Polymorphism in Single-Acid Triglycerides of Positional and Geometric Isomers of Octadecenoic Acid[1]

J.W. HAGEMANN and **W.H. TALLENT**, Northern Regional Research Laboratory[2],
Peoria, Illinois 61604, and **J.A. BARVE, I.A. ISMAIL**, and **F.D. GUNSTONE**,
Chemistry Department, St. Salvators College, The University of St. Andrews, Scotland

ABSTRACT

The polymorphism of 25 glycerol trioctadecenoates with double bonds ranging from $\Delta 4$-$\Delta 17$ was investigated by differential scanning calorimetry. Triglycerides with *cis* bonds in odd positions $\Delta 7$-$\Delta 13$ exhibited three intermediate melting (β'-) forms, but those with *cis* bonds in even positions, except *cis* $\Delta 4$, lacked β'-forms. Among the *trans* compounds, only $\Delta 11$, 13, and 14 showed β'-forms. The *cis* and *trans* $\Delta 5$ triglycerides were unusual, because they readily assumed low melting (α-) forms that were not easily converted to high melting (β-) forms. β-Form mp of compounds in each series (*cis* or *trans*) alternated depending upon double bond position; an even position correlated with high mp. Heats of fusion (ΔH_f) for β-forms, likewise, fluctuated with double bond position but nonuniformly; *trans* $\Delta 6$ had the highest ΔH_f (43 cal/g), *cis* $\Delta 12$ the lowest (21 cal/g).

INTRODUCTION

Unsaturated triglycerides are thought to occur in crystal forms analogous to those of saturated triglycerides because of similarities in X-ray patterns (1) and IR spectra (2). Accordingly, melting profiles for unsaturated triglycerides should, and do, show sharp endotherms that are characteristic of polymorphic forms now well established for saturated triglycerides; namely α, lowest melting; β, highest melting; and β', intermediate melting (3-5). An earlier study of single-acid triglycerides (6) via differential scanning calorimetry (DSC) presented evidence that certain saturated and unsaturated molecuels can assume more than one β' conformation. In this earlier study, unsaturated triglycerides that had *cis* double bonds in odd positions appeared normal because α-, β'-, and β-forms were observed, but, at the same time, they exhibited unusual behavior, because multiple β'-forms also were detected. No β'-forms were found, however, with trielaidin, in which the *trans* bond occurs at an odd position nor were any intermediate forms found for unsaturated triglycerides that had *cis* or *trans* bonds in even positions. Realizing the limitations of previous data in correlating lipid properties with structural effects, we now offer a study that more clearly describes the thermal behavior of isomeric single-acid unsaturated triglycerides.

MATERIALS AND METHODS

Almost all the octadecenoic acids were synthesized by Gunstone and coworkers (7,8). A *trans* impurity was removed from methyl esters of the *cis* $\Delta 13$, 14, and 15 acids before triglyceride preparation by preparative thin layer chromatography (TLC) (9) on a 20% $AgNO_3$ plate with benzene as the eluting solvent.

Each triglyceride was prepared by reaction of the appropriate acid chloride with glycerol. Ca. 50 mg acid was treated with 1.2 parts by wt of oxalyl chloride (Aldrich Chemical Co., Milwaukee, Wisc.) for 3 days (10) after which excess oxalyl chloride was removed under vacuum; all operations were conducted at room temperature. The acid chlorides were dissolved in 0.5 ml distilled *n*-hexane, and to this solution was added glycerol in pyridine (125 g/liter) to a 1:4 molar ratio, glycerol:acid chloride. The reaction mixture was allowed to stand 24 hr, and then triglycerides were purified by preparative TLC on Silica Gel G in 70:30 *n*-hexane:ethyl ether; all were at least 95% pure by gas liquid chromatography (GLC) (11) and TLC.

DSC procedures were as described previously (6), except for modifications to measure temperatures on rapid scans and to determine heats of fusion (ΔH_f) for certain unusually stable β'-forms. Melting temperatures of transient forms detected during scans faster than 10 C/min were corrected by a quantity required to make the fast-scan melting temperature of a stable form (generally β) coincide with its slow-scan melting temperature. When two endotherms appeared on the same scan and when the ΔH_f of one was known, a "double endotherm" technique was used to estimate the ΔH_f of the second endotherm. This technique enables ΔH_f's to be determined on polymorphs (primarily β'-forms) that cannot be prepared in pure form or as a single endotherm. The principal condition under which the method is valid requires that an exotherm not appear between the two endotherms, i.e. the scan speed must be greater than the rate of transformation of the polymorph to be measured. With ΔH_f for the β-form known, the amount of sample required to give the measured area of the β-form endotherm was calculated. This wt was subtracted from the total sample wt to give the amount of sample represented by the β'-endotherm, which, with the β' endotherm area, was used to calculate ΔH_f. When DSC sensitivity and recorder chart speed are the same for sample and reference standard, calculations were simplified by applying the following:

$$\Delta H_f \text{ (unknown)} = \Delta H_f \text{ (standard)} \times \text{area (unknown)} \times \text{wt (standard/} \left[\text{area (standard)} \times \text{total sample wt} - \{ \Delta H_f \text{ (standard)} \times \text{area (known)} \times \text{wt (standard)}/\Delta H_f \text{ (known)} \} \right]$$

RESULTS

Melting Points

DSC data on the newly prepared glycerol trioctadecenoates and on those selected for study previously (6) are summarized in Table I. For convenience, we abbreviated triglyceride nomenclature and refer to individual compounds by only their most distinguishing features, i.e. type and position of double bond in the constituent acid. For example, glycerol tri-*cis*-6-octadecenoate is identified as *cis* $\Delta 6$, glycerol tri-*trans*-6-octadecenoate as *trans* $\Delta 6$, etc. Following convention, the highest major melting endotherm for each triglyceride, differing by only a few degrees from the low melting form of the individual fatty acids (8), is assigned to the β-form, and the lowest melting polymorph is the α-form; those melting between α- and β-forms are β'-forms. Subscripts denote decreasing mp (12).

Two β-form mp are reported for three of the *cis* and two of the *trans* samples. The lower melting polymorphs, which appear as shoulders on the principal (β_1-form) endotherms,

---

[1] Presented at the AOCS Fall Meeting, Philadelphia, September 1974.

[2] ARS, USDA.

TABLE I

Melting Points and Heats of Fusion of *cis* and *trans* Triglycerides

Double bond position	Form and mp (C)						Slow cooling exotherm (C)	ΔH_f (β-form) (cal/g)
	α	β_3'	β_2'	β_1'	β_2	β_1		
cis Δ4	5		8,11,14	19,22	32	34	8	25.4
cis Δ5	-6[a]					14	-9	30.2
cis Δ6[b]						28	-2	30.7
cis Δ7	-36[c]	-30	-27	-1	5	7	-39	28.9
cis Δ8	-15[d]					24	-18	25.9
cis Δ9[b]	-34	-12	-8	-5		5	-30	25.8
cis Δ10	-20[e]					27	-7	27.8
cis Δ11[b]		-3	0	3[f]		10	-11	27.1
cis Δ12						32	8	21.2
cis Δ13		9	15	17[g]	24	26	6	21.7
cis Δ14	15[d]					44	19	30.6
cis Δ15						43	29	29.6
trans Δ4	27					53	24,28	38.0
trans Δ5	23[h]					41	19	35.6
trans Δ6[b]						52	32	43.0
trans Δ7	16					39	12	36.0
trans Δ8	-2					49	25	34.1
trans Δ9[b]	15					41	12	39.5
trans Δ10						49	26	34.8
trans Δ11[b]	15		23	i		43	18	38.7
trans Δ12	21					51	26	33.9
trans Δ13	23			37[j]	42	44	27	28.9
trans Δ14	27[e]		k	43[d]	55	58	35	33.8
trans Δ15	37					56	36	33.6
Δ17				43[d]		55[l]	42	32.8

[a] ΔH_f = 15.2 cal/g.

[b] Data from ref. 6.

[c] ΔH_f = 10.1 cal/g.

[d] Estimated from a 40 C/min scan.

[e] Estimated from an 80 C/min scan.

[f] ΔH_f = 21.5 cal/g.

[g] ΔH_f = 20.0 cal/g.

[h] ΔH_f = 19.5 cal/g.

[i] β_1' suggested at 28-29 C on 20 C/min scans.

[j] ΔH_f = 24.1 cal/g.

[k] β_2' suggested at 40 C on 40 C/min scans.

[l] Solvent crystallized material melts at 59 C.

are listed in Table I as β_2-forms and illustrated in Figure 1 for *cis* Δ4. Though there may be a question whether or not the β_2-forms are really β-forms, we report them as such for at least two compelling reasons. First, their mp are close to those of the β-forms and are at least 5 C from the reported β_1'-forms; both conditions suggest structural similarity to β_1. Second, no exotherm was observed between the two β-forms. DSC scans showing both β'- and β-forms usually exhibit an exotherm between the two endotherms unless the β'-form is unusually stable.

Eight of the samples in Table I exhibited no α-form endotherms. In earlier work (6) slow-scan cooling curves were generally exothermic at ca. the mp of the α-form, and the exotherms were, therefore, used in estimating α-form mp for *cis* and *trans* Δ6 and *cis* Δ11. For many samples in our latest study, however, the slow-scan cooling exotherm occurred at a higher temperature than the α-form mp. Therefore, identification of the α-form mp via slow-scan exotherms is not justified for unsaturated triglycerides showing no α-form endotherms.

An mp of 55 C is reported here for thermally prepared Δ17, even though distilled *n*-hexane crystallized material melted at 59 C. Forms obtained from monoacid saturated triglycerides by crystallization from solvent often melt at higher temperatures than those produced by thermal methods (3), sometimes by as much as 3 C (13). Conditioning of Δ17 at temperatures below 55 C did not produce any of the 59 C form.

β_1-Form mp of both the *cis* and *trans* series compounds alternated with double bond position, the even position correlating with higher values in both series. This alternation is shown clearly in Figure 2, which also illustrates the effects of geometric isomerism upon mp and upon amplitude of mp fluctuation with double bond position from Δ4-Δ12.

Multiple β'-Forms

Multiple intermediate melting forms were encountered most often with *cis* triglycerides in which the double bond occupied an odd position near the central portion, Δ7-Δ13, of the constituent acid. When the double bond was near either end of the chain, Δ5 or Δ15, β'-forms were not observed. Likewise, they were not apparent when the *cis* bond occurred in even positions other than Δ4. Though definitely showing intermediate forms, the polymorphism of *cis* Δ4 was quite different from that characteristic of *cis* odd triglycerides. Melting profiles for *cis* Δ4 (Fig. 1) were complex and varied considerably with conditioning time and scan rate, but β'-forms could be detected reproducibly at scan speeds of 2.5-10 C/min. The endotherms are listed in Table I as α, β', or β depending upon their positions relative to exotherms at ca. 8, 16, and 25 C.

In the series of *trans* triglycerides, only Δ11, 13, and 14 exhibited β'-forms. So far as we know, this observation is the first of β'-forms for *trans* single octadecenoic acid triglycerides, albeit β'-forms of glycerol tri-*trans*-13-docosenoate were observed during previous studies (6).

Polymorph Stability

The term "stable" used here to describe polymorphic forms is based upon Lutton and Fehl's (13) definition and pertains to the tendency to transform during thermal transitions. Rate of transformation is qualitatively deter-

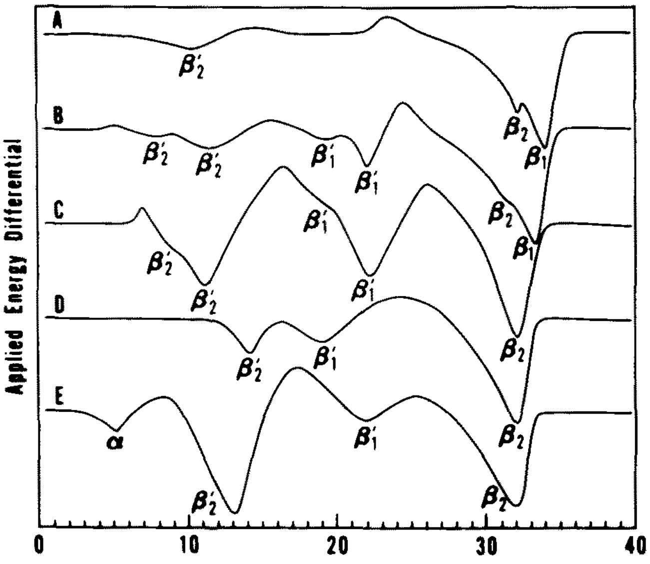

FIG. 1. Differential scanning calorimetry curves of glycerol tri-*cis*-4-octadecenoate with endotherms shown as negative peaks. A. Heating rate 2.5 C/min after holding 1/2 hr at 12 C, then 1/2 hr at 15 C. B. Heating rate 5 C/min after holding 1/2 hr at 12 C, then 1/2 hr at 15 C. C. Heating rate 10 C/min after holding 1 hr at 15 C. D. Heating rate 10 C/min after holding 1/2 hr at 10 C. E. Heating rate 20 C/min after holding 1 hr at 15 C.

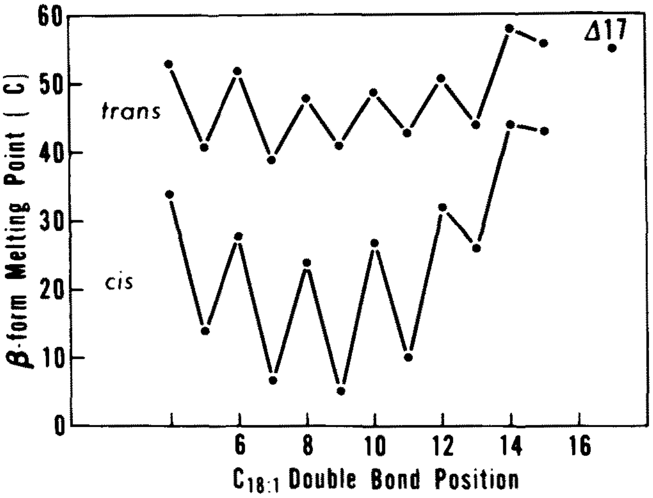

FIG. 2. Triglyceride β mp vs double bond position. β_1-Form mp were used where more than one β-form was observed.

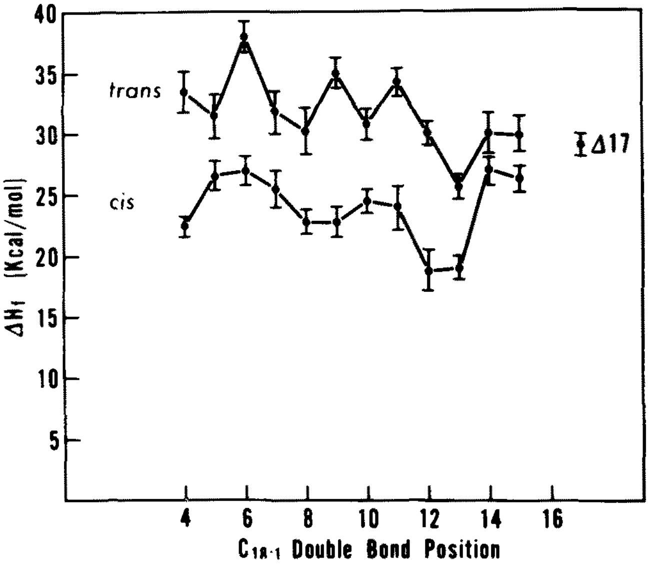

FIG. 3. Triglyceride β ΔH_f vs double bond position. The ht of the vertical bars indicate the 95% confidence interval for the values of ΔH_f.

mined by scan speed; the faster the scan speed required to observe a polymorph, the more unstable the form.

Both the *cis* and *trans* Δ5 samples exhibited an unusually stable α-form, the *cis* being so stable that the β-form could only be prepared by crystallization from distilled *n*-hexane. Conditioning of *cis* Δ5 for up to 4 hr at -6 to -8 C or up to 22 days at 0 C did not produce even a small amount of the β-form. Pure β-form of *trans* Δ5 could be made by holding the sample overnight, ca. 16 hr, at room temperature. Other stable non-β forms were the α-form of *cis* Δ7, the β_1'-forms of *cis* and *trans* Δ13, and the previously reported β_1'-form of *cis* Δ11. The β_1'-form of *trans* Δ13 was observed only in the presence of the β-form.

Heats of Fusion

As shown in Figure 3, ΔH_f values for β-forms of *trans* compounds alternated discontinuously with increasing double bond position, whereas values for *cis* compounds were more uniform throughout the same series of double bond positions. Some interesting, though as yet poorly understood, variations in ΔH_f correlate with certain double bond positions. The *trans* series changes direction of ΔH_f alternation at Δ7 and again at Δ12. Variation of ΔH_f with *cis* double bond position was less for Δ8-Δ11 than when *cis* bonds occurred near the extremes, Δ5-Δ6 and Δ12-Δ13. Both *cis* and *trans* Δ13-Δ15 have similar melting behavior; the *cis* and *trans* Δ13 and the *trans* Δ14 each show two β-forms, and both Δ15 compounds exhibit no β'-forms. Further similar characteristics also are displayed in the ΔH_f pattern of Figure 3, most noteworthy being the rise in value from Δ13 to Δ14 and the leveling off to Δ17. Currently unknown is how the ΔH_f values for Δ16 samples would fit into the pattern. Of even greater interest would be whether the Δ16 triglycerides can assume β'-forms, as did Δ17, or whether they might behave as Δ15, which exhibited no β'-forms.

Although mp may vary with phase preparation as earlier stated, no difference in ΔH_f was found between the 55 and 59 C forms of Δ17. Both the zero ΔH_f difference and the observation that the two forms do not occur simultaneously suggest that the two melting values are not due to two distinct polymorphs.

The ΔH_f values in Table I have a standard deviation, calculated by a pooled analysis of variance, of 1.24 with 91° of freedom. Results determined for samples exhibiting more than one β-form include both forms in the ΔH_f value.

DISCUSSION

Alternation of mp for isomeric *cis* and *trans* triglycerides is analogous to the mp alternation for saturated triglycerides (13), which according to Larsson (14) is due to different packing densities at the methyl end groups. In addition, our results are consistent with Larsson's further conclusion that, in a series of *cis* acids, packing density differences cause mp to alternate as the number of methylene groups between the carboxyl group and double bond increases. Changing double bond position in the tri-*cis*-octadecenoates is similar to varying the number of methylene groups and results in alternation of mp. Similarly, differences in the degree of tilt relative to end group planes, as observed by Lutton and Kolp (15) in X-ray diffraction patterns for a series of *trans* octadecenoic acids, could account for the observed alternation of mp in the tri-*trans*-octadecenoate series.

Endotherms of triglycerides from *cis* unsaturated acids tend to extend over a larger temperature range than those in the *trans* series. That this tendency may be due to an inability of chains containing *cis* bonds to pack as efficiently as those having *trans* bonds is suggested by the following argument: volume/CH_2 group (23.7 Å^3) based upon the triclinic (T//) packing of trilaurin (16) is similar to the orthorhombic (0'//) volume/CH_2 group (23.8 Å^3) of the low melting form of oleic acid (17). Whereas saturated and *trans* unsaturated (trielaidin) triglycerides have similar zigzag geometry (18), it is conceivable that methylene chains in the trioctadecenoates might have similar packing volumes provided *trans* chains pack as T// and *cis* chains as 0'//. However, taking into account that the triglyceride structure around the first three carbons in one of the α-acyl moiety of trilaurin (19,20) does not permit the close packing of which straight chain compounds are capable (T.D. Simpson, private communication), it seems more likely that the packing volume in the tri-*cis*-octadecenoates should be greater than that for the corresponding *trans* compounds.

The number and presence or absence of β'-forms are in general agreement with previous speculations (6) explaining the polymorphism of *cis* unsaturated triglycerides. Double bonds in the even positions divide the chain into two even methylene group segments which may be somewhat less stable than odd group segments; hence, the absence of β'-forms in the even series and the presence of β'-forms in the odd series. Unfortunately, three exceptions, *cis* Δ4, 5, and 15, confront this explanation and are not easily rationalized even though proximity of the double bonds to the glycerol moiety or to methyl end groups might be invoked to account for the behavior in the β' region of these three samples.

Similarly, *trans* Δ11, 13, and 14 oppose the earlier finding that *trans* octadecenoic acid triglycerides exhibit no β'-forms. The presence of β'-forms in the *trans* compounds cannot yet be explained adequately in structural terms.

The correlation between β-form mp and ΔH_f for the trioctadecenoates (Fig. 4) differs markedly from the smooth curve relationship observed for saturated triglycerides (6). Graphic patterns for the two *cis* series are quite similar, but patterns for the *trans* samples are located ca. 10 cal/g higher than the *cis* patterns and are shaped differently. The *trans* odd series even shows a reversal in the arrangement of points, i.e. points Δ5-Δ11 flow clockwise in Figure 4, while the correspoinding *cis* compounds are distributed in a counterclockwise fashion. Whereas the melting patterns of Figure 2 suggest a consistency of structure throughout both series of compounds, the ΔH_f patterns in Figures 3 and 4 indicate variations in structure. Similarly, the polymorphism observed for the trioctadecenoates also reveals the more complex nature of these compounds as opposed to the saturated triglycerides. Considered together, these observations suggest possibilities of greater crystal structure variation then anticipated for unsaturated triglycerides, thus confirming the early X-ray data of Lutton and Kolp (15). These workers in establishing the similarity between all-odd or all-even *trans* Δ6-12 octadecenoic acids, also found differences in fine detail that might be interpreted as evidence of subtle crystal variation with double bond position.

Between the extreme ΔH_f values for *trans* Δ6 and *cis* Δ12 (Fig. 4), four compounds melting near room temperature, *cis* Δ6, 8, 10, and 13, are scattered over a range of 9 cal/g. This distribution and others provide, from a practical standpoint, for a selection of materials with varying heat requirements, melting levels, or processing times, or all three.

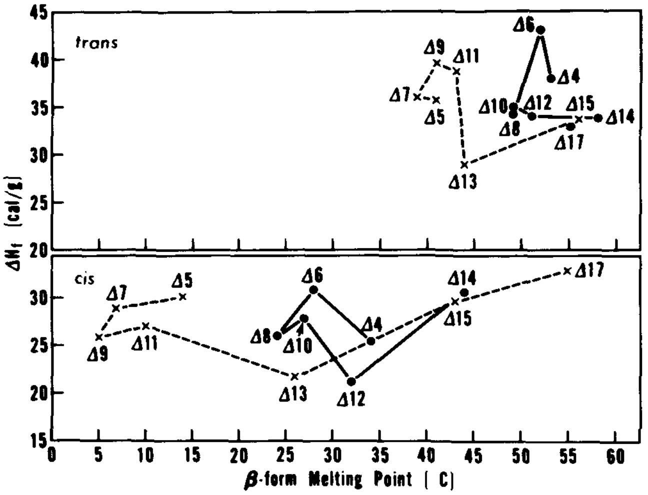

FIG. 4. Triglyceride β mp vs ΔH_f. β_1-Form mp were used where more than one β-form was observed. --- = Odd double bond position and —— = even double bond position.

Almost all the compounds in this study were prepared synthetically, but a good portion of the component fatty acids occur naturally (*21*) and may prove to be important in future research and developments that take advantage of the structure-determined polymorphic characteristics of lipid interactions.

ACKNOWLEDGMENTS

T.D. Simpson and J.A. Rothfus of the Northern Regional Research Laboratory and W.F. Kwolek, biometrician, North Central Region, ARS, stationed at the Northern Regional Research Laboratory, gave helpful suggestions.

REFERENCES

1. Ferguson, R.H., and E.S. Lutton, J. Amer. Chem. Soc. 69:1445 (1947).
2. Chapman, D., JAOCS 37:73 (1960).
3. Chapman, D., Chem. Rev. 62:433 (1962).
4. Larsson, K., Ark. Kemi 23:35 (1965).
5. Hoerr, C.W., and F.R. Paulicka, JAOCS 45:793 (1968).
6. Hagemann, J.W., W.H. Tallent, and K.E. Kolb, Ibid. 49:118 (1972).
7. Gunstone, F.D., and I.A. Ismail, Chem. Phys. Lipids 1:209 (1967).
8. Barve, J.A., and F.D. Gunstone, Ibid. 7:311 (1971).
9. Kleiman, R., C.R. Smith, Jr., S.G. Yates, and Q. Jones, JAOCS 42:169 (1965).
10. Mattson, F.H., and R.A. Volpenhein, J. Lipid Res. 3:281 (1962).
11. Litchfield, C., R.D. Harlow, and R. Reiser, JAOCS 42:849 (1965).
12. Larsson, K., Acta Chem. Scand. 20:2255 (1966).
13. Lutton, E.S., and A.J. Fehl, Lipids 5:90 (1970).
14. Larsson, K., JAOCS 43:559 (1966).
15. Lutton, E.S., and D.G. Kolp, J. Amer. Chem. Soc. 73:2733 (1951).
16. von Sydow, E., Ark. Kemi 9:231 (1955).
17. Abrahamsson, S., and I. Ryderstedt-Nahringbauer, Acta Crystallogr. 15:1261 (1962).
18. Carter, M.G.R., and T. Malkin, J. Chem. Soc. 554 (1947).
19. Larsson, K., Ark. Kemi 23:1 (1965).
20. Larsson, K., Chem. Scripta 1:21 (1971).
21. Gunstone, F.D., "An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides," Second Edition, Chapman and Hall, Suffolk, England, 1967, p.16.

[Received November 14, 1974]