Insight into the Lipophilicity of the Aromatic N-Oxide Moiety

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Purpose. A systematic study to assess the influence of N-oxygenation on the lipophilicity of aromatic weak bases was performed.
Methods. The methods used were experimental (CPC and shake-flask techniques) and computational (AM1 semi-empirical method).
Results. The intrinsic increment in the log P_{oct} system for an oxygen atom added to form an aromatic N-oxide, designated as *diff*(log $P^{N(O)-N}$), was −1.91, but the presence of *para*-substituents markedly affected this value. The good linear relationship ($r^2 = 0.92$) between *diff*(log $P^{N(O)-N}$), and the electronic density on the oxygen atom suggests that H-bond acceptor basicity is the main structural factor responsible for the variations in lipophilicity of aromatic N-oxides. Partition coefficients of aromatic N-oxides in dodecane/buffer and chloroform/buffer systems also support this hypothesis.
Conclusions. The solvent-dependent polarity of the N-oxide moiety is mainly due to its marked H-bond acceptor basicity.

KEY WORDS: lipophilicity; polarity; partition coefficients; N-oxygenation; aromatic N-oxides.

INTRODUCTION

In evolutionary terms, the finality of reactions of biotransformation is to render xenobiotics more hydrophilic (less lipophilic) and thus easier to excrete with minimal loss of water. In pharmacological terms, the differential lipophilicity of metabolites will affect not only their excretion, but also their distribution in the body and thus the tissues where they might act (1). N-Oxygenation is a major metabolic reaction of functionalization, and one that may influence markedly the lipophilicity of metabolites (1).

The physicochemical parameter best correlated with the biodistribution of xenobiotics and metabolites is their distribution coefficient at a given pH (written as log D^{pH} and expressing the relative contributions of all electrical forms of a compound present at that pH), rather than the partition coefficient of their neutral form (expressed as log P) (2). In principle, the polarity of the N-oxide group is expected to produce metabolites of markedly low lipophilicity relative to the neutral form of the parent drugs (i.e., smaller log P). But since N-oxygenation also affects the number of protonable sites in a substrate molecule, its global influence on the distribution coefficient (log D) will depend on the chemical nature of the nitrogen atom being oxygenated. Indeed, the distribution coefficient of an N-oxide metabolite is in fact greater than that of the parent compound in a pH range where the protonated form of the parent drug predominates, and smaller when the neutral form of the parent drug predominates (2). At a physiological pH of 7.4, N-oxygenation increases the distribution coefficient for strongly basic substrates, but decreases it by polar effects when the parent drug is moderately or weakly basic.

While the changes in lipophilicity associated with variations in the ionic character of a drug are well-known and easy to model (3), the effects of N-oxygenation on lipophilicity are documented only by few examples (2,4). Here, we report the results of a systematic study designed a) to assess the influence of N-oxygenation on the lipophilicity of model weak bases of the pyridine type, and b) to identify the major intermolecular forces and intramolecular interactions accounting for the differences observed between such N-oxides.

The difference between the partition coefficients (log P) of the para-substituted pyridines N-oxides 1–9 and the log P of their corresponding pyridines 1a–9a, designated as *diff*(log $P^{N(O)-N}$), was used to characterize the influence of N-oxygenation on lipophilicity (see Figure 1 for chemical structures). To examine the influence of electronic factors on the magnitude of changes in lipophilicity due to N-oxygenation, correlations were searched with electronic parameters calculated by the AM1 semi-empirical method. Because partition coefficients measured in different solvent systems do not encode identical structural information (5–7), the lipophilic properties of N-oxides and their parent amines were also measured in *n*-dodecane/buffer and in chloroform/buffer systems, when experimentally feasible.

The variations in lipophilicity caused by N-oxygenation of aromatic aza derivatives as well as the unusual chemical reactivity of pyridine N-oxides (8) have been attributed to the dual nature of the N-oxide group, which acts both as an electron-donor and an electron acceptor. An improved understanding of intramolecular interactions could serve to revise corrections factors associated with the N-oxide moiety in the CLogP algorithm (9).

MATERIALS AND METHODS

Solvents

Analytical grade *n*-octanol and chloroform were purchased from Fluka Chemie (Buchs, Switzerland). Analytical grade *n*-dodecane was obtained from Aldrich-Chemie (Steinheim, Germany). Morpholino-4-propanesulfonic acid (MPS; Merck, Darmstadt, Germany) and phosphate (Fluka Chemie, Buchs, Switzerland) were used for buffers of pH 7.4 and 9.0, respectively.

Solutes

Pyridine N-oxide 1, quinoline N-oxide 10, quinoline 10a, isoquinoline N-oxide 11, isoquinoline 11a, 4-nitropyridine N-oxide 3, 4-cyanopyridine N-oxide 5, 4-methylpyridine N-oxide 4 and 4-phenylpyridine N-oxide 9 were purchased from Aldrich-Chemie (Steinheim, Germany). 4-Acetylpyridine N-oxide 2, 4-chloropyridine N-oxide 7, 4-dimethylaminopyridine N-oxide 6 and 4-ethoxypyridine N-oxide 8 were prepared by known procedures (10–12). The identity of all synthetized compounds were checked by NMR, IR and MS spectroscopy. The

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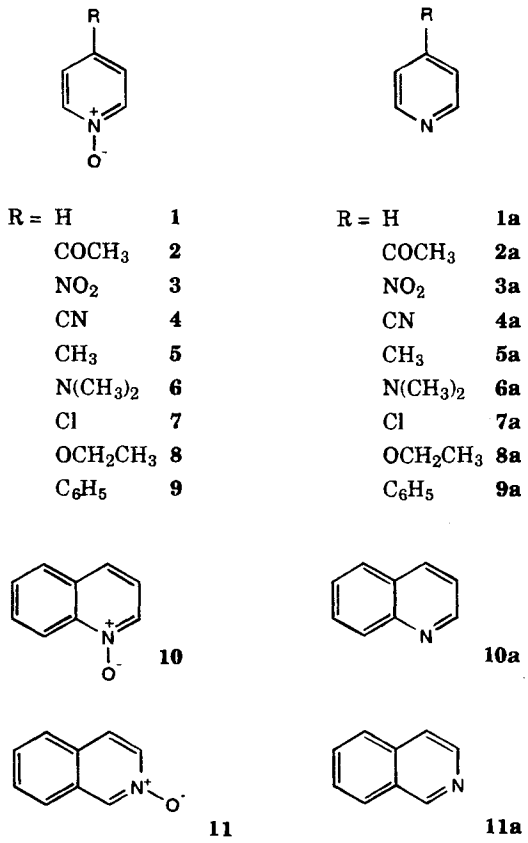

Fig. 1. Chemical structure of investigated model compounds.

purity of the compounds was checked by HPLC, using a Kontron MT1 chromatograph equipped with a MSI T-660 Autosampler, an HPLC pump model 420, a column oven 480, an oven controller 480 and an UV-Vis detector model 430 with variable wavelength (Kontron AG, Zürich-Müllingen, Switzerland). The column was a Supelcosil LC-ABZ (150 × 4.6 mm ID; Supelco, Bellefonte, PA, USA) of 5 μm packing and 100 Å pore size. All the measurements were performed at room temperature, the eluent was methanol/buffer (0.02 M MPS/NaOH pH = 7.4) 70:30 v/v, and the flow-rate was 1.0 ml min^{-1}. Melting points were measured with a Büchi SMP-20 capillary melting point apparatus (Büchi Labortechnik AG, Flawil, Switzerland). Microanalyses for C, H, N were within ±0.3% of expected values (Redox, Cologno Monzese, Italy)

Measurement of Partition Coefficients By Centrifugal Partition Chromatography (CPC)

The partition coefficients in *n*-octanol/buffer (0.02 M MPS/NaOH, pH = 7.4; 0.02 M phosphate pH = 9.0) of compounds 1–8 were determined by flow-through CPC with a coil planet type centrifuge (Ito Multi-layer Coil Separator-Extractor, P.C. Inc., Baltimore, U.S.A.), as described in detail elsewhere (13). With this technique, centrifugal and Archimedean hydrodynamic forces retain the stationary phase and allow genuine liquid-liquid partition coefficients to be measured. The CPC method can circumvent problems inherent to the traditional shake-flask method such as interference of impurities, instability of solutes, and imprecision due to improper volume ratio of the organic and aqueous phase. The measurements were performed using the aqueous phase as eluent (total volume of 265 ml). The partition coefficients were calculated using Eq. 1:

$$\log P = \log \frac{(t_R - t_0) \cdot U}{V_t - U \cdot t_0} \quad (1)$$

where t_0 and t_R are the retention times of the solvent front and solute, respectively, U is the flow rate of the mobile phase, and V_t the total capacity of the column. The value of t_0 was measured using a non-retained solute ($K_2Cr_2O_7$).

Measurement of Partition Coefficients by the Shake-flask Method

The partition coefficients of compounds 9, 10, 11, 9a, 10a and 11a in the chloroform/buffer system (log P_{chf}) were measured at room temperature by the shake-flask method (14). The pH values were chosen to ensure minimal ionization of each solute (see above). The two layers were shaken for about 8 h, separated and centrifuged (10 min). The concentrations of solutes were measured in the aqueous phase by HPLC with the equipment described above. All values were obtained at least in quadruplicate.

Semiempirical MO Calculations

The geometry of the pyridine N-oxides 1–9 were optimized by the quantum-mechanical semi-empirical AM1 method (15) using the program MOPAC 5.0 (QCPE No. 445) running on Silicon Graphics 4D35 Personal Iris or Sun Sparc 2 workstations. The convergence criteria defined by the keyword PRECISE were used.

RESULTS AND DISCUSSION

Intrinsic Lipophilicity of the Aromatic N-Oxide Moiety in the *n*-Octanol/Buffer System

To assess the lipophilicity increment of the aromatic N-oxide moiety, we use here the *diff*(log $P^{N(O)-N}$) parameter, i.e. the difference in log P values between parent compounds and their corresponding N-oxide (4). Table 1 reports the partition coefficients in the *n*-octanol/buffer system, either measured here by CPC (all N-oxides), or taken from the Pomona database (16) (parent compounds 1a–11a). This is a reasonable procedure considering the high quality of the log P values (designated log P*) contained in the Pomona database (16). Note that the log P* values of the N-oxides 3, 5, 10 and 11 can also be found in the Pomona database (16). Our values (Table 1) are in very good agreement with them (mean difference 0.03 ± 0.02).

As shown in Table 1, the pyridine N-oxides are more hydrophilic than their parent compounds by a value ranging from −2.24 to −0.87. For the unsubstituted pyridine N-oxide, the value is −1.91, which can be taken to be the intrinsic increment for an oxygen atom added to form an aromatic N-oxide. In all the other cases the *para*-substituent markedly affects the intrinsic value of the N-oxide moiety, for reasons investigated below. This strong influence renders doubtfull a literature estimate of the lipophilic increment (−1.7) derived from quinoline derivatives (4).

Table 1. Partition Coefficients of Pyridines Derivatives and their N-Oxides in the n-Octanol/Water System

Compound	log P_{oct}	*diff*(log $P^{N(O)-N}$)[c]
1	−1.26[a]	
		1.91
1a	−0.65[b]	
2	−0.93[a]	
		1.41
2a	0.48[b]	
3	−0.54[a]	
		0.87
3a	0.33[b]	
4	−0.91[a]	
		1.37
4a	0.46[b]	
5	−0.88[a]	
		2.10
5a	1.22[b]	
6	−0.90[a]	
		2.24
6a	1.34[b]	
7	−0.44[a]	
		1.72
7a	1.28[b]	
8	−0.53[a]	
		2.10
8a	1.57[b]	
9	0.93[a]	
		1.66
9a	2.59[b]	
10	0.41[a]	
		1.62
10a	2.03[b]	
11	0.26[a]	
		1.82
11a	2.08[b]	

[a] Measured here by CPC.
[b] Taken from the Pomona Database (16).
[c] log $P^{(N\text{-oxide})}$ minus log $P^{(\text{parent compound})}$.

Electronic Effects Influencing the Lipophilicity of Aromatic N-Oxides in the *n*-Octanol/Buffer System

The calculated *diff*(log $P^{N(O)-N}$) values for *para*-substituted pyridines and their N-oxide varies markedly with the nature of the *para*-substituent. Electronic interactions between a *para*-substituent and the N-oxide moiety are postulated to be the main intramolecular effects influencing the partitioning characteristics of N-oxides, in analogy with their effect on chemical shifts in ^{17}O-NMR (17) and ^{15}N-NMR (18). Indeed, the role of these intramolecular interactions is underlined by the correlation (Eq. 2 and Fig. 2) observed between *diff*(log $P^{N(O)-N}$) and the electronic charge on the oxygen atom as calculated by the quantum-mechanical semi-empirical AM1 (15):

$$diff(\log P^{N(O)-N}) = 16.5(\pm 3.7) \cdot Charge_{oxygen} + 5.4(\pm 1.6)$$
$$n = 11;\quad r^2 = 0.92;\quad s = 0.12;\quad F = 100 \qquad (2)$$

Equation 2 with its good correlation coefficient suggests that the electron density on the oxygen atom, as controlled by the electronic effects of the *para*-substituent, is the main structural factor responsible for the variations in lipophilicity of aromatic N-oxides. Although previous investigations of the N-oxide moiety (19) have shown that solvent-independent semi-empirical calculations fail to reproduce satisfactorily the experimental geometry of pyridine N-oxide, the oxygen charge densities calculated here are validated by their good relationships with ^{17}O-NMR chemical shifts (17) (Eq. 3 and Fig. 3):

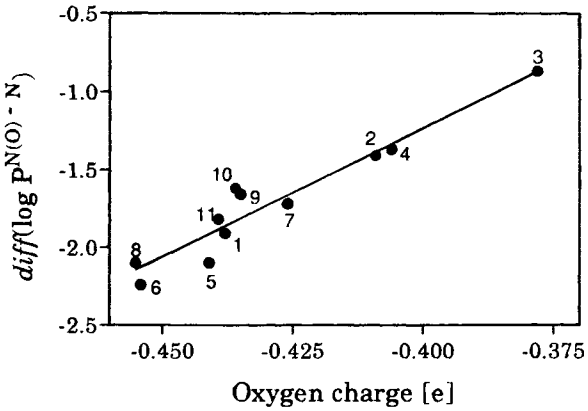

Fig. 2. Relation between *diff*(log $P^{N(O)-N}$) (the difference in log P between N-oxides and their parent compounds) and the atomic charge on the oxygen atom as calculated by the AM1 semi-empirical method. The correlation is described by Eq. 2 in the text.

$$\delta_{^{17}O} = 1356(\pm 426) \cdot Charge_{oxygen} + 938(\pm 180)$$
$$n = 7;\quad r^2 = 0.93;\quad s = 10.6;\quad F = 67 \qquad (3)$$

In fact, a good correlation also exists between *diff*(log $P^{N(O)-N}$) and ^{17}O-NMR chemical shifts (Eq. 4)

$$diff(\log P^{N(O)-N}) = 0.012(\pm 0.004) \cdot \delta_{^{17}O} - 5.93(\pm 1.45)$$
$$n = 7;\quad r^2 = 0.91;\quad s = 0.15;\quad F = 49 \qquad (4)$$

Partition Coefficients of Aromatic N-Oxides in Dodecane/Buffer and Chloroform/Buffer Systems

The above relations between oxygen charge density and decrease in lipophilicity suggest that the H-bond acceptor bacisity of the oxygen atom (as assessed by the β parameter in

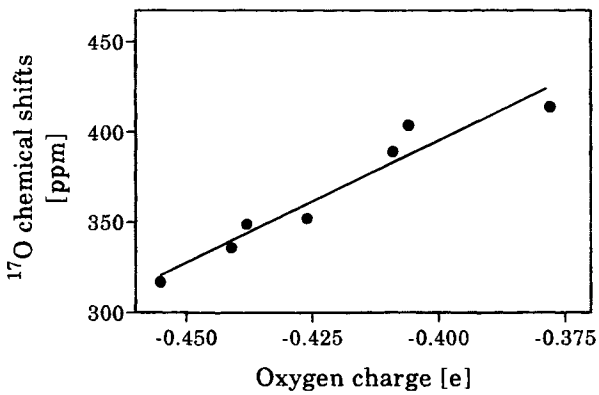

Fig. 3. Relation between the ^{17}O-NMR chemical shifts versus the atomic charge on oxygen calculated by the AM1 semi-empirical method. The correlation is described by Eq. 3 in the text.

solvatochromic analyses (20)) is the major structural factor responsible of the decrease of the partition coefficient of N-oxides relative to their parent compounds. To examine this hypothesis, we measured the partition coefficient of N-oxides in other solvents systems. Indeed, it is well-known that partition coefficients obtained in *n*-octanol/buffer, chloroform/buffer and *n*-heptane/buffer systems encode different relative contributions of a solute's H-bond acceptor basicity, the regression coefficient of the β-parameter in solvatochromic analyses being −3.51, −3.17 and −5.35, respectively (5). Thus, if the H-bond acceptor basicity of the N-oxide moiety is the major factor accounting for its hydrophilic increment, the variation of *diff*(log $P^{N(O)-N}$) from one solvent system to another should reflect these relative contributions.

While the high polarity of pyridines N-oxides restricts their solubility in inert solvents, the most lipophilic N-oxides 9–11 could be measured in *n*-dodecane/buffer and chloroform/buffer to obtain the *diff*(log $P^{N(O)-N}$) values in these solvent systems. The results (Table 2) demonstrate that the variation in lipophilicity is comparable in the *n*-octanol/buffer and chloroform/buffer systems (which encode a similar contribution of the H-bond acceptor basicity β), while the *n*-dodecane/buffer system strongly increases the difference in lipophilicity between the parent compounds and their N-oxides. This result confirms that the lipophicility contribution of an N-oxide moiety is mainly influenced by its H-bond acceptor basicity. Moreover, the log P_{alk} value of N-oxides, when measurable, will represent a more complete measure of their lipophilicity than the log P_{oct} value since it is more strongly dependent on their H-bond acceptor basicity.

Encoding the N-Oxide Moiety in the CLogP Algorithm

From measurements of the *n*-octanol/buffer partition coefficients of eleven aromatic N-oxides, information can be extracted that may serve to refine $f_{N(O)}$, the fragmental value of the N-oxide moiety adopted in the latest version of the CLogP algorithm (9). First, we note that the experimental log P of pyridine N-oxide 1 (Table 1) leads to a value of $f_{N(O)}$ that is similar to the CLogP value (−3.02) (Eq. 5):

$$f_{N(O)} = \log P_{oct} - 5 \cdot f_C - 5 \cdot f_H = -3.01 \quad (5)$$

Second, substituent effects as large as those found here (Table 1) necessitate a well-parametrized pair of constants $\sigma_{N(O)}/\rho_{N(O)}$ (21) to be efficiently taken into account in the CLogP algorithm. The σ parameter represents the ability of a functional group to behave as 'inducer' in an electronic interaction, whereas the ρ parameter expresses the sensitivity of a functional group to the electron effects of remote substituents. The coefficients $\sigma_{N(O)}$ and $\rho_{N(O)}$ of the independent variables were calculated by multilinear regression analysis from the N-oxides 1, 2, 3, 4, 6 and 8 using as dependent variable the experimental log P value minus the sum of all fragmental constants. The values of the dependent and independent variables are compiled in Table 3. The resulting correlation is shown as Eq. 6:

$$\log P - \Sigma f = 1.48(\pm 0.15) \cdot \sigma_R + 0.16(\pm 0.17) \cdot \rho_R$$
$$n = 6; \quad r^2 = 0.98 \quad s = 0.08; \quad F = 64 \quad (6)$$

These new values of $\sigma_{N(O)} = 0.16 \pm 0.17$ and $\rho_{N(O)} = 1.48 \pm 0.15$ indicate clearly that the main factor controlling the lipophilicity of the N-oxide moiety is its susceptibility to the electronic effects of remote substituents (as deduced from the large value of the coefficient $\rho_{N(O)}$), whereas the negligible coefficient of $\sigma_{N(O)}$ (statistically not different from 0) indicates that the N-oxide function cannot influence the properties of remote groups. The predominance of the $\rho_{N(O)}$ term is also demonstrated by the correlation between the Hammett constant σ (22) and Δlog $P_{[N(O)-N]}$, thus proving the sensitivity of the N-oxide moiety to both electron-donating and electron-withdrawing influences (Eq. 7):

$$diff(\log P^{N(O)-N}) = 0.79(\pm 0.34) \cdot \sigma_{Hammett} - 1.76(\pm 0.17)$$
$$n = 6; \quad r^2 = 0.88; \quad s = 0.21; \quad F = 28 \quad (7)$$

The chloro derivative 7 was an outlier in Eq. 6 and was removed from the derivation of the constants $\sigma_{N(O)}/\rho_{N(O)}$. This is compatible with the lack of electronic effects of a chloro substituent, as already noted by Katritzki et al. (23) in their comparative study of the mesomeric moments of *para*-chloropyridine and its N-oxide. It must also be stressed that Eqs. 2–4, 6 and 7 being based on few compounds have a limited extrapolative value. However, their main interest is to suggest an orderly influence of electronic factors on the difference in lipophilicity resulting from N-oxygenation.

Table 2. Chloroform/Water and *n*-Dodecane/Water Partition Coefficients of Pyridines and Their N-Oxides

Compound	log P_{chf}[a]	*diff* (log $P^{N(O)-N}$)	log P_{alk}[c]	*diff* (log $P^{N(O)-N}$)
9	1.38		−2.36	
		1.60		3.94
9a	2.90		1.58	
10	0.78[b]		−2.05	
		1.75		3.31
10a	2.53		1.26	
11	0.83		−2.51	
		1.89		3.62
11a	2.72		1.11[b]	

[a] Measured by the shake-flask method except when indicated otherwise. S.D. ≤ 0.05 except for 9a (S.D. = 0.10).
[b] Taken from the Pomona database (16).
[c] Measured by CPC except when indicated otherwise.

CONCLUSIONS

In conclusion, this study brings quantitative information on the increment in lipophilicity (or rather in hydrophilicity) resulting

Table 3. σ, ρ, and $\sigma_{Hammett}$ Values of Substituents (21,22)

Compound	log P − Σf	σ	ρ	$\sigma_{Hammett}$
R = H	0.00	0.00	0.00	0.00
R = $COCH_3$	0.86	0.51	0.27	0.50
R = NO_2	0.94	0.60	0.00	0.78
R = CN	0.88	0.65	0.00	0.66
R = $N(CH_3)_2$	0.13	0.00	0.61	−0.83
R = OCH_2CH_3	0.25	0.17	0.50	−0.24

from N-oxygenation of pyridine-type aromatic azaheterocyclic compounds. For pyridine itself, the increment is −1.91 in the octanol/buffer system, but ring substituents strongly influence this value *via* electronic interactions. Such variations allow the mechanism of lipophilicity decrease upon N-oxygenation to be unraveled, but render very difficult the derivation of an incremental value for the aromatic N-oxide moiety.

The small but carefully selected series of solutes examined here indicate that the polarity of the aromatic N-oxide moiety is mainly due to its marked H-bond acceptor basicity. As a consequence, the lipophilicity increment of the aromatic N-oxide moiety is much more negative in the alkane/buffer system (ca. −3.5 to −4.0) than in the octanol/buffer system, the former encoding a larger contribution of H-bonding than the latter. The capacity of such N-oxides to permeate or not across biological membranes will thus depend heavily on the H-bond donating capacity of these membranes.

In contrast to aromatic N-oxides, the incremental value for aliphatic N-oxides is expected to be more constant and easier to parametrize, but would yield little information on the underlying mechanism. This hypothesis awaits experimental challenge, especially since aliphatic N-oxides are much more frequent than aromatic N-oxides as drug metabolites. Work is in progress to address this problem.

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