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LIPID COMPOSITION OF *DAUCUS CAROTA* ROOTS

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Key Word Index—*Daucus carota*; Umbelliferae; carrot root; lipids; phospholipids; molecular species.

Abstract—Lipids extracted from *Daucus carota* roots were analyzed and the fatty acid composition of the triglycerides and phospholipids determined. Comparison of two different cultivars showed no significant differences of lipid composition. A method for the separation of several different molecular species from phospholipid classes is reported. Phosphatidylethanolamine and phosphatidylcholine occurred as 8 and 7 molecular species respectively.

INTRODUCTION

In studies of lipids from higher plants often only data on the relative distribution of lipid classes [1] or total fatty acid composition [2–5] are reported. However, to fully characterize the physiological significance of lipids, both separation of individual classes and analysis of their fatty acid composition should be done. In this paper triglycerides and two phospholipid classes from two different varieties of *Daucus carota* are fully analyzed in this respect. To elucidate the involvement of phospholipid classes in cellular membrane properties and functions it is further necessary to fractionate these into molecular species. The procedure was applied to carrot phospholipids with a method recently reported for the separation of molecular species of *E. coli* phospholipid [6, 7].

RESULTS AND DISCUSSION

Lipids were extracted from 10–200 g of material and two different cultivars of *D. carota* were used, Kuroda Gosun and Rote Riesen. From 1 g fr. wt of both materials 0.4–0.5 mg lipids were extracted. These lipids had the same phospholipid:triglyceride ratio of 1:0.7 in both cases and also phosphatidylcholine (PC) and phosphatidylethanolamine (PE) made up *ca* 50% (by wt) of the total phospholipids (Table 1). Phosphatidylglycerol and cardiolipin were also detected as additional phospholipids, and phosphatidylserine and phosphatidylinositol were minor constituents of both cultivars (data not shown). With the methods described no significant amounts of mono- and diclycerides or free fatty acids were found in the analyzed materials.

Table 1. Lipids extracted from carrot root tissue. Data are expressed as μg lipid extracted from 1 g fr. wt of material (average from duplicate experiments)

Material (cultivar)	Triglycerides	Total phospholipids	Phosphatidyl-ethanolamine	Phosphatidyl-choline
'Kuroda Gosun'	213	299	64	68
'Rote Riesen'	154	216	66	42

Table 2. Fatty acid composition of triglycerides and phospholipids extracted from carrot cv 'Kuroda Gosun'. Data are given as wt(%), analytical details are described in Experimental, for fatty acid nomenclature see Table 3

Fatty acid	Triglycerides	Phosphatidyl-ethanolamine	Phosphatidyl-choline
16:0	8.4	24.2	21.9
18:1	3.6	1.0	2.8
18:2	70.8	70.2	72.3
18:3	17.2	4.6	2.9

Fatty acid compositions of triglycerides, PE and PC were analyzed by GLC of their respective Me esters. In both cases and as is known from total fatty acid analyses from other materials from higher plants [3, 10–12], the fatty acid composition of triglyceride and phospholipids was simple (Tables 2 and 3). The main fatty acids were 16:0 (palmitic), 18:1 (octadecanoic, most likely oleic), 18:2 (linoleic), and 18:3 (linolenic acid). Linoleic acid alone accounted for *ca* 70% of total fatty acids, whereas only traces of 14:1, 16:1 and 18:0 were detected. The main fatty acids were the same in both materials and occurred in about the same relative amounts. Fatty acids of a chain length above 18 as well as unusual fatty

Table 3. Fatty acid composition of phospholipids from carrot cv 'Rote Riesen'. Data represent wt(%), for the conditions see Experimental

Fatty acid	Phosphatidyl-ethanolamine	Phosphatidyl-choline
14:1 tetradecanoic acid	0.6	1.5
16:0 palmitic	23.3	19.7
16:1 palmitoleic	0.4	0.1
18:0 stearic	1.3	1.7
18:1 octadecanoic*	2.1	3.2
18:2 linoleic	68.7	71.4
18:3 linolenic acid	3.6	2.4

* Probably oleic acid.

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Table 4. Phospholipid molecular species from carrot cv Rote Riesen. Relative distribution of molecular species of the two major phospholipids. Analytical procedures are described in Experimental

Subfraction		1	1	2	3	4	4	5	6	6
R_f		0	0	0.01	0.03	0.11	0.11	0.29	0.59	0.59
fatty acid	in 1-position	18:3	18:2	18:2	18:1	18:0	16:0	16:0	16:0	16:0
	in 2-position	18:3	18:3	18:2	18:2	18:2	18:2	18:1	18:0	16:0
PE	amount (μg)	trace	1.9	25.8	0.83	—	26.7	0.53	0.03	0.37
	wt %	—	3.4	45.9	1.5	—	47.5	0.9	0.1	0.7
PC	amount (μg)	—	0.7	11.1	1.5	tr	38.3	0.7	—	0.04
	wt %	—	1.3	21.2	2.9	—	73.2	1.3	—	0.1

acids are not included because they only occur in very low proportions in the materials.

Several investigators have studied the biosynthesis of higher plant cellular membrane phospholipids [13–16], but so far not enough interest has been paid to the separation, analysis and metabolism of phospholipid molecular species in higher plants, even though exact knowledge of individual phospholipids and their molecular species is a prerequisite for further studies on factors controlling properties and functions of cellular membranes [17]. For the separation of molecular species, PE and PC were first treated with phospholipase C, the resulting diglycerides acetylated and the obtained monoacetyldiglycerides (MADG) separated by $AgNO_3$–TLC (see Experimental). Each MADG preparation (0.5 mg) was applied to each plate and after chromatography 6 subfractions with the following R_f values were visualized: 0 (origin), 0 01, 0.03, 0.11, 0.29 and 0.59. By GC–MS of the 6 subfractions 9 molecular species were detected and identified (Table 4).

Calculation of the amounts of individual fatty acids from the data in Table 4 shows a good agreement with the fatty acid analysis in Table 2, proving the quality of the applied method; 16:0 and 18:2 in the PE molecular species represented 24.9% and 72.1% respectively; in Table 2 these values were 24.2 and 70.2%. These results show that the methods are capable of separating MADG molecular species from carrot root phospholipids containing up to 6 double bonds.

EXPERIMENTAL

Commercially available carrots were used, *Daucus carota* cv Kuroda Gosun (Chiba Prefecture, Japan) and cv Rote Riesen (Germany).

Extraction of lipids. Plant material (200 g) was homogenized for 2 min with 2 vol. 5% TCA in a blender at max. speed. After centrifugation total lipids were obtained by repeated extraction of the TCA homogenate and solids with $CHCl_3$–MeOH (2:1). The combined $CHCl_3$–MeOH layers were washed with 2 M KCl and H_2O and dried *in vacuo* under N_2.

TLC of total lipids and phospholipids. Performed on Si gel, for analytical (0.25 mm) and preparative (2 mm) separation of total lipids the solvent was petrol–Et_2O–HOAc (80:20:1). The solvent used for phospholipids was $CHCl_3$–MeOH–HOAc (65:25:8). Lipids were visualized with I_2 vapour and the appropriate areas on the PLC plates scraped off and eluted with $CHCl_3$–MeOH (2:1). The effectiveness of this method was proven by checking the recovery of a known amount of ^3H labeled PC from the plate.

Fatty acid analysis. Triglycerides, PC and PE were esterified by treatment with 3% (w/w) methanolic HCl for 2 hr at 100°. Fatty acid Me esters were then extracted with petrol and analyzed by FID GLC with a 0.3 × 250 cm glass column packed with 15% Degs on Neopak AS at 187°. Peaks were identified using appropriate standards.

Analysis of phospholipid molecular species. PE and PC (2–4 mg), suspended by sonication, were hydrolyzed by treatment with 45U of phospholipase C (EC 3.1.4.3, from *Bacillus cereus*) in a final vol. of 1.25 ml 0.01 M Tris–HCl buffer, pH 7.2, shaken vigorously for 30 min at 30°. Completeness of the treatment was checked by TLC ($CHCl_3$–MeOH–HOAc; 65:25:8). The diglycerides extracted from the incubation mixture were dissolved in redist. C_5H_5N and acetylated by treatment with Ac_2O [6]. For the separation of subfractions of MADG, $AgNO_3$–TLC was performed. The Si gel plates were impregnated with 10% $AgNO_3$ in MeCN, activated at 148° and developed for a distance of 17 cm at room temp. with C_6H_6–$CHCl_3$–MeOH (98:2:0.1). MADG subfractions were visualized by spraying with 0.01% Rhodamine 6G (for observation under UV) and by treatment with 20% aq $(NH_4)_2SO_4$ at 180–200° for 30 min. Corresponding areas were marked, appropriate amounts of tricaprylin were added to these zones (as an int. standard for the quantitative analyses of the phospholipid molecular species) which were then scraped from the plates and extracted. GC–MS analysis of the molecular species was done with the MADG extracted from the plates using a 35 × 0.3 cm glass column packed with 2.5% OV-17 [7].

General methods. Pi [8] and glycerol [9] were assayed following published procedures.

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