

The Structure of Lipids and Proteins Studied by Attenuated Total-Reflection (ATR)
Infrared Spectroscopy

I. Oriented Layers of Tripalmitin

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(Z. Naturforsch. 27 b, 780—796 [1972]; received May 24, 1972)

Tripalmitin, oriented bilayers, attenuated total reflection, infrared spectroscopy, temperature dependence

The structure of tripalmitin multilayers prepared by the Langmuir-Blodgett technique has been investigated within room temperature and 62 °C using infrared internal reflection spectroscopy (ATR). It was found that the bilayer structure exhibits crystalline features with pseudo hexagonal structure. The methylene groups of the hydrocarbon chains are in the all-trans-zig-zag conformation, oriented perpendicularly to the germanium internal reflection plate. Increasing temperature produces at first a continuous conformational change in the glycerol part of the molecule. This process is extended over nearly 10° and followed by sudden melting (disordering) of the hydrocarbon chains within 1°.

In comparison microcrystalline tripalmitin shows an identical infrared spectrum and analogous behaviour at increased temperatures. This indicates that the bilayer structure (head-head, tail-tail) also exists in the crystal.

1. Introduction

In this series of papers we wish to report on investigations of the structure of lipids and proteins in artificial and living membranes.

Results of ATPase investigated under biological conditions in the adsorbed state have been reported previously ¹⁻³. Structural changes of this enzyme under the influence of Mg⁺⁺ and electric fields were observed. However, the complexity of this system renders the interpretation of the spectra rather complicated.

As will be discussed in this paper, artificial lipid membranes on the other hand result in relatively simple IR-spectra. From this fact one may conjecture that IR-spectra would yield a sensitive probe for the study of protein lipid interactions, in particular if pure lipid membranes and lipid membranes doped with proteins are compared. Insight of considerable detail into molecular conformation and specific interactions between molecules may be expected to result from such spectra.

Artificial systems have already been investigated by several authors using different methods ^{4, 5}. Investigations of model systems most probably are a

prerequisite for a better understanding of the IR spectra of living membranes. ATR technique as applied here allows to perform experiments under biological conditions.

In this paper we wish to report infrared spectra of a simple lipid, glycerol tripalmitat, ordered in double layers. The use of multiple reflection ATR offers a unique possibility to study the IR spectra of thin layers. Oriented layers have already been investigated by several authors; cf. Ref. ⁶⁻¹¹. Furthermore, it is known from a body of experimental studies that long hydrocarbon chains with or without polar endgroups in the solid state exhibit IR spectra which are characteristic for the all trans zig-zag conformation. This conformation leads to typical sequences of absorption bands, which by analysis of the spectra may be assigned to group vibrations of the polymethylene chain ¹²⁻¹⁹. For a general review the reader should consult Ref. ²⁰. Spectra of polymer chains have recently been used by ZERBI ^{21, 22} to derive information about the stereochemistry and defects (stereochemically irregular polymerisation steps) along the polymer chain by comparison of calculated model spectra and density of states with observed spectra.

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It therefore appears appropriate to study the conformation of polymethylene chains in lipid layers by IR spectroscopy.

Since in this case primary interest concentrates in single, double or on multiple layers, ordinary absorption technique barely would yield sufficient absorption. On the other hand ATR technique tends itself to multiple reflection measurements. As will be shown in this paper that high quality ATR spectra may indeed be obtained and yield a powerful basis for the study of the conformation in the alkyl chain and in the polar part. From the temperature dependence of the spectra it has been found that at room temperature the chain is in the all trans zig-zag form. At higher temperature this regular structure still seems to persist, however, some degrees below the melting point continuous conformational changes in the glycerol part take place. Just below the melting temperature also the longrange order in the CH_2 -chains break down and as the last step of the complicated melting process the framework of the polar groups in the layers is destroyed.

2. Experimental

2.1. Preparation of oriented layers

Oriented tripalmitin layers were deposited on a germanium internal reflection plate by the well known dipping technique of BLODGETT and LANGMUIR^{23, 24}.

The layers of odd number being oriented with the CH_3 -groups away from the germanium, the layers with even number with these groups toward the plate. Such bimolecular lipid lamellas serve as model membranes. An important point for the deposition of the first layer is the cleanliness of the germanium plate. This usually was found sufficient when water drains from the surface and formation of interference fringes is observed. The cleaned plate was dipped into the trough of the film balance before the tripalmitin monolayer was prepared by spreading some drops of a 10^{-3} M solution in hexane-chloroform-methanol (3:1:1). When the solvent was vaporized the film was compressed to 10–15 dyne/cm.

During the upwards movement of the plate the first tripalmitin layer is attached with the polar glycerol heads to the germanium. The second layer was formed by redipping the plate. Successive layers are formed repeating this procedure.

2.2. ATR-Spectra

For a general review of ATR-technique the reader is referred to ref. ²⁵. A typical optical set up is shown in Fig. 1.

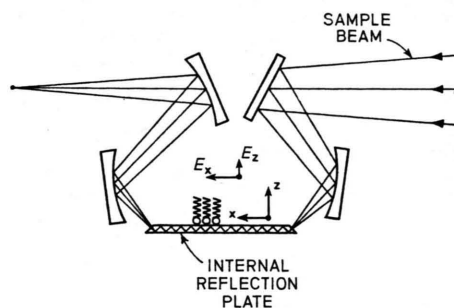


Fig. 1. ATR set up. (Reproduced from ²⁵ with kind permission of the author.)

ATR spectra were recorded on a Perkin Elmer Mod. 225 infrared spectrometer equipped with two ATR attachments (Wilks Sci. Corp., Model 9 and 50) in the sample and reference beam, respectively. The reflection plates were germanium ($50 \times 20 \times 1.5$ mm), supplied by Harrick Sci. Corp. The angle of incidence was 30° , resulting in 30–60 active reflections depending on the plate area coated with the film. 11 to 21 oriented tripalmitin layers were required in order to get a good signal-to-noise ratio. The plates were thermostated to $\pm 0.5^\circ\text{C}$. Temperature inhomogeneity on the plate due to asymmetric heating by the IR-source was smaller than 0.3°C .

Polarization measurements were made with a Perkin Elmer grid polarizer mounted in front of the entrance slit of the monochromator. Thereby the effects of self-polarization of the monochromator are negligibly small.

3. Results

Figs. 2 to 4 show successive scans of the same 21 layers of tripalmitin at different temperatures, whereas in Fig. 5 analogous experiments with microcrystalline tripalmitin, pressed on the reflection plate are presented. Fig. 6 shows an ATR spectrum of tripalmitin crystallized on the germanium plate by evaporating the solvent, (methanol). These investigations shall give information on temperature induced conformational changes in the lipid bilayers. Each spectrum was scanned twice with parallel ($\parallel$) and perpendicular ($\perp$) polarized light respectively. The use of polarized light is a powerful help in the analysis of oriented systems.

An empirical assignment of the absorption bands based on the analysis of polymethylene chain spectra of *n*-paraffins and longchain esters is given in Table I. A more detailed vibrational analysis using normal coordinate calculations is in preparation. Additional support for the assignment was obtained from determination of dichroic ratios from some of

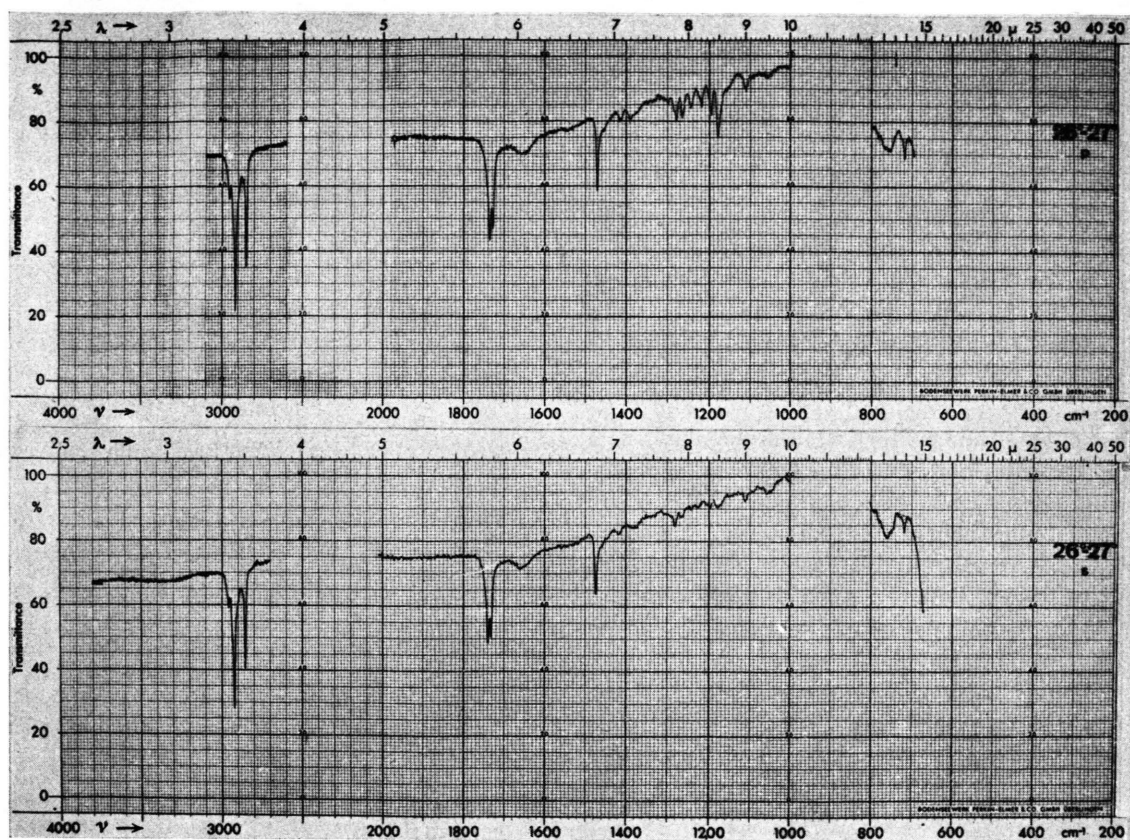


Fig. 2 a.

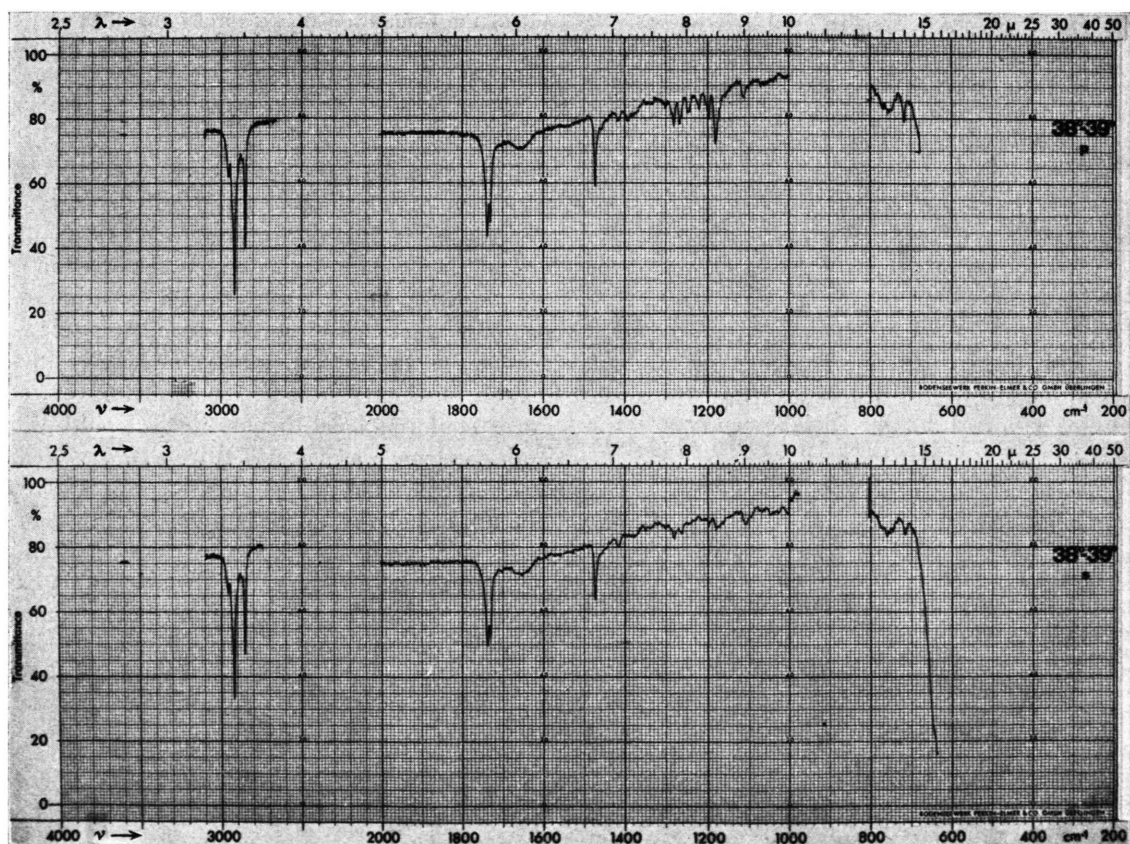


Fig. 2 b.

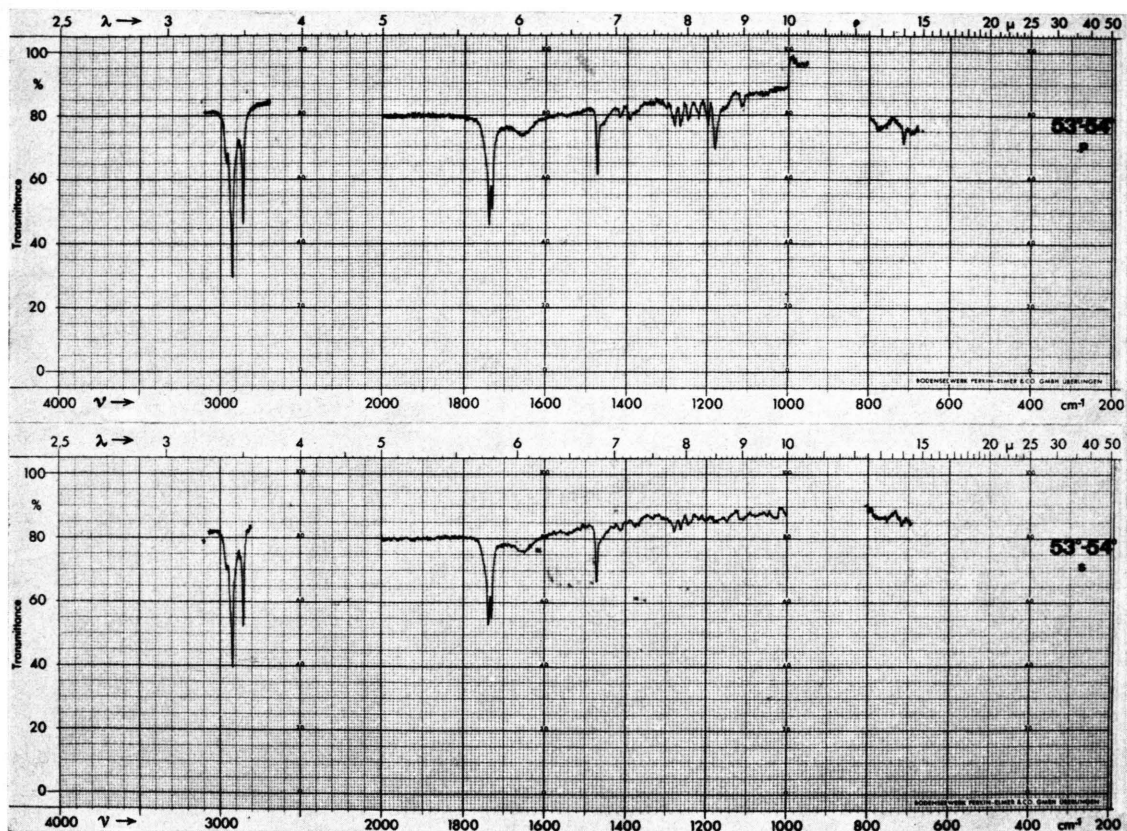


Fig. 2 c.

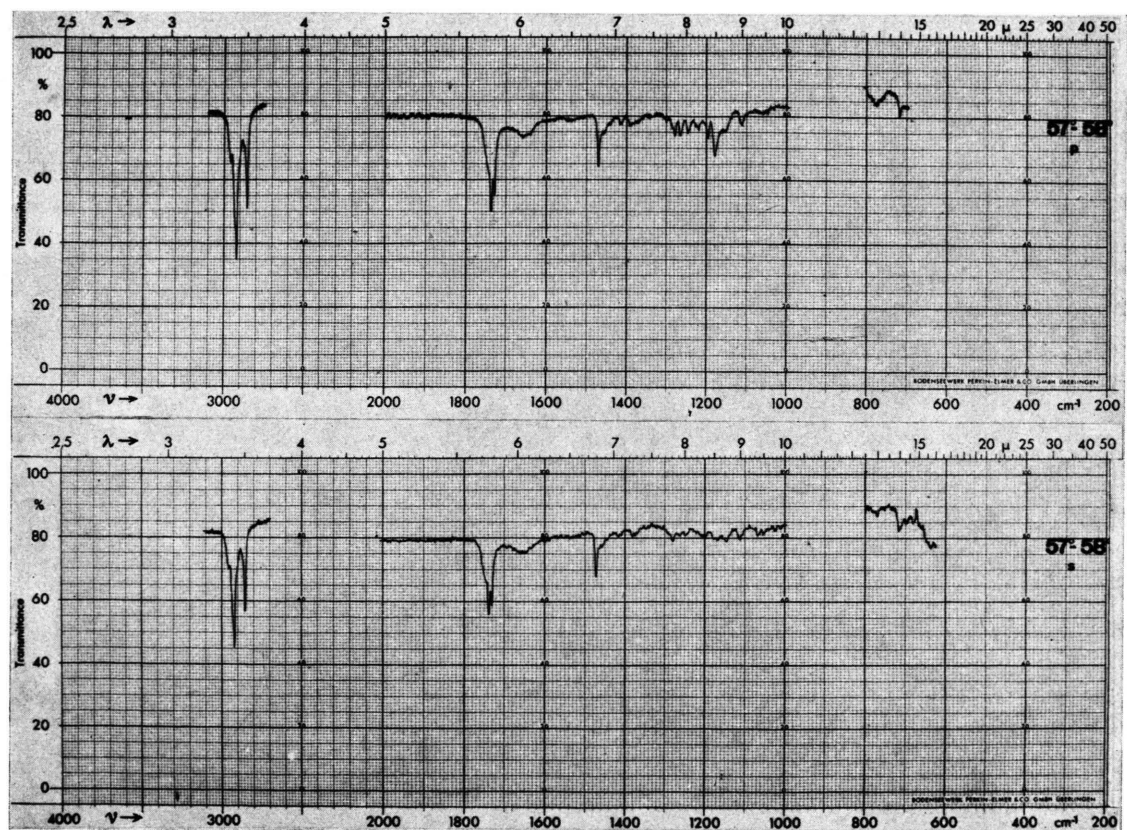


Fig. 2 d.

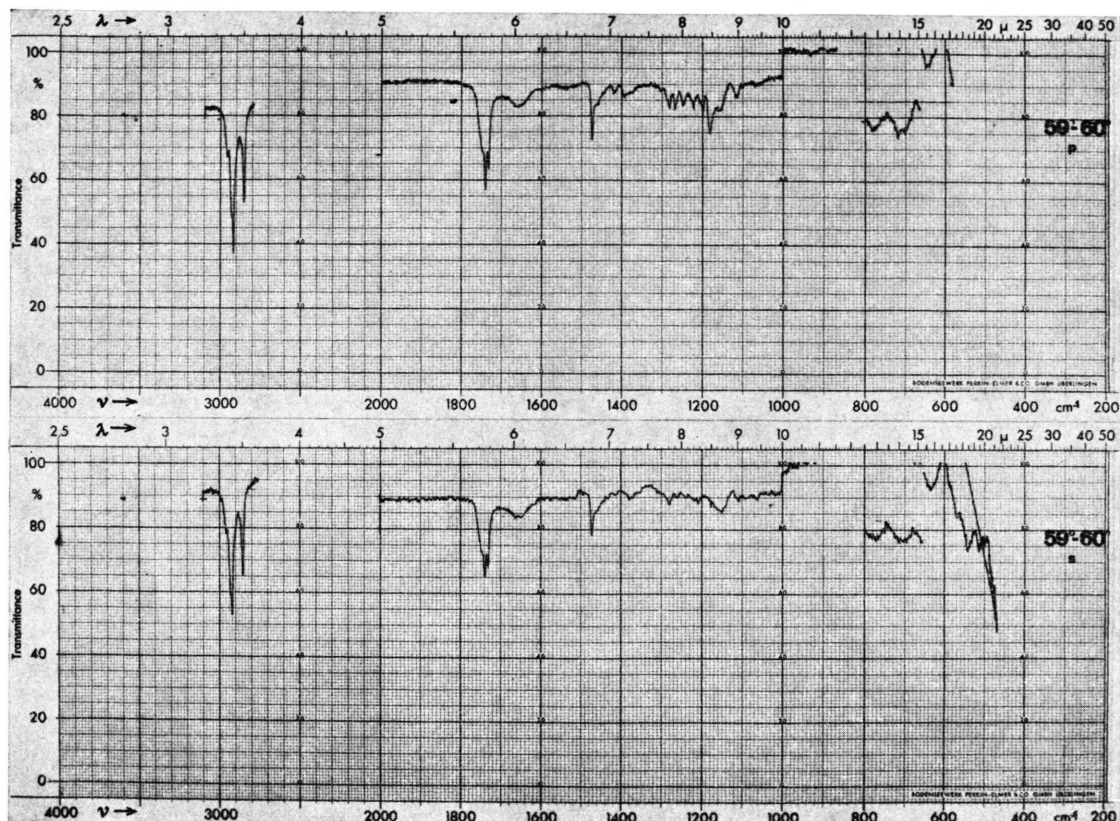


Fig. 2 e.

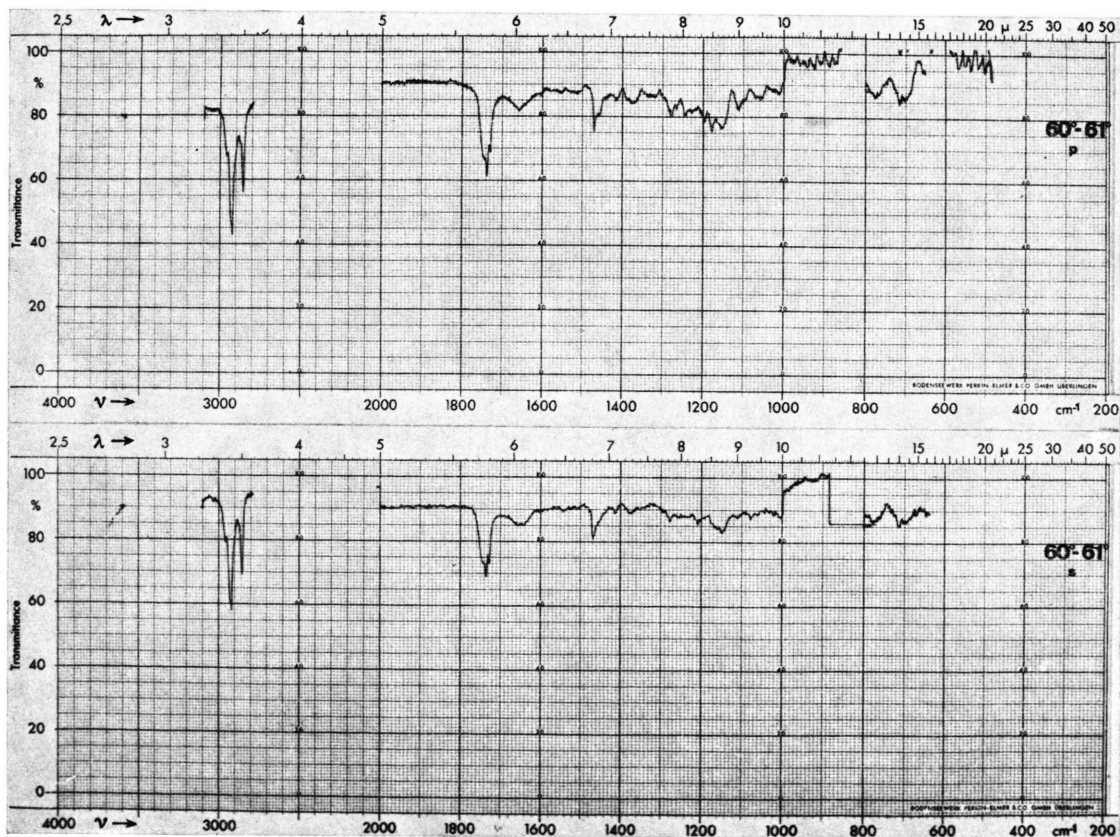


Fig. 2 f.

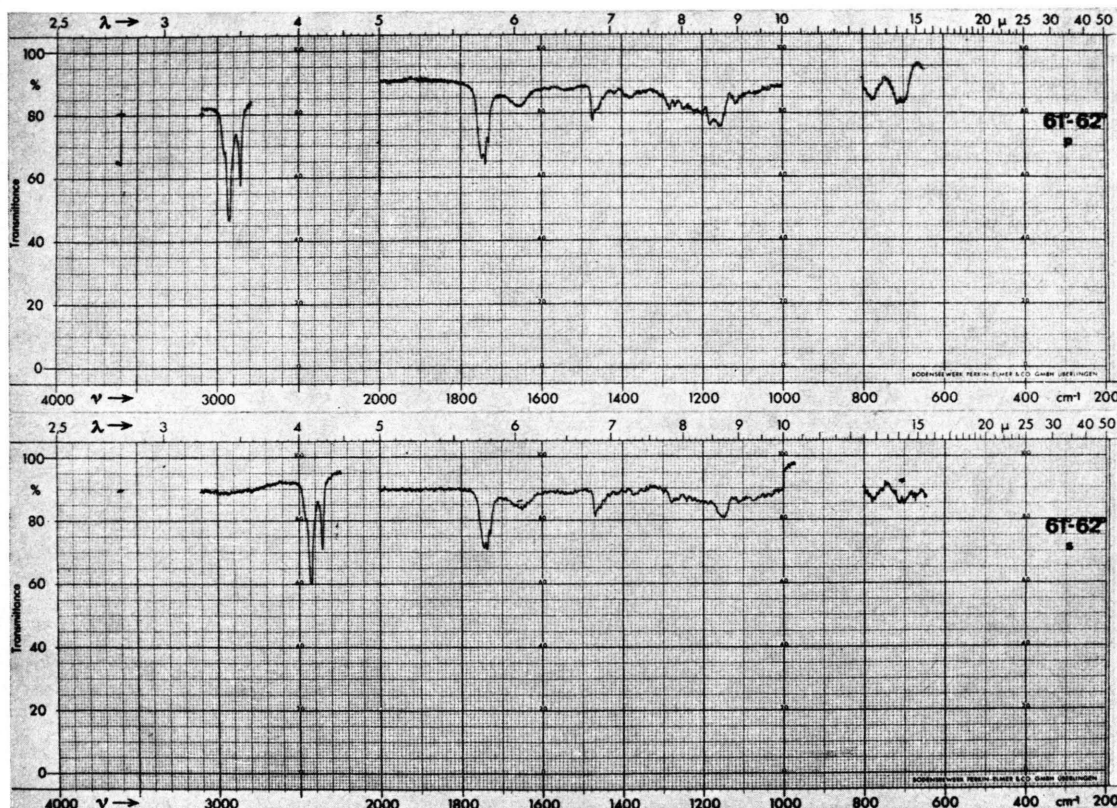


Fig. 2 g.

Fig. 2. Polarized ATR spectra of tripalmitin multilayers: Increasing temperature. *a.* 26–27 °C, *b.* 38–39 °C, *c.* 53–54 °C, *d.* 57–58 °C, *e.* 59–60 °C, *f.* 60–61 °C, *g.* 61–62 °C.

the absorption bands using the definition of ²⁰

$$R = \frac{\ln T_{\perp}^{-1}}{\ln T_{\parallel}^{-1}} = \frac{\alpha_{\parallel}}{\alpha_{\perp}}, \quad (1)$$

where T stands for transmission and α for the absorption coefficient. Eq. (1) holds for transmission measurements with plane (homogeneous) waves. However, analogous formulae can be derived for ATR-measurements essentially under the condition of weak absorption, i. e. $T = e^{-\alpha d_e}$ with $\alpha d_e \ll 1$, where d_e is the effective thickness of the rarer medium. For $\parallel$ - and $\perp$ -polarized light the effective thickness $d_{e\parallel}$ and $d_{e\perp}$ respectively are different ^{25, 26}. By this reason usually only relative dichroic ratios are given in ATR measurements defined by

$$R' = \frac{\ln T_{\perp}^{-1}}{\ln T_{\parallel}^{-1}} \cong \frac{\alpha_{\parallel}'}{\alpha_{\perp}'} = \frac{\alpha_{\parallel} d_{e\parallel}}{\alpha_{\perp} d_{e\perp}}. \quad (2)$$

In Table I also the relative dichroic ratios of tripalmitin ATR bands are collected as well as ap-

proximative directions of transition dipole moments calculated from relative dichroic ratios R' using the semi-empirical method given in section 4.5.

4. Discussion

4.1. Molecular Model

The analysis of the tripalmitin spectra is based on a molecular model with the features

i. the typical spectra of the hydrocarbon chain essentially not influenced by the connection of the ester group to the glycerol skeleton, i. e. it is assumed that the 3 palmitic acid chains are only slightly coupled.

ii. the 3 palmitic acid chains are taken as pseudo-equivalent. This is equivalent to assume a pseudo-trigonal conformation for the non-polar part of the molecule.

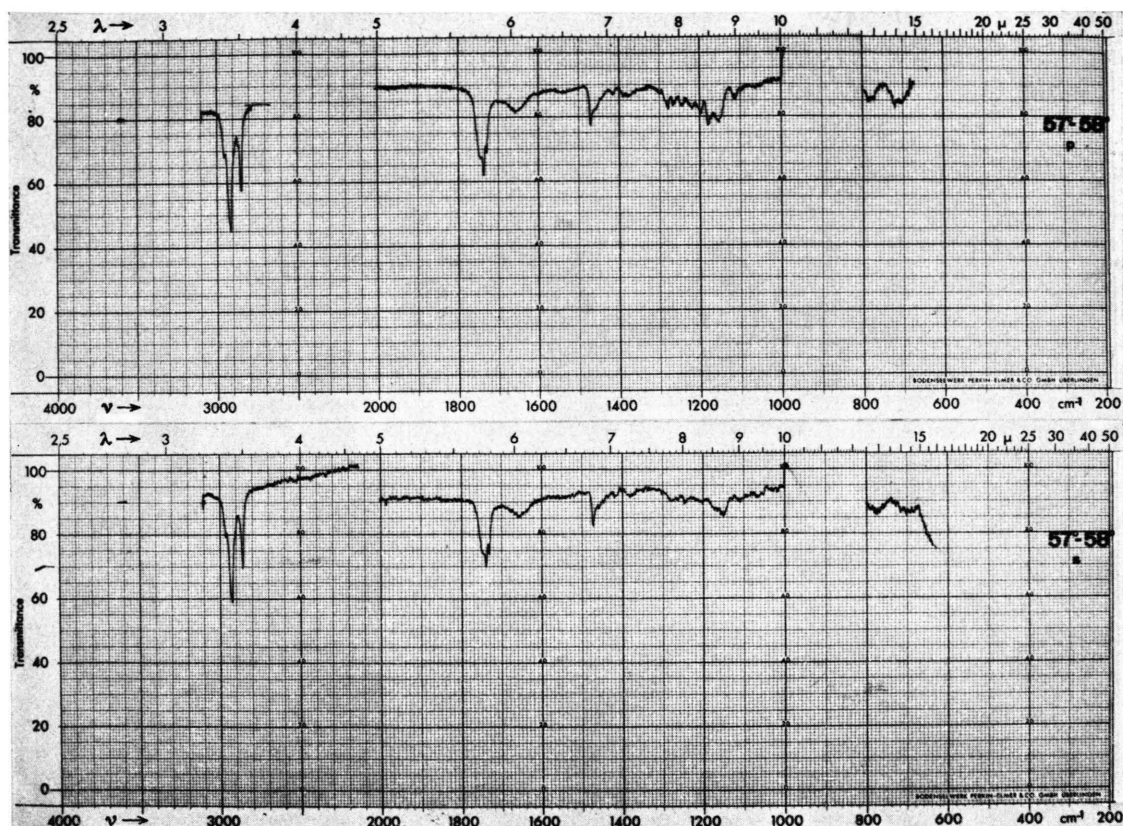


Fig. 3 a.

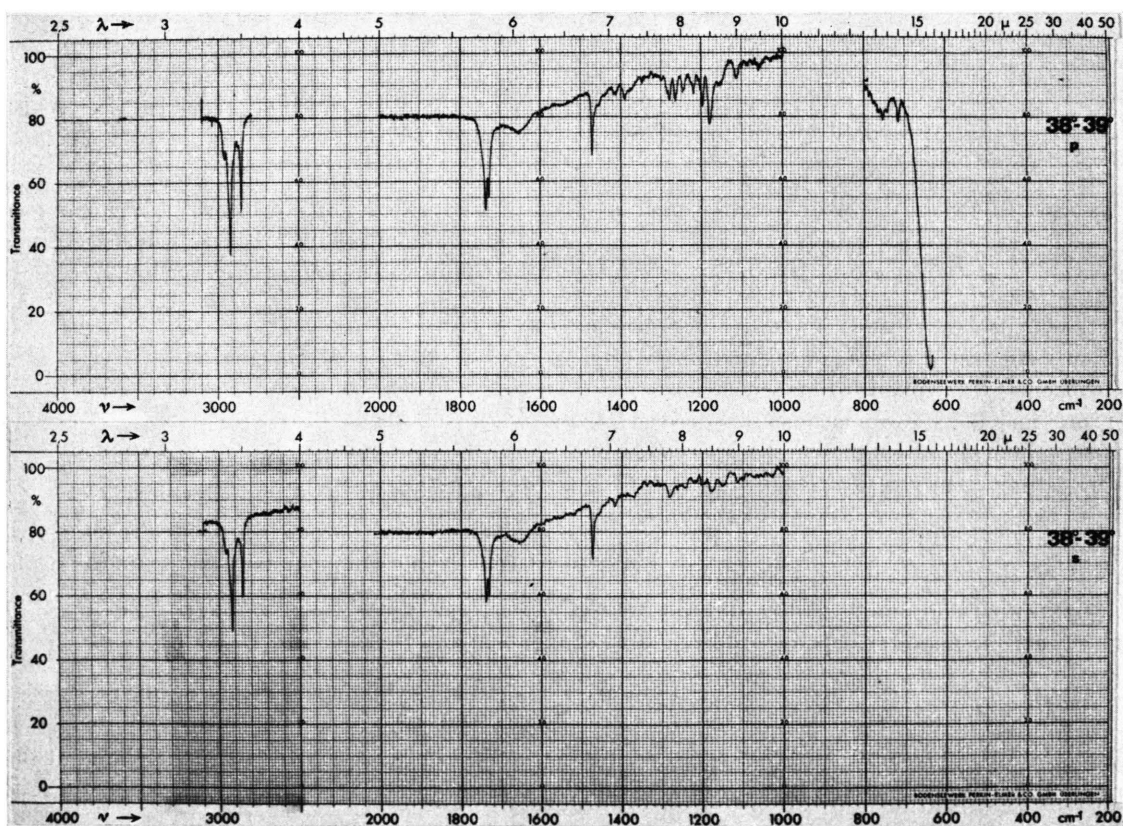


Fig. 3 b.

Fig. 3. Polarized ATR spectra of tripalmitin multilayers: Temperature decreasing from transition point (61°C). a. $57-58^{\circ}\text{C}$, b. $38-39^{\circ}\text{C}$.

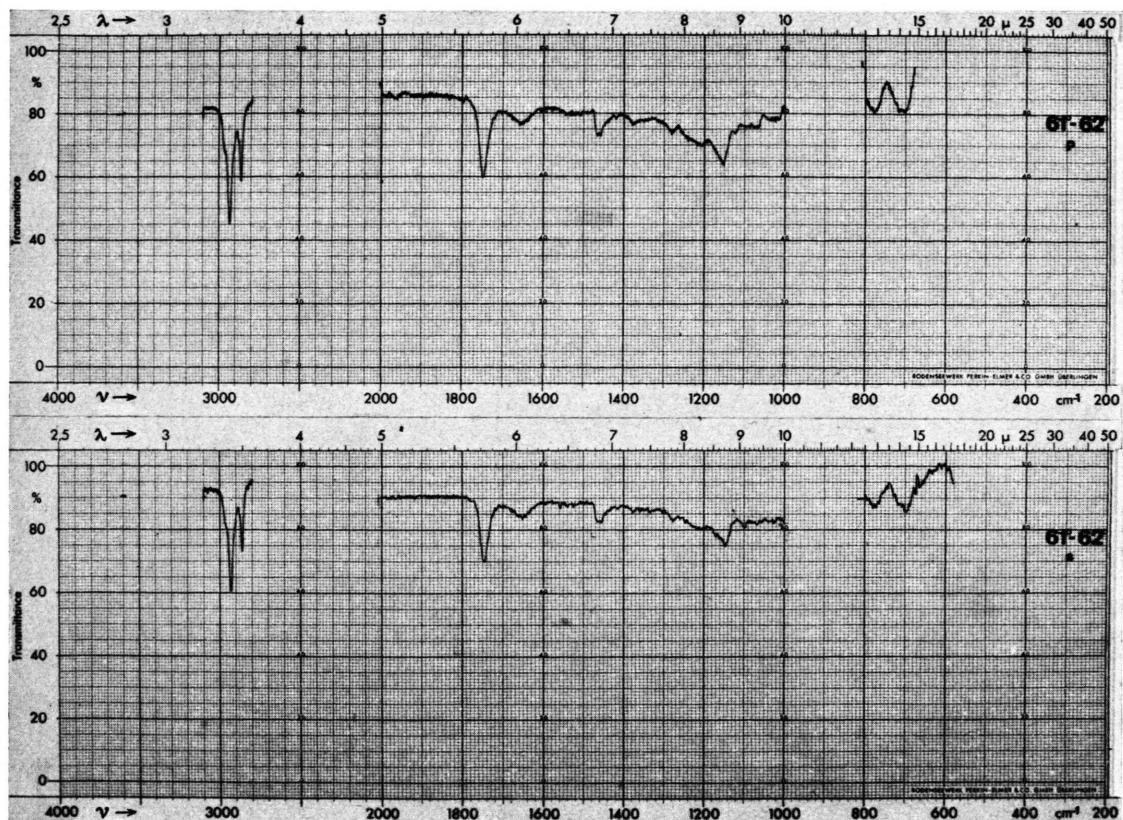


Fig. 4 a.

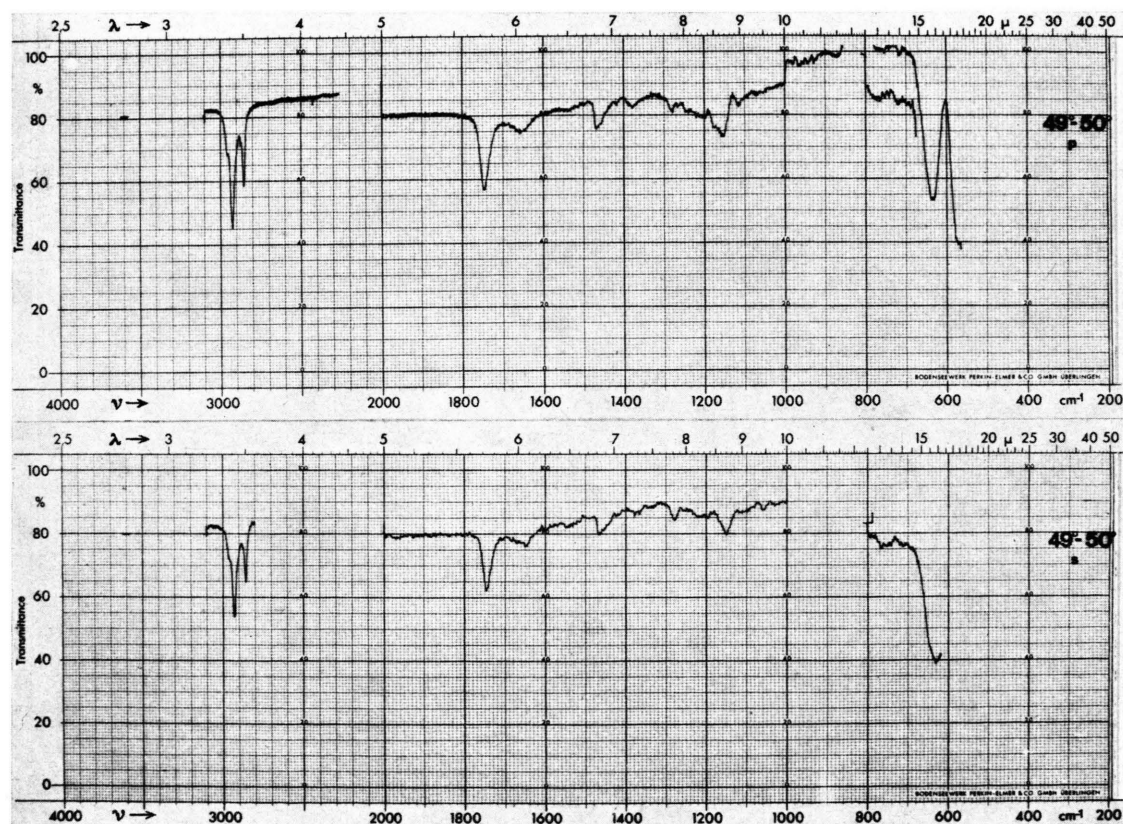


Fig. 4 b.

Fig. 4. Irreversibility effects in the polarized ATR spectra of tripalmitin: a) Effect of reincrease of temperature to transition point (61 °C), b) Effect of redecrease of temperature below transition point.

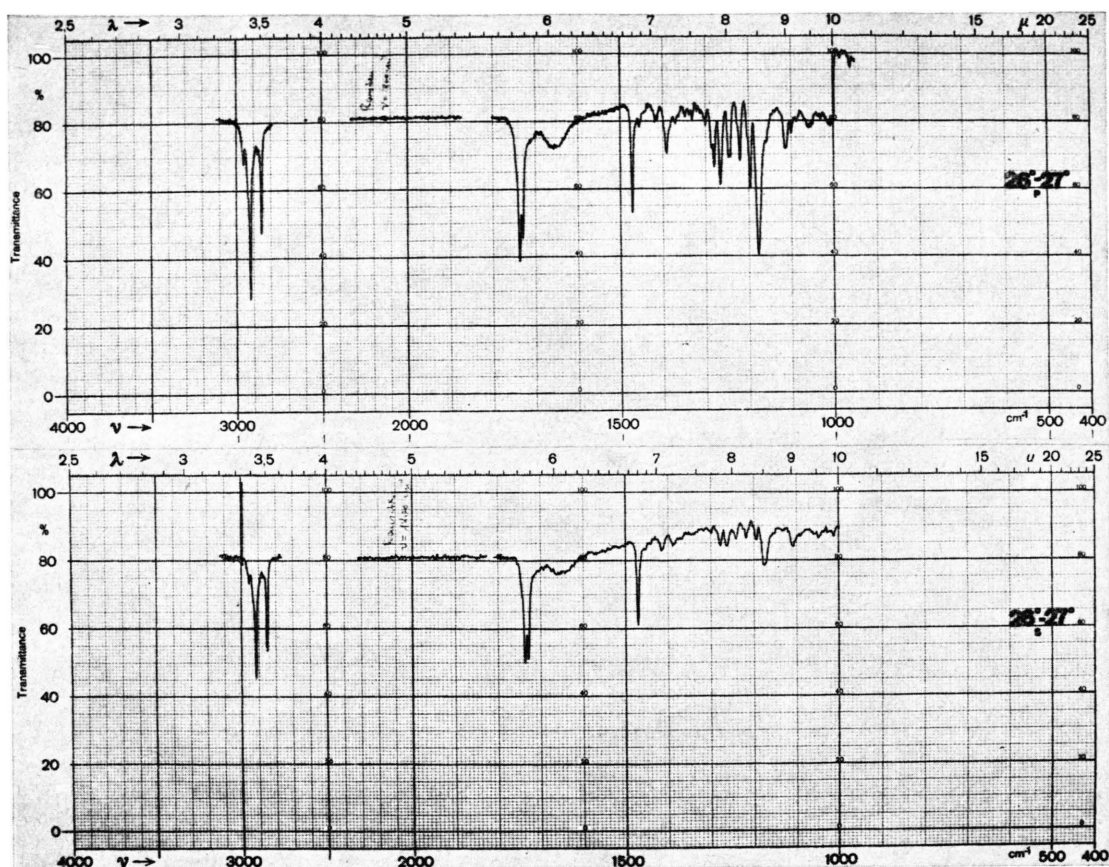


Fig. 5 a.

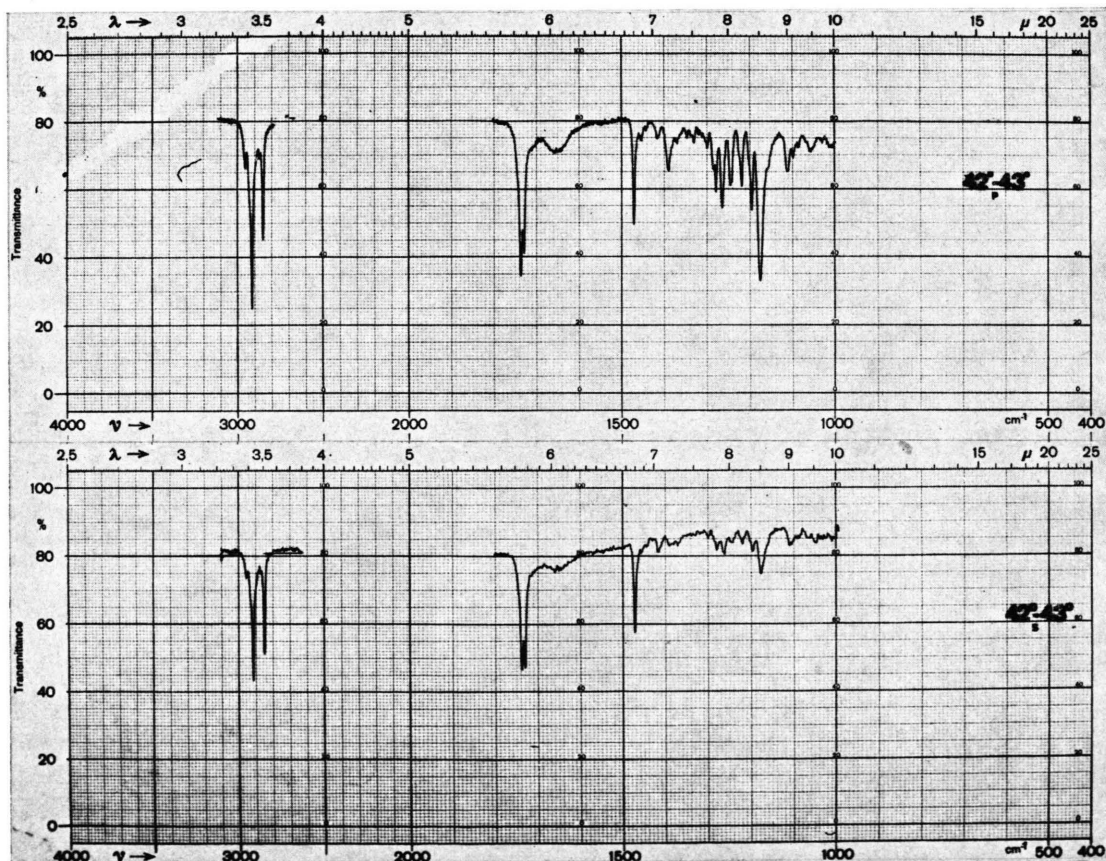


Fig. 5 b.

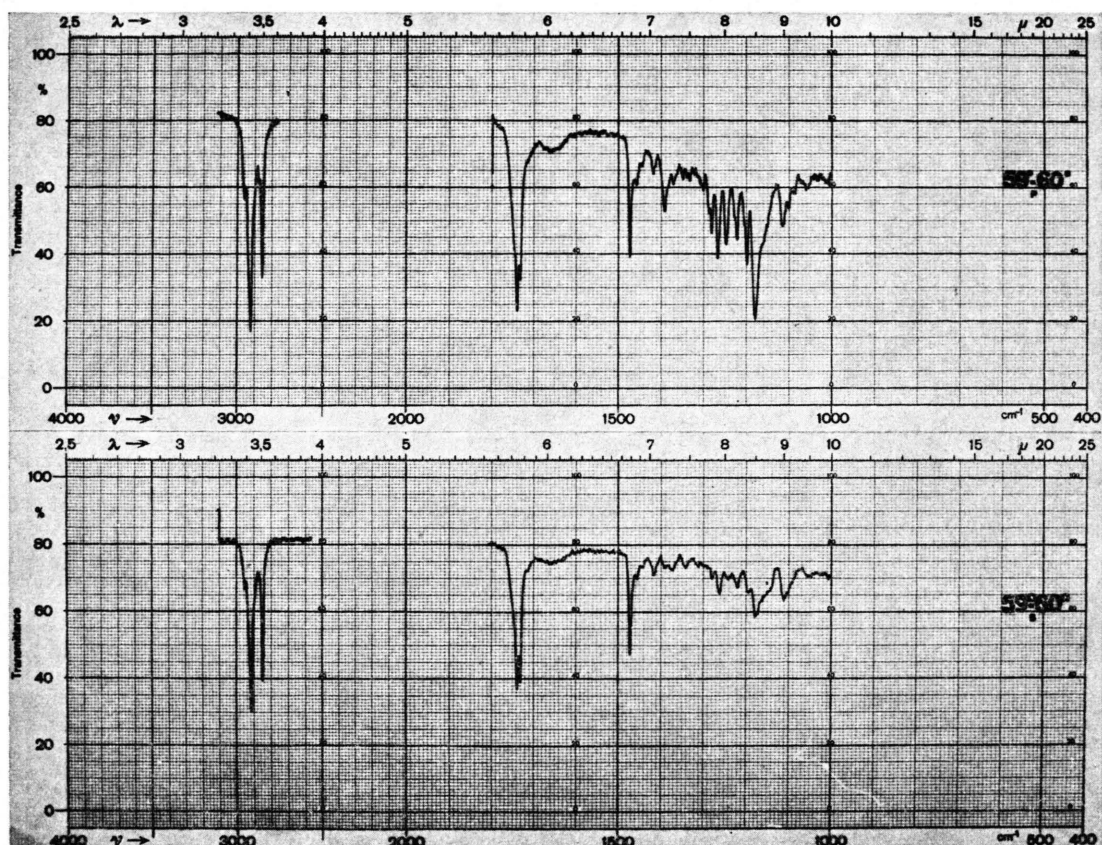


Fig. 5 c.

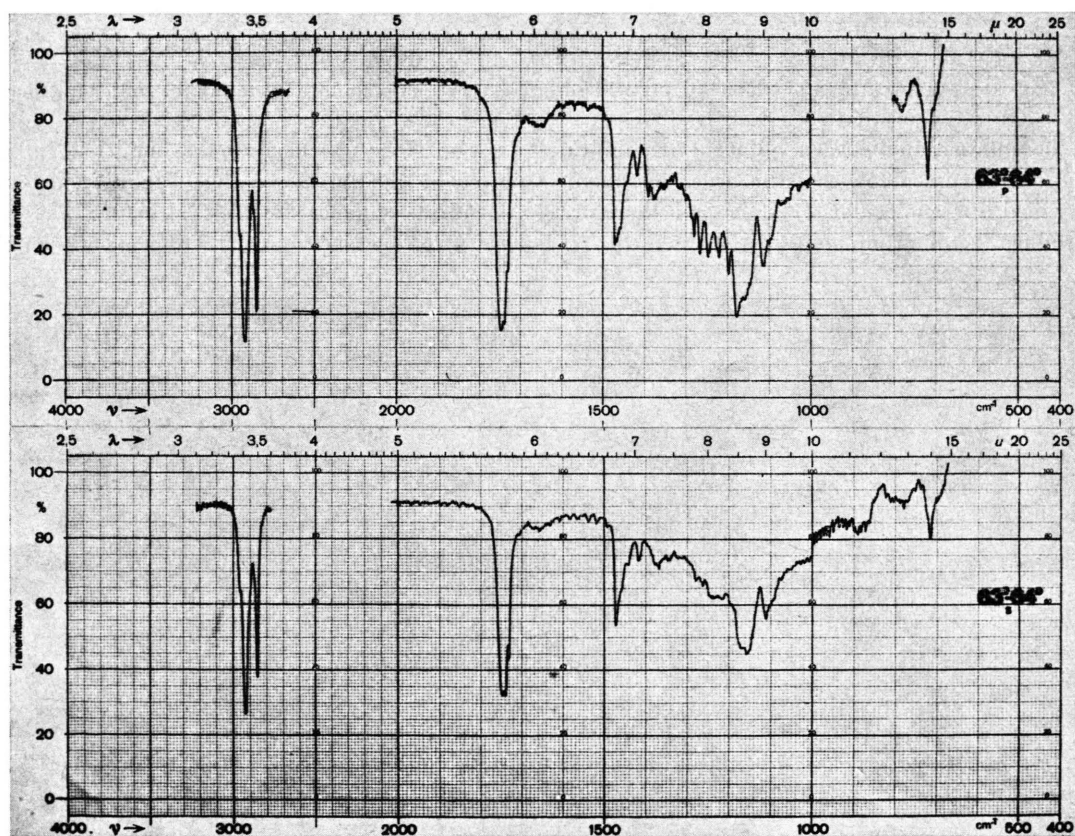


Fig. 5 d.

Fig. 5. Polarized ATR spectra of microcrystalline tripalmitin: Increasing temperature. *a.* 26–27 °C, *b.* 42–43 °C, *c.* 59–60 °C, *d.* 63–64 °C.

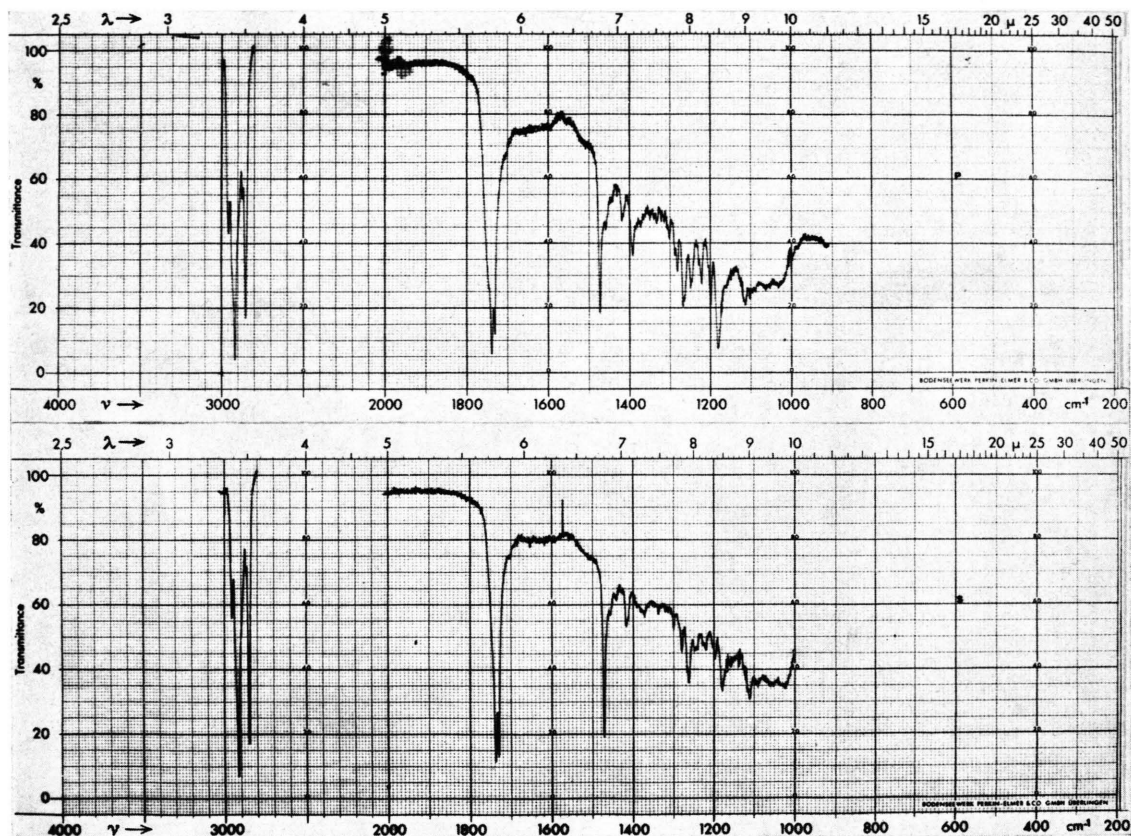


Fig. 6. Polarized ATR spectrum of tripalmitin crystallized on the reflection plate by evaporating the solvent (methanol).

iii. the interaction between adjacent tripalmitin molecules is assumed to be of the order of magnitude typical for molecular crystals and therefore will produce factor group splittings at the most.

As will be shown below this model is fully supported by the spectra of the tripalmitin layers. Independent evidence for this structure has earlier been given by electron diffraction experiments pub-

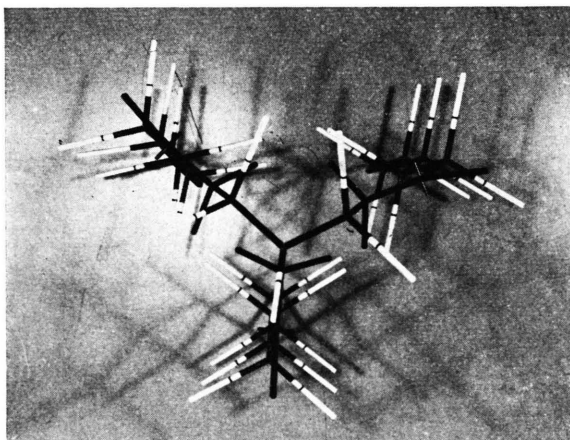


Fig. 7. Molecular frame work model of tripalmitin: Room temperature conformation.

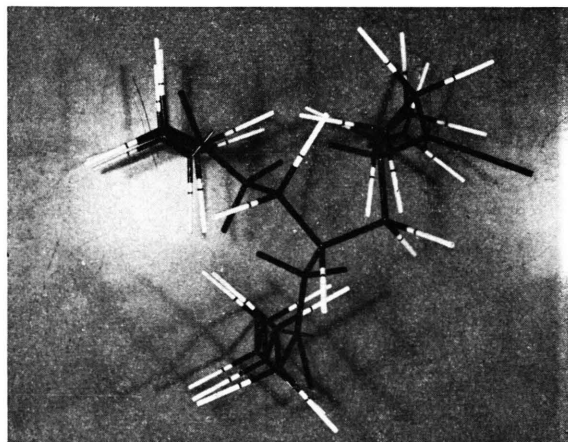


Fig. 8. Molecular frame work model of tripalmitin: Elevated temperature conformation.

lished by DE WAEL and HAVINGA²⁷. These authors suggested a pseudohexagonal packing of the tripalmitin molecules, the latter being located on site of symmetry C_{3v} . Fig. 7 represents a photograph of a molecular framework model taken along the pseudo-trigonal axis of the molecule from the side of the glycerol skeleton. In Fig. 9 a photograph of a CPK model is shown, which shows the proposed hexagonal packing of the tripalmitin units within the layers.

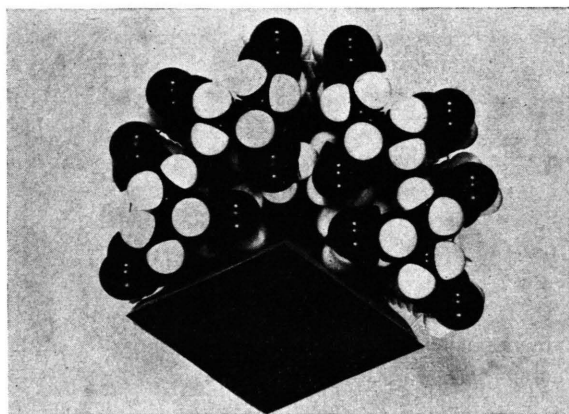


Fig. 9. CPK-Model of hexagonal packing of tripalmitin monolayer. View on the polar glycerol part.

The molecular model of tripalmitin layers described above should be correlated with models derived from X-ray analysis²⁸⁻³⁰. In some of these papers a tuning fork shaped conformation has been suggested for the tripalmitin molecules in the solid state. TRILLAT³¹ reported a bilayer structure for triglycerides. In view of the difficulties associated with the analysis of Debye-Scherrer diagrams of complex crystal structures the hexagonal structure of tripalmitin layers may still be considered compatible with both X-ray and electron diffraction studies of triglycerides³² and related compound like *n*-paraffins³³ and *n*-fatty acid salts and esters^{34,35}.

4.2. Spectral assignment and conformational analysis of room temperature spectra

The following discussion of double layer spectra will be made in the framework of the free oriented gas model. This approach seems to be justified by the fact, that no factor-group splittings have been observed so far.

i. CH-Stretching Modes of CH_2 -Groups (2858, 2922 cm^{-1})

As a main result of the study of these bands it will be shown that the structure of the layers is (spectroscopically) isotropic around the *z*-axis, i. e. normal to the reflection plate, cf. Fig. 1. The electric field in the rarer medium in the general case is anisotropic, $E_x \neq E_y \neq E_z$ ²⁵. Hence, a difference in dichroic ratios of the symmetric and antisymmetric CH-stretching modes of the methylene group should be observed, since the two corresponding transition dipole moments of a single CH_2 -chain are perpendicular to each other. However, no significant difference could be observed in the R' -values of these bands (Table I). This indicates that the tripalmitin molecules are symmetrically arranged around the *z*-axis within experimental error. This conclusion is supported by the fact that no significant change of the spectrum is observed when the layers are deposited on two germanium plates which are oriented with their long sides parallel and perpendicular to the dipping direction, respectively. It, however, should be mentioned that a more detailed model for the vibration dipole moment associated with the CH-stretching modes also leads to the symmetric pattern of the tripalmitin molecules around the *z*-axis.

ii. CH_2 -Wagging and Twisting Modes (1150–1400 cm^{-1})

Vertical orientation of polymethylene chains on the reflection plate.

The existence of the band progression between 1200–1300 cm^{-1} exhibits clearly the crystalline structure of the layers, with the CH_2 -groups in the all-trans-zig-zag configuration¹²⁻²². These typical bands can be assigned as wagging and twisting modes of the methylene groups^{12,17-19} and are considered as nearly undisturbed group motions. For the wagging modes one may expect the direction of the transition dipole moments to lie approximately perpendicular to the H–C–H-plane of the CH_2 -group. The room-temperature spectra, Fig. 2, show that some of the bands between 1200–1300 cm^{-1} vanish nearly completely in the spectrum with E_y -polarized light, indicating that the transition dipole moment of these bands is perpendicular to the *y*-component of the electric field (see section 4.5). Using the isotropic arrangement of molecules around the *z*-axis as derived in section i., this fact can only

be understood, if the polymethylene chains stand perpendicular on the germanium plate.

iii. Carbonyl Stretching Modes:
1730, 1738 cm^{-1}

C_s -symmetry of tripalmitin molecule, pseudohexagonal symmetry of the layer.

Inspection of the spectra scanned at temperatures below 50 °C, cf. Fig. 2, shows the existence of only 2 carbonyl bands. For a isolated tripalmitin molecule of arbitrary conformation 3 distinct carbonyl stretching bands should be expected. From our experiments for preparation of tripalmitin multilayers, we believe the packing of the molecules in the layers on the ATR plate to be equal to that in the surface layer. Both a molecular model with C_s or pseudotrigonal symmetry might be considered compatible with the observation of only two carbonyl stretching bands at room temperature. For a C_s conformation one would expect two a' and one a'' carbonyl stretching mode with M_z , M_x , M_y and M_x , M_y polarization, respectively. On the other hand a pseudotrigonal conformation should exhibit one a and one e mode, with M_z and (isotropic) M_x , M_y polarization respectively⁴⁰. Since no purely M_z (E_z) polarized band has been found, the model of a pseudotrigonal molecular structure is ruled out. However, the dichroic ratio of the two observed bands are apparently different (cf. Table I). Hence, it may be concluded that the M_z component contributes to a higher extent to the intensity of the 1738 cm^{-1} band. Both carbonyl bands have dichroic ratios essentially equal to that of the CH-stretching bands. From this we conclude that the orientation of the molecules in the layer structure should have at least pseudotrigonal symmetry, thereby producing nearly isotropic carbonyl modes. The fact that only two bands were observed at room temperature may nevertheless be considered compatible with the C_s molecular symmetry (cf. Figs. 7 and 9), the $a' - a''$ splitting is assumed too small for observation*. Actually closest packing in the layer structure is achieved by packing with hexagonal symmetry, which is compatible with the conclusion, reached above which lead to at least trigonal layer structure. This is in agreement with the electron diffraction results reported by DE WAEL et al.²⁷ for oriented tristearin layers.

* This point will be further investigated by normal coordinate analysis which also should allow more detailed assignment of the two carbonyl bands.

iv. Carbonyl-Single Bond Stretching Modes: (1155, 1180, 1262 cm^{-1})

From the carbonyl group vibrations in the 1150 cm^{-1} region valuable information about conformation and orientation of the ester groups may be derived. The most prominent band found at 1180 cm^{-1} may be assigned to a combined motion of both CO-single and CO-double bond stretching modes, coupled with the $\alpha\text{-CH}_2$ group³⁸. From consideration of the spectra scanned at temperatures below 50 °C, Fig. 2, one may conclude that the transition dipole moment of this band has nearly perpendicular direction with respect to the reflection plate (E_z polarization). It seems therefore very probable that the C_1 -atom of the glycerol skeleton, the adjacent ester oxygen, the carbonyl group and the $\alpha\text{-C}$ -atom of the chain all lie approximately in the same plane, perpendicular to the reflection plate. This interpretation is supported by the behaviour of the $\alpha\text{-CH}_2$ -wagging mode, which may be assigned to the band at 1390 cm^{-1} and which shows the same strong polarization as the other wagging modes of the chain³⁸. As an alternative interpretation of this band one could also assign this band to the bending modes of the CH_2 -groups of the glycerol part.

v. Further Remarks

Some further remarks concerning the assignment should be made. The practically unpolarized band at 1420 cm^{-1} may be attributed to the $\alpha\text{-CH}_2$ bending of the hydrocarbon chain. Near 1280 cm^{-1} a further nearly non-polarized band is found, which probably originates from CH_2 -wagging modes of the glycerol fragment. Inside the domain of the wagging progression a further unpolarized band at 1262 cm^{-1} is observed, which should originate from a mode involving the ester groups. From spectra of unperfected layers a strong correlation between the polarization behaviours of this band and the $\text{C}=\text{O}$ stretching band has been observed. Finally, it may be mentioned that only the 720 cm^{-1} band of the CH_2 -rocking band progression has been observed so far. Though it appears critical to derive much information from this one band of the rocking progression, the fact that it does not show any splitting appears to have some significance. For n -paraffins in orthorhombic and monoclinic crystals from this band has been found to exhibit factor-group splitting, whereas for triclinic and hexagonal crystal structure no such splitting appears to occur^{15, 36, 37}. Hence, the lack

of splittings of the 720 cm^{-1} band is in agreement with the hexagonal structure of tripalmitin layers as derived above.

4.3. Elevated Temperature Spectra and Induced Conformational Changes

From observation of tripalmitin ATR spectra between room temperature and 62°C information is derived about the melting process. No significant effects in the infrared spectra can be observed up to about 50°C , however, increasing the temperature above this point results at first in characteristic continuous changes of 4 absorption bands. The melting process of the oriented layers is found to extend over nearly 10°C . The first continuous conformational changes are observed in the polar glycerol part, followed by a sudden melting of the hydrocarbon chains. The melting process is completed when the polar framework of the layers breaks down.

i. Carbonyl-Single Bonds and Double Bond Stretching Modes

Above 50°C two new absorption bands appear at 1745 cm^{-1} and 1155 cm^{-1} indicating that the symmetry of the tripalmitin molecule is broken slowly. It seems clear that the first conformational changes take place in the glycerol part, because obviously typical CO-vibrations are affected primarily. The new band at 1745 cm^{-1} belongs to one of the three carbonyl groups of the molecules which have now become unequal. The 1155 cm^{-1} band may be assumed to correspond to the 1180 cm^{-1} band of the low temperature spectra [cf. section 4.2 (iv)]. The 25 cm^{-1} shift probably originates from changes of conformation in the ester part of the molecule. It should be pointed out that the direction of the transition dipole moment of this mode is strongly altered by the conformational change. In the 1180 cm^{-1} band it is nearly parallel to the z-axis, as already mentioned before, while in the 1155 cm^{-1} band of the $61-62^\circ\text{C}$ spectrum it is approximately 45° inclined against the z-axis. This value is evaluated from spectrum g of Fig. 2 by using a semi-empirical calculation described in section 4.5. This fact indicates that the temperature-induced conformational change originates primarily from a change of dihedral angles around the bonds involved in this vibrational mode. Furthermore, this torsion also affects the first few CH_2 groups near the ester group, producing specific effects discussed in ii. below. For

interpretation of the conformational changes around the ester groups it will be proposed that the glycerol part assumes a conformation of lower symmetry than C_s , featuring presumably the glycerol- CH_2 group at C_1 in staggered position with respect to the adjacent tertiary CH bond and the carbonyl group eclipsed to the CH_2 group. However, this new conformation requires a larger area per molecule as demonstrated in Figures 8 and 10. This transition from the C_s conformation of the molecules in the hexagonally packed layers to the less symmetrical conformation is expected to involve considerable increase in internal energy. As seen from Fig. 2 g the 1155 cm^{-1} band has anomalously large bandwidth which indicates the existence of a variety of different angles in the new conformation.

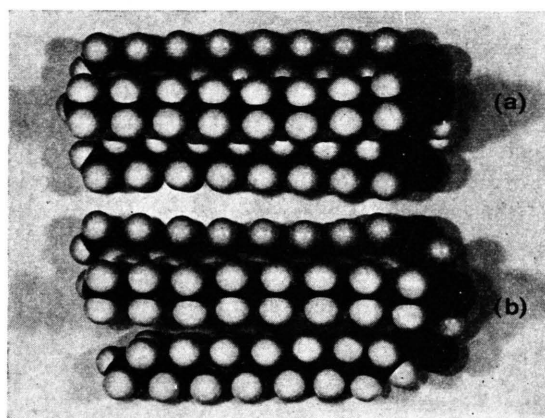


Fig. 10. CPK-Model of tripalmitin molecule. Comparison of the structures (area requirement) at room temperature (a) and at elevated temperature (b).

ii. Methylene Bonding Mode and α -Methylene Wagging Modes $1472, 1390\text{ cm}^{-1}$

Also these two modes exhibit continuous changes under the influence of increasing temperature. Broadening of the long wavelength part of the 1472 cm^{-1} band (CH_2 -bending mode) continuous decrease in intensity of the strongly polarized absorption band at 1390 cm^{-1} (α - CH_2 -wagging or glycerol bending mode) and broadening of the band centered at 1375 cm^{-1} occur simultaneously with changes described in section i. above. The broadening of the methylene bending mode may be traced back to the formation of non-equivalent CH_2 groups by conformation changes of the aliphatic chain (cf. Fig. 8). Similar effects have earlier been observed with cyclic paraf-

fins in the crystalline state. Cyclododecane, e. g., exhibits a complicated contour of the CH_2 -bending band due to a complicated pattern of dihedral angles in the sequence of CH_2 groups around the ring⁴¹. Further support for this interpretation comes from the disappearance of the strongly polarized 1390 cm^{-1} band. If assigned to the $\alpha\text{-CH}_2$ -wagging mode, this behaviour indicates that the $\text{H}-\text{C}-\text{H}$ plane of the $\alpha\text{-CH}_2$ group is no longer parallel to the surface of the reflection element. Similar arguments apply, if the 1390 cm^{-1} band is attributed to the glycerol- CH_2 -bending modes.

As a whole, the behaviour of both the bands of 1472 and 1390 cm^{-1} is in strong favour of the specific continuous intramolecular disordering process described in section 4.3 i. in contrast to first-order phase transitions.

iii. Wagging and Twisting Modes of the Hydrocarbon Chain

Fig. 2 clearly shows that the CH_2 -wagging progression of the aliphatic chains are practically not affected up to temperatures of $59-60^\circ\text{C}$, proving that the continuous disordering process leaves the all-trans-zig-zag conformation of the hydrocarbon chain essentially unchanged. In the temperature interval $60-61^\circ\text{C}$ a radical change of the wagging progression occurs. Hence, the thermal behaviour of the tripalmitin layer in the $60-61^\circ\text{C}$ temperature interval should be classified as a first-order phase transition.

4.4. Microcrystal Spectra

The microcrystal spectra shown in Fig. 5 serve mainly to prove that tripalmitin microcrystals and ordered layers behave by perfect analogy with respect to infrared spectra and thermal processes in the whole range $20-61$ (65) $^\circ\text{C}$. By comparison of the spectra shown in Fig. 2 (layer spectra) and Fig. 5 (microcrystal spectra), one reaches the conclusion that both states of aggregation feature practically identical spectra of corresponding temperatures and therefore one may assume that in both states the tripalmitin molecule exists in the same conformation. This result contradicts those of MALKIN²⁸ and KNOPP et al.^{29, 30}, referred to above in section 4.1 iii. These authors suggest a tuning-fork-shaped conformation for the solid state. However, the results arrived at in this work point to a bilayer type structure also for the crystal.

Finally a remarkable phenomenon observed in preparation of the microcrystal sample should be mentioned. It has been found that samples prepared by the procedure mentioned in section 2.2 show polarized spectra, as proved by Fig. 5. The only explanation for this behaviour at the present time is to assume that microcrystalline tripalmitin by either electrical or mechanical forces is oriented to a high extent during preparation of the sample.

Polarized spectra are also observed with samples produced by evaporation of the solvent from tripalmitin solutions dispatched on to the ATR plate. This appears to indicate a pronounced tendency of this glyceride to spontaneous organisation into double layers.

4.5. Direction of Transition Dipole Moment Determined from Relative Dichroic Ratio

Since the intensity of an absorption band is proportional to the square of the scalar product of the electric field vector $\vec{E}$ and the transition dipole moment $\frac{\delta\vec{\mu}}{\delta q}$ (change of molecular dipole moment with respect to the change of the normal coordinate q)^{40, 45}

$$\alpha \sim \left(\vec{E}, \frac{\delta\vec{\mu}}{\delta q} \right)^2$$

relative absorption coefficients and dichroic ratios can be calculated, when the electric field components are known. If the unit vector of the transition moment is denoted $\vec{m} = (m_x, m_y, m_z)$, one gets the following expression for the dichroic ratio

$$R' = \frac{(\vec{E}_{||}, \vec{m})^2}{(\vec{E}_{\perp}, \vec{m})^2} = \frac{(E_x m_x + E_z m_z)^2}{(E_y m_y)^2} \quad (2)$$

$$\begin{aligned} \text{with} \quad m_x &= \sin \vartheta \cos \varphi \\ m_y &= \sin \vartheta \sin \varphi \\ m_z &= \cos \vartheta \end{aligned} \quad (3)$$

where ϑ is the angle between $\vec{m}$ and the z -axis and φ is the angle between the projection of $\vec{m}$ on the x - y -plane and the x -axis. Introducing (3) into (2) and making use of the fact that tripalmitin molecules are isotropically arranged around the z -axis leads to the following expression for the dichroic ratio

$$R' = \frac{E_x^2}{E_y^2} + 2 \frac{E_z^2}{E_y^2} \cdot \text{ctg}^2 \vartheta. \quad (4)$$

Averaging of the absorption coefficients $\alpha_{||}$ and $\alpha_{\perp}$ over both angles ϑ and φ , respectively, results in

$$\bar{R}'_{x,y,z} = \frac{E_x^2}{E_y^2} + 2 \frac{E_z^2}{E_y^2}. \quad (5)$$

Eq. (5) holds for a thin film with isotropic distribution of the molecules. Such a film must be expected, when the temperature of an oriented layer is increased to some degrees above its melting point. It should however be mentioned that the electric field components in Eqs. (4) and (5) are assumed to be averaged values over the film thickness, which was smaller than 550 Å in all experiments. The ratios of the field components as appearing in Eqs. (4) and (5) may be determined experimentally. First E_x^2/E_y^2 is given by the relative dichroic ratio of absorption bands which have their transition dipole moments parallel to the x-y-plane and are isotropically arranged around the z-axis. As is shown in

section 4.2 the $\nu_a(\text{CH}_2)$ -, $\nu_s(\text{CH}_2)$ - and the $\delta(\text{CH}_2)$ -bands exhibit these features, cf. Table I. The corresponding averaged dichroic ratio becomes then

$$\bar{R}'_{x,y} = \frac{E_x^2}{E_y^2} = 1.24 \pm 0.09.$$

On the other hand the isotropic dichroic ratio from Eq. (5) was calculated from the molten layer spectrum at 67°–68 °C. This spectrum featured the same dichroic ratios as the presented one in Fig. 4 a, resulting in

$$\frac{E_z^2}{E_y^2} = 0.10 \pm 0.05.$$

Introducing E_x^2/E_y^2 and E_z^2/E_y^2 into (4) enables the approximative calculation of ϑ from experimentally determined relative dichroic ratios. For some bands ϑ -values are indicated in Table I.

It should be mentioned that the application of the analytical expressions for the electric field compo-

$\tilde{\nu}$ (cm ⁻¹)	$R' = \frac{\alpha'_{ }}{\alpha'_{\perp}}$	ϑ	Assignment	Remarks
2960	—	—	$\nu_a(\text{CH}_3)$	antisymm. CH-stretching of CH ₃ groups
2880	—	—	$\nu_s(\text{CH}_3)$	symm. CH-stretching of CH ₃ groups
2922	1.27 ± 0.12	69° ± 40°	$\nu_a(\text{CH}_2)$	antisymm. CH-stretching of CH ₂ groups
2858	1.19 ± 0.13	90° ± 40°	$\nu_s(\text{CH}_2)$	symm. CH-stretching of CH ₂ groups
1745	—	—	$\nu(\text{C=O}) + \nu(\text{C-O})$	carbonyl stretching, band appears at temperature above 50 °C
1738	1.23 ± 0.15	90° ± 40°	$\nu(\text{C=O}) + \nu(\text{C-O})$	carbonyl stretching
1730	1.19 ± 0.13	90° ± 40°	$\nu(\text{C=O}) + \nu(\text{C-O})$	carbonyl stretching
1472	1.25 ± 0.18	90° ± 44°	$\delta(\text{CH}_2)$	methylene bending of hydrocarbon chain
1457	—	—	$\delta_a(\text{CH}_3) + \delta(\text{CH}_2)$	combination of antisymm. CH ₃ -bending and CH ₂ -bending of chain ^{36, 38}
1443	—	—	ev. $\delta_a(\text{CH}_3)$	antisymm. methyl bending (?)
1419	1.15 ± 0.43	90° ± 47°	$\delta(\alpha\text{-CH}_2)$	bending of α -methylene groups
1390	strongly z-polarized	approx. 0°	$\gamma_w(\alpha\text{CH}_2)$ or $\delta(\text{glycerol-CH}_2)$	wagging of α -CH ₂ groups or bending of glycerol-CH ₂ groups
1370	—	—	$\delta_s(\text{CH}_3)$	symm. bending of methyl groups
1330, 1300	—	—	—	—
1287, 1267	strongly z-polarized	approx. 0°	$\gamma_w(\text{CH}_2)$	wagging progression of methylene groups of hydrocarbon chain
1248, 1243	—	—	—	—
1228, 1220	—	—	—	—
1203, 1198	—	—	—	—
1280	approx. x-y-polarized	approx. 90°	$\gamma_w(\text{glycerol-CH}_2)$	wagging of glycerol-CH ₂ groups
1262	slightly z-polarized	—	—CH ₂ —COO-group vibration	with CO-single bond stretching involved ^{38, 41}
1180	strongly z-polarized	approx. 0°	—CH ₂ —COO-group vibration	with CO-single bond stretching involved ^{38, 41, 42}
1155	1.87 ± 0.35	45° ± 20°	—CH ₂ —COO—CH ₂ —group vibration	with CO-single bond stretching involved, measured at 60–61 °C ³⁸
1112, 1060	—	—	N(C—C)	Band appears only at temperatures above 50 °C.
720	approx. x-y-polarized	approx. 90°	$\gamma_r(\text{CH}_2)$	stretching of C—C frame ^{38, 43} methylene rocking, long wavelength limit of progression

Table I. Assignment of Absorption Bands of Tripalmitin Oriented Layers. Relative Dichroic Ratios and Angle ϑ between Transition Dipole Moment and z-Axis.

nents given in ¹⁰ leads to results which are in contradiction with the experimental results described in this paper. To get more accurate analytical expressions, straightforward calculations following ⁴⁶ are required. Furthermore, a correct treatment of this problem also requires the knowledge of the internal electric field in the neighbourhood of the absorbing dipole. However, in general it is assumed that this field is not significantly different from the external field in a non-absorbing anisotropic medium.

The authors wish to thank to Prof. Dr. L. C. DE MAEYER (Göttingen), Prof. Dr. M. EIGEN (Göttingen), Dr. H. HOEGL (Geneva) and Dr. W. MÜNCH (Göttingen/Zürich) for their continuous interest in this work. Also the help of Dr. P. FROMHERZ (Göttingen) and Prof. Dr. P. LÄUGER (Konstanz) in the preparation of oriented layers is acknowledged.

This project is supported by the Swiss National Foundation, the Battelle Memorial Institute and the Max-Planck-Gesellschaft.

Furthermore, financial support by Messrs. Sandoz AG. and Messrs. F. Hoffmann-LaRoche, Basle, is gratefully acknowledged.

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