240. The Partition Coefficient of Protonated Antihistamines. Its Calculation and Interpretation in Terms of Hydrophobic Fragmental Constants

by **Bernard Testa** and **Livia Murset-Rossetti**

Laboratoire de Chimie pharmaceutique, Ecole de Pharmacie, Université de Lausanne, Place de Château, CH-1005 Lausanne, Suisse

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Summary

The octanol/water partition coefficient (P_+) of nineteen monoprotonated antihistaminic drugs has been measured. These values are compared with the partition coefficient (P) of the neutral molecules, suggesting the variations in partition coefficient seen upon protonation to obey simple rules. Indeed, the ($\log P - \log P_+$) values are multiples of a constant term 0.28 (*Rekker*'s 'magic constant' c_M[6]), each functional group contributing with a fixed incremental value. The incremental values are confirmed by multiple linear regression analysis, and their physical significance is discussed.

Introduction. – Major determinants in the biological activity of drugs and other xenobiotics are their hydrophilic-hydrophobic properties. These properties are best assessed by the partition coefficient between two nonmiscible solvents:

$$P = \frac{[C]_l}{[C]_w} \tag{1}$$

where P is the partition coefficient, and $[C]_l$ and $[C]_w$ are the total concentrations of the compound in the lipidic and aqueous phase, respectively. For reasons authoritatively discussed in [1], the system water/saturated octanol is to be preferred when P (or rather $\log P$) is used as an input parameter in QSAR (quantitative structure-activity relationships) studies. Saturated octanol indeed closely resembles biomembranes in its physicochemical properties [3], and the $\log P$ (octanol/water) values of many thousand compounds are reported in the literature (*e.g.*, in [4]). The values refer to unionized (neutral) species, and a salient feature is their additive-constitutive character. Thus, the hydrophobic substituent constant π_X has been defined as the '$\log P$' of substituent X, *e.g.*:

$$\pi_X = \log P_{SX} - \log P_{SH} \tag{2}$$

where SH and SX and the unsubstituted and substituted molecule, respectively [5]. Recently, the hydrophobic fragmental constant has been proposed by *Rekker* [2] as

a reliable and versatile means of calculating the log P of a given molecule by simply adding the f_i values of its fragments (*e.g.*, CH_3, CH_2, CH, H, phenyl, NH_2, NH, H, OH, O) and those of some intramolecular interactions (*e.g.*, proximity effects of polar groups, H attached to a negative group, aryl-aryl conjugation, cross conjugation). Using a formalism close to *Rekker*'s, log P can be written as:

$$\log P = \sum f_i + \sum f_j \tag{3}$$

where f_i is the fragmental constant of a given fragment (atom or group of atoms) and f_j is the constant for a given intramolecular effect. Equation (3) thus not only serves to calculate the log P of a given molecule without actually measuring it, but it finds an ever increasing value in assessing intramolecular effects such as conjugation, and interaction of hydration shells. In this context, a far-reaching finding may be the fact that f_j is consistently found to be a multiple of the factor 0.28 designated the 'magic constant', c_M. The number of times the factor 0.28 is operating is designated the 'key number' kn [6]. Equation (3) therefore changes [7] to:

$$\log P = \sum f_i + \sum \mathrm{kn} \cdot c_M \tag{4}$$

The quantifiability of intramolecular interactions in terms of a constant term does not appear as a mere mathematical game, but may have a sound physical meaning. Indeed, a solvent is not a continuum, and the 'magic constant' may correspond to the solvation sphere being increased or decreased by one molecule of solvent. A diminished hydration sphere, or an enlarged solvation sphere of the organic solvent, would result in an increased lipophilicity. The discrete jumps in log P values corresponding to intramolecular interactions can therefore be accounted for by the solvation sphere diminishing or expanding in discrete jumps (*i.e.*, molecules of the solvent).

In the present work, we study the influence of protonation upon the log P values of antihistamines taken as model amines. Our conclusion is that several functional groups influence the log P variation seen upon protonation, and that these variations are quantifiable in terms of the 'magic constant' c_M.

Physicochemical properties of the investigated antihistamines. – Nineteen antihistaminic drugs were investigated in the present study. Their experimentally determined pK_a and log P_+ values (log P of the monoprotonated species) are reported in *Table 1*. Inspection of the (log P – log P_+) values reveals that within experimental errors they can be considered as multiples of c_M (taken as 0.28). The following relationship is thus indicated:

$$\log P = \log P_+ + \mathrm{kn} \cdot c_M \tag{5}$$

Such an equation however may be purely fortuitous, and would indeed be so unless the variations in key numbers (kn, *Table 1*) can be rationalized in terms of functional groups (see next section).

Table 1 also shows more positive than negative residuals ($\bar{x} = 0.04$). If equation (5) is valid as postulated, a simple explanation would be for the true value of c_M to be slightly larger than 0.28 (*e.g.*, 0.280–0.285).

Table 1. *Physicochemical properties of antihistamines*

No.	Compound	pK_{a1} ± SD[a])	pK_{a2} ± SD[a])	log P[b])	log P_+[c])	log P − log P_+	kn[d])	Residual
I	Tolpropamide	8.57±0.08	-	4.88	0.58	4.30	15	0.10
II	Didesmethylpheniramine	9.88±0.06	4.22±0.08	2.34	−0.85	3.19	11	0.11
III	Monodesmethylpheniramine	10.10±0.06	4.02±0.10	2.56	−0.86	3.42	12	0.06
IV	Pheniramine	9.32±0.06	4.03±0.08	2.99	−0.76	3.75	13	0.11
V	Chlorpheniramine	9.26±0.02	3.99±0.05	3.75	0.09	3.66	13	0.02
VI	Brompheniramine	9.79±0.12	3.97±0.03	3.98	0.24	3.74	13	0.10
VII	Diphenhydramine	ND[e])	-	3.29	−0.12[f])	3.41	12	0.05
VIII	Carbinoxamine	8.98±0.04	3.77±0.04	2.67	−0.17	2.84	10	0.04
IX	Rotoxamine (=levo-VIII)	ND	ND	2.67	−0.28	2.95	10	0.15
X	Diphenylpyraline	8.90±0.06	-	3.43	0.23	3.20	11	0.12
XI	Bamipine	8.04±0.11	3.34±0.04	4.20	0.80	3.40	12	0.04
XII	Thenalidine	8.39±0.07	3.36±0.06	3.88	0.50	3.38	12	0.02
XIII	Methaphenilene	8.24±0.11	3.02±0.05	3.74	0.14	3.60	13	−0.04
XIV	Histapyrrodine	7.95±0.02	2.99±0.12	4.76	0.64	4.12	15	−0.08
XV	Cyclizine	8.32±0.02	1.88±0.12	3.06	−0.03	3.09	11	0.01
XVI	Tripelennamine	8.68±0.06	3.90±0.08	3.47	−0.23	3.70	13	0.06
XVII	Cycliramine	8.78±0.02	3.64±0.12	4.50	0.37	4.13	15	−0.07
XVIII	Phenindamine	8.11±0.08	-	4.66	0.73	3.93	14	0.01
XIX	Mebhydroline	7.63±0.04	2.55±0.18	4.18	0.89	3.29	12	−0.07
								$\|\bar{x}\| = 0.07$

[a]) Apparent stoichiometric pK_a at a ionic strength of 0.30.
[b]) Calculated according to [2].
[c]) The experimentally determined log P of the protonated molecules.
[d]) See equation 5.
[e]) Not determined.
[f]) Value reported by *Rekker* [8].

The quantifiability of (log P − log P_+) in terms of functional groups. – The molecules investigated (I-XIX) are quite complex, and a prior examination of simple amines may prove useful. We have therefore considered the partition coefficients (octanol/pH 7.0 buffer) of primary n-alkylamines reported by *Fowler et al.* [9]. The calculated log P_+ values *(Table 2)* show that the protonation of these amines is accompanied by a decrease in log P of 2.26, *i.e.* 8 c_M (c_M = 0.28, residual 0.02). The knowledge of this value has proven of help in attributing incremental values of kn to functional groups. *Table 3* reports the complete structures of compounds I-XIX, together with the proposed attribution of increments. In agreement with the results of *Table 2*, the basic nitrogen atom (of the alkylamino or piperidino group) contributes with 8 c_M to the decrease in log P resulting from protonation. Each *N*-methyl group further decreases log P by 1 c_M, while the presence of a nonconjugated diaryl moiety (aryl = phenyl, *p*-methyl-, *p*-chloro-, or *p*-bromophenyl, benzyl, thienylmethyl) decreases log P by 5 c_M. Cycliramine (XVII) appears as an exception, the diaryl moiety contributing with 8 c_M. In this compound however, the two aromatic rings are conjugated in an unique fashion, being linked by a sp^2 carbon atom.

On the other hand, *Table 3* shows that polar atoms tend to diminish the decrease in log P due to protonation. Thus, a nitrogen or an oxygen atom in the chain con-

Table 2. *The partition coefficient of primary amines*

Compound	pK_a[a])	log P[b])	log P[c])	log P_+[d])	log P $-\log P_+$	kn	Residual
Butylamine	10.61	−1.22	0.90	−1.24	2.14	8	−0.10
Pentylamine	10.63	−0.86	1.43	−0.88	2.31	8	0.07
Hexylamine	10.64	−0.42	1.96	−0.44	2.40	8	0.16
Heptylamine	10.66	0.15	2.48	0.13	2.35	8	0.11
Octylamine	10.65	0.76	3.01	0.74	2.27	8	0.03
Nonylamine	10.64	1.33	3.54	1.31	2.23	8	−0.01
Decylamine	10.64	1.92	4.06	1.91	2.15	8	−0.09
Average					2.26		0.02

[a]) Data from [10].
[b]) Observed log P at pH 7.0 [9].
[c]) Calculated according to [2].
[d]) Calculated from log P' and pK_a.

tributes with $-3\ c_M$, while one or two aromatic nitrogen atoms (of the anilino and/or pyridino group) contribute with $-2\ c_M$.

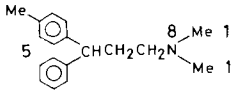
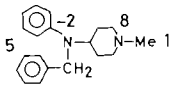
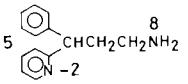
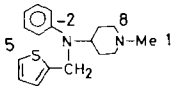
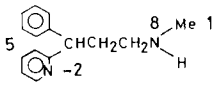
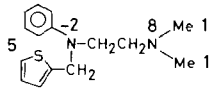
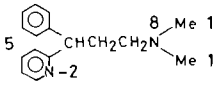
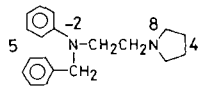
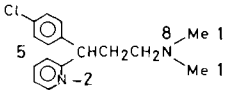
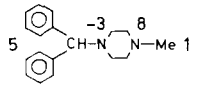
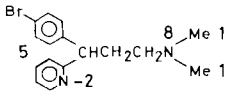
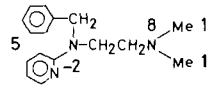
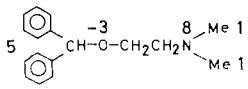
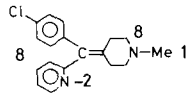
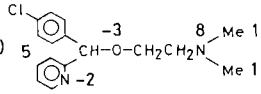
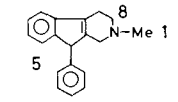
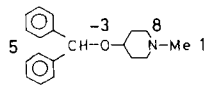
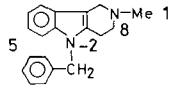
The set of increments drawn in *Table 3* is coherent, *i.e.* functional groups are attributed constant effects. At this stage however, and within the limited number of molecules investigated, the set of increments remains an interpretation and a hypothesis.

Multiple linear regression analysis of the relationship between log P and log P_+. – In order to remove part of the equivocal character of the incremental values attributed to functional groups, the relationship between log P and log P_+ has been analyzed by multiple linear regression. Taking log P as the dependent variable, the following set of independent variables was examined:

- log P_+;
- *N*-SUBST: number of CH_3 or CH_2 units substituting the basic alkylamino or piperidino nitrogen atom;
- DIARYL: indicator variable, presence of a diaryl unit = 1, absence thereof = 0;
- POLAR: indicator variable, presence of a polar atom (O or N) in the chain = 1, absence thereof = 0;
- *N*-ARO: indicator variable, presence of an aromatic nitrogen = 1, absence thereof = 0;
- the nitrogen atom being protonated is present in all observations; it is therefore represented by the constant term and not by a variable.

The seven alkylamines reported in *Table 2* were included in the set of observations, together with 18 out of the 19 antihistamines. Indeed, cycliramine (XVII) was not included in the series, this compound having an uniquely conjugated aromatic region displaying a particular hydrophilic behavior. The statistical study thus uses 25 observations (compounds) and 5 independent variables, a proportion rendering chance correlations negligible.

Table 3. *The quantifiability of* ($\log P = \log P_+ + kn \cdot c_M$)

No.	Compound	Proposed interpretation of hydrophilic effects (number of kn contributed by functional groups)	No.	Compound	Proposed interpretation of hydrophilic effects (number of kn contributed by functional groups)
I	Tolpropamide kn = 15		XI	Bamipine kn = 12	
II	Didesmethyl-pheniramine kn = 11		XII	Thenalidine kn = 12	
III	Monodesmethyl-pheniramine kn = 12		XIII	Methaphenilene kn = 13	
IV	Pheniramine kn = 13		XIV	Histapyrrodine kn = 15	
V	Chlorpheniramine kn = 13		XV	Cyclizine kn = 11	
VI	Brompheniramine kn = 13		XVI	Tripelennamine kn = 13	
VII	Diphenhydramine kn = 12		XVII	Cycliramine kn = 15	
VIII IX	Carbinoxamine Rotoxamine (levo) kn = 10		XVIII	Phenindamine kn = 14	
X	Diphenylpyraline kn = 11		XIX	Mebhydroline kn = 12	

The calculations yielded the following regression coefficients and statistics:

$$\log P = 0.96\,(\pm 0.02)\log P_+ + 0.26\,(\pm 0.02)\,N\text{-SUBST} + 1.44\,(\pm 0.04)\,\text{DIARYL} - 0.81\,(\pm 0.04)\,\text{POLAR} - 0.57\,(\pm 0.04)\,N\text{-ARO} + 2.27\,(\pm 0.03). \quad (6)$$

$n = 25$; $r^2 = 0.996$; $s = 0.073$; $F = 902$; $t(\log P_+) = 46.1$; $t(N\text{-SUBST}) = 12.4$; $t(\text{DIARYL}) = 25.2$; $t(\text{POLAR}) = -18.5$; $t(N\text{-ARO}) = -12.7$; $t(\text{constant}) = 81.2$.

It is apparent than an extremely good correlation has been obtained in equation (6). Of particular interest is the high multiple correlation coefficient, and the minute standard deviations of the regression coefficients. The *Student*'s *t*-test analysis shows that the inclusion of every independent variable and of the constant term into the equation is highly significant (> 99.99).

The regression coefficients (the coefficients of the independent variables) and the constant term in equation (6) assess the contribution of each functional group to the change in log *P* due to protonation. These contributions have thus been determined by two separate approaches: simple deduction (see *Table 3*), and statistical analysis (6).

Rewardingly, the two approaches yield similar values for these contributions, the regression coefficients being the same multiples of c_M (within standard deviation) as those reported in *Table 3*. This identity is best apparent in rewriting equation (6) in the following manner (residuals in parenthesis):

$$\begin{aligned}\log P = {} & 1.00\,(-0.04)\log P_+ + 1\times 0.28\,(-0.02)\,N\text{-SUBST} \\ & + 5\times 0.28\,(+0.04)\,\text{DIARYL} - 3\times 0.28\,(-0.03)\,\text{POLAR} \\ & - 2\times 0.28\,(+0.01)\,N\text{-ARO} + 8\times 0.28\,(+0.03).\end{aligned} \qquad (7)$$

Discussion. – The present study shows that the decrease in log *P* seen upon protonation of amines is under the influence of structural elements within the molecule, and that it obeys apparently simple rules. The calculation of log P_+ from log *P* thus appears feasible within the context of fragmental constants by adding multiples of the 'magic constant' c_M.

Another interest of the present approach is that it allows some insight into the changes in solvophilicity and -phobicity accompanying protonation. The alkylamino- or piperidino-nitrogen atom itself contributes with 8 c_M, while additional methyl groups contribute each with 1 c_M. This indicates that a *N*-methyl group increases the hydrophilicity of protonated amines, a fact already indicated by *Rekker* [11], and fully compatible with the known delocalization of the positive charge. The case of methylene units as in histapyrrodine (XIV) is unclear at present, since a single compound does not suffice to draw any sound conclusion.

More unexpected is what appears as an increased hydrophilicity (or decreased lipophilicity) of diaryl moieties upon protonation. It may be postulated that the hydration sphere of the cationic head interferes with the layers of water molecules surrounding the aromatic region and promotes their organizational state, thereby enhancing the hydro-compatibility of the latter region. This effect should be specific for the aromatic region of molecules and vary with its dimensions. Indeed, a hydrophilicity gain of 5 c_M is noted for diaryl moieties linked by a little-conjugating center, whereas the gain is tentatively estimated at 8 c_M for cycliramine in which the two aromatic rings are conjugated *via* an *exo* double bond. However, more data are needed in order to confirm and to better explain the above effect.

It is also found that polar atoms (N or O) in the chain decrease the hydrophilic gain due to protonation. The hydration sphere of these polar centers is little affected

by the protonation process, but it must prevent by a proximity effect the protonated nitrogen to fully expand its own hydration sphere. Aromatic nitrogen display a similar but less marked effect, their conjugation with the aromatic ring decreasing their polarity and hence their hydration. Future studies should indicate whether the distance (number of atoms) separating the basic nitrogen from the polar atom controls the incremental value.

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Experimental Part

Compounds. The antihistaminic drugs used in this study were kindly donated by the following companies: *Bayer AG* (Leverkusen, German Federal Republic), *Boehringer Mannheim GmbH* (Mannheim, GFR), *Ciba-Geigy AG* (Basle, Switzerland), *Hoechst AG* (Frankfurt a.M., GFR), *F. Hoffmann-La Roche & Co AG* (Basle, Switzerland), *Knoll AG* (Liestal, Switzerland), *McNeil Laboratories* (Fort Washington, Pennsylvania, USA), *A. H. Robins Company* (Richmond, Virginia, USA), *Sandoz AG* (Basle, Switzerland), *Schering Corporation* (Bloomfield, New Jersey, USA), *Smith Kline & French Laboratories* (Philadelphia, Pennsylvania, USA, and Welwyn Garden City, Herts, UK), *Warner Lambert Research Institute* (Morris Plains, New Jersey, USA). The compounds were of pharmaceutical grade, and were used without further purification.

Cyclizine was synthetized and purified according to *Baltzly et al.* [12].

Determination of pK_a *values.* The amines (free base, $3\text{–}5 \times 10^{-4}$ mol) were dissolved in 20 ml 0.05N HCl + 100 ml 2% NaCl-solution. The solutions (ionic strength 0.30) were titrated with 0.1N NaOH using a *Metrohm* E536 potentiograph equipped with a combined glass electrode EA121. The pK_a values were calculated from the titration curves by the method of *Benet & Goyan* [13] based on the two nonlogarithmic equations (8) and (9):

$$Z' = X - M - mH^+ + nOH^- \tag{8}$$

where X and M are the numbers of mol of strong acid and base, respectively, added to the solution, and mH^+ and nOH^- are the numbers of mol of proton and hydroxyde ion, respectively, present in the solution.

$$[H^+] = K_a \cdot \frac{A^0 + Z'}{B^0 - Z'} \tag{9}$$

where A^0 and B^0 are values for the numbers of mol of pure weak acid and base, respectively, present at the beginning of the titration. This method yields the stoichiometric dissociation constant, and it has the advantage of taking into account volume changes occurring during the titration. The calculations were performed using a *Diehl Alphatronic* desk calculator. The reported pK_a values result from triplicate experiments.

Determination of partition coefficients [14]. The solvents used were octanol *(puriss. Fluka)*, and phosphate buffers (0.1M, pH values 2, 3, 4, 5, and 6, ionic strength adjusted to 0.30 by addition of NaCl). Each phase was saturated with the other at RT. The starting concentrations of amines were in the range $10^{-3}\text{–}10^{-4}$M. In the usual experiments, equal volumes (5 ml) of aqueous and lipidic phases were used; the test tubes were shaken mechanically for 1 h at RT. ($21 \pm 1°$) and centrifuged. To 2 ml of the aqueous phase, 3 ml of 0.5N HCl were added, and the concentration of the amine determined photometrically (*Beckman* 25 spectrophotometer). For highly hydrophilic compounds, the volumes of phosphate buffer and octanol were 2 and 20 ml, respectively, whereas they were 20 and 2 ml, respectively, for lipophilic

compounds. Duplicate or triplicate experiments were conducted. From the observed partition coefficients (expressed as P'), the partition coefficient of the monoprotonated amines (expressed as P_+) were calculated using the following equation [15]:

$$P_+ = P'(1 + \text{antilog}[pK_{a2} - pH]). \tag{10}$$

Multiple linear regression analysis. The calculations were carried out on the *Cyber CDC* computer of the Federal Institute of Technology and of the University of Lausanne using the SPSS program [16].

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