A STUDY OF THE STRUCTURE OF PLANT TRIACYLGLYCEROLS BY THE METHOD OF STEREOSPECIFIC ANALYSIS

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The distribution of the acids over the three positions of sn-glycerol in the triacylglycerols (TAGs) of the seeds of Phlomis regelii M. Pop, Ph. oreophilla Kar. et Kir., and Lavandula spica L., family Labiatae, has been established on the basis of the results of a stereospecific analysis.

As the result of an investigation of the fine structure of certain plant triacylglycerols (TAGs), the position stereospecificity of the fatty acids with respect to one of the two primary hydroxy groups of glycerol, which leads to a stereoisomerism of the TAG molecule, has been established [1].

The small number of experimental facts concerning the composition of the acids in the sn-1 and sn-3 positions does not permit a reliable prediction of the nature of the distribution of acyl residues in unstudied TAGs, particularly in those cases where some acid of unusual structure is present among the total fatty acids.

Previously, in a study of the structures of the TAGs of the seeds of 24 species of the family Labiatae, we elucidated some features of the distribution of the acyl residues of unsaturated acids in the sn-2 position that are characteristic for this family [2]. The aim of the present work was to elucidate the distribution of acids over the sn-1 and sn-3 positions of the TAGs of the seeds of three species of the same family and to establish the stereospecies composition of their PAGs.

Stereospecific analysis was carried out by Brockerhoff's method [3] with slight modifications.

In relation to their fatty acid compositions, the triacylglycerols of the seeds of the species under investigation were assigned to the oils containing oleic acid (Phlomis regelii M. Pop), linoleic acid (Phlomis oreophilla Kar. et Kir.), and linolenic acid (Lavandula spica L.). In the TAGs of the two species of Phlomis, in addition to the usual acids, we detected laballenic (Table 1), which is specific for this family.

The pure TAGs were hydrolyzed with pancreatic lipase. The reaction products were isolated from the hydrolysates by preparative GLC in system 1: Uncleaved TAGs, the sum of the 1,2- and 2,3-diacyl-sn-glycerols (sn-DAGs), and the 2-monoacyl-sn-glycerols (sn-2-MAGs) were analyzed for their fatty acid compositions by the GLC method. The equality of the acid compositions of uncleaved and the initial TAGs served as a proof that the lipase hydrolysis was not accompanied by isomerization. The representative nature of the DAGs was confirmed by the agreement of their acid composition with the calculated composition [4].

The sn-DAGs were phosphorylated with phenyl phosphorodichloridate. At this stage, a white amorphous precipitate was formed which it was difficult to separate from the phosphorylation products and distorted the results of analysis. When it was present in appreciable amount, this precipitate would not be separated even by treatment with a solution of Na_2CO_3 and by column chromatography. It was possible to prevent the formation of the precipitate by cooling the reactants and performing the reaction at 0°C.

The mixture of L- and D-phosphatidylphenols was purified by chromatography on a column of silica gel; their purity was checked by TLC on silica gel in system 2.

The phosphatidylphenols were hydrolyzed with phospholipase A, and the phospholipolysis products were isolated by preparative TLC on silica gel in system 2. The best separation was achieved when the silica gel was impregnated with oxalic acid.

The bands of the D-phosphatidylphenols and of the lysophosphatidylphenols were scraped from the plates and were treated with a methanolic solution of KOH, with the subsequent addition of HCl. The methyl esters

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TABLE 1. Distribution of the Acids in the Triacylglycerols (TAGs) of Three Species of the Family Labiatae

Plant	Position	Acid (mole %, GLC)					
		16:0	18:0	18:1†	18:2		18:3
					Δ 9,12	Δ 5,6	
Phlomis regelii M. Pop.	TAG	4,6	0,5	70,5	9,8	13,9	0,6
	sn-1	12,7 (92,0)‡	1,6 (88,9)	63,5 (30,0)	20,7*		1,5 (53,3)
	sn-2	0,9 (6,5)	–	78,7 (37,2)	15,3 (52,0)	4,8 (11,5)	0,3 (16,7)
	sn-3	0,2 (1,5)	0,2 (11,1)	69,3 (32,8)	30,3*		–
Phlomis oreophilla Kar. et Kir.	TAG	2,5	0,6	36,7	50,5	7,1	2,6
	sn-1	3,8 (50,7)	1,0 (55,6)	34,9 (30,8)	59,0* (37,1)		2,3 (25,5)
	sn-2	0,6 (8,0)	–	25,9 (23,5)	72,0 (41,7)		1,5 (19,2)
	sn-3	3,1 (41,3)	0,8 (44,4)	50,3 (45,7)	41,3* (24,2)		4,0 (51,3)
Lavandula spica L.	TAG	3,1	0,5	8,1	9,4		78,9
	sn-1	8,3 (89,2)	1,2 (80,0)	13,4 (55,1)	8,7 (30,9)		63,4 (28,9)
	sn-2	0,4 (4,3)	–	10,7 (44,0)	12,2 (43,3)		76,7 (32,4)
	sn-3	0,6 (6,5)	0,3 (20,0)	0,2 (0,9)	7,3 (25,8)		91,5 (38,7)

*Sum of $\Delta^{9,12}$- and $\Delta^{5,6}$-18:2.
†Together with 16:1.
‡Proportion of the acid in percentages with respect to their total amount on the TAGs [1].

(MEs) obtained in this way were free from the phenol formed in the case of acid methanolysis. The absence of phenols from the methanolysis products was confirmed by gas-liquid chromatography on polar and nonpolar phases. When a model mixture of phenol with fatty acid MEs was chromatographed on the polar phase, phenol and the 16:10 ME issued as a single peak, while on the nonpolar phase the phenol was eluted with the solvent and the amount of 16:0 ME corresponded to its true amount in the total MEs.

Of the phosphorolysis products, the composition of the acids of the lysophosphatidylphenols directly reflected the composition of the fatty acids from the sn-1 position, and the composition of the acids split out that of the acids in the sn-2 position. The set of acids in the sn-3 positions was obtained by two methods of calculation [3]. The results of the two calculations coincided. The compositions of the acids from the sn-2 position determined from the results of two lipolyses were also identical.

The amounts of laballenic acid ($\Delta^{5,6}$-18:2) in the TAGs and sn-2-MAGs of the two species of Phlomis were determined gravimetrically by separating their MEs by preparative TLC on silica gel with the addition of $AgNO_3$ and by GLC analysis. The distribution of the $\Delta^{5,6}$-18:2 acid in the sn-2 position was calculated in this way. The small amount of lysophosphatidylphenols did not permit the amounts of this acid in the sn-1 and sn-2 positions to be determined.

The structures of the TAGs of each plant species were analyzed in duplicate. The results of the stereospecific analysis are given in Table 1. The combined fatty acids of the species studied included, in addition to the acids mentioned in the Table, traces of the 16:1 acid. The saturated and unsaturated acids were distributed differently from the sn-1 and sn-3 positions.

The saturated acids were concentrated mainly in the extreme positions, but in the TAGs of two species (Ph. regelii and Lavandula spica) almost all the saturated acids were concentrated in the sn-1 position, and in the TAGs of Ph. oreophilla more than 1/3 of the saturated acids were also present in the sn-3 position.

The preferential esterification of the sn-1 position by the saturated acids has also been detected in several other plant TAGs [1].

In the TAGs of Ph. regelii in which oleic acid is the predominating one, it is distributed approximately uniformly over the extreme positions, but some excess of it is also found in the sn-2 position. In the other Phlomis species, the 18:1 acid esterifies the sn-3 position to a greater degree, and in Lavandula spica L. the sn-1 position.

In the TAGs of Ph. oreophilla and Lavandula spica, about half the 18:2 acid is bound in the sn-2 position and the remainder is largely included in the sn-1 position, but in the TAGs of Ph. regelii this acid mainly esterifies the sn-3 position.

In the TAGs of two species, the linolenic acid occupies the sn-3 position preferentially, and in Ph. regelii the sn-1 position.

The laballenic acid is distributed in a somewhat unusual fashion in the TAG molecules. With rare exceptions [5], acids of unusual structure have not been detected in the sn-2 position, and they either esterify the

TABLE 2. Stereotypic Compositions of the Triacylglycerols (TAGs) of the Seeds of Three Species of the Family Labiatae

Plant	Type of TAG, mole %						Total number of TAG species
	SSU*	SUS	SUU	UUS	USU	UUU	
Phlomis regelii M. Pop	0.1 (2)†	—	14.1 (9)	0.4 (6)	0.8 (4)	84.6 (15)	36
Phlomis oreophilla Kar. et Kir.	—	1.5 (2)	2.6 (7)	3.9 (9)	0.6 (4)	91.4 (21)	43
Lavandula spica L.	—	—	9.3 (9)	0.8 (5)	0.4 (2)	89.5 (18)	34

*S denotes acyl residues of saturated acids and U those of unsaturated acids.

†Number of TAG species of the given type.

sn-3 position completely or are enriched in this position as compared with the sn-1 [1]. There is no information on the distribution of the $\Delta^{5,6}$-18:2 acid in the acylglycerols. In the seeds of Ph. oreophilla the amount of the $\Delta^{5,6}$-18:2 acid is small, and in the sn-2-MAG fraction of this species no laballenic acid has been detected, which gives grounds for concluding that it is distributed predominantly in the extreme positions. However, this acid is present in the appreciable amounts in the analogous fraction of Ph. regelii (14% in the TAG). On calculating the molar fraction, it was found that about 12% of the $\Delta^{5,6}$-18:2 acid esterifies the sn-2 position, and 88% the extreme positions.

In Ph. regelii the laballenic acid is distributed over all three positions of the TAGs with a predominance in the extreme positions.

On the basis of the results of the spereospecific analysis we calculated the stereotypic composition of the TAGs (Table 2). As can be seen from Table 2, TAGs of the S_3 and US_2 types are absent from these oils.

EXPERIMENTAL

The seeds of Ph. regelii were collected in the environs of Darbaza (Kaz. SSR); those of Ph. oreophilla in the environs of Santash (Kirg. SSR); and those of Lavandula spica in the Tashkent Botanical Garden in 1980.

The triacylglycerols were isolated from the oils by column chromatography on silica gel L 100-160 mesh with elution by a mixture of low-boiling petroleum ether and diethyl ether in a ratio of 98:2.

Thin-layer chromatography was carried out on Silufol and silica gel L 5/40 mesh with the addition of 10% of gypsum in the systems 1) petroleum ether–diethyl ether (6:4); 2) diethyl ether–methanol–ammonia (88:10:2); 3) petroleum ether–diethyl ether (1:1); and 4) benzene. Gas–liquid chromatography was performed on a Chrom-4 instrument with a flame ionization detector in the isothermal regime using a 2.5 m × 4 mm column filled with 17% of ethylene succinate on Chromaton N-AW at a column temperature of 202°C at a pressure of carrier gas (He) of 0.7 kgf/cm^2, and on a 1.2 m × 3 mm column filled with 5% of SE-30 on Chromaton N-AW at a column temperature of 220°C at a pressure of He of 9.65 kgf/cm^2.

The acylglycerols were hydrolyzed with 10% methanolic KOH solution. Lipolysis by pancreatic lipase was carried out by adding to 1 g of TAGs 2 ml of hexane, 4 ml of 1% poly(vinyl alcohol), 1 ml of 22% $CaCl_2$, and 0.3 g of pancreatic lipase from the Olaine chemical reagents factory (previously defatted with acetone and diethyl ether) in 10 ml of ammoniacal buffer having pH 8.0. The reaction was performed at 40°C for 5-20 min, depending on the depth of hydrolysis. The reaction was monitored by taking samples and analyzing them by TLC in system 1.

The initial reagents for the phosphorylation reaction were obtained as described previously [6].

Phosphorylation. A weighed sample of sn-DAGs was dissolved in 0.5 ml of dry diethyl ether and the solution was cooled in ice for 5-10 min. Then, with shaking, the cooled solution of the substance was added dropwise to a previously cooled mixture of dry pyridine and 0.5 ml of freshly distilled phenyl phosphorodichloridate. The reaction mixture was left at room temperature for 12 h.

The isolation and purification of the phosphorylation products was carried out as described by Brockerhoff [3].

Phospholipolysis of the Phosphatidylphenols. To 50 mg of the phosphatidylphenols in 10 ml of diethyl ether were added 0.2 ml of a 0.1 M solution of $CaCl_2$ and a solution of 20 mg of phospholipase A from kufi venom in 1 ml of Tris buffer with pH 8.0. The mixture was left at room temperature for 12 h. After the end

of the reaction (monitored by TLC in system 2), the solvent and the water, in the form of an azeotrope with benzene, were evaporated off at a temperature not exceeding 40°C. The residue was treated with 1 ml of chloroform, 0.5 ml of methanol, and a few drops of water. The resulting solution was deposited on a plate.

The following variant of TLC was used to separate the phospholipolysis products. Two plates (20 × 20 cm) coated with silica gel L 5/40 mesh were impregnated with a 1% aqueous solution of oxalic acid. After the first run in system 3 and visualization with 50% H_2SO_4 followed by heating at 120°C, the band corresponding in its R_f value to the free lipids was removed, and the plates were kept in ammonia vapor for 10 min and were immersed in system 2. The bands of the D-phosphatidylphenols and of the lysophosphatidylphenols visualized similarly were removed from the plates and, without the substances being eluted from the adsorbent, 3 ml of 0.5 M KOH in methanol was added. After 15 min, 2 ml of 1 N HCl was added and the resulting MEs were extracted three times with petroleum ether. After the solvent had been eliminated, the residue of free acids was methylated with diazomethane.

The isolation of the $\Delta^{5,6}$-18 : 2 ME was carried out by chromatographing the MEs from the TAGs and the sn-2-MAGs on silica gel with the addition of $CaSO_4$ and 30% of $AgNO_3$ in system 4. The zone with R_f 0.86 was eluted from the sorbent [7].

SUMMARY

1. In the triacylglycerols of the seeds of three species of the family Labiatae, the saturated acids are distributed either predominately in the sn-1 position or in the sn-1 and sn-3 positions; the distribution of the 18 : 1 acid is characteristic for each oil; the 18 : 2 acid esterifies mainly the sn-2 position and of the two extreme positions, the sn-1; the 18 : 3 acid in two species occupies predominantly the sn-3 position and in one species predominantly the sn-1 position.

2. Laballenic acid esterifies all three positions, with a preference for the extreme ones, in the triacylglycerol of Ph. regelii M. Pop.

3. The phosphorylation of the sn-1,2- and sn-2,3-diacylglycerols is not accompanied by the formation of appreciable amounts of by-products when previously cooled reagents are used.

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