Molecular Geometry and Physicochemical Characteristics of Selected Anilinoquinolines, Indolo[3,2-*c*]quinolines and Tetrahydroindolo[3,2-*d*]benzazepines

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The molecular geometry, acid dissociation constants and partition coefficients of the anilinoquinoline (I), indolo[3,2-*c*]quinoline (II) and tetrahydroindolo[3,2-*d*] [1]benzazepine (III) ring systems have been determined using representative compounds: 7-chloro-4-(*p*-anisidino)quinoline (Ia), 3-chloro-8-methoxy-11*H*-indolo[3,2-*c*]-quinoline (IIa) and 3-chloro-9-methoxy-5,6,7,12-tetrahydroindolo[3,2-*d*] [1]benzazepine (IIIa). Ring systems II and III are cyclic analogues of I. The minimum energy conformation was determined by molecular mechanics. Compound IIa is the most planar and conformationally restricted, followed by IIIa and Ia. The acid dissociation constants (pK_a) were determined by the solubility method. The ring nitrogen of Ia is most basic, followed by that of IIa and IIIa. The partition coefficient (log *P*) was determined between octanol and appropriate aqueous buffers by the shaken flask method. Hydrophobicity decreases in the order of Ia > IIa > IIIa. Factors contributing to the different molecular geometry, pK_a and hydrophobicity of these related compounds are discussed. The present study may contribute to the design of better drugs with ring system I, II or III.

Keywords molecular geometry; physicochemical property; anilinoquinoline; indoloquinoline; tetrahydroindolobenzazepine

In our ongoing research on antimalarial agents, we have synthesized and tested various anilinoquinolines (I), indolo[3,2-*c*]quinolines (II) and tetrahydroindolo[3,2-*d*] [1]benzazepines (III) (Fig. 1).[1,2] The anilinoquinolines (I) are derivatives of the potent antimalarial agent amodiaquine (IV) while II and III are cyclic analogues of I. These compounds have appreciable antimalarial activities.[2] In the design and comparative biological study of these compounds, it is necessary to know their molecular geometry, pK_a values and hydrophobicities (log *P*). We have therefore examined compounds Ia, IIa and IIIa, which are representative members of the corresponding series. The selected compounds do not have a basic aminoalkyl side chain R, which is present in all other members of the series, since it might interfere with the physical (pK_a and log *P*) and geometrical determinations. The observed physicochemical parameters of Ia, IIa and IIIa thus serve to characterize the parent ring systems of I, II and III. As there is a wide spectrum of biological activities observed with derivatives of indolo[3,2-*c*]-quinoline[3-5] and tetrahydroindolo[3,2-*d*] [1]benzazepine,[6] these physical studies may be useful in the rational design of novel derivatives of these ring systems.

Experimental

Materials 7-Chloro-4-(*p*-anisidino)quinoline (Ia) and 3-chloro-8-methoxy-11*H*-indolo[3,2-*c*]quinoline (IIa) were synthesized according to reported methods.[6,7] Their melting points, infrared and nuclear magnetic resonance spectra were found to be in accord with literature values.[6,7] 3-Chloro-9-methoxy-5,6,7,12-tetrahydroindolo[3,2-*d*] [1]benzazepine (IIIa) was synthesized in the laboratory as described below. All reagents used in the partitioning and pK_a determinations were of analytical grade.

Infrared spectra were obtained from presented KBr discs on a Jasco IR-810 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a JEOL 60 SI (60 MHz) spectrometer. Chemical shifts were reported as δ ppm relative to tetramethylsilane. Elemental analyses were performed on a Perkin Elmer Auto Analyser 240. Mass spectra were determined on a Micromass 7035 E double focussing mass spectrometer using a Digital PDP 81a computer system for data capture and processing. Ultraviolet spectra and fluorescence data were obtained from a Perkin Elmer Lambda 4A Controller UV/VIS spectrophotometer and a Perkin Elmer LS-5B luminescence spectrometer, respectively.

Synthesis of 3-Chloro-9-methoxy-5,6,7,12-tetrahydroindolo[3,2-*d*] [1]-benzazepine (IIIa) A mixture of 1.2 g (0.0062 mol) 8-chloro-2,3,4,5-tetrahydro-1-benzazepin-5-one,[6] 1.07 g (0.0062 mol) 4-methoxyphenylhydrazine hydrochloride (Tokyo Kasei), 24 ml of ethanol and 6 ml of concentrated hydrochloric acid was refluxed for 18 h and cooled to room temperature. The precipitated solid was collected by filtration, dried and recrystallized from ethanol to give 0.96 g (52.7%) of the hydrochloride salt, mp 230 °C. The free base, obtained by treating the hydrochloride salt with 25% NaOH, melted at 183.5—185 °C. *Anal.* Calcd for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; N, 9.38. Found: C, 68.19; H, 4.97; N, 9.34. IR (KBr): 3460, 3390 (N–H), 1590, 1480 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 9.11 (1H, s, NH), 7.56—6.72 (6H, m, aryl H), 3.86 (3H, s, OCH_3), 3.45 (2H, t, NCH_2), 3.09 (2H, t, NCH_2CH_2). MS *m/z*: M^+ Calcd for $C_{17}H_{15}ClN_2O$: 298.0873. Found: 298.0870.

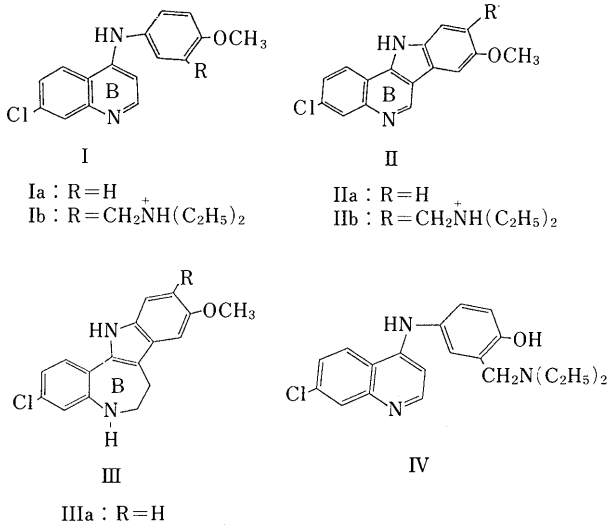

Fig. 1. Structural Formulae of Compounds Ia,b, IIa,b, IIIa and IV

Determination of pK_a The pK_a of compounds Ia, IIa and IIIa were determined by the solubility method.[8] Approximately 4 mg of the hydrochloride salt of the compound was shaken with 10 ml of buffer in a silanized conical flask for 10 h at 29 °C on a mechanical shaker at 200 rpm. The undissolved compound was filtered off and the pH of the filtrate accurately determined with a pH meter. The concentration of the filtrate was determined by UV spectroscopy at a suitable wavelength using previously constructed calibration curves. Two or more determinations were carried out at each of 7 different pH values. The latter covered a range of 0.8 pH unit which included that of the expected pK_a (estimated from preliminary trials). The following buffers were used: Ia, 1/15 M Na_2HPO_4–1/15 M NaH_2PO_4 (pH 6.3—7.9); IIa, 1/15 M Na_2HPO_4–1/15 M citric acid (pH 3.0—4.3); IIIa 0.1 M HCl/KCl (pH 2.1—2.9).

Based on the relationship,[8]

$$S=\frac{[B][H_3O^+]}{K_a}+[B] \quad (1)$$

where S is the sum of solubilities of the free base B and its protonated form, in which [ ] represents the concentration, a plot of S against $[H_3O^+]$ gave a straight line, from which the limiting solubility [B] and dissociation constant K_a were determined from the y-intercept and slope, respectively.

Determination of Distribution Coefficient The distribution coefficients (D) of compounds Ia, IIa and IIIa were determined in 1-octanol/aqueous buffers by the shaken flask method. Octanol and the appropriate aqueous phase were pre-equilibrated before use. The free base of Ia and the hydrochloride salts of IIa and IIIa were used for partitioning. The compound was dissolved in the aqueous phase to give a concentration of 0.001 M. The solution was then shaken with 1-octanol (5:4 or 4:5 volume ratio) in a silanized flask on a mechanical shaker (200 rpm) for 1 h at 29 °C. The 2 layers were left to stand for at least 10 h, separated and centrifuged at 1000 g for 10 min. The concentrations of the solute in the octanol and aqueous phases were determined by UV spectroscopy after dilution with methanol (octanol phase) or buffer (aqueous phase). The concentration of IIIa in the aqueous phase was determined by fluorometry at 418 nm (emission) with excitation at 327 nm. The following buffers were used: Ia, 1/15 M Na_2HPO_4–1/15 M citric acid (pH 3.00); IIa, 0.1 M HCl (pH 1.15); IIIa, 0.5 M H_2SO_4 (pH 1.31). At least 10 determinations were carried out for each compound at a specified volume ratio. The average distribution coefficient (D) was obtained and the partition coefficient (P) of each compound was determined using the following relationships[9]:

$$D=C_o/C_w \quad (2)$$

$$P=D(1+10^{pK_a-pH}) \quad (3)$$

where C_o and C_w are the concentrations of the compound in the octanol and aqueous phases respectively, pH refers to that of the aqueous phase buffer and pK_a is the previously determined value for Ia, IIa or IIIa.

Conformational Studies The minimum energy conformation of the non-protonated form of each compound was determined by using an interactive molecular modelling program, PC Model Version 4[10] which incorporates the MMX force field for molecular mechanics (MM) calculations.

Results and Discussion

Evaluation of Molecular Planarity of Compounds Ia, IIa and IIIa Compounds Ia and IIIa are characterized by aromatic rings linked by a C–N–C bridge. Conjugation of the bridge nitrogen with the rings requires a planar conformation. Planarity can be determined by measuring an appropriate twist angle τ in the minimum energy conformation of Ia, IIa and IIIa (Fig. 2). The appropriate twist angle is that which can best represent the angle of twist of the molecule. Increasing twist angle from zero represents deviation from planarity.

It can be seen from Fig. 2 that Ia has an angle of twist τ 1–2–3–4 = 40.40°, indicating some deviation from planarity. The non-planarity may be attributed to steric interaction between H_x and H_y, and also H_v and H_w. The interatomic distances of these atoms at the planar conformation are less than the sum of their van der Waals radii. Moreover, the partial double bond character of the C–N–C bridge may also increase the rotational barrier about the bridge. Compound IIIa gives an angle of twist τ 1–2–3–4 = 22.01°. Ring strain due to the 7-membered B ring may contribute to the deviation from planarity.

Compound IIa is by far the most planar of the three molecules. The planarity is not surprising as the ring system is aromatic, satisfying the Huckel $4n+2$ rule. MMX computation gives a negligible angle of twist. In terms of molecular geometry, IIa is thus the most planar, followed by IIIa and Ia. However, when conformational lability is considered, IIa is most restricted, followed by IIIa and Ia.

pK_a of Compounds Ia, IIa and IIIa The experimentally determined pK_a values of Ia, IIa and IIIa are given in Table I. These pK_a values are those of the ring B nitrogen of the compounds.

Of the three compounds, Ia is the most basic. Compound Ia is a derivative of 4-amino-7-chloroquinoline, whose pK_a (8.23)[11] may be attributed to the balance between the base-weakening electron-withdrawing inductive effect of the chlorine atom (*meta* to the ring B nitrogen) and the base-strengthening electron-donating mesomeric effect of the 4-amino group. N-Substitution with a *p*-methoxyphenyl group as in the case of Ia (pK_a 7.78), further weakens the basicity of the ring nitrogen. The phenyl ring competes with the quinoline ring for the 4-amino lone pair of electrons. In addition, the base-strengthening effect of the 4-amino group would be influenced by the deviation from planarity of Ia. The presence of an electron-donating *p*-methoxy group is not

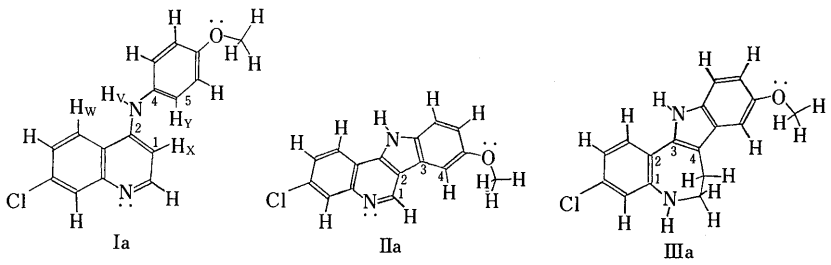

Fig. 2. Twist Angles (τ) of Ia, IIa and IIIa in their Minimum Energy Conformations

a) The twist angle (τ 1–2–3–4) represents the angle of twist of the molecule and was determined from the minimum energy conformations of Ia, IIa, and IIIa using PC Model Version 4.[10]

TABLE I. Dissociation Constants (pK_a) and Hydrophobic Parameters ($\log D$ and $\log P$) of Compounds Ia, IIa and IIIa

Parameter	Ia	IIa	IIIa
pK_a	7.78	3.99	2.62
% protonation at pH 7.4	70.58	0.04	0.002
$\log D$[a]	0.163	1.647	2.904
P[b]	87096 (1209)	30902 (822)	17378 (470)
$\log P$	4.94	4.49	4.24

a) The $\log D$ values were obtained at pH 3.00, 1.15 and 1.31 for compounds Ia, IIa and IIIa, respectively. b) The P values were derived from Eq. 3. Values in parentheses indicate standard error. The P values of Ia, IIa and IIIa are significantly different from each other ($p<0.01$) using Student's t test.

expected to nullify the overall base-weakening effect of the aromatic ring. This can be seen from the lower basicity of *p*-methoxyaniline (pK_a 5.3) as compared to that of ammonia (pK_a 9.3).[12]

Cyclization of Ia to give IIa leads to a great reduction in the pK_a values of the ring B nitrogen, from 7.78 to 3.99. This is not surprising as cyclization transforms the 4-amino substituent of Ia to the weakly basic indolo nitrogen of IIa. The indolo nitrogen contributes its lone pair of electrons to aromatization of the indole ring. The observation that the pK_a of IIa (3.99) is closer to that of 7-chloroquinoline (pK_a 3.85)[11] indicates that the indolo nitrogen of IIa, unlike the 4-amino group of Ia, has no effect on the basicity of the quinolinyl nitrogen.

The pK_a of IIIa (2.62) is much lower than those of Ia and IIa. Unlike IIa, ring B of IIIa is no longer aromatic. The other nitrogen atom in IIIa is present as the indolo nitrogen of the heterocylic ring, and as described earlier would not exhibit any basic property. Thus, the basicity of IIIa has been attributed to the ring B nitrogen and its pK_a would be closer to that of a substituted aniline rather than a quinoline nitrogen. The aniline moiety of IIIa has an *ortho* indolo and a *meta* chloro substituent. From a comparison of the basicity of aniline (pK_a 4.58)[11] and 3-chloroaniline (pK_a 3.50),[11] it is clear that the electron-withdrawing *meta* chlorine substituent is base-weakening. Furthermore, when there is an *ortho* aryl substituent, further electronic delocalization to the second aromatic ring should take place and depress the basicity of the anilino moiety. Thus, there is a lowering of pK_a values from aniline (4.58)[11] to 2-phenylaniline (3.82).[11] Such a conjugation effect should not be influenced by the slight deviation of planarity between the aromatic rings of IIIa. As the anilino moiety of IIIa has *ortho* indolo and *meta* chloro substituents, it is not surprising that the observed pK_a of IIIa is the lowest of the three compounds. Although the alkyl substituent on the anilino nitrogen may be base-strengthening, its effect appears to be largely annulled by the electron-withdrawing character of the other groups.

Partition Coefficients of Compounds Ia, IIa and IIIa The experimentally determined log distribution coefficients ($\log D$) of Ia, IIa and IIIa are given in Table I. They were obtained at different pH values due to the different solubility characteristics of the compounds. However, since there is only one basic center in each compound, the log partition coefficient ($\log P$) of the compounds can be calculated using Eq. 3, based on the assumption that the protonated form is not transferred to the organic phase.

As seen from Table I, the relative hydrophobicity of these compounds, in terms of $\log P$, is in the order Ia > IIa > IIIa. The greater hydrophobicity of Ia compared to IIa, its cyclized analogue, is interesting. An important consequence of ring closure is volume and surface area reduction. Surface area calculations using PC Model Version 4[10] showed that cyclization reduced the surface area of Ia from 270 Å^2 to 252 Å^2 in IIa. As molecular size decreases, the free energy for partitioning becomes increasingly more positive and the free energy for the opposing process of dissolution becomes more negative. Partitioning into the non-polar phase is thus not favored and $\log P$ is expected to decrease. In keeping with this reasoning, IIa was found to be less hydrophobic than Ia by 0.45 $\log P$ unit. It should be noted that cyclization is accompanied by the loss of 2 hydrogen atoms. The loss in $\log P$ is in agreement with similar reductions observed when *n*-pentane is cyclized to cyclopentane ($\Delta \log = 0.39$[13]) for the loss of 2H) and when cyclohexane is oxidized to benzene with a loss of 6H ($\Delta \log P = 0.47$[14]) for loss of 2H).

Compound IIIa is the least hydrophobic of the three compounds. Its lower $\log P$ value at 4.24 is surprising considering that it has one more carbon than IIa. One is reminded, however, of the unique structure of IIIa, which is an indole substituted with an aminoaryl group, while Ia and IIa are essentially derivatives of quinoline. The difference in $\log P$ values must be attributed to the inherent difference in the hydrophobicity of the ring structures involved and cannot be rationalized in any other way from the present results.

Biological Significance of the Physical Studies A knowledge of the physical properties of these three polycyclic ring systems is essential to an understanding of their bioactivity. The pK_a of the ring B nitrogen determines its degree of protonation at a given pH. At the physiological pH of 7.4, the ring B nitrogen of IIa and IIIa is largely unprotonated while that of Ia is partially protonated (Table I). Despite these differences, derivatives of Ia, IIa and IIIa have antimalarial activity.[2] Thus, one may conclude that these compounds interact with their target receptor with their ring B nitrogen in the unprotonated form. A possible mode of interaction would involve hydrogen bonding between the unprotonated nitrogen and a hydroxyl group on the target molecule. Although such functional group interactions are important in drug-receptor binding, physical complementarity between the two components is also essential for effective interaction. For structural analogues such as I, II and III, the conformation of each ring system would be important.

Marquez and co-workers[4] have compared the DNA binding characteristics of Ib and IIb. Compound IIb, which has a planar ring conformation, was found to bind strongly to DNA, unlike Ib which deviates from planarity. However, no such correlation was noted in their antimalarial activity, implying that intercalation with DNA may not form the basis of their antimalarial activity.[4] In our comparative study of I, II and III as antimalarials,[2] we have found that the conformation of

each ring system is important only in so far as it confers a correct $N\cdots N^+$ distance between the unprotonated ring B nitrogen and the protonated nitrogen at the side chain (as in IIb).

The hydrophobicity of Ia, IIa and IIIa was studied because this parameter influences passive transport across biological barriers. In view of the additive-constitutive property of hydrophobic constants, a knowledge of the $\log P$ values of these parent ring systems would also facilitate calculation of the partition coefficients of derivatives and related compounds by the fragment method[14] or using π values.[9,15] As shown in Table I, the three compounds (Ia, IIa, IIIa) have $\log P$ values which are greater than 4. This exceeds the optimal $\log P$ of 2 which is considered ideal for drug absorption and transportation. Thus, in our rational drug design of antimalarials having ring systems I, II and III, it was necessary to introduce polar substituents/side chains into the ring system if biological activity was to be assured. Our approach was based on the introduction of an aliphatic side chain carrying one or more amino functions which would be protonated at physiological pH.[2] Evaluation of *in vitro* antimalarial activity of these compounds has shown that Ia, IIa and IIIa, which lack a polar side chain, were devoid of activity. In the compounds bearing a polar side chain, the $N\cdots N^+$ distance between the unprotonated ring B nitrogen and the protonated side chain nitrogen has been found to be an important determinant of activity.[2] It would appear that the introduction of a polar side chain into the ring systems I, II and III influences activity not only by modulating hydrophobicity but also by participating in drug/receptor interaction.

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