at the lower doses tested; at higher doses, the observed levels were less than expected.

A direct relationship was observed between the logarithm of serum concentration and therapeutic effect in the adjuvant-induced arthritis test in rats. Significant activity in this test (30–50% inhibition) was associated with plateau serum levels in the range 40–120 mcg./ml., given by daily doses of about 5–10 mg./kg. Preliminary volunteer and clinical trials in man were designed on the basis of these observations in the belief that it is more rational to transpose from one animal species to another (in this case, rat to man) on the basis of comparative serum level data (18). Volunteer and patient studies established that doses of 100–200 mg. administered every 12 hr. gave rise to serum levels of fenclozic acid in the approximate range 50–100 mcg./ml. (5). Clinical effectiveness of fenclozic acid (100–200 mg. b.i.d.) was assessed in patients with rheumatoid arthritis in a double-blind crossover trial against aspirin (900 mg. q.i.d.), the results of which (19) lent support to this approach to clinical trial design. The results also allowed evaluation of the rat screening test in terms of human effectiveness.

REFERENCES

(1) B. B. Newbould, *Brit. J. Pharmacol.*, **21,** 127(1963).

(2) W. Hepworth, B. B. Newbould, D. S. Platt, and G. J. Stacey, *Nature (London)*, **221,** 582(1969).

(3) B. B. Newbould, *Brit. J. Pharmacol.*, **35,** 487(1969).

(4) D. M. Foulkes, to be published.

(5) T. M. Chalmers, J. E. F. Pohl, and D. S. Platt, *Ann. Rheum. Dis.*, **28,** 590(1969).

(6) S. Riegelman, J. C. K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57,** 117(1968).

(7) M. Gibaldi, R. Nagashima, and G. Levy, *ibid.*, **58,** 193(1969).

(8) S. Riegelman, J. C. K. Loo, and M. Rowland, *ibid.*, **57,** 128(1968).

(9) J. C. K. Loo and S. Riegelman, *ibid.*, **57,** 918(1968).

(10) T. Teorell, *Arch. Int. Pharmacodyn. Ther.*, **57,** 205(1937).

(11) J. G. Wagner and C. M. Metzler, *J. Pharm. Sci.*, **58,** 87 (1969).

(12) J. G. Wagner, *Drug Intel.*, **3,** 82(1969).

(13) J. M. van Rossum and A. H. M. Tomey, *J. Pharm. Pharmacol.*, **20,** 390(1968).

(14) J. G. Wagner and J. I. Northam, *J. Pharm. Sci.*, **56,** 529 (1967).

(15) P. A. Harris and S. Riegelman, *ibid.*, **58,** 71(1969).

(16) M. Gibaldi, *Chemotherapy*, **13,** 1(1968).

(17) D. M. Foulkes, personal communication.

(18) B. B. Brodie and W. D. Reid, *Fed. Proc.*, **26,** 1062(1967).

(19) T. M. Chalmers, J. H. Kellgren, and D. S. Platt, *Ann. Rheum. Dis.*, **28,** 595(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 9, 1970, from *Pharmaceuticals Division, Research Department, Imperial Chemical Industries Limited, Alderley Park, Macclesfield, Cheshire, England.*

Accepted for publication September 30, 1970.

The author is indebted to Dr. B. B. Newbould, Mr. P. A. Melvin, Mr. W. A. Hiddleston, Mr. D. Dunlop, Mr. R. D. Broad, and their respective staffs for their help in obtaining suitable samples. Thanks are due also to Dr. D. M. Foulkes for allowing use of the serum and urinary data from his radioactive experiment in a human patient; Mrs. J. Siddall, Mrs. A. Winstanley, and Miss S. E. Bithell for their technical assistance; and Mr. S. H. Ellis for his help in understanding the intricacies of pharmacokinetic analysis.

Dissociation Constants of Some Isomeric Aminoquinolines: Determination of the Site of Protonation from Shifts in Electronic Absorption Spectra

S. G. SCHULMAN

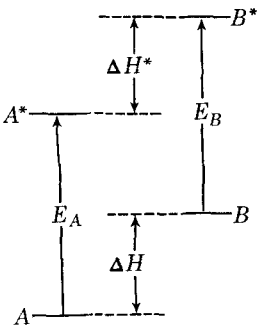
Abstract □ Dissociation constants of four isomeric aminoquinolines were determined spectrophotometrically and are compared with the same constants determined potentiometrically. The shifts in electronic absorption spectra of the compounds studied, upon protonation, are employed to assign the positions of protonation in the aminoquinolines. It was found that, in neutral and dilute acid solutions, a proton is added to the nitrogen atom in the heterocyclic rings in all compounds studied. Addition of a second proton, to the amino nitrogen, occurs only in strong acid solutions. 2-Aminoquinoline appears to exist in solution predominantly as an imino tautomer, while the other aminoquinolines studied exist predominantly in amino forms.

Keyphrases □ Aminoquinolines, isomeric—dissolution constants □ Dissociation constants—aminoquinolines □ Spectrophotometry, UV, visible—aminoquinolines, dissociation constants □ Potentiometry—aminoquinolines dissociation constants

The states of ionization of pharmacologically active substances and of biologically significant molecules are extremely important in determining the action of drugs. If these molecules have more than one group capable of dissociating or accepting protons in solutions, the sites of ionization may be equally important. Consequently, accurate knowledge of the sites of ionization corresponding to pKa values of drug molecules is vital to the study of chemical structure–biological activity relationships. Of several methods available for the determination of pKa values, potentiometry and spectrophotometry enjoy, by far, the greatest popularity. Potentiometry permits rapid and simple pKa determination but, in the case of polyacidic or polybasic molecules, does not permit simple evaluation of the site of dissociation or protonation unless the functional groups have widely differing characteristic pKa values in monofunctional molecules. In the latter case, the site of protonation can usually be inferred from the pH of the buffer regions of the titration curve. Unequivocal assignment of protonation sites, potentiometrically, can only be made by making derivatives of the molecule of interest in which all acidic or basic sites but one are blocked so that the ionizations can be evaluated one at a time. This is a time-consuming and frequently frustrating experience.

Conventional spectrophotometric pKa determination generally suffers from the same problems as potentiometry in assigning sites of protonation. This is due to the fact that, in spectrophotometry, changes in band intensities are used to evaluate base–acid ratios which, in conjunction with pH measurements, yield pKa values (1). Little attention, however, is paid to the shifts in the positions of the band maxima which occur upon protonation.

Shifts in the long wavelengths band maxima of acidic or basic molecules, upon protonation or dissociation, can be related to the difference between the dissociation constant of the molecule in the ground electronic state (pKa) and in the lowest excited singlet state (pKa*), the latter state being the one to which the transition represented by the long wavelength absorption band has occurred. The approximate relationship between excited state dissociation constants and spectral band shifts was developed by Förster (2) on the basis of a thermodynamic cycle and will be discussed briefly here (Scheme I).



Scheme I—The Förster cycle

There are two alternative pathways to reach the excited state of a base starting with the ground state of the conjugate acid. The first pathway is dissociation of the ground state acid (A) to the ground state conjugate base (B). This process is attended by an energy change, ΔH, the enthalpy of dissociation in the ground state. B then absorbs light energy E_B to arrive in the excited state B^*. The total energy absorbed in the process is thus $\Delta H + E_B$. Alternatively, A can first absorb light of energy E_A to arrive in its excited state A^*, followed by dissociation in the excited state with enthalpy change ΔH^*. The total energy absorbed in this process is $\Delta H^* + E_A$. Since this represents a closed thermodynamic cycle, Eq. 1 results:

$$\Delta H + E_B = \Delta H^* + E_A \qquad \text{(Eq. 1)}$$

Now, E_A and E_B are related to the wavelengths of absorption by $E_A = Nh(c/\lambda_A)$ and $E_B = Nh(c/\lambda_B)$, where N is the Avogadro number, h is Planck's constant, c is the speed of light, and λ_A and λ_B are, respectively, the wavelengths of absorption of A and B. If it is assumed that the vibrational structures of A, A^*, B, and B^* are similar, λ_A and λ_B may be taken at the maxima of the acid and conjugate-base long wavelength absorption bands, respectively. By substituting these relationships into Eq. 1 and rearranging,

$$\Delta H - \Delta H^* = Nhc\left(\frac{1}{\lambda_A} - \frac{1}{\lambda_B}\right) \qquad \text{(Eq. 2)}$$

From the second law of thermodynamics,

$$\Delta H = \Delta G + T\Delta S = 2.3\,RT\,\text{pKa} + T\Delta S \qquad \text{(Eq. 3)}$$

in which ΔG and ΔS are, respectively, the Gibbs free energy and entropy of dissociation in the ground state, and T is the absolute temperature, and

$$\Delta H^* = \Delta G^* + T\Delta S^* = 2.3\,RT\,\text{pKa}^* + T\Delta S^* \qquad \text{(Eq. 4)}$$

where ΔG^*, ΔS^*, and pKa* are, respectively, the Gibbs free energy, the entropy of dissociation, and the equilibrium constant for the corresponding dissociation in the excited state.

Substitution of Eqs. 3 and 4 into 2 yields:

$$\text{pKa} - \text{pKa}^* + \frac{T(\Delta S - \Delta S^*)}{2.3\,RT} = \frac{Nhc}{2.3\,RT}\left(\frac{1}{\lambda_A} - \frac{1}{\lambda_B}\right) \qquad \text{(Eq. 5)}$$

If it is now assumed that the entropies of protonation in ground and excited states are equal (a reasonable assumption, since the entropies of protonation in the respective electronic states are predominantly configurational and result in very similar energy differences),

$$\text{pKa} - \text{pKa}^* = \frac{0.625}{T}\left(\frac{1}{\lambda_A} - \frac{1}{\lambda_B}\right) \qquad \text{(Eq. 6)}$$

or, at 25°,

$$\text{pKa} - \text{pKa}^* = 2.10 \times 10^{-3}\left(\frac{1}{\lambda_A} - \frac{1}{\lambda_B}\right) \qquad \text{(Eq. 7)}$$

Equation 7 shows that by knowing the ground state pKa value for a particular dissociation equilibrium and the long wavelength absorption maxima of the acid and conjugate base, the lowest excited singlet state pKa* value for the corresponding equilibrium can be calculated. Since the process of electronic absorption is always accompanied by a change in electric dipole moment of the absorbing species, pKa* is almost always different from pKa. In general, it has been found that aromatic molecules containing —OH, —NH_2, or —SH groups become more acidic in the lowest excited singlet state (3); *i.e.*, pK* < pK. On the other hand, aromatic molecules containing —C(=O)—OH, —C(=O)—R, —(=)N (heteroaromatic nitrogen) groups become more basic in the excited state (pK* > pK). Now, if pKa* < pKa, then $1/\lambda_a > 1/\lambda_b$ and $\lambda_a < \lambda_b$. In other words, protonation of —OH, —NH_2, or —SH to form —OH_2^+, —NH_3^+, or —SH_2^+ always shifts the long wavelength absorption band to shorter wavelengths. Dissociation of the former groups to —O^-, —NH^-, or —S^- shifts the long wavelength absorption maxima to longer wavelengths (this is the reason for the yellow color of phenols in basic solutions while they are colorless in acidic solutions). Similarly, if pKa* > pKa, then $1/\lambda_a < 1/\lambda_b$ and $\lambda_a > \lambda_b$; so protonating —C(=O)—OH, —C(=O)—R, and —(=)N to form —C(=OH^+)—OH, —C(=OH^+)—R, and —(=)N^+—H shifts the long wavelength absorption band to longer wavelengths, while dissociation to yield —C(=O)—O^- shifts the long wavelength band to shorter wavelengths.

In molecules containing two functional groups, where both groups are of the same type (both with pKa* < pKa or both with pKa* > pKa), there has been insufficient study, to date, to determine the overall effect of one group upon the other. However, if one

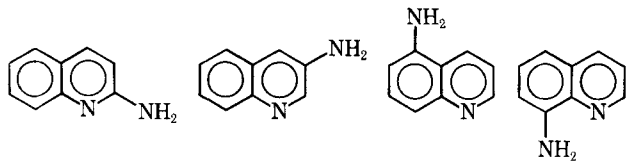
Table I—Long Wavelength Absorption Maxima of the Aminoquinolines

Aminoquinoline	$H_0 = -7.0$ λ, nm.	$H_0 = -7.0$ ε	pH = 1.93 λ, nm.	pH = 1.93 ε	pH = 12.00 λ, nm.	pH = 12.00 ε
2-Aminoquinoline	327	12,500	327	6760	328	5100
3-Aminoquinoline	317	10,600	372	4200	340	4000
5-Aminoquinoline	313	15,500	415	2680	333	3900
8-Aminoquinoline	312	13,900	382	2000	332	4100

Table II—pKa Values of the Aminoquinolines

Aminoquinoline	pKa_1	pKa_2
2-Aminoquinoline	—	7.27 ± 0.08
3-Aminoquinoline	−0.57 ± 0.12	4.96 ± 0.09
5-Aminoquinoline	+0.49 ± 0.08	5.63 ± 0.08
8-Aminoquinoline	−0.52 ± 0.09	3.92 ± 0.04

group has pKa* > pKa and the other has pKa* < pKa, it is almost always found that the values of pKa − pKa* for either group is greater, in absolute magnitude, than for either group alone in a monofunctional molecule. In the present study, the pKa values of four isomeric aminoquinolines, the 2-, 3-, 5-, and 8-isomers (the 8-isomer is a precursor of pamaquine), were determined spectrophotometrically. This is an excellent group of molecules with which to illustrate the foregoing discussion. These compounds each contain an aromatic amino group and a heterocyclic nitrogen atom. Both of these basic functions have similar pKa values in monofunctional molecules (*e.g.*, for pyridine pKa = 5.1 and for aniline pKa = 4.6), although, occurring in the same molecule, the electron-withdrawing effect of the heterocyclic nitrogen should tend to decrease pKa for the amino group while the electron-releasing effect of the amino group should tend to increase pKa for the ring nitrogen slightly (neglecting resonance effects due to positional relationships). As a solution containing any of the aminoquinolines is titrated with acid, it might be expected that the ring nitrogen would be protonated first but that some molecules would be singly protonated on the amino group before all of the ring nitrogens were protonated. Addition of a second proton to all of the molecules should occur at higher acidities, perhaps even in such strongly acidic solutions that potentiometric titration with a glass electrode would be impossible. The foregoing arguments are used here to test this hypothesis.



2-amino-quinoline | 3-amino-quinoline | 5-amino-quinoline | 8-amino-quinoline

EXPERIMENTAL

Spectrophotometric titrations were carried out on 1.00×10^{-4} *M* solutions of the aminoquinolines in buffered solutions with a Cary 15 recording spectrophotometer, using 1-cm. silica cells. Buffer solutions in the acidic region [pH 3 to Hammett acidity − 7.0 (4)] were made from analytical reagent grade perchloric acid[1] diluted with water and standardized against sodium carbonate. In the basic region (pH 10–13), buffer solutions were aqueous sodium hydroxide solutions prepared by diluting the carbonate-free sodium hydroxide with water, purging with and storing under nitrogen, and standardizing against potassium acid phthalate. Buffer solutions in the neutral region (pH 4.01–9.00) were prepared from Beckman standard buffers. 2-Aminoquinoline, 3-aminoquinoline, 5-aminoquinoline, and 8-aminoquinoline[2] were recrystallized from ethanol–water solutions.

Values for pKa were calculated from the relationship:

$$pKa = pH - \log \frac{\epsilon_A - \epsilon}{\epsilon - \epsilon_B} \qquad \text{(Eq. 8)}$$

where ϵ_A and ϵ_B are the molar absorptivities of acid and conjugate-base, respectively, at the wavelength chosen for measurement, and ε is the ratio of absorbance to concentration (*i.e.*, $1.00 \times 10^4 \times$ absorbance) at this wavelength.

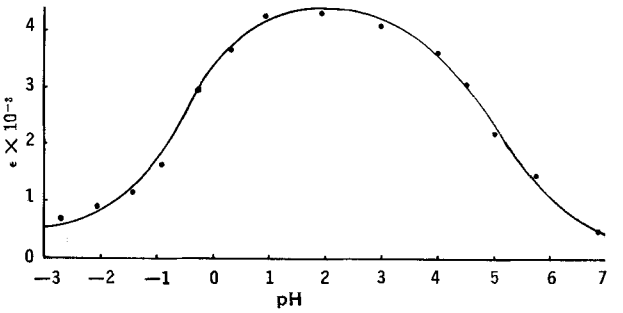


Figure 1—*Variation of the absorptivity (ε) of the 372-nm. absorption band of 3-aminoquinoline with pH. (The pH scale below pH 1.0 is represented by the Hammett acidity scale, H_0.)*

RESULTS AND DISCUSSION

The wavelength absorption maxima of the aminoquinolines in 11.7 *M* $HClO_4$ ($H_0 = -7.0$), 1.17×10^{-2} *M* $HClO_4$ (pH = 1.93), and 1.00×10^{-2} *M* NaOH (pH = 12.00) are listed in Table I. The variation of ε at 372 nm. with pH for 3-aminoquinoline is typical of the titration curves obtained in these studies and is shown in Fig. 1.

Since the absorptions in strong acid and basic solutions should correspond to the doubly protonated and neutral species, respectively, the absorptions in dilute acid solutions must correspond to

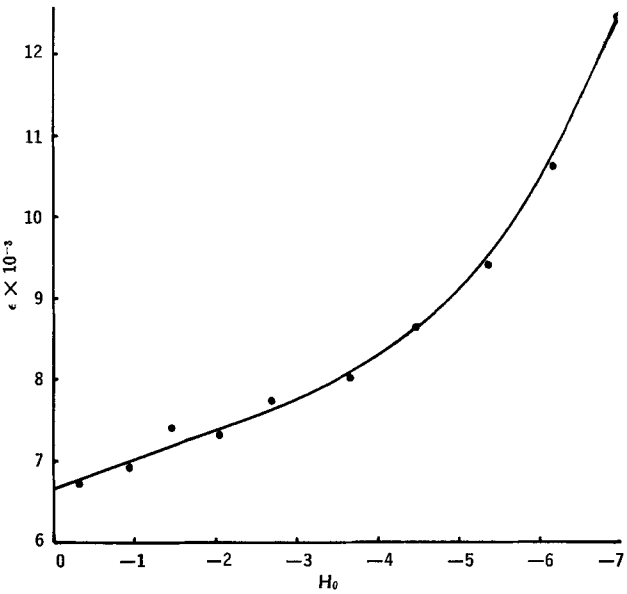


Figure 2—*Variation of the absorptivity (ε) of the 327-nm. absorption band of 2-aminoquinoline with Hammett acidity, H_0.*

[1] Mallinckrodt Chemical Works, Inc., St. Louis, Mo.

[2] K and K Chemicals, Inc., Plainview, N. Y.

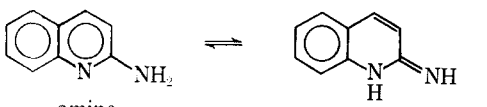
Table III—Values of ΔpKa (pKa − pK*) and pKa* Corresponding to the Spectral Data of Table I and the Titration Data of Table II in Conjunction with Eq. 7

Amino-quinoline	ΔpKa_1	pKa_1	pKa_1^*	ΔpKa_2	pKa_2	pKa_2^*
2-Amino-quinoline	—	—	—	+0.4	7.27	6.9
3-Amino-quinoline	+9.9	−0.57	−10.5	−5.3	4.96	10.3
5-Amino-quinoline	+16.6	+0.49	−16.1	−12.4	5.63	18.0
8-Amino-quinoline	+12.4	−0.52	−12.9	−8.2	3.92	12.1

the singly protonated species. Values for pKa for equilibria between the doubly protonated and singly protonated species (pKa_1) and between the singly protonated and neutral species (pKa_2) were calculated from Eq. 8, the data of Table I, and several solutions of the aminoquinolines at intermediate pH and H_0 values. These results are presented in Table II.

For 2-aminoquinoline, pKa_1 is not given in Table II because, in the most acidic solutions studied here ($H_0 = -7.0$), the titration curve for the formation of the doubly protonated species (or for the disappearance of the singly protonated species) was not completed. Furthermore, the absorbance of the doubly protonated species was increasing at such a rate that at $H_0 = -7.0$, the inflection point of the titration curve had not been passed (Fig. 2). Hence, it may be inferred that for 2-aminoquinoline, $pKa \ll -7.0$. Another striking feature of the 2-aminoquinoline spectra was the similarity at all pH values (Table I). This may be interpreted in terms of a small difference between ground and excited state pKa values, as will be discussed.

In a potentiometric study of the isomeric aminoquinoline, Albert and Goldacre (5) determined the pKa_2 values of the compounds studied here. Their values for pKa_2 and the values in this study are in excellent agreement. In that study it was assumed, but not proven, that protonation occurred on the ring nitrogen. The anomalously high value of pKa_2 for 2-aminoquinoline was attributed to a possible tautomerism between the amino form and the imino (or carbamidine) form (Scheme II). The greater basicity of the ring nitrogen



amino tautomer ⇌ amino (carbamidine) tautomer

Scheme II

in carbamidines relative to aromatic amines or ring nitrogens would account for the anomalously low acidity of the 2-amino isomer. Although no supportive evidence was offered by Albert and Goldacre (5) for this tautomerism, a similar tautomerism is known, from IR studies, to occur for 2-hydroxyquinoline with its 2-quinolone tautomer (6).

In Table III are tabulated the values of ΔpKa (pKa − pKa*) and pKa*, calculated with the help of Eq. 7 and the data of Table II.

The pKa* values of Table III indicate that with the exception of the 2-isomer, for which pKa_1^* cannot be determined in perchloric acid, the aminoquinolines studied here have $pKa_1^* < pKa_1$ and $pKa_2^* > pKa_2$. Since in this type of compound the ring nitrogen becomes more basic in the excited state while the amino nitrogen becomes less basic (or more acidic) in the excited state, pKa_2^* and pKa_2 must correspond to protonation of the ring nitrogen while pKa_1^* and pKa_1 must correspond to protonation of the amino group. Hence, the first protonation of the aminoquinolines, which shifts the absorption spectrum to longer wavelengths, involves protonation of the ring nitrogen only. The second protonation occurs some 5 log units to the acid side of the pH scale and shifts the absorption spectrum to shorter wavelengths. Therefore, pKa_1 must correspond to protonation of the singly protonated species (*i.e.*, at the amino site). Consequently, this experiment supports the speculations of Albert and Goldacre (5) as to the site of the first protonation. It is interesting that the gain in basicity in the excited state of the ring nitrogen is approximately the same as the loss in basicity of the amino group. This implies that the excitation process is accompanied by charge transfer from the amino group to the ring nitrogen.

The excited state pKa* values calculated in this experiment should not be taken literally as thermodynamic dissociation constants. Ground state equilibrium constants are determined not for isolated molecules but for molecules solvated by water in accordance with the distributions of electronic charge in the molecules. The energy of solvation contributes to the chemical potentials of each of the species involved in the dissociation equilibria and, therefore, to the experimentally determined pKa values. Since the process of excitation to the lowest excited singlet state alters the charge distributions in the absorbing molecules, the ground state equilibrium solvent cage is not at equilibrium with respect to the excited species. The absorption of light takes about 10^{-15} sec., while the time required for the solvent to relax to the equilibrium excited state solvent cage configuration is about 10^{-12} sec. Hence, in calculating pKa* values from shifts in absorption spectra, the calculation is performed for excited species in the ground state solvent cage, and the equilibrium energies of the excited species are not truly represented. Nevertheless, the results of absorption shift measurements do give reliable indications of charge distribution changes upon excitation and are perfectly adequate for the scope of the present study. If true pKa* values are required, they are better evaluated from shifts in fluorescence spectra with changing pH or from studies of the variation of fluorescence quantum yields with pH (7). Since the radiative lifetimes of fluorescent molecules are of the order of 10^{-8} sec., thermodynamic properties of excited molecules calculated from fluorescence data will reflect the energies of excited molecules in an equilibrium excited state solvent cage configuration.

The slight increase in the acidity of 2-aminoquinoline with respect to the first protonation, in the excited state, can be explained by two possible mechanisms. First, if protonation in the excited state occurs at the amino group instead of at the ring nitrogen, a shift to shorter wavelengths of the 2-aminoquinoline absorption spectrum is to be expected. However, the shift observed here is anomalously small compared with protonation of the amino groups of the other aminoquinolines. Alternatively, if the 2-aminoquinoline–2-iminoquinoline tautomerism proposed by Albert and Goldacre (5) does occur, the heterocyclic ring nitrogen has some of the character of a secondary amine with respect to the homocyclic ring. Consequently, in this case, protonation of the ring nitrogen might be expected to shift the absorption spectrum to shorter wavelengths (*i.e.*, $pKa_2 > pKa_2^*$) or, at most, only very slightly to longer wavelengths. If an equilibrium mixture of the amino and imino tautomers was present, two spectra, corresponding to the neutral species, should be observed. Protonation of this mixture should shift one spectrum to shorter wavelengths (the imino form) and the other to longer wavelengths (the amino form). Since this is not observed, this possibility is excluded. The best explanation seems to be that 2-aminoquinoline exists entirely in the imino form. The value of pKa_1 of 2-aminoquinoline, which is indeterminate in this experiment, is apparently much more acidic than pKa_1 for any of the other aminoquinolines. This anomaly cannot be explained at present.

REFERENCES

(1) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, England, 1962, p. 73.

(2) T. Förster, *Z. Elektrochem.*, **54**, 42(1950).

(3) A. Weller, *Progr. React. Kinet.*, **1**, 187(1960).

(4) M. Paul and F. A. Long, *Chem. Rev.*, **57**, 1(1957).

(5) A. Albert and R. Goldacre, *Nature*, **153**, 467(1944).

(6) S. F. Mason, *J. Chem. Soc.*, **1958**, 4874.

(7) S. G. Schulman, P. T. Tidwell, J. J. Cetorelli, and J. D. Winefordner, to be published.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 5, 1970, from the *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32601*

Accepted for publication September 24, 1970.