

# Solid Phases of Trimargarin: ✘

## A Comparison to Tristearin

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### ABSTRACT

Data from Raman spectroscopy, X-ray diffraction, infrared spectroscopy and differential scanning calorimetry confirm the existence of four solid state phases for trimargarin. Results of the trimargarin investigation are compared to previous results of tristearin and examined in the light of odd vs even chain-length triglycerides. The conclusion reached is that polymorphic properties of triglycerides result from a balance of three dominant interactions: end-group planar interactions, lateral hydrocarbon chain interactions and glycerol units intermolecular interactions. This contrasts to a general belief that the end-group planar interactions dictate physical behavior of lipids.

### INTRODUCTION

Lipid polymorphism, though still poorly understood at the molecular level, is yielding to improved instrumental analysis and to systematic investigation of the subtle structure-interaction balance that stabilizes discrete solid phases (1).

Larsson related polymorphism to differing packing densities in the methyl end-group planes (2). Different end-group plane conformations arise as a result of inclination of hydrocarbon chain axes with respect to the methyl terminal planes. Stepped angles of chain inclination, first proposed by Knoop and Samhammer (3) and later advanced by Precht (4), are achieved by translation of a hydrocarbon chain by one methylene unit, 1.24 Å, with respect to a neighboring chain.

It remains to be seen whether such end-group changes arise from or cause concomitant differences in overall interplanar interactions. Significant variations in macroscale physical properties, e.g., thermal behavior, volume, and crystal strength, attend such subtle alterations in packing.

Previous work (1) characterized the transition between two intermediate ( $\beta'$ ) phases of tristearin as a minor lateral packing rearrangement of the aliphatic chains. The current study was undertaken to examine closely the intermediate phases of trimargarin and to gain added insight into the disparate thermal behavior of odd vs even chain-length triglycerides.

### MATERIALS AND METHODS

Triheptadecanoin (trimargarin) was purchased as a white crystalline powder from Nu-Chek-Prep, Inc., Elysian, MN and was used without further purification.

Raman spectra were taken with a Spex Ramalab Model RS-2 spectrometer equipped with an FW 130 photomultiplier and a Spectra-Physics 165-08 argon laser. The conditions of spectra procurement have been described earlier (1).

X-ray powder diffraction data were taken using a Warhus camera and sample to film distances of 5 cm for short spacings and 17 cm for long spacings, the latter being calibrated for film shrinkage by preliminary marking of the film. Film spacings were determined on an illuminated film-measuring device.

Infrared (IR) spectra were obtained with a Perkin-Elmer Model 337 grating IR spectrometer. Samples of the phases

of trimargarin were examined as KBr pellets. Spectra were calibrated to the 720  $\text{cm}^{-1}$  band of polyethylene film.

Differential scanning calorimetry data were obtained with a Perkin-Elmer Model DSC-1B. Runs proceeded from room temperature at a heating rate of 5 C/min. Calibration of the instrument was checked against the  $\alpha$  and  $\beta$  melting points of tristearin.

Preparation of specific phases of trimargarin was as follows: a portion of the triglyceride material was packed into the closed end of a Kimax 1.1 mm od capillary tube. The tube was then sealed, and the loaded capillary was placed in a hot water bath for complete melting. Quenching of the capillary immediately in cold water provided the  $\alpha$  phase. Removal of the liquid triglyceride from the hot bath followed by treatment in a heating block at 50.0 C for 20-30 min yielded the  $\beta'_2$  phase. The  $\beta'_1$  phase was obtained by "tempering" the  $\beta'_2$  phase specimen at 60-60.5 C for any period in excess of 60 sec. Attempts were made to obtain the  $\beta$  phase by "tempering" of an alternate phase at 62-65 C for various periods from 5 sec to 5 min, the latter duration resulting in melting of the specimen. The  $\beta$  phase of trimargarin was obtained by recrystallization from chloroform. Hagemann has succeeded in preparing a quantity of the  $\beta$  form by "tempering" material overnight on a DSC (J. Hagemann, private communication). All four phases were stable at room temperature.

### RESULTS

#### Raman Spectroscopy

Raman spectroscopy was the primary means of investigation. This method provided rapid phase identification without sample or phase destruction.

The spectra of the four trimargarin phases were, respectively, identical (within experimental error) to those found for tristearin (1). This work, however, produced a more reliable method for characterizing the four phases of both trimargarin and tristearin. Table I illustrates the comparison of intensity ratios for specific vibrational bands. The phases were found to be independent of one another in the sense that a continuous range of ratio values from one phase to another was not observed. The C-H stretching vibration region of 2800-3100  $\text{cm}^{-1}$  was earlier reported to have no intensity or frequency changes of significance in the study of tristearin (1). Closer examination indicates this observation is in error; the ratio of intensities at 2850  $\text{cm}^{-1}$  to that at 2880  $\text{cm}^{-1}$  does distinguish the triglyceride phases, as do ratios involving the three major vibrational bands in the 1400-1500  $\text{cm}^{-1}$  region. It is conceivable that other more subtle phase changes have been overlooked in the intensity spectrum.

#### X-Ray Diffraction

Figure 1 contrasts the long spacings of the odd-membered trimargarin phases to those for the corresponding phases of nearest-neighbor even-membered triglycerides. Even-membered triglycerides exhibit a linear relationship for

TABLE I

Determination of Triglyceride Phase States by Raman Vibrational Band Ratios

Phase	mp <sup>a</sup> (C)		$R_1 = \frac{I(1420\text{ cm}^{-1})}{I(1438\text{ cm}^{-1})}$	$R_2 = \frac{I(1460\text{ cm}^{-1})}{I(1438\text{ cm}^{-1})}$	$R_3 = \frac{I(2850\text{ cm}^{-1})}{I(2880\text{ cm}^{-1})}$
	trimargarin	tristearin			
$\beta$	65	72	0.10 <sup>b</sup>	0.84	0.45
$\beta'_1$	62	64	0.55	0.58	0.54
$\beta'_2$	60	61	0.40	0.65	0.62
$\alpha$	50	55	— <sup>c</sup>	0.67	0.69

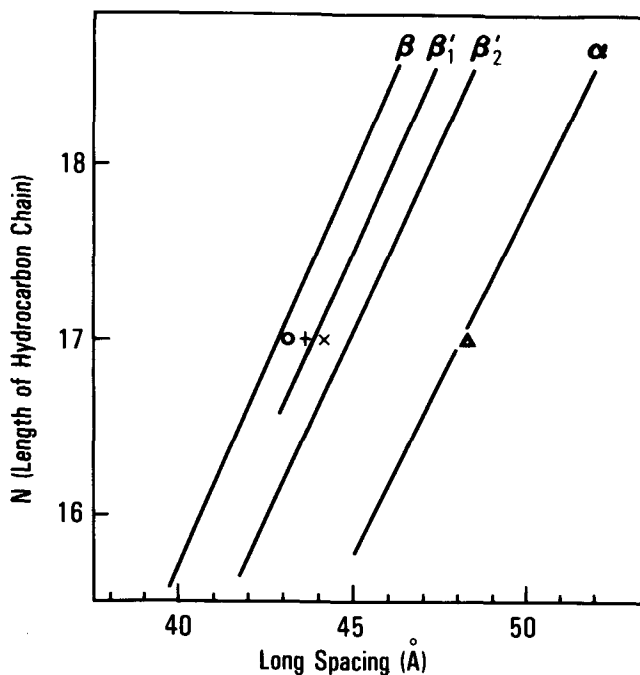
<sup>a</sup>Melting endotherm peak temperatures.<sup>b</sup>Numerator for the  $\beta$  phase is intensity of the 1416  $\text{cm}^{-1}$  band. The intensities measured are the heights of the respective bands from the baseline of the spectrum.<sup>c</sup>The 1420  $\text{cm}^{-1}$  band for the  $\alpha$  phase is not observed.

FIG. 1. Comparison of long spacings for the phases of trimargarin vs respective phase long spacings for even-membered saturated triglycerides. Trimargarin long-spacing values are: 43.2 Å ( $\beta$ ); 43.7 Å ( $\beta'_1$ ); 44.2 Å ( $\beta'_2$ ); and 48.2 Å ( $\alpha$ ).  $\beta$ ,  $\beta'$  and  $\alpha$  long-spacing values of tripalmitin and tristearin are those of ref. 5. The tristearin  $\beta'_1$ , 4.60 Å, is of this work.

X-ray long-spacing vs hydrocarbon chain number,  $C_{12}$  through  $C_{18}$  (5), but only the adjacent chain-length neighbors to trimargarin, tristearin and tripalmitin, are shown here.

Long-spacing measurements were repeated with each of the four phases of trimargarin. The values obtained agree well with Malkin's early work (6), and the 43.7 Å value observed here for  $\beta'_1$  duplicated exactly the  $\beta'$  long spacing found by Malkin. Lutton and Fehl report a value of 43.9 Å for trimargarin  $\beta'$  (7). The method of preparation and  $\beta'$  melting point, 60.1 C, indicate the phase to be  $\beta'_2$ . Their reported long-spacing error evaluation of 0.3 Å permits agreement with the 44.2 Å long spacing for  $\beta'_2$  observed here.

#### Infrared Spectroscopy

Chapman has discussed the means of differentiating the

phases of saturated triglycerides using the 710-730  $\text{cm}^{-1}$   $\text{CH}_2$  rocking mode region of the IR spectrum (5). The hexagonal packed  $\alpha$  phase exhibits only a single band at 720  $\text{cm}^{-1}$ . The  $\beta'$  form provides a doublet at 719 and 727  $\text{cm}^{-1}$  attributable to orthorhombic  $\perp$  packing. The  $\beta$  state has only a single band at 717  $\text{cm}^{-1}$  and the hydrocarbons pack parallel in a triclinic subcell arrangement.

The IR spectra of the four phases of trimargarin in the 720  $\text{cm}^{-1}$  region are distinct from one another. The  $\alpha$  phase spectrum is exactly like that observed for saturated triglycerides (5), whereas the  $\beta$  form spectrum exhibits a single band at 716  $\text{cm}^{-1}$ , within experimental error of that expected (5). More significant are results for the  $\beta'$  forms. The  $\beta'_1$  phase yields a doublet at 719 and 726  $\text{cm}^{-1}$ , again essentially identical to the 719 and 727  $\text{cm}^{-1}$  bands cited by Chapman for the  $\beta'$  phase (5). The  $\beta'_2$  form, confirmed by both Raman spectroscopy and X-ray diffraction, possesses only a single peak at 719  $\text{cm}^{-1}$ .

#### Differential Scanning Calorimetry

The melting points of the four phases of trimargarin were determined by DSC and are presented in Table I. Selection of samples was based on Raman ratios and purity of the phases was confirmed by the thermal analysis. Corresponding phase states of trimargarin and tristearin yield similar shaped thermal heating curves even though phase melting points are lower for the shorter chain-length triglyceride.

#### DISCUSSION

The corresponding phases of trimargarin and tristearin are identical in terms of Raman vibrational band frequencies and intensities. This observation is supported further by the Raman intensity ratios of Table I, which provide means of identifying the saturated triglyceride phases, whether they are tristearin or trimargarin. A conclusion drawn from the striking likeness of vibrational spectra is that the triglyceride molecular lateral packing space, both between chains of a triglyceride and between chains of neighboring molecules, is identical for even and odd hydrocarbon chain species.

X-ray low-angle scattering data provide an additional observation. Comparison of trimargarin long spacings to the even-membered series in Figure 1 confirms the even vs odd physical state dissimilarity. The trimargarin long spacings do not coincide with values interpolated from even-membered triglycerides. A second contrast is also observed. The  $\beta$ ,  $\beta'_1$  and  $\beta'_2$  forms of trimargarin show a much smaller long spacing increment between phases than do those of an even chain length member; 0.5 Å to  $\geq 1.0$  Å. Such small increments in long spacings reveal that the change in the

angle of tilt of the hydrocarbon chain axis is on the order of 1.5 degrees. The calculated values for  $\beta'_2$ ,  $\beta'_1$  and  $\beta$  phases are 66.5, 65.0 and 63.7°, respectively. (The inclination of the chain axis is derived by assuming the unit cell c-axis to equal the  $\alpha$  phase long spacing; the chain axis is considered normal to the unit cell base in the  $\alpha$  phase ( ). The long spacing divided by the c-axis gives the sine of the inclination angle.)

In contrast, Knoop and Samhammer cite inclination angles of 90°, 72.2°, 59.2°, and 50.4° for tristearin phases (3). It would seem unlikely, therefore, that the small change in long-spacing dimension for contiguous phases results from displacement of a hydrocarbon chain by a methylene unit distance, 1.27 Å, with its nearest neighbor chain. This, in turn, implies that the methyl end-group planes cannot be very different between phases. However, to conclude that end-group interactions are not important is a mistake. The changes that do occur between phases in the Raman spectrum occur in regions exhibiting methyl group vibrations.

Complicating the situation further is the physical behavior of supposedly equivalent phases. For tristearin, the  $\beta$  phase is obtainable by appropriate heat treatment as a solid as well as by recrystallization from solution. For trimargarin, heat treatment of the solid state did not produce the  $\beta$  phase. Furthermore, the  $\beta'_1$  form of trimargarin was found to be quite stable at temperatures above the  $\beta'_2$  transition point. This contrasts directly with the tristearin  $\beta'_1$  phase that converts to the  $\beta$  state after 2 min when treated above the  $\beta'_2$  transition temperature.

In conclusion, investigation by Raman spectroscopy suggests that the irregularity observed between odd and even chain triglyceride physical properties arise not predominantly from the interaction of end-group planes, but from a subtle combination of three interactions. These involve the

methyl end-group plane, the lateral packing of the lengthy hydrocarbon chains and the intermolecular interactions of the glycerol units. The first two are best observed in the Raman results, whereas the latter necessitates another means of investigation, perhaps either Fourier transform IR or nuclear magnetic resonance spectroscopy.

The conclusion reached is not unique, in that Mason and Huang (8) propose that the gel phase of saturated symmetric-chain phosphatidylcholine bilayers is dependent upon the bilayer interface region, the conformationally inequivalent terminal ends of the fatty acyl chains, as well as the hydrocarbon chain packing. The present work, therefore, suggests that the behavior of isolated or grouped triglycerides need not be greatly different in host phospholipid membranes.

#### ACKNOWLEDGMENT

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