☙ Effects of Chain Length, Conformation and α-Form Packing Arrangement on Theoretical Monoacid Triglyceride β′-Forms

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Theoretical β′-form monoacid triglycerides of odd and even chain length having a bend in the glycerol region were prepared by bond rotations from selected α-form conformers. The shapes of such β′-forms depend on the bonds rotated and the direction of rotation. Intermolecular minimization procedures determined best-fit positions around a centralized molecule, which revealed unsymmetrical packing among five of nine different energetically-favored orthorhombic subcell arrangements. Methyl gap energy is influenced more by overall triglyceride conformation than by whether the fatty acid chains have an odd or even number of carbon atoms. Total lattice-packing energies varied little between odd and even chain lengths; thus, many combinations of conformation and subcell arrangement are consistent with known X-ray long spacings and polymorphic behavior of β′-forms.

X-ray diffraction (1–3) and thermal analysis (2,4,5) are prime techniques (6–11) applied in investigations of the crystal structure and molecular packing of triglycerides. The former provides the only definitive structure for the highest melting β-form (12). Computer modeling techniques promise concepts of the dynamics of triglyceride polymorphism. They already have been used to examine packing energy values for various subcell arrangements of the lowest melting triglyceride polymorph, the α-form (13). These studies revealed relationships between the subcell arrangement and structure that could not have been obtained from physical measurements. Computer modeling has also been applied to intramolecular conformational analysis of phospholipids (14) and short-chain triglycerides (15,16). Among the three polymorphic forms (α, β′, β), the α-form is most elusive in terms of detailed information on structure and packing arrangement. Alpha-form triglycerides originally were thought to have freely rotating chains (17) as in many other long-chain compounds with hexagonal packing (18). However, there is no bond direction in the polar region of the molecule that is parallel with the chain axis and therefore able to permit rotation without large out-of-plane movements of the chain. NMR studies indicate that free chain rotation may be hindered at glycerol (7). Torsional oscillations about long axes of the chains, which seem to best fit the data, can require as much space as free rotation. The nature of α-form mobility merits detailed investigation because this form fulfills an intermediate or precursor role in triglyceride polymorphism. On the basis of X-ray and Raman data, Hernqvist and Larsson (9) have proposed that the motion occurs primarily in end group regions of β-form-like molecules with chains vertical to end group planes. This motion would be similar to that generally thought to occur in the lamellar liquid crystalline phases. Hagemann and Rothfus (5) quantitated α-form motion during phase transition. They found that α-form thermodynamic data for the odd-even series tricaprylin through tribehenin are best approximated by expressions that represent disruption of the conformational order in two-thirds of each molecule but could not specify which molecular parts experience rotational freedom. Norton et al. (10), however, contend that 80–90% of the α-form molecule experiences rotational freedom upon melting. Such differences suggest that somewhat more precise technology may be needed to accurately characterize the population dynamics of α-phase transitions. Toward this end we are examining the precursor roles of α-forms by better definition of their prospective β′ products and the molecular rearrangements that might be required to achieve them.

The physical properties of β′-form triglycerides depend largely on methylene chain structure; long or short, odd or even. On heating, α-form even chain lengths convert directly to the β-form and the β′-form is generally not observed. By tempering near the α-form melting point, the even chain length β′-form can be observed as a transitory peak by differential scanning calorimetry (DSC) (4) for chain lengths less than C_{22}. Only at longer chain lengths is the even β′-form stable enough to permit thermodynamic DSC measurements (5). In contrast, odd chain lengths convert to stable β′-forms on heating from the α-form, and thermodynamic measurements can be made at all chain lengths. The X-ray long spacings (2) of even chain length β-forms are considerably shorter than those of their β′-form counterparts, but those of odd chain length β- and β′-forms are nearly the same. Knowing that α-form triglycerides convert rapidly to β′- or β-forms, we assume in this work that such polymorphic transitions occur via mechanisms requiring minimal molecular movement and that β′-form molecules are, accordingly, likely to occur in tuning-fork configurations with adjacent molecules alternating between upright and inverted positions. With these restrictions in mind we have modified a modeling procedure for comparing α-form packing arrangements to search for energetically-favored β′-form arrangements in which individual molecules possess a bend near glycerol (19).

EXPERIMENTAL

β′-form structures. The starting α-form triarachidin conformers used to prepare the theoretical β′-form structures were those described previously (13). Bond lengths and angles were based on those reported from single crystal studies on β-form trilaurin (12). A single triarachidin molecule modeled from Minit Model System components (Science Related Materials, Inc., Janesville, Wisconsin) was used to identify rotations required to prepare a β′-form molecule having a bend in the glycerol

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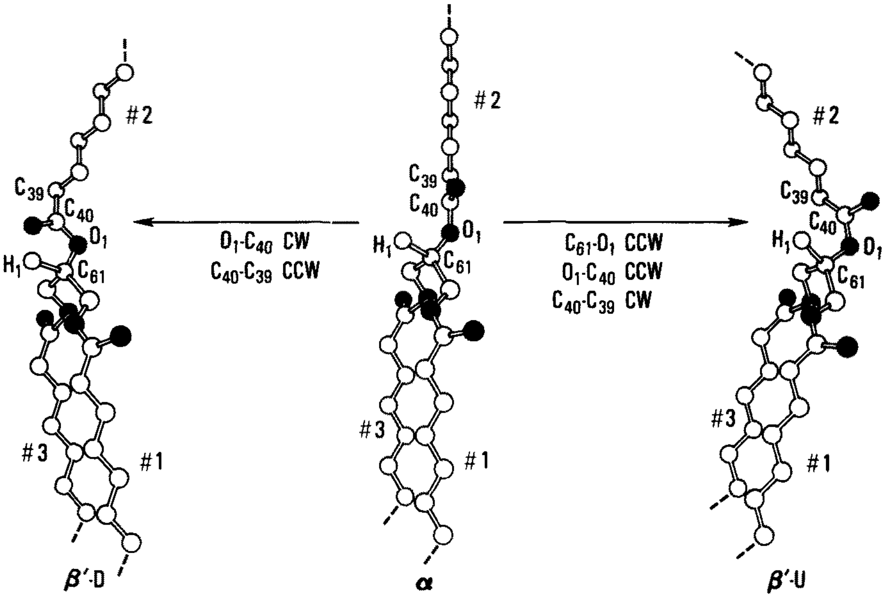

FIG. 1. Triglyceride α- to β'-form transitions by interior bond rotations. Carbon atoms are open circles; oxygen atoms, solid circles; hydrogen atoms, hatched circles. CW, clockwise; CCW, counterclockwise. β'-D: methylene chains extend down when molecule is viewed down the H_1-C_{61} bond; β'-U: methylene chains extend upward when molecule is viewed down the H_1-C_{61} bond.

region (19). Identified bond rotations were performed on a computer model of packed α-form triarachidin molecules until the distance between methyl groups at opposite ends of each molecule approximated the recorded β'-form long spacing (2). The modeling program, operating in a ModComp Model 7870 computer, calculated molecular positions at selected bond rotation intervals and provided a listing of atomic coordinates after the desired long spacing was reached.

Two different β'-form conformers that fit recorded long spacings can be made (Fig. 1) from each α-form depending on the bonds rotated and the direction of their rotation. Clockwise (CW) rotation about the O_1–C_{40} bond and counterclockwise (CCW) rotation about the C_{40}–C_{39} bond produce a bent β'-form whose fatty acid chains extend downward when the molecule is viewed down the glycerol β-hydrogen-C_{61} bond (Fig. 1, β'-D). Similarly, CCW rotation about the C_{61}-O_1 and O_1-C_{40} bonds and CW rotation of the C_{40}-C_{39} bond produce a bent β'-form whose fatty acid chains extend upward when the molecule is viewed down the H_1-C_{61} bond (Fig. 1, β'-U). These theoretical β'-forms, β'-D and β'-U, are similar to the starting α-forms (13) in that the C_{40} carbonyl oxygen can be to the right or left of H_1 as viewed down the H_1-C_{61} bond, and chains 1 and 3 can exhibit an opposite or same carbon zigzag pattern. Rotations illustrated in Figure 1 apply to those starting α-forms that have the C_{40} carbonyl oxygen to the right of H_1 as viewed down the H_1-C_{61} bond (13). When the C_{40} carbonyl oxygen is to the left of H_1, the bond rotations are reversed to make β'-D or β'-U. For example, the O_1-C_{40} CW and C_{40}-C_{39} CCW rotations would make β'-U instead of β'-D when the carbonyl oxygen is left of H_1.

For starting α-forms there are a total of 16 possible β'-form conformers: C_{40} carbonyl, right or left; chains 1 and 3, same or opposite zigzag; chain 2 zigzag, parallel or perpendicular. However, when bond rotations were performed on structures having the zigzag plane of chain 2 approximately parallel to the zigzag planes of the other two chains (13), the resulting β'-forms were the same as after bond rotations of non-parallel chain α-forms. Thus, eight β'-form structures, four with the C_{40} carbonyl oxygen to the right of H_1-C_{61} and four with it to the left, were used as the starting molecules of triarachidin for calculating intermolecular interactions. Six of the eight molecules had the carbon zigzag plane of chain 2 approximately perpendicular to the carbon zigzag planes of chains 1 and 3. The same and opposite zigzag structures of the β'-D conformer with the carbonyl to the left were the only two having the zigzag planes of chains 1, 2 and 3 approximately parallel. Also, for each of the bent β'-form structures, one methylene unit was removed from each fatty acid chain to make β'-forms of an odd chain length monoacid triglyceride, trinonadecanoin. Glycerol regions in the initial trinonadecanoin molecules were the same as those in the initial triarachidin.

Calculation of interaction energies. The Fortran IV computer program used to determine position and lattice energy for pairs of triglyceride molecules in orthorhombic subcell arrangements was essentially as described earlier (13), with the exception that the subprogram producing chain oscillation was eliminated because oscillatory motion about hydrocarbon chain axes is much reduced in more tightly packed structures (8). As before, a minimization program (20) was used to find the minimum energy position of the second molecule with respect to the first utilizing atom-atom interaction potentials of Coiro et al. (21). Reported interaction energies are from modeling results for entire molecules rather than atom pairs (21) and, therefore, are expressed as Kcal/mol.

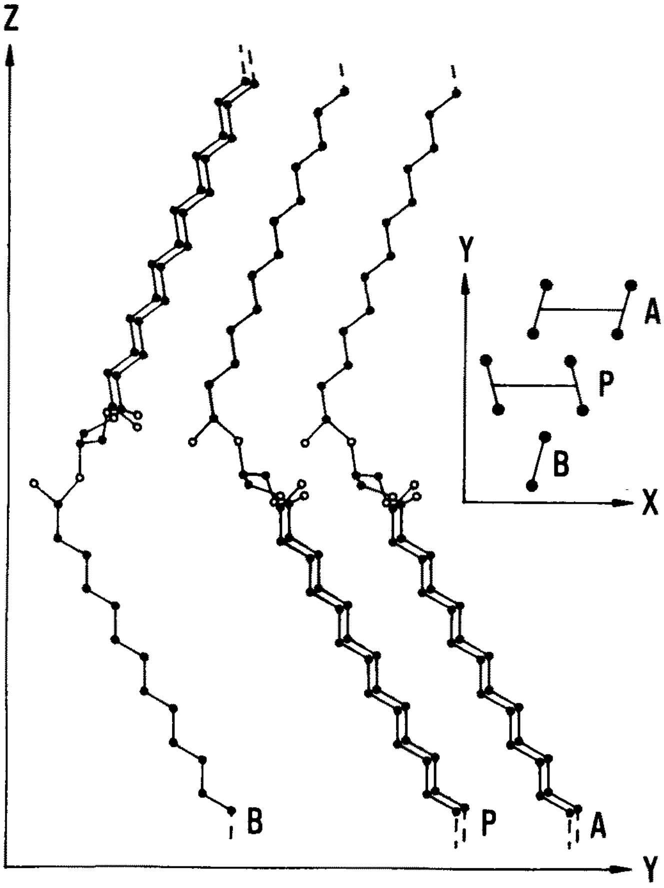

FIG. 2. Unsymmetrical chain packing of triglyceride β'-forms in two views of three closely packed molecules. Carbon atoms are solid circles; oxygen atoms, open circles. Hydrogen atoms are not indicated.

RESULTS

β'-form subcell arrangements. Orthorhombic packing typical of β'-forms requires rows of parallel zigzag planes that intersect approximately perpendicularly with zigzag planes in successive rows. Preliminary results of packing studies on the bent β'-forms revealed that five of a possible nine different subcell arrangements of six molecules around a central molecule had unsymmetrical packing. Figure 2 shows X-Y and Y-Z views of a principle molecule, labeled P, with inverted (B) and upright (A) molecules on opposite sides. The X-Y view is part of the triglyceride arrangement that occurs in five of the nine subcell packings for α- or β'-forms. The computer-generated Y-Z view (Fig. 2) shows that chains 1 and 3 of molecule B are not the same distance from chain 2 of molecule P as chain 2 of molecule B is from chains 1 and 3 of molecule P. Yet the interchain distances between the two upright molecules P and A are approximately the same even though the carbon zigzag planes are oriented differently in each molecule. For the bent β'-forms, the five subcell arrangements having the molecules P and B type spatial orientation were eliminated from further modeling studies.

The remaining four preferred packing arrangements for the bent β'-forms are shown in Figure 3. The P molecule in the middle row of each arrangement is an upright molecule (chains 1 and 3 down) between two inverted molecules (chain 2 down) in the same row. These arrangements are characterized by close packing of upright triglyceride molecules instead of inverted molecules in adjacent rows on either side of molecule P. The upright molecules in rows 1 and 3 are generated during computation as reflections of the central molecule P in the X-direction (X) followed by translations in either the ± Y-direction. Inverted molecules in row 2

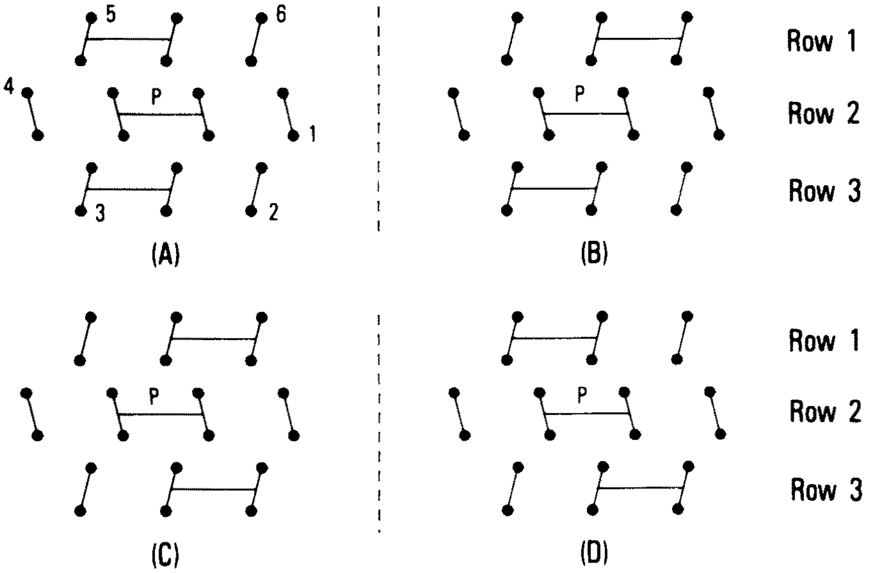

FIG. 3. Preferred packing arrangements of triglyceride molecules in orthorhombic subcell packing. One zigzag period is shown in the direction of the hydrocarbon chains. Connected zigzag periods represent chains 1 and 3 of the same molecule; individual zigzag periods represent chain 2 of separate molecules. Numbered positions (A) are the same for all arrangements. Carbon atoms are solid circles; hydrogen atoms are not indicated. X-axis, horizontal; Y-axis, vertical.

represent reflections of P in the Z-direction (Z), while those in rows 1 and 3 represent both X and Z symmetry operations.

The numbering system used to describe triglyceride positions around molecule P in the β′ subcell arrangements is shown in Figure 3(A). Thus, position 2 represents an inverted molecule in arrangements (A) and (B) and an upright molecule in arrangements (C) and (D). Not shown in Figure 3 are positions 7 to 14 which are for molecules across the methyl gap at both ends of molecule P.

Methyl gap orientations. According to orthorhombic symmetry, hydrocarbon chain-tilt must alternate between opposite directions in successive double layers of chains (8). It is still unknown whether this change in chain-tilt occurs in the glycerol region (19) or at the methyl end group plane (9). Therefore, symmetry operations were performed that enabled examination of both alternating and nonalternating angle of tilt in the methyl gap region. Three methyl gap orientations are possible for alternating and three for nonalternating angle of tilt (Fig. 4), depending on the hydrocarbon chain length and the direction of tilt of the final two carbon atoms of the zigzag chain. Although two of the orientations (II and IV) are generally associated with odd chain length alkanes, even chain length triglycerides can assume these orientations after CW or CCW rotation of chain 2. Likewise, odd chain length triglycerides are capable of exhibiting the end group packings of (I), (III), (V) and (VI) normally associated with even chain length alkanes. For conformers having opposite zigzag patterns on chains 1 and 3, two methyl gap orientations are required to describe methyl gap patterns involved in the calculations. Five hydrocarbon chains were placed across the methyl gap around chains 1 and 3 of molecule P, positions 7 to 11, and three chains around chain 2 of molecule P, positions 12 to 14. All methyl-methyl intergroup distances were set at 4 Å.

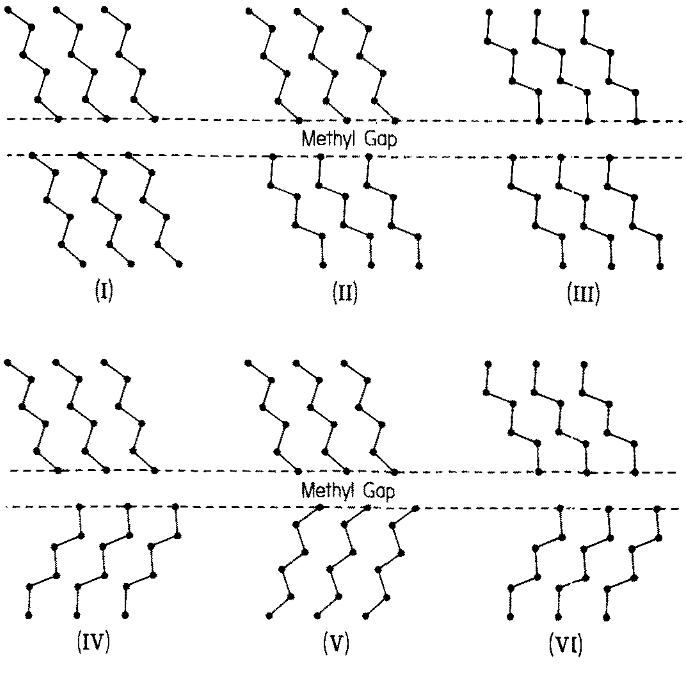

FIG. 4. Methyl gap orientations of triglyceride β′-forms. A nonalternating angle of tilt is represented by (I), (II) and (III), and an alternating angle of tilt by (IV), (V) and (VI). Carbon atoms are solid circles; hydrogen atoms are not indicated.

Trans methyl gap interaction energies calculated for β′-form structures are listed in Table 1. Values ranged from −6 to about −15 Kcal/mol except for the C_{19}-U conformer with opposite zigzag structure, nonparallel chains and carbonyl left; −19.2 Kcal/mol. In general, methyl gap orientations (III) and (VI), in which the final carbon-carbon bond is nearly perpendicular to the end group plane, produce the least negative values and are therefore least likely to be stable. Structures having methyl gap orientations (I) and (V), where the final carbon-carbon bond is much less perpendicular to the end group plane, tend to have less potential energy, but a notable exception is C_{19}-D with nonparallel chains and carbonyl right that produces values similar to orientations (III) and (VI) of C_{20}-D with parallel chains and carbonyl left. Triglyceride conformation appears to affect methyl gap energy more than either odd or even chain length or alternating or nonalternating chain tilt. However, for structures having opposite zigzag patterns on chains 1 and 3, the trinonadecanoin (C_{19}) structures had a greater attractive energy across the methyl gap than did the triarachidin (C_{20}) structures.

Packing arrangement energies. The minimum energy positions for inverted molecules in positions 1 and 4, Figure 3, and for upright molecules in positions 2 and 3 and positions 5 and 6 were found by minimization techniques (20) used in the computer program. The spacings found between molecule P and the inverted molecules in positions 1 and 4 and between molecule P and upright molecules in positions 2, 3, 5 and 6 were used to determine the positions of inverted molecules in positions 2 and 3 and positions 5 and 6. This procedure for locating inverted molecules in positions 2 and 3 was necessary because otherwise the modeling program moved inverted molecules in positions 2 or 3 to a position opposite molecule P, as in the X-Y drawing of Figure 2, or to side packing positions 1 or 4.

The potential energies of interactions between P and an adjacent molecule in each position in Figure 3 were summed to obtain a total value for each subcell arrangement. Results for the triarachidin structures are shown in Table 2 and those for trinonadecanoin in Table 3. Values include results for the appropriate structure from Table 1 for which a non-alternating angle of tilt at the methyl gap was used. Many of the values are exceedingly high, thus indicating unfavorable packing interactions; others are very negative as would be expected of stable arrangements. Because inverted molecules in positions 2, 3, 5 and 6 were not placed by minimization procedures, parts of these molecules often approach parts of molecule P closely. The potential energy function used (13) to calculate interaction energies becomes positive at interatomic distances shorter than optimum spacing. Near complete atomic overlap, the function reverses and becomes very negative. Tables 2 and 3 are therefore noted to indicate positions in which close approach occurred, thus causing suspect values. Values nearer those found for the α-form subcell arrangements (approximately −100 to −220 Kcal/mol) (13) are prob-

TABLE 1

Interactions Across Methyl Gap for β'-forms of Triarachidin and Trinonadecanoin[a]

Fatty acid chain length	Same zigzag on chains 1 and 3		Opposite zigzag on chains 1 and 3	
	NA	A	NA	A
	Parallel chains, carbonyl left on chain 2[b]			
C_{19}-D	-13.6 (I)	-14.7 (V)	-12.4 (I & II)	-13.4 (IV & V)
C_{20}-D	-6.0 (III)	-6.0 (VI)	-7.4 (II & III)	-7.4 (IV & VI)
	Nonparallel chains, carbonyl left on chain 2			
C_{19}-U	-6.1 (II)	-6.1 (IV)	-19.2 (I & II)	-12.2 (IV & V)
C_{20}-U	-10.1 (II)	-10.0 (IV)	-10.9 (II & III)	-10.6 (IV & VI)
	Nonparallel chains, carbonyl right on chain 2			
C_{19}-D	-5.8 (I)	-6.8 (V)	-11.5 (I & II)	-11.4 (IV & V)
C_{20}-D	-6.3 (III)	-6.2 (VI)	-7.1 (II & III)	-7.2 (IV & VI)
C_{19}-U	-9.7 (II)	-10.7 (IV)	-11.0 (I & II)	-11.3 (IV & V)
C_{20}-U	-9.9 (II)	-9.7 (IV)	-8.1 (II & III)	-8.0 (IV & VI)

[a] Interaction energies are expressed in Kcal/mol. Numbers in ( ) refer to methyl gap orientation in Fig. 4.

[b] See text for explanation of β'-form structures. NA, nonalternating angle of tilt; A, alternating angle of tilt across methyl gap.

ably more realistic. Values outside this range do not relate directly to any known thermodynamic quantity, but for comparative purposes they are sensitive to minor conformational and positional variations. These results point out combinations of conformation and subcell arrangements that are less favored representations of β'-form molecular organization.

Of the 16 triarachidin combinations having opposite zigzag patterns in chains 2 and 3, only three combinations have total subcell packing energies in an acceptable range. In contrast, 11 of the 16 combinations with the same zigzag patterns in chains 1 and 3 had acceptable values. This group included all eight of the C_{20}-U combinations. The strongest realistic packing energy (greater negative values) occurred with the same zigzag C_{20}-U conformer with nonparallel chains and carbonyl right. By contrast the same zigzag C_{20}-D conformer with parallel chains and carbonyl left had the highest repulsive (high positive value) energy, which makes it a least likely combination.

Results for trinonadecanoin followed a similar pattern. Only two of the opposite zigzag structures had acceptable total subcell arrangement values. Values for the same zigzag C_{19}-U structures were all normal but slightly lower than those for triarachidin, which is not surprising because the trinonadecanoin computations involved three less methylene units. It is also interesting that none of the parallel chain C_{19}-D combinations had acceptable values, while two combinations of the parallel chain C_{20}-D combinations were in an acceptable range. This is an effect of the odd vs even chain length on molecular spacing because the glycerol regions remained unaltered.

Middle-of-chain energy. Additional insight into the effect of structure/arrangement combinations can be obtained from the contribution of each type of interaction for each atom. A change in the fatty acid chain length from C_{19} or C_{20} would not change the energy contributions of methyl gap and glycerol regions, but the number of methylene units in the central portion of each chain would affect the total potential energy. By adding or subtracting methylene units, the base C_{19} or C_{20} structures were extrapolated to other chain lengths in order to examine potential energy contributions due to central portions of the methylene chains. The central section or middle-of-chain as defined previously (22) consists of methylene units in each chain that have no interactions to oxygen or methyl groups; those that interact only with other methylene units. It was found earlier (22) that middle-of-chain contributions near 60% of the total molecular interaction coincide with n-alkane solid phase changes from triclinic to monoclinic and an increase in α-form stability. Also, a middle-of-chain contribution of 60% occurs at C_{24} for triglyceride α-forms where multiple forms begin to appear on differential scanning calorimetry curves (5).

Results of calculations expressing the middle-of-chain energy as a percentage of total subcell energy appear in Table 4 for even chain lengths and in Table 5 for odd chain lengths. Such calculations were not performed on structure/subcell combinations that have unrealistically high repulsive or attractive energy values. Therefore, some of the combinations in Tables 2 and 3 are not represented in Tables 4 and 5. Energies of subcells for a particular combination varied less than 10%; thus, results for only one subcell are reported where more than one

TABLE 2

Total Interaction Energies of β′-Form Subcell Arrangements for Triarachidin[a]

C_{20}-D

Subcell from Fig. 3	Parallel chains, carbonyl left on chain 2		Nonparallel chains, carbonyl right on chain 2	
	Same zigzag	Opposite zigzag	Same zigzag	Opposite zigzag
A	551820.4^{b}	19818.1^{c}	-166.8	-6114.7^{e}
B	-170.5	-131.2	-172.2	-251.9^{f}
C	4469.6^{c}	4021.1^{c}	4958.0^{c}	17828.2^{c}
D	556460.6^{d}	23969.4^{d}	4963.2^{c}	11965.3^{g}

C_{20}-U

	Nonparallel chains, carbonyl left on chain 2		Nonparallel chains, carbonyl right on chain 2	
	Same zigzag	Opposite zigzag	Same zigzag	Opposite zigzag
A	-182.8	-145.6	-205.7	142.6^{j}
B	-181.6	-72.4^{h}	-201.4	666.3^{k}
C	-190.5	55.5^{i}	-208.7	320.4^{h}
D	-191.7	-17.8^{c}	-213.0	-203.4

[a] Interaction energies are expressed in Kcal/mol. Values include calculations across methyl gaps for nonalternating angle of tilt. See text for explanation of β′-form structures.
[b] High repulsive energy at position 6.
[c] High repulsive energy at position 3.
[d] High repulsive energy at positions 3 and 6.
[e] High attractive energy at positions 2 and 6.
[f] High attractive energy at position 2.
[g] High repulsive energy at position 3, high attractive energy at position 6.
[h] High repulsive energy at position 5.
[i] High repulsive energy at positions 3 and 5.
[j] High repulsive energy at position 2.
[k] High repulsive energy at positions 2 and 5.

TABLE 3

Total Interaction Energies of β′-Form Subcell Arrangements for Trinonadecanoin[a]

C_{19}-D

Subcell from Fig. 3	Parallel chains, carbonyl left on chain 2		Nonparallel chains, carbonyl right on chain 2	
	Same zigzag	Opposite zigzag	Same zigzag	Opposite zigzag
A	259717.8^{b}	13849.9^{d}	-166.0	-5872.5^{g}
B	259723.4^{b}	13652.4^{b}	-170.2	-6595.9^{h}
C	-961.3^{c}	1.59×10^{8e}	5985.6^{e}	59854.8^{e}
D	-966.9^{c}	1.59×10^{8f}	5989.8^{e}	60578.2^{f}

C_{19}-U

	Nonparallel chains, carbonyl left on chain 2		Nonparallel chains, carbonyl right on chain 2	
	Same zigzag	Opposite zigzag	Same zigzag	Opposite zigzag
A	-168.8	-149.7	-188.5	6480.2^{d}
B	-172.8	-141.7	-185.1	2595.4^{i}
C	-182.8	77.2^{e}	-183.8	2324.2^{j}
D	-178.7	69.3^{e}	-187.2	6209.1^{f}

[a] Interaction energies are expressed in Kcal/mol. Values include calculations across methyl gaps for nonalternating angle of tilt. See text for explanation of β′-form structures.
[b] High repulsive energy at position 2.
[c] High attractive energy at position 3.
[d] High repulsive energy at positions 2 and 6.
[e] High repulsive energy at position 3.
[f] High repulsive energy at positions 3 and 6.
[g] High attractive energy at position 2, high repulsive energy at position 6.
[h] High attractive energy at position 2.
[i] High repulsive energy at positions 2 and 5.
[j] High repulsive energy at positions 3 and 5.

subcell for a combination had acceptable values.

For selected even chain length combinations (Table 4), the middle-of-chain contribution for triarachidin ranged from 34 to 48%. This range decreased slightly with extrapolation to longer chain lengths but remained about the same for shorter chain lengths. For most structure/arrangement combinations, no middle-of-chain methylene units exist at C_{10}, which means that the entire chain is influenced by significant methylene interactions with atoms in the methyl and glycerol regions. One exception was a C_n-D parallel chain structure that had a middle-of-chain contribution of almost 5% at C_{10}. At the other extreme, one of the C_n-U structures with nonparallel chains, carbonyl right, and opposite zigzag on chains 1 and 3 had no middle-of-chain contribution at chainlengths C_{14} and shorter because chain 1 had only five middle-of-chain methylene units. At C_{26}, where the orthorhombic subcell is favored in n-alkanes (23), only one of the seven even chainlength combinations (C_n-U) had a middle-of-chain contribution exceeding 60%, although two of the C_n-D structures were but a few percent lower.

Only four of the trinonadecanoin-based structure/arrangement combinations were acceptable for extrapolation to other chain lengths, Table 5, including but one C_n-D structure and none with parallel chains. For odd chain lengths, the middle-of-chain contribution ceased at C_{11} except for one C_n-U structure with nonparallel chains, the carbonyl right, and the same zigzag on chains 1 and 3. At C_{25}, only one combination had a middle-of-chain contribution of 60%; the other three were around 50%.

Detailed comparisons between Tables 4 and 5 are difficult because of chain length differences, but each combination in Table 5 has a reasonable counterpart in Table 4. For the C_n-D combination with nonparallel chains, carbonyl right and same zigzag, percentages for odd chain length structures are lower than those for even chain length homologs with one more or one less methylene per chain. The opposite is true for C_n-U with nonparallel chains, carbonyl right and same zigzag, in which case values for odd chain length structures were generally higher than those for even chain lengths. Odd length C_n-U structures with nonparallel chains, carbonyl left and the same or opposite zigzag exhibit less middle-of-chain interaction than their next higher even homologs if the chains are relatively short, and more if the chains are relatively long, i.e., greater than C_{18-22}. Of the four structures where values are available for the same and opposite zigzag patterns, three show the opposite zigzag structure to have the higher percentages. Only the even chain length parallel chain structure carbonyl left had higher values for the same zigzag conformation.

DISCUSSION

Variables involved in configurational/packing relationships of large molecules are numerous and complex. Most remain unknown or unquantified. Some concessions to rigor must, therefore, be made in studies of this type in order to identify parameters that regulate lipid interactions. The restrictions on β′-forms in this work were derived from previous descriptions of orthorhombic subcells (23), which consist of rows of parallel zigzag planes and alternating zigzag planes in successive rows, Figure 3. A further constraint was taken from the molecular packing of the β-form (12), which has alternating upright and inverted molecules in each horizontal row. Placing chains 1 and 3 in the same horizontal row further restricted the number of possible polymorphs.

Four molecules packed easily around a central molecule, but addition of inverted molecules in packing positions 2, 3, 5 or 6, Figure 3, caused close approach of some molecules creating repulsions that rendered certain arrangements less favorable (Tables 2 and 3). When repulsion between two molecules was in the order of 50 Kcal/mol or less, moving one of the molecules less than a C-H bond distance reduced potential energy of the system to zero; i.e., neither attractive nor repulsive. Molecules in unfavorable subcell arrangements characterized by excessive repulsive or attractive energy could have been displaced some distance to obtain acceptable values. Such deformations or dislocations likely occur in real systems to keep all structure/rrangement combinations within a narrower range of energy values than seen here. But anisotropic distributions are also likely in real populations. Some of the unfavored arrangements identified here may be good candidates for distorted or activated forms that probably occur in normal lipid polymorphic transitions. Subsequent studies will attempt to identify likely transition intermediates. In this work, packing stresses were not relieved in hopes of finding clearly stable and therefore truer representations of β′ subcell arrangements. Indeed, relatively few of the possible arrangements are characterized by reasonable potential energy levels (Tables 2 and 3).

Molecules in adjacent rows cannot slide to different positions to decrease repulsive energy because of the large energy barriers involved. As shown in Figure 5, when an inverted molecule is moved across a stationary upright molecule in the X-direction at a constant Y-axis distance of 7.25 Å, three minima are found at

TABLE 4

Contribution (%) of Middle-of-Chain Energy to Total Energy for β′-Forms of Even Chain Lengths[a]

Fatty acid	C_n-D[b]			C_n-U[b]			
	Parallel chains Carbonyl left		Nonparallel chains Carbonyl right	Nonparallel chains Carbonyl left		Nonparallel chains Carbonyl right	
Chain length (n)	Same zigzag Subcell B[c]	Opposite zigzag Subcell B	Same zigzag Subcell B	Same zigzag Subcell D	Opposite zigzag Subcell A	Same zigzag Subcell D	Opposite zigzag Subcell D[d]
10	0	4.8	0	0	0	0	0
12	14.2	13.2	14.0	4.6	8.8	2.2	0
14	25.1	23.4	24.6	14.8	26.4	13.4	0
16	33.6	30.4	32.8	23.5	33.0	21.0	20.0
18	40.4	36.9	39.5	30.4	42.3	28.4	29.9
20	46.0	41.9	45.1	36.2	48.2	34.2	37.4
22	50.6	46.2	49.8	41.1	53.0	39.2	43.5
24	54.5	49.9	53.7	45.4	56.9	43.4	48.5
26	57.8	53.1	57.1	49.0	60.3	47.1	52.6
28	60.7	55.9	60.0	52.2	63.2	50.3	56.2
30	63.2	58.4	62.5	55.0	65.7	53.2	59.3

[a] Energy calculations for chainlengths other than C_{20} by addition or subtraction of methylene units. See text.
[b] See text for explanation of β′-form structures.
[c] Subcell letters are from Figure 3.
[d] Only five middle-of-chain methylene units on chain #1.

TABLE 5

Contribution (%) of Middle-of-Chain Energy to Total Energy for β′-Forms of Odd Chain Lengths[a]

Fatty Acid Chainlength (n)	C_n-D[b]	C_n-U		
	Nonparallel chains Carbonyl right	Nonparallel chains Carbonyl left		Nonparallel chains Carbonyl right
	Same zigzag Subcell B[c]	Same zigzag Subcell B	Opposite zigzag Subcell B	Same zigzag Subcell B
11	0	0	0	3.1
13	12.5	13.1	18.6	14.3
15	22.2	22.5	28.3	24.1
17	29.8	30.6	39.0	31.7
19	36.4	37.0	46.5	38.1
21	41.8	42.3	52.4	43.4
23	46.4	46.8	57.0	47.9
25	50.3	50.7	60.9	51.7
27	53.6	54.0	64.1	55.0
29	56.6	56.9	66.8	57.9

[a] Energy calculations for chain lengths other than C_{19} by addition or subtraction of methylene units. See text.
[b] See text for explanation of β′-form structures.
[c] Subcell letters are from Fig. 3.

approximately X = -6.0, -0.5 and 4.5 Å, from X = 0 midway between chains 1 and 3 of the upright molecule. Located between the minima are regions of repulsive energy at X = ± 2.5 Å. This curve was obtained with a C_{20}-D structure having parallel chains, carbonyl left, and same zigzag, but similar type curves would be expected with the other β′-form structures. It is, therefore, evident that once molecules in the bent β′-form configuration become closely packed they cannot change subcell arrangement orientations without considerable expenditure of energy. However, when the same structure is in the α-form configuration with synchronous oscillation of the methylene chains (13), the energy curve is almost flat (Fig. 5), showing small minima at approximately X = -3 and 2 Å. In the α-form, much less energy is used for entire molecule movement, and reorientation of subcells could occur to eliminate unsymmetrical packing. During slow crystallization to β′- and/or β-forms above the melting point of the α-form, such sliding movements likely occur, which emphasizes the importance of order in the near-crystalline liquid state and the effect it may have on overall polymorphic behavior.

Based on the present results, there are many combinations of structure and subcell arrangement that would satisfy the β′-form behavior of multiple polymorphs as observed by DSC (5). A broad DSC peak would be expected if all favored combinations were present, but the sharpness of β′-endotherms suggests limitations on the number of existing forms. Variations in chain orientations, resulting from different angles of tilt, could account for temperature and entropy differences between forms. The most favorable total energies for both odd and even chain length were those exhibited by C_n-U structures having nonparallel chains, carbonyl right and same zigzag pattern on chains 1 and 3, Tables 2 and 3. Methyl gap interaction energies for this conformer were

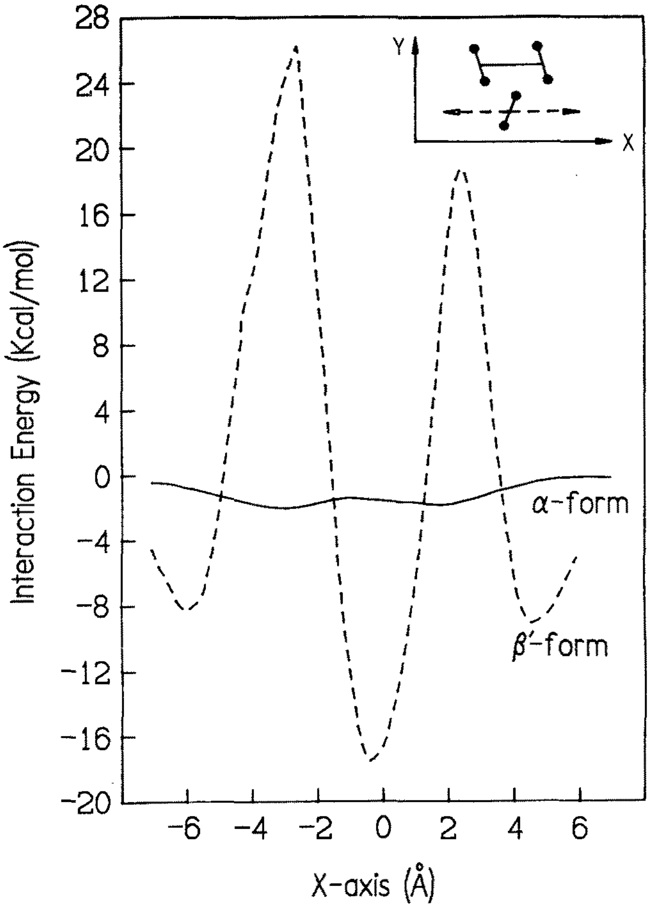

FIG. 5. Energy profiles of lateral movements in α- and β′-forms. β′-Form (dotted line), C_{20}-D with parallel chains, carbonyl left on chain 2, same zigzag on chains 1 and 3. α-Form (solid line), C_{20}, parallel chains, carbonyl left on chain 2, same zigzag on chains 1 and 3, synchronous oscillation of methylene chains (13). Insert depicts an inverted molecule moving on the X-axis across a stationary upright molecule. Carbon atoms are solid circles; hydrogen atoms are not indicated.

of medium value (Table 2), but the middle-of-chain contribution was among the lowest for even chain lengths (Table 4). From the standpoint that orthorhombic structures become stable for even chain lengths at about C_{26} with approximately 60% middle-of-chain contribution, this structure would not seem a good candidate for even β'-forms. It would be more likely for odd chain lengths.

Odd chain length β'-forms are all stable and have melting points, heats of fusion and long spacings (5) similar to those of their corresponding β-forms. The contribution for the middle-of-chain energy may, therefore, not be as critical for odd chain lengths as for even chain lengths, because both β'- and β-forms for the odds are very similar in physical properties. How the proposed unsymmetrical β'-form model (9) fits into the overall picture is still unknown. If the carbon zigzag planes of chains 1 and 2 are parallel, then chain 3 must be nonparallel with chain 1. Disorder between chains 1 and 3 in our study (opposite zigzag on chains 1 and 3) did show a greater middle-of-chain contribution for most structures (Tables 4 and 5) but tended to have a lower total energy (Tables 2 and 3). To satisfy orthorhombic symmetry requirements, the zigzag planes of chains 1 and 3 of the unsymmetrical β'-form (9) would be located in different rows of the subcell (Fig. 3), and the side-chain character of chain 3, a chain-shortening effect, would produce irregularities in the methyl gap region. If polymorphism is as similar between odd and even chainlengths as powder X-ray diagrams suggest, then why would odd chainlength β'-forms be much more stable than their even chain length counterparts? These ambiguities emphasize the distinct possibility of radically different odd and even β'-form structures that are largely dependent on the type of solid/liquid structures that precede them.

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