color of the seed coat. This result was not clearly demonstrated with the Candle variety which had been selected for its lighter seed coat color (Table I) but sinapic content was only slightly less than the other rapeseed cultivars.

Most of the phenolic acids were found in the free or esterified form in the rapeseed and mustard flours. The relative levels of phenolic acids released by hydrolysis of the residues were quite low. These insoluble acids were presumably bound to proteins or carbohydrates and could be released during subsequent processing or cooking to contribute adverse flavors or colors to food products. However, their low concentrations would suggest that solvent extraction procedures, such as those employed to produce protein concentrates and isolates, could be devised to remove most of the phenolic compounds present in the flours.

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Polymorphism and Transformation Energetics of Saturated Monoacid Triglycerides from Differential Scanning Calorimetry and Theoretical Modeling[1]

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ABSTRACT

Differential scanning calorimetry studies on saturated monoacid triglycerides were extended to include most odd and even chain lengths from tricaprylin (C_8) through tritriacontanoin (C_{30}). Two β'-forms were common with triglycerides C_{15} through C_{24}: shorter odd chain length triglycerides (C_9-C_{13}) exhibited only one β'-form; short even chain length triglycerides (C_8-C_{14}) exhibited three. Odd chain length C_{21} and C_{23} triglycerides showed two β-forms. Triglycerides of even chain lengths greater than C_{21} produced two α-forms. Apparent energies of phase excitation for α-form transformations (determined from scans at different heating rates) showed odd-even alternation for short chain lengths, but increased linearly with chain length above C_{14}, evidencing the importance of extended chain conformation and interactions as determinants of polymorph properties. Changes in melting point patterns, particularly for β' and β-forms, at C_{14} correlated with the change in apparent phase excitation energy. Comparisons of X-ray data with dimensions from space-filling models and agreement between observed entropies of fusion and values calculated for probabilistic models also emphasize the importance of extended chain conformation and suggest configurational differences, and possibly different polymorph conversion pathways for odd versus even chain length triglycerides.

INTRODUCTION

Nearly a half-century after Malkin (1) demonstrated that the multiple melting behavior of triglycerides is due to polymorphism, controversy still prevails regarding the structures of possible crystalline phases (2) and the mechanisms for transformation from one phase to another (3). Odd and even chain length saturated monoacid triglycerides are generally known to occur in α-forms (lowest melting), β'-forms (intermediate melting) and β-forms (highest melting) that exhibit distinct X-ray patterns and characteristic physical properties (4, 5), but an unequivocal molecular conformation is known for only the β-form (6). Difficulties inherent in growing single crystals of long-chain triglycerides and the instability of many α- and β'-forms still preclude their thorough characterization.

In a previous differential scanning calorimetry (DSC) study (7) of monoacid triglycerides, two β' endotherms were reported for even chain length samples tripalmitin through tribehenin. At the time, this finding contrasted with earlier work (8–10) except, perhaps, that of Lutton and Fehl (5), who occasionally found "artifacts" during differential thermal analysis of trimyristin. Further examination of this multiple melting behavior by DSC analysis of an extended series, tricaprylin through tritriacontanoin for even chain lengths and tripelargonin through tritrico-

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TABLE I

Polymorphism of Saturated Monoacid Triglycerides

Fatty acid chainlength	ΔH_f (kcal/mol)			Slow cooling exotherm[b] (C)	Polymorphic form (C)						
	α[a]	β'	β		α_2	α_1	β'_3	β'_2	β'_1	β_2	β_1
8	4.1		16.6	−24		−54	−19	−12	0		11
9	8.5	11.8	13.1	−6, −8		−30			7		10
10	13.5		22.0	8, 6, 4		−10	13	18	26		33
11	13.2	17.1	20.2	17		4			28		31
12	17.2		27.8	27, 23		14	30	34	40		46
13	17.8	20.0	23.8	29, 32		23			40		42
14	20.3		32.7	35, 38		31	41	45	51		56
15	23.2	26.0	34.5	42		41		54	56		58
16[c]	24.6		39.6	46		46		53	57		66
17	26.2	30.7	40.4	49		50		60	62		65
18[c]	26.8		45.8	54		55		61	64		73
19	28.8	32.5	44.6	58		60		65	68		72
20[c]	29.2		52.7	61		64		69	71		78
21	30.5	35.8	43.2	64		66		70	73	76	77
22[d]	34.2	36.4	53.5	66	69	70		74	77		83
23	33.6	38.1	45.7	70	71	72		75	76	80	81
24	38.4	37.2	54.1	73	74	75		79	81		86
25											
26	38.8	38.1	56.0	74	78	79		82	85		89
27											
28	27.2	41.5		74	80	82			91		
29											
30	23.0	35.9		75	79	83			93		

[a] Estimated from rapid cooling exotherm for C_8-C_{18}.

[b] Cooling rate = 0.625 C/min.

[c] Data from ref. 7.

[d] Data in part from ref. 7.

sanoin for odd chain lengths, confirms the occurrence of multiple intermediate melting forms. The collected data also allow additional insight into the character of chain conformation effects on form stability and polymorph interconversion.

MATERIALS AND METHODS

Most samples were obtained as the pure triglycerides; others, as acids or esters, some of which were purified further before triglyceride preparation. Acids and their sources were as follows: pelargonic, Emery Industries, Inc., Cincinnati, OH; undecanoic, Delta Chemical Works, Inc., New York, NY; tridecanoic, Eastman Kodak, Rochester, NY; heneicosanoic and tricosanoic, Nu Chek Prep, Elysian, MN; hexacosanoic, Supelco, Inc., Bellefonte, PA; and octacosanoic and ethyl triacontanoate, National Research Council of Canada, Saskatoon, Saskatchewan. Heneicosanoic, tricosanoic and hexacosanoic acids were not further purified. Triglycerides included tricaprylin and tricaprin (Hormel Institute, Austin, MN), and trilaurin, trimyristin, tripentadecanoin, triheptadecanoin, trinonadecanoin and tritetracosanoin (Nu Chek Prep, Elysian, MN). The triglycerides, all certified 99+% pure, were not purified further.

Pelargonic, undecanoic and tridecanoic acids were purified as the methyl esters by gas liquid chromatography (GLC) using an Aerograph Autoprep Model A-700 (Wilkens Instrument and Research, Inc., Walnut Creek, CA) equipped with a 10 ft by 1/8-in. glass column containing 5% ApL. GLC analyses of the purified esters indicated greater than 99% purity. Methyl octacosanoate and ethyl triacontanoate were also purified on the Aerograph Autoprep using a 2 ft by 1/4-in. stainless-steel column containing 3% OV-1. A 98% or better purity of the isolated esters was shown by GLC.

Tripelargonin, triundecanoin, tritridecanoin, triheneicosanoin and tritricosanoin were prepared from the acids via oxalyl chloride as described previously (11). Insolubility of the three long-chain triglycerides trihexacosanoin, trioctacosanoin and tritriacontanoin required a slight modification in method; the acids were dissolved initially in 1mL of benzene, then mixed with oxalyl chloride and allowed to stand for 4–7 days in a desiccator. Triglycerides were isolated by thin layer chromatography (TLC) on 0.25 mm precoated silica gel 60 F-254 plates (EM Laboratories, Inc., Elmsford, NY). For trihexacosanoin and trioctacosanoin, the solvent system was 3:1 chloroform/*n*-hexane and for tritriacontanoin, 4:1 chloroform/*n*-hexane. The tritriacontanoin was further purified on a Model 201 Liquid Chromatograph (Waters Associates, Inc., Milford, ME) containing a 2 ft by 1/8-in. Corasil column of 37–75 μ particle size. The flow rate of 95:5 *n*-hexane/ethyl ether was 2 mL/min and components were found by a differential refractive index detector. The purity of tripelargonin, triundecanoin and tritridecanoin was greater than 96% by GLC, and triheneicosanoin and tritricosanoin showed one spot by TLC. A trace of free fatty acid was observed by TLC in each of the long-chain triglycerides, trihexacosanoin, trioctacosanoin and tritriacontanoin.

DSC and infrared (IR) procedures were as described previously (7) except that triheneicosanoin and tritricosanoin were analyzed on a Perkin-Elmer DSC-2. Following the convention of previous triglyceride polymorphism studies (5, 7, 11), highest melting endotherms were assigned to β-forms; lowest melting, α-forms; intermediate, β'-forms. Subscripts (e.g., α_1, α_2, etc.) denote decreasing melting points (12). For convenience, triglyceride nomenclature is abbreviated to indicate fatty acid chain length: for example, C_{14} = trimyristin.

Molecular models used to study triglyceride conformations were CPK precision models (The Ealing Corp., Cambridge, MA) having a scale of 1.25 cm/Å. A reference model of the β-form of tricaprin was made based on the single crystal X-ray data of trilaurin (6). The α- and β'-forms were simulated by rotations of the long hydrocarbon chains with the accuracy of the particular conformation based primarily on agreement of the long spacing with X-ray data on the various forms (5). X-ray long spacings were approximately equivalent to the height of the model. Therefore, model long spacings of tilted and vertical forms were determined by aligning the methyl carbons on the two parallel chains and then measuring the vertical distance to the methyl carbon at the opposite end of the molecule.

RESULTS AND DISCUSSION

The history of triglyceride polymorphism (8) reflects much confusion concerning the number of distinct crystal forms that should be ascribed to any particular lipid structure. As early as 50 years ago, seven different forms were reported for saturated monoacid triglycerides (13) and at various times since, tristearin, the most frequently studied, has been assigned either two (α and β) or three (α, β' and β) forms. Unfortunately, questions of sample purity and investigative procedures complicate the protracted debate over intermediate (β') polymorphism. Hoerr (14) felt that as little as 2–3% impurity could affect β'-polymorphism markedly, and it is generally accepted that β'-forms are more stable in complex mixtures. Yet recently Simpson and Hagemann (15) selectively prepared two well differentiated β' forms of 99+% pure tristearin, whereas Dafler (3) noted only one β'-form in hydrogenated soybean oil (ca. 85% tristearin). Clearly it would be premature either to exclude intermediate polymorphism as artifacts or to discount possible effects from impurities, but our experience inclines us to accept some of the earliest speculation; namely, that saturated monoacid triglycerides possess the ability to assume multiple yet distinct, perhaps homologously isomorphous, conformations. Considering the practical goal of understanding and manipulating the behavior of solid fats or lipids in organized mixtures, it was gratifying to experience in several ways a high degree of consistency in the data we collected on 20 different monoacid triglycerides from diverse origins.

Melting Points

DSC data on the newly analyzed saturated monoacid triglycerides and on those examined in earlier work (7) are summarized in Table I. Several DSC curves representative of observed thermal behavior appear in Figure 1. DSC curves for 18 of the 20 samples examined clearly showed or suggested more than one β'-form. Simple DSC scans of even-carbon triglycerides [Fig. 1, (C_8), (C_{10}), (C_{12}) and (C_{14})] showed an intriguing intermediate form, labeled β'_1, which melted close to the β-form. As chain length increased from C_8 through C_{14}, the difference between melting points for β'_1- and β-forms decreased rapidly from 11 to 5 C. Attempts to observe this form in simple scans of C_{16} and larger triglycerides were unsuccessful, perhaps for good reason. If the melting point difference between β'_1 and β were to continue decreasing with increased chain length, the corresponding β'_1- form of C_{16} would melt within 4 C of the β-form and might not be resolved easily. Also, as considered in a later section, there is reason to think that, for saturated monoacid triglycerides, C_{13} or C_{14} represents the upper limit of end-group interaction effect on crystal form; i.e., the β'_1 polymorph may reflect a type of end-plane order that becomes less significant above C_{14}. Although the β'_1 forms in Table I for C_{16} and longer triglycerides could be considered a different species than the β'_1 forms of shorter chain lengths, the naming convention was not changed.

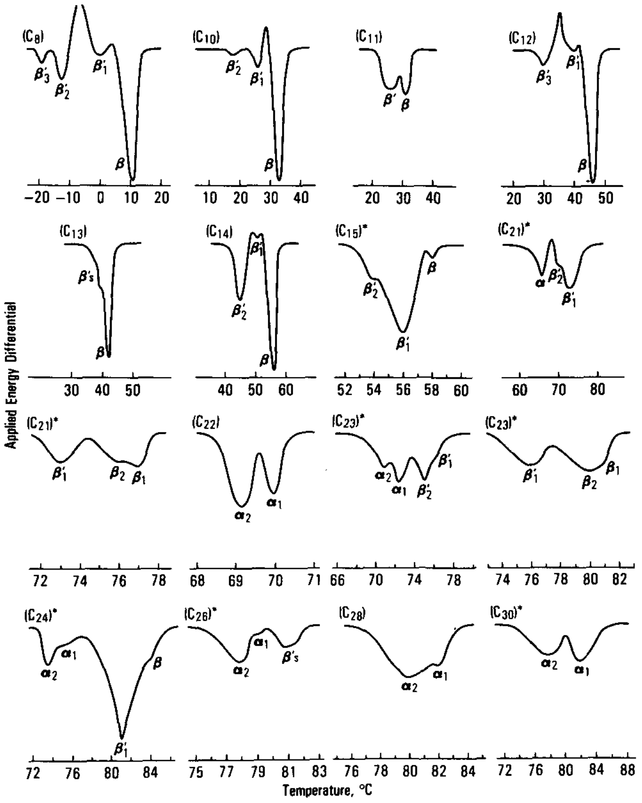

FIG. 1. Selected differential scanning calorimetry curves with endotherms shown as negative peaks. Fatty acid chain length of triglyceride is indicated on left side of curve. (C_8) Heating rate 20 C/min after holding 15 min at −45 C. (C_{10}) Heating rate 20 C/min after holding 30 min at 10 C. (C_{11}) Heating rate 2.5 C/min after holding 1 hr at 14 C. (C_{12}) Heating rate 20 C/min after holding 5 min at 20 C. (C_{13}) Heating rate 2.5 C/min after holding 15 min at 31 C. (C_{14}) Heating rate 10 C/min after holding 30 min at 40 C. (C_{15})* Heating rate 1.25 C/min after holding 60 min at 40 C. (C_{21}, 1st curve)* Heating rate 2.5 C/min after holding 10 min at 65 C. (C_{21}, 2nd curve)* Heating rate 2.5 C/min after holding 2½ hr at 62 C. (C_{22}) Heating rate 2.5 C/min after holding 1 hr at 65 C. (C_{23}, 1st curve)* Heating rate 2.5 C/min after holding 15 min at 72 C. (C_{23}, 2nd curve)* Heating rate 2.5 C/min after holding 2 hr at 71 C. (C_{24})* Heating rate 10 C/min after holding 3 hr at 71 C. (C_{26})* Heating rate 2.5 C/min after holding 17 hr at 73 C. (C_{28}) Heating rate 2.5 C/min after holding 3½ hr at 72 C. (C_{30})* Heating rate 10 C/min after holding 16 hr at 72 C.
*α-Form prepared before holding at the indicated temperature.

By conditioning certain samples, at least two β'-forms were observed for every triglyceride C_8-C_{26} with exception of odd chain lengths C_9-C_{13}. The β' region for C_9-C_{13} as represented by C_{11} in Fig. 1 was often quite broad, however, and might well have contained unresolved endotherms. At much longer chain lengths, odd triglycerides likewise did not exhibit well separated β'-forms [Fig. 1, (C_{21} 2nd curve)* and (C_{23})].

Crystalline states other than the usual α-, β'- or β-forms have been demonstrated amply with fats, e.g., cocoa butter (16) and beef tallow (17). In addition, Hoerr (18) proposed a fourth form, melting between β'- and β-forms, but later (14) attributed this "intermediate" crystal structure and Malkin's β'-form (1) to mixtures. Larsson (12), however, observed a close similarity between X-ray short spacings for the intermediate form and those for the parallel-chain orthorhombic structure of pure 2-methyl-2-ethyleicosanoic acid (19). This observation suggested that unusual configurations need not be unique to triglyceride mixtures. Later, Larsson (2) offered an explanation; namely, that interlayer

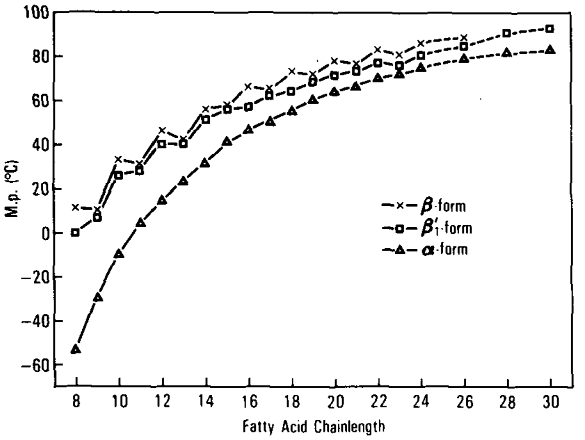

FIG. 2. Melting points vs fatty acid chain lengths of α-, β'- and β-forms of triglycerides. Highest melting form was used for each type where more than one was observed.

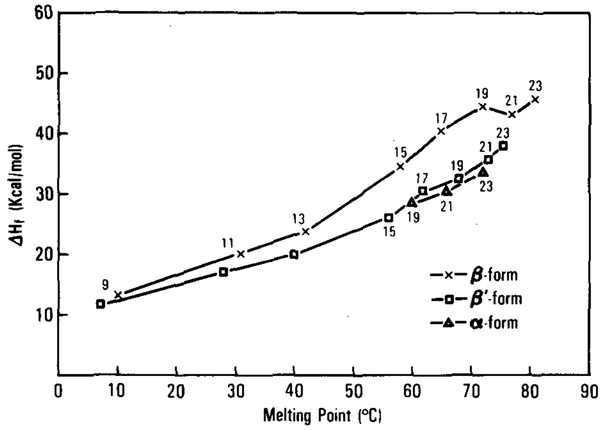

FIG. 4. Odd chain length triglyceride melting points vs ΔH_f. Highest melting form was used for each type where more than one was observed.

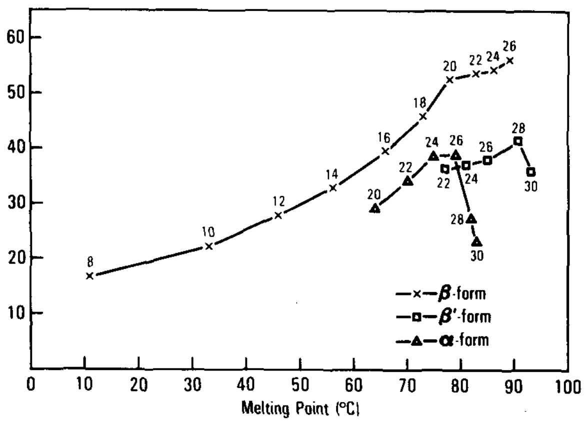

FIG. 3. Even chain length triglyceride melting points vs ΔH_f. Highest melting form was used for each type where more than one was observed.

chain penetration, which would stabilize β'-forms in mixtures, could be relatively absent from pure triglycerides, thereby rendering more difficult the detection of their β'-forms. In this regard, it is interesting that the highest intermediate endotherm for C_8-C_{14} was generally flanked by exotherms [Fig. 1, (C_8), (C_{10}), (C_{12}) and (C_{14})] implying a distinct, albeit relatively unstable, intermediate form. Whatever the nature of this transition, it is different from other β'-form transitions because, to the best of our knowledge, exotherms have not been found previously between multiple β'-forms.

If the temperature difference between β'_1 and β mentioned above were to decrease further one degree per each two carbons beyond C_{14}, the predicted melting points for hypothetical β'_1-forms of C_{16} and C_{18} would be 62 and 70 C, respectively: i.e., exactly the temperatures obtained by Hoerr (18) for "intermediate" forms of C_{16} and C_{18}. Further hypothetical extrapolation of this decreasing difference, β'_1-β, would predict identical melting points for the two forms at C_{24}. Interestingly, this is approximately the chain length at which the favored conformation of even *n*-alkanes (20) changes from triclinic subcell structure (β-form in triglycerides) to an orthohombic subcell (β'-form in triglycerides). Our DSC and IR results with C_{28} and C_{30} were consistent with such prediction.

The C_{28} and C_{30} triglycerides behaved rather differently from the others. Samples crystallized from solvent, therefore presumably β-forms (9), melted in the range expected for β-forms, but their heats of fusion were more consistent with those for the β'-forms of C_{24} and C_{26}. In addition, an infrared spectrum of hexane-crystallized C_{28} contained a doublet at 719 and 727 cm^{-1}, indicative of a β'-form (21, 22). Conditioning these samples for as long as 245 days at 77–80 C failed to produce higher melting forms or greater ΔH_f values. Since possible effects from trace levels of free acid cannot be discounted, we only point out the intriguing possibility that long-chain even-carbon triglycerides, like their shorter odd-chain counterparts, may favor β'-like conformations in their highest melting forms.

Also among longer chain triglycerides, C_{21} and C_{23} each deviated from usual behavior by exhibiting a form melting close to and only partially resolved from the highest melting form [Fig. 1, (C_{21} 2nd curve)* and (C_{23} 2nd curve)*], without an exotherm like those exhibited by the C_8-C_{14} triglycerides. To emphasize this subtle difference, the unusual C_{21} and C_{23} forms were designated β_2 rather than β'_1. Racemic 1-monoglycerides have two β-forms (23), but multiple β-forms have not been reported previously for monoacid triglycerides. Future work will have to decide whether C_{21} and C_{23} are analogous to 1-monoglycerides with which chain-tilt alternation across the methyl gap occurs in one β-form but is absent in the other. Unfortunately, samples of C_{25}, C_{27} and C_{29} were not available to confirm that this β dimorphism is characteristic of long odd chain length triglycerides.

In the α-form region, triglycerides C_{22} and longer regularly exhibited two forms [Fig. 1, (C_{22}), (C_{23} 1st curve)*, (C_{24})-(C_{30})] that tended to melt slightly further apart as the chain length increased. Details of structural relationship between α- and β'-form lipids are not clear, but spectral studies by Chapman (22) and Larsson (9) suggest that they may be quite similar. Raman spectra of hexagonal chain packing (α-form) resemble closely those of orthorhombic chain packing (β'-form) (24). Furthermore, IR bands at 719 and 727 cm^{-1}, corresponding to CH_2 group rocking modes, are present in spectra of both β'-forms and sub-α-forms, which are produced by quenching liquid triglycerides to −70 C (22). Spectra of α-form triglycerides contain an IR singlet at 720 cm^{-1}, but this is not sufficient proof that α- and β'-forms differ significantly because, as Chapman (25) has reported, long-chain molecules can pack in an orthorhombic (β'-form) manner without producing the IR doublet when dimensions of the subcell are larger than normal or when distortions occur in the unit cell. Since α-form hexagonal symmetry in the unit cell accommodates torsional chain oscillation (9), structures that approach hexagonal symmetry may allow distortion required for β'-form-like subcell packings. Accordingly, one might anticipate α-form polymorphism of the type observed for C_{22}-C_{30} (Table I).

The melting point data collected on this series of triglycerides were in other respects self-consistent and compatible with expectations. For example, the α-form melting points increased smoothly with chain length (Fig. 2) as expected of nontilted crystals and the β-form melting points alternated regularly in keeping with common knowledge of odd-even chain length effects in triglycerides. It was rather surprising, however, to see definite changes in the character of β- and β'-melting point alternation at C_{14}. Beyond C_{14}, the amplitude of β-form melting point alternation decreased sharply and the β'-alternation changed even more drastically, suggesting a change in crystal order at this chain length.

Heats of Fusion

Heats of fusion of polymorphic forms are listed in Table I and plotted vs melting points in Figures 3 and 4. Where more than one α-, β'- or β-form was observed, data for the highest melting were used in these plots. Since the multiple forms in any one type polymorph generally did not show baseline separation on DSC, the ΔH_f values in Table I were determined on any one or mixtures of the forms (11).

In this study, ΔH_f values for the β-form of C_{22} were found to be ca. 9 kcal/mol lower than a value reported previously (7). The original scans on chloroform-crystallized material showed slight endothermic baseline shifts following the β endotherm, possibly due to residual solvent. After applying the baseline correction procedure of Guttman and Flynn (26), values from the previous scans agreed well with the present 53.5 kcal/mol. Thus, β-form values in Figure 3 show a gradual rise from C_8 to C_{20} and then almost level off up to C_{26}. Alternation was observed between the odd and even chain lengths.

Forms that produced an endotherm followed immediately by an exotherm were not considered stable for ΔH_f determinations. By this definition, α-form stability occurred at C_{19}, and the corresponding α-form ΔH_f values leveled off in the C_{24}-C_{26} region without clearly suggesting alternation.

For all odd chain length triglycerides analyzed, the β'-forms were observed to be stable with ΔH_f values showing a rise up to C_{23} (Table I and Fig. 4). The difference between β'- and β-form values, 1.3 kcal/mol at C_9, increased with the longer chain lengths and peaked at C_{19}, 12.1 kcal/mol. Even chain length β'-forms became stable at C_{22} and ΔH_f values increased up to C_{28}, then decreased at C_{30}.

Thermal Stability

Scan rates as high as 80 C/min were not sufficient to observe the unstable α-forms of C_8, C_9 and C_{10}. Values reported were taken from exotherms on rapid heating scans after rapid cooling and are in fair agreement with those reported previously (5). For C_{11}, 80 C/min was required to produce an α-form endotherm and for C_{13}, 40 C/min. α-Form instability can also be estimated from the slow cooling exotherm at 0.625 C/min (Table I). The C_8 to C_{15} samples all exhibited slow cooling exotherms above the α-form melting point, indicating transformation from the melt into β'- and/or β-forms. Although only 1 C difference was found at C_{15} between the exotherm and α-form, the heating scan after slow cooling had a single endotherm at 56 C. Above C_{16}, samples solidified on slow cooling into the α-form and exhibited some supercooling properties with increased chain length.

Alpha-Form Excitation

The characteristic thermal properties of specific polymorphic forms for each chain length triglyceride provide a foundation for further characterization of the unusual and perplexing processes by which these compounds undergo interform transitions.

Table II lists energy data collected during thermal scans of triglycerides C_{13}-C_{21} at 10 C/min, 2.5 C/min and 1.25 C/min after cooling at 20 C/min from the melt to below the α-form. All exotherm and endotherm areas were each summed from the cooling and heating scans to obtain the total exotherm/endotherm column values. Overall, caloric inputs and outputs balanced rather well, but there was variation with chain length in the amount of α-form converted to β', β_1 or melt as each triglyceride was heated at the different rates. Longer chain length molecules had no conversion to higher melting forms at rapid scan speeds, but conversion was increased dramatically, especially with the odd chain lengths, as scan rate decreased. The quantity converted to either β' or β at a particular scan rate was estimated by dividing the observed β' or β melting endotherm by the appropriate molar heat of fusion from Table I. After adjusting the transition exotherm in keeping with the actual amount of product, an apparent molar phase excitation energy for the α to β' or β transition was approximated by subtracting the difference between initial and final states $[\Delta H_f(\beta' \text{ or } \beta) - \Delta H_f(\alpha)]$ from the adjusted transition exotherm. For chain lengths above C_{19}, all necessary data were collected directly. For chain lengths shorter than C_{19}, $\Delta H_f(\alpha)$ was assumed to equal the corresponding fast-cooling exotherm. A general agreement between $\Delta H_f(\alpha)$ and the cooling exotherm, where both were available, suggests that this assumption is valid. Converting enthalphy to entropy further reduced the magnitude of error in $\Delta H_f(\alpha)$.

Plots of apparent phase excitation energy vs chain length (Fig. 5) indicate that the rate-limiting process in transforming an α-form triglyceride of odd or even chain length involves different portions of structure depending on molecular size. This is consistent with general consensus that the effect of end groups vs mid-chain groups diminishes rapidly above C_{10} in triglycerides (27).

Our data (Fig. 5) show odd-even alternation in excitation requirements for C_{13} and below. Accordingly, short even-chain α-forms should transform spontaneously or faster than their odd-chain counterparts. Figure 5 further shows a steady increase in apparent phase excitation energy with increasing chain length above C_{13} or C_{14}. Curiously, at these chain lengths, the mid-chain portion (that in excess of 5–6 carbons from either chain end) of the extended acid-glycerol-acid chain, which exists in crystalline β-form triglycerides (2), constitutes 60% or more of the total extended chain length. In this respect, α-form triglycerides resemble hydrocarbons, which possess analogous extended chains and tend to assume crystal forms in which mid-chain interactions contribute 60% or more of the total molecular interaction (28). Mid-chain interaction is apparently a critical structural feature of stable α-form triglycerides above $C_{13/14}$. Accordingly, the difficulties usually encountered in preparing and retaining short-chain α-forms seem appropriate. Furthermore, our data are consistent with recent observations by Simpson and Hagemann (15) that the polymorphs of tristearin differ in lateral interchain interaction.

Our data also suggest a reason for the common absence of β' or β forms from scans of triglycerides C_{24} and above. As seen in Figure 5, the phase excitation energy for an α-form transition increases rapidly with chain length above C_{14}, exceeds the $\Delta H_f(\alpha)$ at C_{19} and, assuming linear ex-

TABLE II

Energy Data from DSC Scans of Saturated Monoacid Triglycerides

Fatty acid chain length	Energy, kcal/mole (heating rate 10 C/min)					Energy, kcal/mole (heating rate 2.5 C/min)					Energy, kcal/mole (heating rate 1.25 C/min)				
	α-form	Trans-formation exotherm	β'- or β-form	Total		α-form	Trans-formation exotherm	β'- or β-form	Total		α-form	Trans-formation exotherm	β'- or β-form	Total	
				Exo.[a]	Endo.				Exo.[a]	Endo.				Exo.[a]	Endo.
13	—[b]	5.9	20.2	23.7	20.2	—[b]	4.8	19.8	22.6	19.8					
14	3.2	14.3	28.6	34.6	31.8	—[b]	7.7	27.1	28.0	27.1					
15	8.7	11.5	25.4	34.7	34.1	1.7	7.0	26.4	30.2	28.1					
16	16.4	23.4	37.6	51.0	54.0	7.8	20.6	38.9	48.2	46.7					
17	23.2	6.7	8.9	32.9	32.1	18.8	24.4	28.5	50.6	47.3					
18	27.6	15.0	13.5	41.8	41.1	22.9	43.4	41.9	70.2	64.8	20.1[c]	30.7	41.4	57.5	61.5
19	29.0	—[d]	—	28.8	29.0	27.9	4.2	4.3	33.0	32.2	27.3	18.2	19.6	47.0	47.0
20	31.5	—[d]	—	29.2	31.5	31.2	36.9	34.2	66.1	65.4	30.8	37.2	42.7	72.1	73.5
21	30.5	—[d]	—	30.5	30.5	25.0	2.1	5.2	32.6	30.2	19.2	8.8	10.8	39.3	30.0

[a] Includes α-form cooling exotherm. See Table I.

[b] α-Form endotherm not present.

[c] Data not collected for chain lengths less than C_{18}.

[d] No conversion to higher melting forms at 10 C/min.

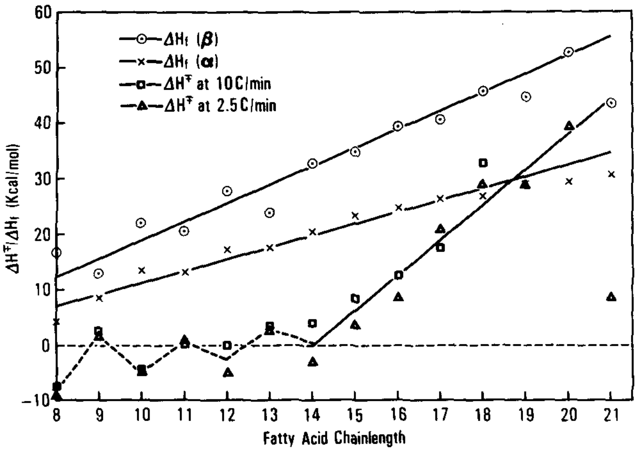

FIG. 5. Comparison of apparent phase excitation energy with heat of fusion of α-and β-forms ΔH^F = apparent phase excitation energy.

trapolation, converges with $\Delta H_f(\beta)$ at about C_{24}. In essence, the energy required to activate α-form molecules as large as C_{24} could be sufficient to melt their other known forms.

The fact that above C_{13-14} α-form phase exictation energies fall along the same line, whether for odd-length triglycerides going to β' forms or for even-length triglycerides going to β forms, is rather surprising. It suggests that the excitation of either odd or even α-form triglycerides greater than C_{13-14} depends upon disruption of the same type of aliphatic chain conformation/interaction. Once activated, odd and even triglycerides collapse, presumably via different transition paths, into forms that are relatively more stable than their α-forms. Conditions that actually determine the path remain obscure. There is little evidence of odd-even differences in α-form melting points or heats of fusion. Yet some variation is apparent in β' melting points, and comparisons between the few heats of fusion that could be determined for β' forms (C_{21-24}) hint that the β'-form is relatively more stable for odds than for evens. Certainly, it is obvious from DSC scans (Fig. 1) that even triglycerides pass readily through transient β'-forms, as if their requirements for β' excitation were minimal compared to those of odd triglycerides. Given, however, substantial differences between odd and even β-form heats of fusion (Table I), we cannot clearly cite order/packing differences in the starting (β') forms or order/packing differences in the products (β forms) as the principal causes of odd-even variation in polymorphic behavior beyond α-form excitation. We can only conclude that the excitation requirement for an even β' to β transition must be quite small relative to the energy difference between the two forms. Conversely, excitation requirements for a similar transformation of odd triglycerides must be large relative to energy differences between β' and β forms. Both our DSC data and earlier X-ray studies (5) indicate that there is less difference between β' and β forms of odd triglycerides than there is between β' and β forms of even triglycerides. With little exergonic advantage from conversion, it is not surprising that the odd triglycerides transform less readily from the β'-form than do even triglycerides.

Molecular Modeling

Since odd and even triglycerides both possess an even number of atoms in extended chains from end to end (or CH_3 to CH_3) through the glycerol center (Fig. 6), it is unlikely that stability differences arise entirely from different orientations at end-group planes, as is thought to account for

TABLE III

Observed and Calculated Entropies of Fusion for C_8-C_{22} Saturated Monoacid Triglycerides

	Entropy of fusion (ΔS_f)								
	α-form (eu)[b]			β′-form (eu)[b]			β-form (eu)[b]		
N[a]	Observed	4.6N	4.6N + 2.3	Observed	4.6N + 4.6	4.6N + 6.9	Observed	6.9N + 2.3	8.1N − 24.3
8	38	36.8	39.1				58	57.5	
9	43	41.4	43.7	42.1	46.0	48.3	46		48.6
10	50	46.0	48.3				72	71.3	
11	53	50.6	52.9	56.8	55.2	57.5	66		64.8
12	58	55.2	57.5				87	85.1	
13	60	59.8	62.1	63.9	64.4	66.7	76		81.0
14	63	64.4	66.7				99	98.9	
15	73	69.0	71.3	79.0	73.6	75.9	104		97.2
16	77	73.6	75.9				112	112.7	
17	80	78.2	80.5	91.6	82.8	85.1	120		113.4
18	82	82.8	85.1				132	126.5	
19	87	87.4	89.7	95.3	92.0	94.3	129		129.6
20	92	92.0	94.3				150	140.3	
21	96	96.6	98.9	103.4	101.2	103.5			
22	100	101.2	103.5	103.9	105.8	108.1	150	154.1	
Average underestimate		1.1	–	–	1.9	–	–	1.8	1.0
Average overestimate		–	1.2	–	–	0.4	–	–	–
Average deviation		±1.8	±1.8	–	±3.4	±3.1	–	±3.0	±3.9

[a] Number of carbon atoms in fatty acid chain.

[b] eu = cal/deg mole.

odd vs even stability differences in hydrocarbons (20). At some stage of crystallization, however, one of the two primary ester groups that could contribute to the extended chain through glycerol must acquire side-chain character that distinguishes it from the remaining primary ester. This event, whenever it occurs, would allow odd triglyceride crystals to differ from evens.

Our interpretation of the results emphasizes the formation of specific rotational conformers; e.g., extended zigzag chains through the glycerol center, as a compelling prerequisite to subsequent lateral chain interaction and crystallization. Thermodynamic quantities associated with the conformational organization of atoms in long-chain molecules outweigh those due to the positioning or orientation of whole molecules relative to one another within the crystal lattice (29). This reality is inherent in empirical expressions that predict melting properties of lipids (27, 30).

If covalent bonds in triglycerides constitute rotational axes of chain twist angles that may assume any of three equally probable conformations in the molten state, then the probability of finding a specific solid-state conformation, such as an extended zigzag chain of n atoms (excluding hydrogen), is $(1/3)^{n-3}$, and the corresponding idealized internal entropy of fusion (30) is

$$\Delta S_{int} = R \ln (1/3)^{n-3} = R\,(n-3) \ln 1/3$$
$$= -2.3\,(n-3)$$

Quantities calculated with variations of this expression for different chain combinations as summarized in Figure 6 compare surprisingly well with fusion entropies that we found for C_8 through C_{22} (Table III).

Using N = number of carbons per fatty acid chain, equations are derived by adding oxygen or carbon atoms through glycerol to the (N−3) term if they are participating in the conformational changes or subtracting atoms in the fatty acid chain that do not participate. A special case is the two independently moving chains in the α-form, where the central glycerol carbon is used for each chain to produce two chains each expressed as 2.3N. Expressions that fit the α-form data for single acid triglycerides are those that require disruption of the conformational order in only two-thirds of each molecule; either (a) in two of the three chains that radiate from the central carbon of glycerol, or (b) in an extended chain through carbons 1 and 2 or 2 and 3 of glycerol.

(a) $$\Delta S_{f(\alpha)} = 2\,(2.3N) = 4.6N.$$

(b) $$\Delta S_{f(\alpha)} = 2.3\,(2N + 1) = 4.6N + 2.3.$$

Overall from C_8 through C_{22} the former expressions (a) tends to underestimate α-form entropies by ca. 1.1 entropy units (eu), while the latter (b) overestimates on the average by 1.2 eu. Average deviation was essentially the same in either case (i.e., ±1.8 eu). The expression that underestimates is probably more realistic because end-group or "methyl gap" interactions might in addition contribute to the total molecular entropy.

The β′ ΔS_f values observed were only slightly higher than those observed for the corresponding α-forms. Thus, the β′ form entropies were best estimated (average underestimate, 1.9 eu; average deviation, ±3.4 eu) by an expression representing an ordered chain through carbons 1 and 3 of glycerol:

$$\Delta S_{f(\beta')} = 2.3\,(2N + 2) = 4.6N + 4.6$$

Reasonable estimates of β-form entropies required different expressions depending on whether the component acids were even- or odd-numbered. Entropies for the even chain triglycerides C_8-C_{22} were approximated (average underestimate 1.8 eu; average deviation, ±3.0 eu) by an expression representing all bonds (excluding those to hydrogen) in an extended chain through glycerol plus those

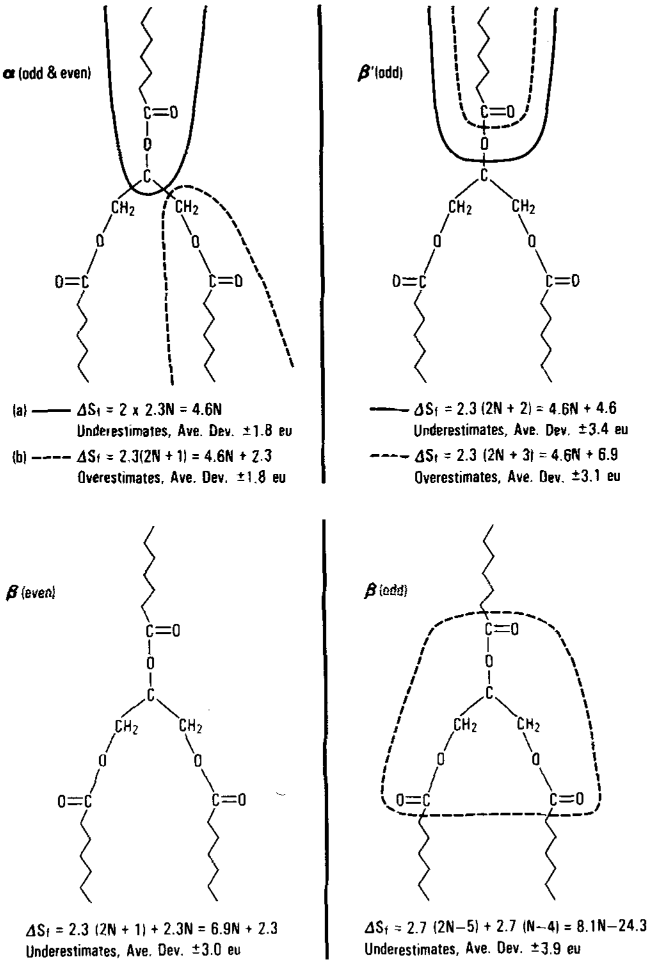

FIG. 6. Expressions and structures utilized for estimated entropies of fusion for α- (odd and even), β'- (odd) and β- (odd and even) forms. N = number of carbons per fatty acid chain. Circled portions (or equivalents) need not participate in melting disorder.

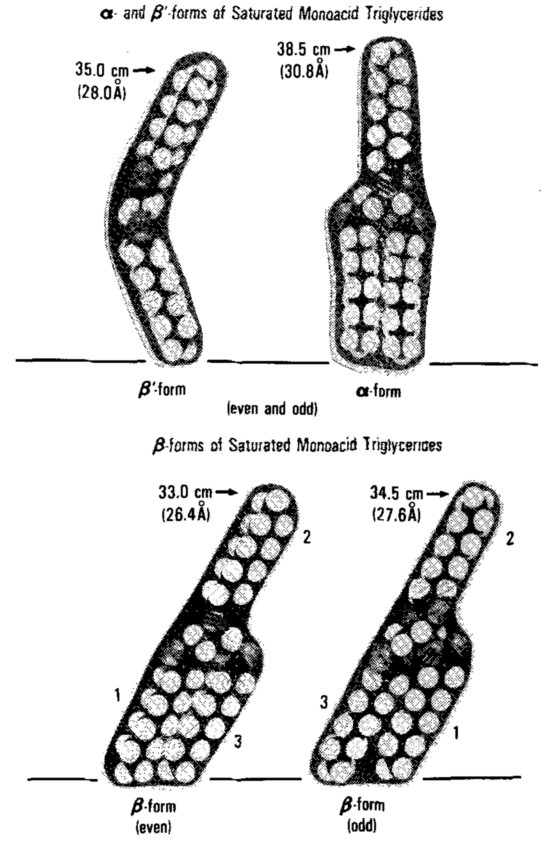

FIG. 7. Molecular models of α-, β'- and β-forms of saturated monoacid triglycerides.

in the remaining side chain:

$$\Delta S_{f(\beta\ \text{even})} = 2.3\ (2N + 1) + 2.3N = 6.9N + 2.3$$

When applied to odd chain length β-form triglycerides (C_9-C_{19}), however, this expression regularly overestimated the observed entropy (average overestimate, 8.8 eu; average deviation, ±8.8 eu). An expression that represents the $\beta_{odd} \rightarrow$ melt transition as disruption of only polymethylene configurational structure fits the β_{odd} melting entropy data much better (average underestimate, 1.0 eu; average deviation, ±3.9 eu):

$$\Delta S_{f(\beta\ \text{odd})} = 2.7\ (2N - 5) + 2.7\ (N - 4) = 8.1N - 24.3$$

This expression contrasts sharply with the others in two ways: (a) the nonparticipation of glycerol and ester-bound atoms in the melting transition; (b) a higher level of entropy per methylene unit, as is more typical of compounds that form monoclinic crystals (30). These differences emphasize the need for definitive crystallographic studies on odd-chain monoacid triglycerides.

In reality, conformation changes produced by melting transitions may not segregate as precisely as these equations

TABLE IV

X-ray vs Molecular Model Long Spacings (A) of Saturated Monoacid Triglyceridesa

Fatty acid chain length	α-Form		β'-Form		β-Form		
	Lit.b	Model	Lit.b	Model	Lit.b	Model Short	Model Long
10	30.2	30.8	27.7	28.0	26.3	26.4	27.6
11	32.3	33.2	30.0	30.2	29.5	28.7	29.9
12	35.5	(35.6)	32.9	(32.4)	31.2	(31.0)	(32.2)
13	37.9	(38.0)	34.2	(34.6)	33.8	(33.3)	(34.5)
14	40.3	(40.4)	36.7	(36.8)	35.5	(35.6)	(36.8)
15	42.9	(42.6)	39.3	(39.0)	39.4	(37.9)	(39.1)

[a] Model long spacing projections based on the C_{10} and C_{11} models are shown in ( ).
[b] Reference (5).

and illustrations (Fig. 6) imply, but the probabilistic models represented by these equations provide an interesting independent frame of reference within which the various polymorphs appear distinctly different yet logically related in the way that chain conformation and interaction could control their formation.

Results with space filling models were consistent with conclusions derived from the probabilistic models; namely, that relatively subtle changes in molecular conformation can account for differences in the physical properties of α, β' and β polymorphs. X-ray data, Table IV (5), reveal that the difference between adjacent α-form long spacings, i.e., C_{10}-C_{11}, C_{11}-C_{12}, etc., is approximately the same as the fatty acid chain length increase and, except for a discrepancy between C_{12} and C_{13}, the same is true for β' long spacings. Considering β-forms, however, odd chain length long spacings do not fall midway between adjacent even carbon spacings and are closer to the next higher even homologue. Also, for odd chain lengths, the β'- and β-form long spacings are almost identical.

Long spacings determined from C_{10} and C_{11} molecular models for α-, β'- and β-forms are given in Table IV along with measurements projected to longer chain lengths. Although many conformations are possible, photographs of tentative structures using C_{10} models appear in Figure 7, in which β-form chains are numbered according to the convention described by Lutton (31). Representative of how model manipulations can affect conformation, an approximate 90° rotation of the α-form chain no. 2 produces the bent β'-form, shown in a side view in Figure 7. β-form type structures can be made from the β'-form via two different routes: a 90° rotation of chains 1 and 3 and a 180° rotation of chain 2, or a 90° rotation of chains 1 and 3 and slight rotation of chain 2. Chain 3 is the branch chain in one of the β-forms but becomes part of the straight portion of the molecule in the other.

In general, the model long spacings are in good agreement with the X-ray data. However, they can be made to vary slightly with small changes in bond angles without distortion of the overall conformation. Of particular interest is that two β-form type structures can be made using the same chain length molecule. These are reported as "short" and "long" under the "β-form" column (Table IV). For even chain length molecules, the model "short" form corresponds to the X-ray measurement, whereas for odd chain lengths, the model "long" form agrees with the X-ray. Therefore, the X-ray data fit the data from the models; i.e., the odd β'- and β-forms have similar long spacings while the even β-form has a smaller long spacing than the β' model.

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