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CONFORMATIONAL STRUCTURE OF GLYCEROL TRIVALERATE AND ITS RELATION TO PHOSPHOLIPIDS: STUDIES BY NMR AND POTENTIAL ENERGY CALCULATIONS

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The structure of a model lipid (glycerol trivalerate, GTV) has been investigated by ^1H and ^{13}C NMR, and energy calculations based on classical potential functions (CPF). The structure of glycerol–ester pivot in GTV is characterized by a dynamic equilibrium between two dominant conformations. One of the two conformations (SA) is similar to the reported crystal structure of triglycerides β-trilaurin and β-tricaprin and has the following conformational angles: $\theta_1 = 52°$, $\theta_3 = 46°$, $\alpha_1 = 208°$, $\beta_1 = 156°$ and $\gamma_1 = 186°$. The other conformer (SB) is similar to the reported crystal structure of β and γ chains in 1,2-dilauroyl-*D,L*-phosphatidylethanolamine and has the conformational angles: $\theta_1 = 287°$, $\theta_3 = 155°$, $\alpha_1 = 195°$, $\beta_1 = 85°$ and $\gamma_1 = 194°$. The ester groups acquire a rigid planar conformation ($\alpha_2, \beta_2, \gamma_2 = 180°$), while the potential energy curves for rotation around the chemical bonds O–CO–C–C (α_3, β_3 and γ_3) indicate a high degree of flexibility. The hydrocarbon chains show a distribution of the *gauche* and *trans* conformations around C–C–C–C bonds with a preference for the *trans* arrangement by almost 0.8 kcal/mol. The conformational picture of the hydrocarbon chains and glycerol moiety in GTV is very similar to that in phospholipids. These results indicate caution in using the reported crystal structure of 1,2-dilauroyl-*D,L*-phosphatidylethanolamine as the only model for the organization of lipid molecules in biological membranes.

I. Introduction

We have recently used quantum chemical theories [1–3] in conjunction with the NMR properties of dipalmitoyl-lecithin in $CDCl_3$, solutions to conclude that the three-dimensional arrangement of hydrocarbon chains (β and γ) near the glycerol backbone in phospholipids can be divided into two families. In terms of notations and nomenclature of Sundaralingam [4], the first set (SA) has a Θ_3 value of 60° while the second set (SB) has Θ_3 value of 180° (fig. 1). The predicted values of α_1, β_2, γ_1, γ_2 are approximately 180° while β_1 shows two minima in potential energy diagrams which are located

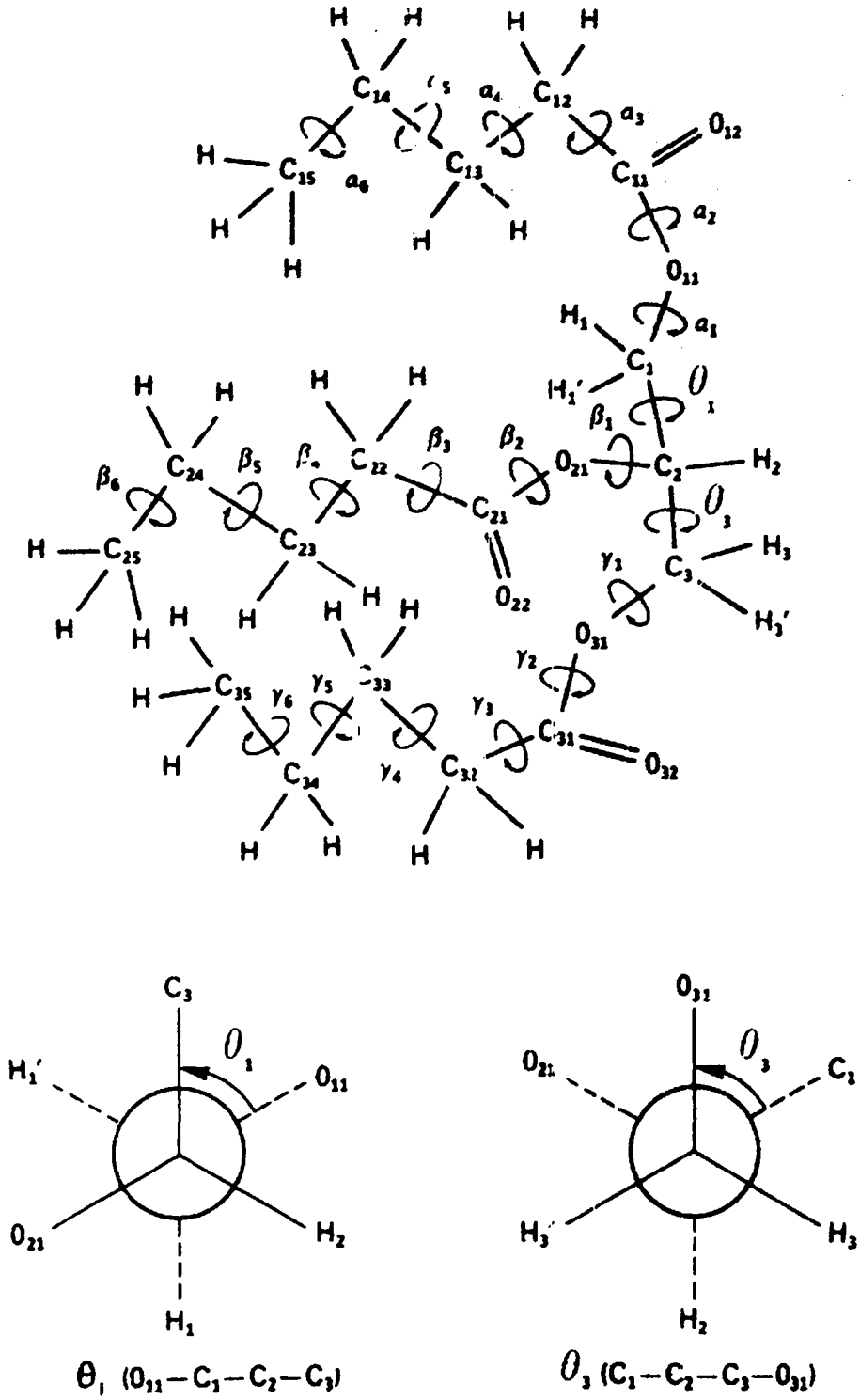

Fig. 1. Molecule of glycerol trivalerate showing α, β and γ chains and nomenclature and notation for dihedral angles and atoms used in this paper. In GTV, α and γ chains are identical. In phospholipids the α chain is replaced by a polar grouping of approximately the same length as in the compound under study. Atom numbers increase as one goes up the chains. The hydrogen atoms are assigned the same numbers as the carbon atom to which they are attached and distinguished whenever necessary, by *H* and *H'*. The torsion angles are considered positive for a right-handed rotation relative to *cis*-planar position and measured as illustrated for angles Θ_1 and Θ_3.

around 100° and 150°. Further down along the hydrocarbon chains, the phospholipid conformations are characterized by a distribution of g^+ (β_n, γ_n – 60°, $n \geq 4$), g^- (β_n, γ_n = 300°) and t (β_n and γ_n = 180°) conformations around C–C–C–C bonds [5,6]. This leads to several distinct conformational states in each set. The stacking of the hydrocarbon chains is a result of interaction between such chains and may be achieved by suitable arrangement with respect to angles β_3 and γ_3 followed by g^+, g^- or t arrangements around the C–C–C–C bonds in the two chains. The t arrangement is preferred over the two g conformations. Conformational energy calculations on phospholipids have also been made using classical potential functions (CPF) and the PCILO method [7–9] and similar conclusions have been reached.

At the time these predictions were made, the crystal structures of two triglycerides [10,11] were known to exist in conformations represented by SA. Since then, the only crystal structure of a phospholipid has been published [12] and has been found to correspond to family SB. Lattice forces in crystals lead to a regular arrangement of molecules and, although the solid state conformation generally has a low potential energy, other structures may coexist in solutions. Indeed, as already stated such a situation is indicated by the NMR properties of dipalmitoyl-lecithin in chloroform solution [2,13]. We have shown by model building [3,14] that both the structural units can be packed in lipid bilayers. Further, a conformational equilibrium between SA and SB may be linked with certain biological properties of biomembranes [14].

Triglycerides (fig. 1) differ from phospholipids in that the non-polar hydrocarbon chain at the α position in the former is replaced by choline or another polar group in phospholipids. Further, in the case of symmetrically substituted triglycerides, we do not have an asymmetric C_2 carbon atom. The α and γ chain are chemically identical and, for each conformer, its enantiomorph (each torsional angle with opposite sign) is equally probable. While this may lead to some conceptual difficulties in relating conformational features of triglycerides to those of phospholipids, the number of unknown conformational parameters in the former are smaller and the interpretation of experimental results becomes simpler. The equivalence of the enantiomorphic forms of triglycerides should be kept in mind since henceforth our discussion and representation of conformational angles will be restricted to the enantiomorph which corresponds to the biologically important *sn* (*L*) stereoisomer of phospholipids.

In this paper we report results on energy calculations and ^1H and ^{13}C NMR of glycerol trivalerate (GTV). The results show that the two families of conformations discussed above for phospholipids are also predicted for triglycerides by CPF calculations and significant proportions of both exist in $CDCl_3$ solutions of GTV.

II. Results of potential energy calculations

A. Details of calculations

In the CPF approach, the interaction energy between a non-bonded pair of atoms is

partitioned into contributions from van der Waals (vW), electrostatic (es) and torsional (tor) potentials. Thus:

$$V = V(\text{vW}) + V(\text{es}) + V(\text{tor})$$

van der Waals energies are estimated from a 6–12 Lennard-Jones potential. Thus:

$$V(\text{vW}) = Vm\, Z^6 (-2 + Z^6)$$

with $Z = R/r$

where r is interatomic distance between the interacting atoms and the summation is carried over all pairs of atoms separated by more than two chemical bonds. Vm and R are Lennard-Jones parameters which depend on the polarizability, effective number of polarizable electrons and the van der Waals radii of the interacting atoms [15–17]. The values for atom-pairs involved in the present calculations are listed in table 1.

Table 1
Lennard-Jones parameters Vm and R for *CPF* calculations

Pair[a]	Vm (kcal/mol)	R (A°)
Cg . . . Cg	0.1197	3.40
Cg . . . Cc	0.1534	3.40
Cg . . . Og	0.1298	3.22
Cg . . . Oc	0.1648	3.22
Cg . . . H	0.1074	2.90
Cc . . . Cc	0.1982	3.40
Cc . . . Og	0.1639	3.22
Cc . . . Oc	0.2092	3.22
Cc . . . H	0.1403	2.90
Og . . . Og	0.1480	3.04
Og . . . Oc	0.1852	3.04
Og . . . H	0.1186	2.72
Oc . . . Oc	0.2322	3.04
Oc . . . H	0.1531	2.72
H . . . . H	0.1222	2.40

[a] Subscript '*g*' denotes C or O atom in glycerol or hydrocarbon chains while '*c*' denotes a carboxyl C or O.

The values for Vm depend on the hybridization of the atom and therefore the C and O atoms in glycerol and fatty acid chains (subscript *g*) have been distinguished from those in C=O groups (subject *c*). Electrostatic interactions are estimated from monopole–monopole approximation by use of charge densities obtained from quantum chemical methods. Torsional potentials are evaluated from empirically assigned barrier heights and potential symmetries based on experimental or theoretical data of

related molecules. In accordance with published reports we chose a three-fold potential:

$$V(\text{tor}) = V1\ (1 + \text{Cos}\ 3\ \phi)$$

with $V1$ = 1.0 kcal/mol for C–C–C–C bond and Θ_1 and Θ_3 rotations and 0.0 kcal/mol for rotations around the bonds represented by $\alpha_1, \beta_1, \gamma_1, \alpha_3, \beta_3$ and γ_3 [17]. A computer program based on the above algorithm and its application to nucleotide conformations has been reported earlier [18]. The program has options to either calculate energy variations as a function of one (potential energy curves) or two neighboring torsion angles (isoenergy contours), or to minimize energy by varying all the torsion angles from a starting structure of the molecule.

The number of calculations involved in energy minimization in a multidimensional space for GTV has been reduced by assuming values of angles for which sufficient information is available from related molecules. The O–C bond in the ester (C–O–CO–C) fragment has a fairly large double bond character which leads to a rigid planar structure for the ester group. Indeed, both quantum chemical calculations and crystal structure data [2,4,7,10–12,17,19] show that the O–C bond in the ester group acquires a planar conformation with α_2, β_2 and $\gamma_2 = 180°$. It is unlikely that these angles differ from the values listed above by more than a few degrees and have been kept constant in these calculations. It is also known that the hydrocarbon chains in long-chain fatty acids and lipids show a preference for the *trans* conformation around C–C–C–C bonds, although a certain population of *guache* states is observed [2,5,6, 20]. Consequently, we have assigned values of 180° for these angles except where the crystal structure data suggest a *guache* conformation. The computational problem is thus reduced to evaluation of angels $\Theta_1, \Theta_3, \alpha_1, \beta_1, \gamma_1, \alpha_3, \beta_3$ and γ_3.

The partial charges on atoms other than those in the ester group are small. Thus, the term V(es) is important only for potential energy behavior with respect to angles α_2, β_2 and γ_2. These angles are, however, kept constant because of the reasons discussed above. In order to save computer time, we have made most of our calculations by neglecting V(es). However, we have checked for several sample cases that the influence of V(es) on the predicted values of angles $\Theta_1, \Theta_3, \alpha_1, \beta_1, \gamma_1, \alpha_3, \beta_3$ and γ_3 is small.

Two starting points have been used in our calculations. These choices have been guided by previous energy calculations [1,2,7,9] and crystal structure data [10–12]. In the first case (referred to as SA and a structure which is similar to the reported crystal structure of triglycerides) we have used $\Theta_1 = 60°, \Theta_3 = 60°, \alpha_1 = 206° \beta_1 = 154°, \gamma_1 = 180°, \alpha_2 = \beta_2 = \gamma_2 = 180°, \alpha_3 = 180°, \beta_3 = 197°, \gamma_3 = 300°, \alpha_4 = \beta_4 = 180°, \gamma_4 = 300°, \alpha_5 = \beta_5 = \gamma_5 = \alpha_6 = \beta_6 = \gamma_6 = 180°$. In the second case (referred to as SB and a structure similar to the reported crystal structure of 1,2-dilauroyl-*DL*-phosphatidylethanolamine) we have used $\Theta_1 = 300°, \Theta_3 = 180°, \alpha_1 = 200°, \beta_1 = 100°, \gamma_1 = 190°, \alpha_2 = \beta_2 = \gamma_2 = 180°, \alpha_3 = 180°, \beta_3 = 280°, \gamma_3 = 160°, \alpha_4 = 180°, \beta_4 = 60°, \gamma_4 = 180°, \alpha_5 = \beta_5 = \gamma_5 = \alpha_6 = \beta_6 = \gamma_6 = 180°$. In each case we have obtained isoenergy contour maps with respect to (Θ_1, Θ_3) and potential energy curves for $\alpha_1, \beta_1, \gamma_1, \alpha_3, \beta_3$ and γ_3. Finally starting from the low

energy regions from these results energy minimization has been carried out to arrive at the minimum energy conformations of GTV. The results are shown in figs. 2–4 and table 2.

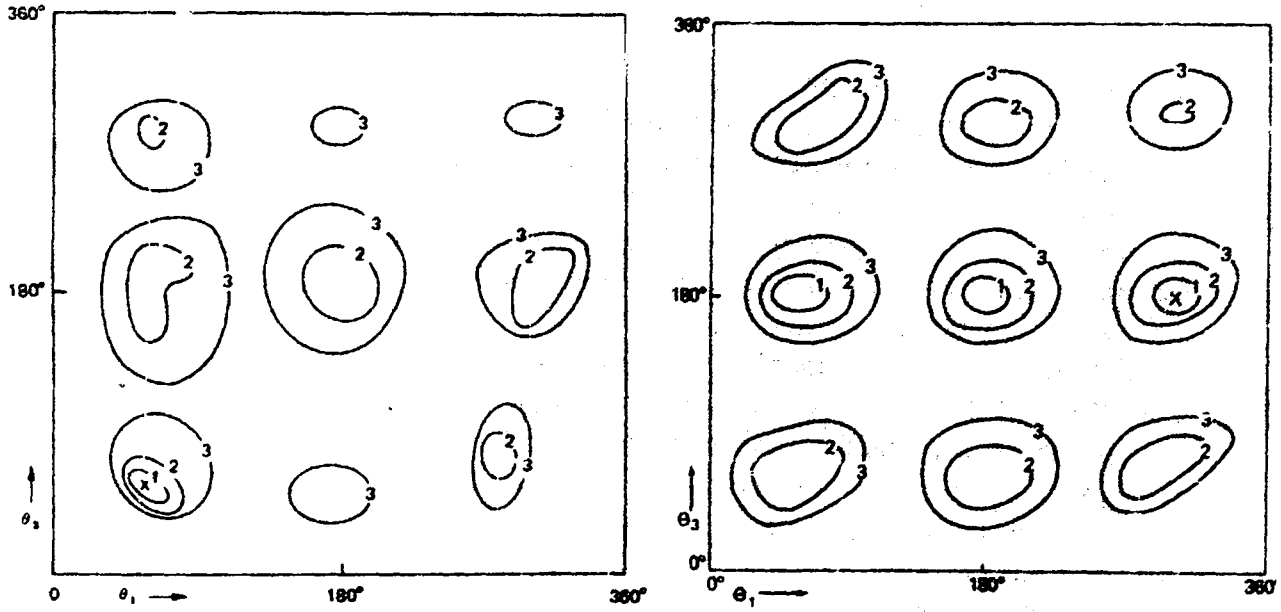

Fig. 2. Two-dimensional isoenergy maps in the (Θ_1, Θ_3) hyperspace. The energies are expressed in kcal/mol relative to the global minimum, which is marked by a 'X' in each case. The contours are drawn by joining points of equal energy in the (Θ_1, Θ_3) space. a: with the remaining dihedral angles as described in the text for SA. b: with the remaining dihedral angles corresponding to the set SB as described in the text.

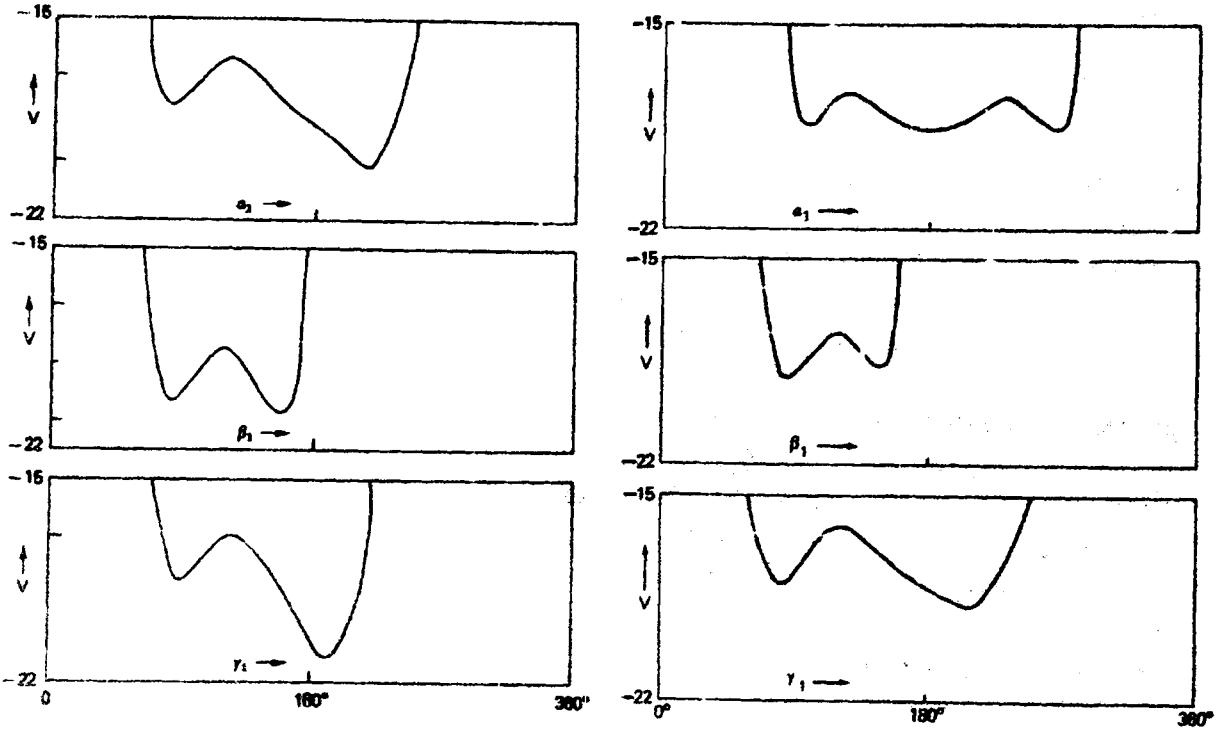

Fig. 3. Potential energy (V) as a function of angles α_1, β_1, γ_1. a: with the remaining torsional angles as in set SA. b: with the remaining torsional angles as in set SB.

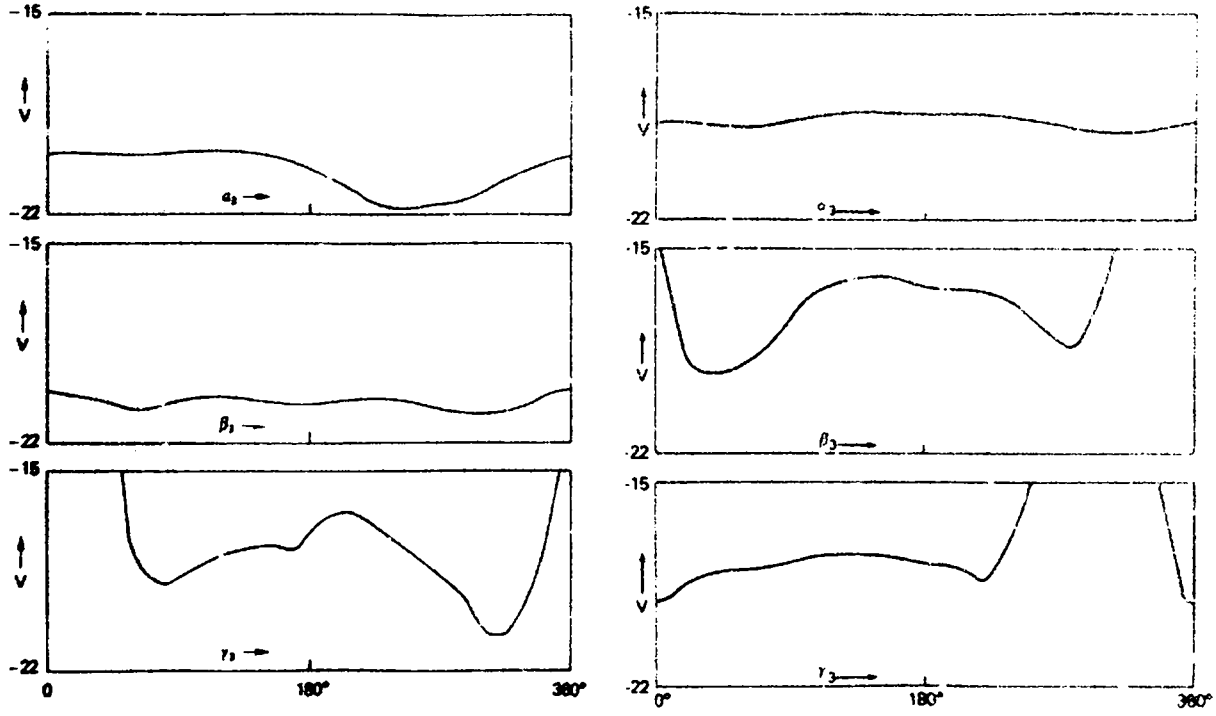

Fig. 4. Potential energy curves with respect to α_3, β_3, γ_3. a: with the remaining torsional angles as in set SA. b: with angles as in set SB.

Table 2
Minimum energy conformations of glycerol trivalerate

Notation	Θ_1	Θ_2	α_1	β_1	γ_1	Energy
CPF theory						
SA	52	46	208	156	186	−22.1
SB	287	155	195	85	194	−20.7
SC	297	69	170	160	160	−22.2
Observed crystal structures						
β-tricaprin	58	50	206	154	172	
1,2-dilauroyl-*DL*-phosphatidyl-ethanolamine	310	182	211	105	190	

All angles are in degrees. Energies are in kcal/mol. As seen from fig. 4 the differences in conformational energies between various staggered conformers with respect to angles α_3, β_3 and γ_3 are small. For the structures listed above the minimum energy conformers have (α_3, β_3, γ_3) values of: SA, 162°, 194°, 300°; SB, 307°, 282°, 147°; SC, 300°, 60°, 60°. The values of α_2, β_2 and γ_2 are restricted to values close to 180° while *trans* conformers are preferred over the *gauche* conformers by about 0.8 kcal/mol for rotations around the C–C–C–C bonds in the hydrocarbon chains.

B. Conformation of the glycerol fragment

The (Θ_1, Θ_3) isoenergy maps with either the SA or SB family of conformations are characterized by a relatively low barrier of rotation. The general features in the two cases are remarkably similar. One observes minimum energy for the 9 staggered conformations with respect to the two C–C bonds (referred to as 'local' minima to distinguish from the deepest or global minimum). The relative energies of the various conformational minima in the two maps are different. For SA type structures the global minimum corresponds to (Θ_1, Θ_3) = (60°, 60°). Other local minima have energies of at least 2 kcal/mol. Starting with the conformational angles of set SB one finds a global minimum at (300°, 180°) and two local minima within 1 kcal/mol located at (60°, 180°) and (180°, 180°). Other local minima in this case also are significantly higher in energy.

C. Conformation with respect to α_1, β_1 and γ_1

The potential energy curves with respect to α_1, β_1 and γ_1 are reminiscent of what has been observed in case of phospholipids [2,7,9]. With respect to α_1 and γ_1 the minimum occurs very close to 180°. In each case local minima with α_1 and γ_1 values of 60° are observed. For the set SB, a minimum around α_1 = 300° is also observed. The behavior with respect to α_1 and γ_1 thus corresponds to the classical *gauche* and *trans* (staggered) conformations of substituted ethanes. In each case, the *trans* arrangement is more stable. On the other hand, the behavior of β_1 curve is significantly different. In both cases (SA and SB), the potential energy curves show a double minimum with β_1 values around 100° and 150°. Of the two cases, global minimum occurs at β_1 = 160° for set SA and around 90° for set SB in agreement with the reported crystal structures of tricaprin [10], trilaurin [11] and 1,2-dilauroyl-*DL*-phosphotidylethanolamine [12].

D. Conformation with respect to α_2, β_2 and γ_2

The structure of the carboxylic ester groups has been extensively studied both experimentally and theoretically [2,4,7–12,19] and values of 180° for these angles are indicated from these findings. As already stated we have accordingly kept these angles fixed in our calculations.

E. Conformation with respect to α_3, β_3 and γ_3

The rotations α_3, β_3 and γ_3 are characterized by a low barrier of rotation and they have only marginal preference for either the *gauche* or *trans* conformation. This observation is consistent with expectation for rotation around a C–C bond attached to a sp^3 hybridized carbon. The position of global minimum varies in each case owing to long range interactions.

F. Conformation around hydrocarbon chains

The conformations around C–C–C–C bonds in hydrocarbon chains have been extensively studied in a recent paper [20]. Both *gauche* and *trans* conformers correspond to local minima but the *trans* states are slightly favored leading to a relatively extended structure in such chains.

G. Energy minimization

Combining the 9 staggered conformations in (Θ_1, Θ_3) space, and two possible values of β_1 (90° and 150°), and other conformation angles chosen as discussed above, we have carried out energy minimization with respect to conformational angles Θ_1, Θ_3, α_1, β_1, γ_1, α_3, β_3 and γ_3. We have found three minimum energy conformational structures within 2 kcal/mol of the global minima. While there are several alternative structures with energies greater than 2 kcal/mol, such structures are not likely to contribute to a significant extent to the structure in solutions. The three conformers are listed in table 2 along with the reported crystal structures for triglyceride and phospholipid [10–12]. It is seen that one of the minimum energy structures (SA) has conformational angles very close to those found in the crystal structure of β-tricaprin. The other structure (SB) has conformational angles for the β and γ chain very similar to the observed crystal structure of 1,2-dilauroyl-*DL*-phosphatidylethanolamine. The third low energy structure (SC) corresponds to the global minimum for phospholipids obtained by the PCILO method [9] but has not been detected so far in any crystal structure.

The minimum energy values of α_3, β_3 and γ_3 are likely to be affected by solvent interactions and crystal forces because of the relatively small conformational preference around these bonds indicated by theory.

III. Experimental

A. Sample preparation

NMR experiments have been made on GTV labeled with ^{13}C at carboxyl carbons as well as on unlabeled material. Both samples were prepared by the condensation of valeric acid with glycerol. The labeled acid was prepared by slowly pouring Grignard mixture, *n*-BuMgBr, on solid $^{13}CO_2$. The mixture was treated with concentrated HCl and then with NaOH and the aqueous phase containing the labeled acid was separated and acidified with HCl. The acid was then washed and dried over $CaCl_2$. The esterification was done by refluxing glycerol with valeric acid in xylene solution using naphthalene sulphonic acid as catalyst. After 8 hr, xylene was distilled out and the residue treated with NaOH and $NaHCO_3$, washed with water and vacuum distilled. The fraction boiling at 123° C at 0.05 mm has been used for NMR.

B. NMR measurements

The ^1H NMR was obtained using a 220 MHz NMR instrument. The ^{13}C NMR was obtained on a 68 MHz instrument using the pulsed Fourier Transform (FT) technique and quadrature phase detection. In each case tetramethylsilane (TMS) was used as internal standard for the measurement of chemical shifts. Positive numbers denote deshielding with respect to TMS. The spectra were recorded in 30% solution in $CDCl_3$ at 20° C, −30° C and in neat liquid. The parameters changed only slightly with solvent and temperature. The results in table 3 give NMR parameters in $CDCl_3$ solution at 20° C.

Table 3
NMR parameters of glycerol trivalerate

(a) ^1H chemical shifts (from tetramethylsilane)

$\delta(H_2)$	= 5.273	$\delta(HA)$	= 4.297	$\delta(HB)$	= 4.152
$\delta(H_{12})$	= 2.327	$\delta(H_{13})$	= 1.611	$\delta(H_{14})$	= 1.345
$\delta(H_{15})$	= 0.925	$\delta(H_{22})$	= 2.332	$\delta(H_{23})$	= 1.611
$\delta(H_{24})$	= 1.355	$\delta(H_{25})$	= 0.925		

(b) ^{13}C chemical shifts (from tetramethylsilane)

$\delta(C_1)$	= 62.179	$\delta(C_2)$	= 69.040	$\delta(C_{11})$	= 173.205
$\delta(C_{12})$	= 33.804	$\delta(C_{13})$	= 27.034	$\delta(C_{14})$	= 22.297
$\delta(C_{15})$	= 13.82	$\delta(C_{21})$	= 172.812	$\delta(C_{22})$	= 33.968
$\delta(C_{23})$	= 27.034	$\delta(C_{24})$	= 22.297	$\delta(C_{25})$	= 13.732

(c) $^1H-^1H$ coupling-constants

$^2J(A,B)$	= −11.9	$^3J(A,H_2)$	= 4.3	$^3J(B,H_2)$	= 5.9
$\Sigma^3J(H_{12}-H_{13})$	= 15.0	$\Sigma^3J(H_{13}-H_{14})$	= 14.4	$^3J(H_{14}-H_{15})$	= 7.2
$\Sigma^3J(H_{22}-H_{23})$	= 14.8	$\Sigma^3J(H_{23}-H_{24})$	= 15.1	$^3J(H_{24}-H_{25})$	= 7.2

(d) Carboxyl $^{13}C-^1H$ coupling-constants:

$^3J(C_1-HA)$	= 2.5	$^3J(C_{11}-HB)$	= 2.8	$^3J(C_{21}-H_2)$	= 3.4
$^2J(C_{11}-H_{12})$	= −7.1	$^2J(C_{21}-H_{22})$	= −7.3	$^3J(C_{11}-H_{13})$	= 4.2
$^3J(C_{21}-H_{23})$	= 4.4				

The reported values are the sum of the two three-bond coupling-constants ($J_{AB} + J'_{AB}$) in the methylene $-CH_2CH_2-$ (AA'BB') fragment. The NMR parameters of ^{13}C and ^1H in α chain are identical with the parameters of the corresponding nucleus in γ chain.

IV. Results of NMR investigations

The ^1H and ^{13}C NMR have been recorded both for unlabeled and ^{13}C labeled GTV.

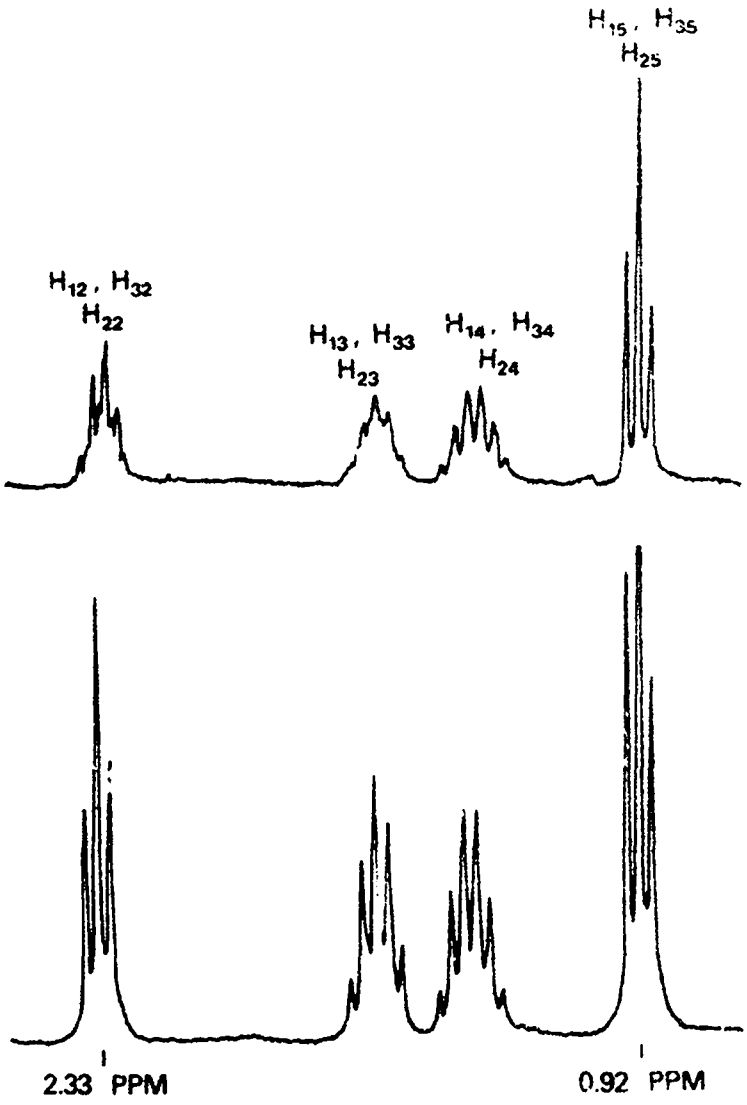

Fig. 5. 220 MHz ^{1}H NMR of the alkyl region in glycerol trivalerate (top trace) and valeric acid (bottom trace). The additional structure in the GTV spectrum arises from the non-equivalence of protons in the β and γ chains.

Typical spectra are shown in figs. 5–7. Of some interest from the viewpoint of NMR is the ^{13}C fine structure in the carboxyl region (fig. 7b) which shows all the long range (two- and three-bond) coupling-constants for α and β carboxyl groups. These couplings also manifest themselves in the proton NMR (fig. 6a) as wings on the resonances from the unlabeled species and therefore a complete assignment of these coupling-constants is possible. The values of two- and three-bond ^{1}H–^{13}C coupling-constants are consistent with those found for related compounds [21]. These coupling-constants along with the proton–proton coupling constants allow several important conclusions on the structure of triglyceride in chloroform solution.

The NMR parameters are listed in table 3.

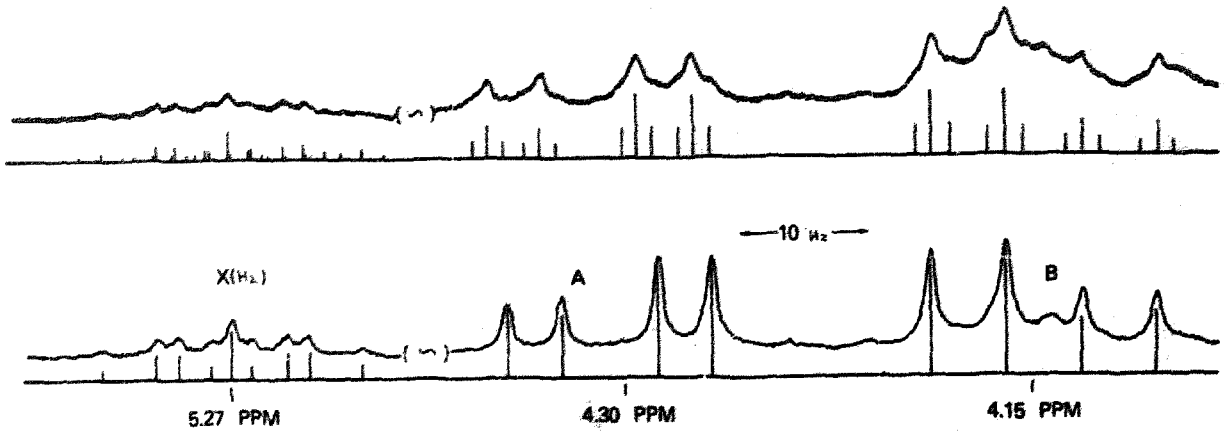

Fig. 6. 220 MHz ^{1}H NMR of the protons in the glycerol moiety of glycerol trivalerate. The top spectrum is for the ^{13}C enriched sample while the bottom spectrum is for the unlabeled sample. The sticks show the calculated spectra on the basis of NMR parameters of table 3.

A. Dynamic motions in GTV

For any of the three conformers listed in table 2, the instantaneous environment of a particular nuclear (^{1}H or ^{13}C) site in the α chain is different from the environment of the corresponding site in the γ chain. Further, since the α and γ chains have identical chemical structure, each conformer can be represented by two equivalent three-dimensional structures which differ by an exchange of the labels for the two chains. We observe identical chemical shifts and coupling-constants for a nucleus in the α chain and the corresponding nucleus in the γ chain. In other words NMR parameters for nuclear pairs such as (C_1, C_3), (C_{11}, C_{31}), (C_{12}, C_{32}), (H_{12}, H_{32}) etc. are identical. This indicates that the α and γ chains flip at a rate which is fast on the NMR time-scale and one observes an average of NMR signals from the two equivalent structures discussed above (flip rates greater than approx. 10^3/sec). It may also be noted that if more than one conformer exists in solution, then there are fast internal rotations around the appropriate chemical bonds such that the observed NMR spectrum is time-averaged over the rotational isomers. The two molecular motions (chain flips and internal rotations) are of fundamental importance in the interpretation of the NMR properties of GTV discussed below and lead to an 'apparent symmetry' in the molecule. Some of the NMR signals from the β chain are resolved from the corresponding signals in α and γ chains showing a non-equivalent physicochemical environment of nuclear sites in the β chain, even in the presence of motions discussed above.

The molecular mechanisms responsible for chain flipping and averaging over conformational states around various chemical bonds are likely to be very similar. Low barriers to internal rotations around several chemical bonds in GTV are probably responsible for both types of averaging. For the convenience of the following discussion, we find it useful to distinguish between the two types of averaging.

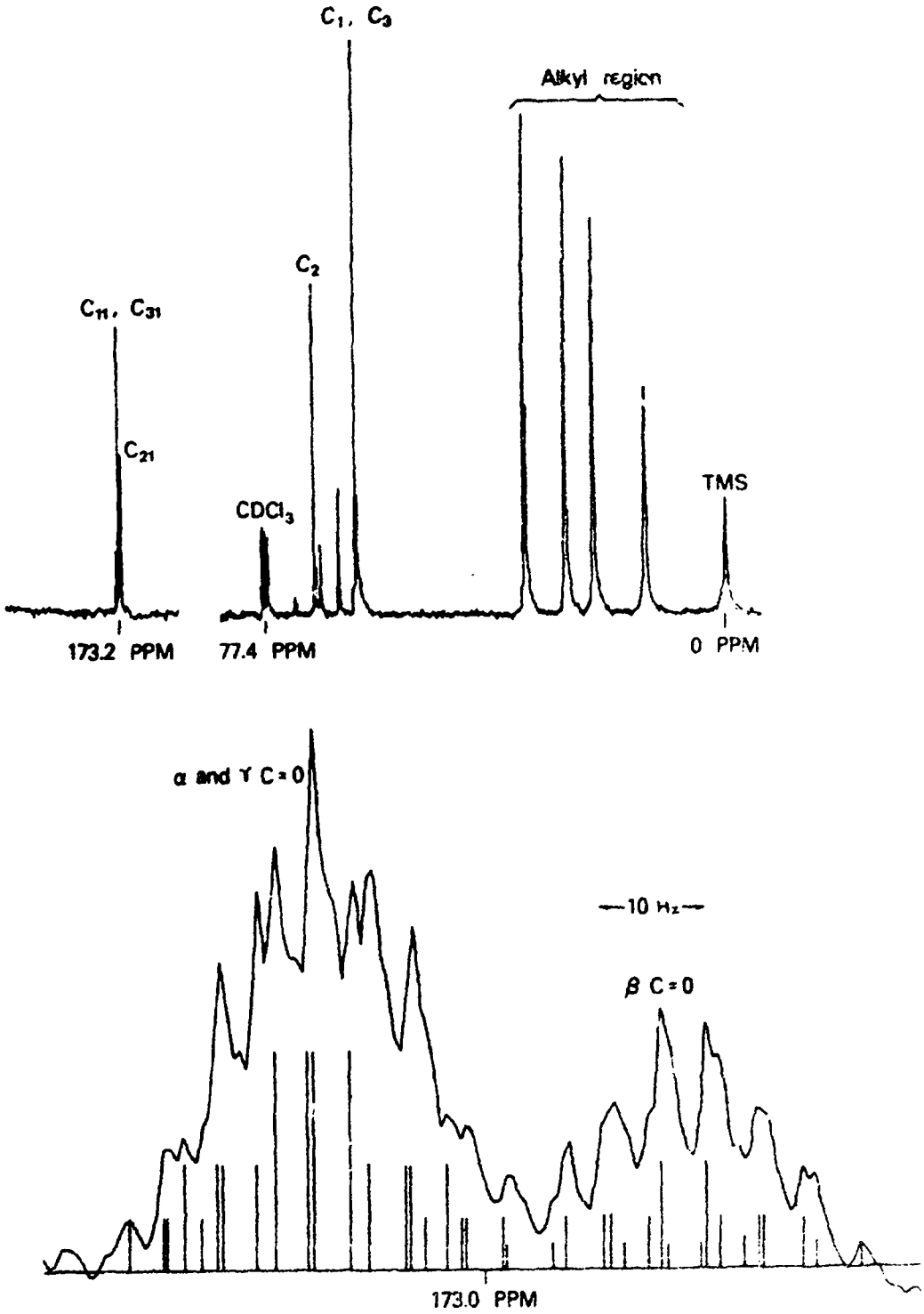

Fig. 7. 68 MHz ^{13}C NMR for glycerol trivalerate. a: complete ^{13}C spectral region for unlabeled sample. b: an expansion of the carboxyl ^{13}C region for GTV enriched with ^{13}C at both α and β carboxyl positions.

B. Structure with respect to (Θ_1, Θ_3)

The conformational structure with respect to these two rotations is the most important factor in deciding the overall lipid structure since these two angles control the relative orientation of the α and β chains both in triglycerides and phospholipids. Information on these angles can be obtained by looking at the ^{1}H NMR of the glyceroi fragment (the 5-spin system consisting of protons on C_1, C_2 and C_3). Averaging over chain flipping and internal rotations leads to a NMR spectrum which corresponds to a A_2B_2X system with H_3 and H_3' being identical to H_1 and H_1', respectively, but pairs (H_1, H_1') and (H_3, H_3') are non-equivalent. Analysis of the spectra by the standard LAOCOON method [22] gives the chemical shifts and coupling-constants involving protons A,B and X. The calculated and observed ^{1}H spectra of the glycerol fragment are shown in fig. 6. While the assignment of X to H_2 is straightforward, it is difficult to decide whether the pair (H_1, H_3) correspond to A and (H_1',H_3') to B (referred to as assignment 1), or vice versa (assignment 2). For this reason, no assignments have been made for A and B in table 3.

The observed NMR pattern of the 5 proton spins in the glycerol fragment can be explained in terms of chain flipping and conformational equilibria through the help of Newman projection diagrams for the three staggered structures (g^+, t and g^-) around the two C–C bonds (Θ_1 and Θ_3) (fig. 8). There is a very interesting vicinal relationship between the projections with respect to Θ_1 and Θ_3, under the conditions of rapid chain flips (which result in equivalence of atom-pairs (O_{31}, O_{11}) and (C_1, C_3)). Under these conditions, one finds that a g^- projection with respect to Θ_1 is identical to a g^+ projection with respect to Θ_3 (labeled I). Similarly, a g^+ projection with respect to Θ_1 is identical to a g^- projection with respect to Θ_3 (labeled III). The t projections with respect to the two angles are identical (labeled II). If there was a free rotation around the two C–C bonds or if the three staggered forms were equally populated then one expects an equivalence of proton pairs (H_1, H_1') and (H_3, H_3'). A non-equivalence in these geminal protons indicates conformational preferences around these bonds.

The three-bond proton–proton coupling-constants, $J(H_1, H_2)$ and $J'(H_1', H_2)$, for each of the three structures (I, II and III) in fig. 8 can be estimated using rules governing the influence of electronegative substituents on proton pairs in *gauche* and *trans* relationships [13,23]. On this basis the values of component coupling-constants are given in fig. 8. The values of these empirically derived component coupling-constants are consistent with those experimentally found in systems where bond torsion angles are known precisely [23–25]. While there is difficulty in assigning the two magnetically non-equivalent protons [H and H'] in the NMR spectra to A and B above, it is evident that the observed results cannot be reconciled with the presence of a single conformation in solutions. The observed crystal structure of β-tricaprin, or the theoretically calculated low energy conformer SA_3, has a g^+ conformation with respect to both Θ_1 and Θ_3. Such a structure leads to equal contributions from projections I and III. The expected values for the coupling-constants for this structure are therefore

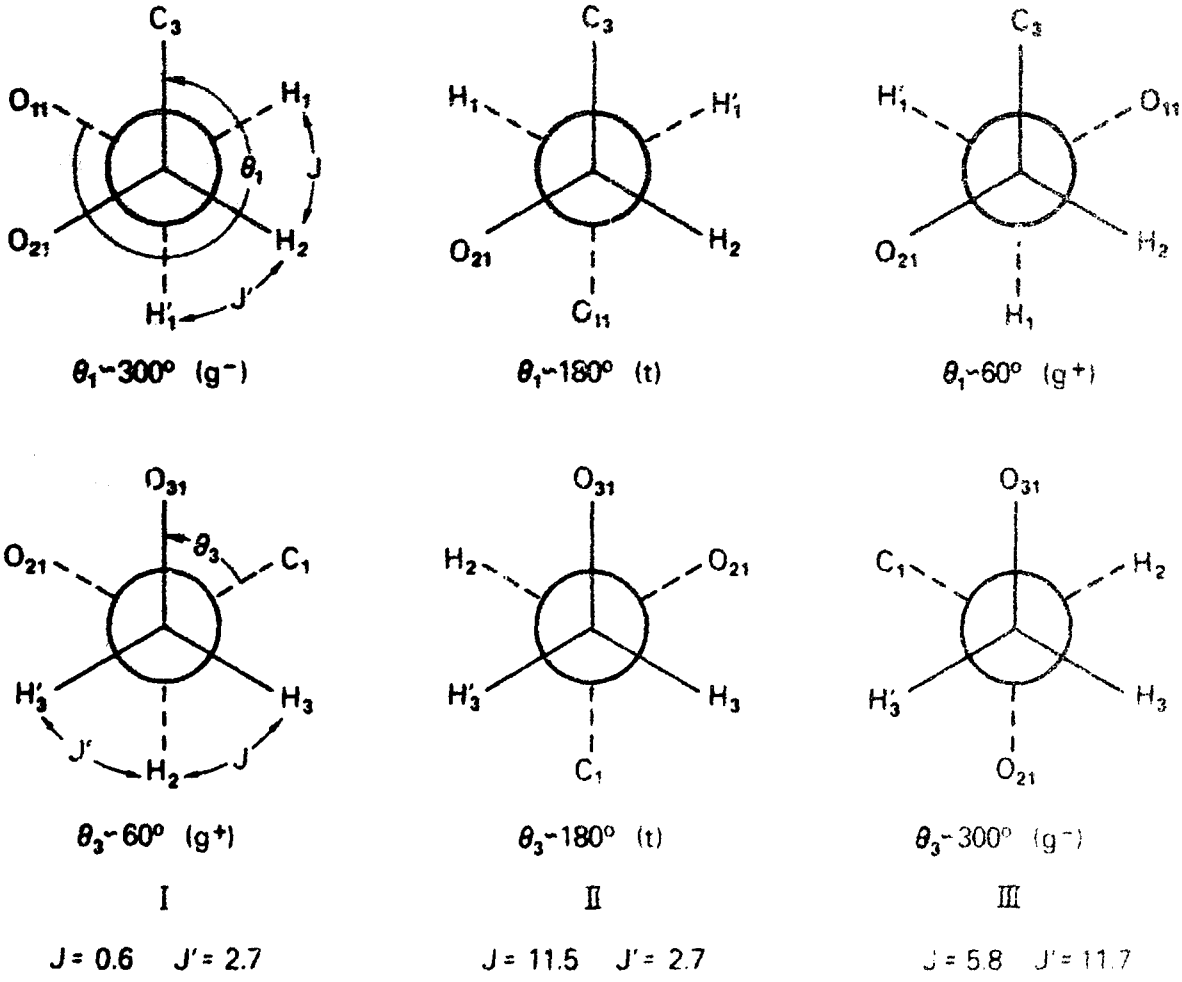

Fig. 8. Newman projections around the bonds Θ_1 and Θ_3 in GTV and values of component coupling-constants. The projections with respect to Θ_1 and Θ_3 are related and the projections can be transformed by simply putting $\Theta_1 = -\Theta_3$ and replacing atoms on γ chain by corresponding atoms on α chain.

3.2 Hz for J and 7.2 Hz for J'. On the other hand, structure SB, or the crystal structure conformation of dilauroyl phosphatiydylethanolamine, has a g^- conformation with respect to Θ_1 and t conformation with respect to Θ_3 (projections I and II respectively). The expected values of J and J' for SB are, therefore, 6.1 and 2.7 Hz, respectively. The third low energy structure (SC) predicted by theory has both α and γ chains as depicted in projection I and the coupling-constants expected from this structure are 0.6 and 2.7 Hz. The observed values of coupling-constants are J = 4.3 Hz and J' = 5.9 Hz (assignment 1) or J = 5.9 Hz and J' = 4.3 Hz (assignment 2). In either case, the experimental data indicates that the amount of SC is negligible, and significant populations of SA and SB are present. Using standard equations relating the observed time-averaged coupling constants to the fractional populations of SA and SB and the coupling-constants for these structures, one can calculate the relative popula-

tions of SA and SB. Assignment 1 gives SA : SB 70 : 30 while the alternative assignment gives SA : SB 30 : 70. Knowledge of proper assignment can help to determine which of the two forms is more populated in $CDCl_3$ solutions. However, the important and unquestionable fact which emerges from these results is that significant amounts of both SA and SB are present while SC is absent in solutions. It is possible to determine the actual assignment of A and B protons using three-bond coupling-constants between C_1 and the two protons under discussion. This in turn requires labeling at the C_1 position.

It may be pointed out that the above analysis of NMR results is based on two reasonable assumptions: (1) that the values of component coupling-constants can be predicted with confidence using the rules used here, and (2) that only the three low energy conformers predicted by CPF theory are important in the conformational equilibria. Errors in the first assumption can lead to differences of a few per cent in the estimated population distributions but will not invalidate the important conclusion made here that structures SA and SB coexist in solutions with significant populations while the population of SC is small. Support for the second assumption comes from energy calculations and reported crystal structures. However, alternative interpretations of the NMR results are possible if other conformers in the (Θ_1, Θ_3) space are present in significant proportion in solutions.

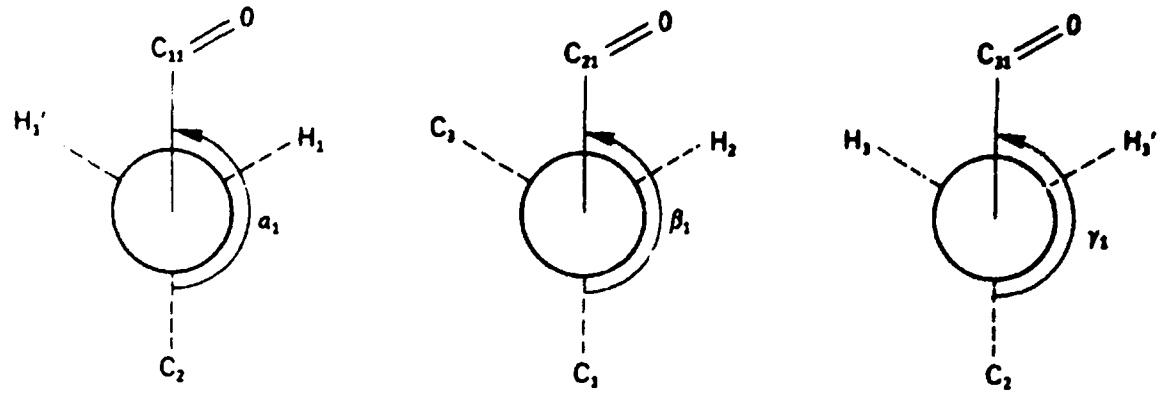

Fig. 9. Newman projections with respect to bonds α_1, β_1 and γ_1 showing stereochemical relationship between various atoms.

B. Structure with respect to α_1, β_1 and γ_1

Information about these angles may be obtained using the three-bond coupling-constants involving carboxyl ^{13}C and ^1H (A,B or H_2) as indicated in fig. 9. These coupling-constants are obtained by combining information from the ^1H and ^{13}C NMR of enriched GTV. Figure 7b shows the proton undecoupled ^{13}C NMR in the carboxyl region for the enriched sample of glycerol trivalerate. Because of chain flipping the α and γ carboxyl groups resonate at a common frequency but the β C=O resonates at a

slightly higher field. The hyperfine splittings of the two group of lines contain information on all the two- and three-bond $^{1}H-^{13}C$ coupling-constants involving carboxyl ^{13}C. The complete assignment is aided by the proton spectrum of the labeled GTV. For example, in the A_2B_2X spectrum of the glycerol fragment (fig. 6b), the ^{13}C satellites appear as wings of the ^{12}C component and the three-bond coupling-constants with these protons can be estimated. It is found that the protons A and B show slightly different coupling-constants. In a similar fashion the remaining two- and three-bond coupling-constants can be estimated from a comparison of the proton and ^{13}C NMR of the labeled and unlabeled samples. A comparison of the calculated and observed NMR spectra with the coupling-constants thus estimated is shown in figs. 6 and 7b.

The magnitudes of three-bond carboxyl $^{13}C-^{1}H$ coupling-constants and their stereochemical dependence has been studied recently [26,27]. Lemieux et al. [26] have obtained a calibration curve from which dihedral angles can be estimated for a rigid conformation. Esperson and Martin [27] have estimated $Jg = 1.3$ and $Jt = 9.8$ Hz for amino acids. Qualitatively, it is clear that the observed three-bond α carboxyl $^{13}C-^{1}H$ coupling-constants for the protons A and B of 2.5 and 2.8 Hz are consistent with values of torsion angles α_1 and γ_1 close to 180°. Such a conformation places both the protons in a situation where the two couplings are expected to be low and equal. As seen from fig. 9, large deviations from this angle will result in unequal and significantly different values for the three-bond coupling-constants. We have examined several possibilities to explain the slightly larger observed J values than the values of 1.3 Hz expected for α_1 and γ_1 of 180° on the basis of observed values in amino acids [27]. Using values of α_1 and γ_1 predicted by CPF theory for structures SA and SB and time-averaging does not predict values very different from the estimates for α_1 and $\gamma_1 = 180°$. On the other hand a contribution of approximately 16% from conformational states having α_1 and γ_1 of 60° (the other low energy regions predicted by theory) can explain the observed results quantitatively. It may however be pointed out that at this stage information on the substituent effects on three-bond $^{1}H-^{13}C$ coupling-constants is not yet available, and for this reason one cannot derive precise quantitative information from such data. At the same time, it is clear that the time-averaged values of α_1 and γ_1 are not very different from 180°.

Conclusions regarding β_1 from NMR data are less definitive since there is only one three-bond coupling-constants – $J(C_{21}-H_2)$ which can be used to follow the stereochemistry around this bond. From Lemieux curve [26], a coupling-constant of 3.4 Hz corresponds to a dihedral angle ($H_2-C_2-O_{21}-C_{21}$) of ± 30° or ± 140°. These values correspond to 4 values of β_1: 90°, 150°, 260° and 340°. Conformations with β_1 values of 260° or 340° are sterically hindered and such states are unlikely to occur. On the other hand, β_1 values of 90° and 150° correspond very closely to the two minimum energy conformations SA and SB predicted by theory. In some of the previously reported lipid models a β_1 value of 120° has been suggested. Such a value is equivalent to a dihedral angle of 0° along the $H_2-C_2-O_{21}-C_{21}$ pathway (fig. 9). The value of coupling-constant expected from such models (6–7 Hz) is considerably higher than the experimental value of 3.4 Hz.

C. Conformational angles α_1, β_2, γ_2, α_3, β_3, γ_3

No information about these rotations can be obtained from the coupling-constant data.

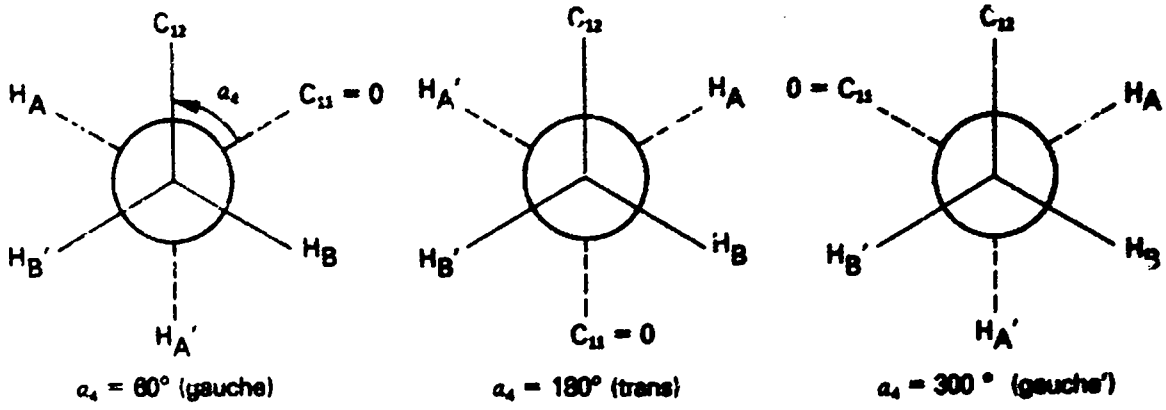

Fig. 10. Projection diagrams for α_4. The two *gauche* forms are similar. In the NMR spectrum one measures the time-average of the sum of couplings between protons on vicinal carbon atoms labeled A, A', B and B' and referred to in text as $J + J'$. Similar projection diagrams can be drawn for staggered conformations around other C–C–C–C bonds in the hydrocarbon chains of GTV.

D. Structure with respect to α_4, β_4 and γ_4

It is possible to obtain information on these bond rotations from three-bond ^1H–^1H coupling-constants, via the H–C–C–H pathway, as well as from the three-bond ^{13}C ^1H coupling-constants via the C–C–C–H (fig. 10) pathway. Significant differences in chemical shifts are noticed as one goes along the hydrocarbon chains and all 4 groups of methylene and methyl resonances are well separated from one another at 220 MHz. As is expected the chemical shifts for the 4 methylene resonances for α and γ chains are identical but differ slightly for the β chain. The spectra are fairly complex but analyzable, particularly since one can use the spectrum of valeric acid as a guide. It is found that each of the methylene group resonances is a superimposition of two components, one from the α and γ chains and the other from the β chain. The two groups have slightly different chemical shifts and coupling-constants. The 4 protons in the CH_2–CH_2 fragment form a AA'BB' type component of a 9-spin system arising from the methylene and methyl protons in each chain. The value of the sum of the two three-bond ^1H–^1H coupling-constants ($J + J'$) with respect to the bonds α_4 and β_4 could be obtained since the β and α protons of the different methylene groups are sufficiently separated. The values of these coupling-constants were found to be 14.8 Hz in the β chain and 15.0 Hz in the α chain. These values are very close to the measured values for valeric acid (14.9 Hz). Using the electronegative rules [13,22] one expects $J + J'$ to be 18.1 Hz (fig. 10) in a CH_2–CH_2 fragment if the conformation with respect to the C–C–C–C bond is *trans* (α_4 or β_4 =

180°) and 10.2 Hz if the conformation is *gauche* or *gauche'* (α_4 or β_4 = 60° or 300°). In absence of any conformational preference one expects equal (33%) contributions from the *guache, trans* and *guache'* conformer leading to a time-averaged value of 12.8 Hz for $J + J'$. The observed values of $J + J'$ correspond to a 58% population of *trans* conformer with respect to β_4 and 60% population with respect to α_4. The values of three-bond carboxyl $^{13}C-^{1}H$ coupling-constants give similar results. For a *trans* conformer the expected three-bond $^{13}C-^{1}H$ coupling-constants are 1.3 and 1.3 Hz [27] while the values are 1.3 and 9.8 Hz for *gauche* or *gauche'* conformers. The observed values in β and γ chains (4.2 and 4.4 Hz) indicate presence of approximately 69% of *trans* conformer for rotations represented by α_4, β_4 and γ_4. These results therefore indicate a definite preference for the *trans* arrangement along the C–C–C–C bonds with a free energy difference of about 800 cal/mol. Such a free energy difference is comparable to the observed values in liquid *n*-pentane [28,29], polythene [20] and phospholipids [5].

E. Structure with respect to α_5, β_5 and γ_5

Again information on these rotations can be obtained from the three-bond coupling-constants in the methylene fragments. As in the case of α_4 and β_4 the value of the observed $J + J'$ is 15.2 Hz indicating a population of 62% for the *trans* structure with respect to these rotations.

F. Structure with respect to $\alpha_6, \beta_6, \gamma_6$

The terminal methyl proton resonance for α, β and γ chains shows a single triplet with a coupling-constant of 7.2 Hz. This value is very close to the expected value of 7.5 Hz [13] for equal populations of the three staggered forms around these bonds.

V. Conclusions

The NMR and energy calculations presented here lead to the following conclusions for the conformation of GTV. (1) One finds two families of conformations, SA and SB, for glycerol fragment in GTV characterized by (g^+, g^+) and (g^-, t) arrangement with respect to angles (Θ_1, Θ_3). The existence of the two families of conformations is therefore a common property of GTV and phospholipids. (2) The torsional angles α_1 and γ_1 are close to 180° while β_1 is close to 150° in SA and 85° in SB. (3) The hydrocarbon chains in GTV can acquire both *gauche* and *trans* conformation with respect to C–C–C–C bonds but the *trans* conformers are preferred by almost 0.8 kcal/mol. (4) A close similarity between the conformations of phospholipids and triglycerides in $CDCl_3$ solutions is indicated, contrary to the inferences which may be drawn from the limited data on crystal structures of these lipids.

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