Lipophilicity and Solvation of Anionic Drugs

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Abstract: This paper first gives a brief review of the main techniques used to measure the lipophilicity of neutral and ionic drugs, namely the shake-flask method, potentiometry, and cyclic voltammetry at liquid–liquid interfaces. The lipophilicity of 28 acidic compounds with various functional groups was studied by potentiometry and cyclic voltammetry in the *n*-octanol/water and 1,2-dichloroethane/water systems in order to complement our understanding of the lipophilicity of neutral and ionized acids and to clarify the solvation mechanisms responsible for their partition. The parameter *diff*($\log P^{N-A}_{dce}$) (i.e., $\log P$ of the neutral acid minus standard $\log P$ of the conjugated anion in 1,2-dichloroethane/water) was shown to depend not only on intramolecular interactions and conformational effects in the neutral and anionic forms, but also on the delocalization of the negative charge in the anion, confirming the ability of Born's solvation model to describe qualitatively the effect of the molecular radius on the lipophilicity of ions.

Keywords: anions · cyclic voltammetry · drug design · lipophilicity · solvent effects

Abbreviations and symbols:
$\Delta^{w}_{o}\phi$: Galvani potential difference between the aqueous (w) and the organic (o) phases.
$\Delta^{w}_{o}\phi^{O}_{i}$: standard potential of transfer of ion *i* between the phases w and o.
$\Delta^{w}_{o}\phi^{1/2}$: half-wave potential of ion *i* between the phases w and o.
$\Delta G^{O,w\rightarrow o}_{tr,i}$: standard Gibbs' energy of transfer of ion *i* from phase w to phase o.
$\log P^{N}_{oct}$: partition coefficient of the neutral form of an ionizable solute in the *n*-octanol/water system.
$\log P^{N}_{dce}$: partition coefficient of the neutral form of an ionizable solute in the 1,2-dichloroethane/water system.
$\log P^{I}_{dce}$: apparent partition coefficient of the ionic form of an ionizable solute in the 1,2-dichloroethane/water system.
$\log P^{O,I}_{dce}$: standard partition coefficient of the ionic form of an ionizable solute in the 1,2-dichloroethane/water system.
$\Delta\log P^{N}_{oct\text{-}dce}$: difference between $\log P^{N}_{oct}$ and $\log P^{N}_{dce}$.
diff($\log P^{N-I}_{dce}$): difference between $\log P^{N}_{dce}$ and $\log P^{O,I}_{dce}$.
r_{vdw} (V_{vdw}): van der Waals molecular radius (volume).

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Introduction

The environment plays a key role in influencing the properties and reactivity of compounds in condensed phases. The complexity of chemical phenomena in solution has made it necessary to develop a variety of models and computational techniques to simulate molecules in solution and understand their behavior.[1, 2] Relevant solvent properties include polarity, polarizability, H-bonding capacity, acidity/basicity, and hydrophobicity/hydrophilicity.[3–5] These properties have been translated in empirical linear free-energy relationships in order to gain an understanding of the influence of the solvent on various chemical phenomena including solubility, partition, and chemical equilibria.[6, 7]

The partition coefficient ($\log P$) of a given solute between two immiscible solvents is a measurement of its relative affinity for the two phases. Since $\log P$ is related to the free energy of transfer of the solute between the two solvents, it encodes information on the differential solvation effects[8, 9] and has been correlated with biological and pharmacological processes such as adsorption, transport through cell membranes and hydrophobic binding.[3, 10]

Various techniques, such as the classical shake-flask method, allow the $\log P$ of a neutral compound to be measured but, until recently, none was well-adapted to the study of electrically charged species.[11] Recent work has shown that cyclic voltammetry at the interface between two immiscible electrolyte solutions (ITIES) is the method of choice to study the lipophilicity of cations,[12–14] in particular drugs that can be protonated.[15] In contrast, relatively little has been under-

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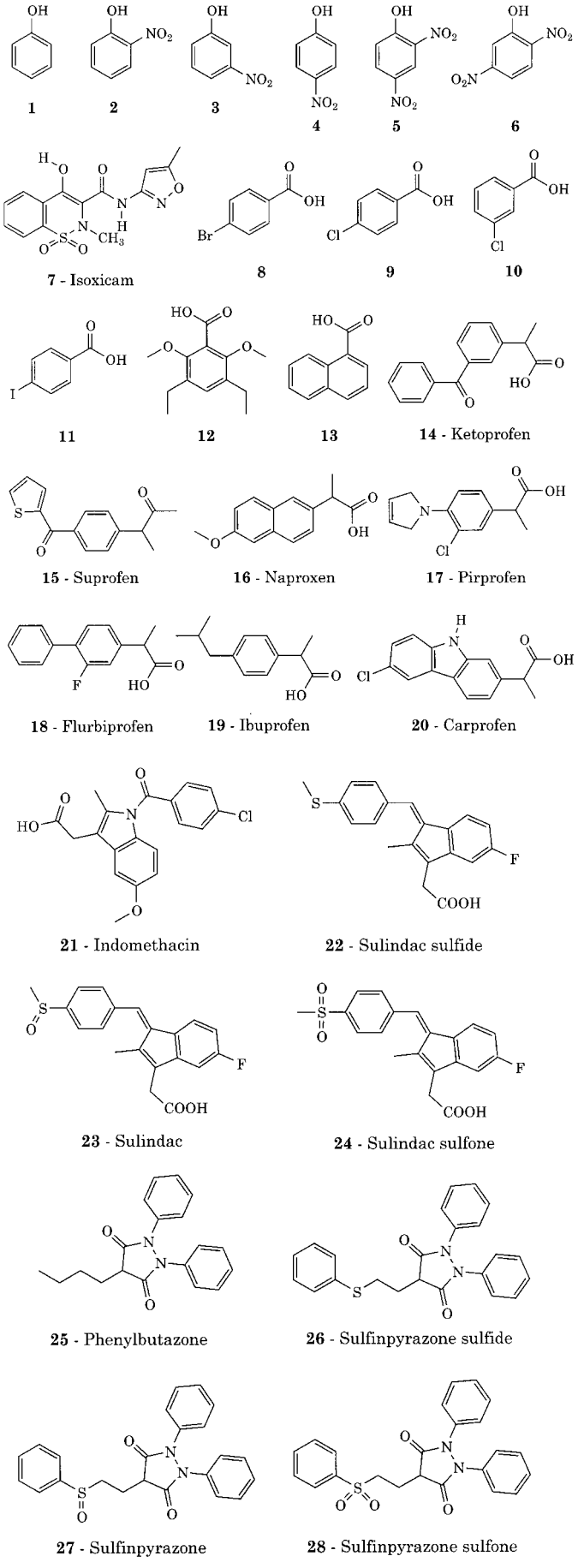

taken concerning the lipophilic behavior of anions. In the present work the lipophilicity of 28 acidic compounds with various functional groups has been studied by potentiometry and cyclic voltammetry in the *n*-octanol/water and 1,2-dichloroethane/water systems. The objective of this study was to gain more information on the lipophilic behavior of neutral and ionized acids in order to clarify the solvation mechanisms responsible for their partition.

Measuring log*P* values: a brief overview

Definitions: The partition coefficient (P^N) of a neutral solute N is given by Equation (1):

$$\log P^N = \log\left(\frac{a_o}{a_w}\right) \quad (1)$$

in which a_o (a_w) is the activity coefficient of N in the organic (aqueous) phase and $\log P^N$ is a function of the temperature and solvent system.[3]

For an ionic solute I, both an apparent and a standard partition coefficient can be defined [Eq. (2)].[16]

$$\log P^I = \log P^{0,I} + \frac{z_I F}{RT \ln 10}\Delta_o^w\phi \quad (2)$$

Here $\log P^I$ ($\log P^{0,I}$) is the apparent (standard) partition coefficient of I, and z_I is its charge. F is the Faraday constant (96500 C mol^{-1}), R is the gas constant (8.31 J K^{-1} mol^{-1}), T the temperature in Kelvin and $\Delta_o^w\phi$ is the Galvani potential difference between the aqueous and the organic phase in volts.

In Equation (2), $\log P^{O,I}$ depends on the temperature and on the chemical structure of the ion (i.e., its nature, volume, and charge). In contrast, the second term is proportional to $\Delta_o^w\phi$ and thus depends on the temperature, the volume of each phase, and all species in the system (i.e., their concentration and intrinsic lipophilicity). This difference in the definition of $\log P$ for neutral [Eq. (1)] and ionic solutes [Eq. (2)] is fundamental to the understanding of drug partitioning and for the correct experimental determination of $\log P$ values.

Solvent systems: Hansch chose *n*-octanol as the reference solvent for $\log P$ measurements because of its superficial similarity to lipids: a long alkyl chain plus a functional group having both hydrogen-bond-accepting and -donating characteristics.[3] The effectiveness of $\log P_{oct}$ in correlating biological properties has been extensively investigated, and a multiplicity of factors have been found to concur. However, the $\log P_{oct}$ lipophilicity scale alone is not sufficient to model membrane permeation, due to major differences in biophysical properties. More recently 1,2-dichloroethane has been extensively used to replace alkanes since it presents similar properties and a better dissolving capacity.[17] The 1,2-dichloroethane/water interface being polarizable, this system is also well-suited for cyclic voltammetry studies.[18]

The shake-flask method: The traditional technique for measuring partition coefficients is the so-called "shake-flask method". By controlling the pH of the aqueous phase, the $\log P$ of neutral and ionic solutes can be measured. However,

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the shake-flask method suffers from a number of practical limitations, such as the precision of phase-volume ratio, solute stability or volatility, solute impurities, formation of microemulsions, and time consumption.[19]

Moreover the $\log P$ values measured for ionized solutes are apparent values (see Equation (2)) and thus strongly dependent on experimental conditions such as phase volume, nature of the buffer, and ionic strength; this renders their use in structure–property and structure–activity relationship studies less reliable.

The potentiometric method: The potentiometric technique allows the ionization constants and the $\log P$ values of ionizable solutes to be measured.[20] The method is based on the observation that when an ionizable compound in aqueous solution is titrated in the presence of an organic phase, the titration curve shifts to the right for acids and to the left for bases. This shift is related to the pK_a and the lipophilicity of both the neutral and ionized forms of the solute, and to the volume ratio of the two phases.[20] In the simple case of a monobasic drug B and by neglecting the partition of protons, this relation is given by Equation (3).

$$pK_a^{app} = pK_a - \log\left(\frac{1 + r\cdot P^B}{1 + r\cdot P^{BH^+}}\right) \quad (3)$$

Here pK_a^{app} is the apparent pK_a measured in the presence of the organic phase, r is the phase ratio and P^B (P^{BH^+}) is the partition coefficient of the neutral (protonated) drug. Thus determination of the $\log P$ values of a monobasic (or monoacidic) drug involves the knowledge of its pK_a and a minimum of two titrations in the presence of two different organic-to-aqueous ratios r to yield two different values of pK_a^{app}. By assuming that $\log P^{BH^+}$ is a constant, the resolution of the system given by Equation (4) (two equations, two unknown parameters) yields $\log P^B$ and $\log P^{BH^+}$.

$$pK_a^{app1} = pK_a - \log\left(\frac{1 + r_1\cdot P^B}{1 + r_1\cdot P^{BH^+}}\right) \quad (4)$$

$$pK_a^{app2} = pK_a - \log\left(\frac{1 + r_2\cdot P^B}{1 + r_2\cdot P^{BH^+}}\right)$$

The advantage of the potentiometric method is the possibility of using a variety of solvents and measuring a large range of $\log P$ values.[18] However, interpreting the results is not always straightforward.[21] In particular, the main limitation comes from the assumption that $\log P^{BH^+}$ is a constant since Equation (2) shows clearly the dependence of $\log P^{BH^+}$ on experimental conditions, particularly phase volumes. When $P^B \gg P^{BH^+}$, pK_a shifts obtained from Equation (3) do not depend on $\log P^{BH^+}$, and the potentiometric method yields reliable $\log P^B$ values. But when P^{BH^+} is not negligible relative to P^B, the results given by potentiometric titrations must be considered with caution.

Cyclic voltammetry at the ITIES: Cyclic voltammetry has recently been introduced in medicinal chemistry to determine the lipophilicity of ions and study their mechanisms of transfer at the interface. This method requires the use of a polarizable interface, excluding the *n*-octanol/water system. Given its interesting properties, the system usually used is 1,2-dichloroethane/water,[17] but it must be handled carefully.[22] The principle of cyclic voltammetry experiments is described in references.[13, 23]

The main advantage of cyclic voltammetry at the ITIES is that, in contrast to all other techniques that do not control the Galvani potential difference, the potentials are here controlled; this provides standard $\log P$ values independent of experimental conditions except temperature and solvents.[11] Moreover, the physicochemical parameters so obtained allow ionic partition diagrams to be drawn and the mechanisms of transfer at the interface to be understood.[23, 24]

Results and Discussion

Lipophilicity of neutral acids in *n*-octanol/water: The experimental $\log P^N_{oct}$ values measured by potentiometry are compared in Table 1 with $\log P$ values calculated by the 2D-CLOGP algorithm.[29]

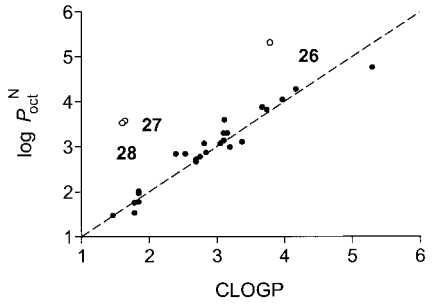


Figure 1. Relationship between $\log P^N_{oct}$ and CLOGP values for compounds **1**–**28**; ○: compounds excluded from Equation (5), ●: compounds included in Equation (5). The dotted line represents the identity line.

Figure 1 shows that for all acids except sulfinpyrazone and its analogues (**26**–**28**), the CLOGP algorithm gives a reliable estimate of $\log P^N_{oct}$ (see Equation (5) obtained by excluding compounds **26**–**28**).

$$\log P^N_{oct} = 0.94(\pm 0.05)\cdot \text{CLOGP} + 0.21(\pm 0.15) \quad (5)$$
$$n = 25,\ r^2 = 0.94,\ s = 0.22,\ F = 353$$

In these 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 test.

The deviant behavior of **26**–**28** can be explained. A previous study[30] has shown that a) in the folded conformers of **27** and **28**, both the S-containing moiety and the pyrazolidine-dione ring are prevented from expressing their full polarity due to the masking effect of the phenyl groups, and b) in the folded conformers of compound **26**, masking of polarity involves only the pyrazolidine-dione ring, since the sulfide group is not polar. Such a masking effect on the polarity of the pyrazolidine-dione ring is not described by the 2D-CLOGP algorithm; this results in an underestimated $\log P$ of compounds **26**–**28**. In contrast, these masking effects do not exist in phenylbutazone (**25**) due to the absence of a third phenyl group, and the CLOGP algorithm gives a good estimate of its $\log P^N_{oct}$.

  0947-6539/02/0815-3480 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 15

Table 1. Physicochemical parameters of acidic compounds.

N	pK_a[a]	CLOGP	$\log P^N_{oct}$[b]	$\log P^N_{dce}$[b]	$\Delta\log P^N_{oct\text{-}dce}$[c]	$\Sigma Frag_\alpha$[d]	$\log P^{O,N}_{dce}$[e]	diff($\log P^{N-A}_{dce}$)[f]
1	9.99	1.47	1.46	0.61	0.85	0.60	−2.3[g]	2.9
2	6.92	1.85	1.77	2.81	−1.04	0	−2.0[g]	4.8
3	8.10	1.85	2.00	0.92	1.08	0.79	−2.4[g]	3.3
4	6.90	1.85	1.96	0.72	1.24	0.82	−2.5[g]	3.3
5	3.96	1.79	1.37	2.46	−0.79	0	−1.7[g]	4.2
6	4.97	1.79	1.75	2.49	−0.74	0	−2.3[g]	4.8
7	3.93[h]	2.40	2.83[h]	3.89	−1.06	0.33	−1.0	4.9
8	4.15	2.85	2.86	1.04	1.82	0.59	−5.0	6.0
9	3.93	2.70	2.66	1.06	1.60	0.59	−4.8	5.9
10	3.82	2.70	2.71	0.97	1.74	0.59	−5.0	6.0
11	3.87	3.11	3.13	1.59	1.54	0.59	−4.7	6.3
12	2.82	3.20	2.98	2.39	0.59	0.59	−5.1	7.5
13	3.64	3.06	3.06	1.80	1.26	0.59	−4.9	6.7
14	4.25	2.76	2.77	2.38	0.39	0.60	−4.0	6.4
15	4.05	2.54	2.83	2.36	0.47	0.60	−4.3	6.7
16	4.18	2.82	3.06	2.57	0.49	0.60	−4.2	6.8
17	4.01	3.12	3.58	2.78	0.80	0.60	−4.2	7.0
18	4.21	3.75	3.81	2.91	0.90	0.60	−3.2	6.1
19	4.31	3.68	3.87	2.87	1.00	0.60	−3.7	6.6
20	4.45	3.98	4.04	2.58	1.46	0.87	−3.6	6.2
21	4.42	4.18	4.27	2.87	1.40	0.60	−2.8	5.7
22	4.88[i]	5.31	4.76[i]	4.26	0.50	0.60	−2.2	6.5
23	4.03[i]	3.16	3.29[i]	2.82	0.47	0.60	−4.8	7.6
24	4.16[i]	3.11	3.29[i]	3.64	−0.35	0.60	−4.4	8.0
25	4.61[i]	3.38	3.10[i]	4.72	−1.62	0	−1.6	6.3
26	2.55[i]	3.80	5.31[i]	7.70[h]	−2.39	0	0.9	6.8
27	2.37[i]	1.65	3.56[i]	4.69[h]	−1.13	0	−1.6	6.3
28	2.09[i]	1.61	3.51[i]	5.53[h]	−2.02	0	−0.1	6.1

[a] Measured by potentiometry. [b] $\log P$ of the neutral acids measured by potentiometry. [c] $\Delta\log P^N_{oct\text{-}dce} = \log P^N_{oct} - \log P^N_{dce}$. [d] Calculated by using Systhal 1.0.[28] [e] Measured by cyclic voltammetry. [f] *diff*($\log P^{N-A}_{dce}$) $= \log P^N_{dce} - \log P^{O,I}_{dce}$). [g] Taken from [32]. [h] Taken from [33]. [i] Taken from [30].

Lipophilicity of neutral acids in 1,2-dichloroethane/water: The lipophilicity of the 28 acidic compounds was also measured by potentiometry in the 1,2-dichlororethane/water system, and the results were used to calculate the compounds' $\Delta\log P^N_{oct\text{-}dce}$ parameter (see Table 1). This parameter is of interest in pharmacokinetics since it describes the H-bond-donor capacity of drugs and is negatively correlated to membrane permeability.[18, 31] The H-bond-donor capacity ($\Sigma Frag_\alpha$) of each acid was also calculated (see materials and methods). Since compounds **25**–**28** are mostly diketonic in water,[30], their ketonic tautomer was used for the calculations. H-Bond-donor compounds ($\Sigma Frag_\alpha \neq 0$) such as benzoic acids (**8**–**13**), arylpropionic acids (**14-20**), and compounds **21**–**23** had a positive $\Delta\log P^N_{oct\text{-}dce}$. They are more attracted by *n*-octanol than by 1,2-dichloroethane due to the greater solubility of water in *n*-octanol and to the large H-bonding capacity of *n*-octanol, relative to dichloroethane. The behavior of compound **24** remains unclear since it is slightly more lipophilic in 1,2-dichloroethane/water than in *n*-octanol/water, in spite of its H-bond-donor capacity. Compound **24** cannot fully express its H-bonding-donor properties in 1,2-dichloroethane, probably because of an intramolecular H-bond.

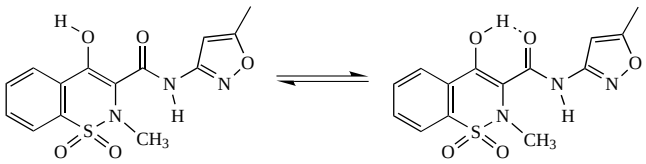
The case of the phenolic acids (compounds **1**–**6**) was studied in detail.[32] They usually behave as H-bond donors (compounds **1**, **3**, and **4**), but for compounds **2**, **5**, and **6** the formation in 1,2-dichloroethane of an internal H-bond between the hydroxy and nitro groups induces a negative $\Delta\log P^N_{oct\text{-}dce}$. In the same way, Isoxicam (**7**) is stabilized in 1,2-dichloroethane by the formation of a H-bond between the hydroxy group and the amide oxygen,[33] inducing a negative value of $\Delta\log P^N_{oct\text{-}dce}$ (see Scheme 1).

The relationship between the $\log P^N_{oct}$ and $\log P^N_{dce}$ of the 28 acids is shown in Figure 2 and compared with the results of the linear solvation Gibbs' energy relationship (LSER) analysis of the 1,2-dichloroethane/water system.[17] This analysis has recently been extended to more lipophilic non-H-bond-donor compounds[34] to give the following results for H-bond-donor [Eq. (6)] and non-H-bond-donor compounds [Eq. (7)], respectively.

$$\log P^N_{oct} = 0.92(\pm 0.05)\cdot\log P^N_{dce} + 0.95(\pm 0.07) \quad (6)$$
$$n = 19,\ r^2 = 0.95,\ s = 0.28,\ F = 309$$

$$\log P^N_{oct} = 0.91(\pm 0.05)\cdot\log P^N_{dce} - 0.43(\pm 0.14) \quad (7)$$
$$n = 32,\ r^2 = 0.92,\ s = 0.43,\ F = 359$$

Figure 2 shows that enolic acids (**25**–**28**), as predicted by the parameter $\Sigma Frag_\alpha$, display no H-bond-donor capacity [Eq. (7)]. In contrast, H-bond donors with a positive $\Sigma Frag_\alpha$ value, such as benzoic and arylpropionic acids (compounds **8**–**20**), follow Equation (6). The results obtained here for acids confirm that the parameter $\Sigma Frag_\alpha$ calculated with Systhal 1[28] gives a good estimate of the H-bond-donor capacity of drugs.



Scheme 1. Intramolecular H-bond in compound **7**.

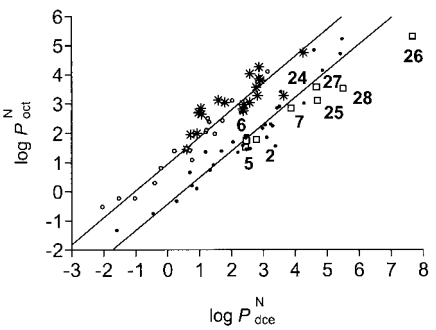


Figure 2. Relationship between $\log P^N_{oct}$ and $\log P^N_{dce}$ values; ○: H-bond-donor compounds, ●: non-H-bond-donor compounds,[17, 34] □: acids with $\Sigma Frag_\alpha = 0$, *: acids with $\Sigma Frag_\alpha \neq 0$.

Lipophilicity of anions: The standard partition coefficient of anionic species (noted $\log P^{N-A}_{dce}$) was measured by cyclic voltammetry (see Table 1). The present study deals only with lipophilic acids with corresponding $\log P^{O,A}_{dce}$ values higher than -6, since the transfer of more hydrophilic anions is outside the potential window of cyclic voltammetry.[35]

The parameter *diff*($\log P^{N-A}_{dce}$) represents the difference between $\log P^{N}_{dce}$ and $\log P^{O,A}_{dce}$ (see Table 1). This parameter mainly depends on the nature of the acidic group (see below). In congeneric series, moreover, the parameter *diff*($\log P^{N-A}_{dce}$) can decrease or increase due to conformational effects and intramolecular interactions in the neutral or/and ionic compounds. For example, the mean value of *diff*($\log P^{N-A}_{dce}$) of phenolic acids is around 3.2 ± 0.2, excluding **2**, **5**, and **6**. The mean ($\log P^{N-A}_{dce}$) value of these three compounds is increased by an intramolecular H-bond that no longer exists in the corresponding anion (see Figure 3).

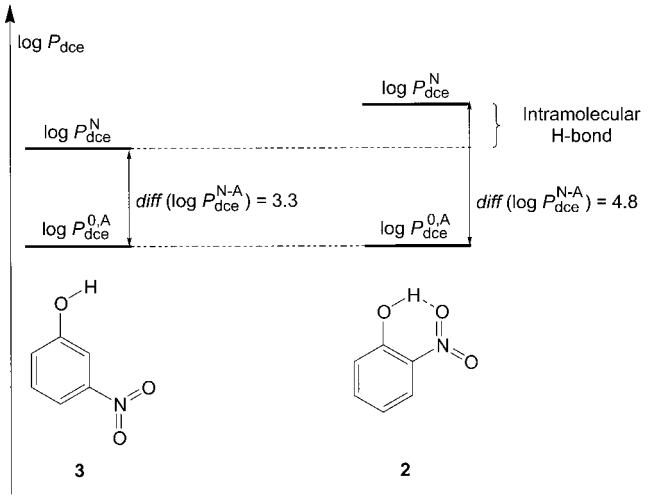


Figure 3. Schematic representation of intramolecular effects acting on the lipophilicity of compounds **2** and **3**. The $\log P^{N}_{dce}$ of compound **2** was increased by an intramolecular H-bond, inducing a value of *diff*($\log P^{N-A}_{dce}$) higher than for its analogue **3**.

For benzoic acids, the mean value of *diff*($\log P^{N-A}_{dce}$) is 6.2 ± 0.3, excluding compound **12** whose $\log P^{N}_{dce}$ is increased by an intramolecular H-bond between the hydroxy group and one of the two methoxy groups. For enolic compounds, the mean value of *diff*($\log P^{N-A}_{dce}$) is 6.4 ± 0.3. For arylpropionic acids (**14**–**20**) and the other carboxylic acids (**21**–**24**), the mean value of *diff*($\log P^{N-A}_{dce}$) is 6.7 ± 0.6. These results suggest that, in the absence of intramolecular interactions and conformational effects in the neutral and anionic forms, the charge effect on the lipophilicity of anions is also a function of the nature of the functional acidic group, as discussed below.

Solvation of anions: Born's solvation model describes the ion as a rigid sphere with radius r_0 (equivalent to the crystallographic radius of the ion) and charge z_i. The solvent, which is polarized in the vicinity of an ion, is represented by a structureless continuum of uniform dielectric constant ε_r, corresponding to its bulk value. In spite of its limitations (Born's theory neglects the dielectric saturation and assumes that the dielectric constant around the ion is equal to that in the bulk, resulting in a overestimation of ion–solvent interaction values), Born's equation provides good estimates of ionic solvation energies. Moreover, a recent paper[15] has shown that since partition coefficients represent the difference in solvation energy between two solvents, the $\log P$ difference between a neutral and a charged species in the 1,2-dichloroethane/water system can be written as Equation (8):

$$diff(\log P^{N-I}_{dce}) = \frac{\Delta G^{dce}_{IS} - \Delta G^{w}_{IS}}{RT \ln 10} \quad (8)$$

$$= \frac{z^2 e^2 N_A (\varepsilon^{dce}_r - \varepsilon^{w}_r)}{8\pi\varepsilon_0 \varepsilon^{dce}_r \varepsilon^{w}_r \cdot RT \ln 10} \cdot \frac{1}{r_0}$$

in which ΔG^{dce}_{IS} (ΔG^{w}_{IS}) is the ion–solvent interaction energy in 1,2-dichloroethane (water), z and r_0 are, respectively, the charge and the molecular radius, N_A is the Avogadro constant, and ε^{dce}_r (ε^{w}_r) is the dielectric constant of 1,2-dichloroethane (water).

Born's solvation model has been successfully applied to studying the lipophilicity of various compounds such as phenol derivatives,[32] quaternary ammonium cations,[11] and benzodiazepine derivatives.[36]

Figure 4 shows the relationship between the parameter *diff*($\log P^{N-A}_{dce}$), the acidic group and the molecular radius calculated from Equation (8), in the absence of intramolecular interactions and conformational effects.

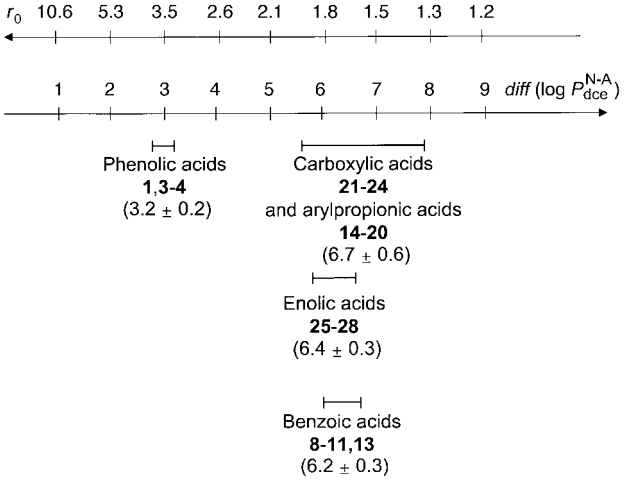


Figure 4. Schematic representation of the relationships between the parameter *diff*($\log P^{N-A}_{dce}$) and the molecular radius of the anion (r_0) calculated from Equation (8). For each functional group, the mean value of *diff*($\log P^{N-A}_{dce}$) is given in brackets.

The relatively small value of *diff*($\log P^{N-A}_{dce}$) obtained for phenolic acids corresponds to a mean molecular radius of 3.3 Å, which is approximately equal to the molecular radius of the phenolate calculated from the van der Waals volume ($r_{vdw} = 3.0$ Å). The molecular electrostatic potential (MEP), obtained by AM_1 calculation (Spartan 5.0, Wave function Inc., Irvine USA) is displayed in Figure 5 in order to localize the charge. The latter indicates that the negative charge of phenolic anions is strongly delocalized on the aromatic ring, inducing the stabilization of the anion and relatively high $\log P^{O,A}_{dce}$ values for compounds **1**–**6**.

0947-6539/02/0815-3482 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 15

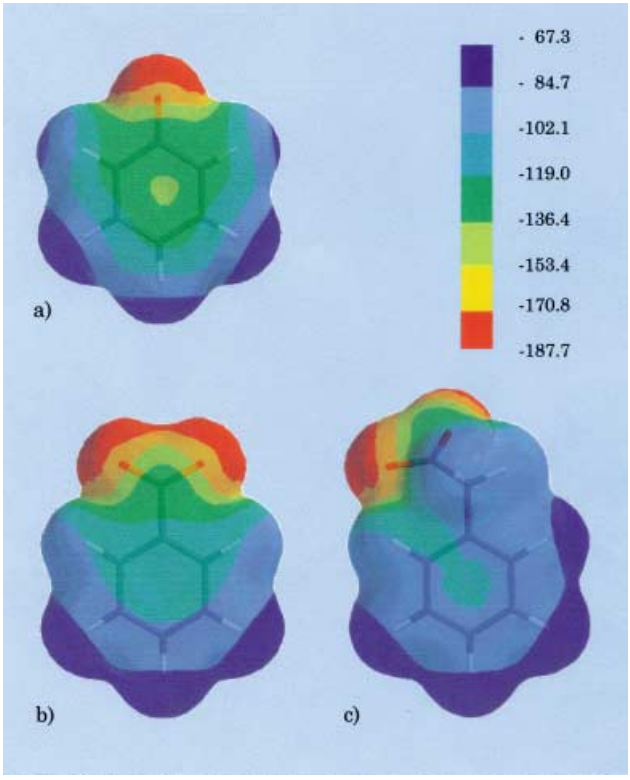


Figure 5. AM_1 semiempirical calculation of the molecular electrostatic potential surrounding deprotonated a) phenol, b) benzoic acid, and c) phenylacetic acid. The electrostatic potential increases from red to dark blue.

In contrast, the higher $diff(\log P_{dce}^{N-A})$ values obtained for benzoic acids (**8**–**11**, **13**) correspond to a mean molecular radius of 1.7 Å; this suggests that the negative charge is more localized on the carboxylate group and only partially delocalized onto the aromatic ring, as seen in Figure 5. The mean value of $diff(\log P_{dce}^{N-A})$ obtained for compounds **14**–**24** is slightly higher than for the benzoic acid derivatives; this indicates that their negative charge stays localized on the carboxylate group exclusively, as illustrated by the MEP of anionic phenyl acetate (Figure 5c). In the same way, the negative charge of enolic acids (**25**–**28**) may be slightly delocalized on the pyrazole ring, but stays mainly on the enolic group.

Conclusion

This study of 28 acidic compounds containing different acidic groups has shown the variable lipophilic behavior of anions. In particular, the parameter $diff(\log P_{dce}^{N-A})$ depends not only on intramolecular interactions and conformational effects in the neutral and anionic forms, but also on the delocalization of the negative charge on the anion. The value of this parameter decreases when the delocalization of the negative charge increases, due to the increased stabilization of the anion in the organic phase.

Experimental Section

Compounds and reagents: All compounds were purchased from Fluka (Buch, Switzerland), except **22**–**24** (kindly donated by Merck Research Laboratories, Rahway, NY, USA) and **26**–**28** (kindly donated by Novartis Pharma, Basel, Switzerland). 1,2-Dichloroethane (Romil, Cambridge, UK) was used without further purification and handled with all necessary precautions.[22] BTPPATPBCl (bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate) was prepared by metathesis of potassium tetrakis(4-chlorophenyl)borate (Fluka) and of bis(triphenylphosphoranylidene)ammonium chloride (Aldrich, Milwaukee, USA). All other chemicals were of analytical grade and supplied by Fluka.

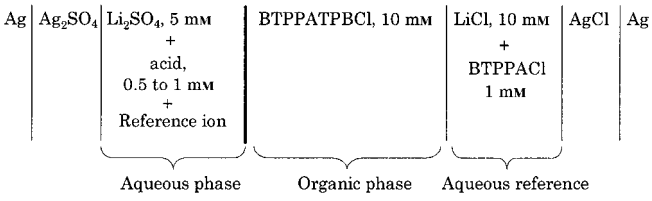
Potentiometric determination of ionization constants and partition coefficients: The ionization constants and partition coefficients of neutral acids were determined by potentiometric titration by using the GLpK_a apparatus of Sirius Analytical Instruments (Forest Row, East Sussex, UK).[25] All titrations were conducted under an argon atmosphere at $25 \pm 0.1\,^{\circ}C$.

The ionization constants were determined in water or, for compounds with low solubility, in mixtures of water and methanol. For the latter, the apparent pK_a values were extrapolated to zero cosolvent by the Yasuda–Shedlovsky procedure.

The partition coefficients in the *n*-octanol/water and 1,2-dichloroethane/water systems were measured by titrating drug solutions in the presence of different volumes of organic phase (volume ratio organic/water between 0.02 and 1.5). The $\log P$ values were estimated from difference Bjerrum plots and refined by a nonlinear least-squares procedure by including previously determined pK_a values as unrefined contributions. The detailed experimental procedures can be found elsewhere.[26, 27]

Cyclic voltammetry: The experimental set-up used was a home-made four-electrode potentiostat with ohmic drop compensation, as described in reference [11]. The scanning of the applied potential was performed by a waveform generator (VA-scanner E612, Metrohm, Herisau, Switzerland), coupled to an X-Y recorder (Bausch & Lomb, Rochester, NY, USA). Both the cell and the four-electrode potentiostat were housed in a Faraday cage. All experiments were carried out at room temperature.

The following electrochemical chain was used (cell 1).



1,2-Dichloroethane and water were mutually saturated. The drugs were dissolved in the aqueous phase. The pH of the aqueous solution was adjusted to the desired value with HCl or LiOH. All half-wave potentials ($\Delta_o^w\phi^{1/2}$) were referred to the half-wave potential of the tetramethyl ammonium cation (TMA) or tetrapropyl ammonium (TPA).[11] The standard transfer potential ($\Delta_o^w\phi_i^O$) of ion *i*, its standard Gibbs' energy of transfer ($\Delta G_{tr,i}^{O,w\rightarrow o}$) and its standard partition coefficient ($\log P_{dce}^{O,i}$) were calculated as previously described.[11]

Calculation of the H-bond-donor capacity of acids: Polar hydrogen atoms and lone pairs able to form H-bonds were identified, and fragmental values were assigned to them by using the fragmental system Systhal 1.0.[28] The total capacity of a solute to donate H-bonds ($\Sigma Frag_\alpha$) is then given by the sum of the donor capacities of its constitutive H-bonding elements.

All calculations were performed on a Silicon O_2 R5000180 workstation by using the SYBYL 6.6 molecular modeling package (Tripos Associates, St. Louis, MO, USA).

Acknowledgements

P.-A.C., H.H.G., and B.T. are indebted to the Swiss National Science Foundation for support, as well as to the "Fond de recherche en génie biomédical" of the three Lemanic Universities.

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Received: February 6, 2002 [F3856]

  0947-6539/02/0815-3484 $ 20.00+.50/0