12. *Infrared Spectra and the Polymorphism of Glycerides. Part I.*

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The infrared spectra of the different polymorphic forms of some 1- and 2-monoglycerides have been obtained between 3500 and 650 cm.$^{-1}$. Information about the polymorphic transitions and about the molecular structure of the polymorphic forms has been obtained, *e.g.*, the stability of the α-form and the crystallinity of the sub-α-form of the 1-monoglycerides.

Although it is known that abrupt changes can occur in the infrared spectra of the crystalline phase when polymorphic transitions occur (Kendall, *Analyt. Chem.*, 1953, **25**, 382; Tyler and Ehrhardt, *ibid.*, p. 390; Keller and Halford, *J. Chem. Phys.*, 1949, **17**, 26), little use has been made of this to investigate polymorphic transitions themselves, in molecules such as fatty acids, esters, and glycerides. The present paper reports an investigation of the polymorphism of some monoglycerides carried out by means of infrared spectra.

Whilst infrared spectra of some monoglycerides have been reported (Kuhrt, Welsh, Blum, Perry, and Weber, *J. Amer. Oil Chemists' Soc.*, 1952, **29**, 261; Barcelo and Martin, *J. Phys. Radium*, 1954, **5**, 403; O'Connor, Dupre, and Feuge, *J. Amer. Oil Chemists' Soc.*, 1955, **32**, 88), none of this work was concerned with the various polymorphic forms, and the spectra given there are mainly solution spectra or those of the stable β-form. Up to the present, spectra of 2-monoglycerides have not been reported.

Experimental.—Samples of 1-monostearin (m. p. 81°), 1-monopalmitin (m. p. 77°), and 1-monolaurin (m. p. 63°) were obtained by the action of the corresponding acyl chloride (from pure fatty acids) on redistilled *iso*propylideneglycerol and recrystallised slowly from acetone or ether–acetone by Mr. M. Dallas of this laboratory. The samples of 2-monolaurin (m. p. 51·0°), 2-monomyristin (m. p. 61·3°), 2-monopalmitin (m. p. 68·5°), and 2-monostearin (m. p. 75·5°) were presented by Dr. A. G. Tallentire of Bristol University.

The infrared spectrometer was a Grubb-Parsons S.3 double-beam spectrometer with a rock-salt prism. The spectra were obtained between 3500 and 650 cm.$^{-1}$. A heated infrared cell was used for the investigation of the liquid and for the polymorphic transitions which occur on heating or cooling. A thermocouple was arranged and calibrated so that the temperature of the material could be determined to $\pm 1°$. Capillary thicknesses of the glycerides between rock-salt flats were investigated. The β'- and the β-forms of 1-monoglycerides were obtained by rapid and slow crystallisation from light petroleum respectively. The spectra of these forms were obtained by dispersion in Nujol. The identities of the sub-α, β'-, and β-forms were confirmed by m. p. and X-ray data. The β-forms of the 2-monoglycerides were obtained by crystallisation from the melt and also by solvent crystallisation.

Results and Discussion.—1-*Monoglycerides.* The pioneer work of Malkin and Shurbagy (*J.*, 1936, 1628) showed that 1-monoglycerides exist in three different solid modifications, *viz.*, a low-melting α-form and two higher-melting forms, β' and β. The α-form is thought to be one in which the chains are vertical and rotating. The X-ray powder photograph of this form shows only one strong side spacing at 4·12 Å and it is uniaxial. Hydrocarbons (Müller, *Proc. Roy. Soc.*, 1932, *A*, **138**, 514), alcohols (Bernal, *Z. Krist.*, 1932, **83**, 153), and esters (Malkin, *Trans. Faraday Soc.*, 1933, **29**, 977) possess analogous α-forms
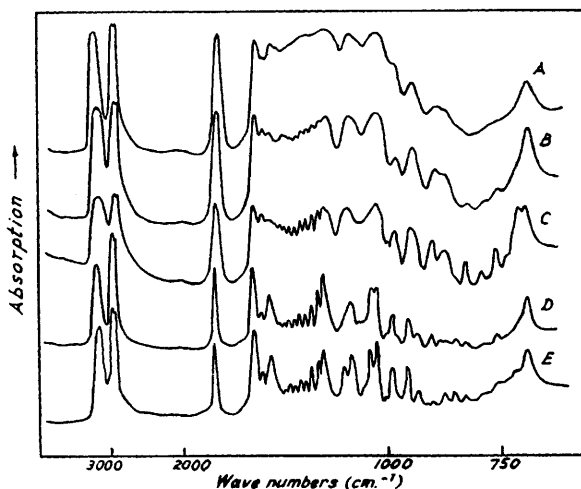

Fig. 1.   *Polymorphic forms of 1-monostearin.*

A, Liquid; *B*, α; *C*, sub-α; *D*, β'; *E*, β.

with vertical rotating chains. They all have a side spacing of 4·12 Å and are uniaxial. Lutton and Jackson (*J. Amer. Chem. Soc.*, 1948, **70**, 2445) have claimed the existence of a fourth form, sub-alpha. They suggest that the α-form is reasonably stable down to a lower transition temperature at which point it changes reversibly into a new crystalline modification, sub-α, giving a distinctive set of short spacings. Controversy exists, however, whether the sub-α-form is or is not crystalline, whether the sub-α to α-transformation is genuinely reversible and whether in fact the α-form is stable down to the lower transition temperature (Malkin, " Progress in the Chemistry of Fats and Other Lipids," Pergamon Press, London, 1954, Vol. II).

1-*Monostearin liquid form.* The infrared spectra of 1-monostearin in the liquid state and in the various polymorphic forms are shown in Fig. 1.

In the spectrum of the liquid the bands are typically broad and have a tendency to smear into each other. The OH absorption band at 3453 cm.$^{-1}$ indicates that hydrogen bonding of the hydroxyl groups is occurring. The C=O stretching absorption band occurs at 1706 cm.$^{-1}$.

Assignment of other bands in the spectrum is more difficult. Both C–O stretching and O–H deformation vibrations of the alcohol groups in the glycerides might be expected to give rise to strong absorption bands since most alcohols do in fact give rise to two bands. However, confusion exists even in the spectra of alcohols as to their correct assignment (Bellamy, " The Infrared Spectra of Complex Molecules," Methuen, London, 1954, p. 94).

A broad strong band at 1174 cm.$^{-1}$ may however be assigned to a C–O stretching vibration of the CO_2R group since a band at this frequency occurs in all glycerides, whilst bands at 1048 and 975 cm.$^{-1}$ are probably associated with primary alcohol groups. A band near 935 cm.$^{-1}$ is probably associated with secondary alcohol groups (cf. O'Connor *et al.*, *loc.cit.*). A band at 719 cm.$^{-1}$ can be certainly associated with a methylene rocking mode.

1-Monostearin α*-form.* When the liquid is cooled to the α-melting point, crystallisation was visually observed and a change occurred in the spectrum (see Fig. 1). This form is the α-form. A series of bands now occurs in the 1180—1340 cm.$^{-1}$ region, which can be assigned to CH_2 wagging modes by analogy with similar bands which have been observed in the solid state of *n*-paraffins (Brown, Sheppard, and Simpson, *Discuss. Faraday Soc.*, 1950, **9**, 261), long chain fatty acids (Jones, McKay, and Sinclair, *J. Amer. Chem. Soc.*, 1952, **74**, 2575), αω-polymethylenedicarboxylic esters (Gunthardt, 1st. Reunion Molecular Spectroscopy, Paris, 1953), and alkyl bromides (Brown and Sheppard, *Trans. Faraday Soc.*, 1954, **50**, 535) and shown by the latter to arise from CH_2 wagging modes. The OH absorption band shifts to 3360 cm.$^{-1}$ but remains broad and the carbonyl absorption band shifts to 1721 cm.$^{-1}$. From this it can be deduced that the OH group becomes more strongly hydrogen-bonded in the α-form than was the case in the liquid state, but that the reverse occurs with the carbonyl group. This suggests that in the liquid state hydrogen bonding occurs between OH and C=O groups but that in the solid form bonding is preferentially between OH groups.

The strong bands near 1111 and 1048 cm.$^{-1}$ remain relatively unchanged, but slightly increased resolution of other bands occurs and a number of new weak bands appear.

The band at 719 cm.$^{-1}$ assigned to a CH_2 rocking mode becomes more intense and sharper than the same band in the liquid spectrum. Since the general broadness of bands observed in the spectrum is more typical of a liquid than a normal crystalline solid, the spectrum of the α-form is consistent with the existence of rotating hydrocarbon chains in this form.

On further cooling, little change occurs in the α-form spectrum apart from a slight increase in resolution of the bands, until 47° is reached. The α-form is therefore reasonably stable from the α-form melting point down to the sub-α-form melting point and this is in agreement with the X-ray observation by Lutton and Jackson (*loc. cit.*).

*1-Monostearin sub-*α*-form.* At this point the sub-α-form is obtained and a change occurs in the spectrum. The major change is the splitting of the single band at 719 cm.$^{-1}$ of the α-form spectrum, into two (at 727 and 719 cm.$^{-1}$). An analogous situation occurs with solid *n*-paraffins in this region of the spectrum and throws some light on the situation. In the liquid state of the paraffins a single band is observed near 720 cm.$^{-1}$; this band increases in intensity but remains single at the solidification point. At a lower transition point two components occur (Robert and Favre, *Compt. rend.*, 1952, **234**, 2270). Between the solidification and transition points the paraffins exist in a hexagonal form and rotation of the hydrocarbon chains about their axes takes place. This can be compared with the α-form of the monoglycerides. Below the transition point the crystal system becomes orthorhombic (Müller, *loc. cit.*). In Polythene an analogous band (725 cm.$^{-1}$) is single in the amorphous or liquid state, but double in the crystalline form. A more detailed examination of the doublet has been given recently by Stein and Sutherland (*J. Chem. Phys.*, 1954, **22**, 1993) who showed that the splitting arises in *n*-paraffins and Polythene in the solid form only when these materials are in a certain crystalline state. Stein (*ibid.*, 1955, **23**, 734) describes the doublet in terms of interaction between nearest-neighbour CH_2 groups, the CH_2 rocking frequency in the isolated molecule ν_0 being split into two components, the out-of-phase component being at a frequency very slightly higher than ν_0, and the in-phase component at a considerably higher frequency. This definitely suggests that the sub-α-form has a crystalline lattice (or at least a very ordered system), and this is in agreement with Lutton and Jackson's conclusion (*loc. cit.*). It might also be predicted that the unit cell in the sub-α-form is smaller than that in the α-form (cf. Stein, *loc. cit.*).

Other changes between the spectra of the α- and the sub-α-form are that the OH band shifts from *ca.* 3360 cm.$^{-1}$ in the α-form to *ca.* 3342 cm.$^{-1}$ in the sub-α-form, and the carbonyl

band shifts from 1721 to 1730 cm.$^{-1}$. The OH group therefore again becomes more strongly hydrogen-bonded whilst the reverse occurs with the carbonyl group. The bands between 852 and 727 cm.$^{-1}$ also become better resolved. The spectrum in general suggests some orientational freedom since the general sharpness of bands typical of the spectrum of normal crystalline material does not occur, and this orientational freedom appears a little greater nearer the melting point of the sub-α-form than at room temperature. This is consistent with the work of Crowe and Smyth (*J. Amer. Chem. Soc.*, 1950, **72**, 4427), who observed orientational freedom in the sub-α-form and suggested that orientation of various molecular segments occurred in order to explain the wide distribution of relaxation times observed in the sub-α-phase despite the order observed by *X*-ray methods.

The sub-α-form was observed to be quite stable at room temperature, in agreement with the observation of Lutton and Jackson (*loc. cit.*). Further, by heating the sub-α-form to 47° the α-form was again observed. This transformation from one form to the other was observed to occur repeatedly. The reversible effect with the α- and sub-α-forms and indeed the stability of the α-form itself may, however, depend on the presence or absence of sufficient β′- or β-nuclei.

Although a transition temperature at 42° is reported (Malkin, *op. cit.*), little change occurred in the spectrum of the sub-α-form below 47° except for a slight increase in resolution of some bands.

1-*Monostearin* β′- *and* β-*forms*. The β′- and the β-spectrum obtained from a dispersion in Nujol are very similar to each other and are more typical of the type of spectrum normally obtained with true crystalline materials, *i.e.*, bands are narrow and sharp. The β′-form was obtained by rapid crystallisation. The rapid-crystallisation technique sometimes gives a mixture of forms although the β′-form is generally predominant. The *X*-ray powder photograph can indicate whether any β-form is present since the short spacings of the β′- and the β-form are quite different, but it is difficult to ascertain whether any sub-α-form is present because of the similarity of the short spacings.

In the β′-form spectrum the OH absorption band is split into components at 3342 (the main component) and 3243 cm.$^{-1}$. The carbonyl band occurs at 1736 cm.$^{-1}$, and the broad band at 1050 cm.$^{-1}$ observed in the sub-α-form spectrum is split into two components (at 1062 and 1047 cm.$^{-1}$). The 1181 cm.$^{-1}$ band is now quite sharp and a band at 1100 cm.$^{-1}$ is almost resolved into different components. A number of small bands occur in the region between 850 and 729 cm.$^{-1}$, which may be due to rocking modes of the methylene groups by analogy with hydrocarbon spectra (Brown and Sheppard, *loc. cit.*). There is only one component at 719 cm.$^{-1}$.

In the β-form spectrum the OH stretching band is again split into two components. The main component is at 3243 cm.$^{-1}$ with the other at 3307 cm.$^{-1}$. Near 1100 cm.$^{-1}$ there are clearly three components and the bands at 1063 and 1048 cm.$^{-1}$ are slightly more split than in the β′-form spectrum. There is again only one component of the methylene rocking absorption band, at 719 cm.$^{-1}$. The spectra of the β-form have been discussed in more detail by Barcelo and Martin (*loc. cit.*). It is of interest that whilst with *X*-ray powder photographs it is easy to distinguish between β- and β′-forms but difficult to distinguish between the β′- and the sub-α-forms, with infrared spectra it is easy to distinguish between sub-α- and β′-forms but difficult to distinguish between β′- and β-forms.

It is perhaps significant that the OH stretching band moves to lower frequencies (showing that an increase in the strength of the hydrogen bonding is occurring) in the order liquid ⟶ α ⟶ sub-α ⟶ β′ ⟶ β. This is also the order of stabilities of the polymorphic forms. The existence and stability of these polymorphic forms may therefore be bound up with the hydrogen-bonding schemes possible, and the strength of the hydrogen bonding to the hydroxyl groups.

1-*Monopalmitin*. Similar results were obtained with 1-monopalmitin. The α-form gives rise to a spectrum similar to that observed with 1-monostearin. The α-form was quite stable down to the sub-α-transition temperature, at which point the sub-α-form was observed, giving rise to a spectrum similar to that of the sub-α-form of 1-monostearin. This sub-α-form was stable at room temperature, but did change during several weeks at this temperature into the β′-form. When the sub-α-form was heated above the transition

temperature the α-form could be obtained. The β'- and the β-form were obtained by crystallisation from solvents.

1-*Monolaurin.* With 1-monolaurin the sub-α-form was not observed since the transition temperature for this glyceride was below room temperature. At room temperature, transition to the stable β-form was observed to occur within a few hours (Fig. 2).

The spectra of monoglycerides of different chain length in the same polymorphic form were quite similar, except for the number of methylene wagging bands which increase as the chain length increases. This is similar to the situation with spectra of crystalline fatty acids (Jones *et al., loc cit.*).

2-*Monoglycerides.* The only thermal investigation of 2-monoglycerides is due to Daubert and Clarke (*Oil and Soap,* 1945, **22**, 113) who concluded that they were not polymorphic. Malkin (*op. cit.*), however, suggests that 2-monoglycerides probably separate from the melt in the α-form but change extremely rapidly into the β-form. X-Ray data, however, of only one form have been obtained (Filer, Sidhu, Daubert, and Longenecker, *J. Amer. Chem. Soc.*, 1946, **68**, 167).

FIG. 2. *Polymorphic transitions of 1-monolaurin.*

A, Liquid; *B*, α; *C*, α ⟶ β'; *D*, β' ⟶ β.
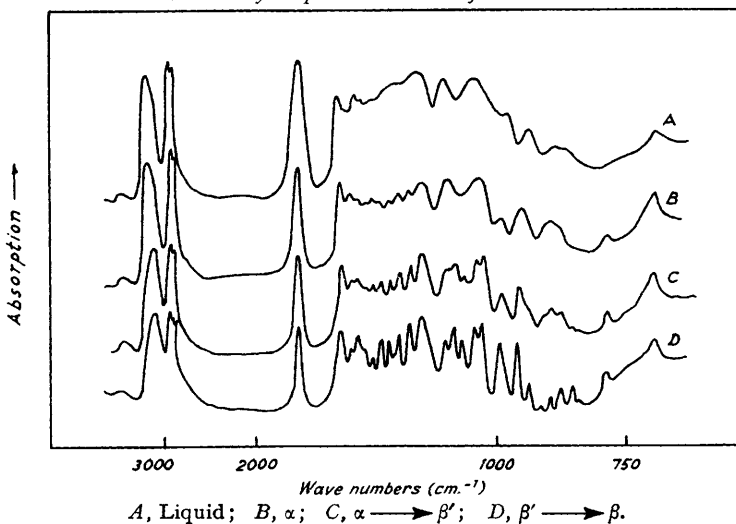
2-*Monomyristin liquid.* The OH stretching absorption band in the liquid form of 2-monomyristin is at 3527 cm.$^{-1}$ and so the OH group is not very strongly bound in the liquid state. The carbonyl absorption band is at 1715 cm.$^{-1}$.

A band at 1175 cm.$^{-1}$ may be assigned to a C–O stretching vibration of the CO_2R group. Bands occur at 1048 and 975 cm.$^{-1}$ (cf. 1-monoglycerides) which are perhaps associated with the primary alcohol groups. A band at 720 cm.$^{-1}$ can be assigned to a methylene rocking vibration.

2-*Monomyristin β-form.* As the liquid 2-monoglyceride cooled, the first form to crystallise was the stable β-form and no evidence for an α-form was observed. (The spectrum of the material crystallised from solvent and shown by X-rays to be the β-form was observed to be identical with this.)

The OH frequency in the β-form shifts to 3347 cm.$^{-1}$, showing that the OH group has become more strongly hydrogen-bonded than in the liquid state, whilst the C=O absorption band is at 1733 cm.$^{-1}$, showing the carbonyl group has become less strongly bonded (cf. 1-monoglycerides). In view of the difference in frequency of the OH stretching absorption bands in the β-form of 1- and 2-monoglycerides it is of interest that the 2-monoglycerides, which on grounds of symmetry might have been expected to melt higher than the 1-isomers, melt several degrees lower. The frequency is higher for the 2- than for the 1-monoglycerides, and therefore the hydrogen bonding to the OH groups is stronger in the

latter.   A regular series of bands between 1344 and 1184 cm.$^{-1}$ (cf. 1-monoglycerides) can be assigned to methylene wagging modes.

A band at 975 cm.$^{-1}$ is greater in relative intensity compared with the 1048 cm.$^{-1}$ band in the spectrum of the β-form of 2-monoglyceride than is the case with the corresponding 1-mono-glyceride (there are two primary alcohol groups in the 2-monoglycerides, and one primary alcohol group in the 1-monoglycerides).   The region from 780 to about 700 cm.$^{-1}$ is unusual in that a broad absorption extends over the whole of this range with bands at 736 and 720 cm.$^{-1}$ (assigned to methylene rocking modes), apparently superposed on the general absorption.   This does not occur in the spectra of other glycerides or esters.

The spectra of 2-monoglycerides of different chain length are very similar (Fig. 3), the main difference being the number of the bands in the 1350—1184 cm.$^{-1}$ range.   The
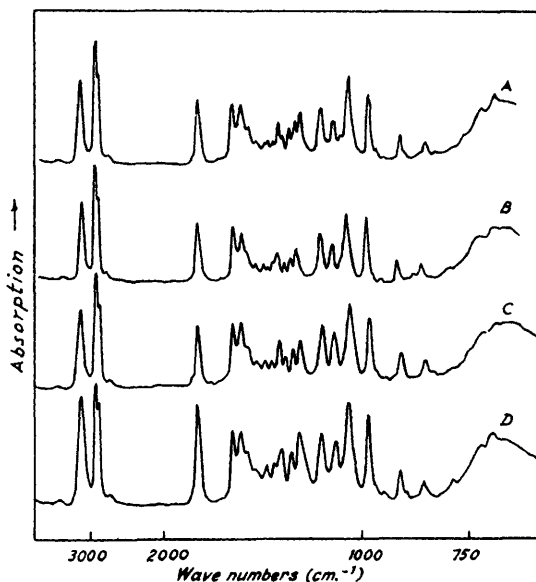

Fig. 3.   *2-Monoglycerides.*
   A, 2-Monostearin.
   B, 2-Monopalmitin.
   C, 2-Monomyristin.
   D, 2-Monolaurin.

relative intensity of the OH compared with the CH stretching bands decreases as the chain length increases.

Whilst heating the 2-monoglycerides it was observed that a transition to the corresponding 1-monoglyceride took place.   Acyl migration in 2-monoglycerides is known to take place in the presence of a catalyst (Stimmel and King, *J. Amer. Chem. Soc.*, 1934, **56**, 1724) but not by the action of heat alone.   If, however, *e.g.*, 2-monolaurin is held a few degrees above its melting point for three hours, the infrared spectrum shows that almost complete conversion into the 1-monolaurin takes place.   A similar change was observed to occur with the other 2-monoglycerides examined.

In conclusion, the investigation has shown that infrared spectra can provide a good standard for observing and following polymorphic transitions   This method for studying polymorphic transitions has some advantages over the X-ray method, notably the speed of obtaining the spectra and the information from them about the bonding of the groups.   As a supplement to the X-ray technique it is of obviously great potential value.

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