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ISOMERIZATION AND SATURATION OF MONOUNSATURATED ACYL MOIETIES DURING CATALYTIC HYDROGENATION INFLUENCED BY THEIR POSITION IN TRIACYLGLYCEROLS

M.M. PAULOSE *, K.D. MUKHERJEE and I. RICHTER

Federal Center for Lipid Research, Piusallee, D-4400 Münster, GFR

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1, 2, 3-Tri-(Z)-9-octadecenoylglycerol (triolein) and 1, 2, 3-tri-(Z)-13-docosenoylglycerol (trierucin) were partially hydrogenated using a palladium catalyst. The unsaturated acyl moieties at the 2-position of 1, 2, 3-triacylglycerols were reduced at a slower rate than those at the 1, 3-positions. The extent of geometrical isomerization was distinctly higher and the double bonds were somewhat more scattered in the acyl moieties at the 2-position than those at 1- and 3-positions.

I. Introduction

Little is known about the reactivity of unsaturated acyl moieties at various positions of triacylglycerols during catalytic hydrogenation. Earlier investigators who hydrogenated mixtures of triacylglycerols, such as randomized soybean oil and olive oil, concluded that hydrogenation of an acyl moiety is not influenced by the position it occupies in a triacylglycerol [1, 2]. More recently, however, the linoleoyl moiety of sunflower oil has been reported to hydrogenate faster in the 1,3-positions than in the 2-position [3].

We subjected pure symmetrical triacylglycerols, 1,2,3-tri-(Z)-9-octadecenoylglycerol (triolein) and 1,2,3-tri-(Z)-13-docosenoylglycerol (trierucin), to partial hydrogenation and analyzed the acyl moieties in the 2-position and 1,2,3-positions of the triacylglycerols formed. We found that the extent of saturation as well as geometrical and positional isomerization of acyl moieties during hydrogenation are indeed dependent on the position they occupy in triacylglycerols.

II. Experimental

A. Material

(Z)-9-Octadecenoic acid (oleic acid) and (Z)-13-docosenoic acid (erucic acid) were purchased from Nu-Chek-Prep, Elysian, Minn. 56028, USA. Olive oil of DAB

* Present address: Regional Research Laboratory, Hyderabad-9, India.

7 grade was purchased locally. Palladium chloride/barium sulfate containing 10% Pd, porcine pancreas lipase as well as all other reagents of analytical grade were obtained from E. Merck AG, D-6100 Darmstadt, GFR.

B. Methods

1,2,3-Tri-(Z)-9-octadecenoylglycerol and 1,2,3-tri-(Z)-13-docosenoylglycerol were prepared by esterification of the respective acids with glycerol using *p*-toluenesulfonic acid as the catalyst, and purified by adsorption chromatography on silica gel. These compounds were found to be more than 96% pure as determined by adsorption and argentation thin-layer chromatography, gas chromatography and reductive ozonolysis followed by gas chromatography (described later).

Hydrogenations were carried out in a 20 ml screw-capped reaction tube provided with a magnetic stirrer. The tube was fitted with a teflon-lined septum, through which two stainless steel needles were inserted to serve as inlet and outlet of hydrogen. Palladium chloride/barium sulfate, 10 mg, was suspended in 8 ml hexane in the reaction tube and reduced to the active catalyst by bubbling hydrogen through the reaction mixture under stirring at ambient temperature for 30 min. The reaction tube was purged with nitrogen and the triacylglycerol, 100 mg, dissolved in 2 ml hexane, was added to the catalyst suspension. Hydrogenation was started by bubbling hydrogen through the reaction mixture under stirring at 25°C. Partially hydrogenated samples were withdrawn from the reaction mixture after 15 min and 60 min and centrifuged to separate the catalyst.

The triacylglycerols used as starting materials, as well as the partially hydrogenated products, were analyzed as follows.

An aliquot of the triacylglycerols was converted to methyl esters by transesterification. The methyl esters were analyzed by gas chromatography using flame ionization detectors. The separations were carried out on a column, 6 ft by $\frac{1}{8}$ in., packed with 10% EGSS-X on Gas-Chrom P, 100–120 mesh (Applied Science Laboratories Inc., State College, Pa. 16801, USA). The proportions of the various components in each mixture were calculated as percentage area of the respective peak, measured by triangulation.

The remaining part of the methyl esters was fractionated into (Z)-and (E)-isomers by argentation chromatography [4] on a layer of silica gel containing 10% silver nitrate. The plates were developed twice with hexane–diethyl ether (90:10). The methyl esters were visualized by spraying with 0.1% ethanolic 2′, 7′-dichlorofluorescein and extracted with water-saturated diethyl ether. A definite amount of methyl eicosanoate was added as internal standard to aliquots of the (Z)-and (E) isomers and the ratio of these isomers was determined by gas chromatography, as described above.

An aliquot of the methyl esters, ca. 100 μg, was dissolved in pentane and ozonized at −70°C using a Supelco micro-ozonizer [5,6]. The reaction products were evaporated to dryness at room temperature, dissolved in 40 μl carbon disul-

fide, and the ozonides reduced using 1–3 mg of triphenylphosphine. After standing at room temperature for 30 min, the mixture of aldehydes and aldehyde esters was analyzed by gas chromatography using a column, 6 ft by $\frac{1}{8}$ in., packed with 10% OV-17 on Gas-Chrom Q, 80–100 mesh (WGA, D-4000 Düsseldorf, GFR), coupled with another column, 2 ft by $\frac{1}{8}$ in., packed with 3% OV-225 on Supelcoport (Supelco, Inc., Bellefonte, Pa. 16823, USA). The temperature was programmed from 50°C to 270°C, 5°C/min [7,8]. Identification of fragments was by a homologous series of aldehydes and by ozonolysis of monounsaturated methyl esters of known positional configuration. Quantitative results were obtained from peak areas, measured by triangulation; response factors were applied to correct for lack of response in the flame ionization detector by carboxyl and carbonyl carbon atoms [9].

A part of each sample of triacylglycerol was hydrolyzed with porcine pancreatic lipase [10]. The 2-acylglycerols formed were isolated by preparative thin-layer chromatography on silica gel G containing 8% boric acid [11] with hexane–diethyl ether–acetic acid (50:50:1) as the developing solvent. As described above, the 2-acylglycerols were converted to methyl esters, which were analyzed by gas chromatography. The methyl esters were then fractionated into (Z)- and (E)-isomers by argentation thin-layer chromatography. The fractions obtained were subjected to reductive ozonolysis and the fragments were analyzed by gas chromatography.

III. Results and discussion

In order to assess the extent to which acyl migration might occur during partial hydrogenation of 1,2,3-tri-(Z)-9-octadecenoylglycerol and 1,2,3-tri-(Z)-13-docosenoylglycerol, the triacylglycerols of olive oil were partially hydrogenated under similar conditions. As criteria for acyl migration, we followed the levels of hexa-

Table 1
Fatty acid composition (%) of olive oil and partially hydrogenated products

	Acyl moieties *					
	16:0	16:1	18:0	18:1	18:2	18:3
0 min hydrogenation (starting material)						
1,2,3-Triacylglycerol	11.1	1.0	2.8	74.9	9.3	0.9
2-Acylglycerol	0.6	0.8	0.1	82.1	15.6	0.8
15 min hydrogenation						
1,2,3-Triacylglycerol	11.3	0.8	14.6	72.8	0.3	0.2
2-Acylglycerol	1.1	0.7	11.3	85.8	0.9	0.2

* Number of carbon atoms : number of double bonds.

decanoic acid and hexadecanoic acids in the tricylglycerols as well as in the corresponding 2-acylglycerols, derived by lipase hydrolysis before and after hydrogenation. The results given in table 1 show that after the reduction of ca. 22% of the double bonds, randomization between 2- and 1,3-positions of the triacylglycerols occurred to an extent of approximately 15% only. The extent of randomization was estimated as follows. A complete randomization would yield, at a level of 12.1% of fatty acids with 16 carbon atoms in triacylglycerols, 12.1/3 = 4.03% of these acids in the 2-acylglycerols. But the actual amount of these fatty acids present in the 2-acylglycerols before hydrogenation is 1.4%. Therefore, in the case of complete randomization the content of these fatty acids would increase by 4.03 − 1.4 = 2.63%. After hydrogenation, the content of fatty acids with 16 carbon atoms increased by 1.8 − 1.4 = 0.4% in the 2-acylglycerols, which implies randomization to an extent of approximately (0.4/2.63) X 100 = ca. 15%. Since acyl migration is a prerequisite for randomization, we assume that the overall extent of acyl migration was also of the same order of magnitude.

The fatty acid composition of the partially hydrogenated products from 1,2,3-tri-(Z)-9-octadecenoylglycerol and 1,2,3-tri-(Z)-13-docosenoylglycerol and of the 2-acylglycerols derived therefrom by lipase hydrolysis is given in tables 2 and 3. Apparently, even after 60 min much less double bonds were reduced in each of these triacylglycerols, compared to partially hydrogenated olive oil (table 1). We assume, therefore, that acyl migration between 2- and 1,3-positions, if any, occurred to an extent less than 15% during the hydrogenation of 1,2,3-tri(Z)-9-octadecenoylglycerol and 1,2,3-tri-(Z)-13-docosenoylglycerol.

The results given in tables 2 and 3 show a higher concentration of saturated fatty acids in the triacylglycerols than in the 2-acylglycerols derived therefrom by lipase hydrolysis. Moreover, the concentration of (E)-isomers is significantly lower

Table 2
Fatty acid composition (%) of partially hydrogenated products derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol

	Acyl moieties *			
	18:0	18:1 isomers		
		(Z) + (E)	(Z) **	(E) **
15 min hydrogenation				
1,2,3-Triacylglycerol	3.7	96.3	95.1	4.9
2-Acylglycerol	3.2	96.8	93.0	7.0
60 min hydrogenation				
1,2,3-Triacylglycerol	4.2	95.8	94.5	5.5
2-Acylglycerol	2.8	97.2	92.6	7.4

* Number of carbon atoms : number of double bonds.
** % total 18:1.

Table 3
Fatty acid composition (%) of partially hydrogenated products derived from 1,2,3-tri-(Z)-13-docosenoylglycerol

	Acyl moieties *			
	22: 0	22:1 isomers		
		(Z) + (E)	(Z) **	(E) **
15 min hydrogenation				
1,2,3-Triacylglycerol	4.4	95.6	88.2	11.8
2-Acylglycerol	3.1	96.9	84.2	15.8
60 min hydrogenation				
1,2,3-Triacylglycerol	6.6	93.2	82.9	17.1
2-Acylglycerol	3.5	96.5	75.7	24.3

* Number of carbon atoms : number of double bonds.
** % total 22:1.

in the triacylglycerols compared to that in the corresponding 2-acylglycerols. It is thus evident that the rate of hydrogenation is lower and the rate of geometrical isomerization is higher in the 2-position of triacylglycerols than in the 1,3-positions.

It is interesting to note that at nearly the same level of reduction, 4.4% and 4.2% saturates, respectively, the content of (E)-isomers in the partially hydrogenated product from 1,2,3-tri-(Z)-13-docosenoylglycerol as well as in the 2-acylglycerols derived therefrom by lipase hydrolysis was more than twice as high as in the corresponding products obtained from 1,2,3-tri-(Z)-9-octadecenoylglycerol.

The composition of (Z)-and (E)-isomers in partially hydrogenated products derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol and 1,2,3-tri-(Z)-13-docosenoylglycerol, which was determined by reductive ozonolysis and gas chromatography, is given in tables 4 and 5, respectively.

The results given in table 4 show that in the partially hydrogenated products derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol after 15 and 60 min, corresponding to 3.7 and 4.2% reduction, respectively, the proportion of the (Z)-9-isomer in the triacylglycerols is almost as high as in the starting material, whereas the content of this isomer in the 2-acylglycerols, obtained by lipase hydrolysis, is considerably lower. It is therefore evident that the acyl moieties in 1,3-positions of the triacylglycerols are almost exclusively comprised of (Z)-9-octadecenoate, whereas some scattering of double bonds occurred predominantly in the 2-position.

In contrast, the double bonds in the (E)-isomers derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol are widely scattered; however, there is no significant difference in the pattern of distribution of (E)-isomers in the 2- and 1,3-positions of the triacylglycerols. It is interesting to note that, in the acyl moieties, both in 2- and 1,3-positions of triacylglycerols the migration of double bonds is preferentially

Table 4
Composition (%) of (Z)- and (E)-isomers in partially hydrogenated products derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol

		Position of double bonds (Δ)													
		2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 min hydrogenation															
1,2,3-Triacylglycerol	(Z)		0.1	tr	tr	0.3	0.2	1.8	96.5	0.1	0.5	tr	tr	tr	tr
	(E)		1.0	0.9	0.4	1.7	2.2	27.8	55.3	2.1	7.7	0.4	tr	0.4	
2-Acylglycerol	(Z)	2.6	2.2	1.3	3.2	0.9	0.5	3.7	82.5	1.6	0.1	1.0	0.2	0.2	0.1
	(E)	1.4	3.7	3.7	0.9	6.0	1.6	18.9	55.6	2.3	2.8	1.1	1.5	0.6	tr
60 min hydrogenation															
1,2,3-Triacylglycerol	(Z)				tr	tr	tr	1.8	97.8	0.2	0.2	tr	tr		
	(E)		1.3	1.0	1.1	1.6	2.8	15.0	63.2	3.1	8.7	0.4	0.8	0.4	0.8
2-Acylglycerol	(Z)		1.1	0.7	0.1	5.7	1.2	9.0	81.6	0.8	tr	tr			
	(E)	0.8	2.5	1.9	0.8	4.8	1.8	24.4	59.5	2.1	1.2	tr	tr	tr	tr

Table 5
Composition (%) of (Z)- and (E)-isomers in partially hydrogenated products derived from 1,2,3-tri-(Z)-13-docosenoylglycerol

		Position of double bonds (Δ)											
		6	7	8	9	10	11	12	13	14	15	16	17
15 min hydrogenation													
1,2,3-Triacylglycerol	(Z)	0.1	0.3	tr	tr	0.1	0.4	0.9	97.2	0.2	0.6	0.1	
	(E)	0.1	0.2	0.7	0.6	0.7	0.9	15.5	59.5	18.5	2.4	0.9	
2-Acylglycerol	(Z)	0.1	0.2	0.1	0.1	0.2	0.1	1.5	93.4	0.7	3.4	0.3	tr
	(E)	0.4	0.8	0.7	0.6	1.0	2.1	19.2	51.7	17.2	4.0	1.3	0.5
60 min hydrogenation													
1,2,3-Triacylglycerol	(Z)	0.1	0.1	0.1	0.1	0.1	0.2	1.6	93.0	0.9	3.7	0.1	0.1
	(E)	0.1	0.2	0.3	0.4	0.6	1.6	15.8	54.3	18.4	5.9	1.8	0.6
2-Acylglycerol	(Z)	0.1	0.2	0.1	0.2	0.2	0.1	1.2	93.7	0.6	3.4	0.1	0.1
	(E)	0.2	0.8	0.6	0.6	0.9	1.9	18.8	54.4	16.8	3.0	1.5	0.5

directed towards the 8-position rather than the 10- position.

The results given in table 5 show that in the partially hydrogenated product derived from 1,2,3-tri-(Z)-13-docosenoylglycerol after 15 min, corresponding to 4.4% reduction, the proportion of (Z)-13-isomer in the triacylglycerols is high, whereas the proportion of this isomer in the 2-acylglycerol obtained by lipase hydrolysis, is slightly lower. However, in the product obtained after 60 min of hydrogenation, corresponding to 6.6% reduction, such differences are not found. It seems that in the partial hydrogenation of 1,2,3-tri-(Z)-13-docosenoylglycerol there are no pronounced differences in the scattering of double bonds in the (Z)-isomers, regardless of their position in the triacylglycerols.

The (E)-isomers in the triacylglycerols derived from 1,2,3-tri-(Z)-13-docosenoylglycerol have the double bonds as widely scattered as in the corresponding products derived from 1,2,3-tri-(Z)-9-octadecenoylglycerol. Again, there is no significant difference in the pattern of distribution of (E)-isomers in the 2- and 1,3-positions of the triacylglycerols. In contrast to the partial hydrogenation of 1,2,3-tri-(Z)-9-octadecenoylglycerol, in which the double bonds preferentially migrated into the vicinal position towards the methyl end, in the hydrogenation of 1,2,3-tri-(Z)-13-docosenoylglycerol, the neighbouring positions of the original double bond are occupied to an almost equal extent.

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