Influence of Molecular Conformation on the Solid State Packing of 1,2-Diglycerides

Study of 1,2-Dipalmitin and Some Structural Analogs by Electron Diffraction, X-Ray Diffraction, and Infrared Spectroscopy

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Diglycerides, Crystal Structure, Electron Diffraction, X-Ray Diffraction

Diffraction studies on natural 1,2-dipalmitin and on analogs, including those based on the configurational isomers of cyclopentane-1,2,3-triol reveal that the 1,2-diglycerides crystallize from solvent with chain methylene packing identical to the monoclinic form of even-chain alkanes. The chains probably are folded back in "hairpin" fashion as found in phospholipid crystal structures. Acyl shifts are observed to occur in the crystalline solid state at room temperature to give the 1,3-diglyceride. Analogs based on the above-mentioned cyclitols show that isomers with adjacent chains *trans* to the ring (possibly extended chain packing) or with chains *cis* to the ring ("hairpin") crystallize readily. Both possibly extended chain configurational isomers have the α-form as well as β'-forms and a β-polymorph. The hairpin isomers each give a β'-polymorph but only the all-*cis* isomer gives an α-form.

Introduction

In considering the possible conformations of glycerol lipids which have two adjacent hydroxyl groups esterified to long chain fatty acids, it is seen that the bulk and position of the "polar group" at the third hydroxyl region of the glycerol backbone might play a significant role in determining the molecular geometry. This is indicated, for example, by the only crystal structure determination of a phospholipid reported to date [1] in which the long acyl chains are forced back by the esterified ethanolamine phosphate into a "hairpin" arrangement with two polymethylene chain axes of a single molecule parallel to one another. A similar result was seen in the crystal structure determination of a tosylated 1,2-diglyceride [2] where the acyl chain conformation again is fixed by the bulk of the toluene sulfonate moiety.

If, on the other hand, the size of the polar region is greatly reduced to the bare hydroxyl group itself, other conformational possibilities may exist for the acyl chains when the molecules pack in the solid state. A crystal structure determination on a long-chain diester of ethylene glycol [3] reveals a molecular packing with extended acyl chains. Thus, at first glance, it is uncertain whether the bulk of a hydroxymethyl group on the glycol diester is enough to force a hairpin conformation in a 1,2-diglyceride when it packs in the homogeneous environment of a crystal grown from organic solvent, although such a conformation is expected at a polar/nonpolar interface.

No complete X-ray crystal structure of a 1,2-diglyceride has been published. A preliminary report of such a determination on *sn* glycerol 1,2-dipalmitate [4] suggested that the acyl chain packing of 1,2-diglycerides mimics that found in biological membranes, perhaps implying that these molecules should also assume the hairpin conformation in crystals. A similar assertion is made by Larsson [5] in his explanation of π-A diagrams of 1,2- and 1,3-diglycerides. The uncertainty in the present understanding of 1,2-diglyceride solid state packing has motivated combined electron and X-ray diffraction determinations of acyl chain conformation in crystalline glycerol 1,2-dipalmitate as well as a study of the influence of both acyl chain conformation and

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perturbation of the hydroxymethyl polar group on molecular aggregation.

Rationale for analytical methodology

It is well known that many long chain lipids of biological interest are not readily crystallized into sizes suitable for single crystal X-ray crystallographic structure analysis. This is evidenced by the report of only one phospholipid crystal structure to date [1]. The reason for this difficulty is largely due to the relative van der Waals forces which favor the side to side packing of long alkyl chains. These forces promote the rapid growth of thin (mono- or bi-) layers with long chain axes oriented nearly normal to the layer surface in contrast to the much slower lamination of these layers responsible for thickening the crystals. The energetics of this process are well characterized [6–8].

Given the ready growth of very thin lipid microcrystals from dilute organic solvents and the greatly enhanced cross-section of matter for electrons over X-rays [9], transmission electron diffraction experiments on single microcrystals are justified for the characterization of acyl chain packing in these compounds. As has been mentioned [10, 11], the presence of elastic bends and other defects in these thin laminated crystals often limit the coherent diffraction to the alkane chain packing alone. In other words, polar group scattering rarely contributes to the diffraction intensity. An unknown lipid can be subjected to a reflection electron diffraction experiment, which only probes the methylene subcell packing at the uppermost crystal surface [12], to define the degree of chain tilt to the surface normal. The goniometer stage then can be set at the determined tilt for a transmission diffraction experiment. The translation controls of the specimen stage are used to search several of the many microcrystals on the grid surface where the tilt axis corresponds to the crystallographic axis of inclination, as described before [13]. Diffraction patterns are obtained with the electron beam parallel to the chain axes and the characteristic (001) diffraction pattern of the subcell is recorded. Fortunately, there are only a few common methylene subcells [14] and thus the procedure can be used effectively to identify the acyl chain packing for these materials.

Powder X-ray diffraction is an important supplement to the electron diffraction procedure outlined above. It is apparent that the average crystalline laminates are thicker in a bulk polycrystalline sample than they are in the microcrystalline array used in the electron diffraction experiments (which are grown from dilute solution). Thus X-ray powder diffraction patterns readily give the useful "long spacing" of the prominent crystalline form of the bulk sample, as well as giving corroborative wide angle spacings typical of the prominent methylene subcell packing. This "long spacing" is related simply to the long unit cell length for the lipid. Another corroborative check of prominent subcell packing can be obtained from another bulk experiment on the material, *i. e.* infrared spectroscopy. It has been shown [15] that the splitting of a peak in the 720 cm^{-1} region, which is due to the CH_2 rocking mode, is dependent on the parallel or perpendicular arrangement of adjacent polymethylene chain planes.

In summary, electron diffraction can be used to give an *a priori* characterization of the chain tilt and methylene subcell for an unknown microcrystalline long chain lipid. Because of the local "microclimates" on an electron microscope grid surface during a crystallization process, various polymorphic forms of the material often are crystallized and can be characterized. X-Ray diffraction and infrared spectroscopy both corroborate the methylene subcell packing of only the major component in the bulk material.

Little about the conformation of long chains pendant to polar groups (*e. g.* glycerol) can be determined *a priori* using these techniques on the natural lipid alone. A desirable means of fixing the conformation of glycerol containing lipids can be realized with the use of lipid analogs based on the configurational isomers of cyclopentane-1,2,3-triol, instead of glycerol. As can be seen by consideration of Fig. 1 a, esterification or etherification of adjacent hydroxyl groups for these isomers can mimic glycerol lipid conformations where adjacent groups on the ring are in opposition to one another (extended) or where they point in the same direction (folded back or "hairpin"). If the perturbation of the extra ethylene group of the cyclopentane ring (in relation to glycerol) is small, then the analog of a lipid which gives the closest diffraction pattern to the natural material should be expected to define the polar group conformation of the unknown lipid. This has been shown to be true in a control experiment on an even-chain triglyceride and its cycitol analogs [16], where all hydroxyl groups on the rings are esterified to long chains.

Experimental

Sample preparation

Analogs of glycerol 1,2-dipalmitate and glycerol 1,3-dipalmitate based on the configurational isomers of cyclopentane-1,2,3-triol were synthesized as described earlier [17] (see Fig. 1 for nomenclature). Purity of the materials was evaluated by thin-layer chromatography [18]. Two isomers which are expected to be least susceptible to acyl migration [19], *i.e.* 1,3/2 1-OH, and 1,2/3 3-OH (Fig. 1) were found to migrate as single bands. Of the two isomers expected to undergo acyl shifts, *viz.* 1,2/3 1-OH and 1,2,3/0 1-OH, only the former was found to migrate on the TLC plate as two separate spots. The ratio of 1,2 : 1,3 isomer was approximately 85 : 15. Microcrystalline samples were prepared on carbon-Formvar covered 400 mesh electron microscopy grids by rapid evaporation of dilute solutions in *n*-hexane, anhydrous ethanol or *n*-pentanol.

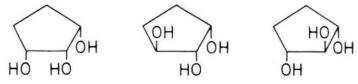

Fig. 1 a.

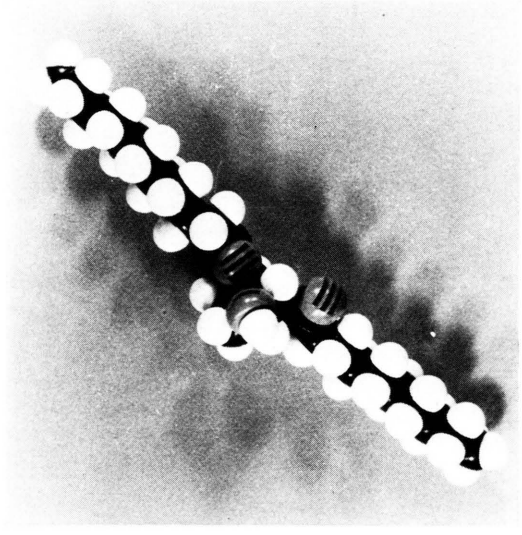

Fig. 1 b.

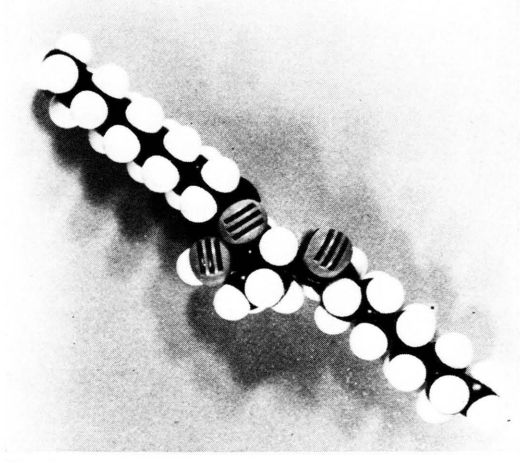

Fig. 1 c.

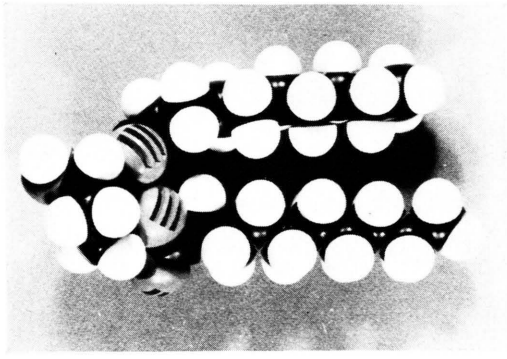

Fig. 1 d.

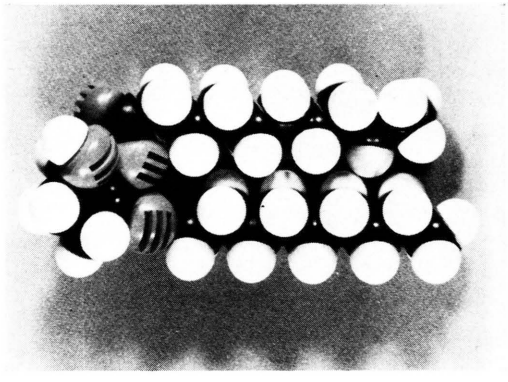

Fig. 1 e.

Fig. 1. "Pseudodiglyceride" analogs based on configurational isomers of cyclopentane-1,2,3-triol. (a) Representation of configurational isomers of the triol: 1. 1,2,3/0; 2. 1,2/3; 3. 1,3/2. CPK molecular models of pseudodiglycerides, roughly to show overall geometry (not to be confused with actual crystal structures). (b) Extended chains 1,3/2 1-OH; (c) extended chains 1,2/3 1-OH; (d) folded-back chains 1,2/3 3-OH (hairpin); (e) folded-back chains 1,2,3/0 1-OH (hairpin).

Commercially available materials were similarly prepared with solvents listed in the following tabulation:

Material	Source	Solvent
sn glycerol 1,2-dipalmitate (99% pure)	Sigma Chemical Co., St. Louis, Missouri	abs. ethanol
glycerol 1,3-dipalmitate		abs. ethanol
rac-glycerol 1,2-dipalmitate	Nutritional Biochemical Div., ICN, Cleveland, Ohio	abs. ethanol
1,2-ethylene dipalmitate	Serdary Res. Labs. Inc., London, Ontario	*n*-pentanol
1,2-propylene dipalmitate		*n*-pentanol

The stock crystalline diglycerides were stored desiccated at 0 °C.

Electron diffraction and microscopy

Most transmission experiments described here were carried out on a JEOL JEM-100U electron microscope equipped with a 30° tilt stage and operated at 100 kV, although some results on diglycerides were obtained with a Philips EM-300 electron microscope (100 kV) or a Siemens Ia electron microscope (80 kV). Diffraction experiments on the JEM-100U utilized an effective selective area of 16 μm diameter. Bright field diffraction contrast images were obtained by first isolating the transmitted central beam of the diffraction pattern with the objective aperture and then focusing the intermediate lens on the image plane of the objective lens. Usual precautions of low beam current and fast photographic emulsion were taken to minimize the radiation dose to the sample [10]. Diffraction camera lengths were calibrated against an orthorhombic paraffin spot pattern [10] or a gold powder pattern. Electron diffraction intensities were measured as areas under peaks in scans of diffraction patterns with a Joyce Loebl MkIIIC flat bed microdensitometer.

Reflection diffraction measurements were done at 75 kV on glycerol 1,3-dipalmitate using a Hitachi HU-11 electron microscope equipped with a HE-1 High Resolution Electron Diffraction Holder. The diffraction standard was aluminum.

X-Ray diffraction

Powder X-ray diffraction patterns were obtained with a Jarrell-Ash slit-collimated (single-mirror) Franks low-angle diffraction camera. Ni-filtered CuKα X-rays came from a microfocus X-ray tube operated at an accelerating voltage of 38 kV and a filament current of 5 mA. Samples were mounted as microcrystalline powders in thin-walled glass capillary tubes.

Infrared spectroscopy

Infrared spectra were obtained from KBr dispersions of the samples on a Perkin Elmer 521 recording infrared spectrophotometer.

Results

Acyl migration of 1,2-diglycerides

It is well known that 1,2-diglycerides will undergo acyl migration in the solid state to form the symmetric 1,3-isomer when the material is held some 3 °C below the melting point [20, 21]. The same phenomenon was found by us to occur at room temperature when either the optically active or racemic glycerol 1,2-dipalmitate was crystallized onto electron microscope grids and allowed to stand in a covered petri dish for several weeks.

Freshly crystallized samples of *sn* glycerol 1,2-dipalmitate gave either an hk0 electron diffraction pattern (*vide infra*) identical to those found for monoclinic paraffins [22] or waxes [23] and B-form fatty acids [13] or the hexagonal diffraction pattern characteristic of rotationally disordered (α-form) polymethylene chains [24]. In our hands, the racemic mixture gave only the hexagonal α-form, as described before [25]. After allowing the thin microcrystals of either material to stand for some weeks, a single new electron diffraction pattern was observed as shown in Fig. 2. Systematic absences ($h+k=2n+1$) indicate the plane group of the projection to be cmm (rather than pgg or p6 as before). Freshly crystallized samples of glycerol 1,3-dipalmitate give an identical diffraction pattern as revealed by the cross correlation of corresponding intensities with those from the transformed 1,2-diglyceride (Fig. 3). A solid state acyl migration at room temperature is therefore indicated.

Acyl chain packing of 1,2-diglycerides

The acyl chain packing of crystalline *sn* glycerol 1,2-dipalmitate is readily determined from electron diffraction patterns. Fig. 4 shows an (hk0) pattern from an untilted crystal of the monoclinic form. This band pattern is characteristic for compounds isostructural with the monoclinic form of even-chain *n*-paraffins [22] as shown by the comparison of observed structure factors with those obtained from

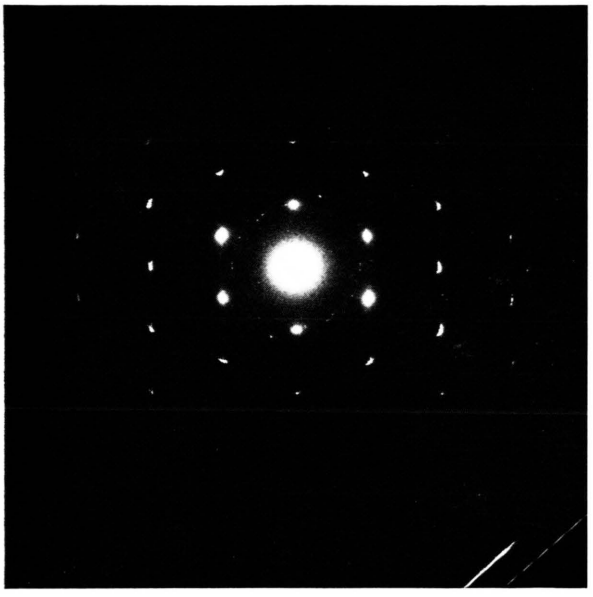

Fig. 2. Electron diffraction pattern (hk0) from transformed *sn* glycerol 1,2 dipalmitate or glycerol 1,3-dipalmitate. Spacings measured from patterns $d_{100}=4.25$ Å, $d_{010}=9.42$ Å.

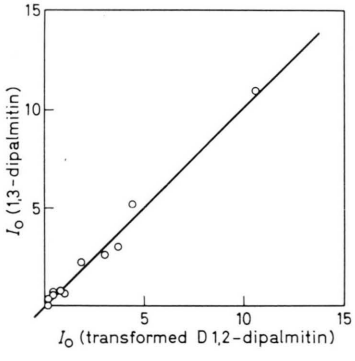

Fig. 3. Cross correlation of corresponding observed diffraction intensities for glycerol 1,3-dipalmitate and transformed *sn* glycerol 1,2-dipalmitate.

monolayer crystals of cetyl palmitate [26]. Acyl chains are therefore inclined to the methyl end plane by about 60° and pack in the $O_\perp$ methylene subcell.

Independent verification of this has been given by a single crystal X-ray structure determination of the optically active diglyceride which is being carried out. Unit cell constants are: $a=5.49$ Å, $b=7.52$ Å, $c=86.67$ Å, $\beta=93.68°$. The space group is $P2_1$. Patterson maps generated from h0l intensity data clearly indicate vectors due to the long polymethylene chains inclined to the methyl end plane by *ca* 60°. Rosen [27] also obtained similar

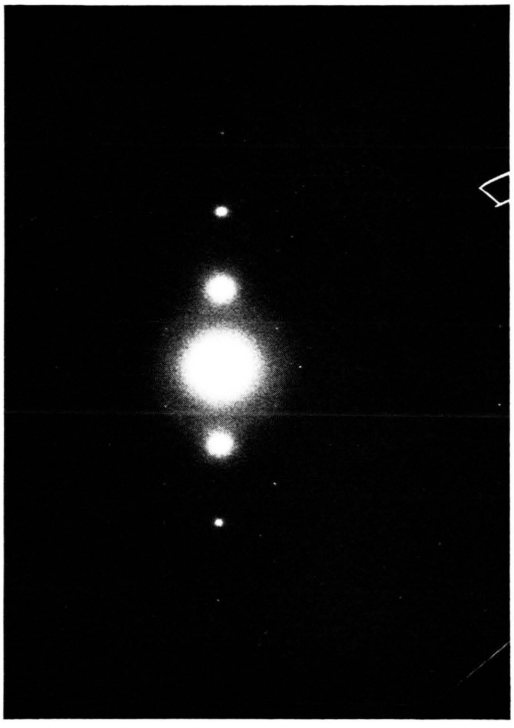

Fig. 4. Electron diffraction pattern (hk0) from untilted crystal of *sn* glycerol 1,2-dipalmitate (fresh sample). Assuming $\beta \cong 90°$, $a=5.46$ Å $b=7.56$ Å.

data from single crystals of the racemic mixture, and Albon *et al.* [28] have verified this chain packing for the optically active material.

Information related to the conformation of the acyl chains about the glycerol is seen from existing published information. Two crystal structures of heavy atom substituted 1,3-diglycerides [29, 30] reveal the chain packing to be partially extended such that the two chain axes intersect at an angle near 90°. Although neither structure is isostructural to the natural β-forms, they are thought to be similar [29]. Analogous results are also found with N-tetracosonyl phytosphingosine [31]. Regarding the acyl shift mechanism, an intermediate crystal form for 1,3-diglycerides is evidenced by crystal data from glycerol 1,3-di(15-bromopentadecanoate) which has short cell edges and methylene subcell identical to the monoclinic 1,2-diglycerides [32]. Thus, in the observed solid state process of acyl chain migration, the chains of the 1,2-diglyceride which pack in the $O_\perp$ methylene subcell and which are inclined to the crystal surface by 63° revert to a 1,3-diglyceride having "herring bone" chain packing in the $T_{\|}$

methylene subcell, with the chain axes inclined to the crystal surface by either 72° or 66°, depending on which β-form appears [33]. (Preliminary reflection electron diffraction results indicate average chain tilts near 70° and 60° for the 1,3-diglyceride.) The conformation of the acyl residues on the glycerol cannot be predicted unequivocally from this information since a mechanism for acyl shift can be proposed for either case. With an extended chain conformation, the acyl shift would take place in a single layer. A hairpin conformation, on the other hand, would demand a reaction across a hydrogen bonded interface.

Perturbations to free hydroxyl group in 1,2-diglycerides-effect on overall chain packing

It remains to be seen whether the acyl chain conformation in the crystal structure of glycerol 1,2-dipalmitate is structurally similar to that of the long chain diesters of ethylene glycol [34] which pack with extended chains [3]. We have verified the congruence of ethylene dipalmitate crystal structure with that reported earlier for the "homologous" ethylene di-11-bromoundecanoate [3] by showing that the hk0 electron diffraction patterns are identical to Fig. 4.

Because disorders in lipid microcrystals often preclude coherent scattering from polar regions of the molecule [26], the orientation of the free primary alcohol group on the molecule is very difficult to determine directly from electron diffraction studies. It is known from X-ray structural studies on the 1,3-diglycerides [29, 30] that the hydroxyl groups are involved in infinite hydrogen bonded chains through the crystal.

It is of interest to study the stabilizing effect of the polar group on the molecular packing. 1,2-Propylene dipalmitate has about the same molecular volume as glycerol 1,2-dipalmitate but is missing the primary alcohol function. Single microcrystals grown from *n*-pentanol give hk0 electron diffraction patterns (Fig. 5) identical to those obtained from the C-form of fatty acids [34] or the γ_4-form of fatty alcohols [35]. The angle between {110} faces of the crystals is 56°, also coincident with the value found for crystalline C-form acids [36]. Low angle X-ray data give a long spacing of 39.6 Å consonant with a chain tilt of 53°. High angle X-ray data confirm the $O_\perp$ methylene subcell.

By analogy to the fatty acids where Malta *et al.* [37] found the Kitaigorodskii [38] molecular packing coefficient k to be 0.690 for the C-form *vs* 0.701 for the B-form, the altered packing due to loss of the polar group in the diglyceride represents a slightly higher crystal potential energy. The crystals themselves are largely "molecular" in nature, the packing mainly predicated by the van der Waals interactions of the long polymethylene chains. The directional polar group interaction can stabilize one of two tilted chains forms which pack with the same $O_\perp$ polymethylene subcell.

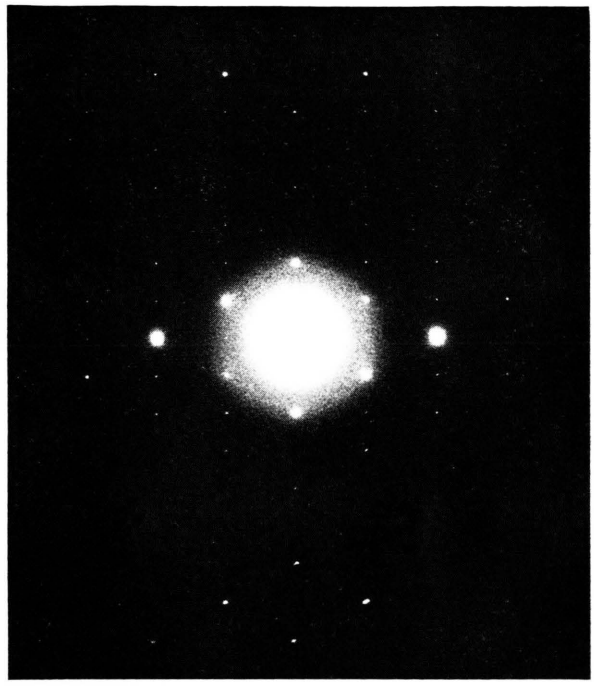

Fig. 5. Electron diffraction pattern (hk0) from untilted crystal of 1,2 propylene dipalmitate. Assuming $\beta \cong 90°$, $a=4.95$ Å, $b=8.99$ Å.

Conformationally-fixed analogs of 1,2-diglycerides based on cyclopentane-1,2,3-triol

a. Extended chain isomers. Two "extended chain" forms of "1,2-pseudodipalmitin" are synthesized from the 1,2/3 and 1,3/2 configurational isomers of cyclopentane-1,2,3-triol. An α-form with the hexagonal hk0 diffraction pattern (Fig. 6) has been obtained for the 1,3/2, 1-OH isomer from anhydrous ethanol. The best solvent for many of the cyclitol lipids in *n*-pentanol. This solvent gives a crystal packing of this isomer with untilted chains, as in the orthorhombic paraffins [39]. The most commonly obtained 1,3/2, 1-OH polymorph has a rectangular unit cell projection (Fig. 7), sometimes

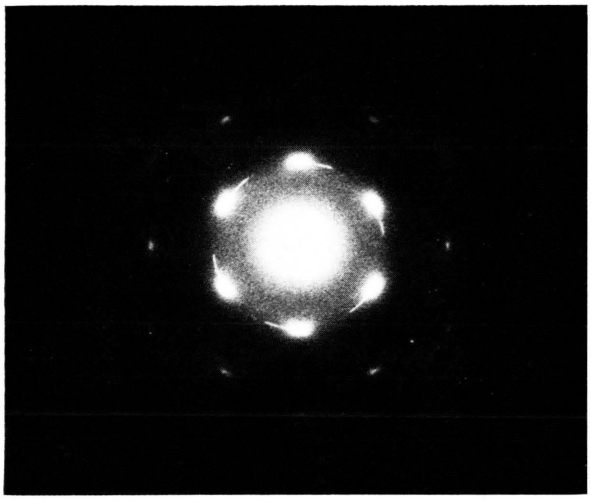

Fig. 6. Hexagonal hk0 electron diffraction pattern from α-form of D,L-(1,3/2)-2,3-di-O-palmitoylcyclopentane-1,2,3-triol (labelled 1,3/2, 1-OH in text and Fig. 1) $d_{100}=4.10$ Å.

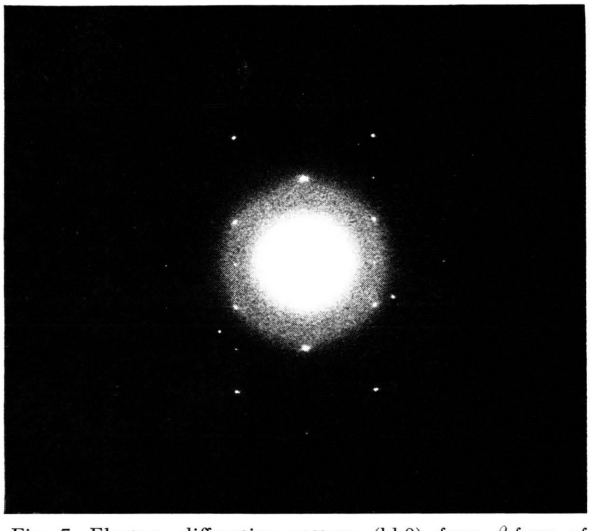

Fig. 7. Electron diffraction pattern (hk0) from β-form of D,L-(1,3/2)-2,3-di-O-palmitoylcyclopentane-1,2,3-triol (or 1,3/2-1-OH); $d_{100}=8.31$ Å, $d_{010}=5.19$ Å. Superlattice trebles d_{010}.

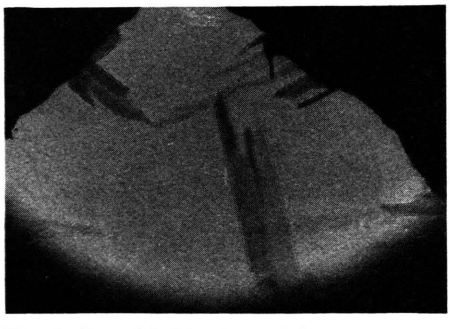

Fig. 8. Crystal habit giving diffraction pattern in Fig. 7.

giving indications of a disordered superlattice. The crystals are irregular flat blades (Fig. 8). Patterns of similar appearance have been obtained from *rac* glycerol 1-monopalmitate [34], but these have slightly different cell dimensions ($d_{100}=8.93$ Å, $d_{010}=5.04$ Å) and correspond to the β-forms which pack in the $M_{||}$ subcell [40].

Powder X-ray diffraction patterns from the bulk material give high angle spacings corresponding somewhat to the hk0 electron diffraction data (Table I). The strong reflections in the hk0 electron diffraction pattern of Fig. 7 are $d_{020}=4.16$ Å and $d_{110}=4.40$ Å. In the powder pattern the 4.16 Å spacing would superimpose with the hexagonal (100) spacings if both polymorphs are present in the bulk. Whether the 4.27 Å spacing seen in the X-ray pattern is close enough to be identical to the (110) spacing in Fig. 7 is uncertain. By analogy to natural diglycerides [15], the long spacing for the compound (Table I) corresponds to a chain tilt of about 62° in contrast to the β-form of the monoglyceride which has a chain tilt of 55°. The intensity of the 4.59 Å powder diffraction line is not large enough to assign comfortably the $M_{||}$ subcell to the structure [41]. Whatever is the non-hexagonal methylene subcell for this compound, the infrared singlet at 720 cm^{-1} indicates that it involves an acyl chain packing with parallel planes of the carbon zig-zag [15].

Table I. Powder X-ray diffraction data from 1,2-dipalmitates of cyclopentane-1,2,3-triol.

Extended chain isomers		"Hairpin" chain isomers	
(1,2/3,1-OH)	(1,3/2,1-OH)	(1,2,3/0,1-OH)	(1,2/3,3-OH)
39.8 Å (s)	42.4 Å (s)	42.9 Å (s)	49.4 Å (s)
35.4 (s)	21.2	38.7 (s)	24.7
13.3	10.6	21.4	16.4
11.8	7.07	19.3	12.3
9.95	5.01	14.3	9.87
6.87	4.81	12.9	8.77
6.34	4.59	9.91	8.35
5.99	4.27 (s)	8.39	6.69
5.74	4.16 (s)	4.85	6.28
5.46	4.02 (m)	4.54	5.96
5.06	3.91 (s)	4.33	5.75
4.15	3.79	4.09 (s)	5.51
4.05		3.86 (s)	5.25
3.97 (s)		3.77 (m)	4.88
3.84 (s)			4.56
3.67 (m)			4.08 (s)
3.53			3.94
			3.78 (s)
			3.69
			3.63

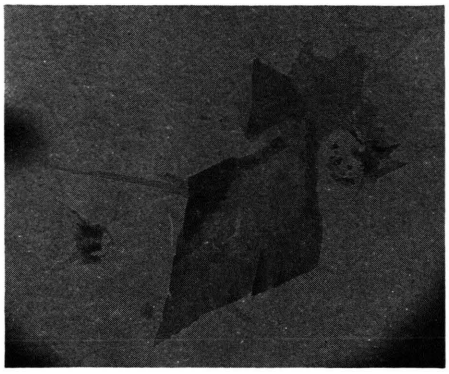

Fig. 9. Crystal habit of β'-form of D,L-(1,2/3)-2,3-di-O-palmitoylcyclopentane-1,2,3-triol (or 1,2/3, 1-OH) $d_{100}=9.79$ Å, $d_{010}=8.92$ Å.

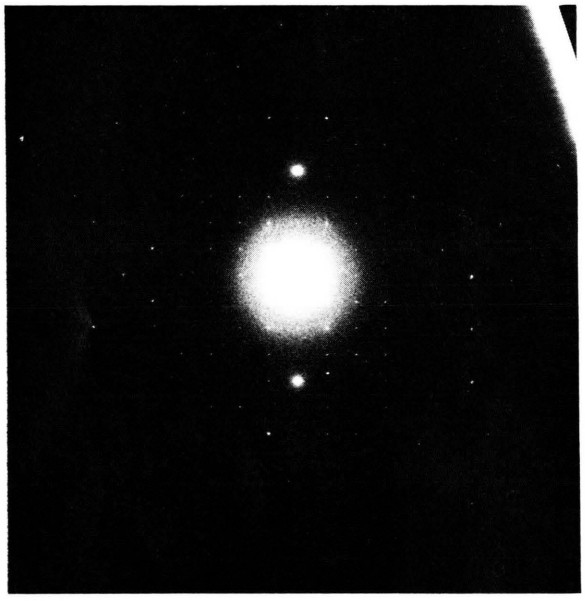

Fig. 10. Electron diffraction pattern (hk0) from crystals as in Fig. 9.

Many polymorphic forms are obtained from the other extended chain isomer 1,2/3, 1-OH. Since this form has the potential of undergoing acyl migration, as evidenced by the migration of two close bands on TLC plates, care must be taken to identify patterns which may be due to crystalline 1,3-pseudodiglyceride. As in the case of the extended chain 1,3/2 compound, the hexagonal α-form patterns, untilted chain $O_{\perp}$ methylene subcell patterns, and even the possibly $M_{\parallel}$ form have been identified for this material. The latter two crystal habits are flat needles.

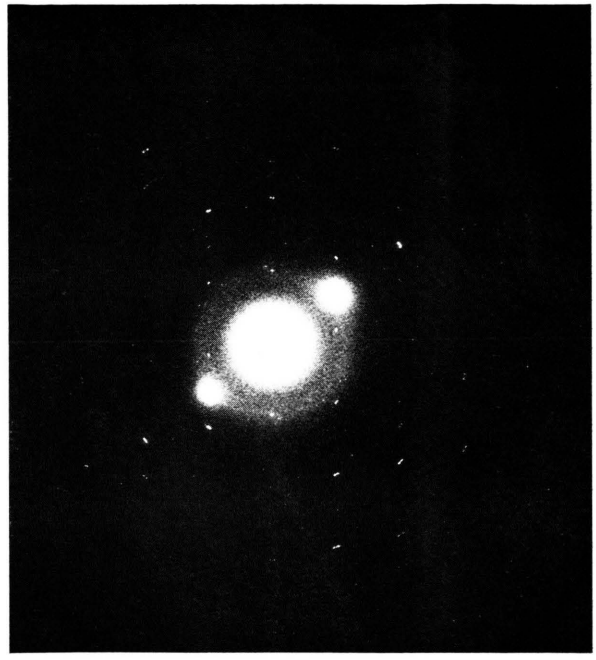

Fig. 11. Triclinic diffraction pattern, perhaps from D,L-(1,2/3)-1,3-di-O-palmitoylcyclopentane-1,2,3-triol (or 1,2/3 2-OH). Unit cell parameters for the projection: $d_{100}=9.41$ Å, $d_{010}=5.30$ Å, $\gamma^*=78.8°$.

The most often observed habit, however, is the losenge type shown in Fig. 9 which has angles between {110} faces ca 57° in accord with those found for C-form fatty acid crystals [36]. Electron diffraction patterns also resemble those obtained from C-form fatty acids (Fig. 10), but, as in the case of triglyceride analogs based on this cyclitol [16], a superlattice pattern is seen which doubles the d_{100} spacing of this C-form packing. The molecular transform sampled by the reciprocal lattice is very similar to that seen in Fig. 5.

A triclinic diffraction pattern is sometimes seen (Fig. 11) which superficially resembles those obtained from β-triglycerides [42]. By analogy to the case of acyl shifts in the crystalline 1,2-diglycerides, since these triclinic patterns have been most often found for samples which have been on the shelf for several days, they are probably due to the 1,3-dipalmitate of 1,2/3 cyclopentane-1,2,3-triol. This will be verified when the 1,3-diglyceride analog is synthesized.

The presence of two isomers due to acyl shift, as was established by thin layer chromatography, also is indicated clearly by a doublet of low-angle bands (Table I). The strongest low angle reflection

at 39.8 Å corresponds to a chain tilt of 54°, if one compares it to the long spacing for the α-form of 1,2-dipalmitin [15], and is consistent with the C-form fatty acid packing [37]. High angle powder data indicates the supposed $O_\perp$ methylene subcell of this form to be distorted since the presumed d_{110} = 3.97 Å is somewhat short. Yet the infrared spectrum gives a doublet at 720 cm^{-1} consistent with the nearly normal acyl chain planes required for the subcell.

b. Hairpin or folded-chain isomers. Two possible "1,2-pseudodipalmitins" with hairpin conformations of the acyl groups to the ring are synthesized from the 1,2/3 and 1,2,3/0 isomers of cyclopentane 1,2,3-triol. The former 1,2/3, 3-OH material crystallizes very easily from *n*-hexane, anhydrous ethanol or *n*-pentanol giving only one crystal habit (Fig. 12). The diffraction patterns (Fig. 13) are those of an untilted polymethylene chain packing in the $O_\perp$ subcell. Agreement between observed structural factors with calculated kinematical structure factors for this subcell [9] is generally very good, with an *R*-value *ca* 0.24.

Powder X-ray diffraction data from the bulk material (Table I) are entirely consistent with the electron microscope observations. The low angle spacing, in comparison with α-1,2-dipalmitin [15], confirms a chain packing with axes normal to the methyl end plane. The intense high angle powder spacings are in agreement with those accepted for the $O_\perp$ subcell and the infrared doubled at 720 cm^{-1} adduces further evidence for this.

From the 1,2,3/0 1-OH isomer 1,2-pseudodipalmitin, two polymorphic forms are seen; the largest crystals being formed from the alcohols. A hexagonal α-form was obtained from anhydrous ethanol. The salient crystal form from *n*-pentanol gives an (hk0) electron diffraction pattern very similar to the B-form of fatty acids or *sn* glycerol 1,2-dipalmitate (Fig. 4) with unit cell spacings $a = 5.60$ Å, $b = 7.62$ Å (assuming a monoclinic β angle near 90°). Tilting these crystals by 27° about the *b* axis gives the expected (001) projection of the $O_\perp$ methylene subcell [13]. The agreement of measured structure factor magnitudes from the diffraction pattern (Fig. 14) with calculated kinematical values is also good with an *R*-value of 0.23. Microcrystals of this form are flat laths (Fig. 15).

Although preparations of this isomer, which is susceptible to acyl shift, could not be resolved into two components on a TLC plate, the presence of the two products is indicated in the powder X-ray pattern as two intense low angle lines. The line at smallest angle (Table I) corresonds to the chain tilt of 61°, consistent with the assigned monoclinic paraffin type chain packing [22]. Intense high angle lines verify the $O_\perp$ subcell, but for some reason the 720 cm^{-1} region in the infrared spectrum is a singlet

Fig. 12. Crystal habit of β-form D,L-(1,2/3)-1,2-di-O-palmitoylcyclopentane-1,2,3-triol (or 1,2/3, 3-OH).

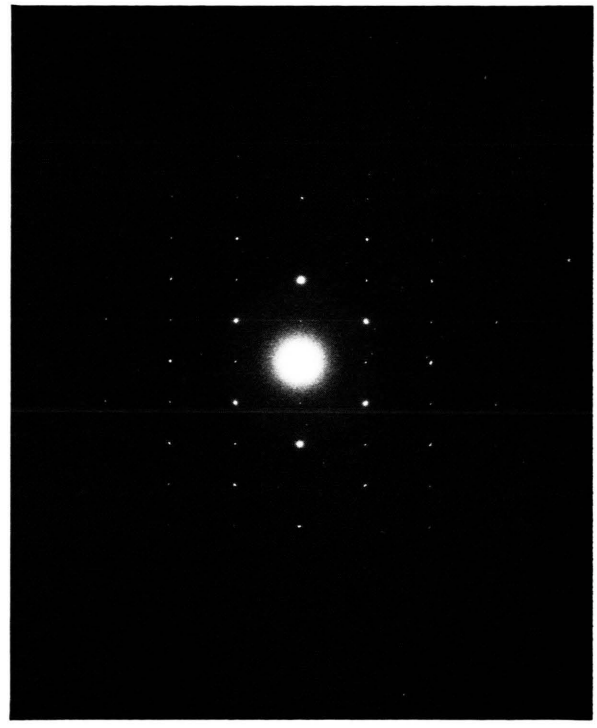

Fig. 13. Electron diffraction pattern (hk0) as from untilted crystals of Fig. 12; $d_{100} = 7.50$ Å, $d_{010} = 4.90$ Å.

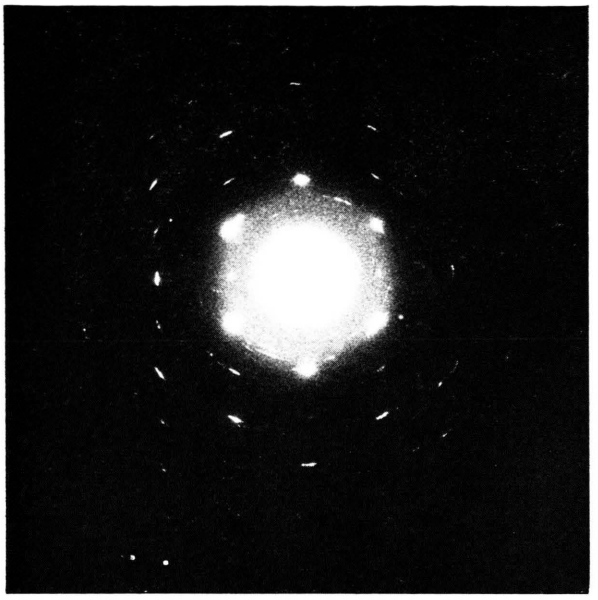

Fig. 14. Electron diffraction pattern (hk0) from crystals of D,L-(1,2,3/0)-2,3-di-O-palmitoylcyclopentane-1,2,3-triol (or 1,2,3/0, 1-OH) tilted 27° about monoclinic *b* axis. Subcell spacings: d_{100}=7.60 Å, d_{010}=4.92 Å.

Fig. 15. Untilted crystals of compound in Fig. 14.

instead of the anticipated doublet. Perhaps there is enough disorder of the subcell packing to cause the disappearance of a well-defined doublet [43].

Implications for the solid state packing of 1,2-diglycerides in biological systems

The two possible chain conformations of 1,2-diglycerides are of importance in a discussion of their molecular geometry in various physiological situations. When both chains are *cis* to the glycerol backbone (*i. e.* when the diglyceride has the "hairpin" conformation), then the molecular geometry is that expected for a polar/nonpolar interface such as a biological membrane. As expected, the cyclopentanetriol analog with the unsubstituted hydroxyl group *trans* to the acyl group is the most easily crystallized because it readily posits the best amphiphile. Such a diglyceride geometry is expected in biological membranes which, when attacked by phospholipase C, supposedly maintain much of their bilayer structure [44–46], even though neutral diacyl glycerols do not form a homogenous phase with phospholipids [47].

It is interesting to discover that the hairpin conformation with the hydroxyl group *cis* to the long chains is the only analog to crystallize with chain packing identical to the natural 1,2-diglycerides. By analogy to our earlier study of triglyceride analogs [16], this implies that this form may be the conformation favored in the solid state. Preliminary X-ray crystal structure analysis confirms this because it is very difficult to rationalize the measured unit cell length with an extended chain packing in the mode of monoclinic paraffin.

1,2-Diglycerides with extended chain conformations (as grown from homogeneous nonpolar solvents) may be of importance in biological systems when a homogeneously hydrophobic medium is encountered – *e. g.* at the interior of fat globules. Larsson [5] proposed an extended-chain intermediate to explain the "flip-flop" motion of phospholipids across biological membranes. However, his model was based on pressure area observations on a collapsed 1,3-diglyceride monolayer and the observed hump (due to the extended chain form) on the dynamic compression curve was absent if any amount of 1,2-diglyceride were present.

The actual presence of a true extended chain crystal packing for these cyclitols having only two *trans* vicinal hydroxyl groups esterified to long fatty acids must be investigated further. The crystal structure of prostaglandin A_1, for example [48], represents a structure where two long acyl chains are substituted *trans* to one another on neighboring carbon atoms of a cyclopentene ring. In this crystal structure the ω-chain contains a *gauche* carbon-carbon interaction at the $C_{15}-C_{16}$ single bond to cause it to fold back in the same direction as the α-chain, *i. e.* to give another "hairpin" conformation. On the other hand when there is a double bond linking

the two ring carbons [49] (*e. g.* in prostaglandin B_1) the pendant chains are extended somewhat like the 1,3-diglycerides [30]. In this paper we have assumed the extended chain conformation for the *trans* substituted cyclitol analogs to 1,2-dipalmitin without proof and it must be recognized that the bulk of the ring may posit a hairpin prostaglandin type structure instead. Nevertheless, the fact that an isomer with the two chains substituted *cis* to the ring is the only one to give a crystal packing like the 1,2-diglycerides, is very significant and probably gives a true insight into the actual crystal structure of the β-form of these compounds.

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