SOME PHYSICOCHEMICAL PARAMETERS OF 11H-INDOLO[3,2-c]QUINOLINE

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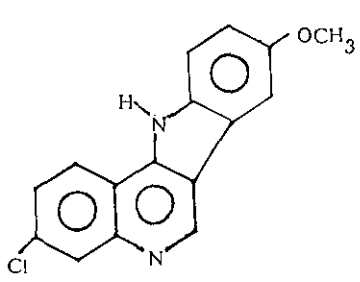
Abstract - The hydrophobicity (log P), pKa and limiting solubility of 3-chloro-8-methoxy-11H-indolo[3,2-c]quinoline (I) , a representative 11H-indolo[3,2-c]quinoline, were determined. A significant difference was observed between the basicity of I and its non-cyclized analogue, 7-chloro-4-N-(p-methoxyphenyl)aminoquinoline (II). I was found to be a weaker base (pKa 3.99) than II (pKa 7.59), which suggested that the indolo N, unlike the 4-amino group of II, did not affect the basicity of the quinolyl N. I was also much less hydrophobic and more water soluble than II, which could only be attributed partly to a reduction of surface area on ring formation. Based on the present determinations, the unique hydrophobicity of the 11H-indolo[3,2-c]quinoline ring system was calculated to have a log P value of 2.22.

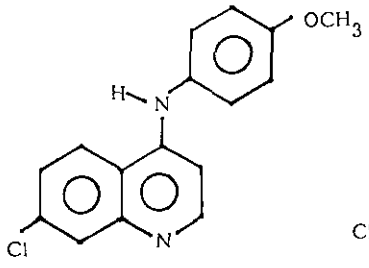
Interesting pharmacological activities have been reported for several derivatives of 11H-indolo[3,2-c]quinoline. For example, 8-methyl- and 8,9-dimethyl-11H-indolo[3,2-c]quinolines were among several polycyclic aromatic hydrocarbons which have been screened for photodynamic activity and zoxazolamine hydroxylase inducing activity.[1,2] The DNA binding characteristics and RNA polymerase inhibitory activity of 3-chloro-8-methoxy-11H-indolo[3,2-c]quinoline-9-N,N-diethylmethanamine have been found to be greater than that of its non-cyclized analogue, amodiaquine, possibly due to the planar conformation of the former.[3] More recently, derivatives of 11H-indolo[3,2-c]quinoline-1,4- and -7,10- diones were found to be cytotoxic to leukemia cells.[4]

Antimalarial activity has been reported among the 5-oxides of N,N-disubstituted 11H-indolo[3,2-c]quinoline-11-ethanamines.[5] In the course of our study on similar activity with some 3-chloro-8-methoxy-11H-indolo[3,2-]quinoline-methanamines, we are struck by the paucity of information relating to the physicochemical

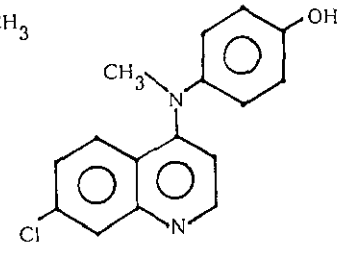
characteristics, viz. hydrophobicity, solubility and dissociation constant, of the parent indolo[3,2-c]quinoline ring. Since these parameters are important modulators of biological activity, some knowledge of their magnitude would be useful in the area of drug delivery and design. To this end, we have determined the hydrophobicity (log P), limiting solubility (So) and dissociation constant (pKa) of 3-chloro-8-methoxy-11H-indolo[3,2-c]quinoline (I), which was chosen as a representative indolo[3,2-c]quinoline, and also because of its structural relationship to the compounds we were investigating. Similar determinations were also made for 7-chloro-4-N-(p-methoxyphenyl)aminoquinoline (II), which may be considered as the non-cyclized precursor of I, and 7-chloro-4-N-methyl-4-N-(p-hydroxyphenyl)aminoquinoline (III), which, in contrast to the planar and rigid I, assumes a non-planar conformation due to steric hindrance. It is noted that II and III are structural isomers with the same number of C and H atoms.



I



II



III

EXPERIMENTAL

Chemical synthesis: I was obtained by refluxing 4-keto-7-chloro-1,2,3,4-tetrahydroquinoline (1.84 g)[6] with p-methoxyphenylhydrazine hydrochloride (1.76 g, Tokyo Kasei) in 22 ml of ethanol and 5.2 ml of concentrated HCl for 18 h. I HCl was obtained as a yellow solid on standing, and recrystallized from methanol (2.1 g, 68% yield, mp 319-320 °C). Anal. Calcd for $C_{16}H_{11}ClN_2O$. HCl: C,60.19; H,3.76. Found: C,60.13; H, 3.67. II and III were synthesized and purified according to

reported methods.[7,8]

Partition coefficient (P) measurements: P was determined in 1-octanol and an aqueous buffer of pH 3.0 (0.01 M citric acid - 0.02 M Na_2HPO_4). Both phases were optically transparent above 240 nm and had been pre-equilibrated. The free base was dissolved in the aqueous phase to give a concentration of 0.001 M and equilibrated with 1-octanol at 3 different volume ratios (5:5, 4:6, 6:4) in 10 ml silanised flasks on a mechanical shaker for 1 h at 28°C. The 2 layers were separated, centrifuged (1000 g, 10 min) and assayed by ultraviolet (uv) spectroscopy at appropriate wavelengths after dilution with 95% ethanol (octanol phase) or pH 3.0 buffer solution (aqueous phase). The partition coefficients at various volume ratios were calculated from eq. 1 and averaged.

$$P = Co/Cw \quad \ldots\ldots\ldots\ldots \quad (1)$$

Co and Cw represent the concentrations in octanol and aqueous phases respectively.

pKa and solubility measurements: Problems of poor solubility precluded the use of potentiometry for pKa determination. Instead, the more tedious solubility method was employed.[9] At constant ionic strength, both pKa and limiting solubility (So) of a compound can be calculated from a plot of its solubilities at different pH. The solubility of each compound was determined in duplicate at 6-7 different pHs within the range of pka±1 (estimated from trial). For each solubility determination, approximately 4 mg of the HCl salt was shaken with 10 ml of buffer (1/15 M NaH_2PO_4 - 1/15 M Na_2HPO_4 for I; 1/15 M citric acid - 1/15 M Na_2HPO_4 for II, III) in a silanized flask for 12 h at 28°C on a mechanical shaker (300 rpm). Excess compound was filtered and the pH of the filtrate was accurately determined with a pH meter (Radiometer PHM 62). The concentration of the filtrate at each pH was determined by uv spectroscopy at suitable wavelength after dilution, using previously constructed calibration curves. From eq. 2, a plot of the hydrogen ion concentration $[H^+]$ against solubility (S) gave a straight line with an ordinate intercept of Ka and a gradient of Ka/So.

$$[H+] = KaS/So - Ka \quad \ldots\ldots\ldots\ldots \quad (2)$$

In this way, the pKa and limiting solubility (So) of the compound were simultaneously determined. No less than three pKa/solubility determinations were carried out for each compound.

RESULTS AND DISCUSSION

Table 1 lists the pka, limiting solubility (S_0), apparent partition coefficient at

pH 3.0 (log P app.), and true partition coefficient (log P) values of I - III.

Table 1 Physicochemical paratmeters of I, II and III.

	I	II	III
pKa	3.99 (0.132) [a]	7.59 (0.0839)	6.65 (0.0296)
% Protonation at pH 3.0 [b]	90.77	99.99	99.96
Log P app.	1.75 (0.037)	0.14 (0.010)	0.34 (0.017)
Log P [c]	2.78	4.72	3.98
So (x 10^{-6} M)	3.63	1.50	1.71

a) Values in parentheses represent standard deviation for n = 4 for pKa determinations, and n = 10 for log P app. determinations.
b) % protonation = 100 / [1 + antilog(pH - pKa)].....(3)
c) P = P app (1 + [H+]/Ka) .....(4)

pKa values of I - III: A comparison of the pKa values of II, III and that of 4-amino-7-chloroquinoline (pKa 8.23)[10] is interesting as it indicates the effect of substitution at the 4-amino function on the basicity of the quinoline N. Girault et al.[11] have earlier shown that methyl and dimethyl substitution of the side chain amino function of 4-aminoquinoline caused a progressive decrease in the quinoline N basicity. Thus, 4-methyl- and 4-dimethylaminoquinoline have pKa values of 9.06 and 8.39 respectively, compared to 9.17 which is the pKa of 4-aminoquinoline. A larger decrease was observed on dimethylation (Δ pKa = 0.78) which was attributed to steric inhibition of conjugation. A similar pattern was also observed when comparing the pKa of II, III and that of 4-amino-7-chloroquinoline, except that a larger fall in pKa value (Δ pKa=0.6) was noted between 4-amino-7-chloroquinoline and II. This is possibly due to the phenyl substituent which competes with the quinoline ring for the amino lone pair of electrons. An even larger fall (Δ pKa=0.9) was evident on going from II to III, which cannot be attributed to the electronic effect brought about by functional group changes (i.e. -OMe → -OH and -NH → -NMe). A comparison of the Hammett constants[12] of -OMe (σ_p = -0.27) and -OH (σ_p = -0.36) indicates that the latter is more electron-donating. Furthermore, a methyl substituent on the N atom is also electron-donating. The net electronic effect of these functional group changes from II to III is expected to be base-strengthening, rather than base-weakening as has been observed. Thus one can attribute the sharp reduction in pKa values from

II to III to the steric effect introduced by the methyl substituent in III. Indeed, construction of the Drieding model of III demonstrated that the phenyl and quinoline rings were twisted out of plane due to the presence of the N-methyl group. Delocalisation of the amino lone pair of electrons to the quinoline ring is thus unfavourable as its orbital is no longer perpendicular to the plane of the ring. The quinolyl nitrogen of III is thus less basic due to this unfavourable stereoelectronic effect.

The lower pKa of I, when compared to those of the 4-aminoquinolines II and III, suggests that the indolo nitrogen, unlike the 4-amino group of II, does not affect the basicity of the quinolyl N. Indeed, the aromaticity of the indole ring requires the participation of the nitrogen lone pair of electrons to satisfy the 4n+2 rule. Indole per se is known to have very low basicity (pKa = - 2.4)[10] since protonation would destroy aromaticity. One may conclude that the basicity of the 11H-indolo[3,2-c]quinoline ring system is very much a function solely of the basicity of the quinoline ring, subjected as usual to the influence of any substituent(s) present. It is noted that the pKa of I is lower than that of quinoline (pKa 4.9), being closer to that of quinoline derivatives with electron withdrawing groups.[10]

Log P and solubility values of I - III: At pH 3.0, log P app. values of I - III are in the order of I > III > II. This sequence is antiparallel to the % protonation of these compounds at pH 3.0, which is a function of their basicities (Table 1). Increased protonation is expected to reduce hydrophobicity.

The advantage of the true log P value over the apparent log P value is that it measures the partitioning of the undissociated species (viz. free base). Being free from the association phenomenon and the complications arising from the partitioning of ionised and non-ionised species, it gives a more accurate representation of the hydrophobic character of a molecule. Thus the relative hydrophobicity of these compounds, as judged by their true log P values, is in the order of II > III > I.

A comparison of the hydrophobicities of I and II is interesting as I may be considered as the ring-closed analogue of II. I is less hydrophobic than II and, in keeping with this observation, its limiting aqueous solubility is greater than II. The lower hydrophobicity of I is puzzling. It can partly be attributed to a reduction in molecular volume (and surface area) on ring formation. As molecular size decreases, the free energy for partitioning becomes increasingly more

positive whereas the free energy for the opposing process of dissolution in water becomes more negative. It is noted that the reduction in hydrophobicity on cyclization of n-hexane to cyclohexane is 0.5,[13] which is in keeping with the loss of 2 H atoms in the cyclized molecule. A far greater reduction in hydrophobicity (Δ log P = 1.3) is observed when cyclohexane is oxidized to benzene,[14] which is attributed to a loss of 6 H atoms. Yet the reduction of hydrophobicity from II to I (Δ log P =2.0) is much greater than can be accounted by a mere loss of 2 H atoms on ring formation. The hydrophobicity of the 11H-indolo[3,2-c]quinoline ring system is thus unique in this respect.

Even though II and III are structural isomers, a direct comparison of their hydrophobicity values is complicated by functional group differences that exist between them. Thus the effect of conformation, if any, on the hydrophobicities of II and III can only be ascertained by a comparison with their computed log P values respectively. Using the fragment method of Leo et al.,[14] the log P values of I,II and III were calculated and compared with their observed values (Table 2). A surprising good correlation was observed with II. There was a difference of only 0.13 between the observed and computed log P values. However, a larger deviation (0.33) was noted for III. Perhaps the higher observed hydrophobicity of III can be attributed to the lack of planarity in its conformation . Thus conformation is an important factor in hydrophobicity calculation, a fact well noted elsewhere in the literature.[15]

Table 2 Calculated[a] and observed log P values

	Calc. log P (A)	Obs. log P (B)	(B)-(A)
II	f CH_3 + f(-O-) + f Ph + f (NH<) + f quinoline + f Cl + 4fb = 0.89 + (-0.57) + 1.90 +(-0.18) + 2.09 +0.94 + 4(-0.12) = 4.59	4.72	0.13
III	f OH + f Ph + f(-N<) + f CH_3 + f quinoline + f Cl + 4fb = (-0.4) + 1.90 +(-1.29) + 0.89 + 2.09 + 0.94 + 4(-0.12) =3.65	3.98	0.33
I	Calc. log P of II - 2fH + (ring closure) = 4.59 - 2(0.23) + (-0.09) = 4.04	2.78	-1.26
IV	Obs. log P of I - f Cl - f(-O-) - f CH_3 - 2fb + 2fH = 2.78 - 0.94 -(-0.57) -0.89 - 2(-0.12) +2(0.23) = 2.22		

a) Hydrophobic constants used in the above calculations were obtained from refs. 14 and 15.

The extremely poor correlation between the observed and calculated log P values of I is possibly the result of the unique hydrophobicity characteristics of the 11H-indolo[3,2-c]quinoline ring system noted earlier. Fortunately the hydrophobicity of the ring can be calculated from the observed log P value of I by eliminating the contributions made by the substituents. Computation in this manner using the fragment method of Leo et al.[14] gave the log P value for 11H-indolo[3,2-c]quinoline (IV) as 2.22 (Table 2) a useful value for calculating the hydrophobicity of other molecules containing the same ring system.

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