

**$^{13}\text{C}$  NMR RELAXATION OF TRILAUROIN AND ITS CYCLOPENTANOID ANALOGS**

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**1. Introduction**

This laboratory has embarked on a program of synthesis of cyclopentanoid analogs of glycerides [1] in an attempt to assess the relationship between molecular conformation and the behavior of triglycerides in biological and model systems, with respect to physiological properties and enzyme-catalyzed reactions. These new compounds are formal analogs of different rotational states of the natural glycerides. The backbone of each series is one of the three isomeric cyclopentane-1,2,3-triols (fig.1). These analogs incorporate restricted rotational freedom about the carbon-carbon bonds of the backbone, with flexibility within these rotameric restrictions, and may permit the evaluation of the effect of the rotameric state on various properties of the lipids. The polymorphic behavior of these analogs has been studied [2]. In this report we extend the studies to a comparison of the molecular motions of the natural glycerides and their cyclopentanoid analogs.

$^{13}\text{C}$  Spin-lattice relaxation times ( $T_1$ ) which are inversely related to the correlation time ( $\tau$ ) for

molecular motion, are potentially of considerable value in studying these systems, since the measurements are sensitive to molecular motion on the time scale of interest and involve no chemical modification of the lipid. For dipalmitoyl lecithin,  $T_1$  values have been reported for 6 of the 16  $^{13}\text{C}$ -resonances of the fatty acid chain. The  $T_1$  values of the carbon nuclei increase from the carboxyl end towards the terminal methyl of the fatty acid chains [3,4] and these values can be explained simply in terms of increasing motion about C—C bonds in the alkyl chains [5,6]. In other cases where the rate of overall rotation is comparable to internal motion, nearly equivalent  $T_1$  values are obtained for each carbon atom in the molecule [7].  $^{13}\text{C}$  spectra and  $T_1$  values for phospholipids have been extensively studied but neutral lipids have received very little attention until recently [8,9]. We report here the  $T_1$  values for trilauroin and its cyclopentanoid analogs. These results suggest that the glyceride backbone of triglycerides possesses restricted rotational freedom.

**2. Materials and methods**

Trilauroin was obtained from NuChek Inc. The cyclopentanoid analogs of trilauroin [1] were kindly supplied by Dr S. M. Greenwald. Pulsed Fourier transform  $^{13}\text{C}$  NMR spectra were recorded on a Varian XL-100-15 spectrometer with a 16K 620/L computer, locked on solvent deuterium ( $\text{CDCl}_3$ ), with proton noise decoupling. Excitation pulse power,  $16\ \mu\text{s}$   $90^\circ$  pulse, was large enough to uniformly irradiate the spectral window of 5000 Hz [10,11].  $T_1$  was measured at  $30^\circ$  by the fast inversion recovery sequence (FIRFT) [11], the  $T_1$  values being obtained

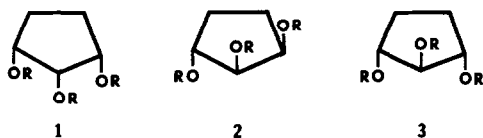


Fig.1. Analogs based on the three diastereoisomeric cyclopentane-1,2,3-triols. The tris lauroyl derivatives **1**, **2** and **3** ( $\text{R} = -\text{C}(=\text{O})(\text{CH}_2)_{10}\text{CH}_3$ ) are referred to throughout the text as the (1,2,3/0), DL (1,2/3) and (1,3/2) analogs respectively.

by a least squares fitting of a plot of  $\ln(S_\infty - S_\tau)$  versus  $\tau$ , where  $S_\infty$  is the signal intensity at equilibrium and  $S_\tau$  is the signal intensity at time  $\tau$ . The slope of this plot is negative  $1/T_1$  [10]. Sets of 9–14  $\tau$  values were used for the  $T_1$  determination; correlation coefficients were greater than 0.99 except for three cases in which values of 0.97 or 0.98 were obtained. Chemical shift assignments were based on those reported for methyl laurate [8] and the isomeric cyclopentanetriols [12]. We have assumed that  $T_1$  for a protonated carbon atom is dominated by dipole–dipole interaction with the attached proton(s), and that  $T_1$  is directly related to the inverse of the effective correlation time and the number of directly attached hydrogen atoms,  $N$ . The product,  $NT_1$ , is indicative of the motion of an individual carbon atom [13].

### 3. Results and discussion

The values of  $T_1$  for trilauroin are given in table 1.

The resonances of C-3 to C-12 in the outer and inner fatty acyl chains are unresolved, and the values given for these are the weighted averages for the carbon atoms in all three chains. Moreover, among these resonances there was coincidence of the resonances of C-5 with C-9 and of C-7 with C-8; thus these  $T_1$  values are each averages for 6 carbon atoms. The gradation of  $T_1$  values found for the acyl carbons of trilauroin is similar to that observed for decanol [13]. In that case,  $T_1$  values varied from 0.65 s for C-1 to 3.1 s for C-10. In the case of decanol, it has been suggested that overall reorientation is comparable to internal motion and the observed  $T_1$  values reflect both processes. The results for trilauroin lead to the following conclusions:

- (1) The larger  $NT_1$  value for the carbon atoms of the  $\text{CH}_2\text{O}$ -groups, i.e.,  $T_1$  times 2, than for the 2-methine group and the larger  $NT_1$  value for the C-2 carbons of the 1,3-acyl chains than for the C-2 carbon of the 2-acyl chain, indicates that the 1,3-chains are more mobile than the 2-acyl chain.

Table 1  
Relaxation times for trilauroin and cyclopentanoid analogs in  $\text{CDCl}_3$  (0.5 M) at 30°C

Carbon	Trilauroin	$T_1$ (s) <sup>a</sup>		
		1,3/2	1,2,3/0	1,2/3
Backbone—C-1	0.33	0.55	0.49	0.73
C-2	0.47	0.34	0.45	0.54
C-3	0.33	0.55	0.49	0.95
C-4		0.48	0.45	0.49 <sup>b</sup>
C-5		0.48	0.45	0.47 <sup>b</sup>
Chain—C-2—(outer chains)—	0.73	0.59	0.59	0.67
C-2—(middle chain)—	0.62	0.56	0.56	0.61
C-3	0.86	0.69	0.87	0.86
C-4	0.96	0.88	0.89	0.94
C-5,9 <sup>c</sup>	1.39	1.08	1.35	1.25
C-6	1.28	1.05	0.95	1.10
C7,8 <sup>d</sup>	1.40	1.28	1.46	1.44
C-10	2.78	2.17	2.68	2.51
C-11	3.18	3.26	3.21	3.33
C-12	3.76	3.42	3.42	3.64

<sup>a</sup> Estimated error in  $T_1$  values  $\pm 10\%$ . To compensate for the different numbers of directly bonded protons, the experimental  $T_1$  values can be multiplied by the following factors:  $\text{CH}_3$ ,  $T_1 \times 3$ ;  $\text{CH}_2$ ,  $T_1 \times 2$ ;  $\text{CH}$ ,  $T_1 \times 1$ ; i.e., to give  $NT_1$

<sup>b</sup> The relative assignment of C-4 and C-5 for this isomer is uncertain

<sup>c</sup> Average value of C-5 and C-9

<sup>d</sup> Average value of C-7 and C-8

- (2) Mobility along the chains fits the pattern observed for phospholipids [5] in which there are only small increases in mobility along the major part of the chain, with a pronounced increase in mobility near the terminal methyl group.

Since the structural difference between trilauroin and the cyclopentanoid analogs is just two additional methylene groups in the analogs and examination of molecular models indicate that these molecules have substantially the same shape, it is assumed here that the rotational motion of these molecules is similar and the differences in  $T_1$  values between the carbon atoms of these molecules are mainly due to differences in segmental motion. Also, the gross molecular size and the rotational correlation times of the cyclopentanoid analogs are probably similar to each other since the chains are not greatly restricted regardless of the configurational differences of the rings. Molecular models show that *trans*-vicinal and *cis*-vicinal fatty acyl chains can approach each other about equally well, depending, in large measure, on the rotational angles about the (ring carbon)-(ester oxygen) bond and the  $C_2-C_3$  bonds of the chains. These expectations are borne out since the  $T_1$  values for C-3 to C-12 of the fatty acyl chains of trilauroin and the cyclopentanoid analogs are similar and the major relaxation differences among these molecules are found in the carbon atoms of the backbone (table 1).

### 3.1. (1,3/2) Isomer

The motion of the ring carbon atoms of this isomer is not isotropic, since  $NT_1$ , for the ring  $CH_2$ -groups, C-4 and C-5, i.e.,  $T_1$  times 2, is more than double that of C-2 and significantly larger than  $NT_1$  values of C-1 and C-3 (table 1). This could be explained if pseudorotation in the cyclopentane ring were biased toward keeping the movement of the substituted carbon atoms at a minimum, i.e., the pseudorotational twisting motion occurs largely at C-4 and C-5 and to a lesser degree at C-1 and C-3. In this model, very little twisting motion would occur at C-2. It is interesting, in this regard, that the C-4 and C-5

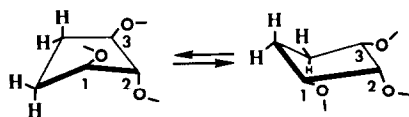


Fig. 2.

methylene groups of this isomer exhibit more motional freedom than C-1 and C-3 of the glycerol portion of trilauroin, even though the motion of the C-4 and C-5 methylene groups is restricted by the constraints of the cyclopentane ring.

### 3.2. (1,2,3/0) Isomer

For this isomer the values of  $NT_1$  are similar to those of the 1,3/2 isomer and trilauroin. Within the ring, the  $NT_1$  values for C-1, C-2 and C-3 are more similar. This may indicate that the pseudorotational twisting motion at C-2 is not quite as restricted in this isomer as in the 1,3/2 isomer.

### 3.3. (1,2/3) Isomer

In this isomer there is a dramatic increase in the mobility of the substituted carbon atoms. The  $NT_1$  values for C-3 and C-1 are nearly the same as C-4 and C-5, indicating that pseudorotation around the ring is almost completely free, although, in this case also, the C-2 carbon is the least mobile. A *cis*-vicinal interaction between the chains at C-1 and C-2 may be the basis for the  $NT_1$  value at C-1 being smaller than that for C-3.

## 4. Discussion

The  $NT_1$  values for the C-4 and C-5 methylene groups of the three isomers are considerably greater than those for the oxymethylene groups of trilauroin, while the  $NT_1$  values for C-2 are comparable. Since the pseudorotational motion of the cyclopentane ring is probably the major contributor to the segmental motion of these carbon atoms and only approximately a  $60^\circ$  dihedral angle is swept out by the methylene hydrogens during a pseudorotational cycle, this suggests that the rotational motion about the C-C bonds of the glycerol moiety of triglycerides may be largely restricted to small angular displacements. Almost certainly, full ( $360^\circ$ ) rotation about these bonds does not occur readily. Possibly the sheer weight of the fatty acyl chains restricts the motion of the carbons to which they are attached (ponderal effect) or some type of interaction between the chains of trilauroin may be involved. Hydrophobic interactions between the chains of dipalmitoyl lecithin in  $CDCl_3$  apparently accounts for the large fractional population of gauche rotamers [14]. This question

may be investigated by measuring the mobility of the glycerol carbons of short chain triglycerides, in which these effects may be lessened. Such a project is planned.

The similarity in rotational mobility of the backbone between triolein and the three analogs suggests that these analogs may be sufficiently similar to natural glycerides to be either substrates or inhibitors of the enzymes of glyceride metabolism. In this regard, Greenwald et al. have found that cholesterol esterase hydrolyzes the cyclopentanoid analogs at rates comparable to the natural substrates [15].

### Acknowledgements

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