Glycerol Conformation and the Crystal Structure of Lipids

II. A Further Study of Tripalmitin and Conformationally Fixed Analogs by Small-Angle X-Ray Diffraction and Reflection Electron Diffraction

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The utility of analogs to glycerol-containing lipids based on the configurational isomers of cyclopentane-1,2,3-triol for *ab initio* crystal structure analysis *via* electron diffraction is assessed further. Such analogs of tripalmitin are examined with the natural triglyceride *via* low angle X-ray diffraction. The 1,3/2 (all-*trans*) and 1,2/3 (*cis-trans*) analogs give long spacing dimensions some 23 Å greater than found for the β-2 form of the natural compound, consistent with the long spacing observed for a β-3 form. The 1,2,3/0 (all-*cis*) analog gives a long spacing near that of the α-form of the triglyceride. Reflection electron diffraction measurements on the 1,3/2 and 1,2/3 analogs reveal a chain tilt near 60° for both and untilted chains for 1,2,3/0. A more accurate tilt determination from X-ray long spacings of the homologous series of 1,3/2 pseudotrilaurin to 1,3/2 pseudotripalmitin confirm the 67° tilt expected for the β-3 form. Therefore, given the same $T_{\parallel}$ methylene subcell, the molecular packing is very close to natural triglycerides. The subtle influences of the cyclopentane ring are overcome for 1,3/2 analogs based on stearic and arachidic acids. This emphasizes the utility of these structural analogs for *ab initio* crystal structure determinations of glycerol containing lipids.

Introduction

Several recent papers [1–3] have discussed the use of three structural probes in the comparison of triglyceride crystal packing with that of three conformationally-fixed analogs based on the configurational isomers of cyclopentane-1,2,3-triol. Of the three probes, transmission electron diffraction proves to be the most efficacious for identifying the coexistent polymorphic crystalline forms of a given isomer in the bulk samples. This capability arises from the very small amount of material needed to form a single crystal transmission electron diffraction pattern and is contrasted to much larger bulk samples required to give a usable signal for powder X-ray diffraction.

It has been shown [3] that the 1,3/2 tripalmitate of cyclopentane-1,2,3-triol is organized in a crystal structure which most resembles that obtained from solution-grown tripalmitin. This isomer (Fig. 1) mimics the "tuning-fork" conformation found in the X-ray crystal structures of even-chain triyglycerides [4–9] more closely than those from the 1,2/3 and 1,2,3/0 isomers. However, the agreement is not exact. Evidence of a superlattice structure was found for the 1,3/2 cyclopentanoid triglyceride analog [3] giving a near doubling of the d_{100} spacing (21.9 Å *vs* 11.8 Å found for tripalmitin). The phenomenon is thought to be a perturbation to the overall molecular packing due to the ethylene moiety on the cyclopentane ring.

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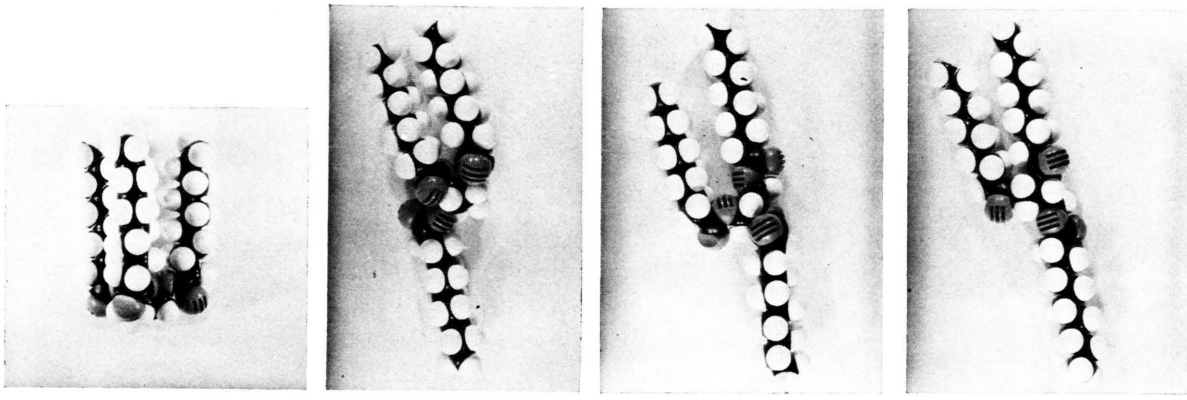

(a) (b) (c) (d)

Fig. 1. CPK molecular models of cyclopentane-1,2,3-triol analogs of tripalmitin in comparison to tripalmitin (a) 1,2,3/0; (b) 1,2/3; (c) 1,3/2; (d) tripalmitin.

Thus, further experiments are required to ascertain the degree of validity of the analogy in the solid state between the natural triglycerides and the synthetic lipids. In lieu of a complete X-ray crystal structure determination, an experiment which must await the growth of suitable large single crystals, we have done low angle X-ray diffraction and reflection electron diffraction experiments to procure more information about crystal packing in projections normal to the long acyl chain axes. Since both techniques sample larger areas than transmission electron diffraction, interpretation is made subject to the known coexistence of polymorphic forms [3].

Experimental

Sample preparation

Cyclopentane-1,2,3-triol analogs of tripalmitin and other homologous triglycerides from C_{12} to C_{20} were prepared and purified as described in an earlier paper [1]. Natural tripalmitin (99%) was purchased for this study from Sigma Chemical Co., St. Louis, Missouri, and was stored desiccated at 0 °C before use. Tripalmitin and its analogs were used, as obtained from purification procedures in the synthesis, for the powder X-ray diffraction studies. Microcrystalline bulk samples were packed in thin-walled glass capillaries. For reflection electron diffraction work, thin layers were formed on a polished brass stud by evaporation of dilute solutions in warm *n*-pentanol.

X-Ray diffraction

Powder X-ray diffraction measurements were made using a Jarrell-Ash slit-collimated Franks low angle X-ray diffraction camera. The Jarrell-Ash generator powered a microfocus X-ray tube operated at an accelerating voltage of 38 kV and a filament current of 5 mA. Incident radiation to the sample was Ni-filtered CuKα X-rays.

Reflection electron diffraction

For reflection electron diffraction experiments, an Hitachi HU-11 electron microscope equipped with an HE-1 high resolution electron diffraction holder was operated in the electron microscopy laboratory at ManLabs Inc., Cambridge, Mass. The accelerating voltage for all experiments was 75 kV. Camera length of the microscope for this configuration was calibrated with an Al° powder standard (transmission diffraction) mounted at the same position as the specimens used in the reflection experiments.

Results

Tripalmitin

Low angle X-ray diffraction patterns from tripalmitin are similar to those reported earlier. Chapman [10] gives a long spacing of 40.6 Å for the β-form of tripalmitin, in accord with our results.

Reflection electron diffraction measurements are consistent with earlier work on long paraffinic-chain

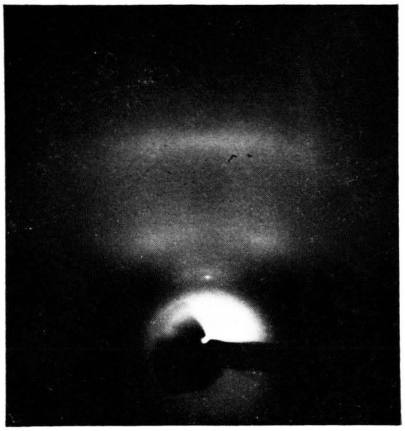

Fig. 2. Reflection electron diffraction pattern from tripalmitin layer on polished brass surface.

materials. The pattern often obtained from tripalmitin (Fig. 2) is typical of a polycrystalline tilted-chain sample for which the crystallites are randomly oriented about the surface normal [11]. Since it is possible to measure the chain inclination directly from arced bands in the diffraction pattern, we employed an analysis given by Karle and Brockway [12] using the diffraction band intercepts on the vertical y-axis of the diffraction photograph.

For all the compounds discussed here we have been able to record the first order reflection diffraction band. This spacing r_{meas} is used in the expression derived from Karle and Brockway's paper [12]

$$\psi = \cos^{-1} \frac{r_{meas.}}{r_T} . \qquad (1)$$

Here ψ represents the chain tilt away from the surface normal (the complement to the chain tilt to the methyl end plane), r_{meas} is the intercept of the first order band with the y-axis in Å and r_T is the axial C – C separation along the zig-zag chain, here taken as 2.54 Å. Since just the uppermost surface of the crystal is excited by the incident beam, only the methylene scattering is observed.

That the spacings d_{001} and $2d_{002}$ differ for tripalmitin indicates a distribution of chain tilts [12]. Equation (1) gives the average value with the spacing of the first order diffuse band *i.e.* 29° or a chain tilt to the end plane of 61°. A small spot is often seen on the y-axis near the diffuse band corresponding to a chain tilt of 53° and is thought to be due to another crystalline form of the triglyceride.

Tripalmitate of (1,3/2) cyclopentane-1,2,3-triol

Measured powder X-ray diffraction lines for the 1,3/2 all *trans* tuning-fork isomer are given in Table I. The first four lines in Table I represent order 1, 2, 3, 5 along c* of a 63.8 Å spacing. High angle spacings are consonant with those measured by Greenwald *et al.* [2] for solution grown material.

Table I. Measured powder X-ray diffraction data for triglyceride analogs.

1,3/2	1,2/3	1,2,3/0
63.8 Å (s)	66.2 Å (s)	46.8 Å (s)
31.9	33.1	15.6
21.3	22.1	11.7
12.8	13.2	7.80
8.28	7.47	7.55
8.08	5.44	7.24
6.96	5.16	6.68
5.14	4.37 (s)	6.38
5.03	4.12 (s)	6.10
4.60 (s)	3.73 (s)	5.86
4.52		5.59
4.34		4.71
4.22		4.61
4.08 (s)		4.26
3.88 (s)		4.16 (s)
3.80 (s)		3.92 (s)
		3.62 (s)

Reflection electron diffraction patterns for this triglyceride analog are similar in appearance to those obtained from tripalmitin, and are evidently from a tilted chain aggregate. Using Eqn (1) and the measured value of the first order arc at the y-axis, the derived chain tilt is 61°. A more accurate estimate of this chain tilt was found from low angle X-ray diffraction patterns from three 1,3/2 analogs of homogeneous even-chain triglycerides based on lauric acid, myristic acid and palmitic acid, all of which have long spacings approx. 1.5 times that of the natural triglyceride. A plot of long spacing *vs* number of carbon atoms in the long chains [18] reveals the tilt to be 67°.

Tripalmitate of (1,2/3) cyclopentane-1,2,3-triol

Powder X-ray diffraction lines for the *cis-trans* 1,2/3 tuning-fork isomer are given in Table I. The first four lines are orders 1, 2, 3, and 5 of a 66.2 Å spacing. High angle spacings are in agreement with those found by Greenwald *et al.* [2].

Reflection electron diffraction patterns from this

material are very faint but are very similar in appearance to Fig. 2. Using Eqn (1) and the measured y-axis intercept of the first order arc, the average chain tilt is estimated to be about 60°.

Tripalmitate of (1,2,3/0) cyclopentane-1,2,3-triol

The all *cis* 1,2,3/0 isomer gives X-ray diffraction lines as listed in Table I. The first four reflections represent orders 1, 3, 4 and 6 along c* of a 46.8 Å spacing. High angle spacings are again in agreement with previous measurement [2].

Fig. 3 shows a typical reflection electron diffraction pattern for this isomer. It is quite different in appearance from Fig. 2 and represents the surface diffraction from an untilted chain [11 – 13]. The diffuse spots on the first layer lines represent an interchain side spacing of 4.6 Å in accord with the observation of hexagonal transmission diffraction patterns from this material [3].

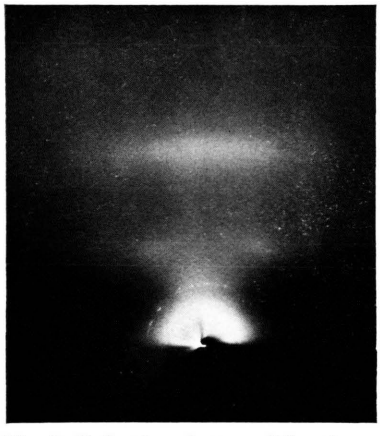

Fig. 3. Reflection electron diffraction pattern from tripalmitate of (1,2,3/0) cyclopentane-1,2,3-triol on polished brass surface.

Discussion

As discussed in an earlier paper [3], the use of analogs of glycerol-containing lipids based on the configurational isomers of cyclopentane-1,2,3-triol in studies of biological activity is hoped to give conformational information for these compounds when they encounter various physical environments. For example, the 1,3/2 isomer in the pseudo-triglyceride series best mimics the glycerol conformation in natural triglycerides crystallized from organic solvents [4 – 9], whereas the 1,2,3/0 isomer is imagined to simulate the glycerol conformation when a triglyceride encounters a polar/nonpolar interface [14].

Although our earlier transmission electron diffraction study on these compounds [3] shows that the 1,3/2-cyclopentanoid analog of tripalmitin gives a diffraction pattern most similar to the natural crystalline material, the near doubling of the d_{100} spacing of the 1,3/2 analog in relation to the hk0 diffraction pattern from tripalmitin indicates some perturbation of the packing from the cyclopentane ring. Additional low-angle X-ray information listed in Table I adduces evidence for this perturbation in that the long spacing of the 1,3/2 analog is some 1.5 times that of the natural triglyceride. On the other hand, reflection electron diffraction data indicate the chain tilts of tripalmitin and of the 1,3/2 analog to be nearly the same, and high angle X-ray diffraction data on both compounds verify the presence of the $T_{||}$ methylene subcell.

An explanation of these apparent contradictions can be found in recent X-ray diffraction studies on natural triglycerides [15 – 18]. The most com-

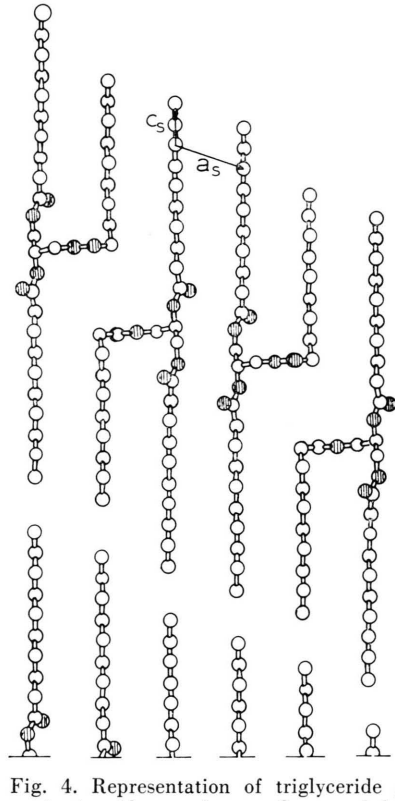

Fig. 4. Representation of triglyceride β-2 packing, (010) projection (drawn after van Soest and de Jong [17], Fig. 1). Subcell axes are indicated. In crystal, a_s is parallel to the plate surface.

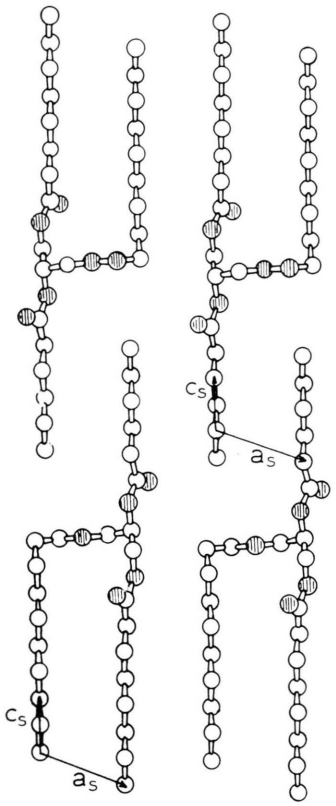

Fig. 5. Representation of triglyceride β-3, packing, (010) projection (drawn after de Jong and van Soest [15], Fig. 4). Subcell axes are indicated. In crystal, a_s is parallel to the plate surface.

monly found polymorph of homogeneous (and some mixed chain) even chain triglycerides is the β-2 * form, as characterized in the cited crystal structure analyses. The molecular packing of this form is shown in Fig. 4, given the orientation of two $T_{||}$ subcell axes with respect to the crystal structure. It should be noted that the glycerol regions of neighboring molecules pack near one another. Buchheim [19] gives 61° as the value of the chain axis tilt for this polymorph and the a_s axis of the subcell (Fig. 4) is parallel to the crystal surface. Another β-3 packing is described [15, 18] which is commonly found for certain mixed acid even chain triglycerides and is shown in Fig. 5. If q is a fatty acid chain length, then the requirement [18] for chain lengths of triglycerides in this form is $C_p C_q C_r$ where q is greater than p by at least four and $r = p$, $p+2$, $p+4$ or $p+6$. The β-3 packing again has the $T_{||}$ subcell (Fig. 5), a chain axis tilt of 67° (a_s in Fig. 5 again parallel to the crystal surface) but a unit cell length 1.5 times that of the β-2 packing. The difference in unit cell length is due to the non-proximity of glycerol groups in the β-3 packing shown in Fig. 5.

It appears that the 1,3/2 analog of tripalmitin must have a crystal packing very similar to a β-3 polymorph since the chains are in the $T_{||}$ subcell and the unit cell length is 1.5 times that of β-2 tripalmitin. Also the more accurate determination of chain tilt from low angle X-ray diffraction patterns of 1,3/2 analogs of trilaurin, trimyristin and tripalmitin is consistent with this polymorph.

Yet the analog is a compound formed from a single fatty acid. The ring perturbation may cause the β-3 packing due to the slightly greater size of cyclopentane *vs* n-propane (of glycerol) and due to chain conformational twists which would cause the methyl end plane terrace proper for this polymorph [15, 18]. It is interesting to note that the 1,3/2 cyclitol analogs of tristearin and triarachidin give X-ray long spacings in accord with the respective triglycerides, implying that the longer chain lengths may overcome perturbations from the cyclitol ring. Analogous behavior can be cited for the case of potassium soaps. In the shorter chain soaps [22] ionic forces cause anomalous chain packings whereas in long chain compounds [23] van der Waals between the hydrocarbon chains dictate the molecular packing.

When all chains are forced in the same direction, as is found with all *cis* isomer, the long spacing is more in accord with the value found for the α-form of the triglyceride [10]. (A comparison of the original model postulated for the 1,3-diglycerides [20] to the actual crystal structure [21] emphasizes that it is very difficult to guess *a priori* the chain conformation of a glycerol lipid from long spacing information alone.) The side to side packing is disturbed, as indicated by supercell spots in the hk0 electron diffraction pattern [3]. The reflection diffraction data are consistent with all other information.

The nature of the crystal packing in the 1,2/3 analog remains an enigma. Reflection diffraction indicates an average chain tilt about 60°, perhaps related to the subcell giving a doublet of 4.39 Å spacing in the (001) projection [3]. A study on a

* For polymorph notation see ref. [15–19]. The appellation β-2 corresponds to Buchheim's β_{III}, written β_3 in ref. [3].

homologous fatty acid series of the analog is in progress.

Despite the perturbation of the ring, these diffraction experiments, in conjunction with our earlier studies [3], indicate that the use of the cyclopentanoid analogs in the study of lipid crystal structure is justified, at least when all hydroxyl groups are substituted. Only the all *trans* isomer gives a crystal packing which resembles the natural crystalline even-chain triglycerides, as has been verified further in this paper.

Recent studies [22] on corresponding cyclopentanoid analogs of diglycerides assess the importance of ring perturbations to crystal packing for partially esterified molecules. In this case the final fatty acid chain directions may not be those defined by the original configuration of vicinal hydroxyl groups on the cyclitol ring. This is also indicated by a recent crystal structure of a prostaglandin [24]. Even with this restriction, the knowledge of glycerol polar group conformation, which is posited by the ring configuration [25], will be of assistance in defining the active molecular geometry when the glycerol lipid in question encounters an enzyme active site [26].

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