The Role of X-Ray Diffraction in Studies of the Crystallography of Monoacid Saturated Triglycerides[1]

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Abstract

The contribution that x-ray diffraction has made to the understanding of triglyceride polymorphism is reviewed. The crystal structure of these compounds is explained in terms of molecular orientation in the crystal lattices. At the present time only the crystal structure of the monoacid saturated triglycerides has been reasonably well defined. Mixed triglycerides and mixtures thereof have not yet been fully investigated. This review stresses the need for systematic correlation of the crystal structure of fats with the molecular composition and configuration.

Introduction

THE MULTIPLE MELTING POINT BEHAVIOR exhibited by triglycerides was first observed over a century ago. In 1849 Heintz (1) observed that, when molten tristearin was rapidly solidified, it melted at 52 C and, upon further heating, it resolidified and exhibited a second, higher melting point at 65 C. In 1853 Duffy (2) reported that tristearin exhibited three melting points at 52, 64.2 and 69.7 C. Still later Berthelot (3) in 1853–54, Guth (4) in 1902 and Lantz (5) in 1913 observed only two melting points. Othmer (6) in 1915 rediscovered three melting points for tristearin, but Joglekar (7) in 1930 again observed only two. In 1932 as many as seven different melting points were claimed by Weygand and Gruntzig (8). At the time it was speculated that this multiple melting behavior was due to the occurrence of some form of isomerism. These early investigations produced considerable confusion, and no definite basis for understanding the melting behavior of triglycerides evolved until 1934. In the period from 1934 to 1939, Malkin and his co-workers in England published the results of a series of x-ray diffraction powder studies which clearly demonstrated that the multiple melting phenomenon was due to polymorphism, i.e., the occurrence of different crystal forms for a single compound, which are distinguishable by characteristic x-ray diffraction powder spectra. Later in 1945 similar investigations were initiated by Lutton, Daubert and their co-workers in the United States. The publications of these groups on x-ray diffraction studies during the period from 1945 to 1955 helped to clarify much of the confusion caused by the earlier thermal studies. They pointed out the relationship of melting behavior to specific polymorphic forms.

Although x-ray powder photography aided considerably in revealing the nature of triglyceride polymorphism, confusion recurred. Reviews by Lutton (9) in 1950 and Malkin (10) in 1954 illustrated this confusion and described the differences in opinions which arose; at this time their viewpoints prevailed. All other ideas about the polymorphism of triglycerides were merely modifications of these two major interpretations. Several attempts to compromise the two views were made by Ralston (11), Vaeck (12), and Hoerr and Paulicka (13,13A) but these failed to correlate all the inconsistencies.

In recent years new techniques beyond x-ray powder photography, primarily x-ray single-crystal methods and infrared spectroscopy, have been applied to the problem of glyceride polymorphism. Reviews of the subject in which the state of knowledge is clearly and critically summarized were given by Chapman (14) and by Larsson (15). The deadlock in opinions between Lutton and Malkin appears finally to be broken.

In the present discussion we will indicate briefly the main features of the controversy as they pertain to monoacid saturated triglycerides and will examine the extent to which they have been resolved.

Discussion

Nomenclature

The use of two different schemes of nomenclature to designate crystal forms is responsible for much of the confusion in this field. Malkin characterized the crystal forms in order of increasing melting points as vitreous, α, β' and β. Lutton characterized these forms by their x-ray short spacings measured from powder photographs. Since these different schemes of nomenclature were based on different principles, but the same terms were used to designate crystal forms, contradictions occurred. Furthermore, both schemes have limitations. A weakness in Malkin's scheme is that homologously isomorphous members will have different names in a series wherein the relative thermal stability of two forms changes. On the other hand, although Lutton's scheme reveals crystal form similarities, it oversimplifies the variations which are possible for glycerides. The short spacings for α, β' and β in Lutton's scheme correspond to the strongest sub-cell reflections of the hexagonal, orthorhombic and triclinic chain packings, respectively. However, data for the triclinic and orthorhombic sub-cells given by von Sydow (16) and Abrahamsson (17) show that the dimensions of the sub-cell, and consequently the short spacings, vary considerably and make identification of crystal form from short spacings alone unreliable.

The confusion which resulted from the existence of these alternate schemes and the manner in which it was resolved will be illustrated in the description of the polymorphic behavior of even and odd monoacid saturated triglycerides.

Even Monoacid Saturated Triglycerides

Even monoacid saturated triglycerides (those having fatty acid acyl groups containing even numbers of carbon atoms) which constitute the most complete homologous series studied are also the center of the greatest controversy. Disagreement centers on the association of x-ray diffraction powder patterns with thermal data, the number of possible forms which exist, and the existence of a vitreous form. Malkin claimed these triglycerides exhibited four melting points, while Lutton maintained they exhibited only three. Both investigators found three distinct x-ray powder patterns but associated them

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TABLE I

Even Monoacid Saturated Triglycerides

Melting point	Designated Name and Short Spacing in A	
	Malkin	Lutton
Highest	β –4.6	β –4.6
Lower	β'–3.8, 4.2	β'–3.8, 4.2
Lower	α –4.15	....
Lowest	vitreous 4.15 (diffuse)	α –4.15

with different melting points. They agree on association of x-ray and thermal data for only the highest melting form. These disagreements are illustrated in Table I which compares the x-ray and melting point data for even monoacid saturated triglycerides.

Malkin supported the validity of his scheme with the following arguments: (a) There is no sharp delineation between the crystalline and the noncrystalline state. In paraffin-like structures, when layer structure diminishes below a certain size, long spacings disappear, but a diffuse short spacing may still persist owing to lateral diffraction (short spacing diffraction) by hydrocarbon chains which is independent of layer structure. Consequently, Lutton's assignment of crystallinity to the lowest melting form on the basis of the existence of a diffraction band at 4.15 A is questionable. (b) A solid crystalline form of pure monoacid saturated triglycerides, which is obtained by crystallization from solvent, has a higher melting point than the form which is obtained by thermal transformation of lower forms, or by crystallization from the melt, and is regarded by Lutton as the most stable. Since the same crystal form of a pure compound cannot have two different melting points, Lutton's scheme is incomplete. (c) The relationship between melting point alternation and tilting of hydrocarbon chains, which is established by consideration of methyl group packing in crystals of long-chain molecules, indicates that, where chains are tilted, melting points alternate and, where chains are vertical, melting points do not alternate. Long spacings of the crystal form characterized by short spacings at 3.8 and 4.2 A in powder patterns are consistent with tilted and not vertical chains. This is inconsistent with the series of non-alternating melting points proposed by Lutton for this form in a homologous series.

Lutton supported the correctness of his scheme by offering the following arguments against the points raised by Malkin: (a) The question is not whether a form actually crystalline is termed vitreous or a glass, but rather what properties are associated with the lowest melting form. This form is not a glass in the usual sense, since it has a sharp melting point, sharp diffraction lines, birefringence or anisotropy and discontinuities in dilatation and heat content during melting. (b) A pure long-chain compound may exhibit variation in melting point within the same crystal form as a result of differences in degree of stabilization of the form. (c) The relationship between melting point alternation and tilting of hydrocarbon chains is only a theory established by Malkin. The theory is of limited use if experimental facts show that it is not completely valid. Since the essential points of the controversy apply to all saturated triglycerides, an extensive deadlock occurred which lasted until recent years.

TABLE II

Data for β Form of Trilaurin

Unit cell: triclinic, 2 molecules/unit cell, space group P$\bar{1}$		
a = 12.31 A	b = 5.40 A	c = 31.77 A
α = 94°16'	β = 96°52'	γ = 99°12'

This difference in opinion was finally resolved, at least qualitatively, by the introduction of infrared spectroscopy to the study of glyceride polymorphism. Chapman (14) suggested that the CH$_2$ rocking mode vibration which appears at approximately 720 cm^{-1} in the infrared absorption spectra of these compounds can be used to determine the nature of the chain packing. It should be noted here that the nomenclature of Lutton will be used hereafter unless stated otherwise. A single band occurs at 720 cm^{-1} in the spectrum of the α-form; a doublet at 719 and 727 cm^{-1} occurs in the spectrum of the β' form; and a single band is observed at 717 cm^{-1} in the spectrum of the β form.

By comparison with other long-chain compounds (esters, fatty acids, etc.), the polymorphic forms of the monoacid saturated triglycerides are classified according to their hydrocarbon chain packing. When spectra of paraffin-like chains in the solid state are obtained, a number of bands are observed to be split into doublets. When the chains are packed in the orthorhombic arrangement, the band at 720 cm^{-1} associated with the CH$_2$ rocking mode is particularly prominent and is split by approximately 6 cm^{-1}. This doublet arises from interaction between the two chains present in the unit cell with hydrocarbons, or in the sub-cell with long-chain derivatives (18,19). If the chains are hexagonally packed, only a single band at 720 cm^{-1} is observed. In this form there is only one chain per sub-cell, and the inter-chain distance is greater than in the orthorhombically packed form. Finally, with triclinic packed chains, a single band is observed at 717 cm^{-1} consistent with the occurrence of one chain per unit or sub-cell. Therefore, in the case of monoacid saturated triglycerides the α form is hexagonal, the β' form orthorhombic and the β form triclinic. The chain planes of the β form are all parallel, while in the β' form every second chain is perpendicular to the plane of the others.

This chain packing or sub-cell symmetry makes plausible the classification of triglyceride crystal forms from x-ray short spacings in powder patterns as proposed by Lutton. However, details of the sub-cell symmetry which can only be obtained by other means, such as infrared or single crystal analyses, must be known before such classification is certain.

The most significant and detailed evidence for the classification of saturated triglyceride crystal forms into general types based on sub-cell symmetry comes from x-ray diffraction analyses of single crystals. The first study of a monoacid saturated triglyceride by this technique was reported by Vand and Bell (20) who analyzed hydrocarbon chain packing in a single crystal of β form trilaurin grown from solution in benzene by evaporation at room temperature. The data they reported are shown in Table II.

Details of the molecular configuration were replicated by conformal or Fourier mapping of electron density. Projection of the Fourier map along the b axis indicated that the chain arrangement in the molecule corresponds to the modified "tuning-fork" or "chair" model. The chains in the 1- and 3-position of the glycerol residue point opposite to the chain in the 2-position as is seen in Fig. 1.

The sub-cell is also triclinic and contains two CH$_2$ groups per unit cell. The angle of tilt between the

TABLE III

Data for β Form of Tricaprin

Unit cell: triclinic, space group P$\bar{1}$		
a = 12.10 A	b = 5.50 A	c = 27.03 A

chains and the basal a-b plane is 62° 7′ which is in good agreement with the angle predicted from long spacing data for the even series of saturated triglycerides (61° 35′). Later studies by Turnbull and Cormia (21) and Larsson (22) reported good agreement with the data of Vand for β-trilaurin. Jensen and Mabis (23) reported on the single crystal study of β-tricaprin crystals grown from hydrocarbon solution by evaporation at 0 C. He obtained the data shown in Table III.

At about this same time, Larsson (24) also reported details of the β form of tricaprin which are in agreement with those of Jensen. As can be seen, the results for β-tricaprin differ from trilaurin only in the c dimension as would be expected from chain length differences. Otherwise, details of the structure of the β forms are similar. Larsson (15) has also reported the results of a single-crystal study of the β′ form of trilaurin. His findings are illustrated in Table IV.

Space group assignment was inconclusive. The chain packing is orthorhombic with the basal a-b plane having sub-cell indices (111). Due to orthorhombic symmetry, the tilt of the hydrocarbon chains alternates between opposite directions in successive double layers. The tuning fork model is assumed by molecules in the β′ form.

Larsson also reported that hexagonal-type chain packing exists in the α-form, and chains are orientated vertical to the end group plane. Larsson stated that, since rapid α to β transition occurs, it is reasonable to assume that the tuning fork arrangement occurs in the α form also.

The above evidence shows that if Malkin's interpretation and criteria of nomenclature for the crystal forms of even monoacid saturated triglycerides are rejected, a consistency of x-ray and infrared data is obtained. Other studies of the lowest melting form also are consistent with this conclusion. Dielectric studies reported by Crowe and Smythe (25) and polarized-light microscopy studies reported by Quimby (26) show that the lowest melting form of monoacid saturated triglycerides exhibits properties which are inconsistent with its being glassy or vitreous. Lutton's assignment of crystallinity to this lower form is undoubtedly correct.

Although all the evidence discussed thus far allows little doubt about the incorrect association of x-ray data with melting points for monoacid saturated triglycerides, the question of how many polymorphic forms are possible still remains. Many authors have reported that the stable form obtained from solvent has a higher melting point than the same form obtained by transformation, whereas the x-ray powder patterns are the same. Chapman (14) points out that attempts to attribute these melting point differences (observed to be as high as 2–3 C) to variations in crystalline size and perfection appear unreasonable. He maintains that differences of this magnitude might more reasonably be explained as an effect of variations of chain alignment or of methylene group orientation within the chain. Similar behavior has been observed with polymers, but no accepted explanation for this behavior exists.

TABLE IV

Data for β′ Form of Trilaurin

Unit cell: orthorhombic		
a = 5.69 A	b = 23.6 A	c = 58.8 A

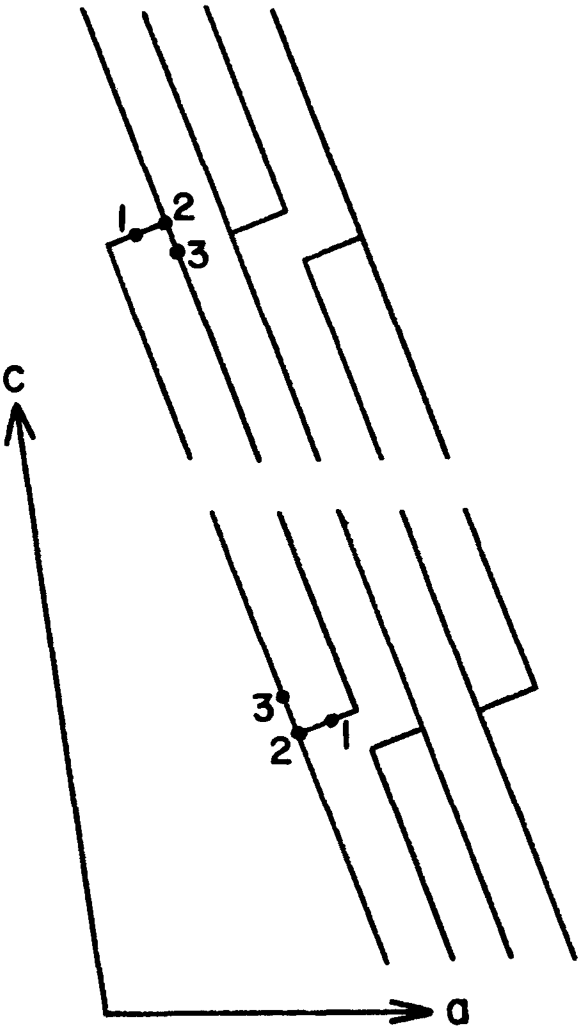

FIG. 1. Orientation of paraffin chains in the beta crystal form of trilaurin (after Vand). Projection along the b axis.

There is evidence that an additional form exists for monoacid saturated triglycerides. Jackson and Lutton (27) reported the appearance of a very diffuse band at 3.8 A in x-ray powder photographs of tristearin at −50 C which they designated as the sub-α form. They further demonstrated that the transition for α to sub-α was reversible or enantiotropic. Malkin challenged the correctness of this interpretation by pointing out that at such low temperatures ice condenses on the specimen, thus giving rise to an x-ray spacing in this region. However, Chapman (14) confirmed Jackson and Lutton's observation by employing an evacuated low-temperature cell to measure the infrared absorption spectrum of tristearin. He found that the main CH_2 rocking mode vibration near 720 cm^{-1} gradually changes from a single band to a doublet as the temperature approaches the range from −50 to −70 C. Chapman proposed that this transition α to sub-α is a second order one. This is clearly confirmed by Larsson (15). Unfortunately, Jackson and Lutton have introduced the term sub-α into their classification scheme for monoacid saturated

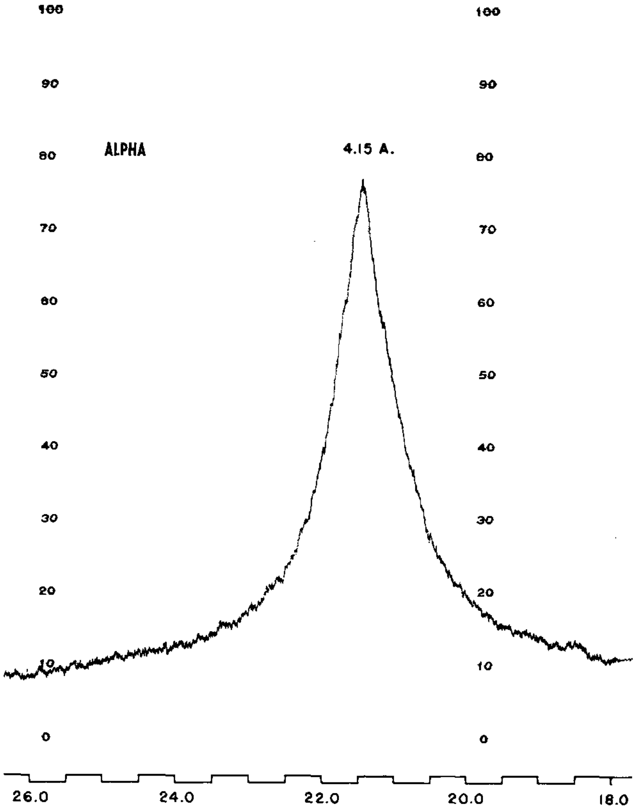

FIG. 2. X-ray diffraction powder pattern of alpha crystal form.

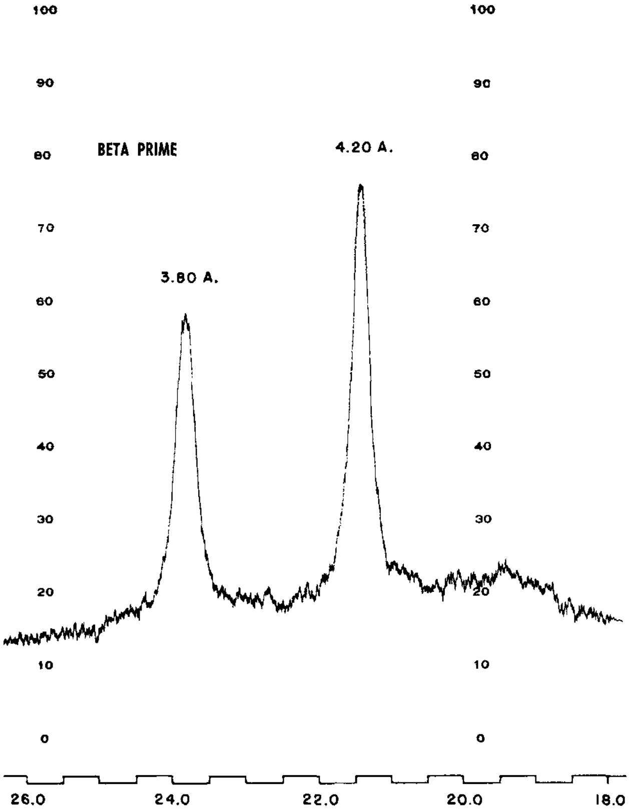

FIG. 3. X-ray diffraction powder pattern of beta prime crystal form.

glycerides. Larsson correctly refers to it (in the sense that it is related enantiotropically to the α form) as a property of the α form. Therefore, the term sub-α should be eliminated in the classification of these glycerides which are monotropic.

The correct interpretation of the polymorphic behavior of monoacid saturated triglycerides with an even number of carbon atoms in the acyl chain is that given by Larsson (15). They exhibit three crystal forms. The lowest melting α form is obtained usually on rapid crystallization from the melt. This form transforms monotropically into the β′ or β form. Only the highest melting β form crystallizes from solvents. The intermediate melting β′ form is obtained when the melt is maintained immediately below the β′ melting point.

Odd Monoacid Saturated Triglycerides

The series of monoacid saturated triglycerides with an odd number of carbon atoms in the acyl chain have been studied less extensively than the even ones. Clarkson and Malkin (28) reported that even and odd monoacid saturated triglycerides behave similarly. However, their results suffer from the same errors as Melkin's data on even triglycerides. Chapman (14) has clearly reported the existing polymorphism for trimargarin. A sub-α form was detected with these compounds by infrared spectroscopy which is related enantiotropically to the α form. Spectra obtained for α, β′ and β forms were analogous to those observed for the even triglycerides. The β′ form, however, was found to be thermodynamically most stable. Chapman's data were not sufficiently complete to give a full description of the polymorphism of these glycerides since he examined only trimargarin.

Larsson (15) has extended the study of the polymorphism of odd members to tripentadecanoin, tritridecanoin and triundecanoin. By employing diffraction pattern versus temperature diagrams, he observed that the stability relations of different crystal forms change with different members in the series. A transition from α to β was observed only for tripentadecanoin. A transition α to β′ and the α to β occurred in tritridecanoin. Triundecanoin, at its α melting point, transformed mainly to the β′ form, but a small amount of the β form was also observed. For this glyceride, the melting point of the β′ form (31.0 C) was higher than that of the β form (28.5 C). This variation in stability was also observed by Larsson in forms produced by slow crystallization from solvents. Only the β form of tripentadecanoin appeared, while both β′ and β forms of tritridecanoin crystallized together. Only the β′ form was obtained for triundecanoin. The data of Chapman and Larsson together clearly show that the β′ form is the most stable form in certain odd triglycerides, whereas the β form is most stable in others. The molecules are arranged in double-chain length layers with chains directed according to the tuning-fork model. These data are further useful in illustrating the limitations and confusion of Malkin's scheme. In instances where the β′ form (after Lutton) is higher melting than the β form, Malkin's scheme would require association based on melting behavior and the β′ and β forms (after Lutton) would be designated β and β′, respectively. This is further reason for rejecting Malkin's scheme.

Alternation in Physical Properties in Monoacid Saturated Triglycerides

The final question, which at this point remains to be resolved in the controversy between Malkin and

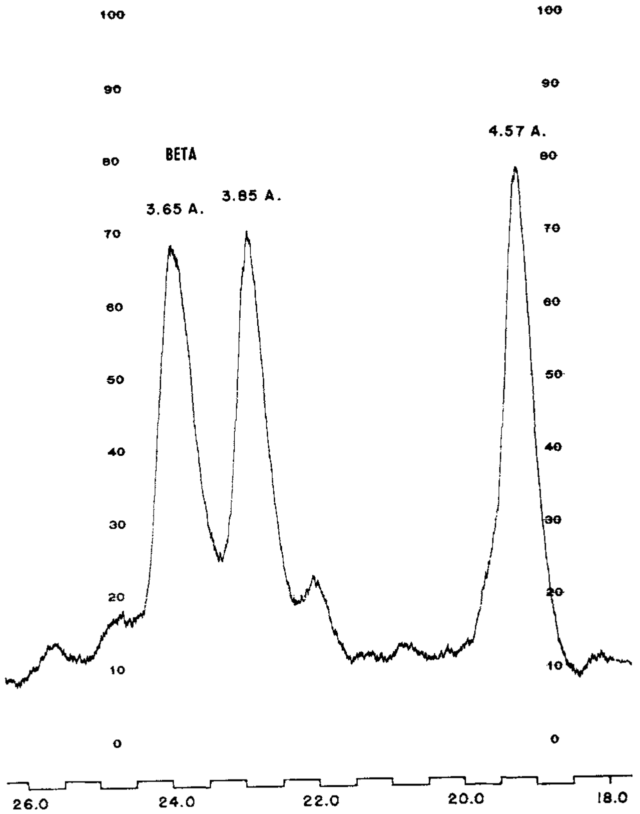

FIG. 4. X-ray diffraction powder pattern of beta crystal form.

Lutton before a clear description of the polymorphism of monoacid saturated triglycerides can be given, is related to the well known existence of alternation in melting points between even and odd members in homologous series of long-chain compounds. According to Malkin, the hydrocarbon chains in non-alternating series are vertical with respect to terminal and group planes, while in alternating series they are tilted. Contradictions to this hypothesis arose when the non-alternating β' form of monoacid saturated triglyceride, having odd numbered carbon chains, was found to have tilted chains. Malkin concluded that the β' form possesses vertical chains on the basis of his hypothesis. On the other hand, Lutton and Chapman have pointed out that the occurrence of tilt of the chains in the β' form is an argument against Malkin's hypothesis. Larsson finally reconciled this apparent contradiction by clearly explaining the occurrence of exceptions to Malkin's hypothesis. He states that the same structure is exhibited by even and odd members if the chain planes are all parallel and if (only if) the projection of the chain axes on the end group planes are perpendicular to the intersections of the chain planes with the end group planes. In packing structures where every second chain plane is perpendicular to the others it is also possible to get a similar end group structure for even and odd members. When the methyl group planes have the indices (011) in the orthorhombic sub-cell, even and odd members should give non-alternating melting points.

Current Views on the Polymorphism of Monoacid Saturated Triglycerides

The most acceptable criteria of nomenclature which provide a consistent and coherent classification of the different crystal forms of monoacid saturated triglycerides are those proposed by Larsson. His criteria are stated as follows according to our modified interpretation: (a) A form which crystallizes from the melt and exhibits a single strong x-ray short spacing band at 4.15 A shown in Fig. 2 is termed α. A form related to the α form by enantiotropic transition is termed sub-α, irrespective of its x-ray short spacing. (b) A form which exhibits two strong x-ray short spacing bands at 4.2 and 3.8 A shown in Fig. 3 is termed β'. (c) A form which exhibits three strong x-ray short spacing bands at 4.6, 3.9 and 3.7 A shown in Fig. 4, is termed β.

It should be pointed out that in Larsson's review his third criterion was stated more generally as: "A form which does not satisfy criteria 1 and 2 is called β." In his review, Larsson reports in detail the polymorphic behavior of saturated tri-, di- and monoglycerides. The x-ray short spacing bands which characterize the β form are different for di- and monoglycerides. Consequently, the more general statement of the third criterion was required to provide a consistent description of the polymorphism of all three classes of compounds. We have been more specific since we are discussing only monoacid saturated triglycerides. Larsson's scheme, however, includes our statement of the third criterion. These three criteria are consistent with those proposed by Lutton.

Further studies, primarily x-ray single-crystal studies, are required to bring our knowledge of the polymorphic behavior of di- and tri-acid saturated, unsaturated and mixed saturated and unsaturated triglycerides to that level of understanding which exists for mono-acid saturated triglycerides. Finally, extensive investigation is required before our knowledge of the behavior of pure glycerides can be applied to describing the polymorphism of naturally occurring and chemically modified fats and oils.

REFERENCES

1. Heintz, W., Jahresber. *2*, (1849).
2. Duffy, P., J. Chem. Soc. *5*, 197 (1853).
3. Berthelot, M., Liebig's Annalen der Chemie *88*, 304 (1853); *92*, 301 (1854).
4. Guth, F., Z. Biol. *44*, 78 (1902).
5. Lantz, H., Z. Phys. Chem. *84*, 611 (1913).
6. Othmer, P., Z. Anorg. Chem. *91*, 237 (1915).
7. Joglekar, R. B., and H. E. Watson, J. Indian Inst. Sci. *A13*, 119 (1930).
8. Weygand, C., and W. Gruntzig, Z. Anorg. Chem. *206*, 304 (1932).
9. Lutton, E. S., JAOCS *27*, 276 (1950).
10. Malkin, T., "Progress in the Chemistry of Fats and Other Lipids, Vol. 2, Academic Press, Inc., New York, 1954.
11. Ralston, A. W., "Fatty Acids and Their Derivatives," p. 545, John Wiley and Sons, New York, 1948, p. 545.
12. Vaeck, S. V., Mededel. Vlaam. Chem. Ver. *10*, 225 (1948).
13. Hoerr, C. W., J. Phys. Chem. *60*, 1265 (1956); JAOCS *37*, 539 (1960); Norelco Reporter *7*, 153 (1960).
13A. Paulicka, F. R., and C. W. Hoerr, "Encyclopedia of X-Rays and Gamma Rays," G. L. Clark, editor, Reinhold Book Corp., New York, 1963, pp. 1081–1087.
14. Chapman, D., Chem. Rev. *62*, 433 (1962); "The Structure of Lipids," John Wiley and Sons, New York 1965.
15. Larsson, K., Arkiv Kemi *23*, 5 (1964).
16. von Sydow, E., Arkiv Kemi *9*, 231 (1956).
17. Abrahamsson, S., Arkiv Kemi *14*, 65 (1959).
18. Stein, R. S., and G. Sutherland, J. Chem. Phys. *22*, 1993 (1954).
19. Chapman, D., J. Chem. Soc., 4489 (1957).
20. Vand, V., and I. P. Bell, Acta Cryst. *4*, 465 (1951).
21. Turnbull, D., and R. L. Cormia, J. Chem. Phys. *34*, 820 (1961).
22. Larsson, K., Arkiv Kemi *23*, 1 (1964).
23. Jensen, L. H., and A. J. Mabis, Nature *197*, 681 (1963).
24. Larsson, K., Proc. Chem. Soc. *87*, March (1963).
25. Crowe, R. W., and C. P. Smythe, J. Amer. Chem. Soc. *72*, 5281 (1950).
26. Quimby, O. T., J. Amer. Chem. Soc. *72*, 5063 (1950).
27. Jackson, F. L., and E. S. Lutton, J. Amer. Chem. Soc. *72*, 4519 (1950).
28. Clarkson, C. E., and T. J. Malkin, J. Chem. Soc. 666 (1934).