Mechanisms of Liposomes/Water Partitioning of (p-Methylbenzyl)alkylamines

Roberta Fruttero,[1] Giulia Caron,[2] Elisa Fornatto,[1] Donatella Boschi,[1] Giuseppe Ermondi,[1] Alberto Gasco,[1] Pierre-Alain Carrupt,[2] and Bernard Testa[2,3]

Received April 15, 1998; accepted June 15, 1998

Purpose. The objective of this study was to compare and interpret the variations in lipophilicity of homologous (*p*-methylbenzyl)alkylamines (MBAAs) in isotropic (octanol/water) and anisotropic (zwitterionic liposomes/water) system.

Methods. Two experimental approaches were used, namely the pH-metric method to measure lipophilicity parameters in octanol/water and liposomes/water systems, and changes in NMR relaxation rates to validate the former method and to gain additional insights into the mechanisms of liposomes/water partitioning.

Results. For long-chain homologues (N-butyl to N-heptyl), the octanol/water and liposomes/water systems mostly expressed hydrophobicity. In contrast, the lipophilicity of the shorter homologues (N-methyl to N-propyl) in the two systems expressed various electrostatic and polar interactions.

Conclusions. The study sheds light on the molecular interactions between zwitterionic liposomes and amphiphilic solutes in neutral and cationic form.

KEY WORDS: lipophilicity; liposomes; intermolecular forces; ionic bonds; hydrogen bonds; hydrophobic interactions; pH-metric method; NMR relaxation rates.

INTRODUCTION

The biological activity of drugs depends on their interaction with biomembranes both in a pharmacodynamic and a pharmacokinetic context (1). In turn, the properties of such membranes depend on their dynamic behavior as influenced by their interactions with various xenobiotics.

Cationic amphiphilic drugs (CADs) are a class of compounds sharing some common chemical features, namely a lipophilic ring and a hydrophilic side-chain with a charged amino group. CADs used in therapy include cardiovascular agents (amiodarone and propranolol), antipsychotics (chloropromazine, imipramine and promazine), and antidepressants (trimipramine and chlorimipramine) (1). Such compounds can affect the properties of biomembranes with which they interact, for example by causing phospholipid storage disorders (phospholidosis) (1).

The log P values of neutral species are known to be comparable in octanol/water and liposomes/water systems, whereas charged species (in particular cations) partition significantly better into anisotropic lipid bilayers than into octanol, presumably due to the formation of ionic bonds with the anionic phosphate headgroups (2–5).

To unravel the complex mechanisms governing the interactions between CADs and biomembranes, homologous (*p*-methylbenzyl)-alkylamines (MBAAs) were prepared and examined for their partitioning in and interactions with zwitterionic liposomes (3,6,7). Two experimental approaches were used. Potentiometry (8,9) yielded the partition coefficients of the neutral and cationic forms in the *n*-octanol/water and liposomes/water systems. Changes in NMR relaxation rates in the presence of liposomes (10) were used to validate the potentiometric results and gave additional insights into the mechanisms of interaction between solutes and liposomes.

MATERIAL AND METHODS

Chemicals and Synthesis of (*p*-methylbenzyl)alkylamines

All reagents and deuterated solvents were supplied by Aldrich Chemical Co. (Milwaukee, WI, USA). Methanol, dichloromethane and diethyl ether were from Merck (Bracco, Milan, I). Analytical grade *n*-octanol was purchased from Fluka Chemie (Buchs, CH). Sodium phosphate (Fluka Chemie) was used as buffer.

The synthesis of the (*p*-methylbenzyl)alkylamines **1–11** was carried out by known procedures (11,12). *p*-Toluylaldehyde was converted into the imines with the corresponding alkylamines and the imines were reduced with $LiAlH_4$. The identity of all synthetized compounds was checked by ^{1}H-NMR spectroscopy in DMSO-d_6 at 200 MHz with a Bruker AC-200 spectrometer. Melting points (supporting information) were determined on a Büchi 530 apparatus (Büchi Labortechnik AG, Flawil, CH) and are uncorrected. Elementar analyses were within ± 0.4% of expected values (REDOX, Cologno Monzese, I).

Preparation of Liposomes

Egg-phosphatidylcholine grade I was purchased from Lipid Products (S. Nutfield, Surrey, UK). The liposomes were prepared by the extrusion method. The first batch was prepared as described in (13), except for a 0.15M KCl solution (instead of buffer) which was added to the dried film without detergent dialysis. The second batch of liposomes, to be used in NMR measurements, was prepared using deuterated water.

All liposomes were stored at 4°C under nitrogen and the lipid concentration of liposomes were determined as reported (13). The size distribution of liposomes was measured by dynamic light scattering (13).

Conformational Analysis and Calculation of Lipophilicity Ranges Using the Molecular Lipophilicity Potential (MLP)

A simplified conformational search strategy using Quenched Molecular Dynamics (QMD) was adopted (14,15). This procedure is able to describe efficiently the main valleys of a conformational space (see (16) for a complete description of the method). The Solvent-Accessible Surface Area (SASA)

[1] Dipartimento di Scienza e Tecnologia del Farmaco, via P. Giuria 9, I-10125 Torino, Italy.

[2] Institut de Chimie Thérapeutique, Section de Pharmacie, Université de Lausanne, CH-1015 Lausanne, Switzerland.

[3] To whom correspondence should be addressed. (e-mail: bernard.testa@ict.unil.ch)

0724-8741/98/0900-1407$15.00/0

(17) of the conformers generated by QMD was used as the space for integrating the MLP back to log P_{oct} as described (18). The MLP yields log P values for individual conformers (so-called virtual log P values), thus revealing the lipophilicity range accessible to a given solute by conformational adaptation (19).

All calculations were run on Silicon Graphics Personal, Indigo R4000 or Indy R4400 workstations. The SYBYL 6.2 molecular modeling package (Tripos Associates, St. Louis, MO, USA) was used. The MLP calculations were performed with the CLIP 1.0 software (20).

Measurement of Partition Coefficients

The partition coefficients in *n*-octanol/water and in liposomes/water were determined by the pH-metric method using a PCA101 apparatus (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK). Briefly, the pH-metric technique is based on two successive titrations. First, the solute in water is titrated against standard acid or base to deduce the ionization constant. Then the titration is repeated in the presence of a water-immiscible organic solvent and a new ionization constant is determined. In the presence of the dual-solvent mixture, the pK_a value shifts in response to the partitioning of some of the substance into the organic phase, giving an apparent constant called p_oK_a. The shift in pK_a is used in the calculation of log P, since the two are related. The principles of the pH-metric method for pK_a and log P measurement have been explained in detail (8). The general procedure described in (16) was used for the *n*-octanol/water system.

Recently the potentiometric method to measure partitioning coefficients was also extended to liposomes/water systems (21). Although the experimental procedure remains the same, the use of an anisotropic phase requires additional care, as detailed in Section 3.3.1. Here, we used phospholipid concentrations ranging from 1.24 to 12.6 mg/ml and phospholipid/solute ratios ranging from 5 to 40.

NMR Experiments

All NMR spectra were recorded at 200 MHz on a Bruker AC-200 NMR-spectrometer in a deuterated 0.03 M phosphate buffer of pD = 7.5 at 25°C. The constant solute concentration was $1.26 \cdot 10^{-2}$ M. The phospholipid/ligand concentration ratio ranged from 0.03 to 0.14, thus avoiding the interference of proton signals from the phospholipids.

The method used is mainly based on the changes in spin-spin ($1/T_2$) and spin-lattice ($1/T_1$) relaxation rates of the molecule spin systems, in the presence of liposomes. Under the experimental conditions used, the assumption holds that $1/T_1 = 1/T_2$ [10,22] and that a rapid exchange occurs between free and liposomes-bound ligand as expressed by Eq. 1:

$$\frac{1}{T_{2obs}} = \alpha \cdot \frac{1}{T_{2bound}} + (1 - \alpha) \cdot \frac{1}{T_{2free}} \qquad (1)$$

where α is the fraction of ligand bound, $1/T_{2bound}$ is the proton relaxation rate for the liposomes-bound ligand, $1/T_{2free}$ is the proton relaxation rate for the free ligand, and $1/T_{2obs}$ is the observed relaxation rate, representing a weighted average of the free and bound species of the ligand. The variation of $1/T_{2obs}$ is mainly due to liposomes-ligand interactions and increases linearly with liposome concentration. As a result, changes in proton relaxation rates broaden the proton signal according to Eq. 2

$$\Delta\nu_{1/2} = \frac{1}{\pi \cdot T_{2obs}} \qquad (2)$$

where $\Delta\nu_{1/2}$ (in Hz) is the line width of the observed signal at half-peak height. In this work, the values of $1/T_{2obs}$ were obtained by monitoring the broadening of suitable isolated spin systems of the solutes, namely the benzylic CH_2 and the aromatic CH_3 groups. The slopes obtained by plotting the observed $\Delta\nu_{1/2}$ versus liposomes concentration were used as an indicator of the degree of interaction of the solute with liposomes. Broadening was determined for at least 5 different phospholipid concentrations.

RESULTS AND DISCUSSION

Conformational Analysis

The conformational space of the neutral form of compounds **2–11** (compound **1** was not included given its very restricted conformational space) was investigated by computations in vacuo. Extended conformers were energetically favored over folded ones for compounds **2, 3**, and **4**, whereas the opposite was true for compounds **5** to **11**.

Partitioning in *n*-octanol/water

Calculated Log P Values

As described elsewhere (16) a comparison of calculated log P values obtained by various previsional methods is useful to avoid errors due to the poor parametrization of some fragments. Table 1 shows the log P values of compounds **1–11** in neutral form as calculated by the CLOGP algorithm (23) as well as their lipophilicity range (virtual log P of probable conformers) obtained by the MLP (17).

Experimental Log P Values

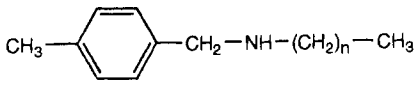
The partition coefficients of the neutral and cationic forms (log P^N and log P^C, respectively) and related parameters of compounds **1–7** are shown in Table 2. For compounds **8–11**, no satisfactory value of partition coefficients could be measured because the poK_as values did not vary regularly when increasing amounts of octanol were added. This suggests that a competitive mechanism acts simultaneously with partitioning. A likely explanation is that the length and folded conformation of the N-alkyl substituent in compounds **8–11** act together to favor their accumulation at the octanol/water interface (surfactant behavior, see Fig. 1). This in turn would increase the number of protons in the water phase, generating electrode artifacts.

A good correlation (Eq. 2) exists between experimental log P^N_{oct} and the calculated CLOGP value of compounds **1–7**:

$$\log P^N_{oct} = 1.08(\pm 0.12) \cdot \text{CLOGP} - 0.29(\pm 0.50)$$
$$n = 7;\ r^2 = 0.99;\ s = 0.16;\ F = 374 \qquad (3)$$

In this and the following equations, 95% confidence limits are given in parentheses; n is the number of compounds; r^2 the

Table 1. Partition Coefficients and Related Parameters of Compounds **1–11** in Octanol/water[a]



*	pK_a	CLOGP[b]	Virtual log P_{MLP}[c]	log P^N[d]	Increment[e]	log P^C[f]	Increment[g]	*diff*(log P^{N-C})[h]	log $D^{7.5}$[i]
1 (0)	9.93	2.01	1.95	1.96		—[k]		—	−0.43
					0.42		—		
2 (1)	10.04	2.54	2.75–2.81 (0.24)	2.38		−0.85		3.23	−0.11
					0.58		0.50		
3 (2)	9.98	3.07	3.08–3.44 (0.36)	2.96		−0.35		3.31	0.54
					0.53		0.52		
4 (3)	9.98	3.60	3.39–3.97 (0.58)	3.49		0.17		3.32	1.07
					0.77		0.60		
5 (4)	10.08	4.13	3.83–4.50 (0.67)	4.26		0.77		3.49	1.73
					0.70		0.54		
6 (5)	10.17[j]	4.65	4.22–5.09 (0.87)	4.96		1.31		3.65	2.33
					0.16		0.25		
7 (6)	10.02[j]	5.18	4.36–5.59 (1.23)	5.12		1.56		3.56	2.52
8 (7)	9.47[j]	5.71	4.91–6.24 (1.33)	—[k]	—	—[k]	—	—	—
9 (8)	9.48[j]	6.24	5.28–6.72 (1.44)	—[k]	—	—[k]	—	—	—
10 (9)	9.48[j]	6.77	5.73–7.25 (1.52)	—[k]	—	—[k]	—	—	—
11 (10)	9.46[j]	7.30	6.11–7.81 (1.70)	—[k]	—	—[k]	—	—	—

* Compound number (number of—CH_2— groups).
[a] Measured by potentiometry; the volume ratios of octanol and water were: 0.067; 0.499, 0.995.
[b] Taken from the Pomona database (23).
[c] Limits of virtual log P_{MLP}. In parentheses the range. See for definition (17).
[d] log P of the neutral form; n = 4; S.D. < 0.03.
[e] Increment in log P^N for the addition of a CH_2 group.
[f] Experimental log P of the cationic form; n = 4; S.D. < 0.03.
[g] Increment in log P^C for the addition of a CH_2 group.
[h] Experimental log P^N minus log P^C.
[i] Calculated by the equation: $D = P^N \cdot \left(\frac{1}{1 + 10^{pK_a - pH}}\right) + P^C \cdot \left(\frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}}\right)$
[j] The Yasuda-Shedlowsky approach (at least 4 points) was used.
[k] Not measurable, see text.

Table 2. Partition Coefficients and Related Parameters of Compounds **1–7** in the Liposomes/Water System[a]

*	log P^N[b]	Increment[c]	log P^C[d]	Increment[e]	*diff*(log P^{N-C})[f]	log $D^{7.5}$[g]
1 (0)	3.09		2.54		0.55	2.54
		−0.03		−0.28		
2 (1)	3.06		2.26		0.80	2.27
		0.01		−0.15		
3 (2)	3.07		2.11		0.96	2.12
		−0.02		−0.57		
4 (3)	3.05		1.54		1.51	1.58
		0.45		0.30		
5 (4)	3.50		1.84		1.66	1.89
		0.70		0.59		
6 (5)	4.20		2.43		1.77	2.48
		0.20		0.28		
7 (6)	4.40		2.71		1.69	2.77

* See Table 1 for the chemical structure of the investigated compounds.
[a] The lipid/water ratios were 1.24, 4.65, 12.6 (in mg/ml); lipid/solutes ratios varied from 5 to 40.
[b] Experimental log P of the neutral form; n = 5; S.D. < 0.05.
[c] Increment in log P^N for the addition of a CH_2 group.
[d] Experimental log P of the cationic form; n = 5; S.D. < 0.05.
[e] Increment in log P^C for the addition of a CH_2 group.
[f] log P^N minus log P^C.
[g] Calculated by the equation: $D = P^N \cdot \left(\frac{1}{1 + 10^{pK_a - pH}}\right) + P^C \cdot \left(\frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}}\right)$

squared correlation coefficient, s the standard deviation and F Fischer's test.

A good relation also exists between the octanol/water log P values of the neutral and cationic forms (log P^N_{oct} and log P^C_{oct}, respectively) (Eq. 4 and Fig. 2a). The value of the Y-intercept (ca. 3.3) is in agreement with the general assumption assigning 3 units to the difference between neutral and cationic forms (designated *diff*(log P^{N-C})in Table 1) (24).

$$\log P^N_{oct} = 1.17(\pm 0.10) \cdot \log P^C_{oct} + 3.34(\pm 0.10) \quad (4)$$
$$n = 5;\ r^2 = 0.99;\ s = 0.07;\ F = 802$$

Thus, the good agreement between experimental log P^N_{oct} and calculated data for compounds **1–7** (Eq. 3), and between log P^N_{oct} and log P^C_{oct} observed for **2–7** (compound **1** was too hydrophilic for its log P^C_{oct} value to be obtained by potentiometry), indicate that no peculiar intramolecular effect exists for these compounds in *n*-octanol/water. The smaller increment between **6** and **7** (see Table 1) suggests that compound **7** is already slightly affected by the surfactant behavior postulated for compounds **8–11**.

Lipophilicity Behavior in Liposomes/Water

Experimental log P_{lip} Values

Table 2 shows the liposome/water partition coefficients of the neutral and cationic forms (log P^N_{lip} and log P^C_{lip}, respectively) of compounds **1–7**. Due to their very low solubility, compounds **8–11** could not be examined.

Interestingly, the relation between log P^N and log P^C is more complex in liposomes/water than in octanol/water (Fig. 2b). This is due to the two parameters varying differently as a function of N-alkyl length (Fig. 3a and 3b).

A similar conclusion is obtained when comparing the log $D^{7.5}$ values (distribution coefficients measured at pH 7.5) in the two systems, which express mainly the contributions of the cationic forms. The relation is a bilinear one of good statistical quality (Eq. 5 and Fig. 2c):

$$\log D^{7.5}_{lip} = -0.97 \cdot \log D^{7.5}_{oct} + 31.3 \cdot \log(0.02 \cdot 10^{\log D^{7.5}_{oct}} + 1) + 1.95 \quad (5)$$

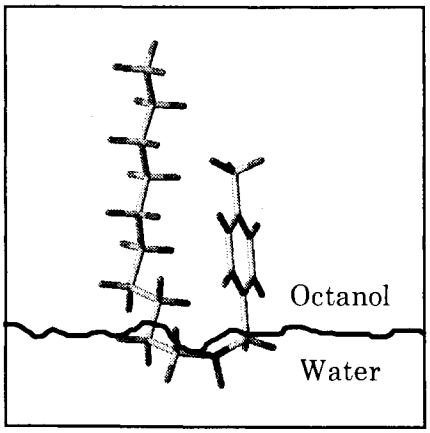


Fig. 1. Postulated interfacial position of higher N-alkyl homologues. The conformer represented here is one of lowest energy as calculated by Quenched Molecular Dynamics in vacuo.

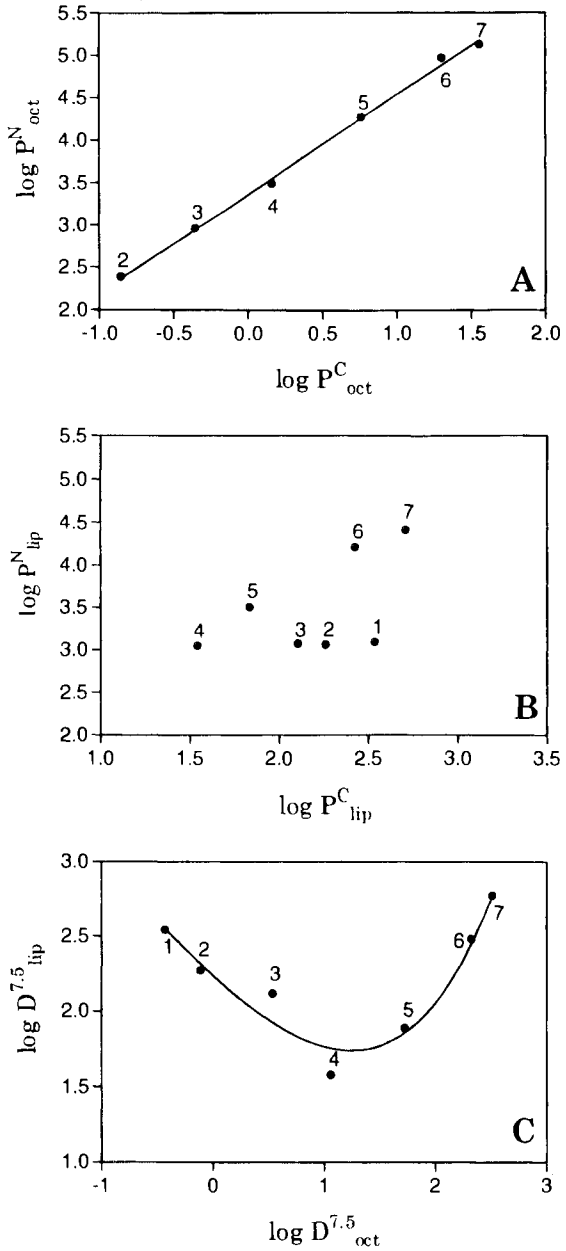


Fig. 2. Compared lipophilicities of compounds **1–7**. (**A**) Relation between partition coefficients of neutral and cationic forms (log P^N and log P^C, respectively) in the octanol/water system. (**B**) Relation between partition coefficients of neutral and cationic forms (log P^N and log P^C, respectively) in the liposomes/water system. (**C**) Bilinear relation between log $D^{7.5}$ in octanol/water and liposomes/water.

$$n = 7;\ r^2 = 0.92;\ s = 0.15$$

For the larger compounds (**5–7**), the positive slope of the bilinear regression (Fig. 2c) indicates that the two log D values increase together. This implies that hydrophobicity should be the major intermolecular force governing the partitioning of compounds **5–7** in the two systems (5). In contrast, the negative slope observed for the smaller (*p*-methylbenzyl)-alkylamines (**1–3**) (Fig. 2c) can only mean that ionic forces control their partitioning in the liposomes/water system. The low log $D^{7.5}_{oct}$ and log $D^{7.5}_{lip}$ values of **4** indicate that for this compound both ionic

and hydrophobic interactions are weak, hence its low affinity for liposomes.

The above interpretation is per force a preliminary one since the pH-metric method does not yields univocal evidence about the origin of pK_a shifts induced by liposomes (interfacial electrostatic field effect and/or hydrophobic anchoring). Independent methods must thus be used to obtain corroborative evidence.

T_2 NMR Results and Their Correlation with Lipophilicity

Table 3 reports the slopes of the linear regressions obtained by plotting the observed line width (in Hz) of the benzylic methylene group as the dependent variable and liposome concentration (in mg · ml^{-1}) as the independent variable. These results were obtained at pD 7.5 and thus express mainly the contribution of the cationic forms of compounds **1–7**. Interestingly the variation in the degree of interaction as a function of increasing chain length is not monodirectional. Indeed, the slope is large for **1**, decreases to reach a minimum for **4**, and increases up to compound **7**.

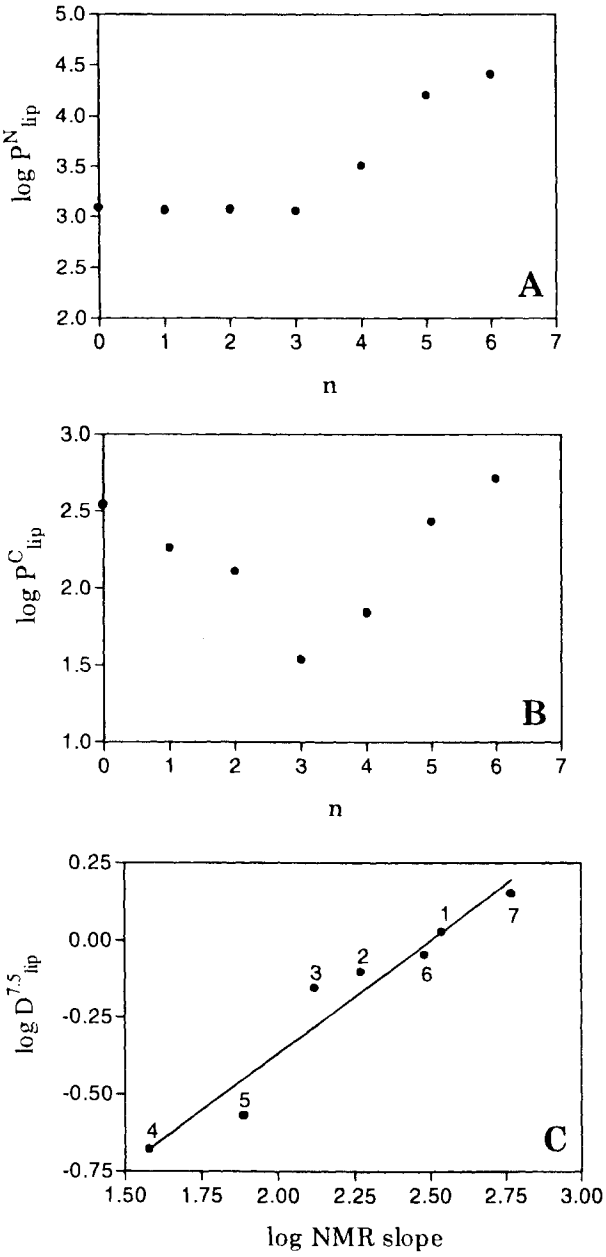


Fig. 3. Relation between lipophilicity of compounds **1–7** and other properties. (**A**) Variation of log P^N_{lip} with the number of methylene groups (n). (**B**) Variation of log P^C_{lip} with the number of methylene groups (n). (**C**) Linear regression between log NMR slope and lipophilicity parameters in the liposomes/water system.

Table 3. Slopes and Statistical Parameters of the Linear Regression Obtained by Plotting the Observed Line Widths as the Dependent Variable and Liposomes Concentrations as the Independent Variable[a]

	N[b]	slope[c]	S.D.	r^2	F
1	6	1.07	0.05	0.99	489.8
2	5	0.79	0.07	0.98	126.6
3	6	0.70	0.10	0.90	46.4
4	6	0.25	0.04	0.92	34.4
5	6	0.27	0.04	0.92	43.5
6	6	0.90	0.12	0.94	59.8
7	6	1.42	0.16	0.95	79.6

[a] Phospholipids/solute concentration ratio (see text for details) varied from 0.03 to 0.14.
[b] Number of experiments with different phospholipid/solute ratios.
[c] Slope obtained by plotting the observed $\Delta\nu_{1/2}$ (in Hz) versus liposomes concentration (in mg/ml).

Log $D^{7.5}$ values (Tables 1 and 2) were used to examine the relation between the lipophilicity parameters of compounds **1–7** and the NMR slope of their methylene group as measured at a pD of 7.5. A bilinear relation was apparent between log $D^{7.5}_{oct}$ and log NMR slope (not shown). In the case of log $D^{7.5}_{lip}$, a highly significant linear relation was found with log NMR slope (Eq. 6 and Fig. 3c):

$$\log \text{NMR slope} = 0.73(\pm 0.21) \cdot \log D^{7.5}_{lip} - 1.84(\pm 0.47) \quad (6)$$
$$n = 7;\ r^2 = 0.93;\ s = 0.09;\ F = 72$$

Despite differences in the experimental conditions, this result is taken as an independent and compelling confirmation of the log D values measured in the liposomes/water system by the pH-metric method.

Figure 4 shows the remarkable line broadening of the $-CH_2-$ signal of compound **2** taken as example, compared to that of the $-CH_3$ group, in the presence of increasing concentrations of liposomes. The line broadening of the $-CH_3$ group was modest in all solutes, the corresponding slope ranging from 0 to 0.20 (results not shown). As already observed by Seydel (10), the charged amino group is likely to be the primary anchor in solute-liposome interactions. This is in line with the present observation that the signal of the $-CH_2-$ group undergoes much larger line broadening than that of the $-CH_3$ group, the former being closer to the anchoring group than the second and hence having its mobility much more decreased by the interaction with liposomes.

When the alkyl chain of (*p*-methylbenzyl)alkylamines is long enough (n > 4), it can be expected to elicit additional hydrophobic interactions with liposomes. This however cannot be seen in the NMR spectra of MBBAs since no isolated spin

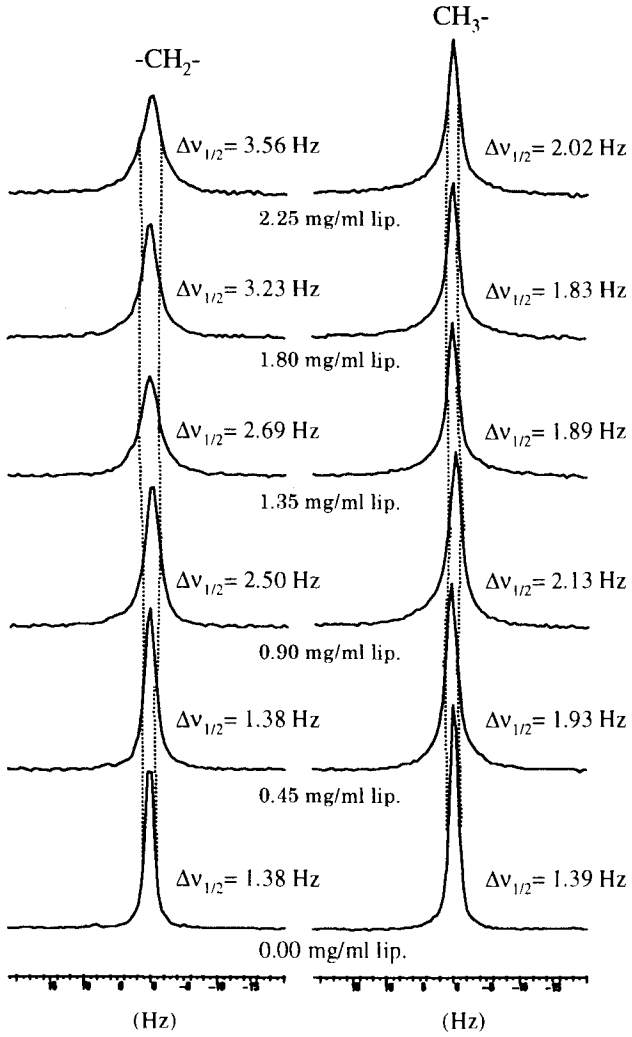


Fig. 4. Comparison between the peak broadening of the $-CH_2$ and $-CH_3$ protons in compound 2.

system other than the benzylic methylene is present which would be sensitive to hydrophobic interactions only.

Mechanisms of Interaction Between (p-Methylbenzyl)-alkylamines and Liposomes

The bilinear trends shown in Fig. 2b and 2c indicate that the length of the N-alkyl group critically influences the mode of interaction of (*p*-methylbenzyl)alkylamines with liposomes. In agreement with the conformational results reported above, and as suggested by others (10), it appears that, for an N-alkyl group in excess of 4 methylene groups ($n > 4$), both the neutral and cationic form of (*p*-methylbenzyl)alkylamines assume folded conformations whose partitioning is controlled by strong hydrophobic anchoring into the phospholipids.

For shorter N-alkyl homologues ($n < 4$) where such strong hydrophobic anchoring is not possible, a more superficial contact with dominating electrostatic forces must occur: ionic bonds for cations, and hydrogen bonds plus van de Waals forces for neutral molecules. This model is compatible with the results in Fig. 3a and 3b. Indeed, the interaction of cations with liposomes must involve the superficial phosphate groups and should decrease with increasing steric bulk at the ammonium group, as seen in Fig. 3b when the side-chain grows from N-methyl to N-propyl. For the neutral forms, a partial penetration into liposomes is necessary to interact with carbonyl moieties by H-bonds (and perhaps other dipole-dipole forces), explaining a constant lipophilicity of the N-methyl to N-propyl homologues (Fig. 3a).

To summarize, three types of intermolecular forces are postulated to control the interaction of (*p*-methylbenzyl)alkylamines with liposomes: a) an interfacial ionic bond between the ammonium and phosphate groups; b) partly internal H-bonds between the N-H groups of the neutral forms and carbonyl moieties; and c) internal hydrophobic interactions between the hydrocarbon groups in solutes and phospholipids. All three (for the cations) or two (for the neutral solutes) types of interactions will occur simultaneously, but their relative contribution will differ depending on the length of the N-alkyl group.

This balance of forces allows the (*p*-methylbenzyl)alkylamines examined here to be grouped into four classes (Table 4) based on their modes of interaction with zwitterionic liposomes: the cationic form of compounds **1–4** (class 1), their neutral forms (class 3), the cationic form of compounds **5–7** (class 2), and their neutral form (class 4). However, a more precise assignment of relative contributions was not possible since, as discussed above, no experimental technique is able to discriminate the relative contributions of ionic and hydrophobic forces.

CONCLUSIONS

Homologous amphiphilic (*p*-methylbenzyl)alkylamines were examined here for their lipophilic behavior in isotropic (octanol/water) and anisotropic (liposomes/water) systems. The solutes with very long N-alkyl groups (N-octyl and beyond) displayed surfactant properties that prevented reliable measurements of their lipophilicity. For the N-methyl to N-heptyl homologues, insights into their mechanisms of interaction with zwitterionic liposomes were obtained for the neutral and cationic forms. A biologically meaningful result is that, in liposomes/water as contrasted to octanol/water systems, little difference exists between the log P of the protonated and neutral form of a given amine. The difference is particularly modest for the shorter homologues in the series. The pharmacokinetic implications of such finding are obvious, given that biomembranes are anisotropic organic phases.

ACKNOWLEDGMENTS

BT and PAC are indebted to the Swiss National Science Foundation for support. The authors gratefully acknowledge Dr. Stefanie Krämer, Dr. Cornelia Ottiger and Prof. Heidi Wunderli-

Table 4. Forces Believed to Control Drug-Liposomes Interactions

	Ionic bond	H-bonds interactions	Hydrophobic interactions
Small cations (class 1)	++	−	−
Large cations (class 2)	+	−	++
Small neutral compounds (class 3)	−	++	+
Large neutral compounds (class 4)	−	+	++

Allenspach (ETH, Zurich) for their generous gift of liposomes. Thanks are also due to Dr. Alex Avdeef (pION, Brookline, MA, USA) for helpful advice and discussion.

REFERENCES

1. U. P. Kodavanti and H. M. Mehendale. Cationic amphiphilic drugs and phospholipid storage disorder. *Pharmacol. Rev.* **42**:327–354 (1990).
2. R. P. Mason, D. G. Rhodes, and L. G. Herbette. Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. *J. Med. Chem.* **34**:869–877 (1991).
3. R. P. Austin, A. M. Davis, and C. N. Manners. Partitioning of ionizing molecules between aqueous buffers and phospholipid vesicles. *J. Pharm. Sci.* **84**:1180–1183 (1995).
4. Y. Boulanger, S. Schreier, L. C. Leitch, and I. C. P. Smith. Multiple binding sites for local anesthetics in membranes: characterization of the sites and their equilibria by deuterium NMR of specifically deuterated procaine and tetracaine. *Can. J. Biochem.* **58**:986–995 (1980).
5. F. Barbato, M. I. La Rotonda, and F. Quaglia. Chromatographic indices determined on an immobilized artificial membrane (IAM) column as descriptors of lipophilic and polar interactions of 4-phenyldihydropyridine calcium-channel clockers with biomembranes. *Eur. J. Med. Chem.* **31**:311–318 (1996).
6. *Liposomes. A Practical Approach*. New, R. R. C. (ed.). IRL Press, Oxford, 1990.
7. B. I. Escher and R. P. Schwarzenbach. Partitioning of substituted phenols in liposome-water, biomembrane-water, and octanol-water systems. *Environ. Sci. Technol.* **30**:260–270 (1996).
8. A. Avdeef. pH-Metric log P. Part 1. Difference plots for determining ion-pair octanol-water partition coefficients of multiprotic substances. *Quant. Struct.-Act. Relat.* **11**:510–517 (1992).
9. A. Avdeef. Assessment of distribution-pH profiles. In V. Pliska, B. Testa, and H. van de Waterbeemd (eds.), *In: Lipophilicity in Drug Action and Toxicology* Vol. VCH Publishers, Weinheim, 1996, pp. 109–139.
10. J. K. Seydel, H. P. Cordes, M. Wiese, H. Chi, N. Croes, R. Hanpft, H. Lüllmann, K. Mohr, M. Patten, Y. Padberg, R. Lüllmann-Rauch, S. Vellguth, W. R. Meindl, and H. Schönenberger. QSAR and multivariate data analysis of amphiphilic benzylamines and their interaction with various phospholipids determined by different methods. *Quant. Struct.-Act. Relat.* **8**:266–278 (1989).
11. W. R. Meindl, E. von Angerer, H. Schönenberger, and G. Ruckdeschel. Benzylamines: synthesis and evaluation of antimycobacterial properties. *J. Med. Chem.* **27**:1111–1118 (1984).
12. J. V. Braun and A. Friedsam. Haftfestigkeit organischer Reste (VII. Mitteil.). *Chem. Ber.* **639**:2407–2412 (1930).
13. C. Ottiger and H. Wunderli-Allenspach. Ideal partition behaviour of acids and bases in a phosphatidylcholine liposome/buffer equilibrium dialysis system. *Eur. J. Pharm. Sci.* **5**:223–231 (1997).
14. P. Gaillard, P. A. Carrupt, and B. Testa. The conformational-dependent lipophilicity of morphine glucuronides as calculated from their molecular lipophilicity potential. *Bioorg. Med. Chem. Lett.* **4**:737–742 (1994).
15. C. Altomare, S. Cellamare, A. Carotti, G. Casini, M. Ferappi, E. Gavuzzo, F. Mazza, P. A. Carrupt, P. Gaillard, and B. Testa. X-ray crystal structure, partitioning behavior, and molecular modeling study of piracetam-type nootropics: insights into the pharmacophore. *J. Med. Chem.* **38**:170–179 (1995).
16. G. Caron, P. Gaillard, P. A. Carrupt, and B. Testa. Lipophilicity behavior of model and medicinal compounds containing a sulfide, sulfoxide, or sulfone moiety. *Helv. Chim. Acta* **80**:449–462 (1997).
17. P. A. Carrupt, B. Testa, and P. Gaillard. Computational approaches to lipophilicity: methods and applications. In D. B. Boyd and K. B. Lipkowitz (eds.), *In: Reviews in Computational Chemistry* Vol. 11, Wiley-VCH, New York, 1997, pp. 241–315.
18. P. Gaillard, P. A. Carrupt, B. Testa, and A. Boudon. Molecular lipophilicity potential, a tool in 3D-QSAR. Method and applications. *J. Comput.-Aided Mol. Design* **8**:83–96 (1994).
19. B. Testa, P. A. Carrupt, P. Gaillard, F. Billois, and P. Weber. Lipophilicity in molecular modeling. *Pharm. Res.* **13**:335–343 (1996).
20. CLIP1.0. Institute of Medicinal Chemistry, University of Lausanne, 1996.
21. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, and K. Y. Tam. pH-metric log P. 10. Determination of vesicle membrane-water partition coefficients of ionizable drugs. *Pharm. Res.* **15**:209–215 (1998).
22. J. J. Fischer and O. Jardetzky. Nuclear magnetic relaxation study of intermolecular complexes. The mechanism of penicillin binding to serum albumin. *J. Am. Chem. Soc.* **87**:3237–3244 (1965).
23. DAYLIGHT Software 4.41. Daylight Chemical Information System, Inc., Irvine, California, 1995.
24. M. Recanatini. Partition and distribution coefficients of aryloxypropanolamine β-adrenceptor antagonists. *J. Pharm. Pharmacol.* **44**:68–70 (1992).