

PMR SPECTRA OF TRIGLYCERIDES: DISCRIMINATION OF  
ISOMERS WITH THE AID OF A CHEMICAL SHIFT REAGENT

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Investigation of triglyceride structure requires tedious enzymatic hydrolysis and fatty acid analysis (1). PMR spectroscopy has been of little utility in these investigations due to the relatively featureless spectra displayed (2). The advent of chemical shift reagents (3) such as  $\text{Eu}(\text{fod})_3$ , i.e., tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium (III) has permitted the "resolution" of overlapped multiplets corresponding to protons along the chain in relatively simple esters and other materials (4).

In this study the three triglycerides glycerol 1,2,3-tripalmitate (PPP), glycerol 1,3-dipalmitate 2-oleate (POP), and *rac*-glycerol 1,2-dipalmitate 3-oleate (PPO) were examined by PMR (60 MHz) in the presence of  $\text{Eu}(\text{fod})_3$  shift reagent (Figure 1). There are two distinct triplets in the spectrum of PPP at 8.5  $\delta$  ( $J = 7.5$  Hz, 2 protons) and 9.0  $\delta$  ( $J = 7.5$  Hz, 4 protons), corresponding to the  $\alpha$ -methylene protons of the fatty acid moieties. We have assigned the high field triplet to the  $\alpha$ -methylene protons of the 2-position fatty acid chain, while the low field triplet is assigned to the  $\alpha$ -methylene protons of the 1- and 3-position fatty acid chains. The total  $\beta$ - and  $\gamma$ -methylene proton resonances of all three chains are observed as symmetrical multiplets at 6.6  $\delta$  and 3.8  $\delta$ , respectively, with relative areas of 6 protons each. The 1- and 3-position glyceryl proton resonances are displayed as two strongly coupled AB doublets at 13.5  $\delta$  and 16.3  $\delta$  (geminal coupling,  $J = 12$  Hz, 2 protons each). The separation of the geminal proton resonances is extremely large (168 Hz) but reasonable since we have seen a slight separation (13.5 Hz) of the AB doublets in the PMR spectra of triglycerides in the absence of chemical shift reagents. Spectra of triglycerides ordinarily display two overlapped quartets corresponding to the 1- and 3-position glyceryl proton resonances (2,5). Upon the addition of small quantities of  $\text{Eu}(\text{fod})_3$ , relative to the

amount of lipid present, two sets of quartets of almost equal intensity were resolved. The geminal and vicinal coupling constants were 12 and 6 Hz respectively. Upon addition of larger relative quantities of  $\text{Eu}(\text{fod})_3$ , significant broadening occurred which presumably obscured the observation of vicinal coupling. In Figure 1 the width at half height of each of the AB apparent doublets is approximately 20 Hz. Thus it appears that  $\text{Eu}(\text{fod})_3$  magnifies the difference between the environments of the geminal protons. The 2-position glyceryl hydrogen resonance is seen as a broad singlet at  $20.2 \delta$  (1 proton).

The spectrum of POP is essentially the same as that for PPP except for the occurrence of the resonance of the double bond hydrogens at  $5.4 \delta$  (2 protons). Again the two triplets representing the  $\alpha$ -methylene protons and the two AB doublets representing the 1- and 3-glyceryl protons are evident.

Unlike PPP and POP, PPO exhibits a degree of dissymmetry. Although this dissymmetry is not apparent in the normal proton spectrum of PPO, it does become evident under the influence of the shift reagent. Thus, there are three different resonances (2 protons each) corresponding to the six  $\alpha$ -methylene protons of the fatty acid chains. At  $8.84 \delta$  there is a quartet resulting from two overlapping triplets at  $8.80 \delta$  and  $8.88 \delta$  ( $J = 7.5 \text{ Hz}$ , 4 protons). These correspond to the 1- and 3-chain  $\alpha$ -methylene protons. Appropriate deuterium substitution experiments have indicated that the low field triplet in PPO corresponds to the  $\alpha$ -methylene protons of the oleate moiety. The 2-chain  $\alpha$ -methylene proton resonances are displayed at  $8.4 \delta$  as a triplet ( $J = 7.5 \text{ Hz}$ , 2 protons). It appears that the 1- and 3-glyceryl protons are affected by the unsymmetrical environment as well. Specifically, peaks associated with the 1- and 3-glyceryl protons are represented as two broad singlets at  $13.5 \delta$  and  $16.3 \delta$ , rather than two AB doublets as seen in the spectra of PPP and POP.

In like manner, rac-glycerol 1,2-dipalmitate 3-linoleate (PPL), rac-glycerol 1,2-dioleate 3-palmitate (OOP) and rac-glycerol 1-stearate 2-palmitate 3-oleate (SPO) exhibit three distinct  $\alpha$ -methylene group triplets and broad singlets for the 1- and 3-glyceryl hydrogens. However, the totally saturated lipid rac-glycerol 1,2-dipalmitate 3-stearate (PPS) exhibited a spectrum essentially identical with that of PPP.

Although discussion of the nature of the interaction between the lipid and the shift reagent and the spatial arrangement of the interacting species in solution would be highly conjectural at this point, there is no doubt that there are differences in the interactions for the dissymmetric compared to the symmetric lipids in the partially saturated species. Thus, with the aid of a simple fatty acid analysis and a PMR spectrum, a structural analysis can be performed on any of the aforementioned triglycerides. The different environments of the chain are most apparent in the  $\alpha$ -methylene hydrogen triplet resonances, presumably because of close proximity of these protons to the region of interaction with  $\text{Eu}(\text{fod})_3$ . It should be emphasized that the spectral differences of the dissymmetric compared to the symmetric lipids cited above are not due to the slight differences in the chain lengths of the fatty acid moieties. This is evident from the spectrum of PPS.

The long range effect of the 9,10 double bond on the  $\alpha$ -methylene protons, in the presence of the shift reagent, is quite startling in light of the fact that the double bond resonance position is unaffected by  $\text{Eu}(\text{fod})_3$ . Apparently, for the aforementioned compounds in the presence of the shift reagent, three types of  $\alpha$ -methylene proton resonances are distinguishable only when the molecules are dissymmetric and one of the fatty acid chains on the primary position of glycerol is saturated and the other is unsaturated.

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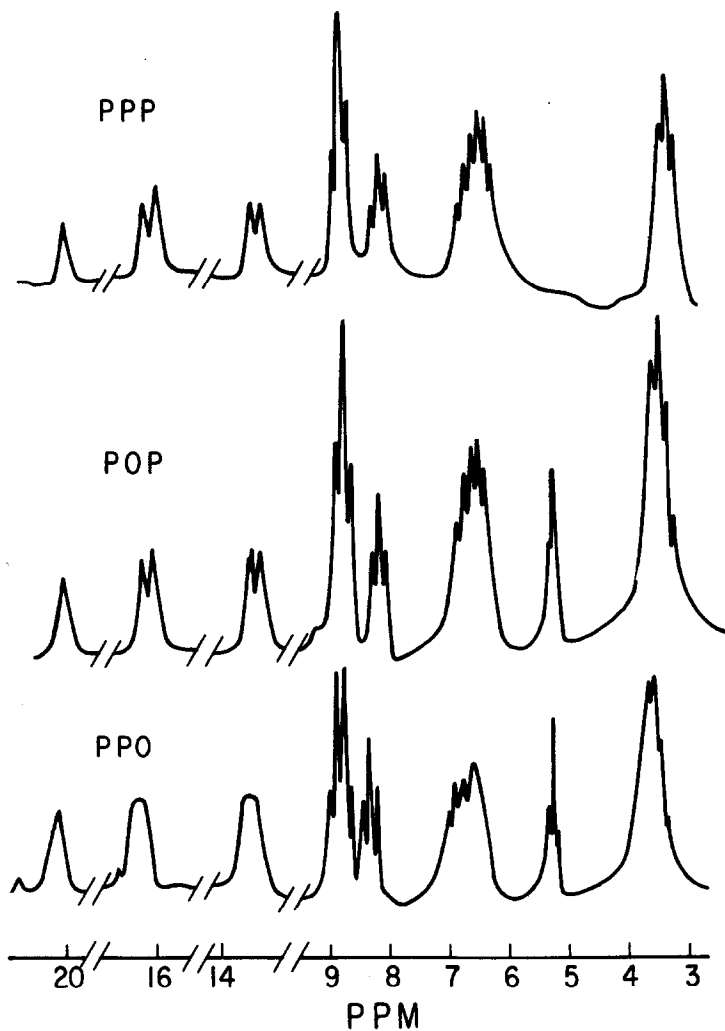


Figure 1.  $\text{Eu}(\text{fod})_3$ -induced shifts. Each sample contains  $2.85 \times 10^{-5}$  moles of triglyceride and  $6.62 \times 10^{-5}$  moles of  $\text{Eu}(\text{fod})_3$  in  $360 \mu\text{l}$  of  $\text{CCl}_4$ . All shifts are relative to TMS as internal standard.