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SUBSTITUTED ACRIDINES AS POTENTIAL TERDENTATE LIGANDS—I

SYNTHESIS AND PROTONATION CONSTANTS

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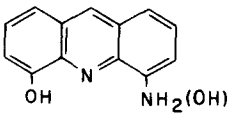
Abstract—Some potentially terdentate acridine ligands have been synthesized and their protonation constants determined in 50 per cent v/v dioxane–water by potentiometric and spectrophotometric methods. The order of protonation of the donor sites has been determined and the effect of steric hindrance on protonation behaviour assessed.

INTRODUCTION

THE PROTONATION constants of polyfunctional chelating ligands are of interest not only in applied problems in analytical chemistry but also in studies concerned with the fundamentals of metal chelate formation. In the present paper, the protonation constants and the order of protonation of basic sites of the new potentially terdentate ligands, 4-amino-5-hydroxyacridine and 4,5-dihydroxyacridine, have been determined. For purposes of comparison, 4,5-diaminoacridine, 4-amino-5-methoxyacridine, and 4-hydroxyacridine were also studied. In the



following paper, the effect of the structural constraints of these ligands on metal chelate formation is described.

Several experimental methods exist for determining the order of protonation of basic sites in a polybasic compound. The more important of these have been summarized by Sudmeier and Reilley[1], who demonstrated the use of proton NMR to elucidate the protonation of polyamine and aminocarboxylate ligands.

In the present work, the protonation order was determined from the electronic absorption spectra of the ligands. The protonation constants were determined by potentiometric and spectrophotometric methods; because of the low aqueous solubility of the ligands and their metal chelates, the solvent used was 50 per cent v/v dioxane–water.

EXPERIMENTAL

Preparative methods. The ligands were prepared by the following general synthetic route[2]: Ullman condensation of a substituted 2-halobenzoic acid and a substituted aniline to yield a diphenylamine-6-carboxylic acid, cyclization of the acid to an acridone, and reduction of the acridone to the acridine.

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1. J. L. Sudmeier and C. N. Reilley, *Analyt. Chem.* **36**, 1698 (1964).
2. R. M. Acheson, *The Acridines*. Interscience, New York (1956).

All microanalyses were by Alfred Bernhardt, Elbach über Engelskirchen, West Germany.

2-Nitro-2'-methoxydiphenylamine-6-carboxylic acid. 50 g of 2-bromo-3-nitrobenzoic acid[3], 90 ml of *o*-anisidine, 20 g of anhydrous sodium carbonate and 0·5 g of copper powder were heated at 135°C for 2 hr. The mixture was extracted with benzene and the benzene-insoluble sodium salts were dissolved in water. Addition of 6 M HCl precipitated 2-nitro-2'-methoxydiphenylamine-6-carboxylic acid (I). Yield, 24 g (42 per cent). Orange-red crystals from 95 per cent ethanol, mp, 227–228°C.

4-Nitro-5-methoxyacridone(II). 24 g of (I) were dissolved in 150 ml of 98 per cent H_2SO_4. The solution was heated at 100°C for 15 min. and then poured into 1·5 l of water. The resulting suspension was stirred overnight (≈ 60°C).

4-Amino-5-methoxyacridone. 125 g of $SnCl_2$ and 300 ml of conc. HCl were added to (II). The mixture was refluxed for 4 hr, the solid was collected, washed with a few ml of conc. HCl, and dissolved in 1 l of hot 1M NaOH. The solution was filtered and then adjusted to about pH 10 with 6M HCl. The precipitated 4-amino-5-methoxyacridone (III) was filtered and dried in air. Yield, 15 g (74 per cent), based on (I).

4-Amino-5-methoxyacridine(IV). 15 g of (III) in 1·5 l of 50 per cent ethanol-water were reduced with 650 g of 4 per cent sodium amalgam at 70–80°C (3 hr) under CO_2. The mixture was filtered hot and the filtrate concentrated. The precipitated material (IV) was extracted with benzene; removal of solvent and recrystallization from benzene-petroleum ether gave yellow-orange plates. Yield, 3 g (22 per cent), mp, 169–170°C. Calculated for $C_{14}H_{12}N_2O$: C, 74·98; H, 5·39; N, 12·49. Found: C, 74·82; H, 5·55; N, 12·51.

4-Amino-5-hydroxacridine(V). 6 g of (IV) was dissolved in 100 ml of 48 per cent HBr and refluxed for 24 hr. The precipitated hydrobromide of (V) was filtered and dissolved in 0·1M NaOH. Neutralization precipitated (V). Yield, 4 g (72 per cent). Orange-brown needles from 50 per cent ethanol, m.p. 195–197°C. Calcd for $C_{13}H_{10}N_2O$: C, 74·27; H, 4·79; N, 13·33. Found: C, 73·76; H, 5·09; N, 13·08.

4,5-Dihydroxyacridine(VI). A solution of 2·5 g of (IV) in 25 ml of 12M HCl was sealed in a heavy-walled Pyrex tube and heated for 8 hr at 180°C. The contents of the tube were dissolved in water. On neutralization, (VI) precipitated. Light yellow needles from aqueous ethanol, m.p. 265–267°C. The compound could also be prepared from 4,5-diaminoacridine by heating with conc. HCl in a sealed tube at 180°C for 24 hr. Calcd for $C_{13}H_9NO_2$: C, 73·92; H, 4·30; N, 6·63. Found: C, 73·81; H, 4·68; N, 6·82.

4,5-Diaminoacridine. This compound was prepared following the procedures of Goldberg and Kelly[4] and Klein and Lahey[5]. M.P. 178–179°C.

4-Hydroxyacridine. This compound was prepared by the above sequence of reactions, starting with 2-bromo-3-nitrobenzoic acid and aniline. M.P. 115–116°C.

In addition to elemental analysis, the new ligands were further characterized by infrared, NMR and mass spectrometry. The infrared spectra showed absorption bands in the regions characteristic of the functional groups. The proton NMR spectra were consistent with the structures of the ligands and will be reported in detail elsewhere. Mass spectra (recorded using an ionizing potential of 80 eV and an ionizing current of 50 μA) gave m/e ratios of the parent ions which agreed with the falculated molecular weights.

Other reagents. All common laboratory chemicals were either analysed grade or of sufficient purity for the purpose intended. Reagent-grade 1,4-dioxane was purified as described elsewhere[6].

Potentiometric determination of protonation constants. The glass electrode was calibrated as a hydrogen-ion concentration probe in 50 per cent v/v dioxane–water by titrating 0·01M perchloric acid solution (50 ml, 0·2M in $NaClO_4$) in dioxane (50 ml) with 0·1M carbonate-free sodium hydroxide solution. With each addition of base, an equal volume of dioxane was added. At each point on the titration curve, the hydrogen-ion concentration was calculated (assuming complete dissociation of $HClO_4$). In the pH range 2–3 (0–80 per cent neutralization) the correction factor C, where

$$pH + C = \log [H^+] \quad (1)$$

3. P. J. Culhane, In *Organic Synthesis* (Edited by F. C. Whitmore), Vol. VII, p. 12. Wiley, New York (1927).
4. A. A. Goldberg and W. Kelly, *J. chem. Soc.* 595 (1947).
5. E. R. Klein and F. N. Lahey, *J. chem. Soc.* 1418 (1947).
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was $-0{\cdot}08 \pm 0{\cdot}01$, in agreement with previous values[7, 8]. From the same titration, p_cK_w was obtained from data in the range 110–170 per cent neutralization. Each pH value was corrected for sodium-ion error ($\leq 0{\cdot}03$ pH units) and then converted to $-\log[H^+]$. From this value and the corresponding calculated value of $-\log[OH^-]$, the value of p_cK_w was $15{\cdot}33 \pm 0{\cdot}02$, compared to 15·38 reported earlier[7].

The titration procedure for the determination of protonation constants was essentially that described by Freiser *et al*[6]. The final titration solution was 0·1M in $NaClO_4$.

For a ligand H_kL of concentration C_L, the average number of bound protons per molecule is given by

$$\bar{n}_H = \frac{k \cdot C_L + [ClO_4^-] + [OH^-] - [Na^+] - [H^+]}{C_L}.$$

For ligands in which the protonation equilibria did not overlap, the protonation constants were calculated[9] using

$$\log K_n^H = -\log[H^+] + \log\left(\frac{\bar{n}_H - n + 1}{n - \bar{n}_H}\right). \quad (3)$$

Values of $\bar{n}_H$ in the ranges of 0·2–0·8, 1·2–1·8, 2·2–2·8 were used to calculate K_1^H, K_2^H and K_3^H respectively.

For the two phenol groups of 4,5-dihydroxyacridine, the equilibria overlapped. The two protonation constants were obtained from the slope and intercept of the least-squares fit of Equation[4],

$$\frac{\bar{n}_H}{(\bar{n}_H - 1)[H^+]} = \frac{(2 - \bar{n}_H)[H^+]}{(\bar{n}_H - 1)} \cdot K_1^H K_2^H - K_1^H. \quad (4)$$

Values of $\bar{n}_H$ in the ranges of 0·2–0·8 and 1·2–1·8 were used in the calculations. The protonation constants are given in Table 1.

Spectrophotometric determination of protonation constants. Protonation constants at or beyond the limits of the potentiometric method were determined spectrophotometrically, using a Hitachi Perkin-Elmer 139 spectrophotometer.

Recorded spectra (Cary Model 14 spectrophotometer) of all the compounds were obtained at various pH values chosen to yield solutions containing, if possible, only one of the protonated forms of the ligand. Spectra of 4-amino-5-hydroxyacridine and, for comparison, of 4-hydroxyacridine are

Table 1. Protonation constants of acridines in 50 per cent v/v dioxane–water, 25°C, $\mu = 0{\cdot}1$

Ligand	$\log K_1^H$	$\log K_2^H$	$\log K_3^H$
acridine	$4{\cdot}22 \pm 0{\cdot}01$*		
4-hydroxyacridine	$11{\cdot}27 \pm 0{\cdot}01$	$4{\cdot}24 \pm 0{\cdot}01$	
4-amino-5-hydroxyacridine	$11{\cdot}50 \pm 0{\cdot}01$	$2{\cdot}57 \pm 0{\cdot}01$	
		$2{\cdot}51 \pm 0{\cdot}01$[b]	c.a. $-0{\cdot}5$†
4-amino-5-methoxyacridine	$2{\cdot}89 \pm 0{\cdot}03$†	c.a. $-0{\cdot}5$[b]	
4,5-diaminoacridine	$3{\cdot}18 \pm 0{\cdot}03$	$1{\cdot}42 \pm 0{\cdot}05$[b]	‡
4,5-dihydroxyacridine	$12{\cdot}12 \pm 0{\cdot}01$	$10{\cdot}58 \pm 0{\cdot}01$	$2{\cdot}56 \pm 0{\cdot}01$
	$12{\cdot}03 \pm 0{\cdot}05$†		

*The limits shown are the standard deviation.
†Determined spectrophotometrically.
‡Extremely low.

7. S. Takamoto, Q. Fernando and H. Freiser, *Analyt. Chem.* **37**, 1249 (1965).
8. H. M. N. H. Irving and U. S. Mahnot, *J. inorg. nucl. Chem.* **30**, 1215 (1968).
9. H. B. Jonassen, R. B. Leblanc, A. W. Meibohm and R. M. Rogan, *J. Am. chem. Soc.* **72**, 2430 (1950).

shown in Figs. 1 and 2. Spectral data for the remaining compounds are summarized in Table 2. Solutions were prepared in 50 per cent v/v dioxane–water; the required pH values were maintained by acetate or phosphate buffers, or by addition of perchloric acid or sodium hydroxide solutions. The ionic strength of solutions above pH 1 was adjusted to 0·10 by the addition of sodium perchlorate.

Protonation constants were calculated using Equation (5),

$$\log K_n^H = -\log [H^+] + \log\left(\frac{A_{n-1} - A}{A - A_n}\right) \tag{5}$$

where A_{n-1} and A_n represent the absorbances of the pure species having n − 1 and n protons respectively, and A is the absorbance of a solution containing both $H_{n-1}L$ and H_nL.

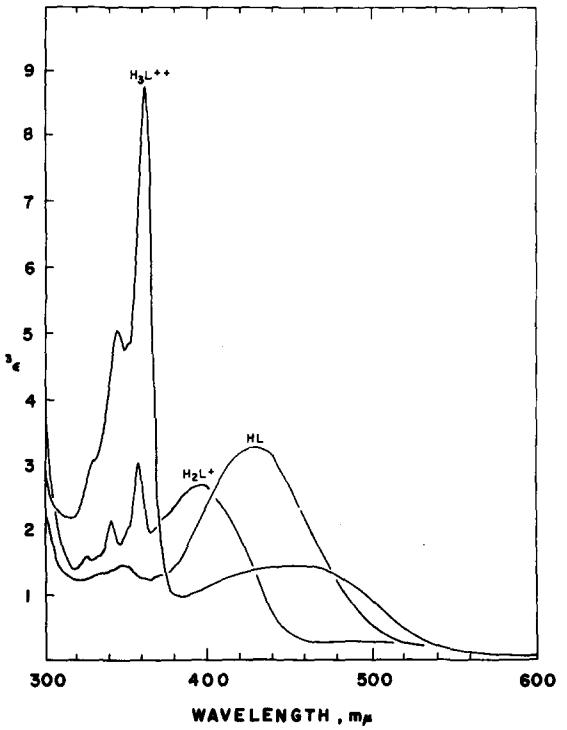

Fig. 1. Absorption spectra of 4-amino-5-hydroxyacridine. H_3L^{2+}, 4M $HClO_4$; H_2L^+, pH1; HL, pH7.

For 4,5-diaminoacridine, the pH ranges for the addition of the first and second protons overlapped; the absorbance of the monoprotonated species was obtained as follows. Equation (5) was rearranged to give

$$A\left(\frac{1 + K_1^H[H^+]}{K_1^H}\right) = A_{HL}[H^+] + \frac{A_L}{K_1^H}. \tag{6}$$

In the pH region in which only the species L and HL exist in appreciable concentrations, a plot of the left-hand side of Equation (6) (using the value of K_1^H obtained potentiometrically) vs. $[H^+]$ yielded a straight line with slope A_{HL}. K_2^H was then calculated from Equation (5) using data in the pH region in which only HL and H_2L exist in appreciable concentrations. Similarly, K_1^H for 4,5-dihydroxyacridine was obtained using the value of log K_2^H determined potentiometrically. Figure 3 shows the typical agreement between the calculated curve and the experimental points using the above procedure.

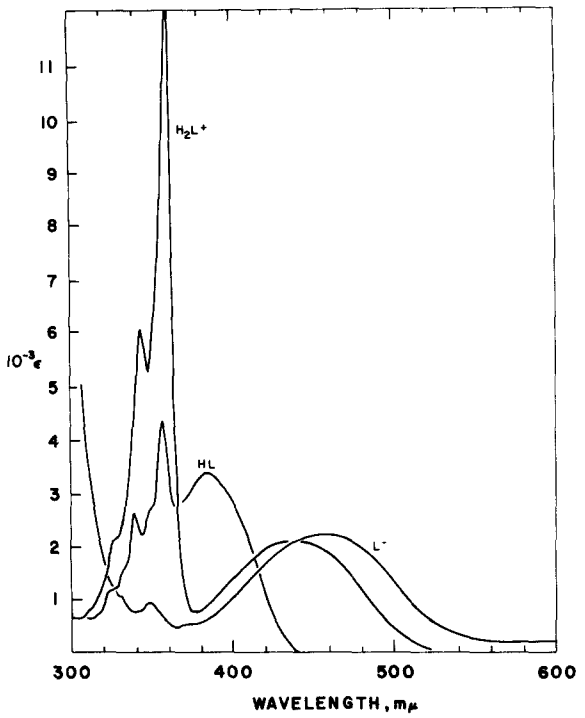

Fig. 2. Absorption spectra of 4-hydroxyacridine, H_2L^+, pH 2; HL, pH 7; L^-, pH 13.

Table 2. Spectral data for ligands, 50 v/v dioxane–water, 25°C

Ligand	Species	pH	Absorption maxima, mμ (log ε in parentheses)
4-amino-5-methoxyacridine	H_2L^{2+}	4M $HClO_4$	343(3·79), 360(4·09), 450(3·32)
	HL^+	2	339(3·40), 354(3·56), 386(3·48)
	L^-	7	424(3·52)
4,5-diaminoacridine	H_2L^{2+}	4M $HClO_4$	337(3·77), 353(3·95)
	HL^+	2	278(4·52), 434(3·36)
	L^-	7	275(4·84), 441(3·60)
4,5-dihydroxyacridine	H_3L^+	1	267(4·73), 279(2·92), 365(3·53), 469(3·23)
	H_2L	7	266(5·07), 400(3·60), 418(sh)
	HL^-	11	282(4·74), 450(3·45)
	$L=$	14	280(4·83), 455(3·62)

RESULTS AND DISCUSSION

For a polybasic ligand, the order of protonation of the donor atoms is of interest, although sometimes difficult to determine.

The order of protonation of aromatic bases containing primary amino groups has been elucidated from electronic absorption spectra. Although primary aromatic amines absorb at longer wavelengths than the parent hydrocarbons because of

conjugation of the amino group with the ring, protonation of the amino group prevents conjugation and gives rise to a spectrum characteristic of the parent hydrocarbon. Thus the spectrum of protonated aniline is almost identical to that of benzene[10]. Using this approach, Craig[11] showed that the primary amino groups of 4,5-diaminoacridine were protonated before the aromatic nitrogen, since the spectrum of the doubly protonated species (obtained in 5M hydrochloric acid) was almost identical to that of neutral acridine. The spectrum characteristic of the protonated aromatic nitrogen appeared in 9M sulfuric acid and was nearly

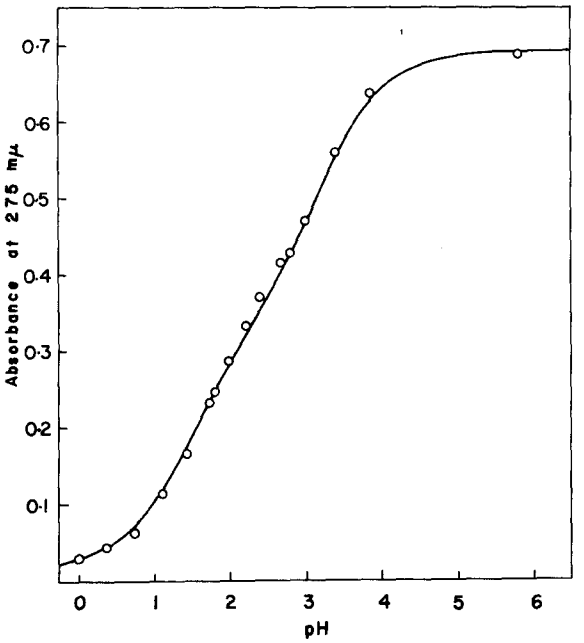

Fig. 3. Absorbance vs. pH for 4,5-diaminoacridine. The circles are experimental points; the solid line is calculated from the ligand concentration and appropriate values of ϵ and K_J^H.

identical to that of the acridinium ion. This behaviour is in contrast to the protonation of the mono-aminoacridines, which were shown by the same technique to protonate first on the aromatic nitrogen[12, 13]. The reversal in protonation order in 4,5-diaminoacridine is due to steric hindrance to solvation of the protonated ring nitrogen. A similar decrease in basicity due to steric hindrance has been observed for 4,5-dimethylacridine[14] (log $K^H = 2{\cdot}88$ compared to log $K^H = 4{\cdot}11$ for acridine) and for 2,6-di-t-butylpyridine[15].

10. C. L. Harberts, P. M. Heertjes, L. J. N. Van der Hulst and H. I. Waterman, *Bull Soc. chim. Fr.* **3**, 643 (1936).
11. D. P. Craig, *J. chem. Soc.* 534 (1946).
12. N. H. Turnbull, *J. chem. Soc.* 441 (1945).
13. D. P. Craig and L. N. Short, *J. chem. Soc.* 419 (1945).
14. A. Albert and R. Goldacre, *J. chem. Soc.* 706 (1946).
15. F. E. Condon, *J. Am. chem. Soc.* **87**, 4494 (1965).

In the present work, the protonation order of the acridine ligands was elucidated by comparison of their electronic absorption spectra at various pH values with the spectra of the model compound, 4-hydroxyacridine. The order of protonation of 4-hydroxyacridine is unambiguous. The spectra of 4-amino-5-hydroxyacridine and 4-hydroxyacridine are compared in Figs. 1 and 2, where the similarities are readily apparent. The spectrum of doubly protonated 4-amino-5-hydroxyacridine (H_2L^+) is almost identical to the spectrum of neutral 4-hydroxyacridine (HL). Similarly, the spectrum of the triply protonated species (H_3L^{2+}) is almost identical to the spectrum of the doubly protonated 4-hydroxyacridine (H_2L^+). These results show that the primary amino group is protonated before the ring nitrogen.

The protonation constants of the acridine ligands (Table 1) can now be assigned. The second protonation constant of 4-amino-5-hydroxyacridine ($\log K_2^H = 2{\cdot}57$) and the first protonation constant of 4-amino-5-methoxyacridine ($\log K_1^H = 2{\cdot}89$) refer to protonation of the primary amino group, and the subsequent protonation constant ($\log K \doteq -0{\cdot}5$ in each case) refers to protonation of the aromatic nitrogen. The lowering of the basicity of the aromatic nitrogen to the point where the amino nitrogen is protonated preferentially is caused by steric hindrance. The basicity of the aromatic nitrogen is then further reduced by electrostatic repulsion between the positively charged centres.

The first and second protonation constants of 4,5-diaminoacridine correspond to protonation of primary amino groups, in agreement with Craig[11]. Electrostatic repulsion causes the decrease in basicity of the second amino group relative to the first, and repulsion from the two protonated amino groups results in the extreme decrease in basicity of the aromatic nitrogen.

In 4,5-dihydroxyacridine, the decrease in basicity of the aromatic nitrogen ($\log K_3^H = 2{\cdot}56$) is caused completely by steric hindrance. The separation between the protonation constants of the phenolate oxygens results from electrostatic repulsion (between the two negative charges of the di-anion), just as is encountered in the di-cation of 4,5-diaminoacridine. Since the charged centres are separated by approximately the same distance, the difference in protonation constants for 4,5-dihydroxyacridine (1·54) is similar to that for 4,5-diaminoacridine (1·76).

In the present work, no attempt has been made to interpret the spectra in terms of band assignments. Recently, an analysis of the spectra of mono-hydroxyacridines has been reported[16].

In summary, sterically hindered protonation of the ring nitrogen occurs in all the 4,5-disubstituted acridines studied, resulting in a significant decrease in the protonation constant. If the substituents are amino groups, they are protonated first. A further decrease in the protonation constant of the aromatic nitrogen then results because of electrostatic repulsion. In the presence of such powerful effects, the role of normal inductive and resonance effects of the substituents is obscured.

In the reaction of the 4,5-disubstituted acridines with metal ions, sterically hindered complexes can be anticipated.

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