

**139.** *Alternation in Long-chain Compounds. Part II. An X-Ray and Thermal Investigation of the Triglycerides.*

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AN interesting and unexplained property of the triglycerides is that usually referred to as their "double melting." As is well known (Meyer-Jacobson, Bd. I, Tl. II, p. 134), crystallised tristearin melts at 71°, but if the specimen has been previously melted and suddenly

\* Experiments with the system monochloroacetic acid-barium monochloroacetate-water indicated that an acid barium salt was not formed below a concentration of 69% acid.

cooled, it first melts at 55°, then solidifies and melts again at 71°. This property appears to be general for the higher triglycerides, but recent work has indicated the existence of still another melting point (Othmer, *Z. anorg. Chem.*, 1915, **91**, 240; Nicolet, *Ind. Eng. Chem.*, 1920, **12**, 741; Loskit, *Z. physikal. Chem.*, 1928, **134**, 135); e.g., with tristearin, melting followed by resolidification has also been observed at 65°. We now find that this property of "triple melting" is general for all the triglycerides that we have examined (tricaprin to tristearin), but whereas the two lower melting points lie on smooth curves (Fig. 3,  $\alpha$ ,  $\gamma$ ), the highest melting points exhibit the well-known property of alternation (Fig. 3,  $\beta$ ).

We considered it probable that the above phenomena might be due to a type of polymorphism similar to that found in the simple esters of fatty acids (Smith, J., 1931, 802; Malkin, *ibid.*, p. 2796; *Trans. Faraday Soc.*, 1933, **29**, 977), in which case X-ray investigation would be expected to disclose the nature of the structural changes.

Examination by this means does, in fact, reveal a close parallelism between the glycerides and the simple fatty esters. The latter have been shown to exist in two modifications, a stable form ( $\beta$ ), in which the long chains are inclined with respect to the planes formed by the terminal methyl groups, and a less stable form ( $\alpha$ ), in which the chains are vertical and rotating (Malkin, *loc. cit.*). Both forms are now found to exist in the triglycerides, a stable  $\beta$ -form with inclined chains, and a monotropic  $\alpha$ -form with vertical chains, the melting points lying respectively on curves  $\beta$  and  $\alpha$ , Fig. 3. In harmony with the views advanced by one of us (*loc. cit.*), the melting points of the inclined  $\beta$ -forms alternate, whilst those of the vertical  $\alpha$ -forms lie on a smooth curve.

The third and lowest-melting form of the triglycerides, which is obtained by rapid cooling of the molten solid, is not truly crystalline, but possesses rather the characteristics of a glass. The X-ray photograph is of a simpler type than that of the  $\alpha$ - and  $\beta$ -forms and shows only a single diffuse side band (see Plate I).

The above results offer a satisfactory explanation of the complex melting phenomena of the triglycerides and also afford strong support for the views on alternation previously advanced.

*Preparation of the Triglycerides.*—The glycerides were made from highly purified acids and glycerol. Tricaprin and trimyristin were made by the method used by Miss Stephenson in the case of tripalmitin, *viz.*, the action of the acid chloride on glycerol in the presence of pyridine (*Biochem. J.*, 1913, **7**, 432). Trilaurin, tripentadecylin, tripalmitin, trimargarin, and tristearin were prepared by heating together 5–10% excess of the theoretical amount of the appropriate fatty acid with glycerol for 5–6 hours. For the first hour the temperature was slowly raised to 160° at ordinary pressure; it was then slowly raised to 180–190°, a vacuum being gradually applied up to 10–15 mm. (essentially the method of Scheij, *Rec. trav. chim.*, 1899, **18**, 169).

Both the above methods gave good yields, but we were unable to obtain satisfactory results from methods involving the use of tribromopropane and silver or sodium salts. We were able to confirm the observations of Bhattacharya and Hilditch (*Proc. Roy. Soc.*, 1930, *A*, **129**, 469) that the direct esterification is accelerated by the presence of aromatic sulphonic acids; e.g., in the presence of 1–2% of *p*-toluenesulphonic acid, palmitic acid and glycerol gave a good yield of tripalmitin after 2½ hours' heating at 150°, the pressure being reduced after one hour to 10–15 mm.

The crude glycerides were freed from excess fatty acid by two or three crystallisations from alcohol, in which the triglycerides other than tricaprin and trilaurin are sparingly soluble. Final crystallisations were as follows: Tricaprin from alcohol; trilaurin from alcohol–benzene; trimyristin and tripentadecylin from benzene; tripalmitin, trimargarin, and tristearin from toluene.

We gratefully acknowledge gifts of specimens of the somewhat inaccessible triundecylin and tritridecylin from Professor Verkade (cf. *Rec. trav. chim.*, 1932, **51**, 850), and of even-acid glycerides from Professor Watson (cf. Joclecar and Watson, *J. Indian Inst. Sci.*, 1930, **13**, *A*, XI, 119) which enabled us to carry out preliminary experiments.

*Thermal Examination.*—Most of the m. p. data of the triglycerides have been obtained by capillary-tube methods. The m. p.'s of the vitreous and of the stable  $\beta$ -form are easily obtained by cooling the molten glyceride rapidly and taking the m. p. in the usual manner. Occasionally there is an indefinite change in appearance at some intermediate temperature, but as a rule a

slightly different procedure is necessary in order to observe the middle m. p. clearly. The m. p. of the vitreous form is first determined by immersing the rapidly cooled glyceride in a bath at a temperature at which it just melts and resolidifies (glassy  $\rightarrow$   $\alpha$ -form). The middle m. p. is then determined in the same way, the bath temperature at which this form melts and resolidifies ( $\alpha \rightarrow \beta$  change) being found by trial. Finally, the highest m. p. is taken in the usual way.

We find that the explanation of the above difference in procedure lies in the fact that the metastable  $\alpha$ -form changes into the stable  $\beta$ -form at temperatures below its m. p., the velocity of the change increasing rapidly as the m. p. is approached. For instance, in the first of the above methods, the heat of crystallisation evolved in the change glassy  $\rightarrow$   $\alpha$ -form, together with the steady rise in the temperature of the bath, invariably brings about the  $\alpha \rightarrow \beta$  change before the m. p. of the  $\alpha$ -form is reached. Consequently, the latter is not observed.

In view of the fact that there does not appear to have been any systematic investigation of the cooling and heating curves of the glycerides, we decided to adopt this method for the determination of the m. p.'s. 0.75—1.0 G. of the triglycerides was taken in small jacketed tubes and heated or cooled in a Dewar flask containing water at the desired temperature, the temperature of the specimen being recorded every half minute by means of a copper-constantan thermocouple and a sensitive millivoltmeter.

The curves for trilaurin, AB, BC, Fig. 1, which are typical, show the m. p.'s of the  $\alpha$ - and  $\beta$ -forms by two well-marked arrests on BC. In this particular case, the first arrest gradually disappears if the specimen is kept at room temperature for some time, thus show-

FIG. 1.

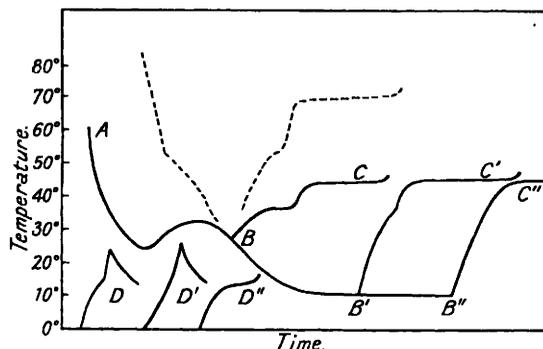
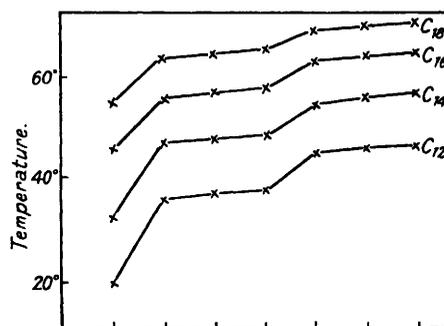


FIG. 2.



ing that the  $\alpha \rightarrow \beta$  change is occurring (B'C' and B''C'' were taken respectively after 1 and 3 hours' standing). Three changes are therefore taking place at the first arrest on BC, *viz.*,  $\alpha \rightarrow$  liquid,  $\alpha \rightarrow \beta$ , and liquid  $\rightarrow \beta$ , the last of these being responsible for the sharp rise in the curve to the second arrest.

The curves for glycerides of odd acids differ slightly in detail from the above owing to the fact that the changes  $\alpha \rightarrow \beta$  and liquid  $\rightarrow \beta$  are considerably slower. This is indicated by a much longer duration of the first arrest, and occasionally, if too high a temperature gradient is used, by complete melting at this point without indication of the second arrest.

The rapidity with which the  $\beta$ -modification is formed depends, not only upon whether the acid chains are even or odd, but also upon the length of the chain, *e.g.*, the  $\alpha$ -forms of trilaurin and tricaprln change into the  $\beta$ -forms with increasing velocity at room temperature, whereas at this temperature the  $\alpha$ -forms of myristin and higher members appear to be indefinitely stable.

This point is conveniently demonstrated by means of a polarising microscope. When a layer of molten glyceride is spread on a microscope slide, the  $\alpha$ -form separates first as spherulites, which between Nicols give characteristic uniaxial crosses. This form is therefore readily distinguished from the stable  $\beta$ -form which does not show this effect. Fig. 5, Plate I, is a microphotograph of the  $\alpha$ -form of trilaurin taken immediately after solidification, and Fig. 6 is the same specimen after  $\frac{1}{4}$  hour. The appearance has completely changed, and all the uniaxial crosses have disappeared. Fig. 7 on the same plate is a microphotograph of the  $\alpha$ -form of tristearin after several days' standing.

The curves D, D', D'', Fig. 1, for trilaurin illustrate the method of determination of the

PLATE I.

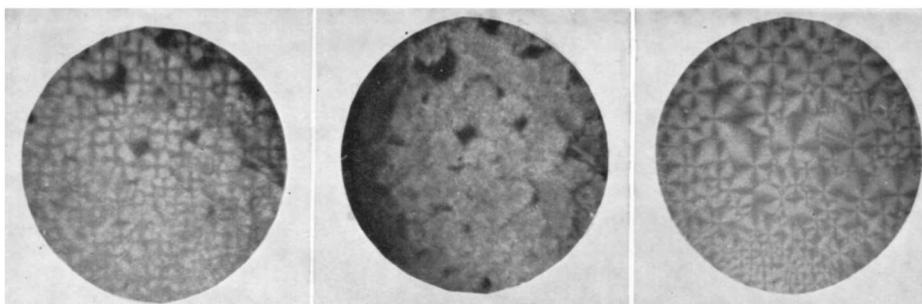


FIG. 5.  
*Trilaurin*  $\alpha$ .

FIG. 6.  
*Trilaurin*  $\beta$ .

FIG. 7.  
*Tristearin*  $\alpha$ .

*Propyl stearate* ( $\beta$ ).

„ „ ( $\alpha$ ).

*Trimyristin* ( $\alpha$ ).

„ ( $\beta$ ).

*Trimargarin* ( $\alpha$ ).

„ ( $\beta$ ).

„ ( $\beta$ ).

*Tripalmitin* ( $\beta$ ).

„ (*glass*).

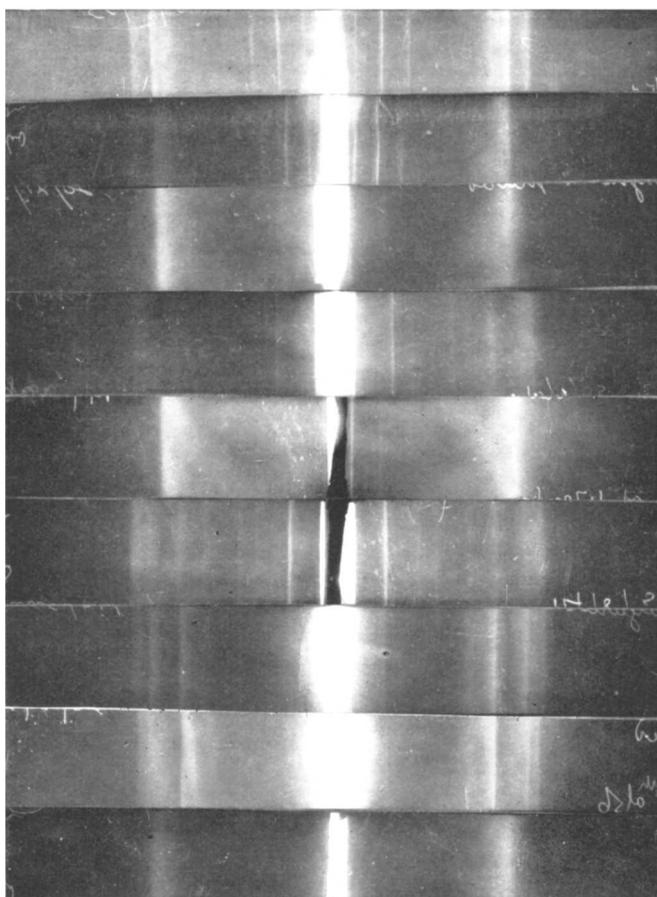


FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

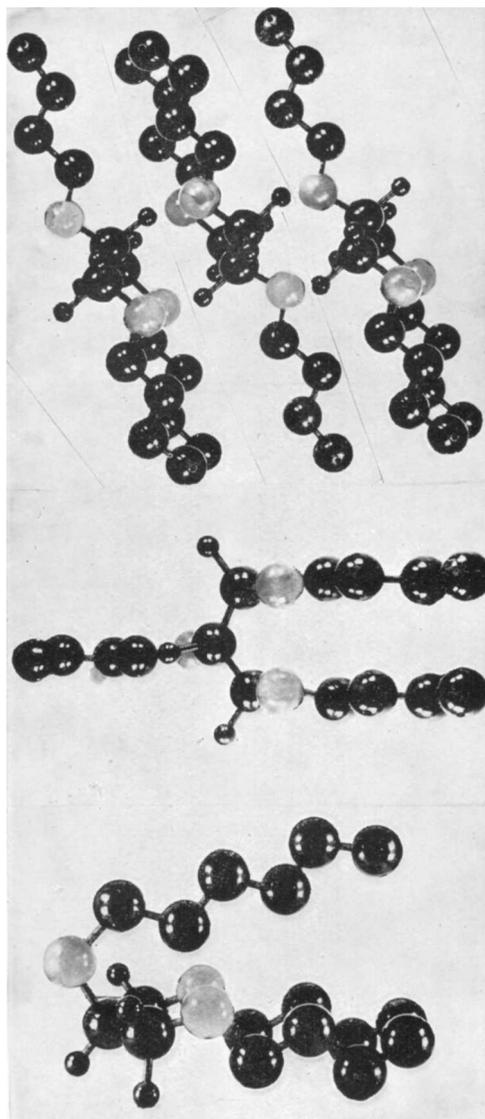
FIG. 13.

FIG. 14.

FIG. 15.

FIG. 16.

PLATE II.



*Grey spheres represent ethereal oxygen atoms of ester groups : ketonic oxygen and hydrogen atoms of acid chains are omitted. Small spheres represent hydrogen atoms of glycerol molecule.*

m. p.'s of the glassy forms. The molten specimen is rapidly cooled in solid carbon dioxide-ether, and placed in a bath at a temperature found by trial to be near the m. p. At temperatures too high, the curve rises rapidly past the temperature of the bath, without showing any marked discontinuity,  $D'$ , whilst at temperatures too low, there is a pronounced arrest before the temperature begins to rise,  $D''$ . The m. p. is taken as the intermediate temperature where the arrest is just sharply noticeable,  $D$ .

For glycerides with longer acid chains than trilaurin, a smaller degree of cooling will bring about solidification in the glassy form. For instance, ice-salt is suitable for glycerides of  $C_{13}$ ,  $C_{14}$ , and  $C_{15}$  acids, and ice for the higher members. The latter, in fact, solidify mainly to a glass if cooled to room temperature, giving curves of the type shown by the discontinuous lines in Fig. 1 (tristearin). Such curves are readily distinguished from those previously described by the coincidence of the setting and melting points, the absence of supercooling, and the unusually steep rise after melting. In these cases a smaller cooling gradient is required to ensure solidification in the  $\alpha$ -form.

The definite, though relatively small, heat absorption during fusion, shown by the curves for the glass, is in agreement with recent work on the specific heats of glasses (Gibson and Giaque, *J. Amer. Chem. Soc.*, 1923, **45**, 93; Parks and Huffman, *J. Physical Chem.*, 1927, **31**, 1843; Parks, Huffman, and Cattoir, *ibid.*, 1928, **32**, 1366; Tammann and Bandel, *Z. anorg. Chem.*, 1930, **192**, 129, 193), and is incompatible with the view that a glass is merely a supercooled liquid. The difference between the crystalline and the glassy state appears to be that, in the former, the molecules have attained a state of minimum potential energy by an ordered neutralisation of the binding forces, whereas in the latter these forces are only partially and irregularly neutralised. In other words, the molecules in a glass have the same disorder as in the liquid state, but have neither the kinetic energy to overcome the attractive forces, nor the mobility to move into a position of minimum potential energy.

Dispersed throughout the glass will be a number of minute crystalline aggregates (nuclei, crystallites), owing to the fact that in the liquid immediately prior to cooling, a certain small proportion of the molecules (dependent on the temperature) occupy positions suitable for crystal formation (cf. Randall, Rooksby, and Cooper, *Z. Krist.*, 1930, **75**, 196).

From the preceding account, it is clear that the nature of the solid separating from a molten glyceride depends on the initial temperature of the liquid, the rate of cooling, and the length of the acid chains. Generally, with temperatures only slightly higher than the m. p. and very slow cooling,  $\beta$ -separation occurs; with higher initial temperatures and rather quicker cooling,  $\alpha$ -separation; and rapid cooling causes glass formation. Of the two crystalline forms, the  $\alpha$ -invariably separates first, provided, of course, that the liquid be maintained below the m. p. of this form. Variation of conditions of cooling results in the separation of mixtures of the various forms, and this we consider is responsible for the results of Weygand and Grünzig (*Z. anorg. Chem.*, 1932, **206**, 304), who claim to have found seven different forms of the triglycerides. Since we find no confirmation of this complexity from our thermal and X-ray work, and since, moreover, details of the determination of the m. p.'s of the different forms are lacking, it will be sufficient to reproduce their m. p.'s in graph form (Fig. 2). These clearly indicate the existence of three main solid modifications.

The m. p.'s obtained by us are given in the following table and plotted in Fig. 3. Values obtained by the more recent workers (*loc. cit.*) are also given. Joclecar and Watson term

	Othmer.			Loskit.			Watson.		Present authors.		
	I.	II.	III.	I.	II.	III.	I.	II.	$\beta$ -.	$\alpha$ -.	Glass.*
Tristearin .....	70·8°	64·5°	54·5°	71·8°	65·4°	55°	71·8°	55°	71·5°	65·0°	54·5°
„ margarin .....									63·5	61·0	50
„ palmitin .....				64·8	55·0	45·4	65·6	46·2	65·5	56·0	45
„ pentadecylin...									54·0	51·5	40
„ myristin .....	55	41·8	32·1	56·5	48·5	47·0	56·5	33	57·0	46·5	33
„ tridecylin .....									44·0	41·0	25
„ laurin .....	44·3	34		46·4		36·4	46·2	18	46·4	35·0	15
„ undecylin .....									30·5	26·5	1·0
„ caprin .....				31·0		25·0	31·6		31·5	18·0	-15

\* These temperatures are not, of course, true m. p.'s, but are the mean of a small melting range. They usually vary by  $\pm 1^\circ$ .

the forms, here given as I and II, the  $\alpha$ - and  $\beta$ -forms; they correspond to our  $\beta$ - and glassy forms respectively.

**X-Ray Investigation.**—The X-ray photographs were taken on a Müller spectrograph, using a Metalix X-ray tube with a copper anticathode (Cu,  $K_{\alpha}$ ,  $\gamma = 1.54 \text{ \AA}$ .), running at  $\approx 8 \text{ mA}$ . The distance of the specimen from the plate was 6 cm.

The pure glycerides, after crystallisation from solvents, were pressed or melted into a thin layer on a glass strip ("pressed" and "melted" layers) and rotated through the X-ray beam. Exposures of 1 and 2 hours were usually necessary for the pressed and melted layers respectively. The former give the spacings of the stable  $\beta$ -form, and the latter give rise to the spacings of the  $\alpha$ - or vitreous form, according to the rate at which the layer is allowed to cool. With the exception of a few of the more rapid transitions of the lower glycerides, changes of form can be followed by means of the X-rays; e.g., a rapidly cooled melted layer of tripalmitin gives the one diffuse side band of the glass (Fig. 16, Plate I); if it is maintained for a few minutes slightly above the m. p. of the glass, the spacings of the  $\alpha$ -form appear; and if, finally, it is maintained for some time at a temperature slightly below the m. p. of the  $\beta$ -form, the spacings of this form take the place of those of the  $\alpha$ -. In this way, the identity of the forms involved in the thermal changes

FIG. 3.  
Melting points of the triglycerides.

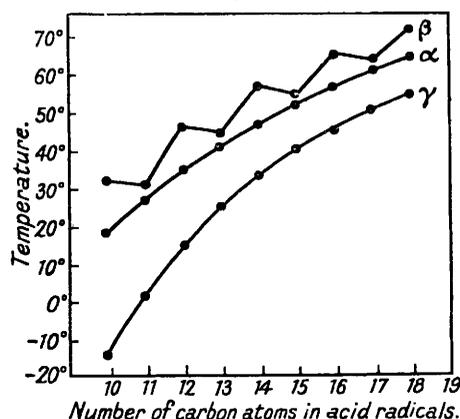
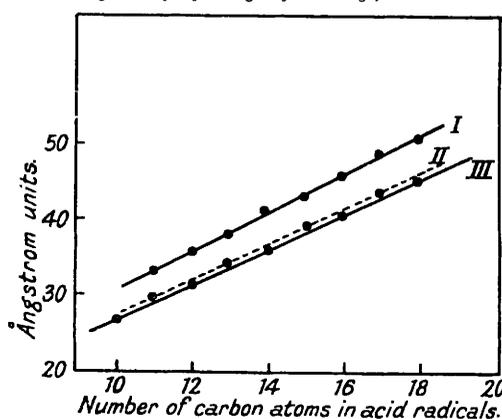


FIG. 4.  
Long X-ray spacings of the triglycerides.



has been established. The long spacings of the  $\alpha$ - and  $\beta$ -forms are given in the following table and plotted in Fig. 4. The side spacings are given in the succeeding table.

Long spacings of the triglycerides ( $\text{\AA}$ .U.).

No. of C atoms in acid	10	11	12	13	14	15	16	17	18
Stable $\beta$ -form	26.8	29.6	31.2	34.1	35.8	38.9	40.6	43.5	45
Unstable $\alpha$ -form *	—	33.0	35.6	37.7	41.2	42.9	45.6	48.5 †	50.6
Vitreous form	No long spacings.								

\* As a rule only two orders appear for this form, and consequently the data are not quite so accurate as those for the  $\beta$ -forms. We consider the error to be less than  $\pm 0.3 \text{ \AA}$ .U.

† The only datum in the literature on synthetic glycerides is a measurement on trimargarin by Trillat, who found  $48.0 \text{ \AA}$ .U. No details were given (*Compt. rend.*, 1925, **180**, 1838).

Side spacings of the triglycerides ( $\text{\AA}$ .U.).

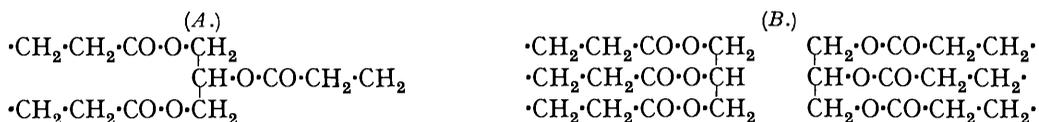
$\beta$ -Form, even acids	3.7 *	3.9 *	4.6	5.3
$\beta$ -Form, odd acids	3.65 *	4.0 *	4.6	5.3
$\alpha$ -Form, and glass		4.2		

\* Plate I, Figs. 14 and 15, shows the slight difference in these two side spacings.

The long spacings fall on three straight lines, the two lower (Fig. 4, II and III) representing the  $\beta$ -forms (odd and even acids), and the upper and steeper line, I, representing the  $\alpha$ -forms. The spacings are much too long to correspond with the length of the acid component, but on the usual assumption of a tetrahedral zig-zag carbon chain, they agree with a molecule of twice this length, lying, in the case of the  $\alpha$ -forms, vertically across the terminal planes, and in the case of the  $\beta$ -forms, inclined at an angle of  $\approx 65^\circ$  to these planes.

The side spacings of the  $\beta$ -form indicate a more complicated structure than is found for most long-chain compounds. In the latter cases, two main side spacings usually appear of 3.7 and 4.2 Å.U. (Plate I, Fig. 8, propyl stearate). Of the four spacings found, one is identical with the spacing of 3.7 Å.U., but the 4.2 Å.U. spacing is absent. When, however, the glyceride is in the  $\alpha$ - or the glassy form, this characteristic spacing of 4.2 Å.U. is now the only one observed (cf. Plate I, Figs. 9, 10, 12, 16). Since the appearance of this single side spacing is now regarded as evidence of rotating carbon chains (see Malkin, *Trans. Faraday Soc.*, *loc. cit.*, for references), this result throws an interesting light, not merely on the structure of the  $\alpha$ - and glassy forms, but also on that of the liquid, in all of which cases it would appear that the long chains are rotating about the oxygen linkages. It is somewhat difficult to visualise rotating chains in the glassy state, where the characteristic property of the molecules is lack of mobility, but in these cases, and in that of the apparently stable  $\alpha$ -forms, the probability is that the chains are "frozen" in the position occupied by rotating chains.

Although it is not possible to deduce an absolute structure of the triglycerides on the basis of the above simple measurements, one which is, at least, highly probable may be suggested. From a consideration of the various factors, the problem appears to resolve itself mainly into a decision between the two arrangements (*A*) and (*B*). Trillat (*Metallog.*, 1930, 9, 1023) has proposed the structure (*B*) on the evidence of the length of the molecule, but on the following grounds we consider (*A*) the more probable.



(1) The structure proposed explains the slightly greater length and lower m. p.'s of the glycerides of odd acids (*i.e.*, alternate loosely and tightly packed terminal methyl planes, Malkin, *loc. cit.*).

(2) In agreement with the data, the distance contributed by the glycerol group to the length is a minimum. This distance is known from the intercept of the curves (Fig. 4) on the ordinate at 0 carbon atoms. Since the spacings are plotted against carbon atoms in the *acid chain*, the intercept is the distance taken up by the glycerol radical and the terminal hydrogen atoms. This intercept is of the order of 4.5 Å.U. It seems almost impossible to accommodate two glycerol molecules (as in *B*) in this distance, even if the space occupied by the terminal hydrogen atoms is neglected.

(3) Arrangement (*A*) requires practically no deviation from the normal tetrahedral arrangement of all the atoms concerned, whereas large deviations are necessary in order to align the three chains together. It may be objected that, in unimolecular films, the three chains are so aligned (Adam, *Proc. Roy. Soc.*, 1922, *A*, 101, 461). We see, however, no necessary parallel, and consider that the polar attraction of the water and the mechanical compression are sufficient to cause the necessary deviation of the oxygen linkages. The models shown in Plate II, which are constructed so as to involve the minimum deviation from tetrahedral angles, illustrate this point. In *A* (1 and 2), the chains are parallel, and the structure forms a very symmetrical unit suitable for crystal building. In *B*, however, the upper chain requires to be forced down considerably in order to bring the chains into alignment. It will be noticed also, that the effective length of this upper chain is less than that of the two lower; this would introduce irregularities at the methyl ends of the chains.

It should, however, be emphasised that a full solution of the structure must await detailed *X*-ray analysis of the type carried out by Müller on single crystals of paraffins and fatty acids.

The investigation is being extended to the mono- and di-glycerides.

Grants from the Government Grant Committee of the Royal Society and the Colston Society are gratefully acknowledged.