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## The Polymorphism of Tristearin and Some of its Homologs

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The "double (and triple) melting" of triglycerides had been long recognized but little understood until the X-ray diffraction studies of Clarkson and Malkin<sup>1</sup> showed that the phenomenon has a basis in crystal structure change like that which underlies the similar thermal phenomena of other long chain compounds. The extensive work of Malkin, *et al.*, on various triglycerides is summarized without essential modification in a general review of triglyceride polymorphism by Ferguson and Lutton.<sup>2</sup> Additional information has been reported on trinonadecoin,<sup>3</sup> on 2-oleoyl-1,3-distearin,<sup>4</sup> on tristearin<sup>5a</sup> and trilaurin,<sup>5b</sup> and on several 2-oleoyl-1,3-disaturated glycerides,<sup>6</sup> but no major advance in the understanding of triglyceride polymorphism has been reported since the fundamental work of Malkin.

The names of the polymorphic forms of triglycerides were based by Malkin upon X-ray diffraction patterns in a manner to relate them logically to corresponding forms of other long chain compounds. It resulted that the forms for tristearin, etc., were called by Malkin gamma (glassy), alpha and beta in order of increasing melting point.

Attempts in this Laboratory to check the classical work of Malkin have confirmed many of his observations. Three forms of tristearin, for example, have been obtained with melting points essentially as he reported them. For the highest melting or beta form, the X-ray diffraction pattern obtained here and that reported by Malkin are practically identical.

There are, however, three essential differences between the observations of this laboratory and those of Malkin<sup>7</sup>:

1. The pattern called alpha by Malkin (from its similarity to patterns of so-called alpha forms of other long chain compounds), attributed by him to the intermediate melting form, is regularly obtained for the lowest melting form of tristearin and other glycerides.

2. For the intermediate melting form of tristearin and its homologs here considered, a pattern, not reported by Malkin for these glycer-

ides, is obtained. It is similar in type to the patterns designated by the name beta prime which Malkin obtained for several mixed glycerides<sup>2</sup> (and probably also for saturated single fatty acid triglycerides with odd number of carbons in the fatty acid chain).<sup>8</sup>

3. The pattern reported by Malkin for glycerides in the lowest melting so-called "glassy" (gamma) form has not been obtained in this laboratory. It may be significant that Malkin's one specific example of the "glassy" form pattern, for tripalmitin (Fig. 16),<sup>1</sup> looks very much like his example of the alpha pattern for trimyristin (Fig. 10).

An inevitable inconsistency in Malkin's nomenclature arises from the faulty association of diffraction pattern and melting point. To overcome this inconsistency it is here proposed that the names assigned to the polymorphic forms of tristearin and its homologs be based on Malkin's reported diffraction patterns as Malkin himself certainly intended they should be.

Table I illustrates the essential differences between the observations of this laboratory and those reported by Malkin and embodies the proposed readjustment of nomenclature.

### Experimental

**Materials.**—The following materials were used:

1. Trilaurin, Eastman Kodak Co., C. P.
2. Trimyristin, Eastman Kodak Co., C. P.
3. Tripalmitin, Neo-fat (1-56) was purified by recrystallization twice from 78% alcohol and distillation at 5 mm. The purified palmitic acid in 10% excess was esterified with glycerol. Excess fatty acid was removed by steam distillation and recrystallization twice from petroleum ether. (This preparation was carried out under the direction of Mr. Eckey of this Laboratory.)
4. Tristearin.—This was prepared by esterification of double distilled glycerol with an excess of purified stearic acid obtained from Eastman Kodak Co. C. P. material. The latter had been recrystallized five times from 95% alcohol and five times from benzene. The crude esterification product was recrystallized once from 1:1 alcohol-petroleum ether, once from 4:1 alcohol-xylene and once from xylene.

As judged by sharpness of melting the first three materials were of comparable purity. The tristearin was of higher purity and should compare with the best described in the literature.

### Methods Employed

**Melting Point.**—Approximately 1 mm. capillary tubes were used. Samples were treated in the tubes as described in later sections.

**X-Ray Diffraction.**—Generally samples were treated in 1-mm. thin-walled capillary tubes of Pyrex glass. When convenient the glass was cracked off the sample, after treatment, to cut down time required for exposure.

The diffraction equipment has been described in previous publications from this Laboratory.<sup>9,10</sup> In most cases

(1) Clarkson and Malkin, *J. Chem. Soc.*, 666 (1934).  
 (2) Ferguson and Lutton, *Chem. Rev.*, **29**, 355 (1941).  
 (3) Woolley and Sandin, *THIS JOURNAL*, **57**, 1078 (1935).  
 (4) Hilditch, *et al.*, *J. Soc. Chem. Ind.*, **60T**, 16 (1941).  
 (5) (a) Ravich, *Compt. rend. Acad. Sci. U. R. S. S.*, **36**, 275 (1942); *C. A.*, **37**, 3660 (1943). (b) Ravich and Tsurinov, *Doklady Akad. Nauk S. S. S. R.*, **38**, 331 (1943); *C. A.*, **37**, 6514 (1943).  
 (6) Daubert and Clarke, *THIS JOURNAL*, **66**, 690 (1944).  
 (7) Agreement with the position here taken with regard to Malkin's conclusions was reported in a paper, "X-Ray Diffraction Investigations on Synthetic Glycerides" by Filer, Sidhu, Daubert and Longenecker, which was presented at a meeting of the American Chemical Society, September 11-15, 1944.

(8) Gruntzig, *Z. anorg. Chem.*, **240**, 313 (1939).  
 (9) Ferguson, Rosevear and Stillman, *Ind. Eng. Chem.*, **35**, 1005 (1943).  
 (10) Ferguson and Nordsieck, *ibid.*, **36**, 748 (1944).

TABLE I  
 COMPARISON OF DATA AND NOMENCLATURES FOR TRISTEARIN

M. p., °C.	This Laboratory			M. p., °C.	Malkin		
	Main short spacings	Long spacings	Name of form		Short spacings	Long spacings	Name of form
54	4.14 Å.	50.6 Å.	Alpha	54.5	4.2 Å. diffuse	.....	Gamma (Glass)
64	3.78, 4.18*	46.8	Beta prime <sup>a</sup>	65.0	4.2	50.6 Å.	Alpha
73.1	3.68, 3.84, 4.61, 5.24	45.15	Beta	71.5	3.7, 3.9, 4.6, 5.3	45	Beta

<sup>a</sup> Beta prime for mixed glycerides according to Malkin—approx. 3.8, 4.2.<sup>3</sup>  
 The situation is analogous for other homologs.

in the present study nickel foil was used to filter out the  $\text{CuK}\beta$  radiation. The various distances from fat sample to film were 2.5 cm. for exploratory tests, 5.0 cm. for routine patterns, and 10 cm. for long spacing study. When low temperature exposures were required samples were maintained at about 3°C. in a "cold block" cooled by circulating water.

**Thermal Curves.**—All melting points were fully confirmed by the important thermal curve method, described and emphasized by Malkin, but since no major advance beyond Malkin was attempted, further report of results is deemed superfluous.

### Results and Discussion

**Alpha Form.**—The lowest melting form, here called alpha, is invariably obtained from the melt by any rate of cooling which is at least moderately rapid. This is because of the great extent of supercooling permitted by the two higher melting forms. The alpha form, however, permits little or no supercooling, as a result of which the clouding point is very close to the alpha melting point.

Since the alpha form tends to transform to the beta prime form with increasing rapidity as the sample temperature is raised, the determination of the alpha melting point involves difficulties. Complete clarification of the melting point sample in some cases may not occur within several degrees of the lowest temperature of first evidence of melting (increased translucence). Considering this group of triglycerides as a whole, the softening point, or first visual evidence of melting, was found to characterize the two lower melting forms better and more reproducibly than a complete clarification point. (Alpha forms of the longer chain length tripalmitin and tristearin did give sharp complete clarification points.)

To determine the alpha softening point a sample in its capillary tube received a pretreatment of melting at 100°, followed by chilling for two seconds at 0°. The sample was then thrust into the melting bath at various trial temperatures. It was noted whether there was or was not increased translucence. The recorded alpha softening point was the average of the lowest temperature where increased translucence definitely was observable and the highest temperature where it was definitely not observable.

For X-ray examination of the alpha form the samples were melted at 100°, then held two seconds or longer at 0°. Pictures of tripalmitin and tristearin could be taken at room temperature. Trilaurin and trimyristin were maintained at 3°. Over a period of three to four years scores of samples were examined. They were generally samples prepared in capillaries, but many were flakes chilled on a variety of different surfaces. Never was the gamma or glassy form pattern of Malkin obtained, always his alpha pattern for this lowest melting form. *The conclusive proof of the correspondence of the alpha X-ray pattern with the lowest melting form is the experimental result that actual samples which gave the alpha X-ray pattern, also exhibited the lowest form melting point when tested immediately after X-ray exposure.* This lowest melting form was called therefore the alpha form, since for legitimate reasons Malkin had given the alpha pattern its name.

**Beta Prime Form.**—The beta prime form, although not the least stable, is in some respects the most fleeting form. When it is prepared by the most obvious procedure, *i. e.*, by obtaining alpha and transforming it near its melting point, the beta prime form has a very short existence at

the temperature of formation, but a longer existence at lower temperatures.

For determining the beta prime softening point, samples in six capillary tubes were pretreated by melting at 100° and chilling two seconds at 0°, then holding for a certain period of time at the alpha melting point (softening point) to promote transformation. The tubes were then kept at 0° to maintain their polymorphic condition until they were tested for softening (*i. e.*, increased translucence), all six tubes being used for a single determination of softening point. The six tubes were then retreated with a different length of time at transformation temperature. A plot was made of softening point *vs.* time at transformation temperature. Softening point rose with transformation time, but an intermediate flattened portion in an inverted s-shaped curve was obtained. (Beyond the flat the curve continued to rise toward the beta form melting point.) The middle of this flat was taken as the beta prime softening point.

Samples for X-ray examination were treated for periods of time corresponding to the mid-points of the flats. The patterns obtained were of the beta prime type reported by Malkin for mixed triglycerides.

It was subsequently found that more cleancut results could be obtained by holding the supercooled melt slightly (0.5°) above the alpha softening point for periods ranging from fifteen minutes for trilaurin to one hour for tristearin, *i. e.*, by avoiding alpha in the preparation of beta prime. The results obtained checked previous results for beta prime softening point and X-ray pattern, and the form was more stable when thus prepared.

**Beta Form.**—Results for the beta form were in satisfactory agreement with Malkin's for both melting point and X-ray pattern. Maximum melting point can be obtained by solvent crystallization, or by melting, chilling two seconds at 0° and storing three weeks between alpha and beta melting points.

The melting points recorded for beta are complete melting points obtained at a heating rate of 0.2° per minute. The beta melting point of the carefully purified tristearin is a full degree above most previously reported results, whether due to differences in degree of purity or method of sample stabilization.

It is undeniable that the beta form will show lower melting points for shorter periods of storage even with the purest triglycerides. Substantially the same X-ray pattern is obtained with melting point variations of a full degree. There are many factors which could possibly account for this troublesome behavior, differences in crystal size being the most obvious. The actual explanation, however, must await proper experimental study.

**Short Spacing Patterns.**—Short spacing data are in good agreement with those of Malkin. There are additional spacings which seem worth reporting in Table II along with the strong short spacings.

TABLE II  
SHORT SPACINGS FOR TRILAURIN, TRIMYRISTIN, TRIPALMITIN AND TRISTEARIN<sup>11</sup>

Alpha	Beta prime	Beta
4.14 Å. v. s.	4.18 Å. v. s.	5.24 Å. m.
2.40 w.	3.78 s.	4.61 v. s.
	3.42 v. w.	3.84 s.
	3.05 w.	3.68 s.
	2.81 w.	2.85 w.—
	2.53 m.—	2.57 m.—
	2.26 m.—	2.38 w.
v. s. = very strong		2.22 w.+
s. = strong		2.20 w.—
m. = medium		2.12 w.—
w. = weak		2.06 w.+
v. w. = very weak		1.95 w.

A new, but weak, spacing is reported for the alpha pattern. It is not inconsistent with the supposed hexagonal structure of alpha if the next most likely sets of planes after the principal sets (of interplanar distance = 4.14 Å.) are considered.

For the beta prime and more notably the beta form a multiplicity of spacings is observed. Some individual spacings seem to be associated with a particular glyceride, *i. e.*, probably relate to the third dimension instead of simply the cross-sectional structure. Only the spacings in common for the homologs are here reported.

**Long Spacing Patterns and Melting Points.**—The long spacings for the various forms of the four homologs are presented in Table III along with melting points. The comparable data, except those for tristearin, are probably less accurate than Malkin's, because the materials were probably less pure than his.

TABLE III  
LONG SPACINGS AND MELTING POINTS

Tri-glyceride	Long spacings, Å.			Melting points, °C.		
	Alpha	Beta prime	Beta	Alpha	Beta prime	Beta
Trilaurin	35.5	32.85	31.15	14	34	43.9
Trimyristin	41.4	37.65	35.45	32	44	55.5
Tripalmitin	46.3	42.3	40.9	44	55.5	65.5
Tristearin	50.6	46.8	45.15	54	64	73.1

Each of the three forms for a given triglyceride has order in the direction of the long chains

(11) Since the manuscript of this paper was accepted for publication another paper covering some of the same ground has appeared: "Thermal Properties of Fats and Oils. IV. Some Observations on the Polymorphism and X-Ray Diffraction Characteristics of Tristearin and a Highly Hydrogenated Cottonseed Oil" by Bailey, Jefferson, Kreeger and Bauer, *Oil & Soap*, **22**, 10 (1945). The writer is essentially in agreement with both their short and long spacing data on tristearin. However, a second short spacing line for the alpha (Bailey's III) form and several additional spacings for the beta prime (Bailey's II) form, seem not to have been observed by Bailey, *et al.*

Comparison of the writer's data for the beta prime form of tristearin with Bailey's for Form I of hydrogenated cottonseed oil reveals considerable similarity, which indicates the latter form is also beta prime. This further suggests that Bailey's Forms I, II and III for hydrogenated cottonseed oil, despite troublesome melting point differences, may actually be one and the same, *i. e.*, may all be beta prime in different stages of "crystal perfection."

(*i. e.*, shows long spacings) as well as in directions perpendicular to the long chains (shown by short spacings). The lowest melting or alpha form has, therefore, a much more highly ordered structure than the so-called "glassy" state described by Malkin on the basis of the faulty observation of a gamma (or glassy) pattern. The alpha form is a "perpendicular form" as concluded by Malkin for the form of alpha pattern. The other two forms, having shorter long spacings are presumably "tilted forms."

**Nomenclature.**—Malkin's proposed names for distinguishing among the polymorphic forms of glycerides on the basis of melting points, in addition to being inconsistent with his nomenclature based on X-ray diffraction patterns, are also in conflict with several earlier proposals for tristearin and homologs as shown in Table IV. It is because (1) the X-ray diffraction work of Malkin was the major contribution in clarifying triglyceride polymorphism, (2) he had excellent precedent for naming the alpha pattern, and (3) his use of the names beta prime and beta for "tilted forms" also followed naturally from the previous practice in the general field of long chain compounds that Malkin's names for the X-ray diffraction patterns of the triglycerides here discussed have been retained and assigned to the proper polymorphic forms. This dispenses with the gamma name and pattern and the so-called "glassy" state of triglycerides.

TABLE IV  
PREVIOUSLY PROPOSED NOMENCLATURE ACCORDING TO MELTING POINT

Form	W. & G. <sup>a,12</sup>	J. & W. <sup>13</sup>	E <sup>14</sup>	Malkin <sup>b</sup>
Lowest melting	III	Beta	Alpha	Gamma (Glass)
Intermediate melting	II	...	Beta	Alpha
Highest melting	I	Alpha	Gamma	Beta

<sup>a</sup> With submodifications to make seven forms in all.  
<sup>b</sup> Also beta prime for mixed glycerides.

Some generally satisfactory rational basis for nomenclature of polymorphic forms of long chain compounds will doubtless some day be proposed. It will depend on more complete understanding of structure than seems forthcoming in the immediate future. A constructive step was taken by Schoon,<sup>15</sup> but he considered only a single type of cross-sectional structure for long chain compounds. This is too limited an approach for triglycerides. Any Greek letter system of nomenclature for polymorphism suffers from the possibility of confusion with other Greek letter systems for position isomerism, etc. However, until a more fundamental approach can be made, Mal-

(12) (a) Weygand and Grüntzig, *Z. anorg. allgem. Chem.*, **206**, 304 (1932); (b) **206**, 313 (1932).

(13) Joglekar and Watson, *J. Indian Inst. Sci.*, **A13**, 119 (1930).

(14) Efremov, *Ann. Inst. Polytech. Oural*, **6m**, 155 (1927).

(15) Schoon, *Z. physik. Chem.*, **B39**, 385 (1938).

kin's nomenclature, based on X-ray diffraction patterns, seems the most promising available to correlate properly the polymorphic forms of a number of different triglycerides and to associate them with corresponding forms of other long chain compounds.

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### Summary

The "even" members of the homologous series of triglycerides, trilaurin through tristearin, have been studied by X-ray diffraction and thermal methods and found to exhibit monotropic trimorphism. This is in line with the conclusions of previous observers, notably Malkin.

Manifestation of different crystal structures by a given triglyceride was clearly established by the classical work of Malkin, *et al.*, as the underlying cause of the multiple melting of triglycerides. The names of polymorphic forms were based by

Malkin upon X-ray diffraction patterns in a manner to relate logically the triglyceride forms to corresponding forms of other long chain compounds. It resulted that the forms for tristearin, *etc.*, were called (by Malkin) gamma (glassy), alpha, and beta in order of increasing melting point. Unfortunately, according to the present study, there appears to have been a faulty association of X-ray diffraction pattern with melting point in the work of Malkin. This association was correct in the case of the highest melting beta form. However, the lowest melting form (called gamma, glassy or vitreous by Malkin) actually exhibits Malkin's alpha pattern and in accordance with Malkin's original intention is therefore named the *alpha form*. The intermediate form (called alpha by Malkin) actually exhibits Malkin's beta prime pattern (not reported by him for tristearin, *etc.*, but for mixed glycerides). It is therefore named the *beta prime form*. The gamma name and pattern and the concept of the glassy state of triglycerides should be eliminated.

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## Separation of Sugar Acetates by Chromatography<sup>1</sup>

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Application of chromatographic methods to the separation of sugars has been made by Hayashi,<sup>4</sup> who reported the separation of D-glucose and sucrose on a charcoal column. Reich<sup>5</sup> employed the colored *p*-phenylazobenzoates of the sugars and this general method has been extended successfully by Coleman and associates<sup>6</sup> to a very considerable number of typical separations. Employing the procedure of fractional elution (liquid or flowing chromatogram), Talley, Reynolds and Evans<sup>7</sup> and Jones<sup>8</sup> have applied chromatographic methods to the separation of sugar derivatives. Bell<sup>9</sup> has utilized the partition (chloroform-water-silica gel) chromato-

graphic procedure of Martin and Synge<sup>10</sup> in the establishment of a new O-tetramethyl-D-glucopyranose end-group assay for polysaccharides. The Molisch reagent was used by Bell to locate the zones on the developed, extruded and oven-dried column.

Although the above-mentioned chromatographic procedures represent a considerable advance over methods previously available for the separation of sugar mixtures, they have disadvantages. We wish to report herein the successful application of the chromatographic brush method established by Zechmeister and collaborators<sup>11</sup> to the separation of the well-characterized sugar acetates. This method consists in extruding the developed, colorless chromatogram and making the zones visible by brushing a line along the length of the column with a brush dipped in a reagent capable of forming a colored reaction product with the adsorbed substance. Such a procedure avoids the previous necessity of either working with colored sugar derivatives or resorting to empirical chromatography. A search for a suit-

(1) Presented before the Division of Sugar Chemistry and Technology at the 108th meeting of the American Chemical Society, New York, N. Y., September 15, 1944.

(2) Hoffmann-La Roche Post-doctoral Fellow of The Ohio State University Research Foundation.

(3) Sugar Research Foundation Post-doctoral Fellow of The Ohio State University Research Foundation.

(4) F. Hayashi, *J. Biochem. (Japan)*, **16**, 1 (1932); *C. A.*, **27**, 8 (1933).

(5) W. S. Reich, *Compt. rend.*, **208**, 589, 748 (1939); *Biochem. J.*, **33**, 1000 (1939).

(6) G. H. Coleman, A. G. Farnham and A. Miller, *THIS JOURNAL*, **64**, 1501 (1942); G. H. Coleman and C. M. McCloskey, *ibid.*, **65**, 1588 (1943); *cf.* J. K. Mertzweiler, D. M. Carney and F. F. Farley, *ibid.*, 2367.

(7) E. E. Talley, D. D. Reynolds and W. L. Evans, *ibid.*, **65**, 573 (1943).

(8) J. K. N. Jones, *J. Chem. Soc.*, 333 (1944).

(9) D. J. Bell, *ibid.*, 473 (1944).

(10) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941); A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *ibid.*, **37**, 79, 86, 92 (1943).

(11) L. Zechmeister, L. de Cholnoky and (Mlle.) E. Ujhelyi, *Bull. soc. chim. biol.*, **18**, 1885 (1936); L. Zechmeister and O. Frehden, *ibid.*, **22**, 458 (1940); L. Zechmeister and W. H. McNeely, *THIS JOURNAL*, **64**, 1919 (1942); L. Zechmeister, W. H. McNeely and G. Solyom, *ibid.*, 1922.