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DETERMINATION OF THE MOLECULAR STRUCTURE OF PLANT PHOSPHATIDYLCHOLINES TAKING CYCLOPROPENOID ACIDS INTO ACCOUNT

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Cyclopropenoid acids (CPAs) in cells lead to an increase in the permeability of biomembranes [1], and in view of this cause definite interest in the study of the fine molecular structure of phospholipids. The molecular structure of phospholipids from microbiological materials taking the CPAs into account has been the subject of an investigation by Japanese workers [2], but there are no similar publications on the phospholipids from plant materials.

We have reported on the localization of the CPAs in the phosphatidylcholine (PC) fraction of the cotton plant of variety 5904-I [3].* In order to determine the positions of the CPAs, the phosphatidylcholines were subjected to enzymatic hydrolysis with phospholipase C [4]. The diglycerides (DGs) obtained were separated on plates coated with silver-impregnated silica gel according to their degree of unsaturation into six zones in system 1. The zones of separation were revealed with a 0.04% aqueous solution of Rhodamine 6G followed by observation in UV light.

The yields of the individual subfractions are given in Table 1. An aliquot of each subfraction was subjected to methanolysis, and the fatty acid methyl esters obtained were hydrogenated (2 h in methanol, Pd–Al catalyst). The completeness of hydrogenation was checked by

TABLE 1. Composition and Position Distribution of the Fatty Acids in the Total Diglycerides and Their Subfractions (in relation to their total amount), wt.%

Fraction	Diglyceride		Fatty acid											
			12:0	14:0	16:0	16:1	18:0	18:1	18:2	CPAs	ΣS	ΣU	ΣCPAs
1 (4,5%)	Total		—	—	0,2	—	—	—	4,3	—	0,2	4,3	—
	Position	1	—	—	0,4	—	—	—	4,1	—	0,4	4,1	—
		2	—	—	—	—	—	—	4,5	—	—	4,5	—
2 (16,1%)	Total		—	—	0,7	—	2,1	—	13,1	0,2	0,7	15,2	0,2
	Position	1	—	—	0,8	—	1,6	—	13,7	—	0,8	15,3	—
		2	—	—	0,6	—	2,6	-	12,5	0,4	0,6	15,1	0,4
3 (25,7%)	Total		0,3	0,2	3,0	0,4	8,3	—	13,5	—	3,5	22,2	—
	Position	1	0,6	0,4	5,4	0,8	7,8	—	10,7	—	6,4	19,3	—
		2	—	—	0,6	—	8,8	—	16,3	—	0,6	25,1	—
4 (31,9%)	Total		0,2	0,3	9,3	—	8,6	0,4	12,8	0,3	10,2	21,4	0,3
	Position	1	0,4	0,4	17,3	—	7,9	0,8	5,1	—	18,9	13,0	—
		2	—	0,2	1,3	—	9,3	—	20,5	0,6	1,5	29,8	0,6
5 (13,8%)	Total		0,3	0,1	5,6	—	0,2	5,6	2,0	—	6,2	7,6	—
	Position	1	0,6	0,2	9,4	—	0,4	3,1	0,1	—	10,6	3,2	—
		2	—	—	1,8	—	—	8,1	3,9	-	1,8	12,0	—
6 (8,0%)	Total		1,1	0,9	3,0	0,3	0,2	2,5	—	—	5,2	2,8	—
	Position	1	1,2	1,8	3,5	0,2	0,4	0,9	—	—	6,9	1,1	—
		2	1,0	—	2,5	0,4	—	4,1	—	—	3,5	4,5	—
Diglyceride	Total		1,9	1,5	21,8	0,7	0,8	27,1	45,7	0,5	26,0	73,5	0,5
	Position	1	2,8	2,8	36,8	1,0	1,6	21,3	33,7	—	44,0	56,0	—
		2	1,0	0,2	6,8	0,4	—	32,9	57,7	1,0	8,0	91,0	1,0

*A repetition of the oxidation of the CPAs [3] with chromium trioxide and a more detailed study of the degradation products by the TLC and GLC methods showed that it is not azelaic but suberic acid that is formed from the dicarboxylic acids.

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GLC analysis of the fatty acids. Qualitative reactions showed the presence of CPAs only in fractions 2 and 4. The remainder of the DGs was subjected to lipolysis to identify the fatty acids in position 2 of the glycerol residue. Lipolysis was carried out with pancreatic lipase from porcine pancreatic gland. The reaction products were separated in a thin layer of silica gel in solvent system 2. The monoglyceride zone was scraped off and subjected to methanolysis, and the resulting methyl esters were analyzed by GLC.

The yields of the fractions and the compositions of the fatty acids in position 2 served as a basis for determining the fatty acids in position 1. From the experimental results on the composition and position distribution of the fatty acids in the subfractions (see Table 1) we determined the molecular species statistically for each subfraction and, by summation, for the sample as a whole (Table 2).

The first subfraction contained only two species, consisting of two acids, 16:0 and 18:2. The second fraction contained mainly 18:2, 18:1, and the 16:0 acids, which form saturated–unsaturated (S–U) and unsaturated–unsaturated (U–U) species, the latter amounting to 88.9%. The cyclopropene acids were also found in this fraction, and in combination with the 18:2 acid the 18:2-CPA species was formed, making up 0.4% of the fraction. The third and fourth fraction, amounting to 97.6% of the initial PCs, contained mainly the species (U–U) and (S–U) and a small amount of the 16:0–16:0 (S–S) species, while the fourth fraction also contained 0.6% of CPAs. The fifth fraction containing mainly the 16:0 and 18:1 acids and, consequently, the 16:0–18:1 species. The sixth fraction consisted of the 16:0, 14:0, and 12:0 saturated acids, present in both positions.

TABLE 2. Molecular compositions of the Phosphatidylcholines, %

Phosphatidylcholine	Subfraction						Total diglyceride
	1	2	3	4	5	6	
12:0–12:0	—	—	—	—	—	0,1	0,1
14:0–12:0	—	—	—	—	—	0,3	0,3
16:0–12:0	—	—	—	—	—	0,5	0,5
16:1–12:0	—	—	—	—	—	+	+
18:0–12:0	—	—	—	—	—	+	+
18:1–12:0	—	—	—	—	—	0,1	0,1
12:0–14:0	—	—	—	+	—	—	+
14:0–14:0	—	—	—	+	—	—	+
16:0–14:0	—	—	—	0,1	—	—	0,1
18:0–14:0	—	—	—	+	—	—	+
18:1–14:0	—	—	—	0,1	—	—	0,1
18:2–14:0	—	—	—	+	—	—	+
12:0–16:0	—	—	+	+	+	0,3	0,3
14:0–16:0	—	—	+	+	+	0,7	0,7
16:0–16:0	—	+	0,1	0,7	1,3	1,4	3,5
16:1–16:0	—	—	+	—	—	+	+
18:0–16:0	—	—	—	+	+	+	+
18:1–16:0	—	+	0,2	0,4	0,5	0,1	1,2
18:2–16:0	—	0,6	0,3	0,2	+	—	1,1
12:0–16:1	—	—	—	—	—	+	+
14:0–16:1	—	—	—	—	—	0,1	0,1
16:0–16:1	—	—	—	—	—	0,2	0,2
16:1–16:1	—	—	—	—	—	+	+
18:0–16:1	—	—	—	—	—	+	+
18:1–16:1	—	—	—	—	—	0,1	0,1
12:0–18:1	—	—	0,2	0,1	0,4	0,6	1,3
14:0–18:1	—	—	0,1	0,1	0,1	1,0	1,3
16:0–18:1	—	0,1	1,8	5,0	5,5	1,8	14,2
16:1–18:1	—	—	0,3	—	—	—	0,3
18:0–18:1	—	—	—	0,2	0,3	0,1	0,6
18:1–18:1	—	0,3	2,7	2,3	1,8	0,2	7,3
18:2–18:1	—	2,2	3,7	1,6	+	0,4	7,9
12:0–18:2	—	—	0,4	0,3	0,2	—	0,9
14:0–18:2	—	—	0,3	0,3	+	—	0,6
16:0–18:2	0,4	0,7	3,4	11,1	2,7	—	18,4
16:1–18:2	—	—	0,6	—	—	—	0,6
18:0–18:2	—	—	—	0,5	0,1	—	0,6
18:1–18:2	—	1,2	5,0	5,0	0,9	—	12,1
18:2–18:2	4,1	10,6	6,6	3,2	—	—	24,5
12:0- CPAs	—	—	—	+	—	—	+
14:0- CPAs	—	—	—	+	—	—	+
16:0- CPAs	—	+	—	0,3	—	—	0,3
18:0- CPAs	—	—	—	+	—	—	+
18:1- CPAs	—	+	—	0,2	—	—	0,2
18:2- CPAs	—	0,4	—	0,1	—	—	0,5

As the results of the investigations showed, the total number of molecular species of PCs was 45, the disaturated and saturated–unsaturated types formed by the combination of the 18:2, 18:1, and 16:0 acids predominating. The cyclopropenoid malvic acid forms molecular types with saturated acids (16:0-CPA) and unsaturated acids (18:1-CPA, 18:2-CPA) to equal extents. It must be noted that the CPAs in the PC molecule are localized only in position 2. The formation of pairs by the CPAs with unsaturated acids leads to an increase in the number of double bonds, which possibly increases the rate of diffusion processes in the cell membranes [8].

EXPERIMENTAL

All the solvents used were purified and made absolute by standard methods [5].

The GLC analysis of the methyl esters of the fatty acids was performed on a UKh-2 instrument with a thermal conductivity detector: for the normal fatty acids at 196-197°C [column 2.5 m long × 4 mm, the stationary phase being 17% of polyethylene glycol succinate on Celite-545 (80-100 mesh)], and for the CP acids at 180°C (column containing 11% of polybutane-1,4-diol succinate on Celite-545); for both cases the carrier gas was helium.

For thin-layer chromatography we used type KSK silica gel treated with nitric acid, water, acetone, and a mixture of chloroform and methanol (less than 150 mesh). We used the following solvent systems: 1) benzene–methanol (92:8) [4], and 2) diethyl ether–petroleum ether (9:4) [6].

Hydrolysis with phospholipase C and with pancreatic lipase, methanolysis, and the separation of the DGs according to their degree of unsaturation were performed as we have described previously [4], and hydrogenation by Gray's method [7].

SUMMARY

The molecular compositions of plant phospholipids have been determined experimentally with the presence of CPAs taken into account for the first time. It has been shown that the phosphatidylcholines of the seeds of the cotton plant of variety 5904-I consist of 45 species, among which the disaturated and saturated–unsaturated types predominate. It has been established that the CPAs form pairs both with the saturated (16:0) and with the unsaturated (18:1, 18:2) acids.

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