

Infrared Spectroscopic Characterization of Glycerides

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GLYCERIDES are usually difficult materials to characterize, in the main because of the variety of possible isomers which can occur. Infrared spectroscopy has however been shown to be a versatile analytical tool with wide applications in this field of investigation. While the greater part of the early infrared spectroscopic work was carried out with the material in solution in either carbon tetrachloride or chloroform, recent studies have shown that much greater qualitative information may be obtained, especially about the configuration of glycerides, by obtaining the spectra of the materials in the crystalline state. This introduces complications arising from the occurrence of polymorphism, *i.e.*, the existence of more than one crystalline form for the same glyceride. However these complications themselves can be turned to good account, and the spectra may be used to provide information about the crystalline nature of the glyceride (1a-e).

In this paper the wealth of information present in the spectra of glycerides is reviewed and discussed.

Experimental

The infrared absorption spectra were measured with a Grubb Parsons S.3 double-beam instrument. The temperature of the spectrometer was maintained at 26°C. Samples were measured in Nujol mulls or KCl discs, or melted on rock salt flats. All the compounds were of a high standard of purity and had been prepared by standard methods by colleagues in this laboratory (excepting samples of aceto-fats which were kindly presented by Miss Audrey Gros of the U.S.D.A., Southern Utilization Research Branch, New Orleans, La., U.S.A.). Heated cell-holders (2) were used to obtain the spectra of some forms and a low-temperature transmission cell (3) for those forms stable only below room temperature.

Discussion

Triglycerides, diglycerides, and monoglycerides have been examined, and their spectra are given.

Triglycerides. O'Connor *et al.* have given the spectra from 2-12 μ of a number of triglycerides obtained from solutions in chloroform and have assigned the main bands to vibrations of the functional groups present (4). We may expect that, except for some small shifts in frequency, these bands will also occur in the spectra of the melt, and this is found to be so. Table I shows the frequencies of the main bands for long-chain triglycerides. Because of the rotation of the methylene groups about the C-C bonds, the spectrum of the solution and liquid will include the spectra of a great many rotational isomers, and bands will generally be broad and smeared into each other.

The spectrum of the randomly oriented crystalline material will also include the major bands resulting from the functional groups present, but splitting of bands and shifts will occur. Furthermore the spectrum will then usually be that of a single rotational isomer. Glycerides may be either saturated or unsaturated in character. Let us first consider the saturated triglycerides. A discussion of the nomenclature

TABLE I

Probable assignment	
C-H stretching (CH ₂ and CH ₃).....	3030-2967 cm. ⁻¹
C=O stretching (COOR).....	1751-1733 cm. ⁻¹
C-H bending (CH ₂ /CH ₃) groups.....	1464-1453 cm. ⁻¹
C-H bend (symmetrical deform of CH ₃).....	1383-1361 cm. ⁻¹
C-H in plane wagging or rocking of CH ₂ groups..	1261-1250 cm. ⁻¹
C-O stretching (COOR).....	1179-1166 cm. ⁻¹
CH ₂ rocking mode.....	730- 717 cm. ⁻¹

used to designate the polymorphic form of glycerides has been given previously (1c).

Saturated Triglycerides. The spectra vary according to the polymorphic form in which the glyceride occurs. The variety of spectra obtained with a single saturated triglyceride in its various polymorphic forms is illustrated in Figure 1 for trimargarin. The major differences between these spectra are similar to those shown by "even-acid" saturated triglycerides, such as tristearin. A single band occurs at 720 cm.⁻¹ in the spectrum of the α_L -form; a doublet at 719 and 727 cm.⁻¹ in the spectrum of the β'^L -form; and a single band at 717 cm.⁻¹ in the spectrum of the β_L -form. (These bands correspond to the main CH₂ rocking mode of the methylene groups.) The lowest form, designated sub- α_L , is obtained by quenching the liquid glyceride to -70°C. and obtaining the spectrum at this temperature. The transition sub- α_L to α_L is observed to be a reversible one. The main CH₂ rocking mode in the spectrum of the sub- α_L form is also a doublet at 719 and 727 cm.⁻¹. These variations have been discussed recently in terms of the packing of the hydrocarbon chains (5): hexagonal in the α_L -form, orthorhombic in the β'^L -form, and triclinic in the β_L -form [cf. also an investigation (6) of crystal-

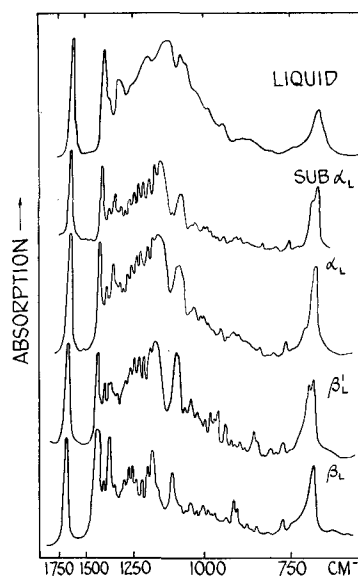


FIG. 1. The spectra of the polymorphic forms of trimargarin. (a) Liquid at 65°C. (b) Sub- α_L form. Glyceride melted, then quenched to -70°C., spectrum obtained at this temperature. (c) α_L -form. Glyceride melted, then allowed to solidify at room temperature, spectrum obtained at this temperature. (d) β'^L -form. Glyceride melted, allowed to solidify at room temperature, then heated to 53°, spectrum obtained at this temperature. (e) β_L -form. Glyceride crystallized from benzene, spectrum of Nujol mull at room temperature.

line n-paraffins]. The packing of the hydrocarbon chains in the sub- α_L form is also probably orthorhombic. For characterization purposes the most suitable form is probably the α_L -polymorphic form, which for triglycerides, of greater molecular weight than trilaurin, is the form usually obtained by solidification at room temperature from the melt. It is therefore fairly easily and conveniently obtained. The spectra of the remaining forms are obtained by suitable thermal treatment or by crystallization from solvent followed by KCl disc or Nujol mull preparation.

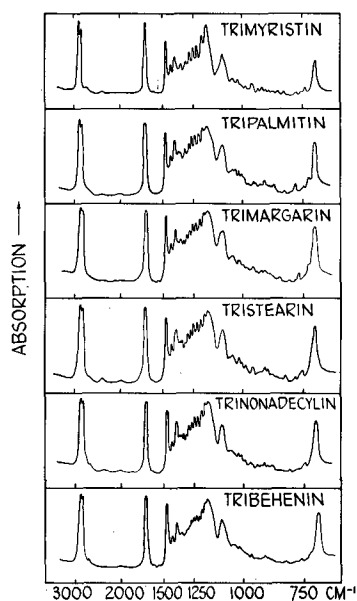


FIG. 2. The spectra of a series of saturated glycerides in the α_L -polymorphic form. All the glycerides were melted and cooled to room temperature; spectra were obtained at this temperature.

The spectra of a series of saturated triglycerides are shown in Figure 2. In each case the glyceride is in the α_L -polymorphic form, and the general similarity of the spectra is apparent. The number of bands in the 1250 cm^{-1} region increases with increasing chain-length. The frequencies of these bands are given in Table II. The number of bands for an even number of carbon atoms in the chain is usually equal to half the number of carbon atoms in the chain $\frac{1}{2}n$ while for an odd number of carbon atoms it is usually equal to half of the number of carbon atoms plus one $\frac{1}{2}(n+1)$. While the number of bands in the spectrum of a triglyceride with an odd-numbered chain is the same as in the spectrum of the next higher even-numbered chain, all the bands are shifted in frequency. The frequency difference between the band of highest frequency and its next lower neighbor is always less than the frequency difference between the other bands. These bands were thought to originate from methylene wagging modes and are analogous to those observed with anhydrous sodium soaps (7a) and fatty acids and esters (7b).

Susi (8) has however pointed out that, in agreement with Ferguson (9), data are not in agreement with the assignment of these bands to wagging and twisting vibrations of coupled $-\text{CH}_2$ groups. He believes from his data as well as those of Corish and Chapman (10) that "all evidence points to the conclusion that we are not dealing with separable vibra-

TABLE II

Saturated triglycerides (α_L -form)	Frequency of bands in the 1250 region (cm^{-1})
Trimyristin.....	1344, 1329, 1300, 1276, 1252, 1229, 1200
Tripalmitin.....	1342, 1331, 1308, 1285, 1265, 1240, 1222, 1199
Trimargarin.....	1334, 1318, 1299, 1276, 1260, 1235, 1219, 1198
Tristearin.....	1341, 1330, 1308, 1289, 1270, 1255, 1232, 1216, 1193
Trinonadecylin.....	1346, 1335, 1323, 1302, 1285, 1266, 1248, 1230, 1213, 1194
Tribehenin.....	1339, 1323, 1310, 1293, 1279, 1265, 1252, 1233, 1222, 1205, 1189
Saturated diglycerides (β_L -form)	Frequency of bands in the 1250 region (cm^{-1})
1:3 Dilaurin.....	1328, 1310, 1298, 1266, 1235, 1211
1:3 Dimyristin.....	1347, 1329, 1306, 1279, 1258, 1231, 1209
1:3 Dipalmitin.....	1346, 1332, 1310, 1290, 1266, 1245, 1224, 1205
1:3 Distearin.....	1340, 1328, 1309, 1292, 1271, 1255, 1231, 1214, 1198
Saturated diglycerides (α_L -form)	Frequency of bands in the 1250 region (cm^{-1})
1:2 Dilaurin.....	1349, 1329, 1299, 1270, 1239, 1210
1:2 Dimyristin.....	1347, 1333, 1306, 1281, 1259, 1231, 1205
1:2 Dipalmitin.....	1343, 1331, 1308, 1286, 1265, 1240, 1221, 1199
1:2 Distearin.....	1342, 1328, 1307, 1290, 1269, 1253, 1232, 1213, 1192
Monoglycerides (β_L -form)	Frequency of bands in the 1250 region (cm^{-1})
1-Mono-olein.....	1333, 1294, 1255, 1213
1-Monocaprylin.....	1327, 1283, 1231
1-Monocaprin.....	1329, 1293, 1256, 1221
1-Monolaurin.....	1337, 1302, 1275, 1242, 1215
1-Monomyristin.....	1332, 1306, 1284, 1258, 1230, 1205
1-Monopalmitin.....	1339, 1314, 1293, 1271, 1248, 1227, 1202
1-Monostearin.....	1332, 1312, 1297, 1277, 1258, 1233, 1219, 1198

tions and that the actual mixed motion probably involves skeletal movements of the carbon chain."

To differentiate saturated triglycerides of mixed chain-length is a little more difficult since a number of complications may now occur. With glycerides such as 1-palmito-distearin the number of bands in the 1250 cm^{-1} region of the α_L -form is the same as that in tristearin while with 1-stearo-dipalmitin the number of bands is the same as in the spectrum of tripalmitin. With some glycerides however the α_L -form is not stable while with other glycerides the most stable form is a β'^L -form rather than a β_L -form [cf. the spectra (1e) of 2-stearo-dipalmitin (PSP) and 2-palmito-distearin (SPS)]. In the spectrum of a triglyceride of considerable difference in chain length, e.g., 1:2-diaceto-3-palmitin, the band at 7.25 μ is of strong intensity corresponding to the methyl groups present, and the CH_2 rocking mode is a single band at 718 cm^{-1} , indicating that the hydrocarbon chains are probably triclinically packed in this form as in the β_L -form of other saturated triglycerides.

Unsaturated Triglycerides. Unsaturated triglycerides can be divided into two main types: those containing *trans* groups and those containing *cis* groups although glycerides containing both these groups also can occur. There is little difficulty in differentiating these two main types since the presence of the *trans* group is clearly shown by a band at 963 cm^{-1} . This band occurs in the spectrum of material in the liquid state, in solution, or in the crystalline state. A considerable amount of work has been published concerning this band (11, 12). The spectra obtained from the polymorphic forms of trielaidin designated α_L and β_L are shown in Figure 3. (The spectrum of a β'^L -form was not obtained.) The strong band near 961 cm^{-1}

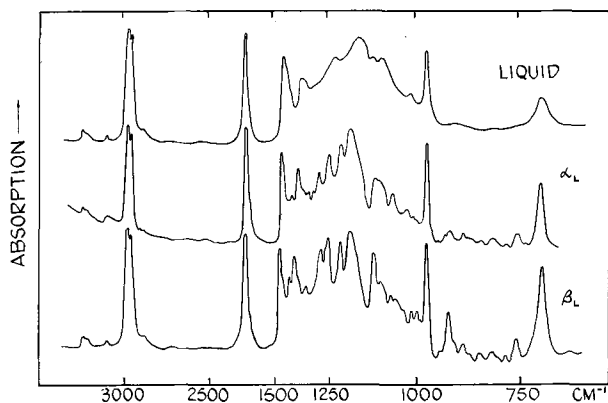


FIG. 3. The spectra of the polymorphic forms of trielaidin. (a) Liquid at 50°C. (b) α_L -form. Glyceride melted, quenched to 0°C, spectrum run at 20°C. (c) β_L -form. Glyceride crystallized from acetone, spectrum of Nujol mull at room temperature.

attributable to the *trans* group in the molecule is apparent in all the spectra. The frequency of the main CH_2 rocking mode is at 722 cm^{-1} in the spectrum of the α_L -polymorphic form, and at 718 cm^{-1} in the spectrum of the β_L -polymorphic form. This is suggestive that the hydrocarbon chains in these two forms are predominantly packed in the hexagonal and triclinic forms, respectively. The relative intensities of the bands in the 1250 cm^{-1} region in the spectra change in a manner analogous to that observed with saturated triglycerides. A strong band at 896 cm^{-1} occurs in the spectrum of the β_L -form as in the spectra of saturated triglycerides.

If a weak band occurs near 1660 cm^{-1} , we may deduce that there is probably a *cis* group present. The band attributable to the bending vibration of the hydrogen atoms in the *cis* group is however not constant in frequency and is very sensitive to crystal structure (13). As with the saturated triglycerides a variety of spectra can be obtained, depending on the particular crystalline form in which the material can exist. (Most unsaturated natural fats exist predominantly in the *cis* form but change to a mixture of *cis* and *trans* forms on partial hydrogenation.)

It is preferable with these glycerides to obtain more than one spectrum if this is possible. Examination of the 1250 cm^{-1} region of the spectrum of the α_L -form will provide information about the chain length. The spectrum of the most stable form, usually obtained by slow crystallization from solvent, can also be very informative, *e.g.*, the most stable form of 2-oleo type mono-oleo triglycerides crystallizes in a form (14) designated β_L while the most stable form of 1-oleo type mono-oleo triglycerides crystallizes in a form designated β'^L . As a result of this, the spectra of the stable forms of these closely related isomers differ considerably. The spectra of 2-oleo-dipalmitin and 1-oleo-dipalmitin in their most stable forms are shown in Figure 4.

The main CH_2 rocking vibration in the spectrum of the 2-oleo isomer is single at 717 cm^{-1} while that of the 1-oleo isomer is a doublet at 729 and 719 cm^{-1} . The spectrum of the 2-oleo isomer also shows a strong band at 690 cm^{-1} , attributed to a vibration of the *cis* group. A strong band does not occur in this region of the spectrum of the 1-oleo isomer. A sub- α_L form showing a doublet at 719 and 727 cm^{-1} is also observed with these glycerides. (This is particu-

larly clear in the spectra of 1-oleo distearin at 0°C.) This large difference observed between the spectra of the 1- and 2-oleo isomers is very useful and has already been used to determine the major glyceride present in cocoa butter (15) and kokum butter (16). Spectra of other natural fats, such as illipe butter, have also been obtained. The major glyceride in all these natural fats except lard appear to have the same 2-oleo type of configuration.

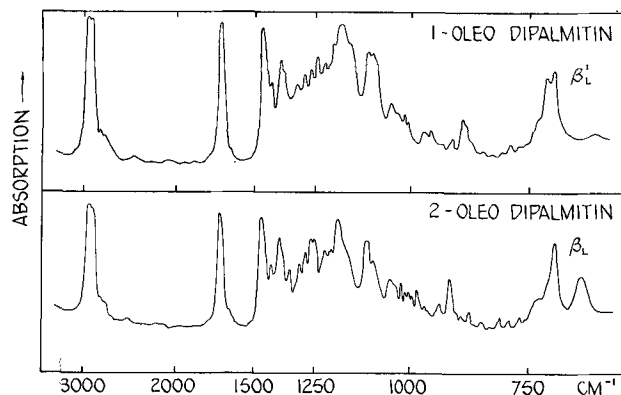


FIG. 4. The spectra of the most stable forms of 1-oleo dipalmitin and 2-oleo dipalmitin (β'^L and β_L , respectively). Both glycerides were crystallized slowly from acetone and dispersed in Nujol. Both spectra were run at room temperature.

Unusual variations may occur when mixed crystal formation occurs. This is particularly observed with mixtures of 2-oleo and 1-oleo distearin. Mixtures of equal amounts of these glycerides, crystallized slowly from solvent and dispersed in Nujol, give rise to a spectrum quite different from that of either glyceride and corresponds to a definite mixed crystal formation.

Spectra obtained from the polymorphic forms of triolein are shown in Figure 5. The strong band near 690 cm^{-1} and the band at 1660 cm^{-1} , attributable to the presence of the *cis* group, are apparent. Of particular interest is the band assigned to the main CH_2 rocking mode near 720 cm^{-1} . In the spectrum of the α_L -form the band is single at 723 cm^{-1} , and in the spectrum of the β'^L -form it is split into two components at 729 and 722 cm^{-1} while in the spectrum of the β_L -form it is single at 722 cm^{-1} . This is closely analogous to the variation of this band observed in the spectrum of the saturated triglycerides. It may perhaps be indicative that, despite the presence of the *cis* groups in the chain, the chains or parts of the chains still pack predominantly in a manner analogous to that observed in saturated triglycerides. The x-ray short-spacings of these forms are consistent with this interpretation (17).

Apart from the presence of bands attributable to vibrations of the particular unsaturated group, the spectra of triolein and trielaidin in corresponding polymorphic forms have many similarities.

Diglycerides. Diglycerides may also exist in two isomeric forms, depending on the position of the chain on the glycerol residue. Thus 1:3 and 1:2 type glycerides occur.

Characterization of the two types is usually quite simple since the spectra of crystalline forms of these two types of glyceride differ considerably as a consequence of the different modes of packing adopted

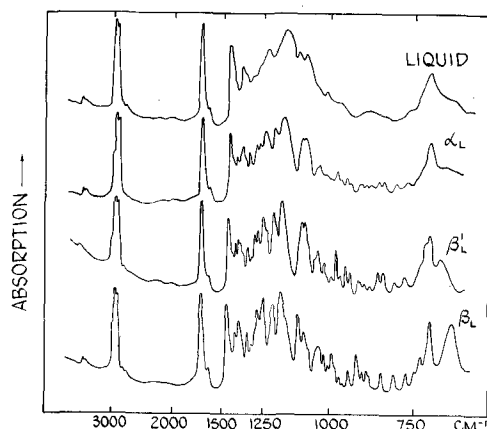


FIG. 5. The spectra of the polymorphic forms of triolein. (a) Liquid at room temperature. (b) α_L -form glyceride quenched to -55°C ., spectrum obtained at this temperature. (c) β'^L -form glyceride quenched to -55°C ., heated to -20°C ., spectrum obtained at this temperature. (d) β_L -form glyceride quenched to -55°C ., heated to -3°C ., spectrum obtained at this temperature.

by the hydrocarbon chains. The 1:3 type of diglyceride, such as distearin and dipalmitin, appears to crystallize (18) in two very similar forms (probably with triclinic packed chains) while the 1.2 type glyceride appears to crystallize in two forms (19), with x-ray short spacings different in type from those of the 1:3 diglycerides. The spectrum of a diglyceride, such as distearin in its most stable form after crystallizing from solvent and dispersing in Nujol, affords a very convenient way to differentiate between the 1:2 and 1:3 diglyceride isomers. The 1:3 type of glyceride from the melt gives rise to a spectrum nearly identical with that obtained by dispersing the solvent-crystallized material in Nujol, *i.e.*, it exists in two β_L -type forms. These glycerides do not appear to crystallize in an α_L -form. The spectra of a series of 1:3 diglycerides are shown in Figure 6. Information about the chain length can be deduced from the 1250 cm^{-1} region. The frequencies of the bands are given in Table II.

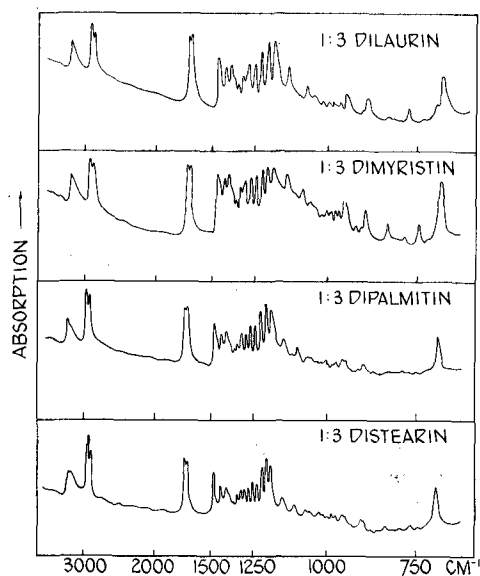


FIG. 6. The spectra of a series of 1:3 diglycerides. The glycerides were crystallized slowly from acetone and pressed in KCl discs. Spectra run at room temperature.

It should be noted that not all 1:3 diglycerides crystallize in the β_L -form, *e.g.*, 1-aceto-3-palmitin crystallizes (20) in a β'^L -form, and its spectrum is very different from that observed with, say, 1:3-dipalmitin. In particular, the main CH_2 rocking mode near 720 cm^{-1} consists of a doublet.

The spectra of the two polymorphic forms (designated $\alpha_L \equiv \alpha_M$ and $\beta'^L \equiv \beta_M$) of the 1:2 diglycerides have marked differences (1e). In the spectrum of the α_L -form the main CH_2 rocking mode is single at 720 cm^{-1} while in the spectrum of the β'^L -form there is a doublet at 727 cm^{-1} and 719 cm^{-1} . This implies that the hydrocarbon chains are predominantly packed in the hexagonal manner in the α_L -form but are orthorhombically packed in the β'^L -form (1e). The spectra of a series of 1:2 diglycerides in the α_L -polymorphic form are shown in Figure 7.

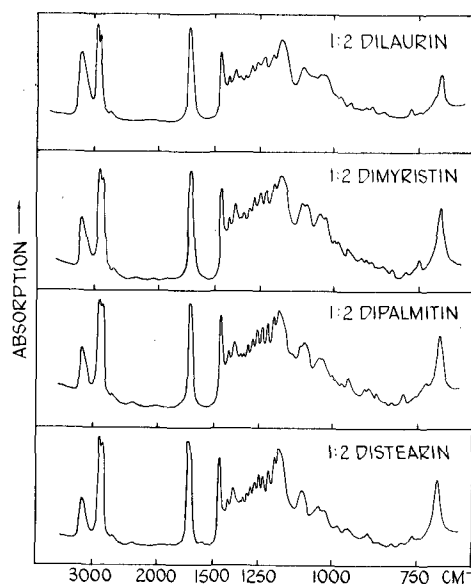


FIG. 7. The spectra of a series of 1:2 diglycerides in the α_L -polymorphic form. All the glycerides were melted and cooled rapidly to 0°C .. The spectra were run at room temperature.

Information about the chain length can be deduced from the bands in the 1250 cm^{-1} region of the spectra. The number of bands observed is equal to half the number of carbon atoms in the chain, $\frac{1}{2}n$ for the "even carbon" diglycerides. The frequencies of the bands are given in Table II.

Monoglycerides. Monoglycerides can be of two main types, depending on where the chain is attached to the glycerol residue. If the chain is attached in either the 1 or 3 positions, the glyceride is designated a 1-monoglyceride since these positions are equivalent. The spectra of the monoglycerides in the liquid state show the bands to be broad (1a, 21) and tending to smear into each other. This is caused by the presence of many rotational isomers from rotation of the methylene groups about the C-C bonds.

A means of differentiating the two types of monoglyceride follows from the fact that different thermal treatment of the 1-monoglycerides gives rise to different spectra, corresponding with the different polymorphic forms in which the 1-monoglycerides can occur (1a). The 2-monoglycerides however exist in only one polymorphic form (22).

The spectra of a series of 1-monoglycerides in the stable β_L -form are shown in Figure 8. The major differences between the spectra lie in the 1250 cm^{-1} region. The frequencies of these bands are given in Table II.

The spectrum of the crystalline form of a 2-monoglyceride is quite distinct from that of the 1-monoglyceride; and this simplifies characterization of the two types (Figure 9).

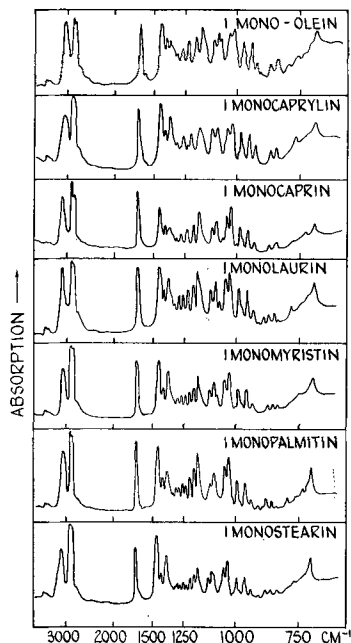


Fig. 8. The spectra of a series of 1-monoglycerides in the most stable form (β_L). 1-mono-olein was melted between rock salts and cooled to room temperature and allowed to stand at this temperature for 4 hrs. The spectrum of the solidified melt was run at room temperature. All the remaining glycerides were crystallized slowly from acetone and dispersed in Nujol. Spectra were run at room temperature.

The practical use of the spectra obtained with the crystalline form of 1-monoglycerides has already been demonstrated by Kuhrt *et al.* (23), who showed that monoglycerides were formed to an appreciable extent when monoglyceride-free fat was used to make bread. It is clear that a great deal of similar spectroscopic work to aid characterization will be carried out in the future.

Summary

The infrared spectra of a number of glycerides of different types, tri-, di-, and monoglycerides in their crystalline forms have been obtained, and the wealth of information present in these spectra has been discussed. In particular, polymorphic form, chain length, type of unsaturation, and configuration are all revealed by the spectra.

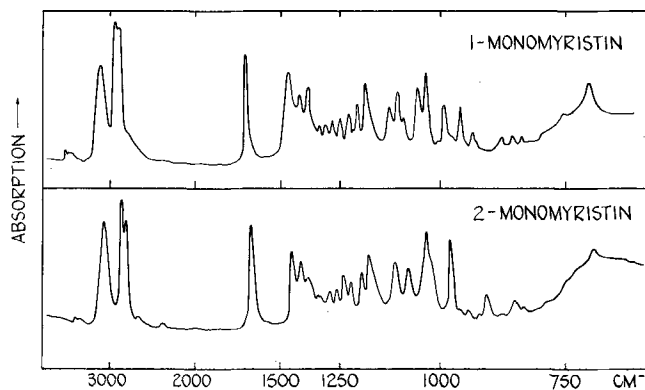


Fig. 9. The spectra of 1- and 2-monomyristin in their most stable form (β_L). 1-Monomyristin was crystallized slowly from acetone and dispersed in Nujol; the spectrum was run at room temperature. 2-Monomyristin was melted between rock salts and allowed to solidify at room temperature. The spectrum was run after allowing the glyceride to stand two days at room temperature.

Acknowledgments

I wish to thank my colleagues of the Research Department and also Miss Audrey Gros for presenting the samples of the pure glycerides, and Misses Coope, McCormack, and Williams for aid in obtaining the spectra. I also wish to thank the directors of Unilever Ltd. for permission to publish this paper.

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[Received September 17, 1958]