Steric Structures of Triacylglycerols in the Seed Oil of Carrot *Daucus carota* L. var. *sativa* DC.

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The steric structures of the triacylglycerols (TGs) in the seed oil of carrot *Daucus carota* L. var. *sativa* DC. were studied by the stereospecific analysis of the acyl distributions using the chiral-phase high-performance liquid chromatography of the monoacylglycerol derivatives. Total fatty acids of the seed oil TGs contained 71 mol % petroselinic acid (6-18 : 1), 12 mol % oleic acid (9-18 : 1), 11 mol % linoleic acid (18 : 2 n-6), and 4 mol % palmitic acid (16 : 0). Fatty acid percents in each sn-1, sn-2 and sn-3 position of the TGs were 6-18 : 1 (38, 23 and 40 %), 9-18 : 1 (21, 66 and 13 %), and 18 : 2 n-6 (20, 65 and 15 %). Structures and estimated contents of the major TG components in the carrot seed oil was found to be (6-18 : 1) (6-18 : 1) (6-18 : 1) 32~48 %, (6-18 : 1) (9-18 : 1) (6-18 : 1) 17~25 %, (6-18 : 1) (18 : 2 n-6) (6-18 : 1) 15~22 %. Petroselinic acid distributes preferentially at the sn-1 and sn-3 positions in the carrot seed oil TGs in analogy with 16 : 0 and 18 : 0 in the ordinary seed oil TGs.

1 Introduction

Seed oils of some plants belonging to the family Umbelliflorae could serve as a source of petroselinic acid *cis*-6-octadecenoic acid (6-18:1)¹. Carrot seed oil is one of a rich source of triacylglycerols (TGs) containing 6-18:1 as a major component similarly to the seed oils of celery, parsley, fennel and coriander¹. The contents of 6-18:1 in three samples of the carrot (*Daucus carota*) seed oil were reported as 70~73 % by Kleiman and Spencer¹. In this study, the stereospecific analysis of the carrot seed oil TGs has been performed by our method²~⁴ using chiral-phase high-performance liquid-chromatography (HPLC) method combined with the high resolution GC, since positional distributions of 6- and 9-18:1 in the carrot seed oil TGs have not been known. Previously, the contents of 6- and 9-18:1 had been determined by gas chromatography (GC) of fatty acids as methyl esters and their scission products with a packed column. In this study, 6- and 9-18:1 have been separated by GC with a high resolution WCOT column. The fatty acid compositions of the carrot seed oil TGs and their fractions have been determined by the GC method. Stereospecific analysis of natural TGs gives only acyl distribution on the three hydroxyl groups of glycerol. Since the natural TGs usually have the complex compositions, the direct informations on the TG compositions cannot be obtained. In this study, the approximate compositions of major TG components could be calculated from the acyl distribution in the carrot oil TGs due to the simple composition of the main fatty acid components.

2 Experiments

2.1 Extraction of seed oil TGs.

Seeds of carrot were bought at the shop in Hakodate. The trade name was Koizumi fuyugoshi gosun ninjin from Kyouwa Seed
and Seedling Co. The seeds 40.5 g were milled with a small electric mill and extracted repeatedly with each 100 mL of diethyl ether five times. The yield of the extract was 6.61 g (16.3 %). The extract 168 mg was fractionated by preparative thin-layer chromatography (TLC) on four Kiesel Gel 60 G plates (20 x 20 cm, thickness 0.5 mm) using n-hexane-diethyl ether (80 : 20, vol/vol) as developing solvent. The TG fraction 154 mg was separated by the extraction of the main band.

2.2 Partial hydrolysis with Grignard reagent

The carrot seed oil TGs (141 mg) was taken into a 10 mL screw cap centrifuge tube, and dry diethyl ether (3 mL) and then 3M ethylmagnesium bromide diethyl ether solution (0.33 mL) were added to the tube. The mixture was shaken for 1 min, and glacial acetic acid (0.1 mL) and water (3.3 mL) were added. The products were extracted with diethyl ether each 3 mL five times. The extract was washed with 2 % sodium hydrogen carbonate aqueous solution and then with water, and dried over anhydrous sodium sulfate. The residue after evaporation under nitrogen flow was 147 mg.

2.3 Separation of 1- and 2-monoacylglycerols

The residue was subjected to preparative TLC on Silica gel 60G plates containing 10 % wt/wt boric acid using chloroform/methanol 98 : 2 vol/vol mixture for developing. The bands containing 1- and 2-monoacylglycerol fractions were scraped and extracted with each four portion of 100 mL diethyl ether respectively.

2.4 Preparation of 3,5-dinitrophenylurethanes (DNPUs)

Dry toluene (5 mL) dehydrated on sodium wire, 3,5-dinitrophenylisocyanate (40 mg, Sumika Chemical Analysis Service, Osaka), and then dry pyridine (50 μL) dehydrated on calcium hydride were added to the 10 mL screw cap centrifuge tube containing the partially hydrolyzed products. The mixture was shaken frequently and left overnight under nitrogen at ambient temperature. The solvent was removed under nitrogen flow without heating.

2.5 HPLC on the chiral phase

HPLC of the 1-monoacylglycerol 3,5-DNPUs was carried out using a Hitachi L-6200 instrument (Hitachi Ltd., Tokyo) equipped with a chiral Sumichiral OA 4100 column (25 cm x 4 mm i.d.) packed with 5 μm particles of N-(R)-1-(α-naphthyl) ethyl amino-(S)-valine chemically bonded to γ-aminopropyl silanized silica. Mobile phase was n-hexane/1,2-dichloroethane/ethanol (40 : 12 : 3, vol/vol/vol). Peaks were monitored at 254 nm with a Shimadzu SPD-1 UV detector, and a Shimadzu C-R3A integrator was used to get the retention data. Flow rate was 1.0 mL/min.

2.6 GC of methyl esters

Methyl esters were prepared from 5 mg of the TG, monoacylglycerols or their 3,5-DNPUs by shaking in a sealed glass tube with 2 mL of anhydrous diethyl ether and 25 μL of 1 M sodium methoxide in methanol solution at 50°C during 30 min. After neutralization with addition of acetic acid 6 μL and removing of solvent under nitrogen flow without heating, the product was extracted with n-hexane and injected to a Shimadzu GC-6AM instrument connected with a FID detector, a Shimadzu C-R 6 A integrator, and a WCOT column (50 m x 0.25 mm i.d.) coated with Silar 5 CP. The column temperature was 160°C, and the injector and detector were 230°C. The carrier gas was hydrogen.

3 Results and Discussion

3.1 Fatty acid composition

Petroselinic and oleic acids are not readily resolved by the GC of their methyl esters with the packed column, and the complex methods, such as silver-ion chromatography or GC of the oxidative products and the NMR method, have to be used for the analysis. Recently, the method for the separation between 6-18:1 and 9-18:1 by GC as isopropyl esters was proposed by Wolff and Vandamme. In our study, the GC analysis was carried out using a capillary column coated with Silar 5CP at relatively low column temperature (160°C), a low flowrate of
hydrogen as carrier gas, and low loading of the methyl ester sample (3 μg after splitting). Fig.-1 shows the separation of 6-18:1 and 9-18:1 peaks as their methyl esters. They have a long time as 60 min and the sufficient resolutions are obtained in the different ratios of 9-18:1/6-18:1, A 0.18 and B 0.52 as shown in Fig.-1 A and B.

The oil content was about 16.3 % of the carrot seed weight. The reported percentages of oils in the seeds of Daucus carota are 18 and 27 % in the dry basis (1). The total fatty acid composition of the carrot seed oil is shown in Table-1. The total fatty acid compositions of the carrot seed oils reported were 16:0 3.6–6.1 %, 18:0 0.2–1.1 %, 6-18:1 70–73 %, 9-18:1 10–13 %, 18:2 n-6 10–13 %, 18:3 n-3 0–0.3 % (1). The percentages of the total fatty acid composition in Table-1 is in accord with the reported data (1). The high content of 6-18:1 over 70 % in carrot seed oil fatty acids is noticeable.

3.2 Stereospecific distribution

The composition (mol %) of the acyl group in each of the sn-1, sn-2 and sn-3 positions was obtained by the Scheme-1 as described in our previous paper (2).

Fig.-2 shows the HPLC resolution of the 1-monoacylglycerol DNPU’s to the sn-1 and sn-3 enantiomers in the analytical and preparative separations.

The fatty acid compositions of total TGs and each monoacylglycerol fraction are shown in Table-1.

The percentages of each fatty acid in sn-1 (3)- and 2-monoacylglycerols (1-MG and 2-MG in the Scheme-1) were corrected for a little differential losses found in the separation of the 1- and 2-monoacylglycerols by the boric acid–impregnated TLC using the next equations (1) and (2).

\[
\text{[sn-1 (3)-MG] (mol %)=3×TG} \\
\times\left[1-\text{MG}/(2×1-\text{MG}+2-\text{MG})\right] \quad (1)
\]

\[
\text{[sn-2-MG] (mol %)=3×TG} \\
\times\left[2-\text{MG}/(2×1-\text{MG}+2-\text{MG})\right] \quad (2)
\]

This correction gives the next relations between [sn-1 (3)-MG] and [sn-2-MG] ;

\[
\text{TG}=\frac{\left([\text{sn-1 (3)-MG}]×2+[\text{sn-2-MG}]\right)}{3}
\]

The differential losses of the unsaturated derivatives in the preparation of the 3,5-
Scheme 1 Procedures for stereospecific analysis of acyl distribution in triacylglycerols by chiral HPLC.

DNPU derivatives and methyl esters of the Scheme-1 lead to some errors in the analysis of the sn-1 and sn-3 monoacylglycerols. Comparison of the loss showed, in general, increasing losses as the unsaturation of the acyl groups increased. The losses were less than 1% for 18:1, and about 3% in the conversion of the monoacylglycerols to the 3,5-DNPU derivatives and about 9% in the conversion of the monoacylglycerols to the methyl esters thorough the 3,5-DNPU for 18:2. The fatty acid compositions of the sn-1- and sn-3-monoacylglycerols, sn-1-MG and sn-3-MG, were corrected with the next equations by considering that the mean of [sn-1-MG] and [sn-3-MG] is equal to [sn-1(3)-MG].

\[
[sn-1-MG] \text{ (mol %)} = \\
2 \times [sn-1(3)-MG] \times \frac{[sn-1-MG]}{[sn-1-MG]+[sn-3-MG]} \quad (3)
\]

\[
[sn-3-MG] \text{ (mol %)} = \\
2 \times [sn-1(3)-MG] \times \frac{[sn-3-MG]}{[sn-1-MG]+[sn-3-MG]} \quad (4)
\]

The corrected mole percentages of total fatty acids, [sn-1-MG] and [sn-3-MG], are shown with [sn-2-MG] in Table-2. The mole percentages of each fatty acids among the three positions in the TGs and shown in Table-3.

Plant TG biosynthesis proceeds through the glycerol 3-phosphate pathway. The sn-3-glycerol-phosphate formed by the action of the enzyme glycerol kinase on free glycerol is acylated sequentially by specific acyltransferases in three steps: glycerol phosphate acyltransferase at the sn-1 position, lysophosphatidate acyltransferase at the sn-2 position, and diacylglycerol acyltransferase at the sn-3 position. The data of Table-2 and 3 reflects the selectivity of the acyltransferases in the acylations at

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**Table-2**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>sn-1-MG</th>
<th>sn-2-MG</th>
<th>sn-3-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:1</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>18:2</td>
<td>40</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table-3**

<table>
<thead>
<tr>
<th>Acyltransferase</th>
<th>sn-1-MG</th>
<th>sn-2-MG</th>
<th>sn-3-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceraldehyde</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Phosphatidate</td>
<td>0.6</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>0.4</td>
<td>0.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>
the sn-1, sn-2 and sn-3 position. The glycerol-3-phosphate acyltransferase and the diacylglycerol-acyltransferase have distinctive preference for 6-18:1 at the sn-1 and sn-3 positions. In the contrast, the lysophosphatidic acid acyltransferase has an increased affinity at the sn-2 position for 9-18:1 and 18:2 n-6.

Usually 18:2 n-6 shows a more affinity than 9-18:1 at the sn-2 position. For example, the mole percentages of each acids at sn-1, sn-2 and sn-3 positions in soybean oil TGs are 29.7, 33.1, 36.7 mol % for 9-18:1 and 29.3, 42.8, 27.9 mol % for 18:2 n-6. The similar affinity for 9-18:1 and 18:2 n-6 at the sn-2 position in the carrot seed oil TGs is remarkable. This unusual fact will be explainable by the influence of the combinations of the high proportion of 6-18:1 with 9-18:1 and 18:2 n-6 in the TG.

### 3-3 Estimation of TG components

In Table-2, distributions of major fatty acids at the sn-1, sn-2 and sn-3 in the TGs are 6-18:1 (79, 48 and 85 mol %), 9-18:1 (8, 25 and 5 mol %), 18:2 n-6 (8, 22 and 5 mol %), respectively. They indicate that the major TGs of carrot seed are the three components shown below. The estimated contents are shown together.

<table>
<thead>
<tr>
<th>sn-1</th>
<th>sn-2</th>
<th>sn-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6-18:1) (6-18:1) (6-18:1)</td>
<td>32~48 mol %</td>
<td>[1]</td>
</tr>
<tr>
<td>(6-18:1) (6-18:2 n-6) (6-18:1)</td>
<td>15~22 mol %</td>
<td>[3]</td>
</tr>
</tbody>
</table>

The maxima of the contents in [1]~[3] are obtained assuming that all of 6-18:1, 9-18:1, and 18:2 n-6 of the sn-2 combine only with 6-18:1 at the sn-1 and sn-3. The minima of the contents in [1]~[3] are calculated assuming that 6-18:1 of the sn-2 combines randomly with each fatty acid at the sn-1 and sn-3 positions. In the TGs of the ordinary seed oils, palmitic and stearic acids distribute exclusively in the sn-1 and sn-3 positions, and 9-18:1 and 18:2 n-6 mainly in the sn-2 positions. In TGs of the carrot seed oil, 6-18:1 distributes predominantly in the sn-1 and sn-3 positions, and 9-18:1 and 18:2 n-6 mainly in the sn-2 position as shown in Table-3. It shows that the distribution of 6-18:1 in the carrot seed oil TGs has a somewhat resemblance to those of the palmitic and stearic acids in the TGs of the ordinary seed oil.

The minor TG components of the carrot seed oil expected are shown below.

<table>
<thead>
<tr>
<th>sn-1</th>
<th>sn-2</th>
<th>sn-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(9-18:1) (6-18:1) (6-18:1)</td>
<td>3.1~7.7 mol %</td>
<td>[4]</td>
</tr>
<tr>
<td>(6-18:1) (6-18:1) (9-18:1)</td>
<td>1.8~4.9 mol %</td>
<td>[5]</td>
</tr>
<tr>
<td>(9-18:1) (6-18:1) (6-18:1)</td>
<td>2.7~6.6 mol %</td>
<td>[6]</td>
</tr>
<tr>
<td>(9-18:1) (6-18:1) (18:2 n-6)</td>
<td>1.9~5.1 mol %</td>
<td>[7]</td>
</tr>
</tbody>
</table>

The maxima of the contents in [4]~[7] are obtained assuming that all of 9-18:1 and 18:2 n-6 of the sn-1 and sn-3 combine only with two moles of 6-18:1 in the other positions. The minima of the contents are calculated assuming 9-18:1 and 18:2 n-6 of the sn-1 and sn-3 positions combine randomly.

with each acid at the other positions.
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References