487. *Infrared Spectra and the Polymorphism of Glycerides. Part II.* 1 : 3-*Diglycerides and Saturated Triglycerides.*

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The infrared spectra of the liquid and the polymorphic forms of some even-membered 1 : 3-diglycerides and even-membered saturated triglycerides have been studied : they enable deductions to be made about the transitions between the polymorphic forms and about their structure. The spectra obtained for the lowest-melting forms of the triglycerides provide a new criterion for the identification of these forms about which considerable controversy exists.

IN the earlier paper on monoglycerides,* it was pointed out that infrared spectra provide a useful means for detecting and following polymorphic transitions. This method has now been applied to the examination of the polymorphic transitions of di- and tri-glycerides.

The literature records considerable study of the polymorphism of these glycerides, by thermal techniques and particularly by *X*-ray powder photography. Although considerable information has thus been obtained,[1] there is still controversy over the correlation of melting points of the triglycerides with the short spacings in their polymorphic forms. Amongst other difficulties the short spacings for various polymorphic forms may not differ greatly.

EXPERIMENTAL

The diglycerides were prepared by treating the acid chlorides (from pure fatty acids) with pure glycerol according to Rose's method.[2] They were recrystallised from ether and then a number of times from hexane; specimens used were 1 : 3-dilaurin, m. p. 56·5°; 1 : 3-dipalmitin, m. p. 73·5°; and 1 : 3-distearin, m. p. 78·7°.

The triglycerides were prepared from pure fatty acids and pure glycerol at 180—200° in the presence of 0·2% of stannous chloride. The crude product was melted and shaken with hot alcohol a number of times to remove the bulk of the free fatty acid and mono- and di-glycerides, the remainder of which was removed by stirring the molten glyceride with hot 0·1N-sodium hydroxide solution and finally with hot distilled water. The resulting triglyceride was dried *in vacuo* and crystallised from toluene. Thus were obtained trilaurin, m. p. 46·4°, tripalmitin, m. p. 65·7°, and tristearin, m. p. 72·5°.

The spectrometer was a Grubb-Parsons S.3 double-beam spectrometer with a rock-salt prism; the glycerides were examined in capillary thicknesses between rock-salt flats; and the spectra were obtained between 3500 and 650 cm.$^{-1}$. A heated infrared cell was used for the liquid forms and for those polymorphic forms which occurred during heating or cooling; and the temperature of the material was determined within $\pm 1°$ by means of a calibrated thermocouple. The spectra of the stable β-forms were also obtained by dispersing the known β-form crystalline materials in Nujol. *X*-Ray and m. p. data were used to confirm the identity of the β-forms of both di- and tri-glycerides.

RESULTS AND DISCUSSION

1 : 3-*Diglycerides.*—The polymorphism of simple diglycerides was first investigated by Malkin, Shurbagy, and Meara,[3] who stated they existed in α-, β′-, and β-forms. Baur, Jackson, Kolp, and Lutton [4] later observed the β-forms for dipalmitin and distearin, but not an α-form. However, Malkin states that, owing to the rapidity of the transition $\alpha \longrightarrow \beta'$, *X*-ray spectra for only the β′- and the β-forms have been recorded but that the identity of the low-melting form as an α-form has been inferred by analogy with the behaviour of other glycerides and by the fact that the melting points are non-alternating.[1]

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* Part I, *J.*, 1956, 55.

[1] Malkin, " Progress in Chemistry of Fats and other Lipids," Vol. II, Pergamon Press Ltd., London, 1954.

[2] Rose, *J. Amer. Chem. Soc.*, 1947, **69**, 384.

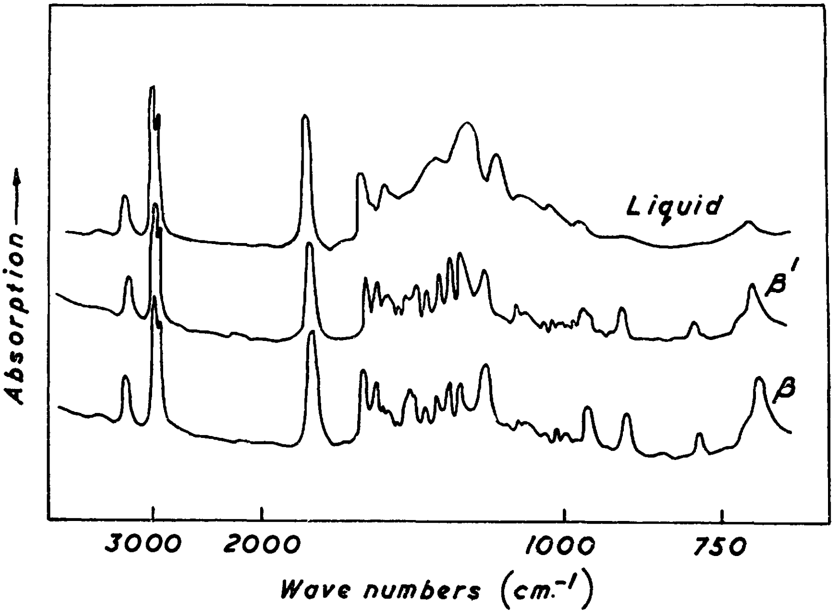
[3] Malkin, Shurbagy, and Meara, *J.*, 1937, 1409.

[4] Baur, Jackson, Kolp, and Lutton, *J. Amer. Chem. Soc.*, 1949, **71**, 3363.

The infrared spectra of liquid, β′-, and β-1 : 3-dilaurin are shown in Fig. 1. No evidence for the existence of an α-form was obtained. A band at 3487 cm.$^{-1}$ is assigned to O–H stretching of the secondary alcohol group. The free unbonded hydroxyl group band usually occurs between 3700—3500 cm.$^{-1}$, so the O–H group in this case does not appear to be very strongly hydrogen-bonded. The carbonyl frequency occurs at 1739 cm.$^{-1}$, whilst a band at 1418 cm.$^{-1}$ is assigned to a C–H bending vibration. A band at *ca.* 1164 cm.$^{-1}$ may be assigned to C–O stretching vibration of the CO_2R group, whilst a band at 720 cm.$^{-1}$ may be assigned to a CH_2 rocking mode.

When liquid 1 : 3-dilaurin cools, the first to crystallise is the β′-form. The spectrum is typical of that obtained with normal crystalline material. No evidence for an α-form was observed. (This is consistent with the work of Crowe and Smyth [5] who observed, using dielectric measurements, that virtually no freedom of orientation existed in the solid first formed from the liquid with either 1 : 3-distearin or 1 : 3-dipalmitin.) The O–H stretching frequency in the β′-spectrum is at 3430 cm.$^{-1}$, a slight shift from that in the liquid, whilst the carbonyl stretching frequency shifts to 1722 cm.$^{-1}$. The carbonyl group therefore appears to be more hydrogen-bonded here than in the liquid state, which is the reverse of the hydrogen-bonding which occurs in 1-monoglycerides. The band near 1420 cm.$^{-1}$ increases considerably in intensity relative to the C–H bending vibration band at 1470 cm.$^{-1}$.

FIG. 1. *The polymorphic forms of* 1 : 3-*dilaurin.*



A number of sharp bands spaced at approximately regular intervals of 30 cm.$^{-1}$ occur in the region between 1210 cm.$^{-1}$ and 1330 cm.$^{-1}$, which may be assigned to wagging modes of methylene groups by analogy with similar bands in fatty acids, alkyl halides, monoglycerides, etc.

A band at 1185 cm.$^{-1}$ is presumably a shifted C–O stretching frequency. The band at *ca.* 940 cm.$^{-1}$ can be associated with the secondary alcohol group present in the molecule by analogy with the assignment of a band in this region in 2-monoglycerides. A band at 717 cm.$^{-1}$ is probably due to a CH_2 rocking mode.

When the β′-form is heated near its melting point or crystallised from a solvent, the stable β-form is obtained (and confirmed by the *X*-ray pattern). The spectrum of the

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[5] Crowe and Smyth, *J. Amer. Chem. Soc.*, 1950, **72**, 5281.

β-form is very similar to, but possesses some features different from, that of the β-spectrum. The O–H stretching absorption band is now split into two components, at 3487 and 3429 cm.$^{-1}$, and there are also two carbonyl stretching frequencies, at 1727 and 1709 cm.$^{-1}$. That there are two carbonyl frequencies may be due to the fact that one carbonyl group is hydrogen-bonded differently from the other, or to the site and unit-cell symmetry. A band near 1420 cm.$^{-1}$ is still prominent but the intensity of the components at 1389, 1376, and 1350 cm.$^{-1}$ is considerably reduced in comparison with similar bands in the β′-spectrum. A new band occurs at 1314 cm.$^{-1}$ but the bands assigned to CH_2 wagging modes are still present in the β-spectrum. Occasional reversal of the intensities of the bands at 1185 and 1139 cm.$^{-1}$ was shown to arise from orientation effects of the crystals. The remainder of the spectrum from 1139 to 717 cm.$^{-1}$ is very similar in both forms.

Similar differences were observed between the spectra of the polymorphic forms of the other diglycerides examined.

Saturated Triglycerides.—This group of glycerides is the most complete homologous series of glycerides that has been studied by thermal and *X*-ray techniques. Clarkson and Malkin [6] have reported that triglycerides exist in four forms—vitreous, α, β′, and β—in order of increasing melting point and stability. The vitreous form obtained by rapid solidification was considered to possess the characteristics of a glass rather than of a true crystal. The α-form was characterised by both its vertical long spacings and its lack of alternation of melting point from odd to even members. The β′-form and the β-form, which show an alternation of melting point, were found to possess tilted chains, the latter at an angle of approximately 65°. Lutton [7] agreed with these findings in the case of the stable β-forms, but found that the lowest-melting form designated vitreous by Clarkson and Malkin gave a diffraction pattern corresponding to the α-form, and that the form designated by Clarkson and Malkin as the α-form gave the β′-pattern. Also, Lutton [7] did not find a glassy or a vitreous form, or a melting point corresponding to that of Malkin's β′-form. An investigation by Crowe and Smyth [5] of the dielectric behaviour of triglycerides in general confirmed Lutton's work by showing that the α-form of tripalmitin and tristearin possesses dipole orientational freedom down to relatively low temperatures; further, the dispersion regions for the triglycerides were different from those usually encountered in crystalline compounds and organic glasses. The results of microscopic work by Quimby [8] also support Lutton's views. Malkin,[1] however, does not accept the interpretation by these American workers, and has recently defended the case for the existence of a vitreous form of triglycerides.

The difficulty attendant on the use of the *X*-ray technique is shown by the fact that according to Clarkson and Malkin [5] the so-called "vitreous" and α-forms give rise to the same side spacings, and it is impossible to state whether or not a specimen giving the α-form spacings contains any vitreous form. It is suggested that a specimen predominantly "vitreous" gives an *X*-ray powder photograph little different from that of an α-form. (Further, to add to the difficulty, the strongest lines given by the α-form and the β′-form are practically the same, 4·19 and 4·22 Å respectively.) It was thought that the infrared spectra of the different polymorphic triglycerides might be sufficiently distinctive to clarify the problem; also, with a specimen which is predominantly vitreous the spectrum would be mainly that of the vitreous form rather than that of the smaller amount of strictly crystalline material present, so that one of the difficulties inherent in the *X*-ray method would be eliminated.

The spectrum obtained for liquid tripalmitin (Fig. 2) is similar in general appearance to that previously obtained [9] for tripalmitin in solution. The carbonyl band occurs at 1739 cm.$^{-1}$ and a band found in all glycerides occurs at 1418 cm.$^{-1}$. A very strong band at *ca.* 1160 cm.$^{-1}$ is assigned to the stretching vibration of the C–O contiguous to the C=O group. A strong band at 720 cm.$^{-1}$ may be assigned to a methylene rocking mode.

When the liquid was rapidly cooled to 0°, a change in the spectrum was observed. This

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[6] Clarkson and Malkin, *J.*, 1934, 666; 1948, 985.

[7] Lutton, *J. Amer. Chem. Soc.*, 1945, **67**, 524.

[8] Quimby, *ibid.*, 1950, **72**, 5064.

[9] O'Connor, Dupre, and Feuge, *J. Amer. Oil Chemists' Assoc.*, 1952, **29**, 261.

new spectrum is shown in Fig. 2 and is assigned in this paper to the α-polymorphic form, m. p. 45·0°. In comparison with the spectrum for liquid tripalmitin, there are shifts of bands and new bands appear. The band originally at 1418c m.$^{-1}$ moves to 1410 cm.$^{-1}$, and the 1372 cm.$^{-1}$ band assigned to symmetrical bending in methyl groups is now less broad. A number of sharply defined new bands between 1335 and 1190 cm.$^{-1}$ occur at intervals of the order of 20 cm.$^{-1}$, and can be assigned to wagging modes of methylene groups. The strong band associated with a C–O stretching vibration at 1160 cm.$^{-1}$ in the

FIG. 2. *The polymorphic forms of tripalmitin.*

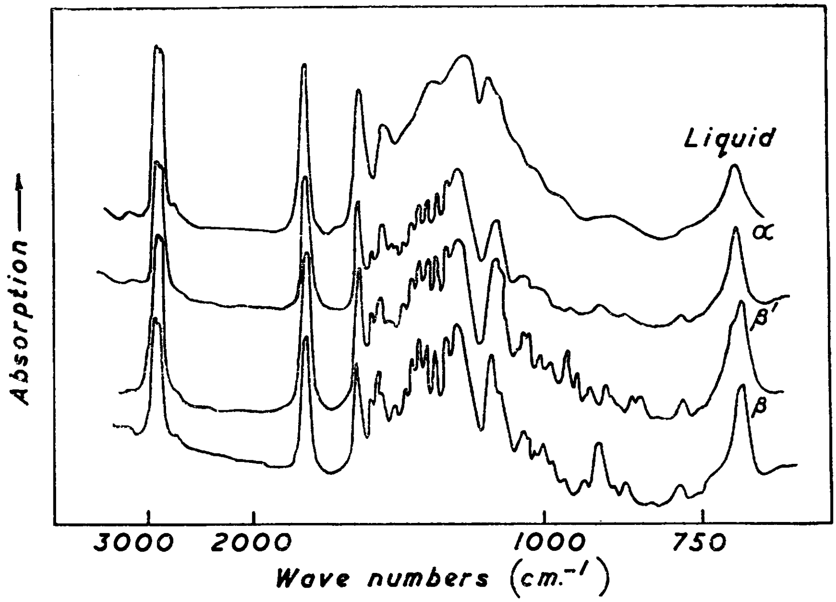
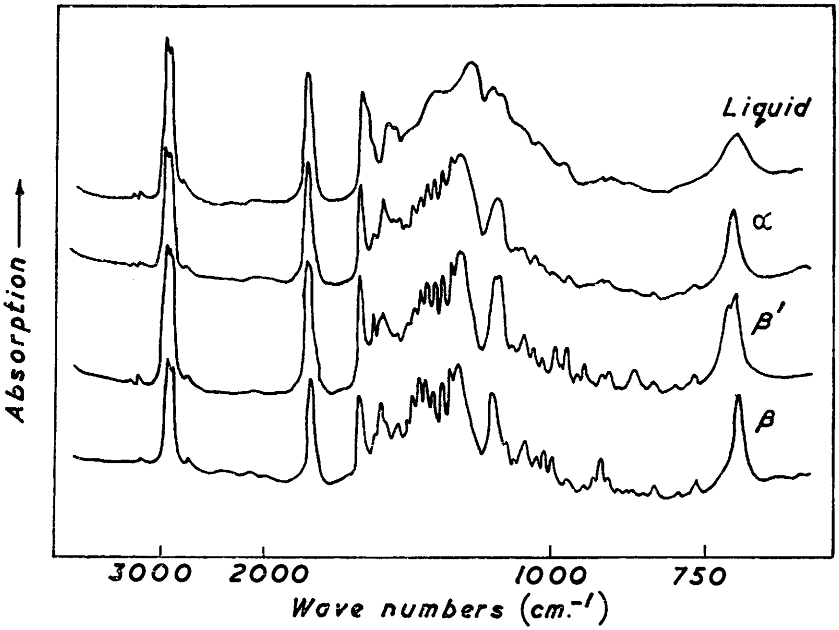


FIG. 3. *The polymorphic forms of tristearin.*



liquid is now found at 1172 cm.$^{-1}$, whilst the strong band at 1112 cm.$^{-1}$ in the liquid form has moved to 1100 cm.$^{-1}$. The 720 cm.$^{-1}$ band assigned to a methylene rocking mode remains strong and single (cf. the α-form of 1-monoglycerides). A number of weak bands between 1100 and 720 cm.$^{-1}$ were observed; these vary in intensity from sample to sample and are considered to arise from the presence of small amounts of the higher polymorphic form.

It is to be noted that Brown and Sheppard [10] found that alkyl halides and lower alcohols, when cooled to their freezing point, gave glasses recognised by their high transmission and by the considerable similarity of their infrared spectra to that of the liquid. Since, with tripalmitin, bands shift position and new bands occur it is logical to deduce that this form is not glassy or vitreous, but that it is more probably an α-form. (Further attempts were made to investigate the " vitreous " form by plunging melts of tristearin or tripalmitin, enclosed between thin rock-salt plates, into liquid air. A polarising microscope showed the presence of tiny crystals. The infrared spectrum obtained was similar to that designated as the α-form, Fig. 2.) However, whether this form is in fact vitreous or not, the infrared spectrum associated with the form having m. p. 45° provides a new criterion for identification of this polymorphic form.

When this form was heated at 45°, rapid transition to the stable β-form was observed (of. Crowe and Smyth [5]). (The new spectrum was similar to that obtained by dispersing the known crystalline β-form in Nujol and the transformed material also melted at the m. p. of the β-form.)

When the liquid triglyceride was cooled slowly and kept above the melting point of the lowest-melting form (45°) for some time (depending on the chain length) a change occurred in the spectrum thus showing the presence of a different polymorphic form. This spectrum is designated β′ in Fig. 2 to agree with the nomenclature proposed by Lutton [7] (although it is questionable whether this nomenclature is the best, in view of a different form also designated β′ by Malkin). This spectrum is similar to that of the α-spectrum but with a number of important differences, notably in the 720 cm.$^{-1}$ region. Whereas a single band occurs at 720 cm.$^{-1}$ in the spectrum of the α-form, there are two components at 725 cm.$^{-1}$ and 718 cm.$^{-1}$ in the β′-spectrum. From this it seems reasonable to deduce that a change of site symmetry of the glyceride molecules in the crystal has taken place. Stein and Sutherland [11] showed that a doublet in this region occurs in *n*-paraffin and Polythene only when these molecules are in a certain crystalline state. Further, the infrared spectra obtained with other molecules changing from an α-form with hexaganol spacings to a more stable modification of different crystalline form shows precisely this change in the methylene rocking band near 720 cm.$^{-1}$. Thus in the spectra of hydrocarbons,[12] ethyl esters,[12] long-chain alcohols,[13] and 1-monoglycerides [14] a single band at 720 cm.$^{-1}$ is observed when these molecules are in the α-form. Below the transition temperature, in the more stable modification, *i.e.*, the β-form for hydrocarbons, ethyl esters, and long-chain alcohols, and the sub-alpha-form for the 1-monoglycerides, two components of this band are observed. It is tempting, therefore, to stretch the analogy to the simple triglycerides, and conclude that this is additional evidence to support the view that this form (m. p. 56·0°) is not the α-form suggested by Malkin. The melting points of this form in the homologous series do not alternate, however, and this form might be expected to possess vertical rather than tilted chains.[1] Nevertheless, the overall evidence is in favour of Lutton's view rather than Malkin's. Other changes which occur in the β-spectrum are that an additional band appears at 1385 cm.$^{-1}$, the splitting of the bands at 1103 and 1095 cm.$^{-1}$ is now increased, and a number of sharp bands appear in the range between 1060 and 778 cm.$^{-1}$ which may be due to methylene rocking mode vibrations.

When the β′-form was heated to 56° and the spectrum was scanned rapidly as the temperature increased, the material was seen to melt and then change rapidly at this

[10] Brown and Sheppard, *Discuss. Faraday Soc.*, 1950, **9**, 146.
[11] Stein and Sutherland, *J. Chem. Phys.*, 1954, **22**, 1993.
[12] Robert and Favre, *Compt. rend.*, 1952, **234**, 2270.
[13] Chapman, unpublished work.
[14] *Idem*, *J.*, 1956, 55.

temperature into a form possessing a spectrum corresponding to that of the stable β-form. Further heating to the m. p. of the β-form (65·7°) and scanning provided no evidence for an intermediate form with melting point between 56·0° and 65·7°. Since the supposed intermediate form (cf. Malkin [1]) in even-acid members is said to be fleeting the above observation does not necessarily preclude its existence. The stable β-spectrum is shown in Fig. 2. It is different from the β'-spectrum in a number of ways. The 1374 cm.$^{-1}$ band in the latter is now reduced in intensity relative to the 1385 cm.$^{-1}$ and there is a change in the relative intensities of the bands (cf. the spectra of the different crystal forms of fatty acids in this region [15]) attributed to methylene wagging modes. The 1266 cm.$^{-1}$ band is now stronger in intensity than the 1245 cm.$^{-1}$ band, and the 1218 cm.$^{-1}$ band is reduced in intensity relative to the latter. A new band occurs at 1157 cm.$^{-1}$ on the side of the 1175 cm.$^{-1}$ band, and the band at 1100 cm.$^{-1}$ in the β'-spectrum is now split into three components, at 1110, 1099, and 1088 cm.$^{-1}$. A new band of medium intensity occurs at 890 cm.$^{-1}$. Only one band now occurs at a frequency of 717 cm.$^{-1}$ (cf. the β-spectrum of 1-monoglycerides [14]). This implies that the site symmetries of the β'- and the

FIG. 4. *The conversion of tristearin β'- ——➤ β-form.*

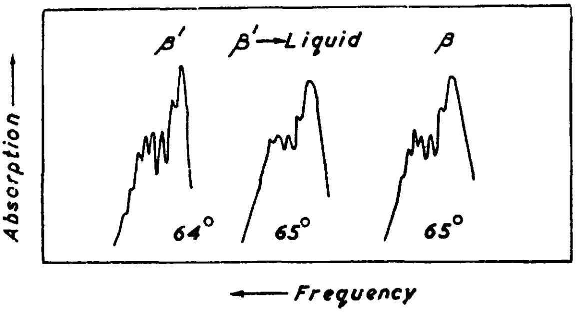
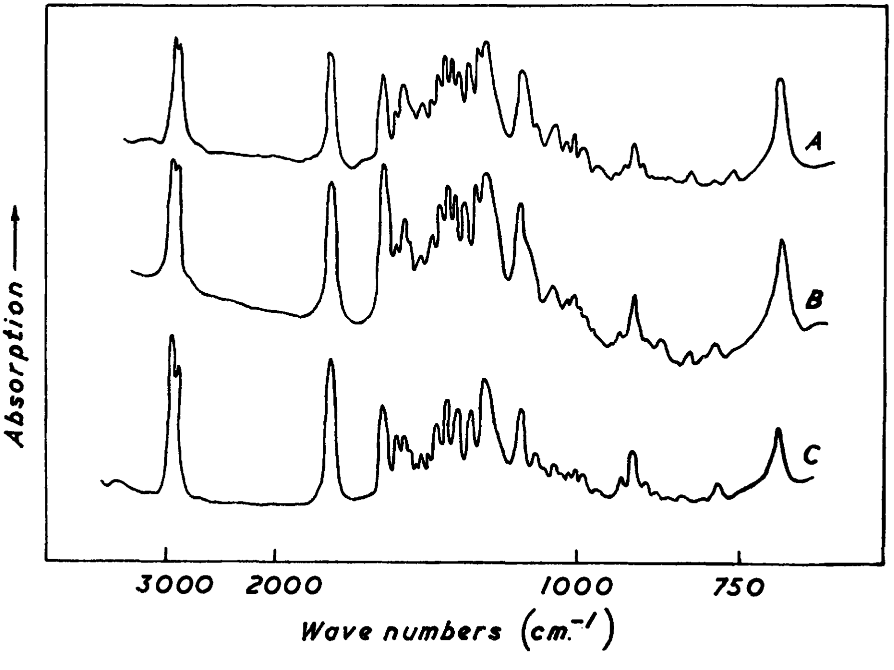


FIG. 5. *The β-forms of the triglycerides (A, tristearin; B, tripalmitin; C, trilaurin).*



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[15] Sydow, *Acta Chem. Scand.*, 1955, **9**, 1119.

β-form are also different. The carbonyl frequencies are the same in both the β′- and the β-form.

Tristearin and Trilaurin.—Tristearin gave results similar to those obtained with tripalmitin (Fig. 3). In this case the α-form changed at 54·5° into the β-form. A part of the spectrum rapidly scanned at different temperatures is shown in Fig. 4, whereon the transition from β′- to β-form with the fleeting intermediate liquid transition at 65° (the β′-melting point) is apparent. With trilaurin, however, the spectrum of the α-form could not be obtained in the normal manner since the melting point (15°) was below the temperature inside the spectrometer, but the spectra of the β′-form (m. p. 35°) and the β-form (m. p. 46·4°) were obtained. The spectra of the stable β-forms of the triglycerides examined are shown in Fig. 5.

The present investigation has shown that infrared spectra can provide an important new means for the identification of the different polymorphic forms of glycerides. It has the following advantages : (*a*) Only thin samples are needed for an adequate absorption spectrum. This is important since the low thermal conductivity of glycerides makes it difficult to obtain homogeneous metastable forms except in thin layers. (*b*) Rapid scanning of the spectrum and graphical recording enable spectra to be obtained whilst heating or cooling the sample. This enables direct observation to be made of transformations whilst they are occurring.

The technique is being extended to a study of the polymorphism of unsaturated glycerides.

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