Crystallographic Study of Tritetracosanoin

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ABSTRACT

Crystallographic examination and computerized structural analysis of the X-ray powder diagram of tritetracosanoin shows the molecule to be isomorphous with saturated triglyceride structures previously established. Cell dimensions are: a = 13.1, b = 5.30, c = 58.9 Å; $\alpha = 94$, $\beta = 98$, $\gamma = 99°$. Space group is $P\bar{1}$. The molecule is turned relative to the unit cell a-axis likely because of acyl chain packing and end group plane interactions. Results indicate that the glycerol unit largely determines the lateral packing of saturated even membered triglycerides. The method of structure solving indicates that saturated triglycerides can be studied by computerized structural analysis.

INTRODUCTION

The solid state phase behavior of long chain triglycerides is not understood yet. Only in the past few years has general agreement been reached on the characterization of the polymorphic crystalline forms of monoacid saturated triglycerides (1-3). However, controversy still prevails regarding the number and nature of intermediate β'-form crystalline phases of even membered saturated triglycerides and their relation to the stable β-form (3,4). Tritetracosanoin (trilignocerin) was investigated to determine the structural conformation of the molecule, and to contribute additional information to the solid state behavior of saturated long chain triglycerides. The additional information will aid simulated molecular model approaches. Analogous compounds, such as saturated long chain hydrocarbons, already have been successfully studied by computerized structural analysis (5,6).

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EXPERIMENTAL PROCEDURES

Tritetracosanoin was purchased as a white crystalline powder from Nu Chek Prep, Elysian, Minnesota. Purity of the compound was 99% as determined by TLC and by gas chromatography (GC) of its methyl esters. Differential scanning calorimetry confirmed the purity of the β-form. The melting point was 86 C. Density of the compound as determined by flotation was 0.96 ± 0.01 g/cm^{-3}. Debye-Scherrer X-ray diffraction film data were gathered with a cylindrical powder camera (radius = 57.296 mm) equipped with Ni filtered Cu radiation (λ = 1.5405 Å). Aluminum powder was used for calibration. Low angle diffraction data were taken with a Warhus camera at a sample to film distance of 29 cm using Ni filtered Cu radiation. The low angle data were not calibrated. Reflection intensitites were visually estimated, and the film spacings were read on an illuminated film measuring device. Computer calculations

TABLE I

X-Ray Powder Diagram Data for Tritetracosanoin

Spacing (Å)	Intensity		Unit cell index (calculated spacing)
	Observed[a]	Calculated	
57.8	(VS)[b]	>100	001(58.1)
28.9	(VW)[b]	---	002(29.0)
19.5	(M)[b]	>100	003(19.4)
13.1	EW	---	-101(12.9), 100(12.8)
11.7	M	46	101(12.1), 005(11.6)
8.37	EW	6	007(8.30)
7.32	W	3	008(7.26)
5.86	W	24	203(5.81), 0010(5.81)
5.33	M	17	0-11(5.23), 0-12(5.21)
5.08	VW	14	-111(5.10), 0-14(5.06)
4.93	VW	10	1-13(5.00), 013(4.92)
4.77	VW	2	014(4.77)
4.57	ES	59	11-1(4.60), 11-3(4.59), 110(4.57), 11-4(4.54)
4.36[c]	VW	24	1-17(4.43), 11-5(4.46), 2-11(4.40), -302(4.31)
4.13[c]	VW	17	-306(4.14), 2-15(4.10), 2-14(4.20)
3.97	M	100	304(3.93), 11-9(3.99)
3.86	VS	20	2-17(3.86)
3.66	S	11	2-19(3.60)
3.47	WM	1	308(3.45)
3.36	VW	2	3-15(3.39)
3.26	VW		NA[d]
2.57	WM		NA
2.34[c]	VW		NA
2.26	WM		NA
2.21	EW		NA
2.15	VW		NA
2.11	EW		NA
2.06[c]	W		NA
1.98	VW		NA
1.93	EW		NA

[a]Intensity scale: S = strong; M = medium; W = weak; WM = weak to medium; ES = extremely strong; EW = extremely weak; VS = very strong; VW = very weak.

[b]Low angle diffraction data.

[c]Wide reflection.

[d]NA = not assigned. These diffraction lines could not be confidently assigned due to the large number of possible reflections.

TABLE II

Saturated Long Chain Triglyceride Cell Dimensions

Dimension	β-Tricaprin[a]	β-Trilaurin[b]	β-Tritetracosanoin
a(Å)	12.176	12.35	13.1
b	5.488	5.44	5.3
c	26.93	31.75	58.9
α(°)	94.65	94.0	94.
β	92.73	96.7	98.
γ	100.72	99.2	99.

[a]See Ref. 7.
[b]See Ref. 8.

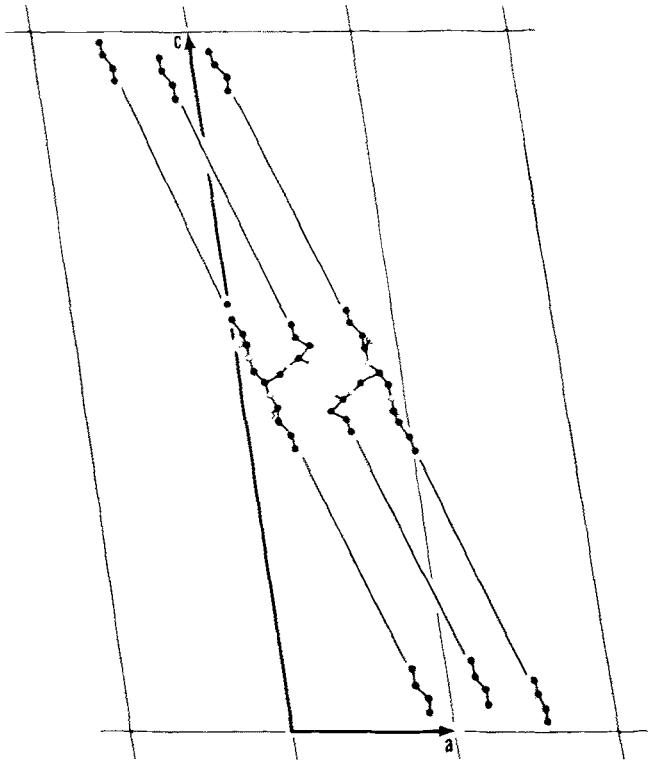

FIG. 1. Projection of the tritetracosanoin molecule and its symmetry related equivalent along the b-axis. • = carbon; ○ = oxygen; a = a-axis; c = c-axis.

were performed on an IBM 1130 (Table I). Crystal structure data are: Unit cell = triclinic, $P\bar{1}$; molecules per unit cell = 2; a = 13.1 ± 0.1 Å; b = 5.30 ± 0.01; c = 58.9 ± 0.3; α = 94 ± 1°; β = 98 ± 2; γ = 99 ± 2; molecular formula = $C_{75}O_6H_{146}$; mol wt = 1144; calculated density = 0.95 g/cc.

RESULTS AND DISCUSSION

Examination of the X-ray powder pattern of tritetracosanoin led to the following conclusions. Comparison of the powder diagram to the linear triglyceride structures previously studied of β-tricaprin (7) and β-trilaurin (8) indicated that the molecule is a linear β-form triglyceride. In addition, major reflections were present at 5.33 (Medium [M]), 4.57 (Very Strong [VS]), 3.86 (VS) and 3.66 Å (Strong [S]). Lutton and Fehl reported (3) that even membered β-form linear triglycerides having acyl chain lengths C_8-C_{22} show similarity in the following short spacings: 5.24 (M), 4.61 (VS), 3.84 (S) and 3.68 Å (S). Both α- and β'-forms exhibit significantly different patterns. The measured crystallographic long spacing is 58.2 Å. The value predicted for tritetracosanoin according to Vand and Bell's (9) linear law (d = pn+q, for the expected calculated long spacing of saturated even membered triglycerides, where n is the number of atoms in the chain) is 58.8 ± 0.5 Å. They gave p = 2.29 ± 0.013 and q = 3.82 ± 0.18 as constants derived from least squares analysis of previous triglyceride results. Lastly, the melting point of 86 C is close to the predicted value of 85.2 C by Lutton and Fehl's (3) equation for even membered β-form saturated triglycerides: (140 - mp) = 2.43894 - 0.0488017 n + 0.00081758 n^2, where n is the number of atoms in the chain.

Indexing of the powder diagram was not practical by Ito's method (10). The multitude of observable reflection possibilities due to the large c-dimension inhibited proper indexing of reflections.

The powder diagram was solved by matching observed reflection intensities to a set of calculated intensities. These intensity values originated from an almost completely constructed triglyceride structure fitted to a series of trial unit cells. Intermolecular packing analysis was used to determine the optimum location of the triglyceride. The potential function was that of Coiro, et al., (11).

Several initial assumptions were considered acceptable. $P\bar{1}$ was the space group chosen because of previous diffraction similarities cited with β-tricaprin and β-trilaurin. The unit cell of β-trilaurin served as the starting point. The c-axis was extended to 58.8 Å while the angles were kept constant. Since the 001, 002, 003, 005, 007, and 008 reflections were easily indexed in this scheme, the lattice cell angles would not change much.

The constructed triglyceride was formed by taking the β-trilaurin single crystal conformation (8) and extending the acyl lengths in a manner so as not to lose the chain plane structure of the triglyceride acyl groups. Hydrogen atoms were added to the glycerol carbons and to the methylene groups on the basis of tetrahedral carbon bonding and a bond length of 0.96 Å. Almost 99% of the mol wt was accounted for by this construction.

In considering the triglyceride position within the larger unit cell, we believed that the tritetracosanoin coordinate location would differ from that of the parent β-trilaurin. Reasoning held that the molecular packing of long chain triglycerides in x-y planes mainly depended on the part of the molecule exhibiting greater lateral dimensions, i.e., the glycerol residue. In comparison, acyl groups exhibited much less lateral intermolecular interaction. Application of the packing analysis to the glycerol residue in the β-trilaurin structure supported this hypothesis.

Simultaneous with these calculations, a series of a- and b-cell dimensions were examined with subsequent calculation of intensity values for data correlation. The intensities were calculated as: $I = (F_{hkl})^2 \times (Lp)$. F_{hkl}, the hkl reflection structure factor, is the amplitude brought about by the diffraction of the proposed molecule located in the unit cell for the hkl indexed reflection. Lp is the Lorentz polarization correction to compensate for the loss in intensity due to polarization of the beam upon reflection:

$$Lp = (1 + \cos^2 2\theta)/(\sin^2 \theta \cos \theta) \quad (I)$$

The cell dimension values were constrained by the expected cell volume of 4019 Å^3 according to the equation:

$$V = N \times M \times (1.66 \times 10^{-24})/D \quad (II)$$

where N = number of molecules per unit cell, M = mol wt, D = density, and 1.66×10^{-24} the number of gm/atomic-wt unit, with the atomic wt of oxygen defined as 16. Subsequent intensity calculations led to a match of observed spacings with calculated spacings.

To improve the comparison between the observed and calculated intensity values, the tritetracosanoin molecule was rotated counterclockwise about a pole through the center glycerol carbon position and perpendicular to the a-b plane. Justification of this procedure was as follows. The a-cell dimension increases while the b- decreases with

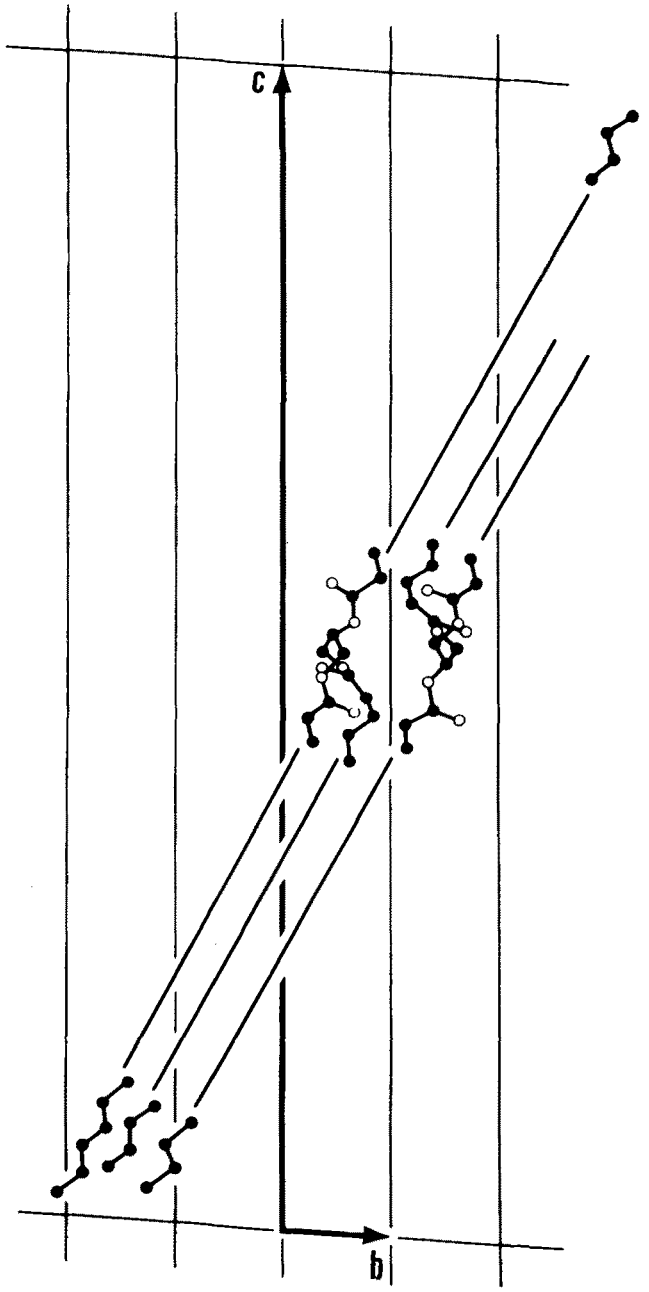

FIG. 2. Projection of the tritetracosanoin molecule along the a-axis. The symmetry related equivalent is shown in the adjacent unit cell to avoid pictorial overlap of the glycerol residues. • = carbon; ○ = oxygen; b = b-axis; c = c-axis.

increasing acyl chain length (Table II). The triglyceride molecules in both structures are tilted with respect to the a-b plane, and the projection of the triglyceride molecules on this plane is in general alignment with the a-axis. Because the resultant tritetracosanoin cell dimensions evidenced an even further a-b plane elongation, the triglyceride molecule should be expected to be placed counterclockwise with its chain vector projection on the a-b plane in closer alignment to the a-axis. Minimum packing energy calculations supported this hypothesis and gave a molecular rotation of 4.5°.

The final triglyceride cell dimensions were obtained by a least squares refinement of the indices assigned.

As in previous triglyceride structures, the tritetracosanoin molecule has a "tuning-form" conformation (Figs. 1 and 2). The acyl groups are parallel and the methyl end groups form 3-membered terraces (8,12).

Subcell dimensions were obtained graphically with the choice of the 001 subcell reflection based upon a high intensity calculated for the -1026 unit cell reflection. Indexing of subcell dimensions is given in Table III; subcell dimensions are found in Table IV.

TABLE III

Tritetracosanoin Subcell Data

Observed spacing (Å)	Subcell index (calculated spacing)
4.57	010(4.55)
4.13	110(4.13)
3.97	100(3.98)
2.57	120(2.58)
2.26	210(2.26), 101(2.25)
2.21	001(2.20)
2.06	1-11(2.07), 202(2.07)
1.98	200(1.99), 111(1.97)
1.93	0-21(1.92)

TABLE IV

Tritetracosanoin Subcell Dimensions

Tritetracosanoin (Å)	Subcell dimensions (°)
a = 4.62	α = 102
b = 5.30	β = 79
c = 2.98	γ = 65

Because of the manner in which the structure was solved, it cannot be expected that the triglyceride chain be parallel to the subcell c-axis. The angle between the two vectors is 6°. The triglyceride chain is tilted 65.8° with respect to the a-b plane. This tilt is in good agreement with the 62.7° angle of tilt found for β-trilaurin (9).

Results and methods of the tritetracosanoin investigation provide the following conclusions. Saturated β-triglycerides of chain lengths 8 to 24 can be considered to have nearly identical conformation and molecular packing. Although three examples are not sufficient to render an absolute conclusion, the three crystallographic triglyceride structures studied do exhibit the transition of unit cell base elongation with increasing chain length. Although the cause of this phenomenon is not resolvable, the method of structure solving does indicate that lateral packing in the a-b plane of saturated even membered triglycerides depends principally on the packing of the glycerol unit. This transition further indicates that elongation of unit cell dimensions must be due to a combination of acyl chain packing interactions and end group plane interactions. Glycerol residue interactions should not differ significantly between structures. Another factor leading to unit cell base elongation may be the thermal motion of the chain ends. The crystallographic thermal parameters of β-tricaprin (7) indicate a whiplike motion of the acyl chains is greatest towards the a-axis direction. Longer chain molecules should exhibit greater terminal motion. If the interpretation is correct, these molecules would increasingly adjust towards an attitude parallel to the a-c plane.

Additional single crystal studies of triglycerides are needed to understand better the solid state intermolecular interactions involved, but it is difficult to obtain long chain crystalline compounds of sufficient size to utilize X-ray single crystal methods. Therefore, the alternative is to examine the problem by computerized structural analysis. This study of tritetracosanoin demonstrates that such an analysis is both reasonable and possible. The benefit of knowledge of such phenomena would increase understanding about the physical nature of triglycerides in emulsions, solutions, and other multiphase systems.

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