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Hydrophobicity Change on N-Oxidation of Some 4-Aminoquinolines

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The change in hydrophobicity on N-oxidation of the ring nitrogen of quinoline and its 4-amino derivatives was determined by the shake-flask method and reverse phase liquid chromatography (RPLC), buffered at an appropriate pH. Hydrophobicity was expressed as log partition coefficient ($\log P$) (shake flask) or log capacity factor in 100% water ($\log k_w$) (RPLC). It was found that the ring nitrogen, when unprotonated, was less polar than the corresponding N^+–O^- fragment of the *N*-oxide. When protonated however, the N^+–H fragment was more polar than the N^+–O^- fragment. Correlationship was observed between the $\log P$ and $\log k_w$ values of six phenolic compounds of the series. The derived Collander-type equation was $\log P = -6.78 + 3.73 \log k_w$ ($n=6$, $r=0.972$).

Keywords——partition coefficient; capacity factor; substituted 4-aminoquinoline; N-oxidation hydrophobicity change

Introduction

The role of N-oxidation in the metabolism and excretion of xenobiotics is well known.[1] The *N*-oxide metabolite may be more potent than the parent molecule, as in the case of *N*-oxides of the antimalarial agents amodiaquine, chloroquine and quinacrine,[2,3] or it may possess activity which is not significantly different from that of the parent compound, as has been noted for the *N*-oxide of tebuquine.[4,5]

The most striking physicochemical change on N-oxidation is an expected decrease in hydrophobicity of the drug molecule, which would obviously influence its absorption, distribution and excretory characteristics. The parabolic relationship between drug hydrophobicity and biological activity is well known.[6] The improved activity on N-oxidation of the antimalarials noted earlier may have been due to a shift towards optimum hydrophobicity leading to a higher concentration of drug at the receptor site.

The hydrophobicity of a molecule is traditionally expressed by the logarithm of its partition coefficient (P) determined between a non-polar solvent and water. It has been shown that the $\log P$ of organic molecules possess an additive-constitutive character if there is no appreciable change in electrical structure on introduction of a substituent.[7,8]

Thus it is possible to calculate the $\log P$ of a molecule by simple summation of the lipophilicity of appropriate molecular fragments, without actual experimental determination. Among the compilations of hydrophobic substituent constants by Leo *et al.*[7] and f fragments by Rekker,[8] there are few data on charged functional groups and none on *N*-oxide. It would be useful in the context of quantitative structure–activity relationship and drug design if the hydrophobicity of an *N*-oxide derivative, whether real or hypothetical, can be computed purely from a knowledge of the hydrophobicity of the parent molecule and a quantitative term expressing the expected change in hydrophobicity on N-oxidation. We would like to report the determination of this quantitative term by the shake-flask method and by reversed phase liquid chromatography (RPLC), using as our examples quinoline and some quinolyl antimalarials.

Materials and Methods

Quinoline (I), quinoline *N*-oxide (III) and 4,7-dichloroquinoline (II) were obtained from Aldrich Chemical Company. Amodiaquine (VI), amopyroquin (VII) and *O*-methylamodiaquine (VIII) were obtained gratis from Warner-Lambert Company as the hydrochloride salts. 4,7-Dichloroquinoline *N*-oxide (IV), *p*-(7-chloro-4-quinolylamino)phenol (V) and its *N*-oxide (IX), amodiaquine *N*-oxide (X), amopyroquin *N*-oxide (XI), *O*-methylamodiaquine *N*-oxide (XII) and *p*-(7-chloro-4-quinolylamino)anisole (XIII) were synthesized according to reported methods.[2,9,10]

The log *P* of V—XII were determined by the shake-flask method using 1-octanol (purified and redistilled) and an aqueous phase of pH 2.8 containing 1% triethylamine (v/v) and orthophosphoric acid. For the purpose of useful comparison, the composition of the aqueous phase has been formulated as closely as possible to that of the mobile phase of the RPLC system described later. Both phases were optically transparent above 240 nm and had been preequilibrated. An amount of the compound (free base), not exceeding 40% of the quantity required to saturate the octanol phase, was weighed, dissolved in octanol and shaken with the aqueous phase for 1 h at 28 °C on a mechanical shaker. The two layers were then separated, centrifuged (10 min) and the amount of compound in each phase determined by ultraviolet (UV) spectroscopy at the appropriate maximal wavelengths after dilution with 95% ethanol (octanol phase) and pH 2.8 buffer (aqueous phase). No less than 10 determinations, using at least two different phase volume ratios (octanol : aqueous = 3 : 3, 4 : 2, 5 : 1), were made for each compound. For I—IV, log *P* was determined using an aqueous phase of pH 7.4 phosphate buffer, with other conditions remaining unchanged. All glassware used were silanised to minimize adsorption.

The capacity factors (k') of V—XII were determined by RPLC using a Waters chromatography system and a pre-packed reverse phase phenylsilica plastic column (μBondapak Rad-Pak Phenyl, 10 μm particles, 10 cm × 8 mm i.d.; Waters Associates). The mobile phase consisted of varying concentrations of chromatographic grade methanol and double distilled water (14%, 18%, 23%, 27%, 32% (w/w) methanol), containing 1% triethylamine (w/w), adjusted to pH 2.8 with orthophosphoric acid.[11] Triethylamine has been included into the RPLC mobile phase to reduce tailing of the emerging peaks. Determinations were carried out at 28 °C, with the flow rate adjusted to 2—5 ml/min depending on the mobile phase composition. Detection was performed by UV (254 nm) spectroscopy. Solutions of V—XII were prepared in the mobile phase (1 mg/ml) and acetone was added as void volume marker. 20 μl was used for injection and determinations were carried out in quadruplets for each mobile phase.

The pK_a of XIII was determined by potentiometric titration[12] using a Radiometer autotitrator assembly (RTS 822). 10 ml of 0.001 M XIII hydrochloride, prepared in freshly deionised water, was titrated with standardised 0.01 M KOH. The titration was performed under nitrogen in a water-jacketed thermostated vessel (25 °C), with stirring provided by a mechanically rotating teflon coated rod. Titrant was added from a 2.5 ml microburet calibrated to 0.001 ml and no less than 10 additions were made in a single run. The pH of 0.001 M XIII hydrochloride remained between 5 and 9 throughout the course of the titration. Proton nuclear magnetic resonance (^{1}H-NMR) spectra of VI and VIII were recorded with a JEOL JNM FX-100 spectrometer with dimethyl sulfoxide (DMSO)-d_6 as solvent and tetramethylsilane as internal standard. The chemical shifts are given on the δ scale in ppm.

Results

log *P* of I—XII was calculated from Eq. 1:

$$\log P = C_{\text{octanol}}/C_{\text{aqueous}} \quad (1)$$

where C_{octanol} and C_{aqueous} are the concentrations of the compound in the octanol and aqueous phases respectively.

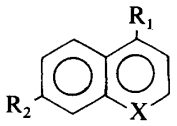
The hydrophobicity change on N-oxidation was determined from Eq. 2:

$$\Delta \log P = \log P_2 - \log P_1 \quad (2)$$

where $\log P_1$ and $\log P_2$ are values of the parent molecule and its *N*-oxide derivative respectively. The computation of $\Delta \log P$ in this way is analogous to that for the determination of Hansch's substituent constant π[7] except that N-oxidation is strictly a chemical transformation of the basic N and not a substitution by a function X for H of the parent molecule as π would imply. Nevertheless, $\Delta \log P$ and π are similar in that the hydrophobicity of the derivative of the parent molecule can be computed if the hydrophobicity contributions of the parent molecule and the derivation process are known.

The log *P* values of I—IV are given in Table I. Since I (pK_a 4.90) and II (pK_a 2.80)[13] are

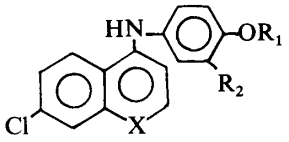
TABLE I. log P of I—IV (Octanol–Buffer pH 7.4)



Parent molecule (X=N)	R_1	R_2	log P_1 [a]	N-Oxide (X=N^+–O^-)	log P_2 [a]	Δ log P [b]
I	H	H	2.09[c] (0.03)	III	0.36 (0.02)	−1.73
II	Cl	Cl	3.57 (0.01)	IV	1.85 (0.01)	−1.72

a) Values in parentheses indicate standard deviation for $n=10$. *b*) $\Delta \log P = \log P_2 - \log P_1$, and represents the difference in hydrophobicity of $\geq N^+$–O^- and $\geq N$: c) Literature value 2.04 (octanol–buffer pH 7.0).[7]

TABLE II. log P of V—XII (Octanol–Buffer pH 2.8)



Parent molecule (X=N)	R_1	R_2	log P_1 [a]	N-Oxide (X=N^+–O^-)	log P_2 [a]	Δ log P [b]
V	H	H	−0.624 (0.066)	IX	1.226 (0.066)	1.850
VI	H	CH_2NEt_2	−1.652 (0.081)	X	−0.534 (0.170)	1.118
VII	H	CH_2N⟨⟩	−2.432 (0.129)	XI	−1.505 (0.122)	0.927
VIII	CH_3	CH_2NEt_2	−2.620 (0.162)	XII	−1.900 (0.043)	0.720
XIII[c]	CH_3	H				

a) Values in parentheses indicate standard deviation for $n=13$—23. *b*) $\Delta \log P = \log P_2 - \log P_1$ and represents the difference in hydrophobicity of $\geq N^+$–O^- and $\geq N^+$–H. *c*) Compound XIII was employed specifically for pK_a determination.

completely non-protonated at pH 7.4, the true partition coefficients of I and II have been determined. In contrast, V—XII are amphoteric compounds with no pH region where they exist exclusively in the non-protonated state. Hence log P of V—XII determined at pH 2.8 (Table II) are apparent values. Nevertheless, Δ log P being the difference of two log P values determined at the same pH would be unaffected whether true or apparent log P values have been used for its computation.

log k' was obtained from Eq. 3:

$$\log k' = \log [(V_s - V_o)/V_o] \quad (3)$$

where V_s and V_o are the retention volumes of the sample and the void volume marker respectively.

Linear regression of log k' of V—XII against mobile phase composition and extrapolation to 100% water gave log k_w of each compound at pH 2.8 (Table III). All regression lines were linear ($r>0.99$) over the eluent composition range indicating system equilibrium and absence of mixed retention mechanisms.[14] The possibility of using log k_w as a hydrophobic parameter was first envisaged and demonstrated by Hulshoff and Perrin[15] for a series of benzodiazepines. More recent studies have added strong evidence that log k_w is more

TABLE III. $\log k_w$ of V—XII (pH 2.8)

Parent molecule	$\log(k_w)_1$ [a]	*N*-Oxide	$\log(k_w)_2$ [a]	$\Delta \log k_w$ [b]
V	1.749 (0.0299)	IX	2.097 (0.0429)	0.348
VI	1.293 (0.0407)	X	1.602 (0.0240)	0.309
VII	1.185 (0.0303)	XI	1.499 (0.0294)	0.316
VIII	1.539 (0.0188)	XII	1.881 (0.0094)	0.342

a) Values in parentheses represent standard error with $n=20$. *b*) $\Delta \log k_w = \log(k_w)_2 - \log(k_w)_1$ and represents the difference in hydrophobicity of $\geqslant N^+-O^-$ and $\geqslant N^+-H$. Mean $\Delta \log k_w = 0.329$ (0.0191).

correlated to log *P* determined in octanol than isocratic capacity factors.[16,17]

The pK_a of XIII, determined by potentiometric titration, was found to be 6.49 ± 0.0075 ($n=20$) as computed from the Henderson–Hasselbalch equation:

$$pK_a = pH + \log C_{acid}/C_{base} \quad (4)$$

where C_{acid} and C_{base} are the stoichiometric concentrations of the conjugate acid and base respectively. This value specifically refers to the quinolyl N which is the only ionisable group present in XIII.

Discussion

At pH 7.4, the quinolinyl N of I and II are non-protonated. $\Delta \log P$ (Eq. 2) thus represents the difference in hydrophobicities of the quinolyl N and the N^+-O^- fragments. There was a predictable fall in hydrophobicity on N-oxidation as indicated by the lower log *P* values of III and IV. The reported negative $\Delta \log P$ values (Table I) indicates a shift to aqueous affinity on N-oxidation by a factor of about 53. It is somewhat surprising to note that $\Delta \log P$ values obtained from I (-1.73) and II (-1.72) are very close despite the presence of an electron withdrawing 4-chloro substituent in II. The aromatic *N*-oxide moiety exerts both an electron donating and withdrawing mesomeric effect, the direction of the mesomeric shift being governed primarily by the position and electronic effect of substituent(s) on the ring.[18] The very similar $\Delta \log P$ values observed here suggests a lack of electronic effect by a 4-chloro function when attached to a π deficient heteroaromatic ring. Similar observations have been noted by Katritzky *et al.*[19] in their comparative study of the mesomeric moments of 4-chloropyridine and its *N*-oxide.

Since V—VIII have more than one ionisable group, it is difficult to determine the pK_a of the quinolyl N in these compounds without interference from the other ionisable groups present. However, compound XIII which is structurally similar to V—VIII has only one ionisable group, *viz.* its quinolyl N. One would expect the pK_a of the quinolyl N of V—VIII to be close to that of the quinolyl N of XIII (6.49). Hence at pH 2.8, the quinolyl N of V—VIII is expected to be completely protonated.

N-Oxidation of a heterocyclic N generally results in a sharp drop in the basicity of the *N*-oxide function. For example, a decrease of approximately 5.4 pK_a units is noted when 4-aminopyridine (pK_a 9.0)[13] is converted to its *N*-oxide (pK_a 3.6).[13] Unfortunately the pK_a of 4-aminoquinoline *N*-oxide has not been reported, but one may expect a decrease of about the same magnitude when 4-aminoquinoline is N-oxidised. The same can be said of V—VIII. Thus, the pK_a of the *N*-oxide moiety of IX—XII is probably nearer 1. It follows that at pH 2.8, the *N*-oxide moiety of IX—XII exists predominantly in the conjugate base (N^+-O^-) form rather than in the protonated N^+–OH form. The computed $\Delta \log P$ values (Table II) thus

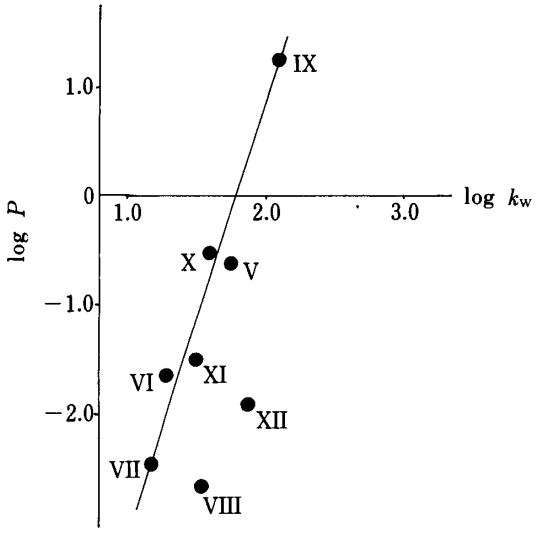


Fig. 1. Regression of $\log P$ on $\log k_w$ Values of V—XII

represents the difference in hydrophobicities of the N^+–H and N^+–O^- fragments in the molecules examined. Since the computed $\Delta \log P$ values are all positive, the N^+–O^- fragment appears to be more hydrophobic than the N^+–H moiety. In the context of drug metabolism, this may imply that though enzymatic N-oxidation may detoxify a base, for certain bases ($pK_a > 8$) which are protonated at physiological pH, the same process may inadvertently result in the formation of less polar *N*-oxides which are less readily excreted.

It is interesting to note that the difference in hydrophobicities of the N^+–H and N^+–O^- fragments as measured at pH 2.8 by the shake-flask method ($\Delta \log P$, Table II) show a wider range than that measured by RPLC ($\Delta \log k_w$, Table III). It is unlikely that the wide variation noted is due to the electronic effect of the 4-substituent of the compounds on the polarity of the *N*-oxide moiety. Probably it reflects the inherent limitation of the shake flask method when dealing with very polar solutes. The narrowness of the spread of the $\Delta \log k_w$ values indicates that $\log k_w$ is a better parameter for the measurement of hydrophobicity of organic molecules than $\log P$, as has been noted by other investigators.[14,16,17]

Regression of $\log P$ on $\log k_w$ of V—XII gave a good correlation in respect to the six compounds which are phenolic, while the two methoxy compounds (VIII, XII) are outliners (Fig. 1). The derived Collander-type[20] equation for the phenolic compounds is as follows:

$$\log P = -6.78\ (\pm 0.719) + 3.73\ (\pm 0.450) \log k_w \qquad (5)$$
$$n = 6,\ r = 0.972,\ s = 0.331$$

The different behavior of VIII and XII may be due to the fact that O-methylation has removed the possibility of strong intramolecular hydrogen bonding between adjacent polar functions with hydrogen bonding capabilities. Compounds with and without intramolecular hydrogen bonding are known to behave differently in RPLC and octanol/water systems.[21]

Evidence showing that O-methylation interfers with intramolecular hydrogen bonding has been deduced from NMR studies of VI and VIII. The NMR spectrum of VI hydrochloride in DMSO at 25 °C showed a broad singlet at δ 4.25 identified as the methylene function (Ar–CH_2–X) of the side chain, thus indicating restricted rotation of the latter. It is possible that the N^+–H of the protonated amino function of VI has hydrogen-bonded with the neighbouring phenoxy oxygen forming a stable six-membered ring. The broad peak at δ 4.25 became sharper when the temperature was raised to 80 °C. A similar NMR study on the hydrochloride salt of VIII, the O-methyl compound, however showed a sharp singlet (–CH_2) at δ 4.28 even at room temperature. This indicates weak intramolecular hydrogen bonding as a result of O-methylation.

It is interesting to note from Table II that VIII is more polar than VI, despite the fact that VIII has an additional methyl function. This apparent anomaly can also be attributed to intramolecular hydrogen bonding. It is generally accepted[7] that solutes which readily form intramolecular hydrogen bonds will adopt this favoured conformation during partitioning and that additivity will certainly be affected because such a phenomenon reduces the affinity of the solute for the aqueous phase. Thus a positive $\Delta\pi$ correction factor is normally incorporated into any computation of $\log P$ when intramolecular hydrogen bonding is present. The magnitude of $\Delta\pi$ depends on the strength of the hydrogen bond,[7] which in the case of VI appears to be quite substantial, probably due to the strong ion-dipole interaction involved. Thus at pH 2.8, the main factor contributing to the hydrophobicity difference between VI and VIII seems to be the protonated side chain amino function which is freely available for hydrogen bonding with the aqueous phase in VIII, but is taken out of play in VI because of intramolecular hydrogen bonding.

The relatively large negative intercept observed in Eq. 5 may be attributed to the low pH at which $\log k_w$ have been determined. Most reported $\log k_w$ values have been measured at pH 7. Braumann[14] have suggested that owing to the weakly acidic character of the residual silanol groups on the RPLC stationary phase, an eluent of pH 7 induces a surface with much higher net charge and thus, having a greater number of water molecules bound to the silica surface. At an acidic pH, the surface consists of more strongly interacting ligands and less water is present in the stationary phase-mobile phase interface. Hence the overall polarity of the stationary phase is lower and this may result in the negative intercept as observed in Eq. 5.

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