Structure-Lipophilicity Relationships of Neutral and Protonated β-Blockers

Part I

Intra- and Intermolecular Effects in Isotropic Solvent Systems

by **Giulia Caron**[a]), **Guillaume Steyaert**[a]), **Alessandra Pagliara**[a]), **Frédéric Reymond**[b]), **Patrizia Crivori**[a]), **Patrick Gaillard**[a]), **Pierre-Alain Carrupt**[a]), **Alex Avdeef**[b]), **John Comer**[c]), **Karl J. Box**[d]), **Hubert H. Girault**[b]), and **Bernard Testa**[a])*

[a]) Institut de Chimie Thérapeutique, Section de Pharmacie, Université de Lausanne, CH-1015 Lausanne

[b]) Laboratoire d'Electrochimie, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne

[c]) pION Inc, 127 Smith Place, Cambridge, MA 02138, USA

[d]) Sirius Analytical Instruments Ltd., Riverside, Forest Row Business Park, Forest Row, East Sussex RH18 5DW, UK

The objectives of this study were to validate new experimental techniques used to measure the log *P* of protonated drugs, and to investigate the inter- and intramolecular forces influencing the partitioning behavior of β-blockers in isotropic biphasic solvent systems. The lipophilicity parameters of a number of β-blockers were measured by two-phase titration, centrifugal partition chromatography (CPC), and cyclic voltammetry (CV) in one or more of the following solvent systems: octanol/water, 1,2-dichloroethane/water, and dibutyl ether/water. CV proved to be a promising technique for measuring the lipophilicity of protonated β-blockers. Derived parameters such as $\Delta\log P$ (difference between log *P* in two different solvent systems, a parameter valid for a given solute in a given electrical form) and *diff* (difference between log *P* of two different electrical forms of a given solute, in the same system) yielded insights into inter- and intramolecular interactions characteristic of β-blockers. The relevance of these parameters in structure-permeation relationships is explored.

1. Introduction. – β-Blockers are widely used in the treatment of various cardiovascular diseases such as hypertension, angina pectoris, and cardiac arrhythmias [1][2] (*Fig. 1*). This series of drugs having the common structural elements of one or more aromatic rings, and a β-aminoethanol or 3-amino-2-hydroxypropoxy side chain (pK_a around 9.5) exists mostly as cations at physiological pH. In many studies, the lipophilic characteristics of β-blockers were examined in connection with their pharmacokinetic properties [3], although the lipophilic contribution of the cationic forms have been neglected [4–7]. Today, however, the significance of the lipophilicity of ionized forms is well recognized, not only in anisotropic media [8–10] but also in isotropic systems [11].

For complex compounds such as most drugs, the traditional octanol /H_2O system is not always a very good indicator of biodistribution, mainly because all biological membranes do not possess the same biophysical characteristics. Thus, four solvents, known as the 'critical quartet', each encoding a different balance of intermolecular forces assessed by the so-called solvatochromic parameters (α = H-bond donor acidity; β = H-bond acceptor basicity; π^* = dipolarity/polarizability, and V_W = calculated *Van*

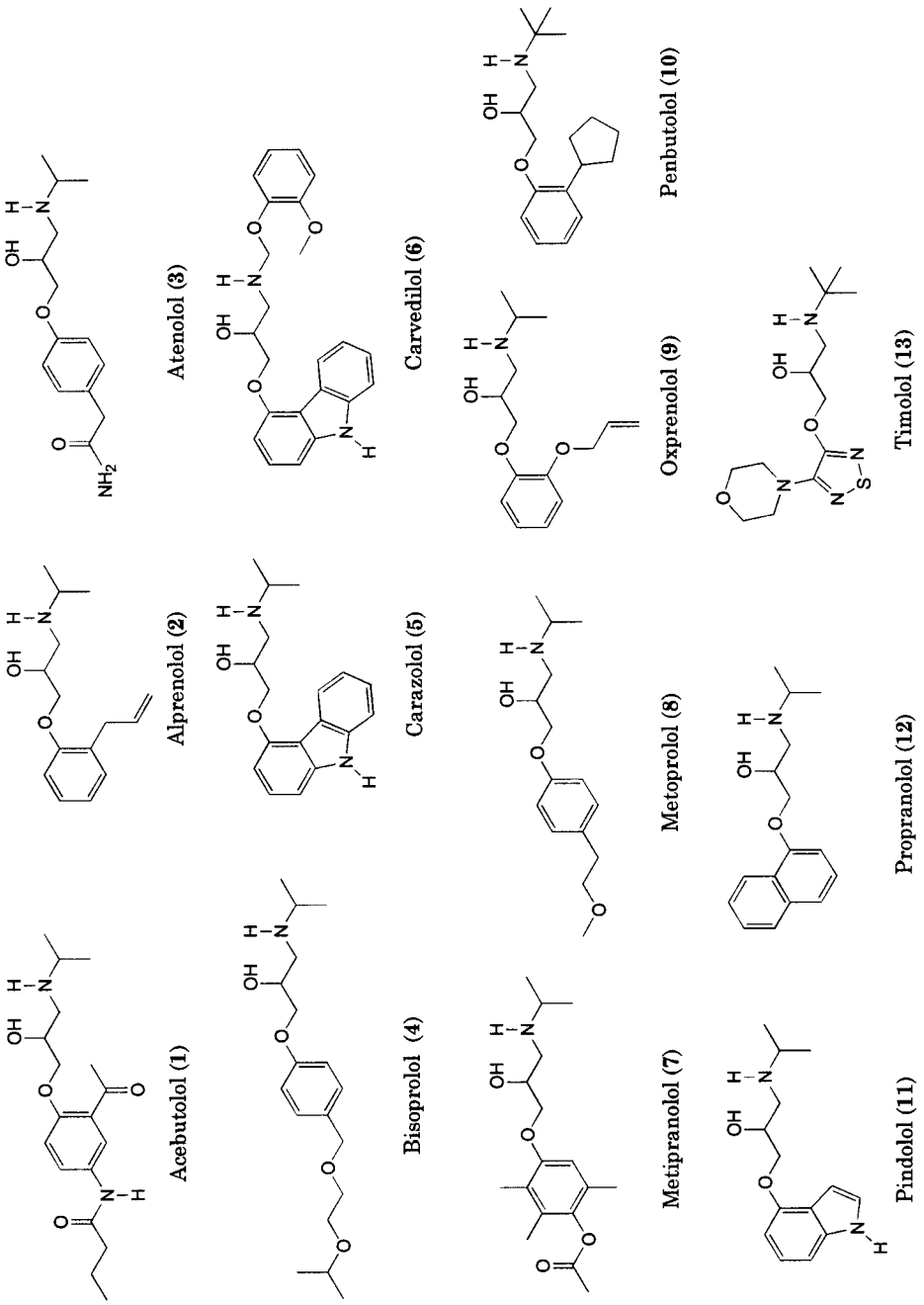


Fig. 1. *Chemical structures of β-blockers under study*

der Waals volume) are routinely used to mimic membrane variability. In particular, we have recently demonstrated [12] that the 1,2-dichloroethane (DCE)/H_2O system is the best inert solvent/H_2O system, since it encodes the same contribution from H-bonding as an alkane/H_2O system, but has far better dissolution properties. The dibutyl ether (DBE)/H_2O system is comparable to DCE/H_2O for partitioning of H-bond acceptors (comparable coefficient for the β term in solvatochromic analyses), but it favors the partitioning of H-bond donors into the organic phase (smaller coefficient for the α term) [13]. The octanol/H_2O system favors the partitioning of both H-bond donors and H-bond acceptors in the organic phase with respect to DCE/H_2O system. The fourth solvent system is CH_3Cl/H_2O, but its use is limited due to poor statistics in solvatochromic equations.

In recent years, new experimental techniques have become available to determine the partition coefficients of ionized forms. These include centrifugal partition chromatography (CPC) [11][14], potentiometry (also called pH-metry or two-phase titration) [15], and, more recently, cyclic voltammetry (CV) in the DCE/H_2O system [16]. These techniques are used routinely in our laboratories. They are compared here and used mainly to obtain the partition coefficients of cationic forms ($\log P^C$) and to unravel the inter- and intramolecular forces which influence the partitioning of neutral and cationic β-blockers in various isotropic solvent systems.

2. Results and Discussion. – 2.1. *Ionization Constants.* All compounds except carvedilol (**6**; pK_a 7.97) had similar pK_a values of around 9.5 (see later, *Table 2*). To rationalize the lower pK_a obtained for **6**, the MedChem database [17] was used to search for structures containing an ether O-atom located β to an amino group. Among the 47 compounds found, most had a pK_a value around 7–8. The origin of the discrepancy in pK_a values between carvedilol (**6**) and other β-blockers was thus attributed to the inductive effect of the β-O-atom which lowers the basicity of the amino group.

2.2. *Partition Coefficients in Various Solvent Systems, and Relevance of the* diff *Parameter*. For a given compound, the difference between the log *P* of its neutral ($\log P^N$) and ionized ($\log P^I$) forms in a given solvent system has been recently termed *diff*($\log P^{N-I}$) [11]. For a series of compounds, the averaged *diff* is the *Y* intercept of the linear regression obtained by plotting $\log P^N$ *vs.* $\log P^I$. For the β-blockers **1**–**13**[1]), the *diff* parameter was determined whenever feasible and is discussed below.

2.2.1. *Lipophilicity of Neutral and Cationic Species in Octanol/Water.* Provisional data of **1**–**13** obtained by the CLOGP algorithm [17] are shown in *Table 1*, whereas

---

[1]) Systematic names of compounds **1**–**13**: **1**: N-*(3-Acetyl-4-{2-hydroxy-3-[(1-methylethyl)amino]propoxy}-phenyl)butanamide*, **2**: *1-[(1-methylethyl)amino]-3-[2-(prop-2-enyl)phenoxy]propan-2-ol*, **3**: *4-{2-hydroxy-3-[(1-methylethyl)amino]propoxy}benzeneacetamide*, **4**: *1-(4-{[2-(1-methylethoxy)ethoxy]methyl}phenoxy)-3-[(1-methylethyl)amino]propan-2-ol*, **5**: *1-(9*H*-carbazol-4-yloxy)-3-[(1-methylethyl)amino]propan-2-ol*, **6**: *1-(9*H*-carbazol-4-yloxy)-3-{[(2-methoxyphenoxy)methyl]amino}propan-2-ol*, **7**: *4-{2-hydroxy-3-[(1-methylethyl)amino]propoxy}-2,3,6-trimethylphenyl acetate*, **8**: *1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol*, **9**: *1-[(1-methylethyl)amino]-3-[2-(prop-2-enyloxy)phenoxy]propan-2-ol*, **10**: *1-(2-cyclopentylphenoxy)-3-[(1,1-dimethylethyl)amino]propan-2-ol,* **11**: *1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)-amino]propan-2-ol*, **12**: *1-[(1-methylethyl)amino]-3-(naphthalen-1-yloxy)propan-1-ol*, **13**: *1-[(1-methylethyl)-amino]-3-{[4-(morpholin-4-yloxy)-1,2,5-thiadiazol-3-yl]oxy}propan-2-ol.*

experimental parameters are listed in *Table 2*. The good agreement (*Eqn. 1* and *Fig. 2,a*) between calculated and experimental data indicates that no strong intramolecular effect operates for the neutral species in the octanol/water system [18].

$$\log P^{N}_{oct} = 1.05(\pm 0.15) \cdot \mathrm{CLOGP} + 0.37(\pm 0.41) \quad (1)$$

$$n = 13;\ r^2 = 0.94;\ s = 0.29;\ F = 180$$

In this and the following equations, 95% confidence limits are given in parentheses; n is the number of compounds; r^2 the squared correlation coefficient, s the standard deviation, and F the *Fischer*'s test.

Table 1. *Calculated Lipophilicity Parameters of Neutral β-Blockers in Octanol/H_2O*

	CLOGP[a])	log P^{high}[b])	log P^{low}[c])	Range[d])
1	1.63	2.27	1.48	0.79
2	2.65	3.29	2.81	0.48
3	– 0.11	0.91	0.34	0.57
4	2.12	3.24	2.18	1.06
5	3.06	2.97	2.47	0.50
6	3.84	4.00	3.01	0.99
7	2.55	3.27	2.50	0.77
8	1.20	2.66	1.87	0.79
9	1.69	3.02	2.41	0.61
10	3.64	4.73	4.09	0.64
11	1.67	1.81	1.34	0.47
12	2.75	3.09	2.48	0.61
13	1.61	1.13	0.55	0.58

[a]) Taken from the *Pomona* database [17]. [b]) Virtual log P of the most lipophilic conformer, as calculated by the MLP. [c]) Virtual log P of the most hydrophilic conformer, as calculated by the MLP. [d]) Lipophilicity range calculated as log P^{high} – log P^{low}.

Table 2. *Dissociation Constants and Partition Coefficients of Neutral and Cationic β-Blockers in Octanol/H_2O*

	pK_a[a])	log P^{N}_{oct}[b])	log P^{C}_{oct}[b])	*diff*(log P^{N-C})$_{oct}$[c])
1	9.52	2.02	– 0.50	2.52
2	9.59	3.10	0.25	2.85
3	9.54	0.22	< – 2.0	–
4	9.57	2.15	– 1.22	3.37
5	9.52	3.73	0.77	2.96
6	7.97[d])	4.11	1.92	2.19
7	9.54	2.81	– 0.26	3.07
8	9.63	1.95	– 1.10	3.05
9	9.57	2.51	– 0.13	2.64
10	9.92[d])	4.62	1.32	3.30
11	9.54	1.83	– 1.32	3.15
12	9.53	3.48	0.78	2.70
13	9.53	2.12	– 0.94	3.06

[a]) Measured by potentiometry; $n = 3$, SD < 0.05. [b]) Determined by potentiometry; $n = 4$, SD < 0.05. [c]) log P^{N} – log P^{C}. [d]) MeOH as cosolvent; $n = 5$, SD < 0.10.

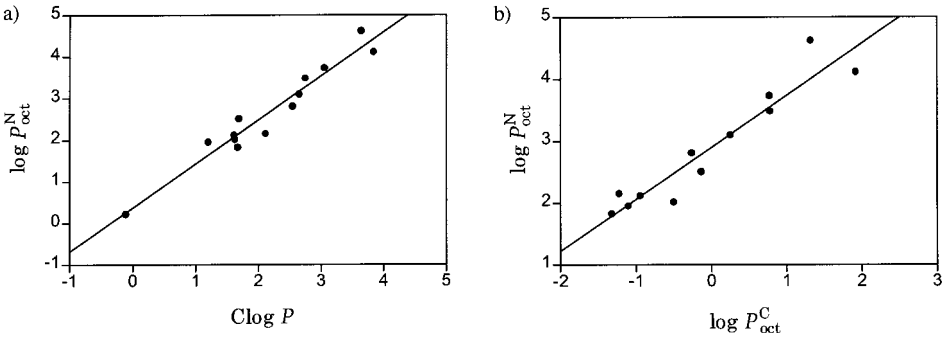


Fig. 2. *Lipophilicity of neutral forms of β-blockers* (log P^N_{oct}) *in isotropic systems:* a) *log* P^N_{oct} vs. *Clog*P; b) *log* P^N_{oct} vs. *log* P *of cationic forms in octanol/H_2O* (log P^C_{oct})

The back-calculation of partition coefficients with the *Molecular Lipophilicity Potential* (MPC) [11][18] was also carried out to investigate the influence of conformational variability on lipophilicity. As shown in *Table 1*, all compounds had approximately the same conformational variability toward lipophilicity (*i.e.*, the same lipophilicity range). The largest range was that of compounds **4** and **6**, which indeed have the greatest potential for flexibility (see *Fig. 1*).

The log *P* values of the neutral (log P^N) and cationic (log P^C) forms were linearly related, as shown by *Fig. 2,b* and *Eqn. 2:*

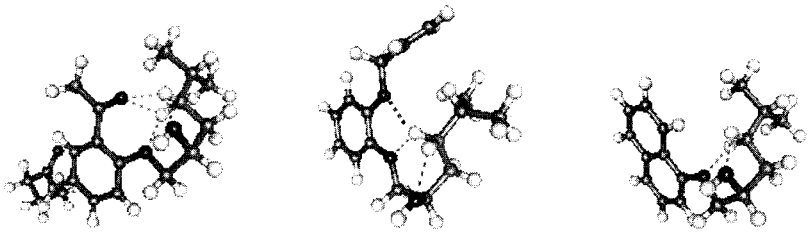
$$\log P^N_{oct} = 0.84(\pm 0.20) \cdot \log P^C_{oct} + 2.90(\pm 0.20) \quad (2)$$

$$n = 12;\ r^2 = 0.90;\ s = 0.31;\ F = 90$$

The value of the Y intercept (2.90 ± 0.20) corresponds to the lipophilicity increment between cationic and neutral species, symbolized by the parameter *diff*(log P^{N-C})$_{oct}$ [11]. This value is in agreement with various literature observations assigning 3 log *P* units for the difference between neutral and cationic forms in octanol/H_2O. Interestingly, compounds **1**, **6**, and **9** showed an abnormally low *diff*(log P^{N-C})$_{oct}$ (average 2.45 for the three solutes, in contrast to the average of 3.06 for the other solutes; see *Table 2*). Because of the good agreement between CLOGP and experimental log P^N (*Eqn. 1*), we can conclude that the cationic form of compounds **1**, **6**, and **9** showed an abnormally high lipophilicity. These three compounds bear an *ortho*-O-atom able to form an intramolecular H-bond with the protonated 3-amino-2-hydroxypropoxy side-chain. The formation of internal H-bonds is well-known to be a factor which increases lipophilicity.

The involvement of a reinforced H-bond between the protonated amino group and the *ortho*-O-atom was confirmed by high-temperature molecular dynamics. Because of the complex structure of carvedilol (**6**), minimum-energy conformations were compared only for the cationic forms of **1** and **9**, with propranolol (**12**) as reference (*Fig. 3*). The *ortho*-O-atom (carbonyl or ether, resp.) in compounds **1** and **9** was involved in a multiple internal H-bonding pattern which afforded additional stabilization of folded conformers relative to compound **12**. The higher virtual lipophilicity of such folded, cationic conformers was demonstrated by MLP calculations [19]. Indeed,

the lipophilicity range of cationic **1** and **9** was extended toward higher virtual log P^C values (lipophilicity range 1.53 and 1.15 for cationic **1** and **9**, resp., relative to 0.82 for **12**).



Acebutolol (**1**) Oxprenolol (**9**) Propranolol (**12**)

Fig. 3. *Minimum-energy conformers of protonated acebutolol* (**1**), *oxprenolol* (**9**), *and propranolol* (**12**). The dotted lines represent reinforced H-bonds.

2.2.2. *Lipophilicity of Neutral and Cationic Species in 1,2-Dichloroethane/Water.* The partition coefficients of compounds **1**–**13** in the DCE/H_2O system are shown in *Table 3*. Two-phase potentiometry was used to investigate the neutral forms (log P^N_{DCE}). Cationic log *P* values (log P^C_{DCE}) were obtained by cyclic voltammetry (CV). This being a recently developed method, we also compared its results with those obtained by an independent approach, but since the pH-metric method was not applicable due to the very low lipophilicity of cationic β-blockers in this solvent system, CPC was used for comparison.

Table 3. *Partition Coefficients of β-Blockers in the DCE/H_2O and DBE/H_2O Systems*

	log P^N_{DCE} [a])	log P^C_{DCE}(CV) [b])	log P^C_{DCE}(CPC) [c])	*diff*(log P^{N-C})$_{DCE}$ [d])	log P^N_{DBE} [e])	Δlog $P^N_{oct-DCE}$ [f])	Δlog $P^N_{oct-DBE}$ [g])
1	0.81	– 2.22	–	–	0.56[h])	1.21	1.46
2	3.26	– 1.84	–	–	2.27	– 0.16	0.83
3	– 1.24[i])	– 5.45	–	–	0.13[h])	1.46	0.09
4	2.37	– 2.38	– 2.40	4.77	0.77	– 0.22	1.38
5	3.03	– 2.03	– 2.09	5.12	2.25	0.70	1.48
6	4.74[i])	– 0.47	– 0.99	–	2.15	– 0.73	1.96
7	3.06	– 1.87	– 1.87	4.93	1.57	– 0.25	1.24
8	1.99	– 2.20	– 2.54	4.53	0.56	– 0.04	1.39
9	2.46	– 1.30	– 2.51	4.97	1.06	0.05	1.45
10	4.50	– 0.65	0.12[j])	4.62	3.91	0.12	0.71
11	1.31	– 3.26	< – 3.0	–	0.27[j])	0.52	1.56
12	3.11	– 2.08	– 1.93	5.04	2.18	0.37	1.29
13	2.13	– 2.89	–	–	0.67	– 0.01	1.45

[a]) Measured by potentiometry; $n = 4$, SD < 0.05. Compounds **3** and **6** were not soluble enough in the organic solvent. [b]) Measured by CV (at least 6 measurements at different pH; SD < 0.30). [c]) Measured by centrifugal partition chromatography (HCl, pH 2); missing values: not determined. [d]) log P^N – log P^C obtained by CPC. [e]) Measured by potentiometry; $n = 4$, SD < 0.05. [f]) log P^N in octanol/H_2O – log P^N in DCE/H_2O. [g]) log P^N in octanol/H_2O – log P^N in DBE/H_2O. [h]) Measured by the shake-flask procedure [11][13]. The aqueous phase was 0.02M HCl, pH 2. [i]) Calculated by *Eqn. 4* (see text). [j]) Measured by the shake-flask procedure [11][33]. The pH of the aqueous phase was adjusted to 12.

The results in the CDE/H_2O system are listed in *Table 3* and are shown in *Fig. 4*. There is good agreement between the two techniques (*Eqn. 3*); however, oxprenolol (**9**) behaved as an outlier and was excluded:

$$\log P^{C}_{DCE}\ (CPC) = 0.74(\pm 0.22) \cdot \log P^{C}_{DCE}\ (CV) - 0.67(\pm 0.46) \qquad (3)$$

$$n = 8;\ r^2 = 0.91;\ s = 0.22;\ F = 66$$

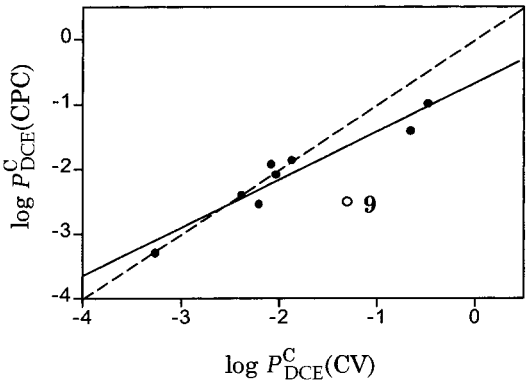


Fig. 4. *Lipophilicity of β-blockers in DCE/H_2O.* Comparison of log P^{C}_{DCE} data: CPC values *vs.* CV values. The dotted line corresponds to the ideal line of slope 1 and intercept 0.

Interestingly, the slope is different from zero due to the fact that the more lipophilic cations (**6** and **10**) showed higher log P^{C} values by CV than by CPC. This is explained by the different counterions used in the two techniques (see *Exper. Part*). The phenomenon is under investigation.

When the experimental partition coefficients of the neutral forms (log P^{N}_{DCE}) were plotted as a function of the partition coefficients of the cationic forms (log P^{N}_{dce}), linear correlations were obtained for the CPC results (*Eqn. 4*). For the CV results, oxprenolol (**9**) and acebutolol (**1**) were excluded as outliers (*Eqn. 5*).

$$\log P^{N}_{DCE} = 0.92(\pm 0.26) \cdot \log P^{C}_{DCE}\ (CPC) + 4.62(\pm 0.59) \qquad (4)$$

$$n = 8;\ r^2 = 0.93;\ s = 0.28;\ F = 74$$

$$\log P^{N}_{DCE} = 1.20(\pm 0.36) \cdot \log P^{C}_{DCE}\ (CV) + 5.30(\pm 0.81) \qquad (5)$$

$$n = 9;\ r^2 = 0.90;\ s = 0.32;\ F = 61$$

Interestingly, the value of the *diff*(log $P^{N-C})_{DCE}$, *i.e.*, the *Y* intercept in *Eqn. 4* (4.62) and *Eqn. 5* (5.30) is larger by *ca.* 2 units than the corresponding value in the octanol/H_2O system (*Eqn. 2*; 2.90). The *diff* value in DCE/H_2O is comparable to values obtained for other localized monocations [20]. The larger *diff* value in DCE/H_2O than in octanol/H_2O implies that, according to the *Born* ion-solvent-interaction model, the molecular radius of ions is larger in octanol than in DCE. This may be because ions with

a localized charge retain more H_2O molecules when they transfer into octanol than into DCE, due to the greater water solubilization and H-bonding capacity of octanol [20].

The deviation of the *diff*$(\log P^{N-C})_{DCE}$ value measured by CV for oxprenolol (**9**) (*diff* = 3.76) and for acebutolol (**1**) (*diff* = 3.03) are in line with the results obtained in octanol/H_2O. The lipophilicity of the cation of these two compounds is increased by the formation of additional intramolecular H-bonds with the *ortho*-O-atom. However, the origin of the discrepancy between values obtained by CV and CPC remains unsolved even if, as in our opinion, CPC data appear to be less reliable when they approach the experimental limit of the technique.

The log *P* of the cation of atenolol (**3**) and carvedilol (**6**) measured by CV was used in *Eqn. 5* to estimate the log *P* of their neutral form (– 1.24 and 4.74, resp.) in DCE/H_2O (*Table 3*).

2.2.3. *Lipophilicity in the Dibutyl Ether(DBE)/H_2O System.* Potentiometry was also used to measure the partition coefficient of the neutral β-blockers in the DBE/H_2O system. The corresponding data are shown in *Table 3*, and their significance relative to other biphasic systems is discussed below. Determining the partition coefficient of the cationic forms in this solvent system proved impossible by pH-metry and by CPC.

2.3. *Comparison of log* P *Values in the Three Solvent Systems to Reveal Intermolecular Effects.* The $\Delta\log P$ parameter is the difference between the log *P* values of a given compound, in a given electrical state, obtained in two different solvent systems [21][22]. As demonstrated in a number of studies, $\Delta\log P$ affords an expression of polar intermolecular interactions, mainly H-bonding.

The $\Delta\log P^{N}_{(oct-DCE)}$ values of the β-blockers are reported in *Table 3* and are represented graphically in *Fig. 5*,*a*, where a comparison is made with a well-distributed series of model compounds [12]. Interestingly, the majority of investigated β-blockers fall between the two regression lines representing H-bond donors ($\alpha > 0$) and pure H-bond acceptors ($\alpha = 0$). This suggests that the H-bond-donor character of β-blockers is poorly expressed in DCE/H_2O. The formation of intramolecular H-bonds between the OH and the neutral NH group of the 3-amino-2-hydroxypropoxy side chain is believed to account for this behavior. When additional H-bond-donor substituents are present, as is the case in acebutolol (**1**), atenolol (**3**), carazolol (**5**), and pindolol (**11**), the solutes behave as regular H-bond donors.

In contrast to their DCE/H_2O values, the log *P* values of β-blockers in DBE/H_2O ($\log P_{DBE}$) are smaller than their $\log P_{oct}$ values, resulting in positive $\Delta\log P^{N}_{(oct-DBE)}$ values (*Table 3*). These data are represented graphically in *Fig. 5*,*b*, together with a well-distributed series of model compounds [13]. Excepting atenolol (**3**), which behaved as an outlier for unknown reasons, *Eqn. 6* demonstrates the good correlation existing between lipophilicity values in octanol/H_2O and DBE/H_2O.

$$\log P^{N}_{oct} = 0.84(\pm 0.18) \cdot \log P^{N}_{DBE} + 1.60(\pm 0.30) \quad (6)$$

$$n = 12;\ r^2 = 0.91;\ s = 0.29;\ F = 106$$

Moreover, the $\Delta\log P_{(oct-DBE)}$ value is even larger for β-blockers than for model compounds with good H-bond-donor capacity [13]. This observation suggests than, like in octanol, the formation of internal H-bonds in the 3-amino-2-hydroxypropoxy

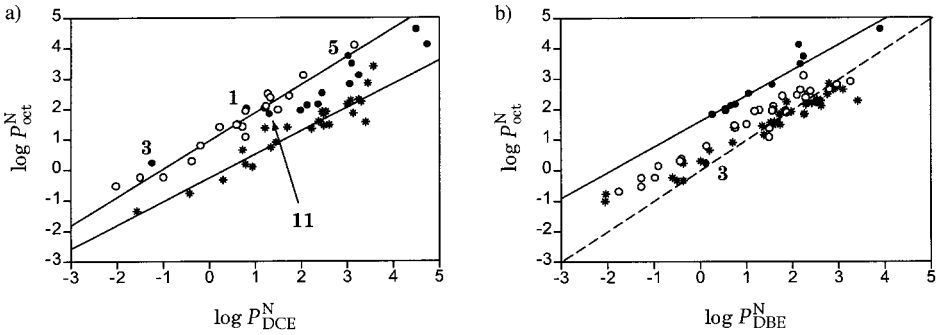


Fig. 5. a) *log* P^N_{oct} vs. *log* P^N *in DCE/H₂O* (log P^N_{DCE}). ○: H-Bond-donor solutes ($\alpha > 0$); ∗: non H-bond-donor solutes ($\alpha = 0$); ●: β-blockers. b) *log* P^N_{oct} vs. *log* P *in DBE/H₂O* (log P^N_{DBE}). ○: H-Bond-donor solutes ($\alpha > 0$); ∗: non-H-bond-donor solutes ($\alpha = 0$); ●: β-blockers.

side chain is not favored in DBE, rendering the substituents available for intermolecular interactions with the solvent. Thus, the β-blockers behaved as regular H-bond donors with lower partition coefficients in DBE/H_2O [13], in compatibility with a solvatochromic analysis that found a large negative coefficient for the H-bond-acceptor capacity (β) and the H-bond-donor capacity (α).

3. Conclusion and Pharmacokinetic Implications. – A congeneric series of β-blockers was used to validate novel experimental techniques to measure lipophilicity parameters. The results demonstrate that CV is an informative technique to measure partition coefficients of ionized compounds. From an experimental log P^C_{DCE} value and an average *diff*(log P^{N-C})$_{DCE}$ value, it is even possible to estimate a log P^N_{DCE}.

The multi-system analysis of the lipophilicity of this series of β-blockers distinguishes ionizable species according to their intramolecular interactions: neutral β-blockers express intramolecular H-bonds mainly in the DCE/H_2O system, but cationic β-blockers bearing an *ortho*-O-atom are able to form internal reinforced H-bonds also in the octanol/H_2O system.

The different information encoded in each system has pharmacokinetic implications. For example, the corneal penetration (expressed of the logarithm of permeability coefficients) of a series of β-blockers at pH 7.65 was related parabolically with their distribution coefficient in octanol/buffer (log $D^{7.65}_{oct}$) [23]. Acebutolol (**1**) was an outlier (*Fig. 6,a*, no curve fitted to the data). Since Δlog $P_{oct-alk}$ is often recognized as a good predictor of permeation and passive diffusion [21][24], we looked for a relation between these corneal permeation data and Δlog $P_{oct-DCE}$ (which encodes the same H-bond-donor contribution as Δlog $P_{oct-alk}$ [12]). As seen in *Fig. 6,b*, compounds having a low Δlog *P* value have high permeability, whereas compounds with a high Δlog *P* value permeate poorly. A linear correlation can be calculated, but it is statistically unsound given the clustered distribution of points.

The corneal permeation data were also examined in relation with log $D^{7.65}_{DCE}$, yielding a sigmoidal relation (*Fig. 6,c*, $r^2 = 0.96$) with no outlier. This suggests that log D_{DCE} is a promising predictor of corneal permeation.

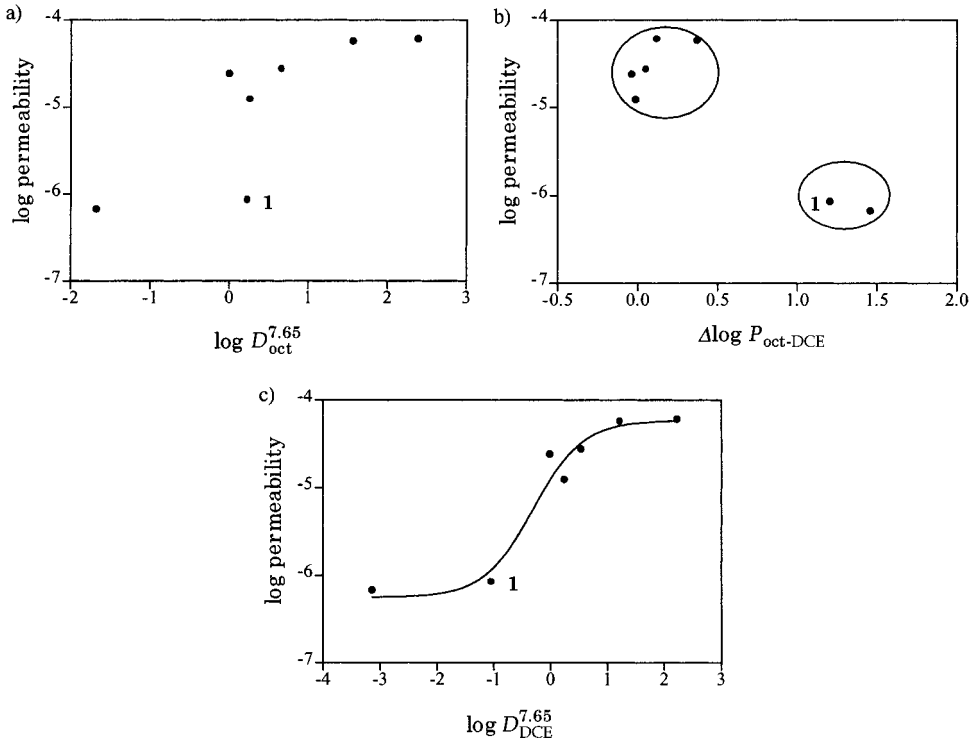


Fig. 6. *Relation between the corneal permeation of β-blockers* [23] (values in cm/s: penbutolol (**10**), −4.22; propranolol (**12**), −4.24; oxprenolol (**9**), −4.56; timolol (**13**), −4.91; metoprolol (**8**), −4.62; acebutolol (**1**), −6.07; atenolol (**3**), −6.17 and various lipophilic descriptors.

a) Corneal permeation *vs.* log $D_{oct}^{7.65}$ calculated from data in *Table 2* and using *Eqn. 7:*

$$D = P^{N} \cdot \left(\frac{1}{1 + 10\,pK_a - pH} + P^{C} \cdot \frac{10\,pK_a - pH}{1 + 10\,pK_a - pH} \right) \tag{7}$$

b) Corneal permeation *vs.* Δlog $P_{oct-DCE}$

c) Corneal permeation *vs.* log $D_{DCE}^{7.65}$ calculated from data in *Table 3* and using *Eqn. 7.*

Experimental Part

1. *Materials.* Acebutolol·HCl (**1**), alprenolol·HCl (**2**), metoprolol tartrate (**8**), oxprenolol·HCl (**9**), pindolol (**11**), propranolol·HCl (**12**), and timolol maleate (**13**) were purchased from *Sigma Chemie* (Buchs, CH). (+)-(*R*)-Atenolol (**3**) was obtained from *Aldrich* (Steinheim, D). Carazolol (**5**), carvedilol (**6**), and metipranolol (**7**) were kindly offered by *Boehringer Mannheim GmbH* (Mannheim, D). Penbutolol sulfate (**10**) was offered by *Hoechst Pharma AG* (Zürich, CH). Bisoprolol hemifumarate (**4**) was a gift from Prof. *G. Cheymol* (Paris).

Anal. grade octanol, dibutyl ether (DBE), and 1,2-dichloroethane (DCE) were purchased from *Fluka Chemie* (Buchs, CH). Anh. Na_2HPO_4 and KH_2PO_4 were purchased from *Fluka Chemie*, and KCl from *Merck* (Dietikon, CH).

2. *Potentiometric Determination of Protonation Constants.* Potentiometric titrations of compounds **1**–**13** were performed with the *PCA101* apparatus [25] (*Sirius Analytical Instruments Ltd*, Forrest Row, East Sussex, UK) as described in [11].

The low aqueous solubility of compounds **6** and **10** required pK_a measurements in the presence of MeOH as cosolvent. At least five separate 20-ml semiaqueous solns. of *ca.* 1 mM, in 20–60 (% *w*/*w*) MeOH were initially acidified to pH 4.0 with HCl. The solns. were then titrated with standardized KOH to pH 10.5. The titrations were conducted under Ar at $25.0 \pm 0.1°$. The initial estimates of the p_SK_a values (the apparent ionization constants in the H_2O/cosolvent mixture) were obtained by *Bjerrum* plots. These values were refined by a weighted nonlinear least-squares procedure. The refined values were then extrapolated to zero by the *Yasuda-Shedlovsky* procedure [26].

3. *Determination of Partition Coefficients.* 3.1. *The Potentiometric Method.* The partition coefficients in octanol/H_2O, DCE/H_2O, and DBE/H_2O were determined by the pH-metric method with the *PCA101* apparatus. Briefly, the pH-metric technique is based on two successive titrations. First, the solute in H_2O is titrated against standard acid or base to obtain ionization constants. Then, the titration is repeated in the presence of a H_2O-immiscible org. 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 org. phase, giving an apparent constant called p_oK_a. This 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 in [25]. At least four separate titrations of compounds **1**–**13** (*ca.* 1 mM) were carried out with the *PCA101* apparatus (see above) in the pH range 1.8 to 12.2, using various volumes of octanol or another org. solvent (volume ratios of organic solvent/H_2O ranging from 1 ml/15 ml to 8 ml/8 ml). The titrations were carried out under Ar at $25.0 \pm 0.1°$ [11][27].

3.2. *Cyclic Voltammetry.* The partition of the cationic forms of β-blockers was studied by CV with a home-made four electrode potentiostat with *iR*-drop compensation. The details on the electrochemical measurements and the theoretical background have been presented in [16][28][29]. The aq. phase was deionized H_2O (*Milli-QSP* reagent water system, *Millipore*) with LiCl (*Fluka*) as aq. electrolyte, and the pH was adjusted to the desired value by the addition of HNO_3 or LiOH (*Fluka*). The org. phase was DCE of the highest available purity (*Merck*) with bis(triphenylphosphoranylidene) ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl) as org. supporting electrolyte. As DCE is a suspected carcinogen [30], it was handled with all necessary precautions to avoid inhalation and skin contact.

3.3. *Centrifugal Partition Chromatography (CPC).* The partition coefficients of cationic β-blockers in the DCE/H_2O system (log P^C_{DCE}) were also determined by flow-through CPC with a coil-planet-type centrifuge (*Ito Multi-layer Coil Separator-Extractor*, *P.C. Inc.*, Baltimore, MD, U.S.A.), as described in detail in [11][14][31]. The pH of the aq. phase was set to 2.00 ± 0.05 by HCl. Because of the hydrophilicity of the compounds, the stationary phase was DCE and H_2O was the mobile phase. $CoCl_2$ was used to determine the dead time.

4.4. *Quenched Molecular-Dynamics (QMD) Calculations and Calculation of Partition Coefficients from the Molecular Lipophilicity Potential (MLP).* The methods are described in [11]. All calculations were run on *Silicon Graphics Indy R4400*, *O2 R5000*, and *Origin2000 R10000* workstations. The Sybyl 6.2 molecular modeling package [32] was used.

B.T., *P.A.C.*, and *H.H.G.* are grateful for support from the *Swiss National Science Foundation*. The authors thank Dr. *Laurent Schio* (*Hoechst Marion Roussel*, Romainville, France) for stimulating discussions.

REFERENCES

[1] B. G. Main, H. Tucker, in 'Progress in Medicinal Chemistry. Vol 22', Elsevier Science Publishers, 1985, pp. 121–164.
[2] B. G. Main, H. Tucker, in 'Medicinal Chemistry', Eds. C. R. Ganellin and S. M. Roberts, Academic Press, London, 1993, Vol. 4, pp. 187–208.
[3] S. Ferrari, T. Leemann, P. Dayer, *Life Sci.* **1991**, *48*, 2259.
[4] D. E. Drayer, *Pharmacotherapy* **1987**, *7*, 87.
[5] G. Cheymol, J. M. Poirier, J. Barré, A. Pradalier, J. Dry, *J. Clin. Pharmacol.* **1987**, *27*, 874.
[6] I. Kobayashi, K. Hosaka, T. Ueno, H. Maruo, M. Kamiyama, C. Konno, M. Gemba, *Biol. Pharm. Bull.* **1997**, *20*, 421.
[7] Y. Yamada, K. Ito, K. Nakamura, Y. Sawada, T. Iga, *Biol. Pharm. Bull.* **1993**, *16*, 1251.
[8] R. Fruttero, G. Caron, E. Fornatto, D. Boschi, G. Ermondi, A. Gasco, P. A. Carrupt, B. Testa, *Pharm. Res.* **1998**, *15*, 1407.
[9] F. Barbato, M. I. La Rotonda, F. Quaglia, *Eur. J. Med. Chem.* **1996**, *31*, 311.
[10] A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, K. Y. Tam, *Pharm. Res.* **1998**, *15*, 209.

[11] G. Caron, P. Gaillard, P. A. Carrupt, B. Testa, *Helv. Chim. Acta* **1997**, *80*, 449.
[12] G. Steyaert, G. Lisa, P. Gaillard, G. Boss, F. Reymond, H. H. Girault, P. A. Carrupt, B. Testa, *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 401.
[13] A. Pagliara, G. Caron, G. Lisa, W. Fan, P. Gaillard, P. A. Carrupt, B. Testa, M. H. Abraham, *J. Chem. Soc., Perkin Trans. 2* **1997**, 2639.
[14] R. S. Tsai, P. A. Carrupt, B. Testa, in 'ACS Symposium Series 593. Modern Contercurrent Chromatography', Eds. W. D. Conway and P. J. Petroski, American Chemical Society, Washington DC, 1995, pp. 143–154.
[15] A. Avdeef, in 'Lipophilicity in Drug Action and Toxicology', Eds. V. Pliska, B. Testa, and H. van de Waterbeemd, VCH Publishers, Weinheim, 1996, pp. 109–139.
[16] F. Reymond, G. Steyaert, P. A. Carrupt, B. Testa, H. H. Girault, *Helv. Chim. Acta* **1996**, *79*, 101.
[17] Medchem95 database. Daylight Chemical Information System, Inc., Irvine, California, 1995.
[18] P. A. Carrupt, B. Testa, P. Gaillard, in 'Reviews in Computational Chemistry', Eds. D. B. Boyd and K. B. Lipkowitz, Wiley-VCH, New York, 1997, Vol. 11, pp. 241–315.
[19] CLIP1.0. Institute of Medicinal Chemistry, University of Lausanne, 1996.
[20] F. Reymond, P. A. Carrupt, B. Testa, H. H. Girault, *Chem. Eur. J.* **1999**, *5*, 39.
[21] R. C. Young, C. R. Ganellin, R. Griffiths, R. C. Mitchell, M. E. Parsons, D. Saunders, N. E. Sore, *Eur. J. Med. Chem*. **1993**, *28*, 201.
[22] R. C. Young, R. C. Mitchell, T. H. Brown, C. R. Ganellin, R. Griffiths, M. Jones, K. K. Rana, D. Saunders, I. R. Smith, N. E. Sore, T. J. Wilks, *J. Med. Chem*. **1988**, *31*, 656.
[23] R. Schoenwald, H. S. Huang, *J. Pharm. Sci*. **1983**, *72*, 1266.
[24] M. H. Abraham, H. S. Chadha, in 'Lipophilicity in Drug Action and Toxicology', Eds. V. Pliska, B. Testa and H. van de Waterbeemd, VCH Publishers, Weinheim, 1996, pp. 311–337.
[25] A. Avdeef, *Quant. Struct.-Act. Relat*. **1992**, *11*, 510.
[26] A. Avdeef, J. E. A. Comer, S. J. Thomson, *Anal. Chem*. **1993**, *65*, 42.
[27] K. Takacs-Novak, A. Avdeef, *J. Pharm. Biomed. Anal*. **1996**, *14*, 1405.
[28] F. Reymond, G. Steyaert, P. A. Carrupt, B. Testa, H. H. Girault, *J. Am. Chem. Soc*. **1996**, *118*, 11951.
[29] F. Reymond, P. F. Brevet, P. A. Carrupt, H. H. Girault, *J. Electroanal. Chem*. **1997**, *424*, 121.
[30] 1,2-Dichloroethane (Environmental Health Criteria N° 176), World Health Organization, Geneva, 1995.
[31] N. El Tayar, R. S. Tsai, P. Vallat, C. Altomare, B. Testa, *J. Chromatogr*. **1991**, *556*, 181.
[32] SYBYL 6.2. Tripos Associates, Inc., St-Louis, Missouri, 1995.
[33] J. C. Dearden, *Environ. Health Perspect*. **1985**, *61*, 203.

Received May 5, 1999