Differential Thermal Analysis of Fats. II. Melting Behavior of Some Pure Glycerides

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A THOROUGH STUDY of some mono-oleyl disaturated glycerides has been undertaken here as part of a program of research into the structure of fats. Before investigating the behavior of mixtures of disaturated triglycerides, it was necessary to characterize the individual pure glycerides. The present paper records the melting characteristics and polymorphism of seven mono-oleyl disaturated glycerides (designated as SOS; POP; OSS; OPP; POS; OPS; and OSP) as determined by differential thermal analysis and X-ray powder photography.

Lutton (1, 2, 3) has studied the polymorphism of all these compounds while Malkin and Wilson (4) have studied the first two: SOS and POP. Daubert and Clarke (5, 6) have reported on the first four: SOS, POP, OSS, and OPP. The disturbing features of their publications are concerned with a) their apparent lack of agreement as to the number of polymorphic forms; and b) in particular, the wide diversity of reported melting points, X-ray data, and nomenclature.

In the present paper the nomenclature of Lutton has been followed where the X-ray data agrees with his. Where there is no agreement, the nomenclature used is made clear in the text. Further, the melting points are approximate values only and have been obtained in a manner similar to that described in Part I of this series.

Experimental

Glycerides Tested. The glycerides used were samples of those prepared by Davies *et al.* and described in another publication (7). They had analytical constants and X-ray patterns in good agreement with those quoted by Lutton (1, 2).

Apparatus and Procedure. The apparatus was essentially similar to that described in Part I, but modified in the following manner. The thermocouples were made of Inconel tubing, with an insulated Ferry wire through the bore. Both were hard-soldered at the measuring point. The thermocouple was centered in the sample holder by means of a polytetrafluorethylene (P.T.F.E.) collar. The copper block (diam. 7.4 cm., height 6.0 cm.) was heated by a 43-ohm Ferry wire heater, fed with 24-30-36 or 42 volts from a constant voltage transformer.

For a 2°C./min. temperature rise at 30°C. the actual heat input was about 36 watts. A Tinsley galvanometer (internal resistance 476 ohms) was used to record the temperature differential.

In this paper the heating rate was 1.5°C./min. except where otherwise stated.

The procedure adopted to study the pure triglycerides was as follows. First, the sample was weighed into the copper cell and melted; the α-form was obtained by chilling in an ice/salt mixture; this then was heated in the copper block until the sample remained completely liquid. In this way the form into which the α-polymorph transformed was obtained. Next, a solvent-crystallized sample was put in the copper cell and heated in the copper block from room temperature. The melt thus obtained was allowed to cool slowly to room temperature over-night, and the solid formed was heated up again to obtain a third melting curve.

The sample was then treated according to previously published methods, which were claimed to yield forms different from those obtainable by the method already described. The forms recorded in our tables however were the only ones actually detected.

As far as possible, second samples were treated in an identical manner and then subjected to X-ray powder photograph, in order to identify the various forms.

A Metro-Vick X-ray unit with Cu-Kα radiation was used; the sample to film distance was 12.5 cm.

Results

2-Oleoyl Distearin (SOS) (Figure 1 and Table I). Three different polymorphic forms have been identified. A solid-to-solid transformation occurred from the α form (obtained by shock chilling) to the sub-β form at about 23°C. when the α form was gradually heated from 0°C. Heating at 1.5°C./min. showed the transformation much more clearly than at 0.5°C./min.

Cooling the melt slowly from 60°C. to room temperature always gave rise to the sub-β form melting at 36.5°C. The stable β form was obtained either from the melt or by crystallization from acetone. The melting points showed good agreement.

2-Oleoyl Dipalmitin (POP) (Figure 2 and Table II). The differential calorimetric method revealed four polymorphic forms for POP. The α form, obtained by shock chilling, transformed in the solid state to a form identical with Malkin's β''(3), when heated at 1.5°C./min. or at 0.5°C./min. This treatment was carried out repeatedly on the same and on different samples of POP, and in all cases the same X-ray pattern was obtained, that of β''. Yet, when the melting point of this form was determined in the calorimeter (Figure 2B) or by the "plunging" method, a value of 26-27°C. was obtained; in one in-

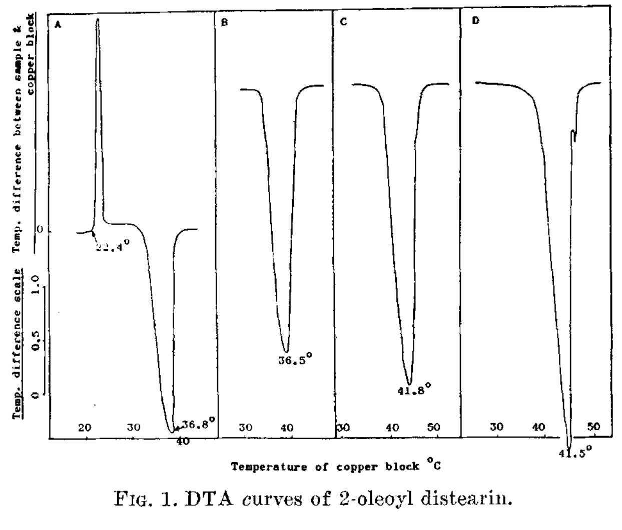

FIG. 1. DTA curves of 2-oleoyl distearin.

TABLE I

2-Oleoyl Dstearin (SOS)

Pretreatment	Melting and transition[a] points	Polymorphic form	Long[b] spacing	Principal short spacings[b]
a) Melted, chilled at −3°C., cooled to −25°C.	T 22.4 36.8	α-3 sub β-3 sub β-3	80.6	4.22 (VS)
b) Melted, cooled slowly to R.T. during 16 hrs., stored at R.T. for 3 days	36.5	sub β-3	72	3.88 (M+), 4.70 (M), 3.60 (W)
c) Melted, chilled at −3°C., stored 123 hrs. at 32°C., cooled to −7°C.	41.8	β-3	65.8	4.56 (S), 3.66 (M+), 4.01 (M) 3.78 (M)
d) Crystallized from acetone	41.5	β-3	64.0	4.59 (S), 3.68 (S), 4.02 (MS) 3.79 (MS)

[a] Where transitions occur, the beginning of the change or the range in which they take place is marked with the letter T.

[b] The X-ray patterns correspond with the lowest polymorphic forms obtained by each pretreatment. The exposures were carried out at room temperature except for the α forms, which were exposed at 0-5°C.

TABLE 2

2-Oleoyl Dipalmitin (POP)

a) Melted, chilled at −3°C., cooled to −10°C.	T 8.8 26.8 32.4	a-2 β″ (Malkin) β″ —	47	4.19 (VS)
b) Melted, cooled at R.T. for 2 hrs., then at 0°C. for 15 min.	26.6 T 26.6-30 32.5	β″ β″ β′-2 β′-2	68 42.4	3.88 (S), 4.73 (M), 4.50 (W+), 5.22 (W), 3.59 (W) 3.95 (VS), 4.29 (S), 4.13 (M),(β′-2)
c) Melted, chilled at −3°C., 17 hrs., at 32°C., cooled to −20°C.	36.4	β-3	60.6	4.55 (VS), 5.39 (M), 4.03 (M+), 3.65 (M+)
d) Crystallized from acetone	35.1	β-3	60.2	4.56 (VS), 4.05 (M), 3.73 (M), 5.40 (W+)

TABLE 3

1-Oleoyl Distearin (OSS)

Pretreatment	Melting and transition points	Polymorphic form	Long spacing	Principal short spacings
a) Melted, chilled at −7°C. for 10 min., cooled to −12°C.	30.6 T 30.6-34.5 39.6	sub α-3 α-3 β′-3 β′-3	84.7	4.15 (VS), 3.75 (S)
b) Melted, cooled during 16 hrs. to room temperature	30.6 40.1	sub α-3 β′-3	70.8	4.04 (S), 3.74 (VS), 4.64 (M), 4.40 (M)
c) Crystallized from acetone	42.2	β′-3	70.8	4.06 (S), 3.79 (MS), 4.64 (M), 4.39 (M)

TABLE 4

1-Oleoyl Dipalmitin (OPP)

a) Melted, chilled 10 min. at 0°C.	18.1 T 18.1-19.4 31.2	α-3 α-3 β′-3 β′-3	79.4	4.20 (VS)
b) Melted, cooled to room temperature during 16 hrs.	31.2	β′-3	67.5[a]	4.06 (S), 3.77 (S), 4.71 (W)
c) Cdystallized from acetone	34	β′-3	65.0[a]	4.03 (S), 3.78 (VS), 4.68 (M)

[a] The difference of 2.5 units in the long spacings of the β′-3 forms in these two instances is within the experimental error of measurement. The photograph, obtained after the pretreatment under (c), had rather diffuse lines because of slight overexposure.

TABLE 5

2-Oleoyl Palmitoyl Stearin (POS)

Pretreatment	Melting and transition points	Polymorphic form	Long spacing	Principal short spacings
a) Melted, chilled in ice 10 min., then cooled to 20°C.	20.8 T 20.8-24.8 30.7	α-2 α-2 β′-3 β′-3	48.0	4.20 (VS)
b) Melted, cooled slowly to R.T., during 12 hrs., cooled to 0°C.	30.8	β′-3	67.5	4.13 (VS), 3.82 (S+), 4.38 (S) 4.56 (M)
c) Melted, chilled at −3°C. for 5 min., then 42 hrs. at 32°C., cooled to −25°C.	35.7	β-3	....	....
d) Crystallized from acetone	35.5	β-3	63.7	4.59 (VS), 4.05 (MS), 3.87 (MS) 3.68 (S)

TABLE 6

2-Palmitoyl Oleoyl Stearin (OPS)

a) Melted, chilled at −3°C. for 15 min.	T 10.4 38.3	α-2 sub β′-3 sub β′-3	—the pattern of the α form was not obtained without the presence of some higher form—	
b) Melted, cooled slowly during 16 hrs. to R.T.	40.0	sub β′-3	68.5	4.10 (VS), 3.78 (MS), 4.35 (M) 4.65 (M)
c) Melted, chilled at −3°C., held at 32°C. for 72 hrs., cooled to −10°C.	39.8	β′-3	....	.... 4.20 (VS), 3.80 (S), 3.71 (S)
d) Crystallized from acetone	38.7	β′-3[a]	68	4.02 (W)

[a] When crystallized from an acetone/petrol mixture, the sub β′-3 form was sometimes obtained; this had a similar melting point.

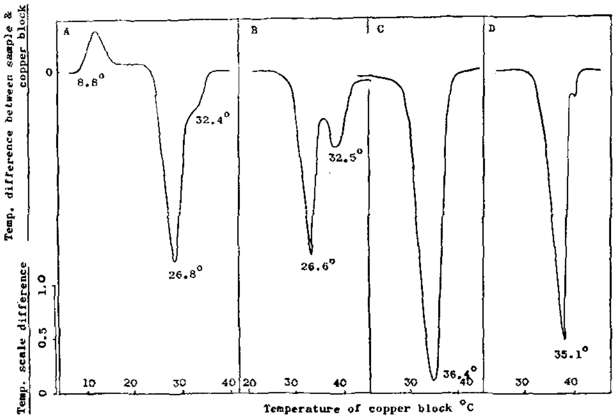

FIG. 2. DTA curves of 2-oleoyl dipalmitin.

stance however a melting point of 30.6°C. was found. This latter value is close to the melting point of Malkin's β″ form (29.0°C.) whereas the former value agrees with that for the melting point of Lutton's sub-β′ form (26.5°C.).

The β″ form, when heated at 1.5°C./min. did not completely change over into a higher form; it did so however at the lower rate of heating, *viz.*, 0.5°C./min. Lutton's β′-2 form was always obtained.

The melting point of 18.1°C. for the α-form, as quoted by Lutton (1), has been confirmed by a "plunging" melting point on the shock-chilled sample but not by differential calorimetry. If the sample was slowly heated from zero°C. in the calorimeter, a solid-to-solid transformation took place at about 9°C. The first melting point then was at 26-27°C.

The stable β-form was obtained either from the melt or by crystallization from acetone.

1-Oleoyl Distearin (OSS) (Figure 3 and Table III). Based on Lutton's data (3) there is evidence for three polymorphic forms of this glyceride. Only two forms however can be detected by differential thermal analysis. On heating a rapidly chilled sample, the first melting point is at 30.6°C. and the second near 40°C. These correspond to the α and β′ forms. Variations in the pretreatment of the sample always resulted in one or both of these two forms. The X-ray data also gave only two polymorphic forms, but these were sub-α and β′. At no time was the X-ray pattern of an α form found. Very slow cooling from the melt to room temperature resulted in crystallization in the β′ form; a small amount of α form was present. Although the melting points of the forms, as obtained respectively from the melt and by crystallization from the solvent, did not agree, X-ray and infrared data showed that the same β′ form was obtained in both cases. This is considered further in the discussion.

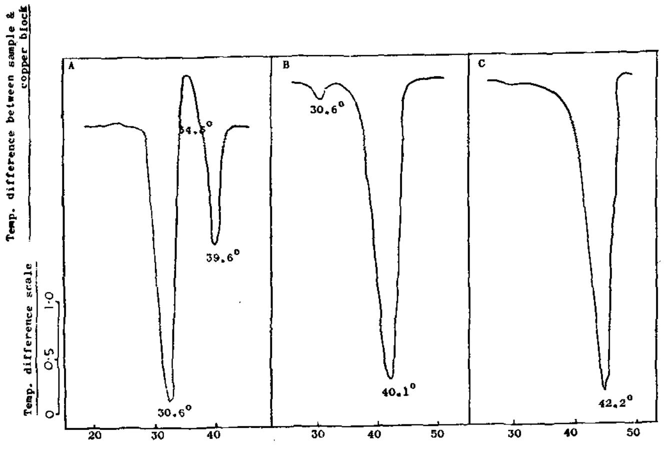

FIG. 3. DTA curves of 1-oleoyl distearin.

1-Oleoyl Dipalmitin (OPP) (Figure 4 and Table IV). Only two polymorphic forms have been found for OPP. As previously, the lowest melting form was obtained by ice-chilling the melt. This form, on heating to 18°C., transformed into the stable β′ form, which melted near 31°C. after further heating. The same form was obtained both by slowly cooling the melt and by crystallization from acetone. Daubert and Clarke (6) have reported three melting points close to those found here, namely 18.5, 29.8, and 34.5°C. They have designated the forms I, II, and III. According to the X-ray data for our sample only two forms were distinguishable.

2-Oleoyl Palmitoyl Stearin (POS) (Figure 5 and Table V). For this glyceride three polymorphic forms have been distinguished. Unlike SOS and POP, there was no evidence of any solid-to-solid transformation. The α form, after some melting, transformed into the β′ form, melting at 30.7°C. This β′ form could be obtained alone by slowly cooling the melt for about 12 hrs. from 50°C. to 18°C. The stable β form was obtained by holding the melt for two days at 32°C., also by crystallization from acetone. The melting points of the samples produced by these two methods were in good agreement.

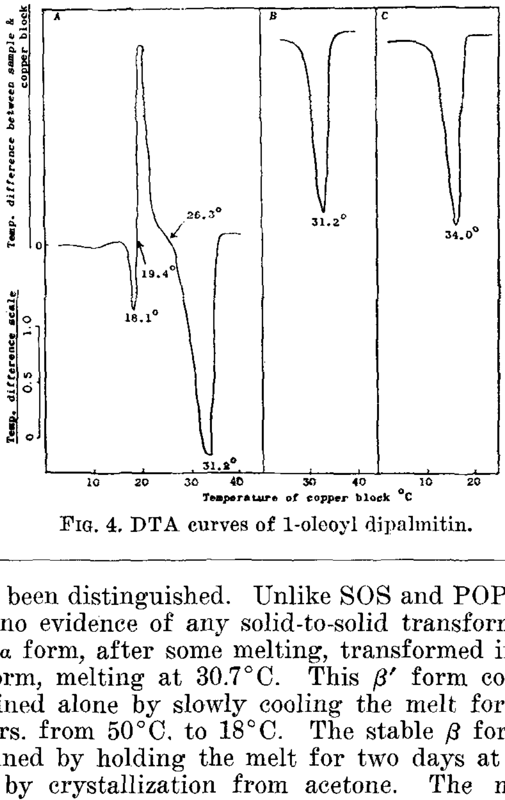

FIG. 4. DTA curves of 1-oleoyl dipalmitin.

2-Palmitoyl Oleoyl Stearin (OPS) (Figure 6 and Table VI). In this case a solid-to-solid transformation occurred when the α form was slowly heated, being similar in this respect to SOS and POP. Three polymorphic forms have been identified for OPS, although one of these, the β′ form, was obtained only with difficulty from acetone. The sub-β′ form was obtained by a) heating the α form, b) cooling the melt slowly, c) holding the melt for 72 hrs. at 32°C., and d) crystallization in some cases from solvent. As for OSP, the melting point of the form obtained from acetone was lower than that of some of the samples obtained by other means.

2-Stearoyl Oleoyl Palmitin (OSP) (Figure 7 and Table VII). Of the seven glycerides studied, this was the only one whose heating curves were not easily interpreted. So far, three polymorphic forms have been identified; but any satisfactory explanation of the heating curves, on the same lines as for the other glycerides, would require the existence of four polymorphic forms.

The curve obtained by heating the rapidly chilled samples at 1.5°C./min. showed three zones of melting and two of heat evolution. The first zone, melting point 25.5°C., has been identified by X-ray study as the sub-α form; the second zone has not been identified with any form; the third zone was associated with the β'-2 form, melting point 37°C. The middle melting zone was obtained by allowing the melt to crystallize at 28°C. for 45 min.; if held much longer, only the β'-2 form was obtained. Although the curve obtained by heating the acetone-crystallized material was very similar to that of the β'-2 form, it represented in fact the β'-3 form and had a slightly lower melting point.

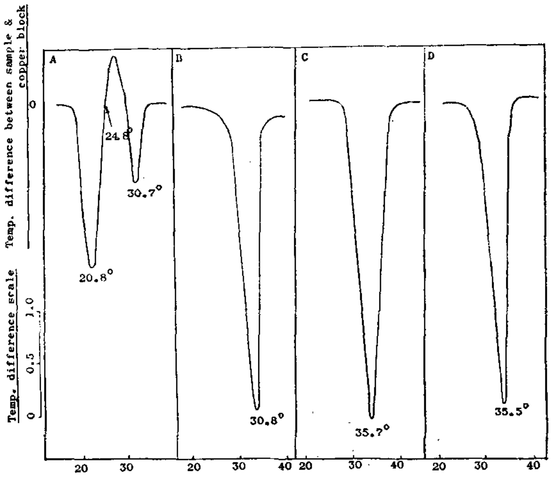

FIG. 5. DTA curves of 2-oleoyl palmitoyl stearin.

Discussion

In general, the results obtained for all seven glycerides were consistent with the data published by Lutton (1, 2, 3), except on three main points, and were in agreement with the pattern of results obtained by Chapman (8) by employing infrared spectroscopy. The three points are given below.

1. Differential thermal analysis showed very clearly a 30°C.-melting form of OSP, which had never before been reported.
2. OSP on rapid chilling crystallized in the sub-α-3 form whereas Lutton *et al.* found sub-α-2.
3. Although Lutton *et al.* and the present author found a 27°C.-melting form of POP, their X-ray patterns do not agree. The pattern given in Table II is in agreement with the β'' form of Malkin and Wilson (4) and is similar to the sub-β-3 type reported by Lutton *et al.* for SOS.

Similarities between certain glycerides were evident, yet in many ways each glyceride was unique. A study of the melting curves of any one glyceride could distinguish it from any of the other six. In fact, a heating curve of the rapidly-chilled melt would in most cases be sufficient.

Certain glycerides showed solid-to-solid transformations on heating the α form, *viz.*, SOS, POP, and OPS while the others exhibited a certain degree of melting before a transformation occurred. The heating rate had some effect on these transformations. For example, as the heating rate was lowered, the solid-to-solid transformation occurred at a slightly lower temperature; and where melting did occur, there was less of it before the transformation set in.

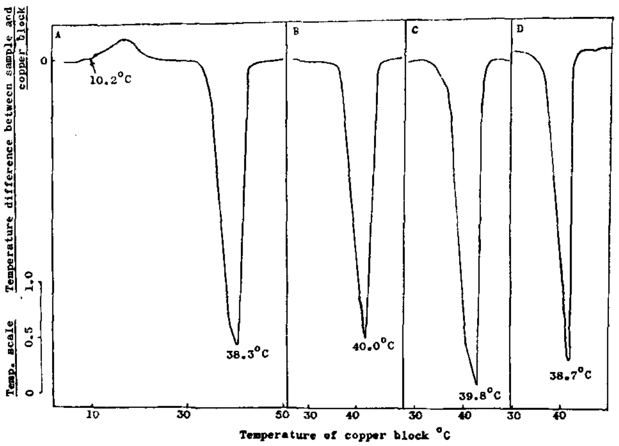

FIG. 6. DTA curves of 2-palmitoyl oleoyl stearin.

For all the glycerides studied, melting began well below the melting point. At least part of this was caused by the design of the apparatus. The sample near the walls begins to melt first and the sample close to the thermocouple last; hence there is a temperature gradient in the cell during the measurements. As melting begins, the sample near the thermocouple is no longer being heated at the predetermined rate. Hence there is an increase in the lag between the test and reference thermocouples, and the heating curve leaves the base line. The presence of impurity also tends to lower the point at which melting begins.

There is a third factor which may cause apparent lowering of the initial melting point, namely, premelting. The theory of catastrophic change, solid—liquid, at one precise temperature is no longer held for complex molecules. It is believed that a gradual change occurs over a range of temperature. The change may not be visible to the eye, but it can be studied by physical means. Ubbelohde (9) has shown that the specific heat of some hydrocarbons increases below the melting point because of premelting. In

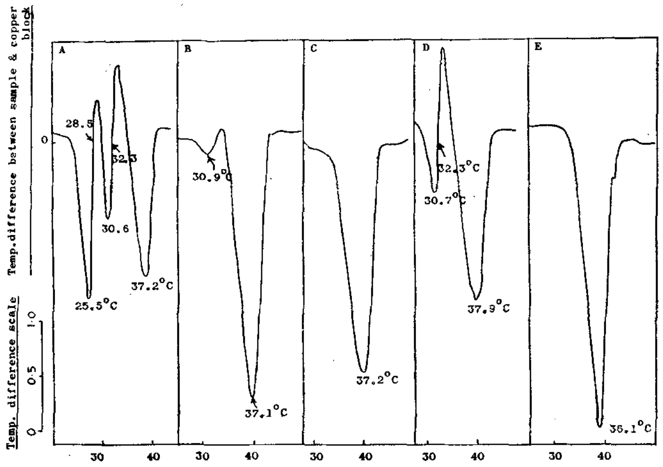

FIG. 7. DTA curves of 2-stearoyl oleoyl palmitin.

TABLE 7

2-Stearoyl Oleoyl Palmitin (OSP)

Pretreatment	Melting and transition points	Polymorphic form	Long spacing	Principal short spacings
a) Melted, chilled at —3°C. for 10 min.	25.5	sub α-3	79	4.18 (VS), 3.81 (M+)
	T 25.5-28.3	....		
	30.6	?		
	T 30.6-32.3	....		
	37.2	β'-2		
b) Melted at 60°C. and allowed to cool slowly to R.T. during 16 hrs.	30.9	?	44.0	4.20 (VS), 3.85 (M), 4.33 (M)
	T 30.9-33.0	....		
	37.1	β'-2		
c) Melted, crystallized at 28°C. for 16 hrs.	37.2	β'-2	44.0	4.20 (VS), 3.85 (M), 4.33 (M)
d) Melted, crystallized at 28°C. for 45 min.	30.7	?	-	....
	T 30.7-32.3	....		
	37.9	β'-2		
e) Crystallized from acetone	36.1	β'-3 (trace β'-2)	68.9	4.23 (S), 4.03 (MS), 3.82 (S) 3.56 (W), 5.48 (MW)

the present apparatus any change in the specific heat of the sample during a measurement will influence the shape of the heating curve.

In our investigation all the α forms were unstable and changed into a higher polymorphic form. This new form, except for POP and OSP, did not transform readily into a third form unless held for some considerable time just below the melting point of this third form. With POP a third form was just indicated at a heating rate of 1.5°C./min. while at 0.5°C./min. it was very obvious.

Although OSP showed three melting zones, it was not comparable with POP. In these three zones only two polymorphic forms could be detected; whereas POP and the other glycerides existed in an α form, OSP had a sub-α form and there was no evidence of an α form. To determine the pattern of the change on heating the sub-α form, a sample was prepared and its X-ray pattern determined at 15-min. intervals while it slowly (in six hrs.) warmed up to 32°C. Only the sub-α and β'-2 polymorphic forms were detected. Since, with the instrument used, only strong lines were detected in this way, it seemed possible that some transient intermediate form might have been missed.

It may well be that a second-order transition takes place in OSP although a transition of this kind has never been recorded in the literature for any triglyceride.

On chilling the OSP melt, the sub-α-3 form was always obtained whereas Lutton (2) claims the most likely form to be sub-α-2.

In general, the melting points of the various forms agreed reasonably well with those published by other workers, notably Lutton. It must be remembered however that in this apparatus melting points cannot be determined with high precision, with the result that in a few cases there was a variation of one or two degrees in our melting points and the literature values. But it seemed significant that the melting point of the same polymorphic form, obtained in two different ways, differed considerably in at least two cases.

For example, Table IV shows that the β'-3 form of OPP had melting points of 31.2°C. and 34°C. Table III shows the β'-3 form of OSS with melting points of 40.1 and 42.2°C.

The great diversity of results, obtained by different workers in the field of polymorphism of glycerides, may result either from variations in the treatment of the glycerides or from the presence of impurity. Of these two, the presence of impurity presents the greater problem for it is not easy to determine which of the samples described in the literature is the most nearly pure.

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Detection and Measurement of Hydroperoxides by Near Infrared Spectrophotometry[1]

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IN A STUDY of the near infrared spectra of fatty acids and related substances (2), unusual absorption maxima were noticed in the spectrum of methyl linoleate hydroperoxide. In order to test whether these maxima are specific for hydroperoxides, spectra of a series of hydroperoxides and related substances have been measured. All the evidence gathered agrees with the assignment of the maxima at 2.07 and 1.46 μ to hydroperoxidic groups. These maxima, although weak, have proven to be useful in studies of the oxidation of unsaturated

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