Dipalmitoyl-lecithin: Assignment of the ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectra, and Conformational Studies

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The ^{13}C resonances of the glycerol and choline groups of dipalmitoyl-lecithin in CD_3OD, $CDCl_3$, and D_2O have been assigned from the $^{14}N-^{13}C$ and $^{31}P-^{13}C$ coupling constants, and proton decoupling studies on the assigned ^{1}H spectra. Well-defined solvent effects are observed, but the chemical shifts for dipalmitoyl-lecithin in bilayers in D_2O are very similar to the shifts of the corresponding resonances in biological membranes. From an analysis of the ^{1}H spectrum of the CH_2 multiplet in the $CH-CH_2OCOR$ fragment, the two vicinal coupling constants were extracted and used to calculate rotamer populations from a Karplus treatment. In a high proportion of the molecules of dipalmitoyl-lecithin in CD_3OD and in $CDCl_3$ the fatty acid chains are arranged *gauche* to each other, suggesting the presence of strong hydrophobic interactions. From an AA'BB' analysis of the choline $-CH_2\cdot CH_2-$ fragment in dipalmitoyl-lecithin observed vicinal coupling constants indicate a similar conformation to that found in choline derivatives where an electrostatic interaction between the N^+ and the O atoms leads to the conformation being exclusively in the *gauche* $^{+}N/O$ form.[1]

RECENTLY measurements have been made of the ^{13}C spin lattice relaxation times (T_1) of all the resolved fatty acid chain carbon atoms (1, 2, 3, 14, 15, 16) of dipalmitoyl-lecithin in micelles in $CDCl_3$ and in bilayers in D_2O.[2] The T_1 values provide information about the chain molecular motion which is sensitive to the structural organisation of the lipid molecules. These relaxation measurements are of potential importance in the application of ^{13}C n.m.r. to phospholipids in biological membranes, in which ^{13}C resonances from specifically enriched phospholipids can be observed.[3] A complete description of the relaxation times of the dipalmitoyl-lecithin carbon nuclei[4] requires assignment of the glycerol and choline carbon resonances, which cannot be assigned with certainty on the basis of chemical shift comparisons with model compounds. Such assignments can be made by use of selective proton irradiation experiments if the ^{1}H resonance spectrum of dipalmitoyl-lecithin has been assigned.

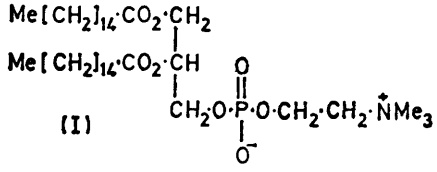
EXPERIMENTAL

The ^{1}H (220 and 100 MHz) and ^{13}C (25·2 MHz) resonance spectra were obtained with Varian HA100D, HR220, and XL100 spectrometers. Selective proton irradiation experiments were carried out with the XL100 Gyrocode and the ^{13}C spectra were accumulated by use of the Fourier transform technique. The lock signal was provided by deuterium in the solvent and the ^{13}C chemical shifts are expressed in p.p.m. from an external dioxan reference (positive shifts are to high field of the reference).

1,2-*sn*-Dipalmitoyl-3-phosphatidylcholine (dipalmitoyl-lecithin), 1,2-*sn*-dipalmitoylglycerol, and *rac*-glycerol 3-phosphate were from Koch–Light; *sn*-glycero-3-phosphorylcholine was prepared from egg lecithin by the method of Chadha[5] and *sn*-3-phosphatidic acid by the action of cabbage phospholipase D on dipalmitoyl-lecithin.[6]

RESULTS AND DISCUSSION

Spectral Assignments.—Assignment of the ^{1}H spectrum of dipalmitoyl-lecithin (I). Figure 1 shows the ^{1}H



(I)

resonance spectrum of dipalmitoyl-lecithin in CD_3OD at 220 MHz. The assignments as indicated on the spectra were made on the basis of chemical shift, coupling constant, and intensity data and supported by homonuclear spin-decoupling experiments. From chemical shift and intensity considerations it is possible to assign directly the terminal alkyl CH_3 (0·90 p.p.m.), the alkyl $[CH_2]_n$ (1·29 p.p.m.), the CH_2CO (2·32 p.p.m.), $CHO\cdot COR$ (5·24 p.p.m.), $^{+}NCH_2$ (3·63 p.p.m.) and $^{+}NMe_3$ (3·20 p.p.m.) protons. Spin-decoupling experiments indicate that the low-field single proton ($CHO\cdot COR$, 5·24 p.p.m.) is coupled to four CH_2 protons in the region 3·9—4·6 p.p.m. which is consistent with these being the glycerol CH_2 protons. One CH_2 group gives a simple eight-line multiplet corresponding to the

[1] C. C. J. Culvenor and N. S. Ham, *Chem. Comm.*, 1966, 537.
[2] J. C. Metcalfe, N. J. M. Birdsall, J. Feeney, A. G. Lee, Y. K. Levine, and P. Partington, *Nature*, 1971, **233**, 199.
[3] J. C. Metcalfe, N. J. M. Birdsall, and A. G. Lee, *FEBS Letters*, 1972, **21**, 335.
[4] Y. K. Levine, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *Biochemistry*, 1972, **11**, 1416.
[5] J. S. Chadha, *Chem. Phys. Lipids*, 1970, **4**, 104.
[6] F. M. Davidson and C. Long, *Biochem. J.*, 1958, 488.

AB part of an ABX type system and these can be assigned to the $CH_2O{\cdot}CO$ glycerol protons because of the

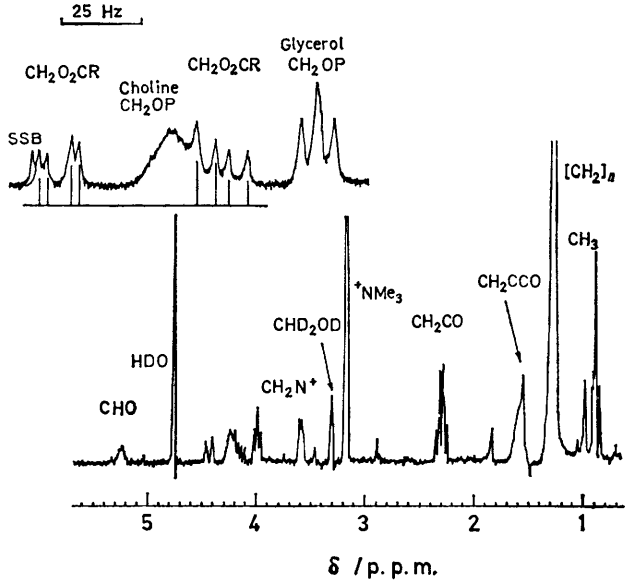


FIGURE 1 220 MHz ^1H spectrum of dipalmitoyl-lecithin in CD_3OD. The insert shows an expanded region illustrating the AB part of the $CHCH_2$–OCOR ABX type system

absence of ^{31}P spin coupling. The CH_2OP glycerol protons resonate at somewhat higher field (4·08 p.p.m.) and are characterised by a ^{31}P–^1H spin coupling constant of 6·8 Hz: this is seen clearly in spectra where the $CHO{\cdot}CO$ proton has been decoupled. The CH_2OP protons are accidentally equivalent which explains the observed doublet (J_{PH} 6·8 Hz) of doublets (J_{HH} 5·8 Hz) with the geminal coupling between the two protons not observed (Figure 1). The remaining resonance (4·25 p.p.m.) is very broad and can be assigned to the choline OCH_2 protons which are expected to be a complex multiplet from spin–spin interactions with their vicinal protons, phosphorus, and nitrogen-14 neighbours. The $^+NCH_2$ multiplet, very similar to that found in certain choline derivatives, has been analysed in terms of an AA′ part of an AA′BB′ spin system, the observed vicinal coupling constants being 7·0 and 2·2 Hz. The CH_2CO protons show separate resonances for the two chains. This can be seen clearly in the 220 MHz spectrum where they appear as two overlapping triplets (deceptively simple spectrum). From a detailed analysis of the glycerol CH_2OCO multiplets the geminal and vicinal proton coupling constants were extracted (J_{AB} 11·9 Hz, J_{AX} 7·0 Hz, J_{BX} 3·2 Hz, and δ_{AB} 0·25 p.p.m.).

There was no ambiguity encountered in transferring these assignments to the ^1H resonance spectrum of dipalmitoyl-lecithin in $CDCl_3$ and the results of these

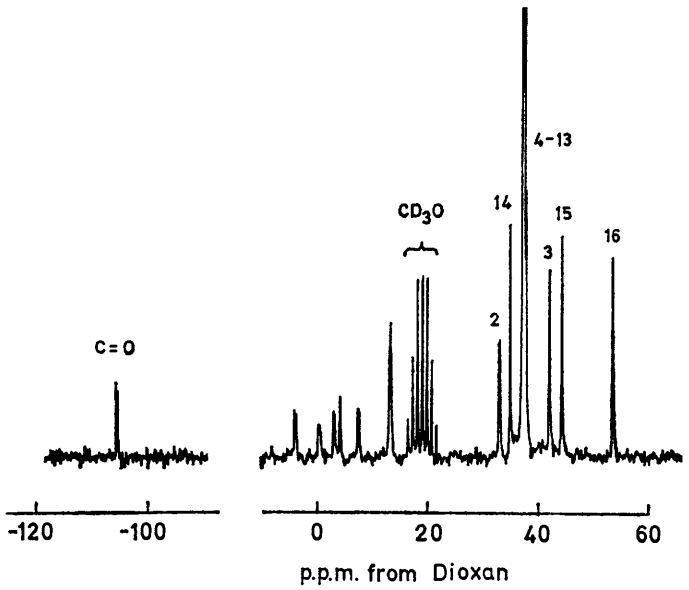


FIGURE 2 25·2 MHz ^{13}C spectrum of dipalmitoyl-lecithin in CD_3OD

experiments are summarised in Table 1. The assignments are essentially in agreement with those of Finer *et al.*,[7] but differ substantially from those of Chapman and Morrison.[8]

Assignment of the ^{13}C *spectrum of dipalmitoyl-lecithin.*

TABLE 1

Compound	Solvent	Choline			Glycerol			Methylene			Methyl
		Me_3N^+	CH_2N^+	CH_2O	1-CH_2O	CHO	3-CH_2O	2	3	4–15	16
Dipalmitoyl-lecithin	$CDCl_3$ [a]	3·32	3·82	4·30	4·3 [b]	5·15 [b]	3·93	2·27, 2·30	1·58	1·27	0·88
	CD_3OD [a]	3·20	3·63 [c]	4·25 [c]	4·30 [d]	5·24 [d,e]	4·08 [e,f]	2·32	1·60	1·29	0·90
1,2-*sn*-Dipalmitoyl-glycerol	$CDCl_3$ [a]				4·27 [g]	5·10 [g]	3·74 [h]	2·34	1·58	1·26	0·89
Dipalmitoyl-*sn*-3-phosphatidic acid	$CDCl_3$ [a]				4·35	5·24	4·02	2·31	1·60	1·28	0·88
sn-Glycero-3-phosphorylcholine	D_2O [i]	3·21	3·65	4·30	3·65	3·90	3·90				
Glycerol 3-phosphate	D_2O pD 8·0 [i]				4·08	4·25	4·25				
	D_2O pD 0 [i]				4·11	4·45	4·45				
Choline bromide	D_2O [i]	3·61	3·90	4·4							

Chemical shifts are expressed in p.p.m. downfield from an internal (*a*) hexamethyldisiloxane (*i*) sodium 4,4-dimethyl-4-silapentane-1-sulphonate standard. [b] J_{gem} 12·0, J_{vic} 7·5, 2·7 Hz, δ_{AB} 0·24 p.p.m. [c] J_{vic} 7·0, 2·2 Hz. [d] J_{gem} 11·9, J_{vic} 7·0, 3·2 Hz, δ_{AB} 0·25 p.p.m. [e] $\frac{1}{2}(J_{AX} + J_{BX})$ 5·8 Hz. [f] J_{PH} 6·8 Hz. [g] J_{gem} 11·8, J_{vic} 5·9, 4·2 Hz, δ_{AB} 0·12 p.p.m. [h] $\frac{1}{2}(J_{AX} + J_{BX})$ 5·2 Hz.

[7] E. G. Finer, A. G. Flook, and H. Hauser, *FEBS Letters*, 1971, **18**, 331.

[8] D. Chapman and A. Morrison, *J. Biol. Chem.*, 1966, **241**, 5044.

The ^{13}C spectrum in CD_3OD recorded at 25·2 MHz under conditions of proton noise decoupling is shown in Figure 2: all six glycerol and choline carbon nuclei are well resolved and show coupling to ^{14}N and/or ^{31}P nuclei for all except one of the carbon nuclei (Figure 3, A). Examination of the ^{13}C spectra of choline bromide, *sn*-glycero-3-phosphorylcholine and *rac*-glycerol 3-phosphate (see Figure 4) allows the ^{14}N–^{13}C and ^{31}P–^{13}C spin coupling constants in these systems to be characterised and Table 2 contains all the ^{13}C coupling constant and chemical shift data for the compounds studied. The ^{13}C spectral assignments for all the model compounds considered were confirmed by selective proton decoupling experiments. In choline analogues the ^{14}N–^{13}C spin coupling to carbons at positions α to the nitrogen is 3—4 Hz while for β-carbon atoms the coupling constants are <1 Hz. For *rac*-glycerol 3-phosphate and *sn*-3-phosphatidic acid the ^{31}P–^{13}C coupling constants involving the glycerol carbons α and β to the phosphate group are 5—6 Hz and 6—8 Hz respectively, with no observable coupling (<1 Hz) from the phosphorus to the γ glycerol carbon atom. ^{14}N–^{13}C and ^{31}P–^{13}C coupling constants very similar to the values measured in these model compounds are observed in the spectrum of *sn*-glycero-3-phosphorylcholine, with additional ^{31}P coupling to both of the choline methylene carbons, so that the CH_2N^+ carbon is split by both ^{31}P and ^{14}N into two partially overlapping 1 : 1 : 1 triplets.

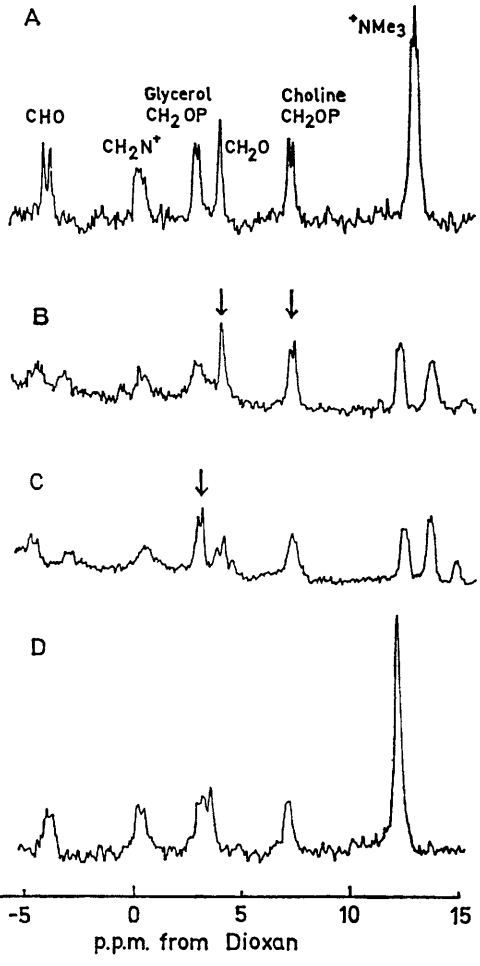


FIGURE 3 A, Glycerol and choline region of the ^{13}C spectrum of dipalmitoyl-lecithin in CD_3OD. B, Dipalmitoyl-lecithin in CD_3OD; selective irradiation at δ 4·30 p.p.m. in the ^1H spectrum. The collapsed triplets at 7·7 and 4·4 p.p.m. are indicated by arrows. C, Dipalmitoyl-lecithin in CD_3OD; selective irradiation at δ 4·08 p.p.m. in the ^1H spectrum. The collapsed triplet at 3·0 p.p.m. is indicated by the arrow. D, Glycerol and choline region of the ^{13}C spectrum of dipalmitoyl-lecithin in $CDCl_3$

The same coupling constants are observed for the glycerol and choline carbons in dipalmitoyl-lecithin in CD_3OD. This allows a preliminary assignment of the $^+NMe_3$, CH_2N^+, and CHO carbon atoms; the $CH_2O{\cdot}COR$ carbon resonance is also assigned because it is the only resonance with no observable coupling. The remaining CH_2OP (glycerol) and CH_2OP (choline) resonances have very similar ^{31}P coupling constants and cannot be assigned solely on the basis of the coupling constant data. The $^+NMe_3$ assignment is unequivocal on the basis of intensity, chemical shift, and selective proton decoupling of the ^{13}C spectrum.

The partial assignment was confirmed and completed by systematic selective proton decoupling and the chemical shifts of the carbon resonances are in Table 2. Irradiation of the proton spectrum at δ 4·30 p.p.m. ($CH_2O{\cdot}COR$ glycerol and CH_2OP choline protons) causes

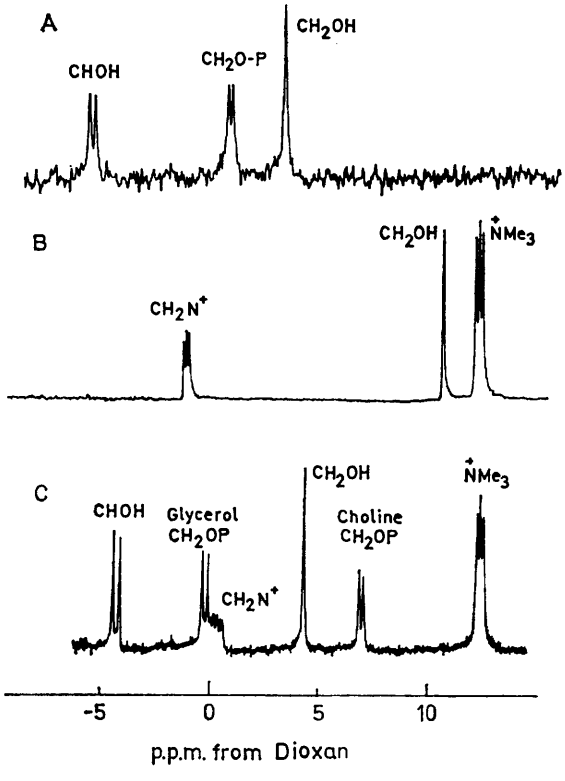


FIGURE 4 A, ^{13}C Spectrum of *rac*-glycerol 3-phosphate in D_2O at pD 8; B, ^{13}C spectrum of choline bromide in D_2O; C, ^{13}C spectrum of *sn*-glycero-3-phosphorylcholine

the collapse of both the $CH_2O{\cdot}COR$ glycerol ^{13}C triplet at 4·4 p.p.m. and the triplet at 7·7 p.p.m. This confirms the assignment of the latter resonance as the CH_2OP choline carbon (Figure 3, B). Similarly, irradiation at δ 4·08 p.p.m. (CH_2OP glycerol proton) collapses the 3·0 p.p.m. ^{13}C resonance (Figure 3, C). Thus the internal consistency of the coupling constant data, and the selective and off-resonance proton decoupling experiments, provide an unequivocal assignment of the ^{13}C spectrum of dipalmitoyl-lecithin in CD_3OD. Figure 3, D shows the proton noise decoupled ^{13}C spectrum at 25·2 MHz of the choline and glycerol carbons of dipalmitoyl-lecithin in $CDCl_3$. This spectrum is similar to that of dipalmitoyl-lecithin in $CHCl_3$ reported by Oldfield and Chapman [9] in which only the $^+NMe_3$ resonance was magnetic susceptibility will be different from that found in CD_3OD solution in which the dipalmitoyl-lecithin molecules are in a dispersed form.

Experiments on biological membranes in which ^{13}C spectra are observed at natural abundance [11] or from specifically labelled nuclei incorporated biosynthetically into membrane phospholipids [3] indicate that the chemical shifts of the ^{13}C resonances in the membrane are very similar to those observed in phospholipid bilayers in D_2O. Thus the assignments reported here can be used to assign the spectra of lecithins in membranes.

Conformational Information.—From the analysis of the $CH_2O{\cdot}COR$ and $^+NCH_2$ multiplets in the ^1H spectrum of dipalmitoyl-lecithin in CD_3OD, vicinal H–H coupling constants can be obtained which provide information

TABLE 2

Compound	Solvent	C=O	Choline			Glycerol			Methylene					Methyl
			Me_3N^+	CH_2N^+	CH_2O	1-CH_2O	CHO	3-CH_2O	2	14	4-13	3	15	16
Dipalmitoyl-lecithin	D_2O	−107·4	12·1	0·1	6·7	3·0	−4·6	3·0	32·0	34·3	36·4	41·4	43·7	52·5
	$CDCl_3$	−106·8 −106·4	12·2	0·2 *d*	7·1	3·5	−4·1 *e*	3·0 *d*	32·0 32·1	34·5	36·7	41·5	43·7	52·4
	CD_3OD	−106·3 −106·0	13·4 *a*	0·6 *b, d*	7·7 *e*	4·4	−3·7 *f*	3·0 *e*	33·1 33·2	35·0	37·3	42·3	44·3	53·4
sn-Glycero-3-phosphoryl-choline	D_2O		12·2 *a*	0·3 *b, f*	6·9 *e*	4·2	−4·3 *f*	−0·2 *d*						
Dipalmitoylphosphatidic acid	$CDCl_3$	−106·8 −106·4				3·9	−3·5 *e*	2·6 *d*	32·0 32·1	34·5	36·7	41·5	43·7	52·4
1,2-*sn*-Dipalmitoylglycerol	$CDCl_3$	−106·7 −106·3				4·2	−5·7	5·0	32·0 32·1	34·5	36·8	41·6	43·7	52·3
Glycerol 3-phosphate	D_2O pD 8·0					3·8	−5·0 *f*	1·4 *e*						
	D_2O pD 4·3					4·0	−4·6 *e*	0·2 *e*						
	D_2O pD 0					4·2	−4·2 *d*	−1·3 *e*						
Choline bromide	D_2O		12·5 *a*	−0·9 *b*	10·9									

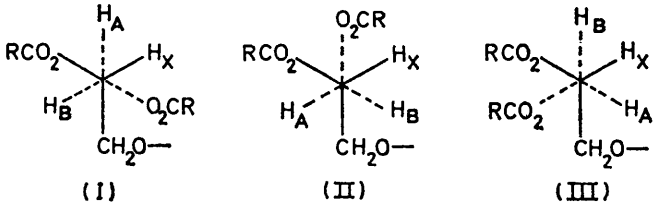
Positive chemical shifts (p.p.m.) are to high field of dioxan external reference. *a* $J(^{14}N-^{13}C)$ 4 Hz. *b* $J(^{14}N-^{13}C)$ 3 Hz. *c* $J(^{31}P-^{13}C)$ 5 Hz. *d* $J(^{31}P-^{13}C)$ 6 Hz. *e* $J(^{31}P-^{13}C)$ 7 Hz. *f* $J(^{31}P-^{13}C)$ 8 Hz.

assigned. However, there is no peak in our spectrum at 9·0 p.p.m. The intensities of the peaks in our spectrum account for all the nuclei in the dipalmitoyl-lecithin structure, the resonances at 3—4 p.p.m. accounting for two carbon nuclei. (A resonance at 9·0 p.p.m. is observed from the CH_2O carbon of ethanol added to $CDCl_3$.) Dipalmitoyl-lecithin in $CDCl_3$ exists as micelles containing 60—70 molecules of dipalmitoyl-lecithin [10] and the ^{13}C resonances are broader than those of dipalmitoyl-lecithin in CD_3OD. In addition, no $^{14}N-^{13}C$ coupling is observed: this may be due to removal of the ^{14}N coupling at the shorter nitrogen T_1 relaxation times which would be expected in the micellar structure. The spectrum was again assigned completely by selective and off-resonance proton-decoupling experiments (Table 2). For dipalmitoyl-lecithin in bilayers in D_2O the resonances of the glycerol carbon atoms are even broader [2,4] and were assigned by comparison with the spectra of dipalmitoyl-lecithin in $CDCl_3$ and CD_3OD.

Table 2 shows that the chemical shifts depend considerably on the solvent. The observed variations in chemical shift of a given resonance measured from external dioxan are consistent with variations in the local diamagnetic susceptibility in bilayers, in micelles, and in solution. For example, the CH_2 groups of the chains of dipalmitoyl-lecithin in micelles and bilayers exist in a hydrophobic environment where the dia-

about the conformation of the glycerol and choline fragments of the molecule.

Glycerol fragment. If it is assumed that rotamers (I), (II), and (III) represent the minimum-energy staggered



conformations for rotation about the C–C bond in the $CH{-}CH_2O{\cdot}COR$ fragment, then by measuring the averaged vicinal coupling constants J_{AX} and J_{BX}, the fractional populations $P_{(I)}$, $P_{(II)}$, and $P_{(III)}$ can be calculated if values of the vicinal coupling constants in the individual rotamers are known. Abraham and Gatti [12] have studied an extensive series of 1,2-disubstituted ethanes and have obtained estimates for the component vicinal coupling constants in these molecules. Our molecules are substituted propanes but because the electronegativity difference between H and C is not large, we have used their component vicinal coupling constants. It was also shown that the component vicinal coupling constants of the rotamers of $X{-}CH_2CH_2{-}Y$

[9] E. Oldfield and D. Chapman, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 949.
[10] O. G. Dervichian, *Progr. Biophys. Mol. Biol.*, 1964, **14**, 263.
[11] J. D. Robinson, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *Biochemistry*, in the press.
[12] R. J. Abraham and G. Gatti, *J. Chem. Soc.* (*B*), 1969, 961.

fragments vary linearly with the sum of the electronegativities of X and Y. Using this relationship we have estimated $J_{AX}^{(I)}$ and $J_{BX}^{(I)}$ for rotamer (I). For rotamers (II) and (III) the vicinal coupling constants of *trans*-2,3-dimethyl-1,4-dioxan [13] have been used. The electronegativities of an $-O-$ and a $-O_2CR$ group are not sufficiently different to affect these vicinal coupling constants [*cf.* the H–H coupling constants in 3,3,4,4,5,5-hexadeuteriocyclohexyl acetate at −110 °C [14] and *trans*-4-t-butyl-3,3,5(axial)-trideuteriocyclohexanol [15]]. Thus, by use of the component coupling constants $J_{AX}^{(I)}$ 5·8, $J_{BX}^{(I)}$ 11·7, $J_{AX}^{(II)}$ 11·5, $J_{BX}^{(II)}$ 2·7, $J_{AX}^{(III)}$ 0·6, and $J_{BX}^{(III)}$ 2·7 Hz the observed averaged vicinal coupling constants are given by equations (1) and (2), where

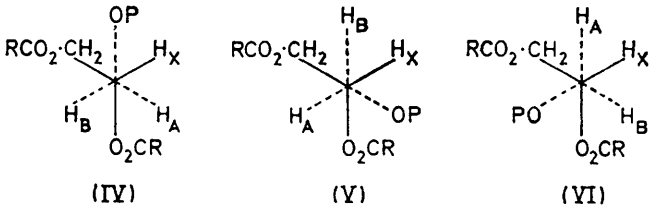
$$J_{AX} = P_{(I)}J_{AX}^{(I)} + P_{(II)}J_{AX}^{(II)} + P_{(III)}J_{AX}^{(III)} \quad (1)$$

$$J_{BX} = P_{(I)}J_{BX}^{(I)} + P_{(II)}J_{BX}^{(II)} + P_{(III)}J_{BX}^{(III)} \quad (2)$$

$P_{(I)} + P_{(II)} + P_{(III)} = 1$, and these equations can be solved to give estimates of the fractional populations. In such an analysis it is not possible to distinguish between rotamers (I) and (II) if H_A and H_B cannot be assigned unambiguously (usually only achieved by selective deuteriation experiments). For dipalmitoyl-lecithin in CD_3OD with J_{vic} 7·0, 3·2 Hz, the two possible results from this analysis are: $P_{(I)} = 0{\cdot}06$, $P_{(II)} = 0{\cdot}56$, $P_{(III)} = 0{\cdot}38$ ($J_{AX} > J_{BX}$) and $P_{(I)} = 0{\cdot}48$, $P_{(II)} = 0{\cdot}01$, $P_{(III)} = 0{\cdot}51$ ($J_{AX} < J_{BX}$), and it is clear that in both analyses rotamer (III), in which the fatty acid chains are in a sterically hindered *gauche* position, has a large fractional population. For dipalmitoyl-lecithin in $CDCl_3$, where the molecules are in a micellar form, $J_{vic} = 7{\cdot}5$, 2·7 Hz, an analysis of these values indicates that the total population of *gauche*-rotamers (II) and (III) is large: for $J_{AX} > J_{BX}$, $(P_{(II)} + P_{(III)}) = 1{\cdot}00$ and for $J_{BX} > J_{AX}$, $P_{(II)} + P_{(III)} = 0{\cdot}47$. An identical analysis of the $CH{-}CH_2{-}O_2CR$ moiety of *sn*-1,2-dipalmitoylglycerol in $CDCl_3$ gives $P_{(I)} = 0{\cdot}17$, $P_{(II)} = 0{\cdot}40$, $P_{(III)} = 0{\cdot}43$ ($J_{AX} > J_{BX}$) and $P_{(I)} = 0{\cdot}36$, $P_{(II)} = 0{\cdot}16$, $P_{(III)} = 0{\cdot}48$ ($J_{AX} < J_{BX}$). Hydrophobic interactions between the chains could lead to this unexpected arrangement of the side chains and it is interesting that a *gauche* arrangement is the most probable conformation in a bilayer structure. Thus the arrangement of the side chains appears to be organized in the correct manner for bilayer formation even when dipalmitoyl-lecithin is in a dispersed form.

[13] G. Gatti, A. L. Segre, and C. Morandi, *Tetrahedron*, 1967, **23**, 4385.
[14] F. A. L. Anet, *J. Amer. Chem. Soc.*, 1962, **84**, 1053.

For the $CH{-}CH_2OP$ moiety in dipalmitoyl-lecithin the vicinal H–H coupling constant of 5·8 Hz is equal to $\frac{1}{2}(J_{AX} + J_{BX})$, where J_{AX} and J_{BX} are the averaged vicinal coupling constants. Because H_A and H_B are coincidentally equivalent, J_{AX} and J_{BX} cannot be extracted individually. The coupling constants can also be expressed in terms of the rotamer populations $P_{(IV)}$, $P_{(V)}$, and $P_{(VI)}$, the same component coupling constants being used as in the previous calculations.



(IV) (V) (VI)

The resulting equations (3) and (4) are underdeter-

$$5{\cdot}8 = 8{\cdot}7P_{(IV)} + 7{\cdot}1P_{(V)} + 1{\cdot}6P_{(VI)} \quad (3)$$

$$P_{(IV)} + P_{(V)} + P_{(VI)} = 1 \quad (4)$$

mined for the solution of individual rotamer populations but by inspection $P_{(VI)}$ must be significant, and limits on its value can be made by putting $P_{(IV)} = 0$ and $P_{(V)} = 0$ respectively. The limits of $P_{(VI)}$ are 0·24—0·41: it is surprising that the conformation (VI), in which the phosphate group is *gauche* to the bulky alkyl chains, is significantly populated.

Choline fragment. An AA'BB' analysis of the $^+NCH_2$ multiplet provides vicinal HH coupling constants in the choline fragment (7·0, 2·2 Hz) which are very similar to those found in acetylcholine perchlorate [16] (J_{HH} 6·9, J_{HH} 2·4 Hz): such coupling constants indicate that the molecules are almost exclusively in the *gauche*-N^+/O conformation. In cholines this conformation is preferred because of the electrostatic interaction between the N^+ and the electronegative oxygen atom.

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