Neutron Diffraction Studies of Liquid and Crystalline Trilaurin

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Neutron diffraction has been employed to investigate the structure of trilaurin in both liquid and crystalline states. The combination of long-wavelength (4.5 Å) neutrons and wide angular ranges of detection ($\approx 6 < 2\theta$ [°] $< \approx 100$) permitted a large range of momentum transfer to be accessed ($\approx 0.15 < Q$ [Å^{-1}] $< \approx 2$) in a single scan of the detector. The chemical technique of selective deuteration of chosen parts of the triglyceride molecules was used to enhance specific aspects of the diffraction pattern and elucidate structures formed. The well established layer structure in the solid phases was confirmed in the diffraction patterns. It was also established that long-range ordering in the solid resulting from chain-chain registry disappeared when the solid melts. However, we found no evidence for long-range ordering in the form of persistence of a layer-layer spacing correlation in the liquid state. We postulate from the diffraction patterns a molecular arrangement resembling the arrangement found in the nematic phase of liquid crystals, where the layer structure is relaxed from that smectic-like arrangement for the crystalline phase. The data also reveal that this lack of order is not dependent on temperature in the liquid phase and persists into the super-cooled region below the normal β melting point.

KEY WORDS: Crystals, layer structures, liquids, neutron diffraction, selective deuteration, triglycerides.

The possibility and existence of order in the liquid state of triglycerides has been discussed on several occasions in the past and certain experimental evidence has been offered by various authors. Measurement of the width of X-ray diffraction reflections led Larsson (1) to propose that, above the melting point, lamellar units reduce in size continuously as the temperature is increased. When the temperature is decreased the lamellar units grow until crystallization finally occurs. Just above the melting point Larsson determined the persistence length in the lamellae to be about 200 Å by using Hosemann's paracrystal concept. Other data exist that support the hypothesis of phase changes in liquid crystalline order in triglyceride melts. A discontinuity in the cooling curve of tristearin at 72°C has been reported (2), and similar discontinuities have been found in the viscosity above the β melting point (3). A ^{13}C nuclear magnetic resonance (NMR) study of liquid tristearin revealed a discontinuity in the spin-spin relaxation time with a shorter T_2 in the "α melt" below 73°C, indicating a more rigid (ordered) structure (4). Norton et al. (5) have observed anomalous crystallization kinetics in triglyceride mixtures, which they attributed to liquid-state de-mixing.

Nevertheless, X-ray diffraction long-spacing data and Raman spectroscopy (6) in the C-C stretching region indicate no evidence for ordering of chains above the melting point. In addition, our own Small Angle Neutron Scattering experiments (7) did not exhibit effects supporting the hypothesis of coherently organized molecules in the liquid form of triglycerides. No reports showing displays of birefringence from long-range structure in the liquid state of

triglyceride are known to the authors. Absence of birefringence does not eliminate the possibility of order, of course; it would, however, suggest an upper limit on the length scale, which is so small that interference with polarized light does not occur. If the structural phase changes, as suggested above, do occur in the "liquid" state they must be weakly energetic, as none of them appear to have been reported as being recorded by calorimetric techniques.

Normally, X-ray diffraction is an excellent method for determining order in liquid and crystalline materials. However, certain difficulties are apparent when X-rays are used to study triglycerides. First, a wavelength of 1.54Å (Cu K$_\alpha$) is the most commonly available in laboratory X-ray equipment (it is possible to achieve slightly longer wavelengths with other targets). A short wavelength is good for observing the high-angle (15° $< 2\theta <$ 30°) reflections from the short spacings, but somewhat lacking when probing the long spacings of 30Å and above. Long spacings produce reflections at low momentum transfer (angles of $2\theta < 5°$), and separation of the diffraction peak from the main beam is difficult. Definition of angular position and shape of peaks is often unreliable. In any case, observation of both angular regions must be performed with different angular resolutions, which renders intensity comparisons between long and short spacing effects subject to questionable reliability. Second, with X-rays the interaction with matter is *via* the electronic structure of the individual atoms. For that reason, the scattering cross-section of an element is related to atomic number. For practical purposes this means that in materials such as lipids the carbon and oxygen atoms dominate the diffraction pattern. Little or no information can be derived on hydrogen positions, because the so-called "form factor" is so small. Third, X-rays are easily absorbed by matter and only small samples can be used before multiple scattering and self-shielding effects begin to deteriorate the quality of information in the scattered beam. Neutron diffraction, on the other hand, offers several distinct advantages over X-rays, which are particularly useful for studying long-chain triglycerides. Neutrons from a reactor source can be selected with wavelengths of any value (with a narrow distribution) up to 10Å or so. The momentum transfer experienced by a waveform during diffraction (or scattering), Q, is given by:

$$Q = 4\pi/\lambda \cdot \sin\theta$$

where λ is the wavelength and 2θ is the angle between incident and scattered beams. The real space dimension, d, observed at such a momentum transfer is:

$$d = 2\pi/Q$$

It follows that, for any given repeat spacing, d, use of radiation of a longer wavelength shifts the angle of observation to higher angles. Hence, by using neutrons with λ = 4.5Å instead of X-rays at λ = 1.54Å, low angle reflections are shifted from about 3°, for example, to 9°. That puts the feature well out of interference from the main beam and allows for the use of a single resolution over the angular region of interest.

Neutrons interact with matter not *via* the electrons but

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via the atomic nucleus. There is no gradual trend in scattering power across the periodic table. On the contrary, elements close together, or isotopes of one element, can have different cross-sections. Hydrogen is an excellent example. Whereas hydrogen and deuterium have identical scattering power for X-rays, they are widely different for neutrons. Some comparisons of scattering power for common elements are shown in Table 1. The feature of large differences in scattering power for the different isotopes of hydrogen can be exploited by tagging specific parts of a protonated molecule with deuterium to enhance diffraction from that particular region.

In addition, there is no form factor for neutrons because the interaction potential is δ-function-like or point-like. For X-rays the form factor itself has an angular dependence because scattering occurs from the extended electronic structure, which has comparable dimension to the X-ray wavelength. The X-ray form factor is therefore different for every element and must be calculated.

Neutron interaction with matter is relatively weak. Therefore, neutrons penetrate deeply into samples before suffering significant attenuation. As a result there is considerably less absorption than with X-rays, which permits the use of relatively large samples in neutron diffraction while still retaining sensitivity to all effects throughout the material under study and not just the surface regions. It also eliminates possible sample size effects when studying order.

It is the objective of this work to ascertain structural effects in liquid and crystalline triglycerides by exploiting features of neutron diffraction that have yet to be used. In particular, we exploit selective deuteration of the triglyceride molecules, thereby changing their appearance. The significance of the difference in selective deuteration of the fatty acid moieties in a triglyceride is shown schematically in Figure 1, wherein comparison is made between the fully protonated and fully deuterated forms.

EXPERIMENTAL PROCEDURES

Materials. Two forms of trilaurin were synthesized. The first form was made from glycerol-H_8 and deuterated lauric acid-D_{23} [$CD_3(CD_2)_{10}COOH$]. A compound is produced with only 5 protons per molecule fixed at the glycerol part. The fatty acid chains were therefore fully deuterated and contain 69 deuterium atoms. This form of trilaurin will be referred to as LLL-HD in the remainder of the paper. The second form of trilaurin was made from fully deuterated glycerol-D_8 and a lauric acid, which was deuterated in the hydrogen positions adjacent to the COO group of the acid [dodecanoic-2, 2-D_2 acid, $CH_3(CH_2)_9$-CD_2COOH]. The resulting molecule is one in which all hydrogen positions in the vicinity of the glycerol were deuterated. The fatty acid chains were protonated beyond the first acyl carbon. This second form of trilaurin will be referred to as LLL-DH. Deuterated chemicals were purchased from Larodan (Malmö, Sweden) and Merck, Sharpe and Dohme (Montreal, Canada). All materials were used as supplied with chemical purities being quoted as better than 98% and isotopic enrichments better than 95%.

Neutron diffraction. The optical principles of diffraction for neutrons are the same as those of X-ray diffraction and will not be further described. The apparatus used for the experiments was the D-16 diffractometer at the Institut

TABLE 1

Neutron and X-Ray Cross-Sections for Coherent Scattering for Some Common Elements

Element	Neutrons[a] $b \cdot 10^{12}$ cm	X-rays[b] $f_x \cdot 10^{12}$ cm
H	−0.374	0.28
D	0.667	0.28
O	0.580	2.25
C	0.665	1.69
Al	0.350	3.65
Si	0.420	3.95

[a]b, Coherent scattering length per atom at a neutron wavelength of 1.08Å. The coherent cross section is given by $4\pi b^2$.

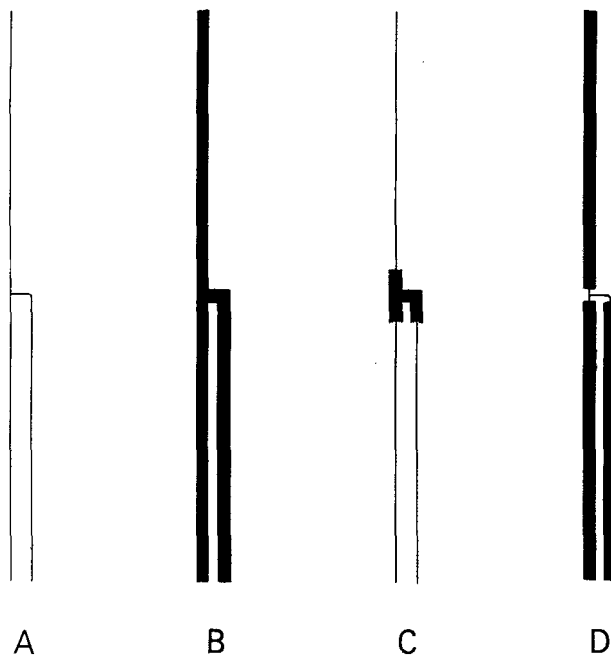[b]The form factor, f_x, for X-rays is given at sin $\Theta = 0$.

FIG. 1. Schematic diagram showing the state of fatty acid and glycerol deuteration in trilaurin. Sketches A and B represent fully protonated and fully deuterated molecules, respectively. Sketch C shows the appearance of deuterated glycerol/protonated fatty acid (referred to as LLL-DH) and sketch D that of protonated glycerol/deuterated fatty acid (referred to as LLL-HD). LLL-HD (which contains 69 deuterium atoms and 5 protons) closely resembles A.

Laue-Langevin (ILL), Grenoble, France (8). D16 is a two-circle diffractometer, which provides a neutron wavelength of $\lambda = 4.5$Å (others are also possible) from a pyrolytic graphite monochromator system. The detector comprises a two-dimensional wire grid system (15.7 cm high × 7.5 cm wide) giving a 64 × 16 matrix. The detector can be placed at various distances from the sample, which it rotates around. The angular range of detection accessible lies between about 3 < 2θ [°] < 120. When placed at 1 m from the sample, this gives a momentum transfer range of about 0.05 < Q [Å^{-1}] < 2. Therefore, d-spacings as large as 125Å can be recorded in the same experiment as those less than 1Å. Such an experimental ability is of par-

132

D.J. CEBULA *ET AL.*

ticular interest to the study of triglycerides, which display two characteristic sets of d-spacings between these extremes.

Control of the diffractometer and data collection was achieved by a Micro-VAX 11 dedicated computer with specially written software. Data reduction was performed on the same computer. For powder diffraction, vertical lines of the detector were straightforwardly summed. A 2θ scan was performed by continuous angular displacement of the detector, and computer software reduced the neutron count rate detected to intensity against angle.

The samples were held in quartz Lindemann tubes of 1 mm diameter. Quartz is essentially transparent to neutrons in comparison to the material of the samples studied here. A beam height of 31.2 mm was used, and neutron collimators defined the beam divergence from the monochromator and at the detector.

Temperature control of the samples was effected by circulating a constant-temperature water/glycol mixture through the sample support in which the Lindemann tube was held. An aluminum foil window was used to surround the Lindemann tube to minimize temperature fluctuation of the sample either by radiation or convection. Thin aluminum is also transparent to neutrons. With this system, mean sample temperatures could be set and controlled to within about ± 0.2°C. Temperature variation along the length of the sample was worse (but not more than about 1°C) at high temperatures (about 60°C, when used). Control was much better at temperatures around 20–30°C.

Experiments performed. The same experiments were performed on each of the two samples. Samples were melted for a few minutes at slightly above 60°C. The temperature was then set at 60°C and a diffraction pattern recorded over a period of approximately 1 hr. The temperature was then reduced to ambient (about 25°C) over several hours to form a solid phase by slow crystallization. A diffraction pattern was then recorded from the solid. The samples were re-melted and then cooled in stages from 60°C. Patterns were recorded at several temperatures, at about 5°C intervals, between 60°C and about 35°C, allowing an hour or so for the diffraction at each. The melting point for the solid in the β phase was ≈45°C, and so, when liquids were cooled below that temperature, the samples were in the supercooled region. The cooling process between each set of temperatures was also slow. From about 35°C down, temperature changes were reduced to ≈1°C until the crystallization point was reached at approximately 32°C. Diffraction patterns were recorded several times at each of the temperatures. After crystallization, the temperature was allowed to fall to ambient. The samples were re-heated slowly from ambient temperature to around 70°C over a period of 18 hr. During this time, diffraction patterns were recorded every 15 min or so, thereby giving diffraction patterns at all temperatures.

RESULTS

The diffraction patterns obtained for LLL-HD in both solid and liquid states are shown in Figure 2. For the low temperature case (ambient temperature of about 25°C) the diffraction obtained is strong in both low and high angle regions. For LLL-HD, the neutron diffraction pattern

resembles the conventional high-angle pattern seen with X-rays. The neutron diffraction pattern has more diffraction lines because, with deuterium, there are more strong scatterers in the unit cell. In the crystal it is known that a layer structure is formed so, in addition, a discreet series of (00l) basal reflections is anticipated, and this is borne out by the data. Reflections from (001) to (004) are clear. The layer spacing from these reflections is about 33Å, which corresponds to a double-layer structure expected for trilaurin. The high-angle reflections are also quite intense and possess the strong diffraction feature at about 4.55Å (2θ approximately 60°), characteristic of the β polymorphic form (9).

For the β phase in triglyceride crystals, the glycerol parts of neighboring molecules reside in a single plane, and fatty acid chains project away from that plane and (broadly speaking) are parallel to one another. For LLL-HD molecules, all the hydrogen atoms of the fatty acid chains are deuterated. Both low and high angle sets of reflections have comparable intensity. As deuterium has such a high cross-section, the diffraction from chain-chain organization, or short-range order, makes a large contribution. Little contrast in scattering is evident in proceeding from one layer to the next parallel to the long spacing.

Consider now the diffraction pattern, shown in Figure 3, of the sample of LLL-DH in the solid state at ambient temperature. Here the glycerol region is deuterated and the remainder protonated, and diffraction is now dominated by the layer organization. A large contrast is evident from layer to layer, owing to the more powerful scattering of the heavily deuterated glycerol and the adjacent fatty acid region relative to the weakly scattering protonated fatty acid chains. Now the scattering power of the molecule is concentrated around the glycerol group. The (00l) reflections due to the layer spacing in crystalline LLL-DH are much more intense than for LLL-HD. The neutron perceives deuterium layers separated by only weakly scattering protonated fatty acid chain regions. A high structure factor for the (00l) series of diffraction lines is apparent for molecules in such an arrangement. The high-angle reflections are virtually absent, even when plotted on the logarithmic scale.

Referring again to Figure 2, the diffraction from the LLL-HD at 61°C is shown. When the solid form of LLL-HD is heated and it melts at about 45°C, the sharp peaks in the diffraction patterns in both the low- and high-angle regions disappear. A broad and much less intense feature is seen for LLL-HD in the high-angle region. The high-angle diffraction resulting is characteristic from pair correlations of atoms in an isotropic liquid with average d-spacings larger than in the solid. Identical patterns to those of the liquid melt at 61°C (shown in Fig. 2) were obtained for LLL-HD when observed in the super-cooled state (below ≈45°C) at 39°C, down to about 33°C just above the crystallization temperature and also just above 45°C on melting of the solid. There is a low-angle feature in the diffraction pattern of the liquid, which is of similar intensity to the high-angle feature. It, too, is broad with a peak position in the region of 15–25Å.

When LLL-DH is melted to 61°C, diffraction at about 60° in 2θ (Fig. 3) is hardly apparent in comparison to that of LLL-HD. This is because of the large cross-section of the deuterated fatty acid chains in the latter case. The broad feature at $2\theta \approx 60°$ indicates general localized order
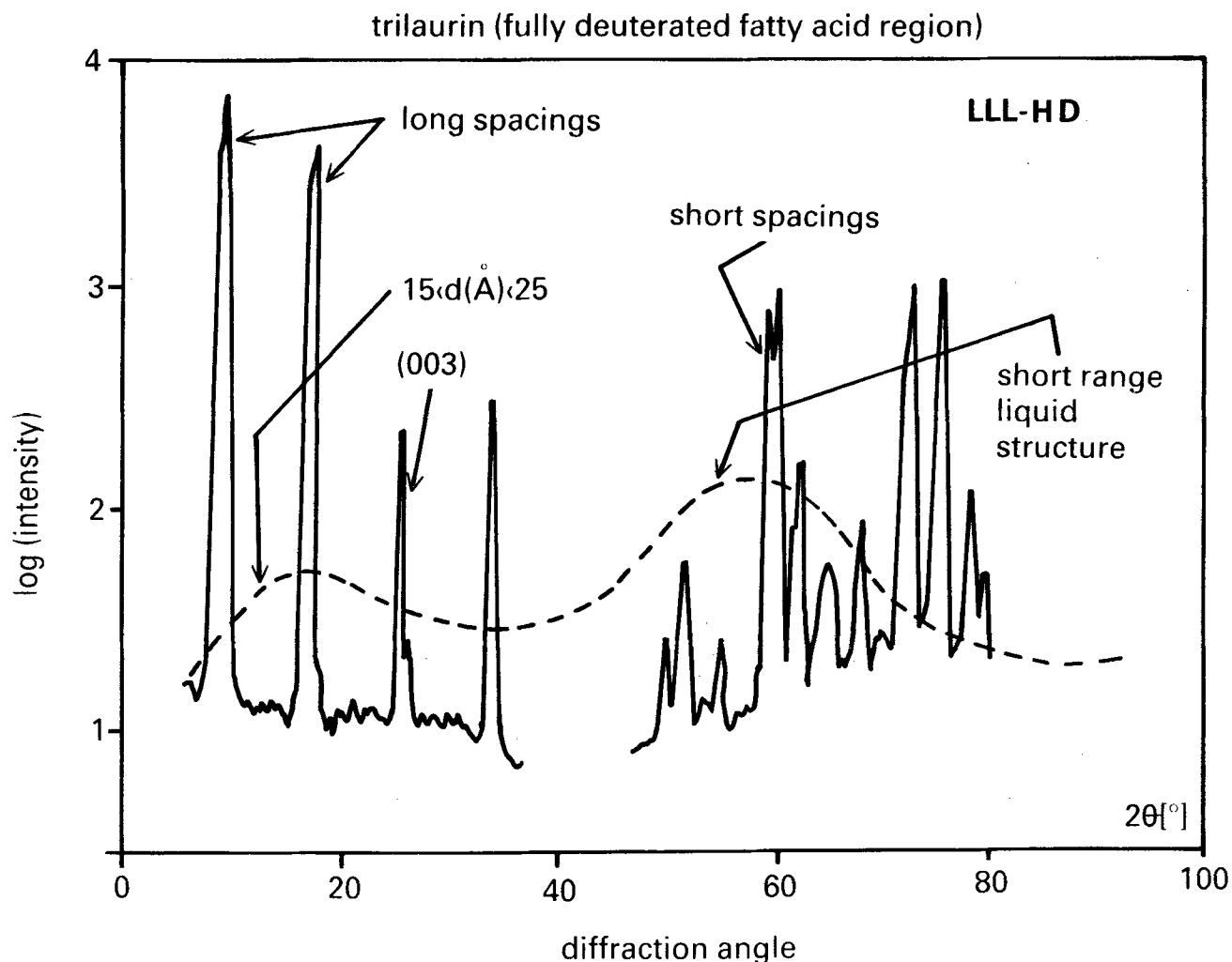
FIG. 2. Neutron diffraction patterns obtained from LLL-HD in the solid state after slow crystallization at room temperature from the melt (full line, sharp reflections), and the molten state at 61°C (broken line, broad feature at about 2θ of 60°). All diffraction patterns obtained from LLL-HD in any of the liquid states were identical.

of the fatty acid chains. The angular position of this broad feature indicates that the average spacing of fatty acid chain-chain correlations is larger in the liquid (reflection at lower diffraction angles) than in the α state. For the α polymorphic form, diffraction shows an intense but broad-winged reflection almost universally at d $\approx$ 4.2Å (2θ in the region of 65° for a wavelength of 4.5Å). This corresponds to a lower density of molecular packing in the liquid as compared to that in the crystalline solid. The position of the broad feature in liquid LLL-HD occurs at about the same position as for the 4.5Å reflection seen in the diffraction pattern of the solid β structures, which is clear in the Figures and is at $2\theta \approx 60°$ for neutrons with $\lambda = 4.5$Å.

The low-angle feature seen for both samples when heated is much more intense for the LLL-DH sample. Its peak position lies between the (001) and (002) reflections obtained for the solid samples. This indicates that the long-spacing structures formed in solid and liquid phases are not like one another. If there was any layer-like structure at all, then glycerol moieties of adjacent molecules

should reside in the same plane parallel to the plane of the layers. Such partition of the glycerol from the fatty acid chains (normally observed in crystalline triglycerides) would produce some form of (00l) series. A layer system might also be expected to show some high-angle (hk) reflections due to glycerol-glycerol organization within the layer. These do not distinguish themselves in any of the data obtained for the liquids.

DISCUSSION

The classical picture of structure proposed for liquid triglycerides is given by Larsson's paracrystal concept (1), in which liquid crystalline smectic structure is postulated. Such a structure cannot be sustained by the neutron diffraction evidence here. If the lamellae postulated by Larsson exist with a well-defined structure, they should produce reflections for both long and short spacings. We can find no evidence for that structure, and any such ordered material must occupy less than 1% of the volume of liquid present. The value is calculated on the basis
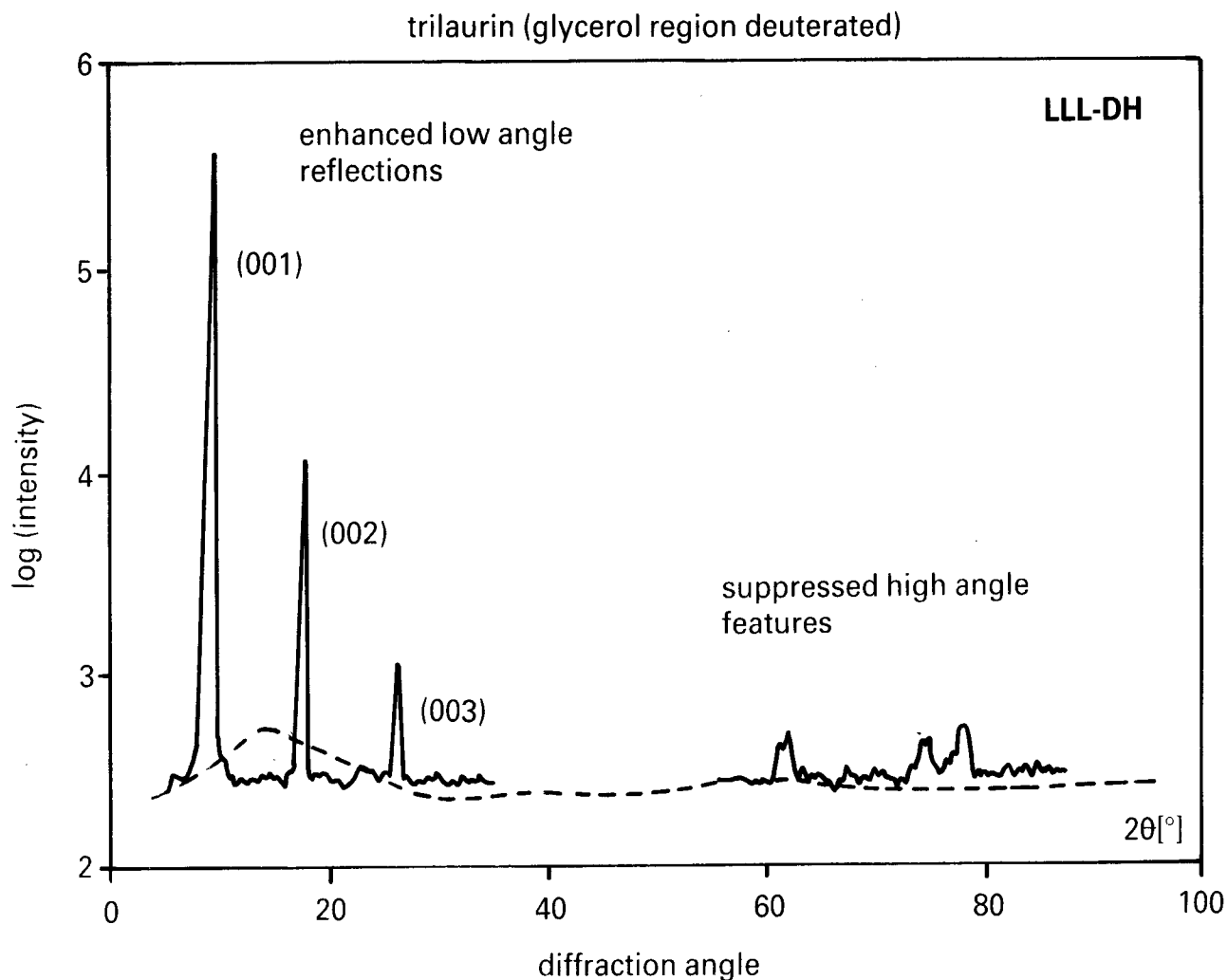
trilaurin (glycerol region deuterated)

FIG. 3. Neutron diffraction patterns obtained from LLL-DH in the solid state after slow crystallization at room temperature from the melt (full line, sharp reflections), and the molten state at 61°C (broken line, broad feature at 2θ of about 14°). All diffraction patterns obtained from LLL-DH in any of the liquid states were identical.

of a comparison of the intensity of the (001) enhanced low-angle reflection from the solid, relative to the baseline, with the peak intensity of the noise level in the signal from the supercooled liquid.

The Hernquist model for the temperature dependence of the size of ordered lamellar regions says that size reduction is progressive as the temperature is raised above the melting point (10). This is suggestive of critical phase behavior whereby "patches" of order develop and grow as the phase change is approached. However, there is no temperature dependence in the neutron diffraction data that were collected at a resolution of no better than ± 0.5°C. The position and size of the liquid features, even with 6°C supercooling (measurement at 39°C), remain unchanged right up to the point of crystallization.

The shift in the low-angle feature in the neutron diffraction from the (001) position to between about 15 and 25Å can be explained only by allowing the glycerol parts of molecules to come much closer to one another than the conventional two-fatty acid layer separation. The high-angle short-range feature is characteristic of organization amongst fatty acid chains, albeit rather poor in comparison to a crystal. Using the parlance of liquid crystal physics (11), we propose that our data obtained for the liquid trilaurin resemble the nematic phase, in which there is some lateral organization of the molecules but absence of discreet layers. The neutron diffraction pattern for the liquid LLL-HD (Fig. 4) is similar to that obtained for other nematic liquid crystals (12). In the nematic model, some molecular registry persists purely because the triglyceride molecules are anisotropic. Correlations are those occurring perpendicular to the axes of the molecules. The reduction in (001) d-spacing could be achieved by the interaction of fatty acid chains from one layer into the adjacent layer. Alternatively or, accompanying an intercalation, a melting of the fatty acid chains could occur. This would cause the deterioration of the high-angle diffraction pattern as observed in comparison to that of the solid. By extending the liquid crystal analogy, the solid α phase in fats (which has strong diffraction reflections from the
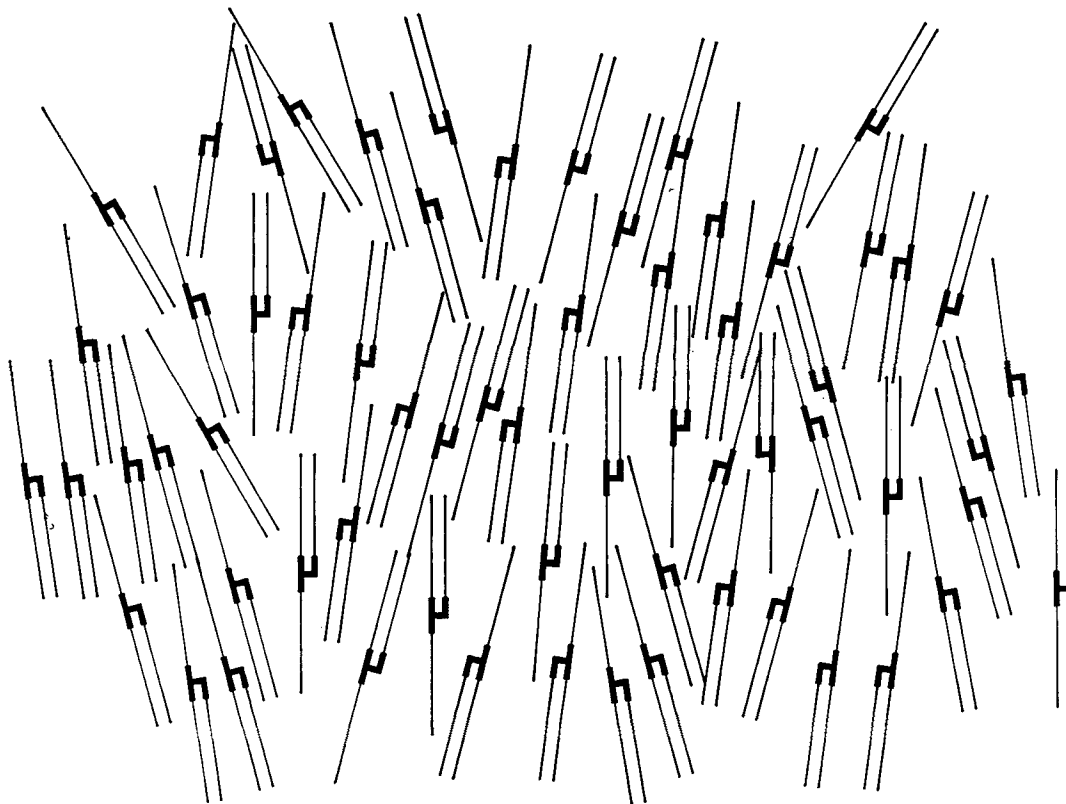
FIG. 4. Schematic diagram of the nematic-like structure of the liquid state for trilaurin in the LLL-DH form. Note the absence of discreet layers. This sketch gives an idea of the spatial arrangement of molecules with respect to one another, rather than emphasizing any internal structure of the trilaurin molecule. Although the fatty acid moieties are depicted as straight lines, that is not to suggest that they are rigid.

layers, but only one strong broad reflection from the poorly organized chain-chain correlation) looks like liquid crystal smectic A (12). Higher polymorphic forms of fats (with more complicated diffraction patterns) resemble the more sophisticated liquid crystal smectic B and C phases, which incorporate molecular tilt. The analogy is limited, it is stressed, to static molecular organization, and no similarity is suggested for macroscopic physical behavior of fats and liquid crystals.

We have performed other experimental measurements to search for a temperature dependence of the liquid state structure. X-ray diffraction was performed across a range of temperatures (36–50°C) for trilaurin (fully protonated). The high-angle diffraction pattern in the angular region of 15–35° did not vary. Abrupt changes only occurred at the crystallization point, indicating a precise phase transition.

The velocity of sound through the liquid trilaurin over the same temperature range was also measured according to the method described by Povey (13). The results showed linearity in the variation of velocity with temperature and without discontinuity, as might be expected at a phase change. The variation in the velocity was 3% at the extremes of temperature, with each measurement accurate to 0.2%. A similar result was obtained for measurements of density by means of the vibrating tube method with a Paar DMA45 densitometer (14). We conclude from these results that no structural changes of a progressive nature

are occurring in trilaurin in the liquid state. At most, a minor density change is evident, reflecting increased molecular energy at the elevated temperatures.

ACKNOWLEDGMENTS

REFERENCES

1. Larsson, K., *Fette. Seifen Anstrichm.* 74:136 (1972).
2. Hoerr, C.V., and H.J. Yarwood, *J. Phys. Chem.* 60:1265 (1956).
3. Phillips, L.W., *Trans. Farad. Soc.* 60:1873 (1964).
4. Callaghan, P.T., and K.W. Jolley, *J. Chem. Phys.* 67:4773 (1977).
5. Norton, I.T., C.D. Lee-Tufnell, S. Ablett and S.M. Bociek, *J. Am. Oil Chem. Soc.* 62:1237 (1985).
6. Lippert, I.L., and W.L. Peticolas, *Proc. Natl. Acad. Sci. USA* 68:1572 (1972).
7. Cebula, D.J., D.J. McClements and M.J.W. Povey, *J. Am. Oil Chem. Soc.* 67:76 (1990).
8. Institut Laue-Langevin, Grenoble, France, *Neutron Research Facilities at the High Flux Reactor, Instrument Manual*, 1983, pp. 32–33.
9. Chapman, D., *The Structure of Lipids*, Methuen & Co. Ltd., London, 1965, p. 266.

10. Hernquist, L., *Fette. Seifen Anstrichm. 84*:297 (1986).
11. Lister, D.J., and Birgeneau, R.J., *Physics Today*, May, 26 (1982).
12. Pynn, R., K. Otnes and T. Riste, *Solid State Communications 11*:1365 (1972).
13. Povey, M.J.W., and D.J. McClements, in *Food Colloids*, edited by R. Bee, P. Richmond and J. Mingins, Royal Society of Chemistry, 1988, pp. 307–322.
14. Kratky, O., H. Leopold, and H. Stabinger, *Calculating Digital Density Meter DMA45*, Anton Paar, Graz, Austria, 1985.