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A semi-empirical conformational analysis of the interaction of *n*-alkanols with dipalmitoylphosphatidylcholine

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The interaction of a homologous series of saturated aliphatic *n*-alkanols (containing 1–13 carbon atoms) with dipalmitoylphosphatidylcholine was studied by a semi-empirical conformational analysis. The minimal conformational energy of the isolated molecule at the hydrocarbon-water interface was calculated as the sum of the contributions resulting from the Van der Waals, torsional, electrostatic and transfer energies. From the conformers of minimal energies were calculated the hydrophilic-hydrophobic balance, the distance between hydrophilic and hydrophobic centres and the energies of interaction between homologous alkanols and between alkanols and lipids. Using these parameters, different modes of conformation, orientation and interaction of *n*-alkanols and dipalmitoylphosphatidylcholine were described. For methanol, ethanol and *n*-propanol, the *gauche* conformers were the most probable interacting only with the lipid polar heads. Only all-*trans* conformers were obtained for alkanols with longer acyl chains. *n*-Butanol to *n*-octanol form clusters in the lipid matrix. Longer *n*-alkanols are randomly distributed in the lipid layer. However, due to the increase in hydrocarbon chain-length, *n*-nonanol and higher alkanols have an interaction energy identical or superior to that found in a pure lipid monolayer, leading to a more ordered alkanol-lipid organization.

Introduction

The molecular mechanisms of general anesthesia are unknown, even though it has been suggested that general anesthetics interact either directly with non-polar sites on excitable proteins [1,2] or affect the dynamic properties of the lipid membrane, which in turn perturb the biological activities of the excitable proteins. The latter received large attention because several theories of general anesthesia point out the correlation of the anesthetic potencies of various unrelated organic molecules with their membrane solubility or their octanol-water coefficient [3,4]. Alkanols have been widely studied in this context. It has been shown that alkanols at nerve-blocking concentrations expand biological membranes [5] or disturb order-parameters associated with the lipid [6,7]. In artificial lipid bilayers, both lipid disorder [8,9] and/or lipid phase transition [10,11] are affected in a continuous and monotonous fashion throughout the series of saturated aliphatic *n*-alkanols, up to $n = 12$, consistent with the anesthetic potency. However, superimposed on a pattern of similarity are also differences among the various substances. These differences require detailed examination of

Abbreviations: C_n, C represents a normal saturated aliphatic alkanol, where n is the number of carbon atoms; DPPC, dipalmitoyl-DL-α-phosphatidylcholine.

particular anesthetics at the molecular level. The mode of interaction of *n*-alkanols and lipids is unknown. Due to the variation of the amphiphile nature of the alkanols as a function of the chain-length, it is likely to change from a one alkanol to the other. This would in turn modify the influence on the lipid dynamics and on the anesthetic potency. The cut-off observed in anesthetic potency and in the effects on lipid dynamics for *n*-alkanols with $n \geqslant 12$ provides evidence for such a phenomenon [9]. Studies of their interaction energy with phosphatidylcholine and of their disturbing effects on the lipid organization provide other indications of discontinuities in the properties of *n*-alkanols in interaction with lipid bilayer [11,12].

In the present communication, the interaction of a homologous series of saturated aliphatic alkanols (C_1-C_{13}) with dipalmitoyl-DL-α-phosphatidylcholine (DPPC) is studied by a semi-empirical conformational analysis. This technique was developed to calculate the conformation of phospholipids [13] and other amphiphilic molecules [14–17], taking into account the anisotropic conditions of the interface. It takes into account not only the Van der Waals energies but also electrostatic and torsional energies. To simulate the lipid-water interface, the electrostatic energy is calculated as a function of changes in the dielectric constant. This procedure makes it possible to calculate at the interface, the hydrophobic-hydrophilic balance, the distance between hydrophobic and hydrophilic centres and the energies of interaction between homologous alkanols and between alkanols and lipids. The ultimate goal is to obtain information on the localization and conformation of alkanols in the DPPC matrix.

Methods

We proceeded in two steps. First, the structure of the isolated molecule was calculated at the lipid-water interface. Second, the alkanol molecule was inserted into the lipid monolayer.

Isolated molecule

The conformation of the isolated molecule and its orientation at the lipid-water interface has been established as described elsewhere [13,16,17]. The total conformational energy is calculated as the sum of the following terms.

(1) The London-Van der Waals energy of interaction between all pairs of non-mutually-bonded atoms. Buckingham's pairwise atom-atom interaction functions have been used

$$E^{\mathrm{VdW}} = \sum_{ij} \left[A_{ij} \exp(-B_{ij} r_{ij}) - C_{ij} r_{ij}^{-6} \right] \tag{1}$$

where $ij = 1,2, \ldots$ are non-bonded atoms, r_{ij} their distances from each other, and A_{ij}, B_{ij} and C_{ij} are coefficients assigned to atom pairs. The values of these coefficients have been reported by Liquori and co-workers [18,19]. Like other quantum-mechanical results, these values emerge in part as the solution of the Schrödinger equation and in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides and lipids [13–17,20,21]. In order to compensate for the decrease of the function E^{VdW} at small r_{ij}, we have imposed an arbitrary cut-off value of

$$E^{\mathrm{VdW}} = 100 \text{ kcal/mol at } r_{ij} < 1 \text{ Å}$$

(2) The generalized Keesom-Van der Waals interaction or electrostatic interaction between atomic point charges:

$$E^{\mathrm{cb}} = 332 \left(\sum_{ij} \frac{e_i e_j}{r_{ij} \epsilon_{ij}} \right) \tag{2}$$

where e_i and e_j are expressed in electron charge units and r_{ij} in Å. ϵ_{ij} is the dielectric constant. The values of atomic point charge are similar to the values used for polypeptides [20,21].

(3) The potential energy of rotation of torsional angles. This rotation around the C-C or C-O bonds was calculated by the equation:

$$E^{\mathrm{Tor}} = \frac{U_{ij}}{2} \cdot (1 + \cos \phi_{ij}) \tag{3}$$

where U_{ij} corresponds to the energy barrier in the eclipsed conformation during the rotation of the angle, and ϕ_{ij} is the torsional angle. U_{ij} is equal to 2.8 kcal/mol for the C-C bond and 1.8 kcal/mol for the C-O bond [22].

(4) The transfer energy of each part of the molecule. The values of the transfer energies used are similar to those determined experimentally by

numerous authors, as summarized elsewhere [23].

In the calculation procedure, six changes of 60° each were first imposed to each of n torsional angles, yielding 6^n conformers. The conformational energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy, i.e., those with a statistical weight of at least 1%. The values used for the valence angles and bond lengths were those currently used in conformational analysis [22]. After systematic analysis, conformations selected for their lowest internal energy were submitted to a simplex minimization procedure [24]. To simulate the membrane interface, we assumed a dielectric constant equal to 3 above the interface, while the atom at the bottom of the lipid configuration was fixed at a plane where the dielectric constant was assumed to be 30. Between these two planes, the dielectric constant was assumed to increase linearly along the z-axis perpendicular to the interface. The molecule is finally oriented with the line joining the hydrophilic and hydrophobic centres of gravity perpendicular to the interface [16,17] (Fig. 1). The hydrophilic centre of gravity ($\vec{C}_w$) is defined by the following equation:

$$\vec{C}_w = \sum_{i=1}^{n} \left(E^+_{\text{transfer}_i} \vec{r}_i\right) / \sum_{i=1}^{n} E^+_{\text{transfer}_i} \tag{4}$$

in which $\vec{r}_i$ are the coordinates of the i atom. The hydrophobic center located in the hydrocarbon domain ($\vec{C}_{Hc}$) is defined by the same equation, except that the negative transfer energies are taken into account. The interface position ($\vec{I}$) is defined by the equation:

$$\frac{\sum_{i=1}^{n} E^+_{\text{transfer}_i}}{\vec{C}_w - \vec{I}} = \frac{\sum_{j=1}^{m} E^-_{\text{transfer}_j}}{\vec{C}_{Hc} - \vec{I}} \tag{5}$$

Conformation of the drug molecule inserted into the lipid monolayer [13,16,17]

The procedure of alkanol insertion can be summarized as follows.

(a) The position of alkanol (orientation of the isolated molecule) was modified along the x-axis. Each distance was equal to 0.5 Å. For each separating distance a rotation angle of 30° was imposed to alkanol around its own z-axis and around the lipid. Among 14 400 possible orientations, only the structure of minimum energy was considered.

(b) Lipid was fixed and alkanol was allowed to move along the z-axis perpendicular to the lipid-water interface. Again only the structure of minimum energy was considered. Alkanol had the possibility of changing its orientation around the z-axis as compared to the lipid. This procedure allowed the probable packing of alkanol and lipid molecules to be defined. Then the packing of these two molecules was maintained, and the orientation of a third lipid molecule around them was considered. Because this was time-consuming, we limited our approach to the number of lipid molecules sufficient to surround the alkanol.

When the configuration of the cluster of m molecules was determined, both areas occupied by each molecule and the intermolecular area were estimated after projection on the x-y plane, and the mean molecular area was calculated. This procedure has been used to evaluate the structure of dipalmitoylphosphatidylcholine organized in bilayers. An excellent agreement [13] was obtained between the predictions and the neutron diffraction data. The position of the lipid molecules was localized with a precision which was within the limit of the experimental error. Calculations were made on a CDC-Cyber 170 Computer coupled to a Calcomp 1051 drawing table with a Pluto draw-

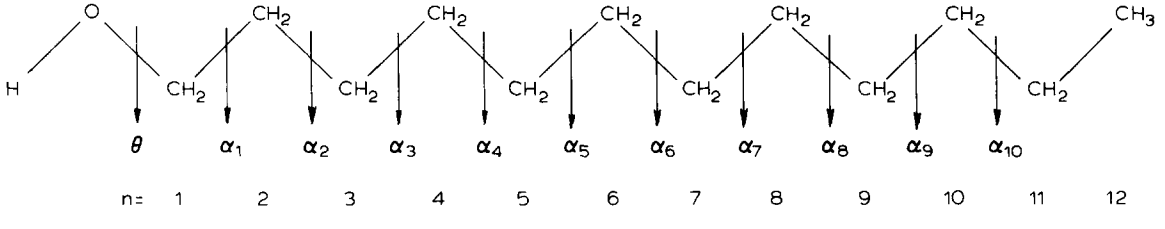

Fig. 1. Chemical formula for the n-alkanols and numbering of the torsional angles.

ing program (Motherwell, S. and Clegg, W. (1978) PLUTO, Cambridge, U.K.).

Results

Isolated molecules

The *n*-alkanols present a number of rotational angles which is a function of the carbon atom number *n* included in the linear hydrocarbon chain. If $n = 2$, the number of torsional angles is equal to 1. For each increase of 1 carbon atom in the hydrocarbon chain, the number of torsional angles increases also by 1 unit as illustrated in Fig. 1,

TABLE I

TORSIONAL ANGLES OF THE MOST PROBABLE *n*-ALKANOL CONFORMERS AFTER SYSTEMATIC ANALYSIS

		θ	α_1	α_2	α_3	α_4	α_5	α_6	α_7	α_8	% [a]	ΔE [b]
C_2	A	120	–	–	–	–	–	–	–	–	54.1	0.0
	B	180	–	–	–	–	–	–	–	–	22.1	0.79
C_3	A	180	120	–	–	–	–	–	–	–	47.2	0.0
	B	180	180	–	–	–	–	–	–	–	26.3	0.164
C_4	A	180	180	180	–	–	–	–	–	–	42.9	0.0
	B	180	180	120	–	–	–	–	–	–	34.1	0.541
C_5	A	180	180	180	180	–	–	–	–	–	42.1	0.0
	B	180	180	180	120	–	–	–	–	–	34.1	0.519
C_6	A	180	180	180	180	180	–	–	–	–	41.5	0.0
	B	180	180	180	180	120	–	–	–	–	34.1	0.495
C_7	A	180	180	180	180	180	180	–	–	–	40.3	0.0
	B	180	180	180	180	180	120	–	–	–	34.1	0.477
C_8	A	180	180	180	180	180	180	180	–	–	39.5	0.0
	B	180	180	180	180	180	180	120	–	–	34.1	0.461
C_9	A	180	180	180	180	180	180	180	180	–	39.3	0.0
	B	180	180	180	180	180	180	120	120	–	34.8	0.458
C_{10}	A	180	180	180	180	180	180	180	180	180	38.8	0.0
	B	180	180	180	180	180	180	180	180	120	35.3	0.452
C_{11}	A	180	180	180	180	180	180	180	180	180	38.6	0.0
	B	180	180	180	180	180	180	180	180	120	35.8	0.458
C_{12}	A	180	180	180	180	180	180	180	180	180	38.4	0.0
	B	180	180	180	180	180	180	180	180	120	36.3	0.444

[a] The probability of existence is given by a Boltzman distribution.

[b] ΔE is the energy above minimal values (kcal/mol)

which gives the numbering of the carbon atoms and of the torsional angles.

A first systematic study was performed on each angle of the hydrocarbon chain (with a maximum of nine torsional angles), enabling two conformers of maximum probability to be designed (Table I). These two conformers represent approx. 75% of all the possible conformers. For alkanols with $n \leqslant 3$, the most probable structure is a *gauche-* (g) conformer containing the g^+- and g^--conformers. In the classical nomenclature, the conformations *trans (t)* and *gauche* (g^- and g^+) correspond to torsional angles in the vicinity of 180°, 60° and 300°, respectively. For alkanols with more than four carbon atoms, the most probable structure is a

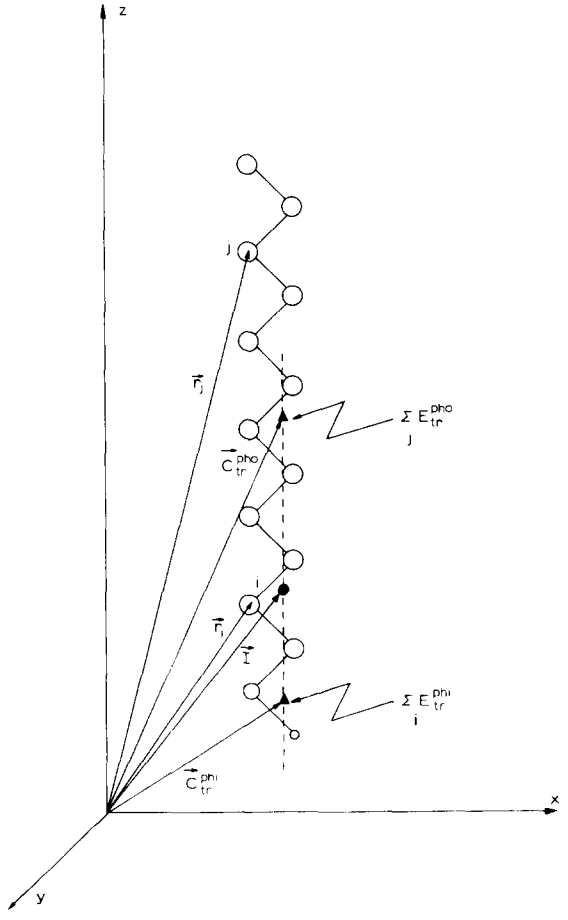

Fig. 2. Definition of the coordinates (x, y, z) of the hydrophobic gravity centre ($\vec{C}_{tr}^{pho}$), the hydrophilic gravity centre ($\vec{C}_{tr}^{phi}$) and the interface, I.

t-conformer. After the minimization procedure and orientation of the alkanol molecule at the hydrocarbon-water interface, it appears (Table II) that the interfacial conditions possibly maintain an equilibrium between g- and t-conformers for n-alkanols with short hydrocarbon chains ($n \leqslant 3$), whereas they eliminate any g-conformer for alkanols with longer hydrocarbon chains ($n > 3$), maintaining in the latter case the t-conformers only. These results are illustrated for n-propanol and n-octanol in Fig. 3. An important consequence of the observed transition between g- and t-conformers is the subsequent change in the distance separating the hydrophobic and hydrophilic centres defined by Eqn. 1. Indeed, this distance is shorter in the g-conformer than in the t-conformer (Fig. 3a). This is further illustrated in fig. 4, where the distance between the hydrophobic and the hydrophilic gravity centres (Δ) is plotted as a function of the length of the hydrocarbon chain and where a sharp transition appears, for $3 < n < 4$. The transition occurs for a value of $\Delta = 2.5$ Å. The importance of this value is discussed below (see discussion) in relation to the hydrophobic-hydrophilic balance calculated according to the following equation:

$$\phi = \log \frac{\sum_{i=1}^{n} E_{tr_i}^{pho}}{\sum_{j=1}^{m} E_{tr_j}^{phi}}$$

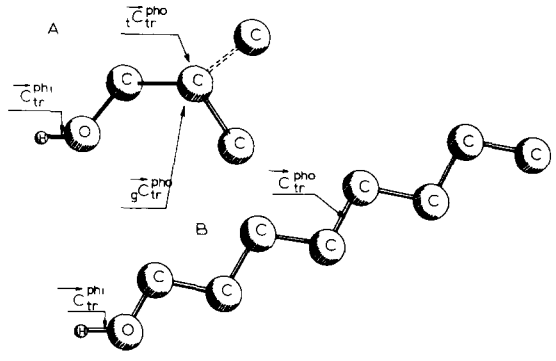

Fig. 3. (A) Conformation of the n-propanol and visualization of the hydrophilic gravity centre ($\vec{C}_{tr}^{phi}$), the hydrophobic gravity centre in the t-conformer ($_t\vec{C}_{tr}^{pho}$) and in the g-conformer ($_g\vec{C}_{tr}^{pho}$). (B) Conformation of the n-octanol and visualization of the hydrophilic gravity centre ($\vec{C}_{tr}^{phi}$) and the hydrophobic gravity centre ($\vec{C}_{tr}^{pho}$)

TABLE II

TORSIONAL ANGLES OF THE MOST PROBABLE *n*-ALKANOL CONFORMERS AFTER MINIMIZATION AND ORIENTATION AT THE SIMULATED HYDROCARBON-WATER INTERFACE

		θ	α_1	α_2	α_3	α_4	α_5	α_6	α_7	α_8	α_9	α_{10}
C_2	A	89	–	–	–	–	–	–	–	–	–	–
	B	182	–	–	–	–	–	–	–	–	–	–
C_3	A	185	78	–	–	–	–	–	–	–	–	–
	B	182	183	–	–	–	–	–	–	–	–	–
C_4	A	181	*	181	–	–	–	–	–	–	–	–
	B	184	*	182	–	–	–	–	–	–	–	–
C_5	A	183	*	*	179	–	–	–	–	–	–	–
	B	184	*	*	185	–	–	–	–	–	–	–
C_6	A	184	*	*	*	181	–	–	–	–	–	–
	B	185	*	*	*	181	–	–	–	–	–	–
C_7	A	181	*	*	*	*	180	–	–	–	–	–
	B	182	*	*	*	*	178	–	–	–	–	–
C_8	A	183	*	*	*	*	*	181	–	–	–	–
	B	185	*	*	*	*	*	182	–	–	–	–
C_9	A	184	*	*	*	*	*	*	179	–	–	–
	B	183	*	*	*	*	*	*	181	–	–	–
C_{10}	A	181	*	*	*	*	*	*	*	178	–	–
	B	179	*	*	*	*	*	*	*	183	–	–
C_{11}	A	178	*	*	*	*	*	*	*	*	178	–
	B	181	*	*	*	*	*	*	*	*	180	–
C_{12}	A	182	*	*	*	*	*	*	*	*	*	177
	B	182	*	*	*	*	*	*	*	*	*	179

*: 180° ± 10.

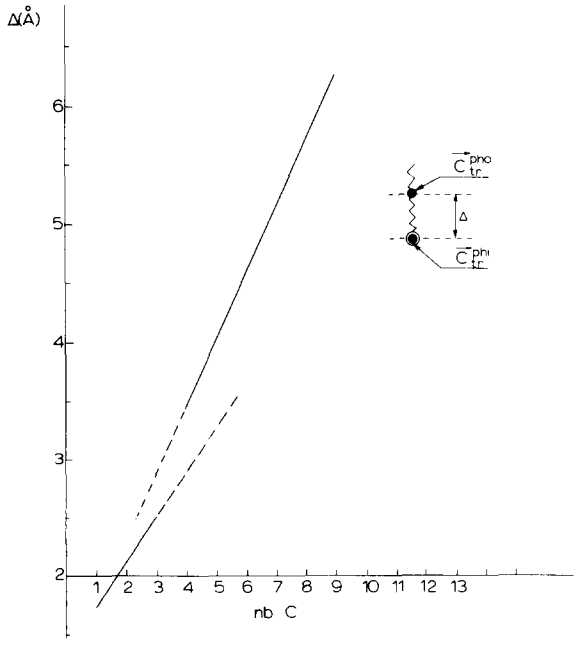

Fig. 4. Distance (Δ, Å) between the hydrophobic gravity centre ($\vec{C}_{tr}^{pho}$) and the hydrophilic gravity centre ($\vec{C}_{tr}^{phi}$) as a function of the hydrocarbon chain-length (nb C), $\Delta = \vec{C}_{tr}^{pho} - \vec{C}_{tr}^{phi}$.

ϕ depends only on the amounts and the nature of the atoms constituting the molecule, but is a monotonous function of the hydrocarbon chain-length (Fig. 5).

(b) Monolayers

Assembling of the most probable conformers (Table II) of homologous alkanols and of a given alkanol and DPPC was conducted as described in Methods. Fig. 6 depicts the evolution of the interaction energy between homologous alkanols (curve Al-Al) and between alkanols and DPPC (curve Al-Lip) as a function of the alkanol hydrocarbon chain-length. For alkanols shorter than *n*-octanol, the alkanol-alkanol interaction energy is more favourable than the interaction energy between the same alkanol and lipids. In this case, the alkanol-alkanol interaction is privileged, and clusters are inserted in the lipid monolayer.

On the other hand, for $n > 8$, the alkanol-lipid interaction energy is the most important; the alkanol-lipid interaction energy is thus privileged

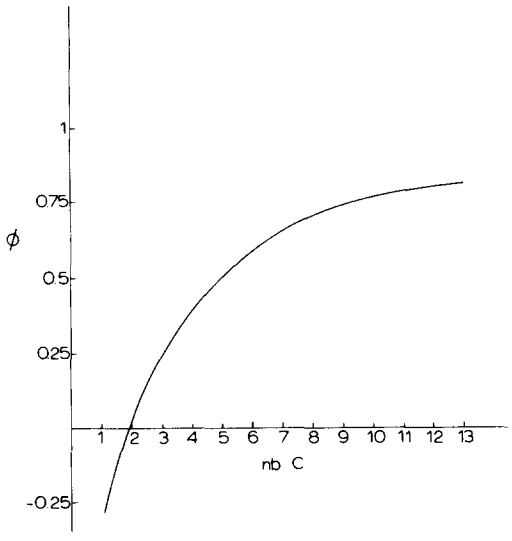

Fig. 5. Hydrophobic-hydrophilic balance (ϕ) as a function of the hydrocarbon chain-length (nb C). ϕ is defined by:

$$\phi = \log \frac{\sum_{i=1}^{n} E_{tr_i}^{pho}}{\sum_{j=1}^{m} E_{tr_j}^{phi}}$$

and the alkanols will then be randomly solubilized in the lipid matrix. As the interaction increases with chain-length, the interaction energy between alkanols ($n > 10$) and lipid becomes equal or superior to the DPPC-DPPC interaction energy (approx. -13 kcal/mol) [13]. The different modes of organization of n-alkanols with a DPPC monolayer are given in Fig. 7, as they are shown by the conformational analysis. For the sake of simplicity, only two DPPC molecules have been represented. Short-chain alkanols like n-butanol (Fig. 7a) do not penetrate the DPPC layer and interact only weakly with the polar head of the lipid. Longer alkanols ($n > 4$) penetrate the non-polar region of the DPPC monolayer but in three distinct patterns according to their interactions. Alkanols like n-propanol aggregate and form clusters embedded in the lipid layer (Fig. 7b), the hydroxyl

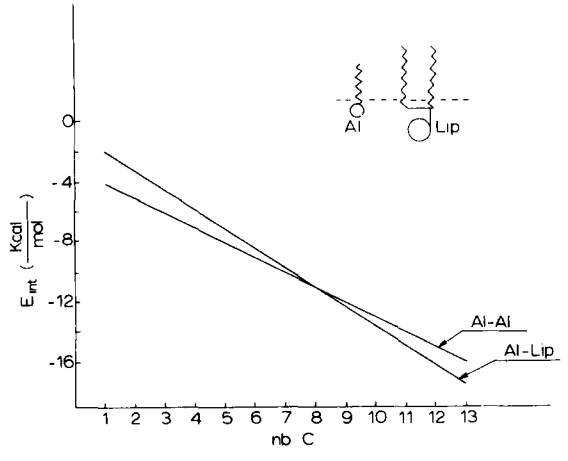

Fig. 6. Interaction energy (E, kcal/mol) between alkanol-alkanol (Al-Al) and alkanol-lipid (Al-Lip) as a function of the alkanol hydrocarbon chain-length (nb C).

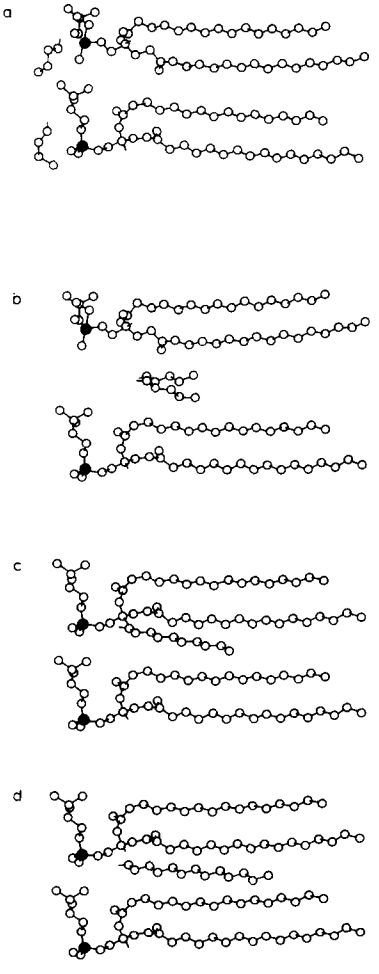

Fig. 7. Schematic visualization of n-alkanols and DPPC organization. Panels a–d represent the conformation of alkanols for $n \leqslant 3$ (a), $4 \leqslant n \leqslant 8$ (b), $9 < n < 10$ (c) and $n \geqslant 11$ (d), respectively. Blackened sphere refers to P atom.

group being located at the level of the ester bonds between glycerol and fatty acids. Fig. 7 must however be considered as a schematic representation; indeed, at the present time, the number of molecules constituting the clusters cannot be calculated from our conformational analysis. It may be argued that clusters made of several alkanol molecules would cause voids between chains in the bilayer. It has been proposed that lipid chains could fill these holes by kinking inwards [25].

Another possibility is the interdigitation of the two monolayers [26]. These two eventualities are presently under investigation using the conformational approach. *n*-Nonanol and *n*-decanol are inserted randomly in the lipid layer (Fig. 7c). They are extended along the hydrocarbon chains of the lipids up to half of the acyl chain. The hydroxyl group is located at the level of the ester bond between glycerol and phosphatidylcholine, i.e., closer to the aqueous phase than shorter alkanols (Fig. 7b). The longer alkanols illustrated by dodecanol in Fig. 7d adopt the same configuration. However their hydroxyl group is located at the level of the glycerol. They are thus more deeply inserted into the lipid layer than nonanol.

Discussion

In the present communication, a semi-empirical conformational analysis is used to study the conformations, the orientations and the interactions of a series of *n*-alkanols which were considered as isolated or inserted into close-packed monolayers consisting of either homologous alkanols or mixed alkanol-DPPC. The interfacial anisotropy conditions were found to induce several patterns of interactions of *n*-alkanols as their hydrocarbon chain-length was increased. For $n \leqslant 3$, the *g*-conformers are the most probable (Table II), yielding a relatively short distance between the hydrophobic and hydrophilic centres of the alkanol molecule (Fig. 3A), giving rise to a water-soluble structure.

These molecules interact only with the polar head groups of DPPC (Fig. 7a). For $n \geqslant 4$, only all-*trans* conformers are obtained (Table II), yielding a longer distance separating the hydrophobic and hydrophilic centres of the molecule (Fig. 3B), giving rise to anisotropic water-insoluble molecular structures. For $n \geqslant 4$, the study of the interactions *n*-alkanols-*n*-alkanols and *n*-alkanols-lipids gives rise to three distinct patterns of interactions according to their hydrocarbon chain-length:

(1) for $4 \leqslant n \leqslant 8$, clusters of *n*-alkanols are formed in the lipid layer (Fig. 7b);

(2) for $9 \leqslant n \leqslant 10$, the *n*-alkanols are randomly distributed in the lipid layer (Fig. 7c);

(3) for $n \geqslant 11$, a grid of lipid and alkanol is formed with a cohesion identical or superior to that found in a pure lipid monolayer (Fig. 7d).

From previous conformational studies on more than 80 molecules, including phospholipids [13], ionophores [14], tumor-promoting agents [15], antibiotics and other various drugs [15,17,27], we were able to classify them on the basis of only two independent parameters: Δ and ϕ (unpublished data). Particularly, a given molecule is able to adsorb at a lipid-water interface only for values of $\Delta > 2.5$ Å and of $\phi > 0.2$.

For the first three alkanols, these conditions are not met, mainly due to the transition observed in the evolution of Δ as a function of chain-length. According to our present data on the conformation of alkanols, this discontinuity must be attributed to the occurrence, for the three alkanols, of the *gauche* structure, which is statistically more probable then a unique *trans* conformation for alkanols with $n \geqslant 4$. Thus, the variation in conformation of alkanols between the *g*-structure ($n \leqslant 3$) and the *t*-structure ($n > 4$) might explain the absence of interactions due to the lack of adsorption of the shorter ones. This could also be related to other experimental observations performed with the same series of alkanols, for instance only the first three alkanols have partition coefficients close to or inferior to 1 (Ref. 26, see also Refs. 7, 9, 27).

It was of interest to correlate the finding of this study with experimental data concerning alkanol-lipid interactions. Jain and Wu [11] found that *n*-alkanols up to butanol did not broaden the transition of DPPC, as measured by differential scanning calorimetry. There is, therefore, no reduction in transition cooperativity and thus no reduction in size of the cooperative lipid clusters due to *n*-alkanols with $n \leqslant 4$. This is consistent with the finding that shorter alkanols ($n \leqslant 3$ in this study) interact only with the polar head of DPPC. Our conclusion concerning the localization of C_1-

C_3 alkanols is, however, different from that of Jain and Wu [11], who positioned these alkanols in the lipid chains between the two leaflets of the bilayer. The absence of reduction in transition cooperativity of dipalmitoylphosphatidylcholine due to the presence of ethanol was confirmed recently by different experimental approaches [29]. The effect of *n*-alkanols up to $n = 8$ on the transition characteristics of DPPC multilamellar vesicles measured as the shift of the temperature at half-height width of the transition peak [11] is consistent with their incorporation into the bilayers (measured as the logarithm of a partition coefficient), whereas extrapolation is not possible for $n = 9$ and $n = 10$ [11], in good agreement with the transition in alkanol organization described in this paper. From the systematic study of the phase transition profile, Jain and Wu [11] suggested that *n*-alkanols with $n > 5$ were located along the lipid acyl chains in a key region included in the first-height methylene C_1-C_8 region, and that longer alcohols would be less effective in disturbing a bilayer, since their alkyl chains can effectively replace the lipid acyl chain in the C_1-C_8 region, where maximal interchain overlap occurs. This corresponds to the interaction of alkanols with $n > 11$ in the lipid layer as described in this study.

Studying the interaction energy between alkanols and DPPC or DPPE monolayers, Villalonga et al. [12] observed a sudden discontinuity for *n*-alkanols with $5 < n \leqslant 9$, independent of whether DPPC or DPPE was used to form the monolayer. It was suggested that the configuration of the molecules and/or entropic factors are responsible for this discontinuity. *n*-Alkanols with $n \geqslant 10$ were again on the straight line extrapolated from the first five numbers of the *n*-alkanol series. The approach used in this paper suggests that *n*-alkanols with $5 \leqslant n \leqslant 9$ form clusters in which the interaction energy per alkanol methylene is different from that observed for randomly distributed molecules.

In addition to effects of *n*-alkanols on microviscosity and/or lipid order [6,11], the general theories of anesthesia differentiate also between change in phase distribution [31] and cooperative fluctuation between phases by critical clusters of lipids [32]. Lipid lateral distribution and interactions will be affected in a very different fashion depending on whether alkanols are organized in clusters ($4 < n \leqslant 8$) or are randomly inserted in the lipid layer ($9 \leqslant n \leqslant 10$). On the other hand, for $n > 11$, alkanols should be much less disturbing for the lipid layer, since they are integrated in the lipid matrix with an alkanol-lipid energy of interaction equal or superior to that of the lipid layer interaction energy which tends to reorganize the DPPC hexagonal matrix. These modifications in anesthetic potencies of *n*-alkanols (taken from Pringle et al. [9], Table I) are reported in Fig. 8 as a function of hydrocarbon chain-length and are tentatively related to the organization of alkanols described in this paper. The anesthetic potency of alkanols up to $n = 8$ is qualitatively consistent with their lipid-water partition coefficient, whereas a deviation is observed for $9 \leqslant n \leqslant 12$. A sudden decrease in potency appears for $n \geqslant 12$. This characterizes the well-known cut-off point in anesthetic potency. From Fig. 8, it appears that the gain in

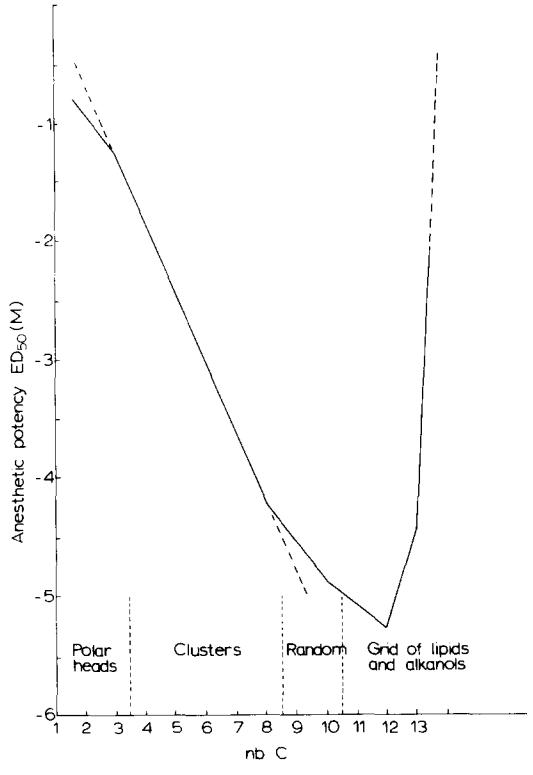

Fig. 8. Comparison of local anesthetic potency of the alkanols as a function of the hydrocarbon chain-length (nb C) and the mode of alkanol-lipid interaction as determined in the present study.

potency is higher for clustered alkanols than for randomly adsorbed alkanols (increased slope). It may be argued that such clusters will destabilize phospholipid-phospholipid interaction much more by increasing the distance between them, whereas isolated alkanol molecules could be integrated in the lipid grid using, at least partially, the free intermolecular space of the grid. This would also explain that branched-chain alcohols have higher perturbability than the straight-chain alkanols [30].

The main result of this conformational analysis study is the clarification to a certain extent, of the molecular reasons for the multiple behaviour of the members of an homologous series of *n*-alkanols. This study provides some stimulating ideas on the mode of interaction of alkanols with lipids. For instance, it points out the importance of the self-association between anesthetic molecules, which, in the case of *n*-alkanols, was well correlated with their anesthetic potency.

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