

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 glycerides: β -trilaurin¹ and β -tricaprin² and one on the phospholipid 1, 2-DL-dilauroyl phosphatidyl ethanolamine³. 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 attempts, using this approach, to study the conformation of phospholipids and triglycerides⁴⁻⁹. 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 ($\text{C}-\text{O}-\text{COR}$) 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 LACON 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 reported⁶ ^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):

$$\begin{aligned} (\text{H}_2) &= 5.15, (\text{H}'_2) = 4.35, (\text{H}_3) = 4.073 \\ (\text{H}_1) &= 3.913 \text{ or } 3.869, (\text{H}'_1) = 3.869 \text{ or } 3.913. \end{aligned}$$

Coupling Constants:

$$\begin{aligned} {}^2\text{J}(\text{H}_2-\text{H}_3) &= -12.01; {}^3\text{J}(\text{H}_3'-\text{H}_2) = 2.78 \\ {}^3\text{J}(\text{H}_3-\text{H}_2) &= 7.21; {}^3\text{J}(\text{H}_2-\text{H}_1) = 5.2 \text{ or } 6.6 \\ {}^3\text{J}(\text{H}_2-\text{H}_1') &= 6.6 \text{ or } 5.2; {}^3\text{J}(\text{H}_1-\text{H}_1') = -11.5 \\ {}^3\text{J}(\text{H}_1-{}^{31}\text{P}) &= 7.3 \text{ or } 6.8; {}^3\text{J}(\text{H}_1'-{}^{31}\text{P}) = 6.8 \text{ or } 7.3 \\ {}^3\text{J}(\text{H}_2-{}^{13}\text{C}) &= 3.4 \\ {}^2\text{J}({}^{13}\text{C}-\text{H}) &= -6.9 \\ {}^3\text{J}({}^{13}\text{C}-\text{H}) &= 3.5 \end{aligned} \left. \begin{array}{l} \\ \\ \\ \end{array} \right\} \text{in the } \beta \text{ chain}$$

$$\begin{aligned} {}^3\text{J}(\text{H}_3-{}^{13}\text{C}) &= {}^3\text{J}(\text{H}_3'-{}^{13}\text{C}) = 2.79 \\ {}^2\text{J}({}^{13}\text{C}-\text{H}) &= -7.18 \text{ and } -7.16 \\ {}^3\text{J}({}^{13}\text{C}-\text{H}) &= 4.31 \text{ and } 4.32 \end{aligned} \left. \begin{array}{l} \\ \\ \end{array} \right\} \text{in the } \gamma \text{ chain.}$$

* With respect to tetramethyl silane (TMS).

† The coupling constants are accurate within $\pm 0.1-0.2$ Hz.

Conformation of glycerol moiety :

The conformation of the glycerol moiety is determined by two dihedral angles θ_1 and θ_2 (See reference 5 and Fig. 1 for rotations). The Newman projection diagrams of the three staggered conformations with respect to θ_1 and θ_2 are shown in Fig. 4. The vicinal ^1H - ^1H coupling constants for such configurations, calculated from electronegativity rules¹¹ 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

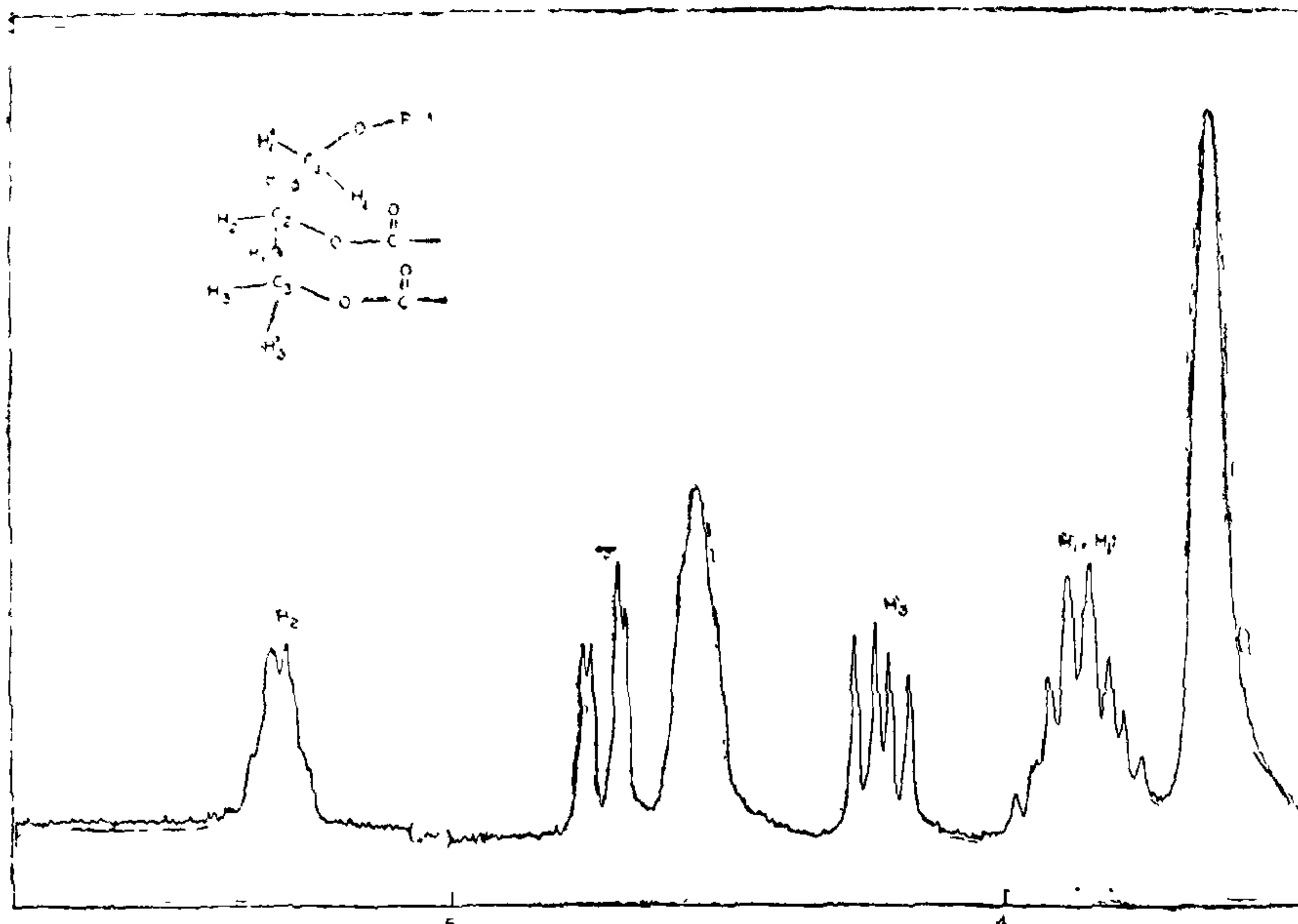


FIG. 1. 360 MHz ^1H FT-NMR spectra of dipalmitoyl lecithin (DPL). DPL was obtained from Sigma Co. Inset is the numbering of the protons on the glycerol fragment (Temperature 20°C).

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

$$\begin{aligned} J_{\text{obs}} &= J_{\theta^-} P_{\theta^-} + J_{\theta^+} P_{\theta^+} + J_{\theta^0} P_{\theta^0} \\ J'_{\text{obs}} &= J'_{\theta^-} P_{\theta^-} + J'_{\theta^+} P_{\theta^+} + J'_{\theta^0} P_{\theta^0} \\ 1 &= P_{\theta^-} + P_{\theta^+} + P_{\theta^0} \end{aligned}$$

where P_{θ^+} , P_{θ^0} , P_{θ^-} are the populations of the three configurations and $(J_{\theta^-}, J'_{\theta^-})$, $(J_{\theta^+}, J'_{\theta^+})$ and $(J_{\theta^0}, J'_{\theta^0})$ 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 θ_1 , if $J_{\text{obs}} = 6.6$ and $J'_{\text{obs}} = 5.2$, one obtains $P_{\theta^-} : P_{\theta^+} : P_{\theta^0} :: 31 : 41 : 28$. On the other hand if $J = 5.2$ and $J'_{\text{obs}} = 6.6$, one gets $P_{\theta^-} : P_{\theta^+} : P_{\theta^0} :: 37 : 20 : 43$. However, it is clear that in either case, all the three staggered configurations are substantially populated. In case of θ_3 , if $J_{\text{obs}} = 2.8$ and $J'_{\text{obs}} = 7.2$ one obtains $P_{\theta^+} \sim 0$ and $P_{\theta^-} : P_{\theta^0} :: 50 : 50$. The alternative assignment, i.e., $J_{\text{obs}} = 7.2$ and $J'_{\text{obs}} = 2.8$ gives $P_{\theta^-} \sim 0$, and $P_{\theta^+} : P_{\theta^0} :: 60 : 40$. Thus, only two conformations with respect of θ_3 coexist in CDCl_3 solutions. Similar conclusions have been obtained in our previous study on glyceryl trivalerate⁶.

Conformations in the Glycerol-Ester Fragment

The relative orientations and the conformations in the α , β and γ chains are determined by the dihedral angles α_1 to α_6 , β_1 to β_n and γ_1 to γ_m . Information has been obtained about the dihedral angles α_1 , β_1 , γ_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 relation¹².

$$J = 16.3 \cos^2 \phi - 4.6 \cos \phi.$$

This gives $J = J' = 1.8$ if α_1 has 't' conformation, $J = 1.8$, $J' = 21.0$, if α_1 has 'g⁻' conformation and $J = 21.0$, $J' = 1.8$ if α_1 has 'g⁺' conformation. With these component coupling constants, if one assigns $J_{\text{obs}} = 6.8$ and $J'_{\text{obs}} = 7.3$, 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 α_1 are substantially populated. We differ from Hauser *et al.* in this conclusion. These authors conclude that the polar group has an exclusively 't'

to motions of the α -chain about an axis perpendicular to the bilayer surface. Such motions have been detected in bilayers and membranes⁷.

Information about β_1 and γ_1 are obtained from 1H - ^{13}C coupling constants; $^3J(H_2-^{13}C)$ for β_1 ;

$^3J(H_3-^{13}C)$ and $^3J(H_1-^{13}C)$ for γ_1 . These coupling constants are identical to the corresponding 3-bond coupling constants observed in glyceryl trivalerate⁶. This implies that DPL has close conformational

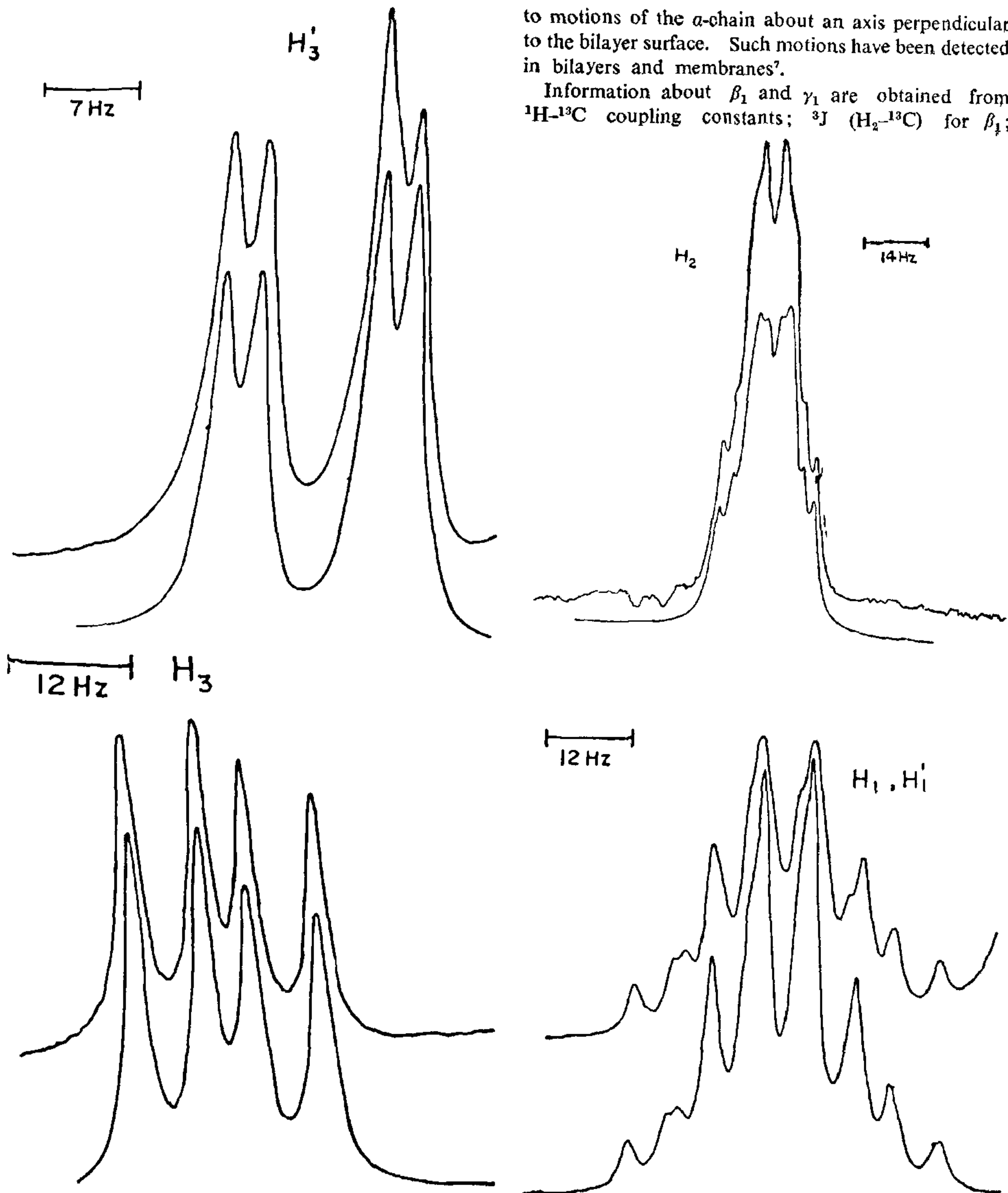


FIG. 2. Experimental and simulated spectra of the different protons. The five protons and the phosphorus nuclei form an ABCDEX system, X being the phosphorous nucleus. The spectra are calculated by the standard LA COON Program.

conformation with respect to α_1 , although their coupling constants are close to the coupling constants reported here. The conformational flexibilities with respect to θ_1 and α_1 , if present in bilayers as well, lead

($H_3-^{13}C$) and $^3J(H_1-^{13}C)$ for γ_1 . These coupling constants are identical to the corresponding 3-bond coupling constants observed in glyceryl trivalerate⁶. This implies that DPL has close conformational

similarities with glyceryl trivalerate with respect to dihedral angle β_1 and γ_1 . A value of 3.44 for $^3J(H_2-^{13}C)$

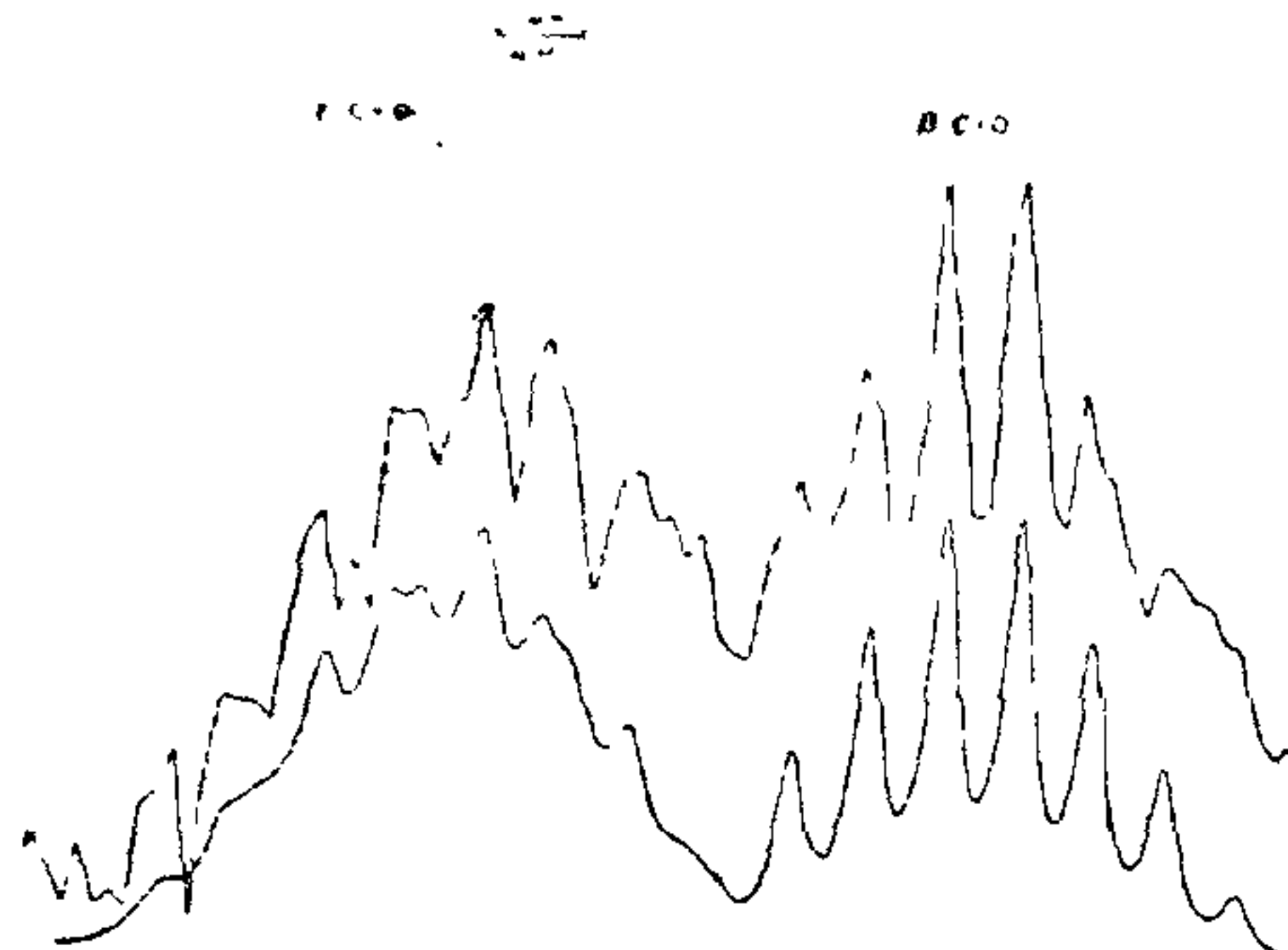


FIG. 3. 1H coupled ^{13}C spectra of the glycerol-ester region $CH-O-^{13}C-CH_2-CH_2$ —system constitutes an XABB'CC' 6 spin system while the $CH_2O-^{13}C-CH_2-CH_2$ constitutes an XABCC'DD' 7 spin system. The two systems have been treated separately and the calculated spectra are then joined together. These two regions are not seen separately in the 25.2 MHz spectrum obtained by Birdsall *et al.*⁴.

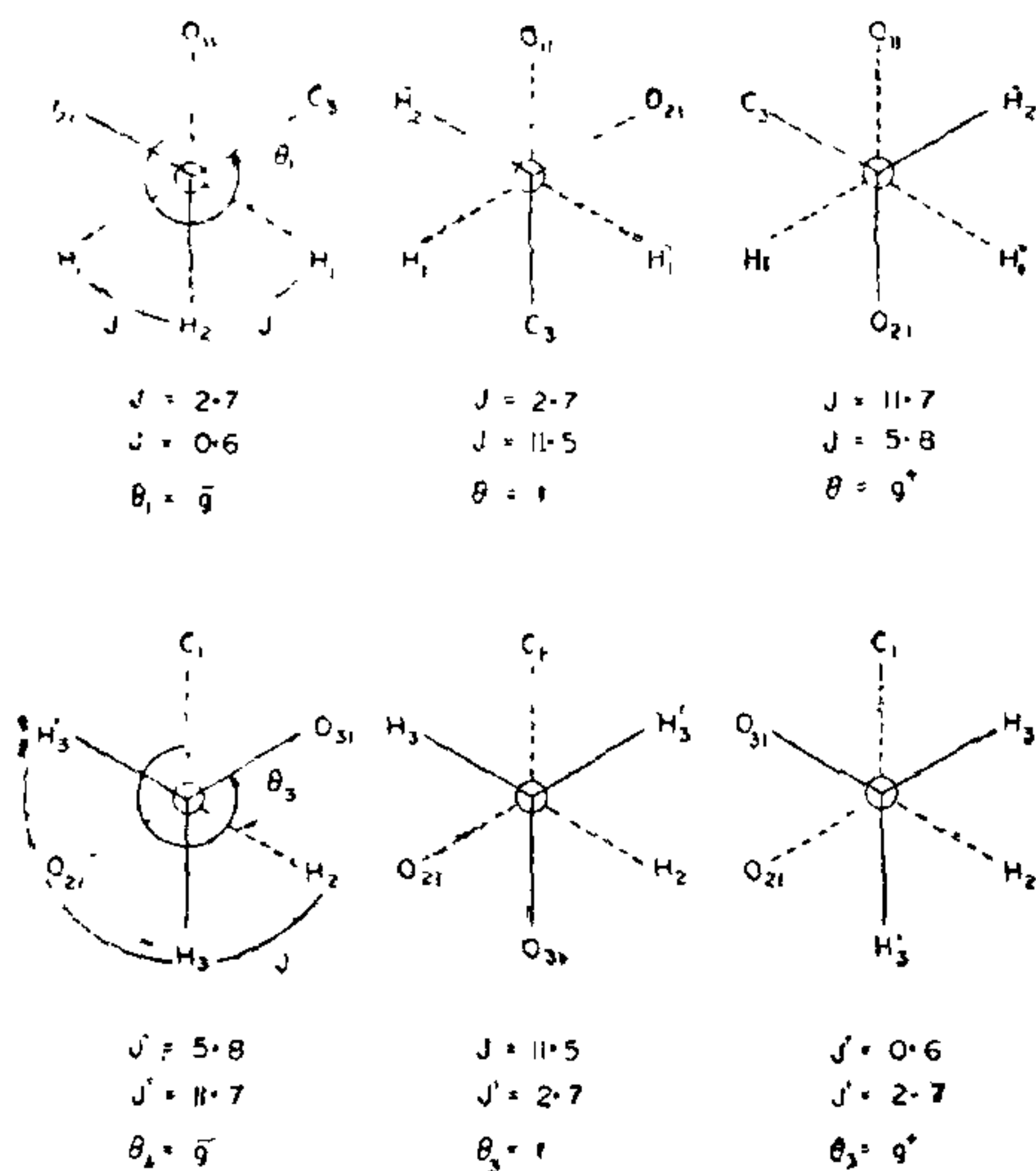


FIG. 4. Newman projection diagrams corresponding to three staggered configurations with respect to θ_1 , θ_2 , θ_3 , α_1 , β_1 and γ_1 . The dihedral angles θ_1 and θ_2 are defined by the sequence $O_{11}-C_1+C_2-C_3$ and $C_1-C_2+C_3-O_{21}$ respectively. The expected 1H coupling constants for these structures 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 curves¹³, this corresponds to four different values of β_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 γ_1 , the low and equal values for the two coupling constants $^3J(H_2-^{13}C)$ and $^3J(H_3'-^{13}C)$, indicates that γ_1 is close to 'trans' conformation⁶.

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 structure³ as the only conformation existing in lipid bilayers⁵.

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- Larson, K., *Ark. Kemi.*, 1964, **23**, 1.
- Jensen, L. H. and Mabis, A. J., *Acta Cryst.*, 1966, **21**, 770.
- Hitchcock, P. B., Mason, R., Thomas, K. M. and Shipley, G. G., *Proc. Nat. Acad. Sci. USA*, 1974, **71**, 3036.
- Birdsall, N. J. M., Feeney, J., Lee, A. G., Levine, Y. K. and Metcalfe, J. C., *J. Chem. Soc. Perkins II*, 1972, p. 1441.
- Gupta, S. P., Govil, G. and Mishra, R. K., *J. Theoret. Biol.*, 1975, **51**, 13.
- Govil, G., Hosur, R. V. and Saran, A., *Chem. Phys. Lipids*, 1978, **21**, 77.
- Seelig, J., Gally, H. U. and Woglemuth, R., *Biochim. Biophys. Acta*, 1977, **467**, 109.
- Levine, Y. K., *Prog. Biophys. Molec. Biol.*, 1972, **24**, 1.
- , Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C., *Biochemistry*, 1972, **11**, 1416.
- Hauser, H., Guger, W., Levine, B., Scrabal, P. and Williams, R. J. P., *Biochim. Biophys. Acta*, 1978, **508**, 450.
- Abraham, R. J. and Gatti, G., *J. Chem. Soc. (B)*, 1969, p. 961.
- Tewari, R., Nanda, R. K. and Govil, G., *Biopolymers*, 1977, **13**, 2015.
- Lemieux, R. U., Nagbhusan, Y. L. and Paul, B., *Can. J. Chem.*, 1970, **50**, 773.

FISSION TRACK AGES AND URANIUM CONTENTS OF SOME INDIAN MUSCOVITES

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ABSTRACT

Fission track ages of muscovite samples from Bihar (984 m.y., 1062 m.y.), Rajasthan (631 m.y., 825 m.y.), Andhra Pradesh (564 m.y.), Nilgiri (810 m.y.) and Delhi (915 m.y.), have been measured and their uranium concentrations have been determined. Samples from Bihar have the lowest uranium content (0.5×10^{-11} atom/atom) and those from Andhra Pradesh have the highest uranium content (16.9×10^{-11} atom/atom). Average measured ages are found to be in good agreement with ages determined by other workers using similar and other radiometric methods and are related to the metamorphic orogenic cycles of the regions.

INTRODUCTION

AGE of muscovite micas has been determined by many workers using the fission track dating technique¹⁻⁴. In many cases the reported fission, track ages are lower than those determined by other radiometric methods. This is known to be due to the loss of "fossil" tracks resulting from the high temperatures encountered during the geological history of the specimen. The fission track age, in fact, gives an idea about the time of crystallisation of the mineral or the time of occurrence of the last overheating event in the history of the specimen since when the tracks have been preserved in it, whichever is later.

In their earlier experiments Fleischer *et al.*² had expressed a doubt that precambrian muscovites older than a few hundred million years, could not be dated by this technique, because of very large fading effects on the "fossil" tracks over the geological times. However by carefully counting the over-etched "fossil" tracks Mehta and Nagpaul⁵ reported the fission track ages of several precambrian muscovites of India and have obtained meaningful age data by applying the fission track density correction as suggested by Mehta and Rama⁶.

Using a similar method, we have also determined the fission track ages and uranium concentrations of several muscovite samples from the three important mica belts of India, *viz.*, Bihar, Rajasthan and Andhra Pradesh and other precambrian muscovites namely from Nilgiri and Delhi (Masoodpur area). The samples at Sl. No. 1, 2, 5 and 6 (Table I), are from the muscovite books already dated by Mehta and Nagpaul⁵ while the other samples are from undated muscovite books obtained from other sources. An inter-laboratory comparison of the results for the common samples dated by the Kurukshetra group has been made and the results have been compared with the known radiometric ages of other pegmatites from the same region. The measured ages are found to correspond to the metamorphic orogenic cycles of the regions.

EXPERIMENTAL DETAILS

The method of sample preparation and other experimental procedures were basically the same as used by Mehta and Nagpaul⁵ and have been described in detail elsewhere⁷.

The fossil track length distribution measurements were carried out after 30 minute etching of the samples in 48% HF at 25° C. These were compared with the track length distribution for the induced fission tracks in the samples after reactor irradiation and similar etching for the purpose of finding the track fading correction. Our separate laboratory track annealing experiments⁷ using the reactor irradiated muscovites have also revealed that practically there is a negligible reduction ($\leq 5\%$) in the track density corresponding to a track length reduction of about 25%. A larger track length reduction requires a track density fading correction to be applied. A normalised track length versus normalised track density curve was plotted to assess the track fading correction. However, in none of our samples, was there a track length reduction of more than 20%. The mean track length of neutron induced fission tracks in muscovite was found to be 10.5 microns and has been used for calculating the uranium contents.

The fossil and induced fission track densities ρ_f and ρ_i respectively, in the muscovite samples were determined after over etching them for three hours in 48% HF at 25° C. The standard glass dosimeters of Fleischer *et al.*⁸, were used to measure the thermal neutron dose. For these dosimeters under standard conditions of etching (5 sec, 48% HF, 22° C), the integrated neutron dose, ϕ , is related to the track density ρ_i , by the formula $\phi = 2.26 \times 10^{11} \rho_i$. The following equations were used for age, A, and uranium content, C(U), calculations :

$$A = 6.57 \times 10^9 \ln \left\{ 1 + 2.1 \times 10^{-11} \frac{\rho_f \rho_0}{\rho_i} \right\} \text{ years}$$

$$C(U) = \frac{3.18 \times 10^4 (\rho_f \phi) \text{ gm/gm}}{10^{11} (\rho_i \phi) \text{ atom/atom}} = 2.534 \times$$