerable progress has been made in the study of molecular species of phospholipids [1-5].

Natural phospholipids form a complex mixture of monotypical compounds differing from one another in their fatty-acid compositions. The present paper gives the results of a structural investigation of the phosphatidylcholines (PC's) obtained from the seeds of three species of plants of the family Cruciferae and forming the main components of the total phospholipids of these plants [6-8].

The analysis of the molecular species of the PC's included the following steps: enzymatic hydrolysis of the PC's with phospholipase C and chromatographic separation of the diglycerides obtained according to their degree of unsaturation on silica gel impregnated with $AgNO_3$, followed by cleavage of the diglycerides obtained with pancreatic lipase to 2-monoglycerides.

After the enzymatic hydrolysis of the samples of the PC's with phospholipase C, the diglycerides obtained were separated on a layer of silica gel impregnated with $AgNO_3$ into individual subfractions (Table 1). The amounts of the subfractions of the phosphatidylcholines proved to be the same in all the plants. Using as an example the phosphatidylcholines obtained from *Crambe amabilis*, we have calculated experimentally the amounts of the various types taking into account the position distribution of the fatty acids. The fractions of the diglycerides of the PC's from *Cr. amabilis* shown in Table 1 were cleaved by pancreatic lipase, which shows a specific action on primary ester groupings in glycerides [9]. The re-

TABLE 1. Total Fatty-Acid Compositions of the Subfractions of the Phosphatidylcholines Isolated from the Seeds of Three Species of Plants of the Family Cruciferae

Diglyceride fractions	Fatty acids												Percent of the total amount of phosphatidylcholines
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:1	S	U	
Diphthychocarpus strictus													
I	—	—	6,4	4,6	—	24,9	32,1	32,0	—	—	6,2	93,8	32,4
II	—	1,1	18,0	2,5	5,2	36,9	36,3	—	—	—	24,3	75,7	18,1
III	—	0,5	25,5	1,8	5,6	24,2	3,8	38,6	—	—	31,6	68,4	18,3
IV	—	5,1	16,9	10,8	10,1	18,1	32,0	7,0	—	—	32,1	67,9	21,3
V	—	1,6	30,4	8,1	8,0	51,9	—	—	—	—	40,0	60,0	9,9
Crambe scaugnana													
I	3,0	2,8	9,5	2,6	—	36,6	39,2	—	6,3	—	21,6	78,4	23,0
II	—	—	22,2	15,8	—	14,0	16,2	31,8	—	—	22,2	77,8	21,0
III	4,4	4,0	8,0	4,0	—	18,0	28,0	4,6	12,0	17,0	28,4	71,6	21,0
IV	4,0	3,0	12,8	4,8	3,6	20,2	5,4	40,0	6,2	—	29,6	70,4	21,0
V	7,8	7,0	25,2	8,7	10,0	41,3	—	—	—	—	50,0	50,0	14,0
Crambe amabilis													
I	—	—	3,6	3,6	—	20,0	32,8	40,0	—	—	3,6	96,4	25,0
II	—	—	5,3	1,4	1,0	22,0	62,5	3,8	—	4,0	6,3	93,7	20,0
III	—	—	10,0	1,4	2,3	34,5	47,7	4,1	—	—	12,3	87,7	20,0
IV	—	—	12,2	1,7	—	22,8	6,4	47,0	2,0	7,9	14,2	85,8	20,0
V	—	—	16,0	2,1	2,5	61,6	5,2	6,9	2,0	3,7	20,5	79,5	15,0

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TABLE 2. Compositions and Position Distributions of the Fatty Acids in Subfractions of the Diglycerides of the Phosphatidylcholines from *Crambe amabilis*

Acid	Fraction I		Fraction II		Fraction III		Fraction IV		Fraction V	
	position									
	1	2	1	2	1	2	1	2	1	2
16:0	6,0	1,5	8,5	1,5	16,0	4,0	20,1	3,8	24,7	7,3
16:1	7,5	—	3,0	—	—	3,6	3,6	—	0,9	4,1
18:0	—	—	2,0	—	4,6	—	—	—	5,0	—
18:1	36,2	3,7	46,8	—	37,1	31,9	14,3	30,1	34,6	88,6
18:2	26,3	38,5	24,5	98,5	34,3	60,5	12,5	—	10,4	—
18:3	24,0	56,3	7,2	—	8,0	—	29,5	66,1	12,8	—
20:0	—	—	—	—	—	—	4,0	—	4,0	—
22:1	—	—	8,0	—	—	—	16,0	—	7,6	—
ΣS	6,0	1,5	10,5	1,5	20,6	4,0	24,1	3,8	33,7	7,3
ΣU	94,0	98,5	89,5	98,5	79,4	96,0	75,9	96,2	66,3	92,7

sulting 2-monoglycerides were hydrolyzed with a methanolic solution of hydrochloric acid and the simultaneously methylated fatty acids were analyzed by GLC [10] (Table 2).

Taking into account the yield of the individual subfractions, we calculated the amount of each acid with respect to the sum of the fatty acids of the initial sample of PC's (Table 3).

The results for the total amounts of each acid in position 2 in the five subfractions were close to those obtained in the hydrolysis of the initial PC's with phospholipase A_2 [6-8], which confirms the specificity of the action of pancreatic lipase. The experimentally determined compositions and position distributions of the fatty acids in the individual subfractions enabled us to establish the molecular species statistically for each fraction of the diglycerides (DG's) and, by summing these figures, for the initial phosphatidylcholine.

The molecular composition of the phosphatidylcholines of the seeds of *Cr. amabilis* were as follows (the types of diglycerides present in amounts of less than 0.1% were not taken into account):

Phosphatidylcholines	Amount, %	Phosphatidylcholines	Amount, %
16:0—16:0	0,5	20:0—18:1	0,7
18:0-16:0	0,1	22:1—18:1	2,0
18:1—16:0	0,9	16:0—18:2	4,9
18:2—16:0	0,4	16:1—18:2	1,1
18:3—16:0	0,3	18:0—18:2	1,0
22:1—16:0	0,7	18:1—18:2	12,9
16:0—16:1	0,3	18:2—18:2	7,5
18:1—16:1	0,4	18:3—18:2	3,0
18:2—16:1	0,2	22:1—18:2	1,5
18:3—16:1	0,1	16:0—18:3	3,4
16:0—18:1	5,5	16:1—18:3	1,4
16:1—18:1	0,3	18:1—18:3	7,5
18:0—18:1	1,0	18:2—18:3	5,3
18:1—18:1	12,6	18:3—18:3	7,2
18:2—18:1	8,9	20:0—18:3	0,5
18:3—18:1	5,2	22:1—18:3	2,1

As can be seen from the figures given above, the species formed from the 18:1, 18:2, and 18:3 acids (18:2—18:1, 18:1—18:1, 18:2—18:2, 18:1—18:2, 18:1—18:3, 18:2—18:3, and 18:3—18:3) predominate in the main. Thus, the molecular compositions of the phosphatidylcholines of *Cr. amabilis* amount to 32 species, of *Cr. schugnana* to 34 species, and of *D. strictus* to 70 species.

On comparing the molecular composition of the PC's obtained experimentally with the composition obtained by calculation [8], it was found that in both cases the results were similar both qualitatively and quantitatively (in the case of the PC's from *Cr. amabilis*) [6] or almost identical (*D. strictus* and *Cr. schugnana*) [7, 8]. In view of the complexity of the experimental determination of the molecular compositions of the individual classes of phospholipids, we consider that for this purpose it is possible to limit oneself to the completely reliable results obtained by calculation. Similar results have been obtained for the case of the phosphatidylcholines of the cotton plant [11].

TABLE 3. Compositions and Position Distributions of the Fatty Acids in the Subfractions of the Diglycerides in Relation to Their Total Amount, wt.%

Acid	Fraction I			Fraction II			Fraction III			Fraction IV			Fraction V		
	initial	position		initial	position		initial	position		initial	position		initial	position	
		1	2		1	2		1	2		1	2		1	2
16:0	0,9	1,5	0,4	1,1	1,7	0,3	2,0	3,2	0,8	2,4	4,0	0,8	2,4	3,7	1,1
16:1	0,8	1,8	—	0,3	0,6	—	0,3	—	0,7	0,3	0,7	—	0,3	0,1	0,6
18:0	—	—	—	0,2	0,4	—	0,5	0,9	—	—	—	—	0,4	0,7	—
18:1	5,0	9,1	0,9	4,4	9,4	—	6,9	7,4	6,4	4,6	2,9	6,0	9,2	5,2	13,3
18:2	8,2	6,6	9,6	12,5	4,9	19,7	9,5	6,9	12,1	1,3	2,5		0,8	1,5	—
18:3	10,0	6,0	14,1	0,7	1,4	—	0,8	1,6	—	9,4	5,9	13,2	1,0	1,9	—
20:0	—	—	—	—	—	—	—	—	—	0,4	0,8	—	0,3	0,6	—
22:1	—	—	—	0,8	1,6	—	—	—	—	1,6	3,2	—	0,6	1,3	—
ΣS	0,9	1,5	0,4	1,3	2,1	0,3	2,5	4,1	0,8	2,8	4,8	0,8	3,1	5,0	1,1
ΣU	24,0	23,5	24,6	18,7	17,9	19,7	17,5	15,9	19,2	17,2	15,2	19,2	11,9	10,0	13,9

EXPERIMENTAL

All the solvents and reagents were of "pure for analysis" grade. As the source of phospholipase C we used the α-toxin of *Clostridium perfringens*, and the pancreatic lipase was obtained from porcine pancreatic gland [9]. We used type KSK silica gel with a particle size of about 125 μ containing 5% of gypsum for the thin layers. The mixtures of methyl esters of fatty acids were separated on a UKh-2 chromatograph with a 2500 × 4 mm column containing 18% of polyethylene glycol succinate on Celite-545 (80-100 mesh) at 197°C with helium as the carrier gas. The pressure of helium at the outlet was 2.5 atm.

Hydrolysis by Phospholipase C. To 100 mg of PC's dissolved in 25 ml of ether were added a solution of 0.4 g of phospholipase C in 0.1 M Tris buffer (pH 8.45) and 0.3 ml of a 0.02 M solution of $CaCl_2$. The mixture was incubated with continuous stirring at 37°C for 2 h. The 1,2-diglycerides obtained were extracted with ether (3 × 15 ml), and the solvent was distilled off in vacuum. The yield of diglycerides was 93-96%.

Fractionation of the Diglycerides. The diglycerides were dissolved in chloroform and separated according to their degree of unsaturation on plates (9 × 24 cm) with a thin layer of silica gel impregnated with an 18% solution of silver nitrate. The plates were prepared by the method of Dyatsovitskaya et al., and before use they were activated at 110°C for 90 min. Upon one plate was deposited 13-16 mg of diglycerides. Chloroform—ethanol (97:3 and 9:1) systems were used. The spots on the chromatogram, after it had been sprayed with a 0.01% aqueous solution of Rhodamine 6G, were marked out in UV light. The subfractions of the diglycerides were scraped off and eluted from the silica gel with mixtures of chloroform and methanol (1:1 and 4:1), and the extracts obtained were washed with water to eliminate the Rhodamine and silver nitrate.

Hydrolysis with Pancreatic Lipase. To 10-12 mg of diglycerides were added, successively, 10 mg of pancreatic lipase, 1.2 ml of 1 M Tris buffer (pH 8.45), 0.3 ml of 22% $CaCl_2$ solution, and 0.4 ml of 0.1% sodium deoxycholate solution. The mixture was heated at 40°C in the water bath with stirring for 10 min. At the end of the reaction, 0.5 ml of 20% HCl was added and the monoglycerides and fatty acids formed were separated by TLC (9 × 24 cm) in the hexane—ether—acetic acid (50:50:1) system. The monoglyceride fraction was subjected to acid methanolysis and the resulting methyl esters of the fatty acids were analyzed by gas—liquid chromatography.

SUMMARY

The molecular compositions of the phosphatidylcholines obtained from the seeds of three plants of the family Cruciferae have been established experimentally.

In view of the similarity of the molecular compositions of the phosphatidylcholines obtained experimentally and by calculation, and also taking into account the complexity of the technique for the experimental determination of molecular species, it is possible to limit oneself to the results of the calculation method.

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THE MONOTERPENES OF THE ESSENTIAL OILS OF CONIFEROUS TREES OF SIBERIA

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In connection with the growing volumes of lumbering operations in the felling areas of our country there is an accumulation of tree verdure which is being used inadequately. The cells of tree verdure contain about 40% of biologically active substances (calculated on the dry matter) [1]. One of the possible products of its processing consists of essential oils which, as is well known, may be effective medicinal agents: antimicrobial, antiviral, bactericidal, and fungicidal effects of coniferous essential oils are known [2, 3].

The essential oils of Siberian coniferous trees are not obtained on the industrial scale, with the exception of fir oil, in spite of the large reserves of raw material. One of the reasons for this is the inadequacy of the study of their compositions. Isolated items of information concerning the composition and physical properties of essential oils from the verdure of some coniferous trees growing in Siberia have appeared in the literature [4-8].

The essential oils from the needles and lopping litter differ from one another [7, 9] and, in addition, the compositions of the essential oils depend on the growth site [10, 11]. It must also be noted that all the investigations mentioned were devoted to an investigation of the essential oils from needles or from lopping litter obtained under laboratory conditions.

We have investigated essential oils isolated from the technical verdure of some coniferous trees of Siberia under industrial conditions. The physicochemical characteristics of the coniferous essential oils from technical verdure were as follows:

Index	*Pinus sylvestris Ldb.*	*Pinus sibirica Mayr.*	*Abies sibirica Ldb.*	*Picea obovata Ldb.*
Density, ρ, g/cm^3	0,8855	1,8782	0,8955	1,8895
Refractive index, n_D^{20}	1,4785	1,4744	1,4730	1,4789
Acid No., g KOH/g	1,19	0,86	0,99	2,62
Saponification No. g KOH/g	82,00	53,27	129,50	65,39
Ester No., g KOH/g	81,81	52,41	128,51	62,77

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