# CONFORMATION OF GLYCEROL MOIETY OF DIPALMITOYL LECITHIN IN $CDCl_3$ SOLUTION BY $^1H$ AND $^{13}C$ NMR

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ABSTRACT

The 360 MHz $^1H$ spectrum and 67·8 MHz $^{13}C$ spectrum of dipalmitoyl lecithin have been analysed by computer simulation to obtain the various $^1H$–$^1H$ and $^1H$–$^{13}C$ coupling constants in the glycerol moiety. The results indicate a dynamic equilibrium between several conformations in the glycerol group. Population analysis using the $^1H$–$^1H$ coupling constants has been carried out.

LIPIDS which form an important constituent of biological membranes have evaded investigation of three-dimensional structure by their peculiar physico-chemical properties. They are amphipathic and are not easily crystallizable. Till today there have been only three crystal structure reports; two on triglycerides: $\beta$-trilaurin[1] and $\beta$-tricaprin[2] and one on the phospholipid 1, 2-DL-dilauroyl phosphatidyl ethanolamine[3]. Nuclear magnetic resonance (NMR), which in conjunction with theoretical studies gives very valuable results, is thus the main source of information for the structure of lipids. There have been several sttempts, using this approach, to study the conformation of phospholipids and triglycerides[4–9] Birdsall *et al.*[4] have assigned the various resonances in the 220 MHz $^1H$ and 25·2 MHz $^{13}C$ spectra of dipalmitoyl lecithin in $CDCl_3$ and $CD_3OD$ solutions. From the analysis of these spectra they have come to the conclusion that the two protons in the $CH_2OP$ portion of the glycerol part are accidentally equivalent. Very recently Hauser *et al.*[10] have studied the conformation of lysophosphatidyl choline in $D_2O$ at 360 MHz and they conclude that the two protons in the glycerol $CH_2OP$ fragment are nonequivalent. In this paper we report 360 MHz $^1H$ and 67·89 MHz $^{13}C$ NMR studies on dipalmitoyl lecithin (DPL) in $CDCl_3$ solutions. Our results are significantly different from those of Birdsall *et al.*, in the sense that we find the two protons in glycerol $CH_2OP$ fragment to be nonequivalent, the chemical shift difference being 0·044 ppm. Further, the $^{13}C$ NMR has given us information about the conformational preferences in the glycerol ester (C – O – COR) groups.

Fig. 1 shows the expanded portion of the 360 MHz $^1H$ spectrum corresponding to the glycerol ester portion of DPL. The spectrum has been analysed using LACOON and the simulated spectrum with the best fit values of chemical shifts and coupling constants is shown in Fig. 2. Fig. 3 shows the $^1H$ coupled $^{13}C$ spectrum of the carbonyl group of the glycerol ester fragment. The previously reported[9] $^1H$ coupled $^{13}C$ O group spectrum of glycerol trivalerate has helped considerably in the analysis of this spectrum. The theoretically simulated spectrum is also shown. All the NMR parameters obtained from this analysis are listed in Table I.

TABLE I

*Chemical shifts* and coupling constants‡ in the glycerol moiety*

Chemical shifts (in ppm):

$(H_2) = 5·15$, $(H'_3) = 4·35$, $(H_3) = 4·073$

$(H_1) = 3·913$ or $3·869$, $(H_1') = 3·869$ or $3·913$.

Coupling Constants:

$^2J\,(H_3–H_3') = -12·01$; $^3J\,(H_3'–H_2) = 2·78$

$^3J\,(H_3–H_2) = 7·21$; $^3J\,(H_3–H_1) = 5·2$ or $6·6$

$^3J\,(H_2–H_1') = 6·6$ or $5·2$; $^3J\,(H_1–H_1') = -11·5$

$^3J\,(H_1–^{31}P) = 7·3$ or $6·8$; $^3J\,(H_1–^{31}P) = 6·8$ or $7·3$

$^3J\,(H_2–^{13}C) = 3·4$

$^2J\,(^{13}C–H) = -6·9$, $^3J\,(^{13}C–H) = 3·5$ } in the $\beta$ chain

$^3J\,(H_3–^{13}C) = {}^3J\,(H_3–^{13}C) = 2·79$, $^2J\,(^{13}C–H) = -7·18$ and $-7·16$ } in the $\gamma$ chain.

$^3J\,(^{13}C–H) = 4·31$ and $4·32$

* With respect to tetramethyl silane (TMS).

‡ The coupling constants are accurate within ± 0·1–0·2 Hz.

*Conformation of glycerol moiety* :

The conformation of the glycerol moiety is determined by two dihedral angles $\theta_1$ and $\theta_3$ (See reference 5 and Fig. 1 for rotations). The Newman projection diagrams of the three staggered conformations with respect to $\theta_1$ and $\theta_3$ are shown in Fig. 4. The vicinal $^1H$–$^1H$ coupling constants for such configurations, calculated from electronegativity rules[11] are also given in the figure. It is clear that the observed coupling constants in the glycerol moiety do not correspond to any of these values. This implies that the glycerol moiety has a dynamic structure involving more than one conformers, and the observed coupling constants

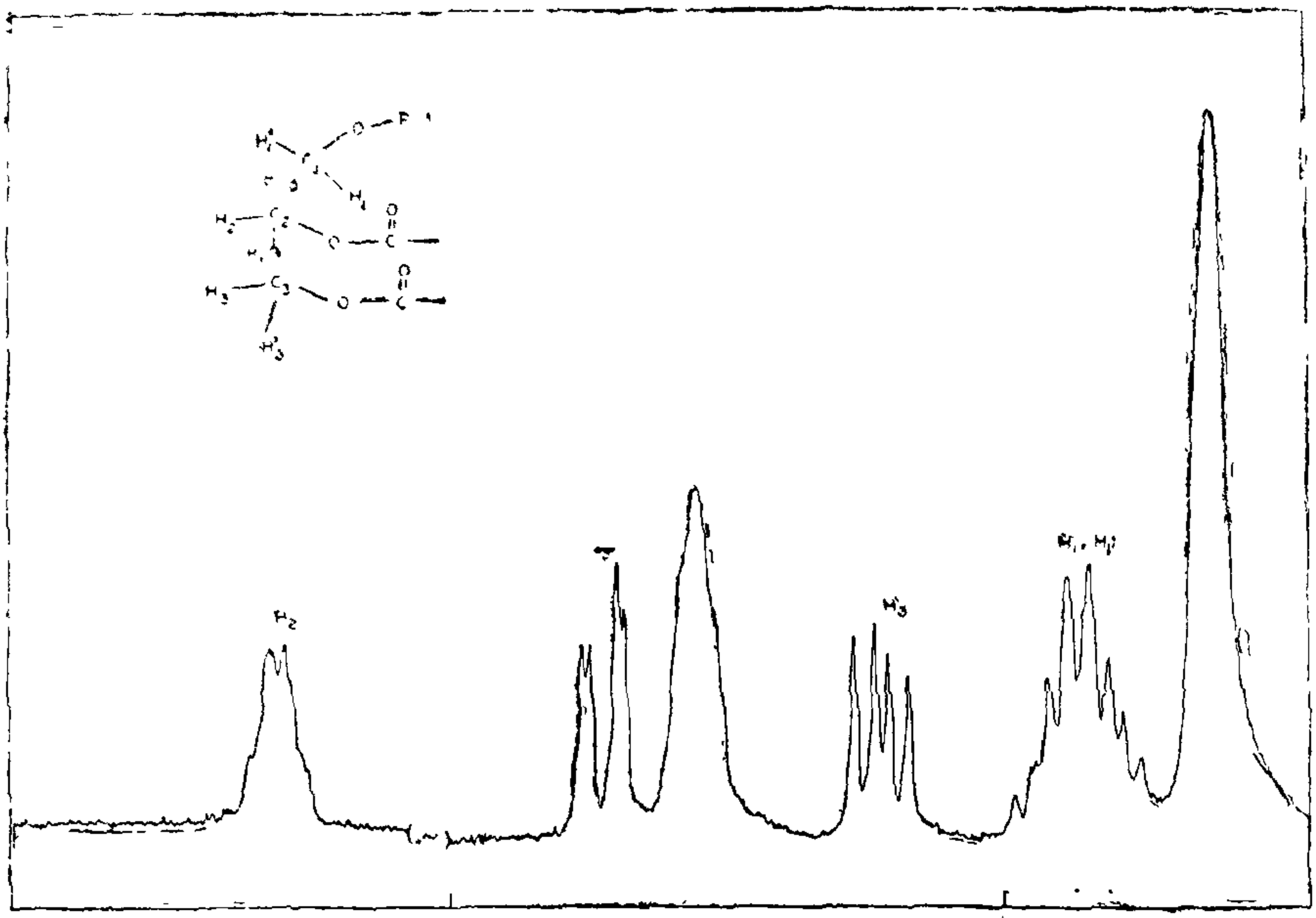



FIG. 1. 360 MHz $^1H$ FT-NMR spectra of dipalmitoyl lecithin (DPL). DPL was obtained from Sigma Co. Inset is the numbering of the protons on the glycerol fragment (Temperature 20° C).

are time averaged values. One can calculate the populations of the three staggered configurations by solving the equations

$$J_{obs} = J_{g-} P_{g-} + J_t P_t + J_{g+} P_{g+}$$
$$J'_{obs} = J'_{g-} P_{g-} + J'_t P_t + J'_{g+} P_{g+}$$
$$1 = P_{g-} + P_t + P_{g+}$$

where $P_{g+}$, $P_t$, $P_{g-}$ are the populations of the three configurations and $(J_{g-}, J'_{g-})$, $(J_t, J'_t)$ and $(J_{g+}, J'_{g+})$ are the corresponding component coupling constants. It may be mentioned that the results one obtains depends upon the assignment of the protons $H_1$, $H'_1$ or $H'_3$, $H_3$ as the case may be. For $\theta_1$, if $J_{obs} = 6{\cdot}6$ and $J'_{obs} = 5{\cdot}2$, one obtains $P_{g-} : P_t : P_{g+} :: 31 : 41 : 28$. On the other hand if $J = 5{\cdot}2$ and $J'_{obs} = 6{\cdot}6$, one gets $P_{g-} : P_t : P_{g+} :: 37 : 20 : 43$. However, it is clear that in either case, all the three staggered configurations are substantially populated. In case of $\theta_3$, if $J_{obs} = 2{\cdot}8$ and $J'_{obs} = 7{\cdot}2$ one obtains $P_t \sim 0$ and $P_{g-} : P_{g+} :: 50 : 50$. The alternative assignment, *i.e.*, $J_{obs} = 7{\cdot}2$ and $J'_{obs} = 2{\cdot}8$ gives $P_{g-} \sim 0$, and $P_t : P_{g+} :: 60 : 40$. Thus, only two conformations with respect of $\theta_3$ coexist in $CDCl_3$ solutions. Similar conclusions have been obtained in our previous study on glyceryl trivalerate[6].

*Conformations in the Glycerol-Ester Fragment*

The relative orientations and the conformations in the $\alpha$, $\beta$ and $\gamma$ chains are determined by the dihedral angles $\alpha_1$ to $\alpha_6$, $\beta_1$, to $\beta_n$ and $\gamma_1$ to $\gamma_m$. Information has been obtained about the dihedral angles $\alpha_1$, $\beta_1$, $\gamma_1$ (Fig. 4) from the present coupling constant data. Population analysis using $^1H$–$^{31}P$ coupling constants gives two results corresponding to two assignments of the protons $H_1$ and $H'_1$. In this case the component coupling constants for the three staggered conformations are obtained using the relation[12].

$$J = 16{\cdot}3 \cos^2\phi - 4{\cdot}6 \cos\phi.$$

This gives $J = J' = 1{\cdot}8$ if $\alpha_1$ has '$t$' conformation, $J = 1{\cdot}8$, $J' = 21{\cdot}0$, if $\alpha_1$ has '$g^-$' conformation and $J = 21{\cdot}0$, $J' = 1{\cdot}8$ if $\alpha_1$ has '$g^+$' conformation. With these component coupling constants, if one assigns $J_{obs} = 6{\cdot}8$ and $J'_{obs} = 7{\cdot}3$, then one obtains the conformer populations as $t : g^- : g^+ :: 45 : 29 : 26$. With the alternative assignment one obtains the conformer populations as $t : g^- : g^+ :: 45 : 26 : 29$. Thus it is clear that all the three staggered conformations with respect to $\alpha_1$ are substantially populated. We differ from Hauser *et al.* in this conclusion. These authors conclude that the polar group has an exclusively '$t$'

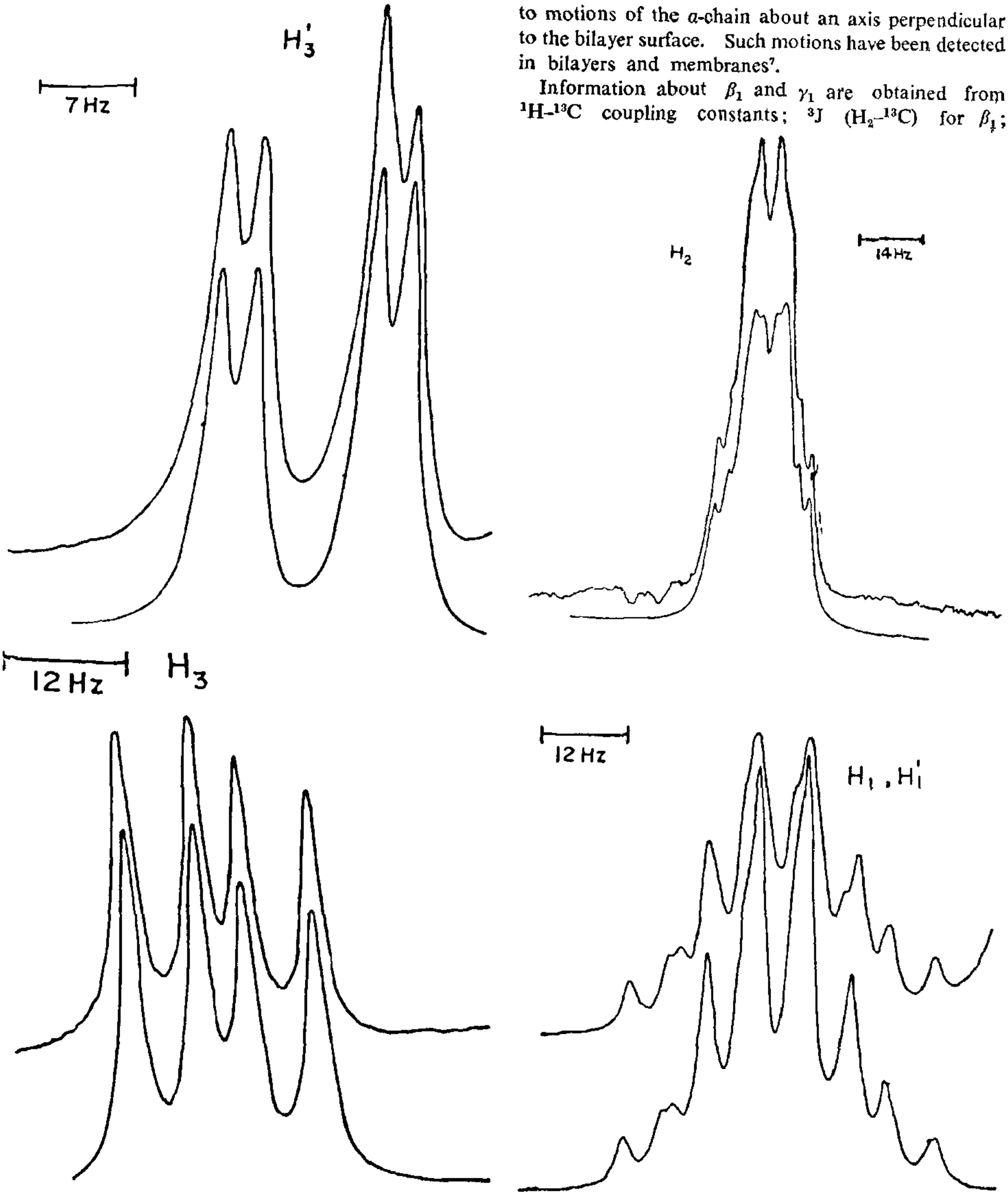



FIG. 2. Experimental and simulated spectra of the different protons. The five protons and the phosphorus nuclei form an ABCDEX system, X being the phosphorous nucleus. The spectra are calculated by the standard LA COON Program.

conformation with respect to $\alpha_1$, although their coupling constants are close to the coupling constants reported here. The conformational flexibilities with respect to $\theta_1$ and $\alpha_1$, if present in bilayers as well, lead to motions of the $\alpha$-chain about an axis perpendicular to the bilayer surface. Such motions have been detected in bilayers and membranes[7].

Information about $\beta_1$ and $\gamma_1$ are obtained from $^1H$-$^{13}C$ coupling constants; $^3J$ ($H_2$–$^{13}C$) for $\beta_1$; ($H_3$–$^{13}C$) and $^3J$ ($H_3$–$^{13}C$) for $\gamma_1$. These coupling constants are identical to the corresponding 3-bond coupling constants observed in glyceryl trivalerate[6]. This implies that DPL has close conformational

similarities with glyceryl trivalerate with respect to dihedral angle $\beta_1$ and $\gamma_1$. A value of 3·44 for $^3J$ ($H_2$–$^{13}C$)

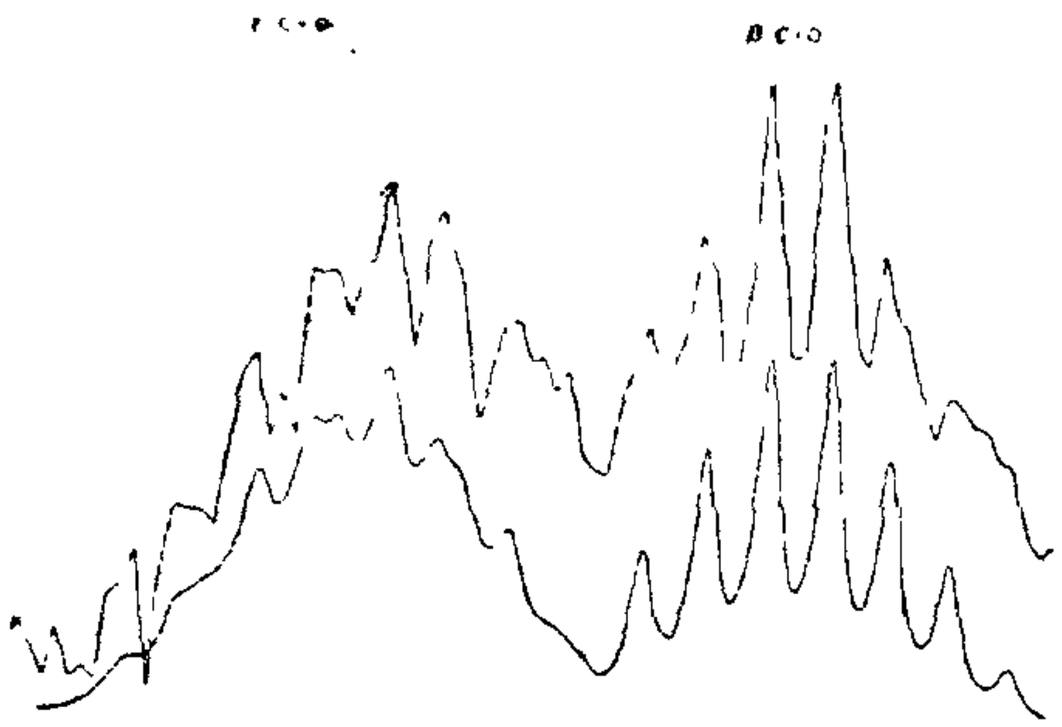
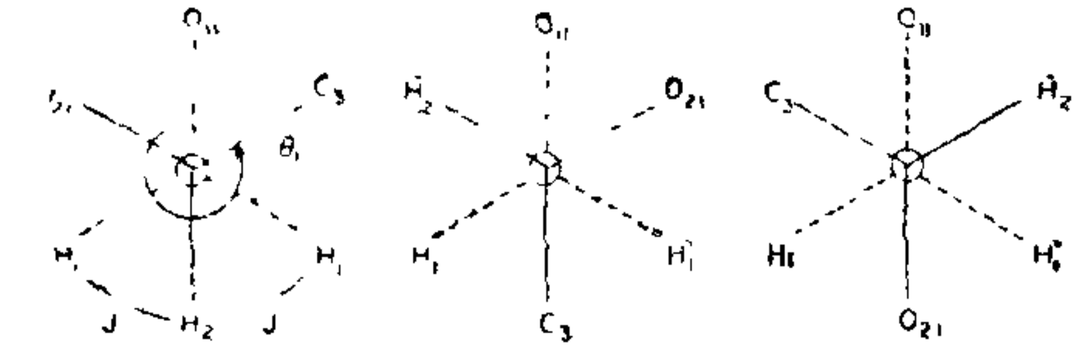


FIG. 3. $^1H$ coupled $^{13}C$ spectra of the glycerol-ester region CH–O–$^{13}$C–$CH_2$–$CH_2$——system constitutes an XABB'CC' 6 spir syetem while the $CH_2O$—$^{13}C$—$CH_2$—$CH_2$ constitutes on XABCC'DD' 7 spin system. The two systems have been treated separately and the calculated spectra are then joined together. These two regions are not seen separately in the 25·2 MHz spectrum obtained by Birdsall *et al.*[4].



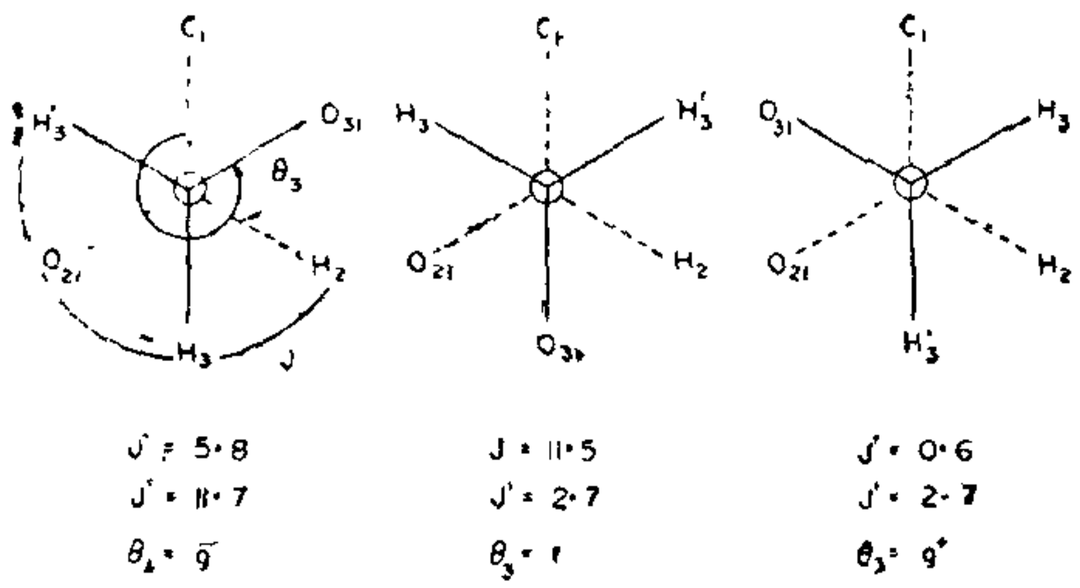




FIG. 4. Newman projection diagrams corresponding to three staggered configurations with respect to $\theta_1$, $\theta_3$, $\alpha_1$, $\beta_1$ and $\gamma_1$. The dihedral angles $\theta_1$ and $\theta_3$ are defined by the sequence $O_{11}$–$C_1 + C_2$–$C_3$ and $C_1$–$C_2 + C_3$–$O_{31}$ respectively. The expected $^1H$ coupling constants for these structure are also indicated.

suggests values of 30° and 140° for the dihedral angles in the sequence of atoms $H_2$–C–O–$^{13}$C. From Lemieux curves[13], this corresponds to four different values of $\beta_1$: 90°, 150°, 260° and 340°. The values of 260° and 340° lead to sterically hindered positions of atoms and hence are unlikely to be present. As regards $\gamma_1$, the low and equal values for the two coupling constants $^3J$ ($H_3$–$^{13}C$) and $^3J$ ($H_3'$–$^{13}C$), indicates that $\gamma_1$ is close to 'trans' conformation[6]).

While the exact populations as estimated by NMR may not be very accurate, the present study establishes considerable flexibilities around the various single bonds in the glycerol moiety of DPL in $CDCl_3$ solutions. This data supports our earlier predictions that the polar head group has considerable flexibility and it may be unwise to use the crystal structure[3] as the only conformation existing in lipid bilayers[5].

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