

Molecular analysis of the β -polymorphic form of trielaidin: crystal structure at low temperature

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This work reports on the structure of trielaidin [EEE, 1,2,3-tri(*trans*-9-octadecenyl)glycerol], a *trans* unsaturated triglyceride present in many refined fatty materials (margarines, chocolate products *etc.*). Firstly, the polymorphism, *i.e.* the existence of different crystalline forms at various temperature ranges, was defined. Secondly, the crystal growth was examined. By developing a particular growing system, monocrystals of the most stable polymorphic form, *i.e.* the β -form, were obtained. To reduce thermal vibrations the X-ray data were collected at low temperature (173 K) and the structure was solved using direct methods. The structure was then analyzed in terms of conformation and crystal packing and compared with those of the other known triglycerides.

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1. Introduction

The complex behavior of natural fats is essentially due to the one particular physico-chemical property, *i.e.* polymorphism – of triglycerides, their main components. Triglycerides may indeed exist in various polymorphic forms, according to different crystal packing. Furthermore, the presence of *cis* and *trans* types of unsaturation influences the existence and stability of the polymorphic forms.

It is thus essential to have a thorough knowledge of the three-dimensional structure and molecular organization of such forms. In this context, single-crystal X-ray diffraction is the most suitable technique to reach the structure at a molecular level.

Some structural information, from single-crystal X-ray diffraction studies, is available. Until now, only four triglyceridic structures have been solved: tricaprïn (Jensen & Mabis, 1963, 1966), trilaurin (Vand, 1951; Larsson, 1964; Gibon *et al.*, 1984), 2–11-bromoundecanoyl 1–1'-dicaprïn (Doyné & Gordon, 1968) and a mixed compound, 1–2-dipalmitoyl-3-acetyl-glycerol (Goto *et al.*, 1992; Table 1).

For the moment, only three-dimensional information is available for the most stable polymorphic form (*i.e.* β - or β' -forms). To obtain more details on metastable forms, and particularly the α -form in which hydrocarbon chains are oscillating, one has to appeal to other experimental techniques, such as differential scanning calorimetry, X-ray powder diffraction and NMR spectroscopy.

2. Data collection

2.1. Thermal measurements

The recording of the differential scanning calorimetric (DSC) curve was performed on a Perkin Elmer DSC-7,

Table 1
Crystallographic data of triglyceridic structures.

	System	Space group	Z	Parameters						R
				a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)	
β -Tricaprin (CCC)	a	$P\bar{1}$	2	12.18	26.93	5.48	87.3	79.3	85.3	0.05
β -Trilaurin (LLL)	a	$P\bar{1}$	2	12.40	31.60	5.52	90.5	101.9	96.2	0.20
2-11-Bromoundecanoyl-1-1'-dicaprin	a	$P\bar{1}$	2	12.35	29.30	5.51	96	102	96	0.19
LML†	M	C_2	8	22.9	66.8	5.7	90	90	91	
β -1,2-Dipalmitoyl-3-acetyl-glycerol (PP2)	M	$P2_1$	2	8.286	42.960	5.375	90.00	93.30	90.00	0.11
β -Trielaidin (EEE)										
Room temperature	a	$P\bar{1}$	2	12.092	45.705	5.446	89.79	100.47	102.09	
173 K				11.665	44.933	5.432	87.03	100.17	89.09	0.11

† Non-solved structure.

assisted by an IBM PS/2 computer and coupled to a TAC 7/3 control instrument (Perkin Elmer). An intracooler allowed measurements at temperatures lower than 273 K. Samples of 3–5 mg were sealed into aluminium pans; a similar empty pan served as a reference.

2.2. Crystallographic measurements

X-ray powder diffraction patterns were recorded on a Philips PW1710 diffractometer (Cu tube, $\lambda = 1.54178 \text{ \AA}$) composed of a camera, equipped with a thermostization unit (HUBER HS-60, TTK-ANTON PAAR) and a temperature control system (Pt 100 probe) connected to a heating device (293 to 573 K) and to a cooling unit, allowing a minimum temperature of 233 K. Nitrogen gas prevented the condensation of water during measurements at low temperatures. The diffractometer was coupled to a Digital Micro-Vax II system.

Single-crystal X-ray data were collected on an Enraf-Nonius CAD-4 diffractometer. Full experimental details are given in Table 2.¹

3. Experimental

3.1. Polymorphism

To follow the polymorphic evolution, trielaidin was first submitted to a dynamic process: melting at 20 K above the melting point, quenching at 233 K and heating again at a constant rate (5 K min⁻¹ for the thermal recording).

By differential scanning calorimetry (DSC), the thermal profile of trielaidin has been recorded. The DSC curve allows the detection of two types of transformation: fusion, which can be visualized by the presence of an ascending peak, and recrystallization, represented by a descending peak. For trielaidin, two melting points and one recrystallization peak have been observed (Fig. 1a). X-ray powder diffraction at different temperatures, measuring the short spacings (from 15 to 30° in 2θ) or short reticular distances, allows the identification of the different polymorphic forms: the α -form spectrum presents

¹Supplementary data for this paper are available from the IUCr electronic archives (Reference: GS0001). Services for accessing these data are described at the back of the journal.

only one peak at 4.1 Å and the β -form shows three peaks at 4.6, 3.9 and 3.6 Å (Fig. 1b). At lower temperature (233 K), the so-called sub- α form (Chapman, 1960) was also detected. The measurement of long spacings (from 1 to 10° in 2θ) or great reticular distances has allowed the prediction of the longitudinal packing in a double hydrocarbon chain length or L-2.

The polymorphism of trielaidin may thus be summarized as follows: sub- α -2 \Leftrightarrow α -2 \rightarrow β -2. This triglyceride is stable in the β -form (appearing from 289 to 314 K) but can

crystallize, at lower temperatures, into two other polymorphic forms: from 233 to 243 K the sub- α is observed and from 243 to 287 K the α -form is present.

From solvent crystallization, the DSC curve only shows one peak, corresponding to the fusion of the most stable crystalline form: the β -form.

3.2. Obtention of monocrystals

According to the literature, numerous attempts have already been made to obtain monocrystals, leading at best to small twins. Many authors have thus been limited to a morphological study (Okada, 1964; Bennema *et al.*, 1992) or,

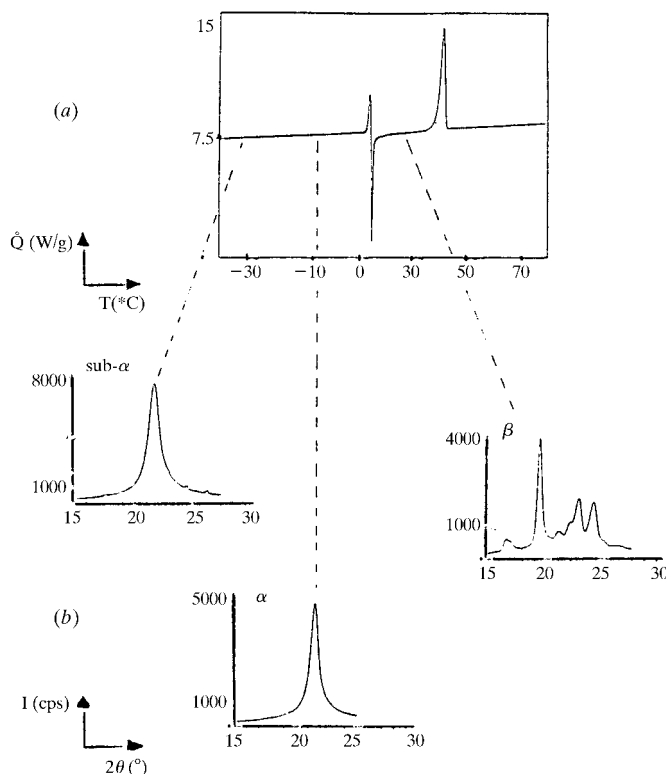


Figure 1
(a) DSC curve and (b) X-ray powder diffraction spectra (short spacings) of trielaidin.

Table 2
Experimental details.

Crystal data	
Chemical formula	C ₅₇ H ₁₀₄ O ₆
Chemical formula weight	885.4
Crystallization solvent	Methanol/chloroform + toluene
Cell setting	Triclinic
Space group	<i>P</i> $\bar{1}$
<i>a</i> (Å)	11.6650 (12)
<i>b</i> (Å)	44.933 (5)
<i>c</i> (Å)	5.432 (6)
α (°)	87.034 (10)
β (°)	100.17 (9)
γ (°)	89.09 (9)
<i>V</i> (Å ³)	2798 (3)
<i>Z</i>	2
<i>D_x</i> (Mg m ⁻³)	1.051
Radiation type	Cu <i>K</i> α
Wavelength (Å)	1.54178
No. of reflections for cell parameters	25
θ range (°)	15–27
μ (mm ⁻¹)	0.502
Temperature (K)	173 (2)
Crystal form	Prism
Crystal size (mm)	0.3 × 0.15 × 0.05
Crystal colour	Colourless
Data collection	
Diffractometer	Enraf–Nonius CAD-4
Data collection method	$\theta/2\theta$ scans
Absorption correction	None
No. of measured reflections	10 414
No. of independent reflections	9523
No. of observed reflections	6467
Criterion for observed reflections	$I > 2\sigma(I)$
<i>R</i> _{int}	0.0340
θ_{\max} (°)	65.04
Range of <i>h, k, l</i>	–13 → <i>h</i> → 13 –52 → <i>k</i> → 52 –3 → <i>l</i> → 6
No. of standard reflections	3
Refinement	
Refinement on	<i>F</i> ²
$R[F^2 > 2\sigma(F^2)]$	0.1116
$wR(F^2)$	0.2009
<i>S</i>	1.197
No. of reflections used in refinement	9523
No. of parameters used	569
H-atom treatment	H atoms riding
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0460P)^2 + 0.5807P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{\max}$	0.03
$\Delta\rho_{\max}$ (e Å ⁻³)	0.273
$\Delta\rho_{\min}$ (e Å ⁻³)	–0.212
Extinction method	SHELXL97 (Sheldrick, 1997)
Extinction coefficient	0.00010 (5)
Source of atomic scattering factors	<i>International Tables for Crystallography</i> (1992, Vol. C, Tables 4.2.6.8 and 6.1.1.4)
Computer programs	
Structure solution	SIR88 (Cascarano <i>et al.</i> , 1988)
Structure refinement	SHELXL97 (Sheldrick, 1997)

in some cases, only to the determination of the space group and cell parameters (Birker *et al.*, 1991).

In the case of trielaidin, the ‘classical’ solvent evaporation (Sato & Suzuki, 1986) has given only small twins. For this

reason, we have focused on more specific crystallization techniques, so-called ‘melt cooling’ (Sato *et al.*, 1989), ‘melt-mediated transformation’ (Koyano *et al.*, 1989, 1991) and ‘solution-mediated transformation’ (Arishima & Sato, 1989). The first two crystallization modes occur from the melted state, by cooling at a controlled rate; the third mode involves growing, from a saturated solution, the most stable polymorphic form.

This latter method, which is being extensively developed for *cis* unsaturated triglycerides (Sato *et al.*, 1989; Koyano *et al.*, 1989, 1991), has been applied to trielaidin. Trielaidin was first dissolved in a minimum of solvent (methanol/chloroform mixture) and this saturated solution was subjected to a well defined cooling (from 313 to 295 K at 0.5 K h⁻¹). Small crystals have been obtained which have been redissolved in a mixture of methanol/chloroform/toluene and resubmitted to the same programmed cooling, as mentioned above. Solutions were then kept at constant temperature (277 K) for 1 week, then taken back to room temperature. After 2 weeks, corresponding to the evaporation of the solvent, small crystals of trielaidin were obtained.

3.3. Resolution of the structure

X-ray data of trielaidin were first collected at room temperature (295 K). Following some disorder problems in the glycerol region during the tentative resolution, a new set of data was collected at lower temperature (173 K) to reduce thermal agitation. By comparison of the parameters at 295 and 173 K (Table 1), we note that the lattice has contracted.

The structure was solved by direct methods, using the SIR88 program (Burlzaff & Hountas, 1982; Cascarano *et al.*, 1988). The programs NRCVAX (Gabe *et al.*, 1989; *International Tables for X-ray Crystallography*, 1992, Vol. IV) and

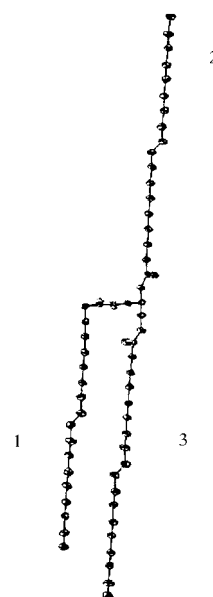


Figure 2

Conformation of the β -trielaidin (each atom represented by its thermal vibrational ellipsoid at 50% probability).

Table 3

 Values of the torsional angles ($^{\circ}$) for the glycerol region of CCC, LLL, EEE and PP2.

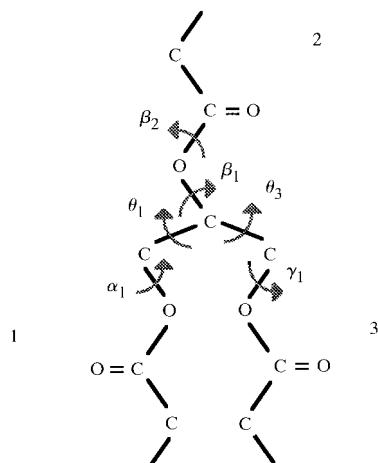
	CCC	LLL	EEE	PP2
θ_1	-58	-57	-56.3 (3)	-55
θ_3	-50	-50	-47.8 (3)	-168
β_1	83	80	84.9 (4)	-103
α_1	154	152	148.0 (5)	-87
γ_1	-172	-172	-174.6 (5)	-126
β_2	163	-180	-179.1 (5)	-175

SHELXL97 (Sheldrick, 1997) were used for the refinement of the structure. The initial model was refined by least-squares techniques. The positions of the H atoms were calculated by the *NRCVAX* program and refined using the riding model method included in *SHELXL96*. Full experimental details are given in Table 2.

4. Results

As with β -tricaprin (CCC) and β -trilaurin (LLL), β -trielaidin (EEE) adopts a chair conformation, with a maximum stretching for chains 2 and 3. Chain 1 is folded at almost 90° with respect to chains 2 and 3 (Fig. 2). This type of conformation, also called a '1-3' conformation (because chains 1 and 3 are parallel to each other), is found for all the known triglyceridic structures, except for the 1-2 dipalmitoyl-3-acetyl-glycerol (PP2), which shows a '1-2' conformation, typical of some glycerophospholipids (Small, 1986). The values of the torsional angles for the glycerol region of EEE are also similar to those of CCC and LLL (Fig. 3 and Table 3).

For the hydrocarbon chains, the conformation is similar to a saturated chain, *i.e.* all *trans*. The double bonds present a 'skew-*trans*-skew' configuration (torsional angles: 120 , 180 and -120°), which can be compared to the 'skew-*cis*-skew' configuration observed around the double bond of the oleic chain of 1-3 distearoyl 2-oleyl-glycerol (Yano *et al.*, 1993).


Figure 3

Numbering for the torsional angles in the glycerol region (Sundaralingam, 1972).

Table 4

 Mean distances (\AA) calculated between one mean plane and one atom belonging to the other parallel chain.

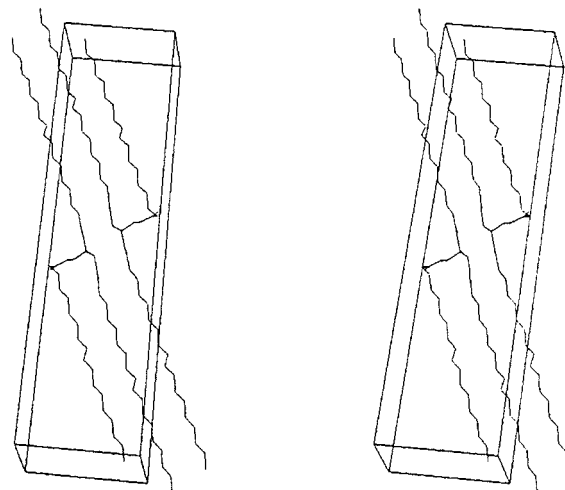
	Distances
Chain 3-Plane 1	3.90 (5)
	3.70 (5)
	3.80 (5)
Chain 1-Plane 3	3.80 (5)
	3.60 (5)
	4.40 (5)

The three hydrocarbon chains are set in parallel zigzag planes from which the C—C bonds are alternately parallel and oblique with regards to the *b* axis (Fig. 4). Along the saturated chain portions, the bond lengths are oscillating between 1.49 and 1.54 \AA . This observation could be related to the alternation of the C—C bond lengths found in the structure of β -tricaprin (Jensen & Mabis, 1966) and β -trilaurin (Gibon *et al.*, 1984), and attributed to the preferential vibrations in different directions or electronic perturbations owing to the presence of the glycerol moiety (de Pryck, 1984; Gibon *et al.*, 1984). Further analysis would be necessary to explain these variations.

The cohesion of the crystal lattice is essentially due to the van der Waals forces between all parallel hydrocarbon chains. To correlate with X-ray powder diffraction, distances were calculated between one mean plane containing one chain (1 or 3) and atoms belonging to the other parallel chain (3 or 1) (Table 4). These mean values correspond well to the values of short spacings (or short reticular distances) which are characteristics of the β -form: 4.60 (5), 3.90 (5) and 3.60 (5) \AA .

5. Conclusions

By the analysis of various crystallization methods, we first focused on a particular crystallization system that provided monocrystals of β -trielaidin.


Figure 4

Stereoscopic view of the crystal packing of trielaidin.

To reduce thermal agitation, single-crystal X-ray data were collected at low temperature (173 K) and the structure was then solved using direct methods.

The β -form of trielaidin presents a chair conformation, the '1-3' conformation being similar to those of other β -monoacid triglycerides (tricaprin and trilaurin). The hydrocarbon chains adopt an all-*trans* geometry, like a saturated chain, except around the double bond which shows a 'skew-*trans*-skew' configuration (comparable to a *cis* unsaturated chain). Interatomic distances between the hydrocarbon chains are comparable with the experimental short spacings obtained by X-ray powder diffraction for the β -form of triglycerides.

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