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 labalienic (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 Na2CO3 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...
TABLE 1. Distribution of the Acids in the Triacylglycerols (TAGs) of Three Species of the Family Labiatae

<table>
<thead>
<tr>
<th>Plant</th>
<th>Position</th>
<th>Acid (mole %, GLC)</th>
<th>16:0</th>
<th>17:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:1</th>
<th>20:2</th>
<th>20:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlomis regeli X. Pomp.</td>
<td>TAG</td>
<td>sn-1</td>
<td>4.0</td>
<td>12.7 (2.9)</td>
<td>0.5</td>
<td>1.1 (0.3)</td>
<td>1.5 (0.9)</td>
<td>70.5</td>
<td>9.5</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-2</td>
<td>0.9 (0.5)</td>
<td>0.2 (0.3)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.2)</td>
<td>20.7</td>
<td>1.5 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-3</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.2)</td>
<td>20.7</td>
<td>1.5 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Phlomis oreophilica Kar. et Klr.</td>
<td>TAG</td>
<td>sn-1</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-2</td>
<td>1.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-3</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>0.5 (0.5)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>Lavandula spica L.</td>
<td>TAG</td>
<td>sn-1</td>
<td>3.1</td>
<td>4.5 (4.5)</td>
<td>4.5 (4.5)</td>
<td>4.5 (4.5)</td>
<td>4.5 (4.5)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-2</td>
<td>0.4 (0.4)</td>
<td>0.4 (0.4)</td>
<td>0.4 (0.4)</td>
<td>0.4 (0.4)</td>
<td>0.4 (0.4)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-3</td>
<td>0.6 (0.6)</td>
<td>0.6 (0.6)</td>
<td>0.6 (0.6)</td>
<td>0.6 (0.6)</td>
<td>0.6 (0.6)</td>
<td>35.7</td>
<td>2.5</td>
<td>0.5 (0.5)</td>
</tr>
</tbody>
</table>

*Sum of Δ9,12- and Δ5,8-18:2.
†Together with 16:1.
‡Proportion of the acid in percentages with respect to their total amount on the TAGs [11].

(MEs) obtained in this way were free from the phenol formed in the case of acid methanalysis. The absence of phenols from the methanalysis 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 phospholysis 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 (Δ5,8-18:2) 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 AgNO3 and by GLC analysis. The distribution of the Δ5,8-18:2 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 18:1 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. regellii and Lavandula spica), almost all the saturated acids were concentrated in the sn-1 position, and in the TAGs of Ph. oreophilica more than 1/2 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. regellii 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. oreophilica 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. regellii 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. regellii 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

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