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Semi-empirical conformational analysis of propranolol interacting with dipalmitoylphosphatidylcholine

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A semi-empirical conformational analysis is used to compute the conformation of (+)-propranolol inserted in dipalmitoylphosphatidylcholine. In a first step, the minimal conformational energy of the isolated molecule at the hydrocarbon-water interface is calculated as the sum of the contributions resulting from the Van der Waals, the torsional, the electrostatic and the transfer energies. Five pairs of conformers of minimal energy are determined. They are compared to data available from other experimental approaches. In a second step, they are assembled with dipalmitoylphosphatidylcholine at the interface. Although propranolol is considered in its protonated form, the electrostatic interaction with dipalmitoylphosphatidylcholine is negligible as compared to the Van der Waals interaction. The area occupied per propranolol molecule is between 0.53 and 0.64 nm^2/molecule. In the most probable modes of insertion of propranolol into the lipid layer, the naphthyl moiety of the compound interacts with the lipid acyl chains. The protonated amino group is located in the vicinity of the phosphate residue possibly causing an electrostatic interaction.

Introduction

Most of the pharmacological properties of propranolol appear to be determined by its beta-adrenergic receptor-blocking properties [1]. On the other hand, propranolol displays a non-specific, membrane-stabilizing effect [1–3] which may interplay with the beta-adrenergic blockade in the complex pharmacological properties of the drug, such as control of schizophrenic symptoms [4], anticonvulsant [5] and antiarrhythmic [6] properties. Studies on the heart include ion flux [7] and electrophysiological measurements in the isolated organ [8].

The membrane-stabilizing effects of propranolol differ from those related to beta-adrenergic blockade in terms of their stereoselectivity and concentration dependence [1,9], the latter being stereoselective and occurring in a lower range of concentrations.

As the membrane-stabilizing effects of propranolol and other beta-adrenergic drugs depend on the organic phase/water partition coefficient, they have been related to interactions with membrane lipid and protein components [2,3]. These are associated with changes in membrane phospholipid and protein conformation as well as alterations in the functional properties of membrane enzymes [10].

Studies using phospholipid model membranes indicated that propranolol reduces the transition phase temperature of dipalmitoylphosphatidylcholine (DPPC) [11,12] and dipalmitoylphosphatidylethanolamine [11] at concentrations comparable to those blocking sodium

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Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, dipalmitoylphosphatidylcholine.

conductance. The drug has only a slight effect on the order parameter of dipalmitoylphosphatidylcholine bilayers [13] as measured with the spin label probe 5-doxylstearic acid. Incorporation of acidic lipids in the neutral matrix increases drug binding [11,13] and ordering of lipids. From these studies, an attempt was made to determine the position, orientation and conformation of propranolol within the DPPC matrix [12,13].

In the present communication, a new semi-empirical conformational analysis is developed to obtain a picture of propranolol interacting with dipalmitoylphosphatidylcholine. The conformational analysis previously developed for insoluble molecules like phospholipids [14] and Ca^{2+} ionophore [15] at the hydrocarbon-water interface is extended to propranolol, which partitions between the water and lipid phases.

Methods

Isolated molecule

The conformation of the isolated molecule and its orientation at the lipid-water interface has been established as described elsewhere [14,15]. The total conformational energy is calculated as the sum of the following terms.

(i) 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:

$$E^{\mathrm{VdW}} = \sum_{ij} \left(A_{ij}\, e^{-B_{ij} r_{ij}} - C_{ij} r_{ij}^{-6} \right) \qquad (1)$$

where i, $j = 1,2,\ldots$ 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 Giglio [16,17] Like other quantum-mechanical results, these values emerge in part as heuristic variables. They have been applied with success to conformational analysis of molecular crystals, proteins, polypeptides, and lipids [18–21,14]. 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:

$$E^{\mathrm{VdW}} = 418\ \mathrm{kJ \cdot mol^{-1}}\ \text{at}\ r_{ij} < 0.1\ \mathrm{nm}$$

(ii) The generalized Keesom-Van der Waals' interaction or electrostatic interaction between atomic point charges is defined in Eqn. 2:

$$E^{\mathrm{cb}} = 1389 \sum_{ij} \frac{e_i e_j}{r_{ij} \epsilon_{ij}} \qquad (2)$$

where e_i and e_j are expressed in electron charge units and r_{ij} in nm. ϵ_{ij} is the dielectric constant. the values of atomic point charges units are shown in Fig. 1 for the propranolol. These values are similar to the values used for polypeptide [18,19].

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

$$E^{\mathrm{Tor}} = \tfrac{1}{2} U_{ij} (1 + \cos \phi_{ij}) \qquad (3)$$

where U_{ij} corresponds to the energy barrier in the eclipsed conformation during the rotation of the angle and ϕ_{ij} the torsional angle. For the C – C

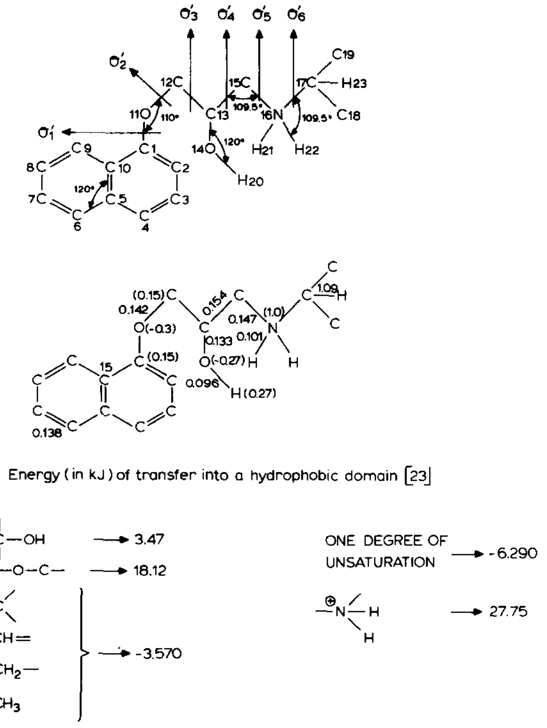


Fig. 1. Schematic formula of propranolol showing the atomic numbering and the notation of the various torsional angles. Values of bond lengths (nm), torsional angles (θ), atomic point charges units and transfer energies are given.

bond U_{ij} is equal to 11.7 $\mathrm{kJ \cdot mol^{-1}}$, 7.5 $\mathrm{kJ \cdot mol^{-1}}$ for C – O bond and 4.2 $\mathrm{kJ \cdot mol^{-1}}$ for C – N bond [22].

(iv) The transfer energy of each part of molecule. The values of the transfer energies used are similar to those determined experimentally by numerous authors, and summarized elsewhere [23]. The values are given for the propranolol in Fig. 1.

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, bond lengths were those, currently used in conformational analysis [22], and summarized in Fig. 1 for the propranolol. After systematic analysis, conformations selected for their lowest internal energy were submitted to a simplex minimization procedure [24]. To simulate the membrane interface, we have 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 is 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 centers of gravity perpendicular to the interface [25]. The hydrophilic center of gravity (C_w) is defined by the following equation:

$$C_\mathrm{w} = \frac{\sum_{i=1}^{n} (E^{+}_{\mathrm{transfer}})_i r_i}{\sum_{i=1}^{n} (E^{+}_{\mathrm{transfer}})_i} \tag{4}$$

in which r_i are the coordinates of the i atom. The hydrophobic center located in the hydrocarbon domain (C_HC) is defined by the same equation, except that the negative transfer energies are taken into account. The interface position, I, is defined by the equation:

$$\frac{\sum_{i=1}^{n} (\mathrm{E}^{+}_{\mathrm{transfer}})_\mathrm{i}}{C_\mathrm{w} + I} = \frac{\sum_{j=1}^{m} (\mathrm{E}^{-}_{\mathrm{transfer}})_\mathrm{j}}{C_\mathrm{HC} - I} \tag{5}$$

Conformation of the drug molecule inserted into the lipid monolayer (Refs. 14 and 25)

The procedure of drug insertion can be summarized as follows. (a) The position of lipid B (orientation of the isolated molecule) was modified along the X-axis. Each distance was equal to 0.05 nm. For each separating distance a rotation angle of 30° was imposed to lipid B around its own Z-axis and around the lipid B (Fig. 2a and b). Among all possible orientations only the structure of minimum energy was considered. (b) Drug A was fixed and drug A was allowed to move along the Z-axis perpendicular to the lipid-water interface (Fig. 2). Again, only the structure of minimum energy was considered. (c) Lipid B had the possibility of changing its orientation around the Z-axis compared with drug A (Fig. 2). This procedure allowed the probable packing of drug 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 drug.

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 bi-

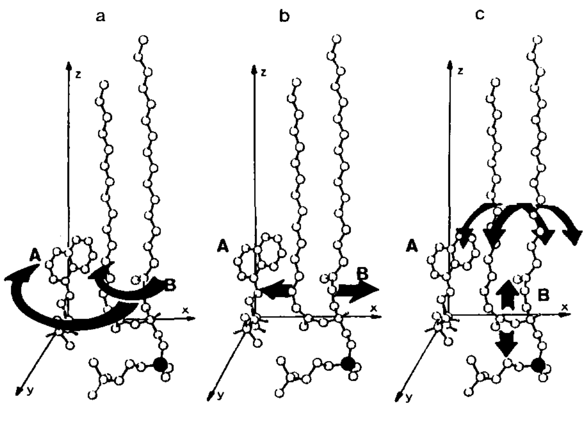


Fig. 2.Schematic presentation of the packing procedure used to assemble molecules in mixed monolayer.

layers. An excellent agreement [14] was obtained between the predictions and the neutron diffraction data. The position of the lipid molecules was localized with a precision which was in the limit of the experimental error.

Results

Isolated molecule

The amino group has been considered in its protonated form. The reported value of pK_a of propranolol (9.45) does, in fact, imply that the molecule is mainly ionized at physiological pH values [11,12]. In a first step, a systematic analysis was performed on the torsional angles θ_1', θ_2', θ_3', θ_4', θ_5', θ_6', (Fig. 1) of propranolol in its protonated form. Successive increments of 60° were imposed on each torsional angle. Among these 46 656 different conformations, five structures with a probability greater than 1% were selected. They represent approx. 50% of all the most probable structures and are close to each other as indicated by the energy above the minimal value which does not exceed 1.55 kJ/mole (data not known). In the classical nomenclature, the conformations *trans* (t), *gauche*$^+$ (g^+) and *gauche*$^-$ (g^-) correspond to torsion angles in the vicinity of 180°, 60° and 300°, respectively. According to this nomenclature, the torsional angles θ_2', θ_3' and θ_5' of all conformers are t, except for the θ_3' in one conformer which is g. Two pairs of conformers (designated as conformers A and B as well as C and D) differ only at the level of θ_6'. The values of θ_6' are of 0° and 120° in the conformers A and C and B and D, respectively. The two conformers A and B differ from the corresponding C and D at the level of θ_1' which takes a value of 120° (conformers A and B) or 240° (conformers C and D). In the last selected conformer, the values of θ_1' and θ_6' are of 240° and 0°, respectively.

In a second step, each structure retained in the preceding systematic analysis was improved by a simplex minimization procedure [24], and orientated at the simulated interface. The minimization procedure was computed with either positive or negative parameters. In these conditions, each of the five structures obtained after the systematic study gave two structures which yielded a total of ten structures after the minimization procedure and orientation at the lipid-water interface.

The sum of the hydrophobic transfer energy was equal to 101.3 $kJ \cdot mol^{-1}$, and the sum of the hydrophilic transfer energy was equal to 49.4 $kJ \cdot mol^{-1}$ (Eqns. 4 and 5).

The torsional angles characterizing these ten structures are listed in Table I along with the

TABLE I

CONFORMERS OF PROPRANOLOL AFTER THE MINIMIZATION PROCEDURE AND ORIENTATION AT THE HYDROCARBON-WATER INTERFACE

Δ is the distance in nanometers between the hydrophobic and hydrophilic centers of gravity.

Conformer	Torsional angles						Δ
	θ_1'	θ_2'	θ_3'	θ_4'	θ_5'	θ_6'	
A	99	184	182	82	177	33	0.217
B	103	185	180	181	183	32	0.233
A'	97	185	185	82	171	86	0.202
B'	100	189	183	179	179	85	0.234
C	260	181	185	82	76	31	0.227
D	260	180	183	180	183	31	0.255
C'	259	180	184	82	174	86	0.216
D'	260	179	184	180	182	85	0.252
E	261	175	286	82	172	32	0.254
F	260	165	287	178	180	32	0.284

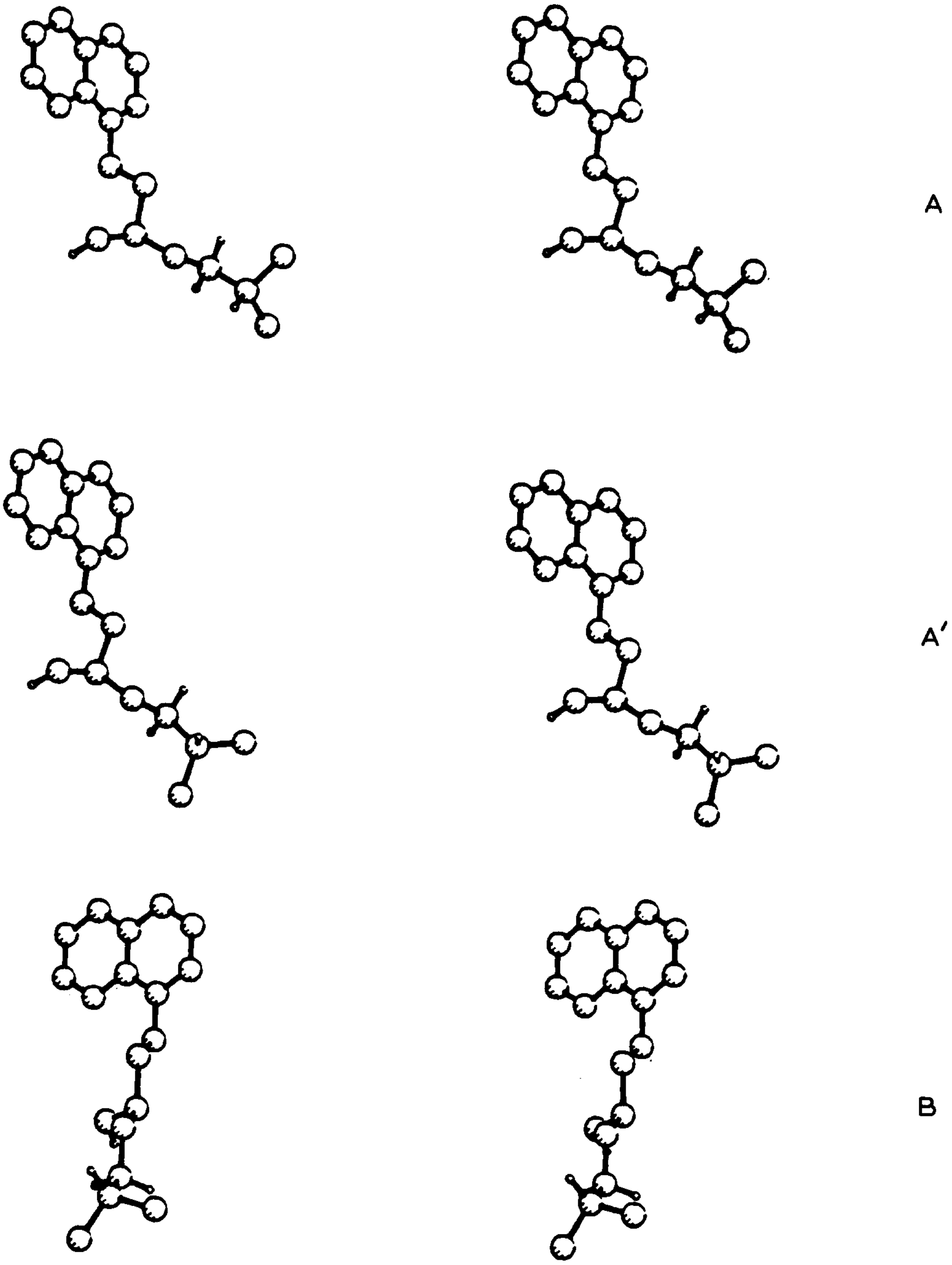


Fig. 3. Stereoscopic views of the most probable conformer for propranolol.

distance (Δ) between the hydrophobic and hydrophilic centers of gravity. A high Δ value corresponds to a largely extended structure.

Fig. 3 shows the stereo view of all conformers listed in Table I.

Monolayer formation

The ten conformers were inserted in a DL-α-dipalmitoylphosphatidylcholine (DPPC) monolayer. The conformation and the orientation of DPPC at the air-water interface had been computed previ-

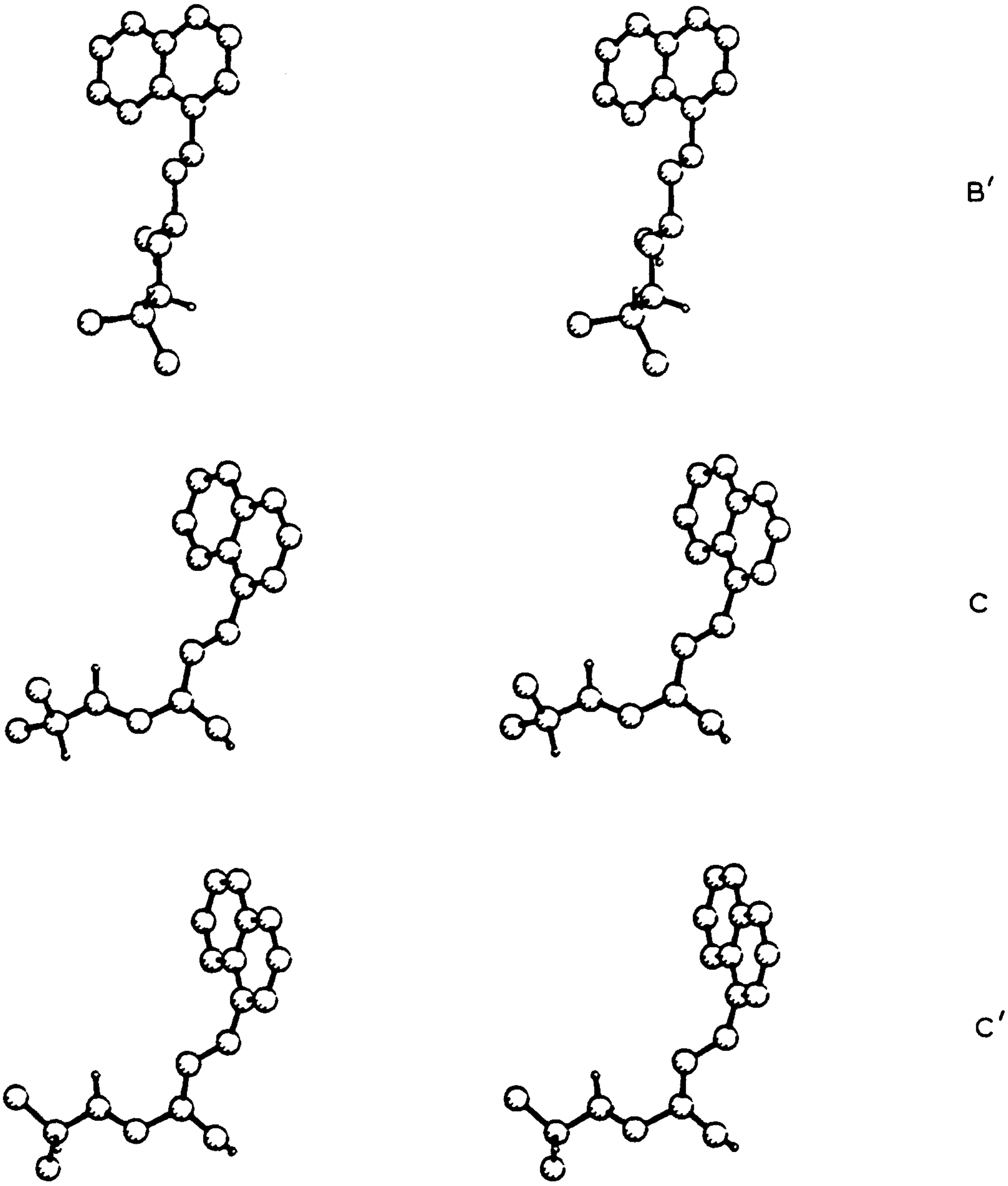
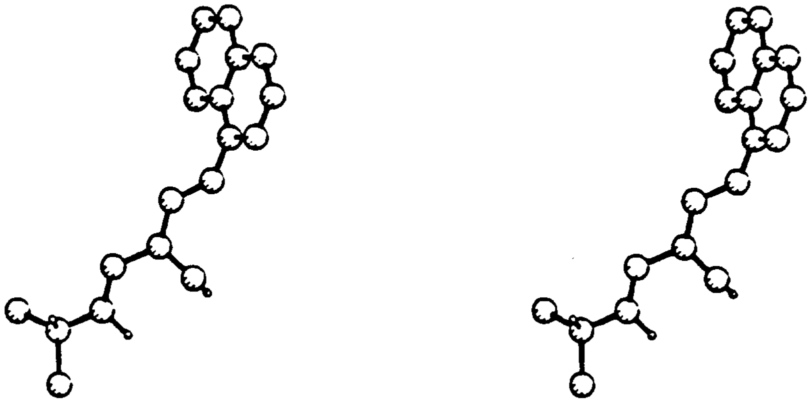


Fig. 3. Stereoscopic views of the most probable conformer for propranolol. (cont.).

ously [14] according to the procedure described in Methods. The total interaction energy between propranolol and DPPC, E_{tot} (sum of the Van der Waals' interaction, E_{VdW}, and the electrostatic interaction, E_{elec}, and the mean area occupied per propranolol molecule in the lipid layer are listed in Table II.

For all conformers, the electrostatic interaction with DPPC is negligible as compared to the Van der Waals' energy, it represents less than 4% of the total interaction energy. The mean molecular areas are included between 0.53 and 0.64 nm^2/molecule. The three most probable structures after insertion in DPPC layer are the structure D′, the structure



D

D′

Fig. 3. Stereoscopic views of the most probable conformer for propranolol. (cont.).

TABLE II

PHYSICOCHEMICAL PARAMETERS OBTAINED USING THE CONFORMATIONAL ANALYSIS DESCRIBED IN THIS STUDY FOR THE DIFFERENT STRUCTURES OF PROPRANOLOL INSERTED IN A LIPID LAYER

E_{tot}, interaction energy between propranolol and lipid (sum of the electrostatic interaction, E_{elec}, and the Van der Waals' interaction, E_{VdW}); A, the mean molecular area occupied by the propranolol molecule inserted in a lipid layer.

Conformer	E_{elec} (kJ·mol^{-1})	E_{VdW} (kJ·mol^{-1})	E_{tot} (kJ·mol^{-1})	A (nm^2·mol^{-1})
A	−0.29	−10.46	−10.76	0.61
B	0.17	−16.70	−16.87	0.54
A′	0.33	−8.58	−8.91	0.54
B′	0.13	−10.46	−10.59	0.54
C	0.04	−19.71	−19.67	0.64
D	0.17	−22.01	−22.18	0.56
C′	0.08	−26.70	−26.62	0.55
D′	0.12	−27.58	−27.70	0.58
E	−0.04	−23.77	−23.81	0.53
F	−0.00	−20.55	−20.55	0.55

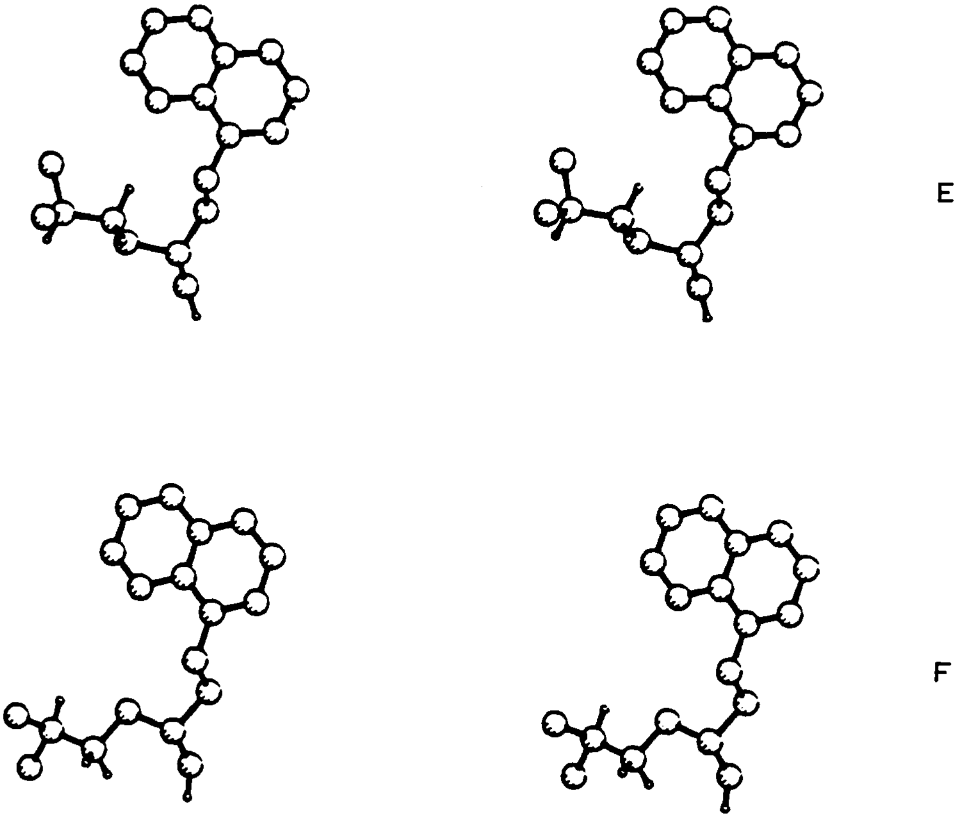


Fig. 3. Stereoscopic views of the most probable conformer for propranolol. (cont.).

C′ and the structure E. Fig. 4 shows the configuration of these three structures inserted in the DPPC layer. For the sake of clarity, only two molecules of DPPC (one to the left and one to the right) of the molecules surrounding the propranolol molecule are shown in this projection. The molecular packing of lipid molecule is only slightly modified by the structure D′ (Fig. 4). The amino residue of one lipid is adjacent to the phosphate residue of the adjacent lipid as in the pure DPPC layer [14]. For the other structures (C′ and E), this organization is broken. For all structures, the naphthyl residue is situated in the hydrocarbon chains of the lipid above the interface plane. In the most probable structure (D′), the protonated amino residue of the propranolol molecule (indicated by an arrow in Fig. 4) is located in the immediate vicinity of the lipid phosphate residue. For the C′ and E structures, the molecular distance separating phosphate and protonated amino residue is larger and the propranolol aminogroup is closer to the glycerol backbone, The naphthyl moiety is maintained in a similar position in the three structures.

Discussion

Minimization of the five structures has little effect on the conformation of the θ_2', θ_3' and θ_5' torsional angles. This introduces, however, profound variations in the other torsional angles θ_1', θ_4' and θ_6'. The values of θ_1' are close to each other for each pair of conformers as is also the case with the value of θ_6'. On the other hand, a difference of approx. 100° is systematically observed for θ_4'. This leads to five pairs of conformers, one of each being more elongated than the other as seen by the systematic increase in Δ (Table I). Data on the conformation of propranolol had already been obtained using various techniques including the theoretical conformational analysis (PCILO analy-

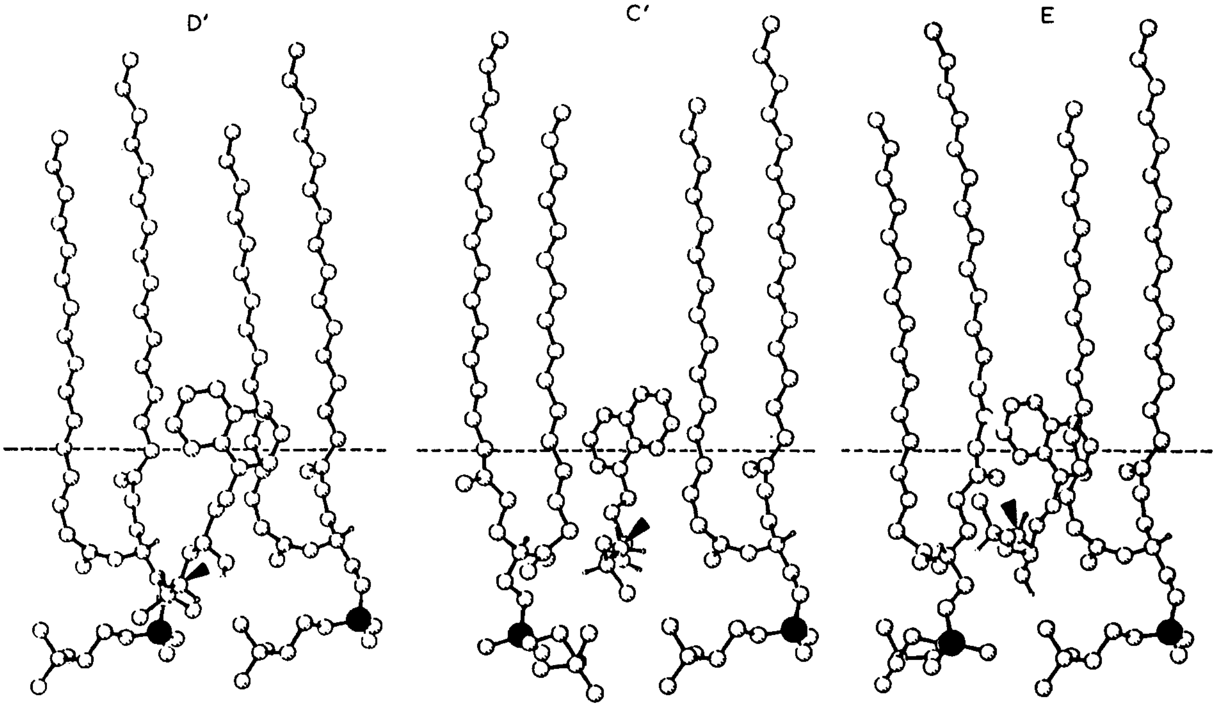


Fig. 4. Configuration of most probable propranolol conformers after insertion in DPPC monolayer. The blackened spheres indicate the phosphorus atoms. The cationic group of propranolol is indicated by the arrows.

sis) [26] and the experimental conformational analysis (X-ray diffraction [27] and NMR analysis in ^2H_2O [26 and in $CDCL_3$ [28]). Comparing the three most probable structures of propranolol obtained after insertion in the lipid (structure D′, C′, E) and the structures obtained for (+) propranolol HCl by X-ray analysis [27] and by PCILO analysis [26], the torsion angles θ'_2 and θ'_5 are fairly constant around a value of 180°. By contrast, θ'_1, θ'_3, θ'_4 and θ'_6 vary according to the methodological approach used. θ'_6 is a torsional angle located at the extremity of the molecule. NMR in ^2H_2O [26] has demonstrated that this angle is not fixed at the extremity of the molecule, all torsional angles being possible between 0° and 360°. θ'_3 and θ'_4 are the torsional angles adjacent to the carbon bearing the hydroxyl group.

They thus condition the orientation of this group with respect to the oxypropranolamino chain. They varied also greatly, considering the data obtained by various experimental approaches [26–28]. However, the values of the torsional angles are close to each other in the most probable structure obtained after insertion in the lipid layer (structure D′) and in the X-ray analysis (27) except for θ'_1. This difference is perhaps due to the properties of the interface, the transfer energy of the residue and the variation in the dielectric constant along the Z-axis perpendicular to the air-water interface.

Direct information on the position and the localization of propranolol in a lipid matrix is scarce. By means of fluorescence spectroscopy, Surewicz and Leyko [12] found that the fluorophore moiety of propranolol is located in a medium characterized by an intermediate dielectric constant value ($\epsilon \approx 40$) as compared to water ($\epsilon \approx 80$) and long hydrocarbon chain ($\epsilon \approx 2$). By a combination of calorimetric and radioisotope measurements, and of neutron diffraction experiments, Herbette et al. [29] concluded that propranolol is solvated in the hydrocarbon core of the lipid layer nearest the phospholipid head group. These observations are in general agreement with the picture obtained in the present study. Accordingly, a scheme for the propranolol-lipid interaction can be proposed in the case of the structure D′. It appears that propranolol can bind to the lipid membrane at two principal binding sites.

First, the naphthyl group is bound to the hydrocarbon chain with the result that the aromatic moiety is highly immobilized. Herbette et al. [30] concluded to a distance of 1.1 ± 0.1 nm between the center of the naphthalene moiety and the center of the DMPC hydrocarbon core. From our conformational analysis this mean distance was estimated to be equal to 1.5 nm for the three calculated structures inserted into a DPPC structure. This distance is in excellent agreement with the value derived from neutron diffraction studies [30]. If one takes into account the difference of two methylene in the DMPC and DPPC acyl chains, indeed for DMPC acyl chains, the calculated distance would be equal to 1.2 nm. The distances between the center of the naphthalene moiety and the charged isopropanolol amino group were in excellent agreement in our work (0.704 nm for the D′ form) and in Herbette et al. evaluation (0.6nm ± 0.1 nm). Under these circumstances, the immobilization of the phosphate residue increases. Second, the protonated amino group of structure D′ is located in the vicinity of the polar head of the lipid layer. There may be a third binding point, namely a hydrogen bond between the β-hydroxyl group of the propranolol and the oxygen in the ester residues in the β-chain of the DPPC. This triple linkage is proposed by Phadke et al. [13]. The structure C′ and E correspond to more globular conformations of propranolol. In these structures, the distance between the center of the naphthyl moiety and the amino group is of 0.528 nm and 0.440 nm for the structures C′ and E, respectively (as compared to 0.704 nm for structure D′).

The naphthyl moiety remaining in the same position in the acyl chains, the formation of an hydrogen bond is no more possible as in structure D′. There is, however, a trend for a direct interaction between the amino group and the glycerol backbone of the phospholipid.

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