Thermodynamics of the Partitioning of 7-Chloro-4-(4′-methoxy)anilinoquinoline and Its Cyclized Analog in Octanol–Buffer and Liposome Systems

Mei-Lin Go,*[,a] Tong-Lan Ngiam,[a] and James A. Rogers[b]

Department of Pharmacy, National University of Singapore,[a] 10, Kent Ridge Crescent, Republic of Singapore 0511, and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta,[b] Edmonton, Alberta, Canada T6G 2N8. Received July 1, 1994; accepted August 18, 1994

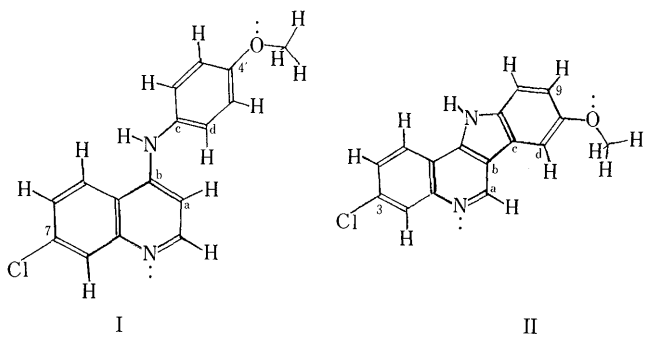
The thermodynamics of the partitioning of 7-chloro-4-(4′-methoxy)anilinoquinoline (I) and its cyclized analogue, 3-chloro-8-methoxy-11*H*-indolo[3,2-*c*]quinoline (II) have been determined in octanol–buffer and liposome systems. Under the conditions of partitioning, the protonated forms of compounds I and II were predominant, but partitioning involved only the non-ionized species. The van't Hoff plots for both compounds were linear in the octanol–buffer system from 11° to 35 °C. The log *P* of compound I increased with temperature, and partitioning was entropically controlled. In contrast, the partitioning of compound II decreased with temperature and was enthalpically driven. The van't Hoff plots of compounds I and II in the dimyristoyl-L-α-phosphatidylcholine (DMPC) liposome–buffer were biphasic. A decrease in log *P* was observed from 13 °C to approximately the T_c of the phospholipid, followed by a subsequent increase in log *P* as temperature increased to about 32 °C. In the case of compound I, partitioning was entropically controlled at temperatures below and above T_c. In contrast, the partitioning of compound II was enthalpically controlled below T_c but entropically driven above T_c. The thermodynamics of the partitioning of compounds I and II in octanol and gel phase phospholipid (below T_c) are similar. This may be attributed to their conformational differences. The planarity and rigidity of compound II allows it to interact well with the ordered matrices of octanol and phospholipid with an expected loss of enthalpy. In contrast, the twisted conformation of compound I would have disrupted the ordered matrices of the octanol and phospholipid phases, resulting in an entropy gain upon partitioning. This study shows that the molecular shape and conformational characteristics of solute molecules are important determinants in the partitioning process.

Key words partitioning thermodynamics; octanol–buffer; liposome–buffer; solute conformational characteristics; anilinoquinoline; indoloquinoline

The hydrophobicity of a drug is an important determinant of biological activity, as this parameter influences the rate and extent of drug absorption, transportation and binding to receptors. The hydrophobicity of a drug can be quantitatively expressed as log *P*, where *P* is its partition coefficient between water and an immiscible non-polar solvent. Considerable interest has been focused on the suitability of synthetic phospholipid layers or liposomes as partitioning models, since in nature phospholipids provide an essential permeability barrier to cells. Several comparisons of partition coefficients obtained from octanol–water and liposome–water systems have been made.[1–6] It has been found that the partitioning of solutes into liposomes is determined by classical hydrophobic forces as well as specific effects (such as lipid packing, interfacial properties and bilayer hydration) which are characteristics of the bilayers but are absent in the bulk solvent phase.[7]

In the present work, the effects of molecular shape and spatial disposition of the solute molecule on its partitioning characteristics in octanol–water and liposome–water systems were investigated using two closely related compounds: 7-chloro-4-(4′-methoxy)anilinoquinoline (I) and its isomeric cyclized analogue 3-chloro-8-methoxy-11*H*-indolo[3,2-*c*]quinoline (II) (Fig. 1). These compounds are the parent molecules of two series of antimalarial agents.[8,9] Although cyclization does not significantly affect antimalarial activities,[8,9] the process does gives rise to significant changes in molecular geometries and physicochemical properties.[10] Compound I has been found to be more hydrophobic, conformationally more flexible, twisted and has a larger surface area than the structurally flat compound II.

Although partition coefficients are good indicators of solute hydrophobicity, they give little information about the actual transfer process. A more complete thermodynamic picture can be obtained by investigating the change in log *P* with temperature, whereby the enthalpic and entropic contributions to the free energy of partitioning can be calculated. In the present work, the effect of temperature on the partitioning of compounds I and II was determined in both the octanol–buffer and liposome–buffer systems. The objective is to determine



I II

Fig. 1. Structural Formulae of Compounds I and II in Their Minimum Energy Conformations

Minimum energy conformations were determined by PC Model Version 4.[13] The twist angles τ a–b–c–d of compounds I and II are 40.40° and 0.06° respectively. An increasing twist angle from zero represents deviation from planarity.

* To whom correspondence should be addressed.

the importance of molecular shape and conformation on the thermodynamics of partitioning between phases.

Experimental

Materials 7-Chloro-4-(4'-methoxy)anilinoquinoline (I) and 3-chloro-8-methoxy-11*H*-indolo[3,2-*c*]quinoline (II) were synthesized according to reported methods.[11,12] Their melting points, infrared (IR) and nuclear magnetic resonance (NMR) spectra were similar to reported literature values.[11,12] Dimyristoyl-L-α-phosphatidylcholine (DMPC, 99%, Sigma Chemical Company) was used as received. All other reagents were of analytical grade.

Octanol–Buffer Partitioning Octanol and aqueous buffers were mutually pre-equilibrated before use. Solutions of compounds I and II were prepared in a 1/15 M phosphate (NaH_2PO_4/H_3PO_4) buffer (pH 3.0, 0.1 mM) and 0.1 M HCl (pH 1.2, 0.125 mM), respectively. Partitioning was carried out by the shake flask method. A volume of 5 ml of octanol and 5 ml of the aqueous phase containing either compound I (0.1 mM) or II (0.125 mM) were placed together in a round bottom flask and agitated for 15 h at a preset temperature (11—35 °C). The phases were then separated and centrifuged (1000 rpm, 10 min), and the concentrations of both phases were determined by UV spectroscopy at appropriate wavelengths. Distribution coefficients (D) were calculated from the mean of seven determinations.

Liposome–Buffer Partitioning Solutions of compounds I and II were prepared in a 1/15 M phosphate buffer (pH 3.0, 0.05 mM) and 0.1 M HCl (pH 1.2, 0.0125 mM), respectively. A volume of 5 ml of a 2 mg/ml solution of DMPC in chloroform was delivered into a 25 ml Quickfit flask. Removal of chloroform by rotary evaporation at 40 °C resulted in the deposition of a thin lipid film on the inside wall of the flask. The latter was subsequently dried under vacuum overnight. Multilamellar liposomes were prepared by adding 5 ml of the aqueous solution containing compound I (0.05 mM) or II (0.0125 mM) to the flask, allowing it to stand at 50 °C for 15 min, followed by swirling on a vortex stirrer for another 15 min. The flask was then shaken for 20 h on a shaking water bath at a preset temperature in the range of 13—32 °C. The lipid and aqueous phases were separated by centrifugation (40000 rpm, 40 min) at the temperature of shaking, except for temperatures above 24 °C, in which case centrifugation was carried out at 24 °C. The possibility of partitioning characteristics changing during the period of centrifugation was considered to be small as centrifugation at such speeds quickly deposits the lipid phase as a small pellet. A reasonable rate of partitioning, on the other hand, is dependent upon the large interfacial area between two phases.

The concentration of compound I or II in the aqueous phase was determined by UV spectroscopy at appropriate wavelengths, while the concentration of liposomally-associated solute was calculated by mass balance. At least 8 determinations were made at each temperature for both compounds I and II.

Conformational Studies The minimum energy conformations of octanol, DMPC and compounds I and II (non-protonated form) were determined using an interactive molecular modelling program, PC Model Version 4,[13] which incorporates the MMX force field for molecular mechanics (MM) calculations. The minimum energy conformations of compounds I and II were superimposed onto the hydrocarbon side chains of DMPC and octanol. The interaction was evaluated from the least squares fit of all the atoms of compounds I or II into the nonpolar region of DMPC/octanol. A favorable interaction is indicated by a small deviation of fit, as calculated by the computer.

Determination of log *P* The distribution coefficient (D) and partition coefficient (P) of each solute between octanol and the aqueous phase were obtained from the following expressions:

$$D = C_o/C_w \tag{1}$$

$$P = D(1 + 10^{pK_a - pH}) \tag{2}$$

where C_o and C_w are the molar concentrations of solute in the octanol and aqueous phases, respectively, pH refers to the pH of the aqueous buffer, and pK_a values are 7.78 (compound I) and 3,99 (compound II) as determined previously at 28 °C.[10]

For the distribution of solute between DMPC liposomes and aqueous buffer, the molal concentration scale was used because of the heterogenous nature of the system under investigation.[14] The molal distribution coefficient (K'_m) is given by:

$$K'_m = \frac{(C_T - C_w)W_1}{C_w W_2} \tag{3}$$

where C_T is the initial aqueous concentration before equilibrium, C_w is the final aqueous concentration after equilibrium and W_1 and W_2 are the weights of the aqueous phase and phospholipid in the sample, respectively.

The partition coefficient (P) was determined from Eq. 2 by replacing D with K'_m. The variation of pK_a with temperature has been considered to be small by various authors.[1,2,15] Thus, the determination of P values at various temperatures was carried out without a corresponding correction of pK_a, and the thermodynamic parameters obtained from the van't Hoff plots for compounds I and II must be taken to be approximate at best.

The temperature dependence of the equilibrium partition coefficient (P) is given by the following relationship:

$$\log P = \frac{\Delta S}{2.3\boldsymbol{R}} - \frac{\Delta H}{2.3\boldsymbol{R}T} \tag{4}$$

where ΔH and ΔS are the enthalpy and entropy changes for the transfer of solute from an aqueous to the octanol or liposomal phase, $\boldsymbol{R}$ is the gas constant (8.3143 J mol^{-1} K^{-1}), and T is the absolute temperature. ΔH can be found from the van't Hoff plot of $\log P$ *versus* $1/T$, where the slope of the line is $\Delta H/2.3\boldsymbol{R}$. The free energy of partitioning (ΔG) at a given temperature is related to P by the expression:

$$\Delta G_{w\to l} = -2.3\boldsymbol{R}T \log P \tag{5}$$

The change in entropy, ΔS, is calculated from Eq. 6 using the known values of ΔH and ΔG at a given temperature:

$$\Delta S_{w\to l} = \frac{\Delta H - \Delta G}{T} \tag{6}$$

Results and Discussion

The partitioning of bases I and II into an octanol or liposomal phase was carried out at pH 3.0 and 1.2, respectively. Under the prevailing pH conditions, the protonated and hence ionic forms of compounds I (pK_a 7.78) and II (pK_a 3.99) would predominate (>99%).[10] The choice of pH was largely determined by experimental factors, since the solubilities of compounds I and II in neutral and alkaline solutions were found to be extremely poor. To minimize experimental error, quantification of compounds I and II in the aqueous phase after partitioning can only be done satisfactorily at an acidic pH.

Partitioning of the solute into the non-polar octanol or liposome phase would normally involve the non-protonated free base, or in some cases, the free base and ion-pairs made up of the protonated species and its counter-ion. Ion-pairs are electrostatically neutral and are known to partition into the non-polar phase. For example, Ahmed *et al.*[1] showed that the log D of chloropromazine between octanol and phosphate buffer (pH 6.0—7.8) increased with concentration, which suggested that the protonated species of chlorpromazine were partitioned as ion-pairs. On the other hand, Betageri and Rogers[2] reported no rise in either the octanol–buffer or liposomal–buffer distribution coefficients of propanolol with increasing concentration. This was consistent with partitioning involving only the free base.

In the case of compounds I and II, there was only a slight variation in log D with concentration (Fig. 2), suggesting that the non-protonated free base is the only species involved in partitioning into the octanol and liposomal phases. Although the non-protonated species of

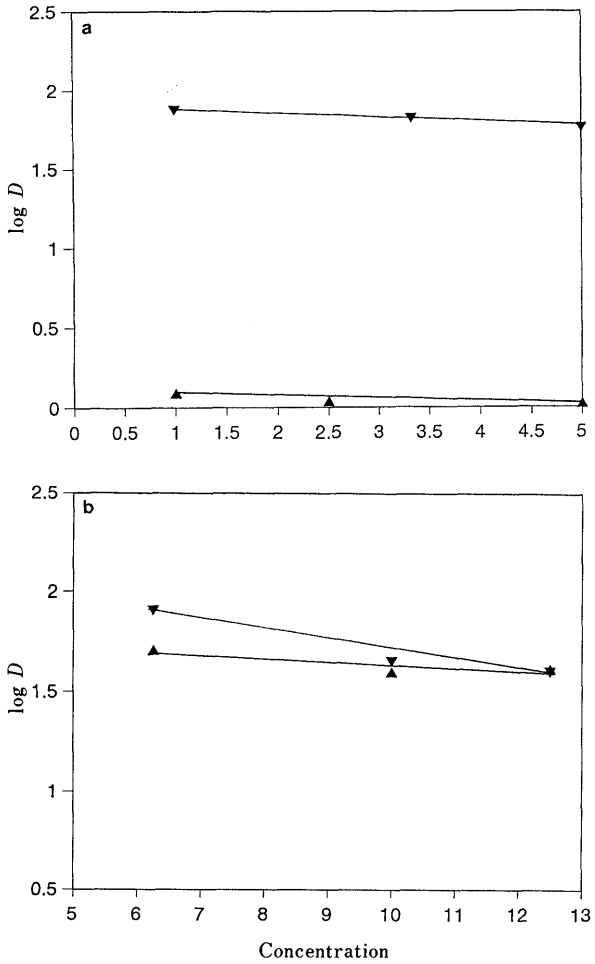


Fig. 2. Change in log D with Concentration of (a) Compound I and (b) Compound II in Octanol–Buffer and DMPC Liposome–Buffer at 29 °C

The pH of octanol–buffer (▲) and liposome–buffer (▼) are 3.0 in (a) and 1.2 in (b). In (a), the concentration of compound I is expressed in terms of 10^{-4} M in octanol–buffer (▲) and 10^{-5} M in liposome–buffer (▼). In (b), the concentration of compound II is expressed in terms of 10^{-5} M in octanol–buffer (▲) and 10^{-6} M in liposome–buffer (▼).

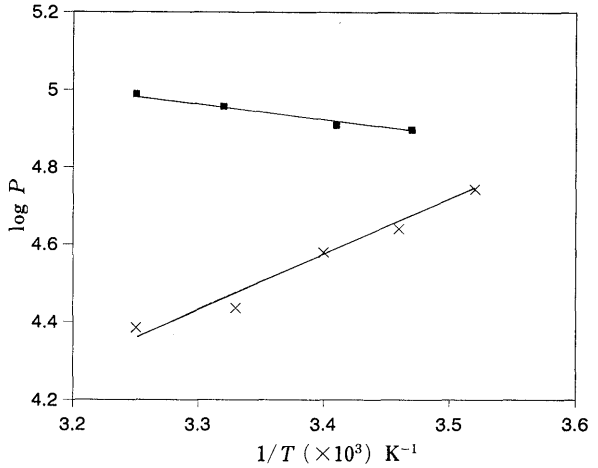


Fig. 3. Van't Hoff Plots of Compounds I and II in Octanol–Buffer

■, compound I; ×, compound II. Compounds I and II were used at concentrations of 0.1 and 0.125 mM, respectively.

TABLE I. Thermodynamic Parameters for the Partitioning of Compounds I and II into Octanol–Buffer

	Compound I		Compound II	
	20 °C	35 °C	16 °C	35 °C
log P	4.91	4.99	4.64	4.39
$\Delta G_{w\to l}$ (kJ mol^{-1})	−27.54	−29.42	−25.68	−25.86
$\Delta H_{w\to l}$ (kJ mol^{-1})	8.67	8.67	−25.82	−25.82
$\Delta S_{w\to l}$ (J mol^{-1} K^{-1})	123.6	123.7	−0.50	−0.13

compounds I or II exist in very low concentrations (< 1%) in the aqueous phase, almost all of the compound has partitioned into the octanol phase in this form at equilibrium, due to its hydrophobic character, as seen from its log P value (Tables I, II).

The variation of log P of compounds I and II in octanol–buffer with temperature is shown by the van't Hoff plots in Fig. 3. It can be seen that log P of compound I increases with temperature, whereas a reverse trend is observed for compound II.

It should be noted that the determination of thermodynamic parameters from the van't Hoff plot is based on the assumption that the mutual solubility of water and octanol is not sustantially affected by temperature.[15] This appears to be so, since the van't Hoff plots (Fig. 3) show reasonably good linearity ($r^2 = 0.9822$, 0.9696 for compounds I and II respectively). In a study on the thermodynamics of the partitioning of chlorpromazine in octanol–buffer, Cheng *et al.*[16] had also assumed that the mutual solubility of the solvent phases was constant over the temperature range studied on the basis that the van't Hoff plots were linear. Nevertheless, in view of this assumption, as well as that concerning the variation of pK_a with temperature (see determination of log P), the interpretation of the thermodynamic parameters reported in this study must necessarily be limited to a qualitative discussion of the overall trends observed.

The linearity of the van't Hoff plots indicates that ΔH is constant over the temperature range studied. Taking 20 °C (compound I) and 16 °C (compound II) as representative temperatures (Table I), it is seen that the partitioning of compound I into octanol is accompanied by a large increase in entropy and a smaller gain in enthalpy. Thus the process is entropically controlled. In contrast, the partitioning of compound II is enthalpy driven, as seen from the large decrease in enthalpy and negligible change in entropy associated with the partitioning process.

The contrasting thermodynamic parameters of compounds I and II in the octanol–buffer system may be explained by their different molecular shapes and conformations. It has been shown that cyclization of compound I to give compound II is accompanied by a reduction in molecular surface area and a decrease in conformational flexibility.[10] The large entropy gain on partitioning of compound I may be attributed to the release of "free water" surrounding the solute molecule as it moves into the non-polar phase, as well as by the subsequent disruption of the orderly octanol phase by the solute molecule. In the case of compound II, fewer "free water"

molecules are released because of its smaller surface area. In addition, compound II, which is planar and conformationally more rigid, may "fit" better in the octanol matrix with less disruption. This would account for the negligible change in entropy observed for the partitioning of compound II into octanol. The reduction in enthalpy is possibly the result of an optimum intermolecular interaction between compound II and the octanol molecules.

Based on the structures of compounds I and II, it is likely that interactions with octanol would involve mainly hydrophobic and van der Waals forces. Hydrogen bonding is expected to occur to about similar extents in both the polar and non-polar phases. Using an interactive molecular modeling program,[13] it was found that the width of compound II (8.811 Å, determined from the distance C3–C9) in its minium energy conformation corresponded more closely to the length of the carbon chain in octanol (8.950 Å) than that of compound I (C4′–C7 = 9.317 Å). The nature of the water-saturated octanol phase is still a matter of conjecture. It has been postulated that octanol molecules are arranged linearly and as cyclic tetramers, and that these are in equilibrium with water centered complex A_4W, believed to have a tetrahedral orientation of hydrogen bonded alcohols (A) around the oxygen atom of water (W).[17] Thus, the octanol matrix is highly ordered. Assuming that van der Waals interactions are key forces developed between the solute and octanol molecules in their various equilibrium states, it would appear that the hydrocarbon portions of compound II and octanol are structurally compatible for optimal interaction. This may account in part for the good "fit" of compound II in the octanol matrix mentioned earlier. Compound I, by virtue of its angle of twist (Fig. 1), would disrupt the octanol matrix rather than being accomodated by it. This may account for the increase in entropy on partitioning.

The zwitterionic phospholipid DMPC is considered to be electrostatically neutral at pH 7.4 because the positive charge of its quaternary trimethylammonium group is counter-balanced by the negatively charged phosphate group (pK_a 3.7).[18] The partitioning of compounds I and II in the liposome–buffer system was carried out at pH 3.0 and pH 1.2 for the same reasons mentioned earlier. Whereas DMPC is largely neutral at pH 3.0, it is positively charged at pH 1.2. The charge difference due to pH may influence the partitioning characteristics of the solutes. Young and Rogers[19] have shown that the apparent partition coefficients of a number of 2-imidazolines were generally higher by about 0.5 log D units in neutral DMPC than in positively charged DMPC liposomes containing stearylamine, due to charge repulsion between the cationic solute and the similarly charged liposomal surfaces. However, the difference was negligible for those cationic imidazolines substituted with chlorine groups.

For compounds I and II, which partition into the hydrocarbon interior of the liposomes as nonprotonated bases, it is unlikely that the positive charge on the surface of the liposomes at low pH would affect log P values. A lower pH may, however, lower the rate of partitioning since the concentration of free base would be reduced accordingly.

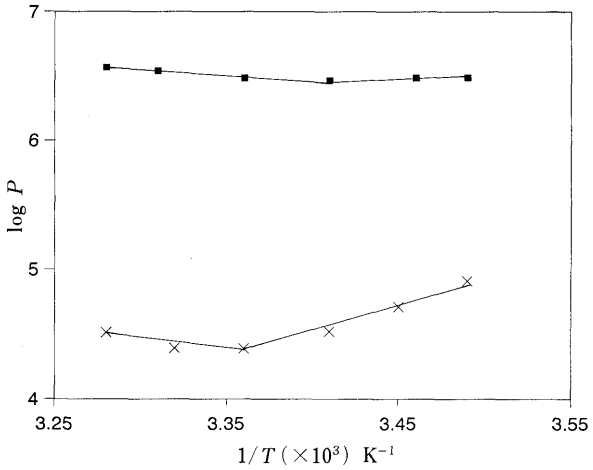


Fig. 4. Van't Hoff Plots of Compounds I and II in DMPC Liposomes–Buffer

■, compound I; ×, compound II. Compounds I and II were used at concentrations of 0.05 and 0.0125 mM, respectively.

TABLE II. Thermodynamic Parameters for the Partitioning of Compounds I and II into DMPC Liposome–Buffer

	Compound I		Compound II	
	16 °C	32 °C	16 °C	32 °C
log P	6.48	6.56	4.71	4.51
$\Delta G_{w\to l}$ (kJ mol^{-1})	−35.88	−38.34	−26.16	−26.36
$\Delta H_{w\to l}$ (kJ mol^{-1})	−5.94	14.91	−82.75	26.54
$\Delta S_{w\to l}$ (J mol^{-1} K^{-1})	103.6	174.7	−195.8	174.3

The change in log P of compounds I and II in liposome–buffer with temperature is shown by the van't Hoff plots in Fig. 4. Unlike the van't Hoff plots obtained in octanol–buffer, the variation in log P with temperature was not linear over the entire temperature range investigated. This is not unexpected as the partitioning characteristics of solutes are generally different at above and below the phase transition temperature (T_c) of the phospholipid,[1-5] which is 23 °C in the case of DMPC. At the T_c, the phospholipid undergoes a transition from a rigid gel state to a more fluid state. The process of chain melting allows for greater uptake of the solutes and it is not uncommon for partitioning into liposomes to be greater above T_c. This is because less energy is required to partition the solute into the liquid crystalline state above T_c than the gel crystalline state below T_c.

In some cases, the T_c of the phospholipid is clearly seen from the van't Hoff plots by an abrupt change in gradient in the vicinity of T_c.[1,4,5] In others,[2] a discontinuity is observed, and this has been attributed to 'freezing out' of the solute and is associated with the gain in enthalpy that arises from a stronger interaction between phospholipid chains.[20]

The log P of compound I showed a slight decrease, from 13 to 20 °C, followed by an increase from 20—32 °C. A similar pattern is observed with compound II, except that a sharper decline in log P was observed, from 13 to 24 °C.

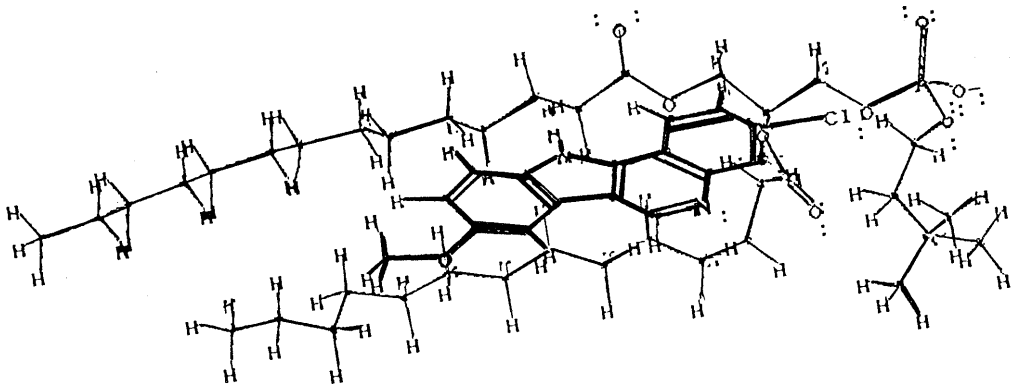


Fig. 5. The Docking of Compound II into the DMPC Molecule as Assessed by PC Model Version 4[13]

Both molecules are in their mimimum energy conformations, and optimal interaction was assessed by a least squares fit of all atoms.

The change in partitioning trends occurred in the vicinity of the T_c of the phospholipid, *viz.* 24 °C for compound II and slightly lower, at 20 °C, for compound I. As shown in Table II, a comparison of the log P values of compounds I and II at 16 °C (below T_c) and 32 °C (above T_c) showed that the log P of compound I is increased at the higher temperature. This may be attributed to a change in the state of the phospholipid (gel to liquid crystalline) with temperature. However, the opposite trend is observed with compound II. There is no good explanation for this anomalous behavior.

The partitioning of compound I into liposomes below T_c is characterized by a small enthalpy loss (heat evolved) and a large entropy gain. Thus, partitioning below T_c is entropically controlled. In contrast, the partitioning of compound II into liposomes below T_c is largely enthalpically driven. Above the T_c, the partitioning of both compounds I and II into liposomes is similarly characterized by a large gain in entropy as well as enthalpy, *i.e.* entropically dominated.

It has been noted that the partitioning of solutes into liposomes may involve large and compensatory changes in enthalpy and entropy which are related to changes in the liposome structure.[3] These changes, if significant, may mask the smaller changes in enthalpy and entropy due to the actual transfer of the solute. In this context, it may be more relevant to compare the partitioning characteristics of compounds I and II in octanol and phospholipid at temperatures below T_c (gel phase), as there is less likelihood of structural changes in the liposomes at a lower temperature. Other researchers have also cited greater accuracy and reproducibility as two advantages of determining the thermodynamic parameters in the gel state of the phospholipid.[4]

It is interesting to observe that the partitioning of compound I into the octanol or liposomal phases at 20 and 16 °C, respectively, is entropically driven. In contrast, the partitioning of compound II into octanol or liposomes at 16 °C is enthalpically controlled. The enthalpy driven partitioning of compound II into octanol has been attributed to its molecular shape and conformation, which vavors a van der Waals interaction with octanol. In order to assess the relative importance of enthalpy in the interaction of compounds I and II with liposomes, the least squares fit of all the atoms of compounds I or II into DMPC was assessed by computer.[13] Compound II was deemed to have a better fit into the DMPC molecule, as seen from its smaller deviation of fit (6.792 Å) as compared to compound I, where a larger deviation (7.029 Å) was observed. The more favorable interaction between compound II with DMPC may be attributed to its planarity and conformational rigidity (Fig. 5). Increased van der Waals interactions between the alkyl chains of DMPC and the hydrocarbon skeleton of compound II, concurrent with the immobilization of a solute molecule within the liposome, would result in a reduction of enthalpy and entropy. On the other hand, the twisted conformation of compound I would have caused considerable disruption of the ordered matrices of either octanol or DMPC, resulting in a large gain in entropy upon partitioning. Thus, by comparing two closely related compounds, it has been possible to show that the molecular shape and conformational characteristics of these solute molecules are important determinants of the partitioning process. The effect of these factors on the thermodynamics of the partitioning of these solutes are similarly manifested in both the octanol–buffer and liposome (gel state)–buffer systems.

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