587. The Polarographic Reduction of Some Aminoacridines.

By R. C. KAYE and H. I. STONEHILL.

In aqueous solution, 1-, 2-, 3-, 4-, and 5-amino- and 2 : 7- and 2 : 8-di-amino-acridines yield polarograms which are distorted by adsorption at the dropping-mercury electrode. The adsorption, confirmed by oscillo-graphic study of the current–time relationship, is less marked than for acridine, and, as with the latter, may be eliminated by adding ethanol. In aqueous-ethanolic solution, the aminoacridines, like acridine, are reduced in two one-electron steps, with the intermediate formation of a semiquinone of apparently great stability. Theoretical equations have been derived to describe the observed relation between half-wave potential and pH for each step. For the aminoacridines which are most active antibacterially (*viz.*, the 2-, 5-, 2 : 7-, and 2 : 8-substituted compounds), the first reduction step requires no hydrogen ions at the biologically important pH of 7; this is not so for the less active aminoacridines, or for acridine itself.

RECENT work (Kaye and Stonehill, *J.*, 1951, 27) on the polarographic reduction of acridine is now extended to a series of aminoacridines, with the aim of correlating electro-reduction characteristics with bacteriostatic activity.

EXPERIMENTAL.

1-, 2-, 3-, and 4-Aminoacridines were samples prepared by Albert and Ritchie (*J. Soc. Chem. Ind.*, 1941, **60**, 120) and kindly presented by Mr. C. L. Bird of Leeds University. Medicinally pure 5-, 2 : 7-, and 2 : 8-amino-substituted acridines were further purified by several recrystallisations from water.

Ethyl alcohol, buffers, apparatus, and experimental technique were as described previously (*J.*, 1951, 27).

RESULTS AND DISCUSSION.

(i) *Detection and Suppression of Adsorption.*—Figs. 1—2 show typical polarograms for all the compounds studied, the same buffer mixture being used as supporting electrolyte in both aqueous and 50% (by volume) aqueous-alcoholic solutions. For 2 : 8-diaminoacridine (Fig. 2, Curves IV) all the polarograms for aqueous solutions (concentration range, 1—8 × 10^{-4} M; pH range 0—14; 10% of alcohol included in solutions at pH >8 where the free base is almost water-insoluble) are anomalous, although less so than those of acridine. Two main reduction waves are identifiable, together with a small anomalous after-wave on the first step, and in some cases a smaller fore-wave on the second step. At the higher concentrations, and over the potential range corresponding to the after-wave on the first main step, abnormal galvanometer oscillations

were observed during polarography, similar to those found with acridine. On the inclusion of 50% of alcohol in the solvent, the anomalies disappeared. The anomalous after-wave on the first step may be attributed, on Brdička's theory (*Z. Elektrochem.*, 1942, **48**, 278), to adsorption of unreduced 2 : 8-diaminoacridine on the dropping-mercury electrode, which is suppressed by the addition of alcohol. Supporting evidence is provided by the oscillographic records (Fig. 3, *A—I*) of the current-time relationship during the first 2 seconds of the life of a single mercury drop. These oscillograms are lettered to correspond with Fig. 2 (Curve IV*a*). For potentials outside the range *A—F* (Fig. 3), normal oscillograms are obtained. Between the potentials of *A* and *F* the current, immediately after the fall of a mercury drop, first rises with abnormal rapidity; this is probably due to adsorption hastening the normally solely diffusion-controlled supply
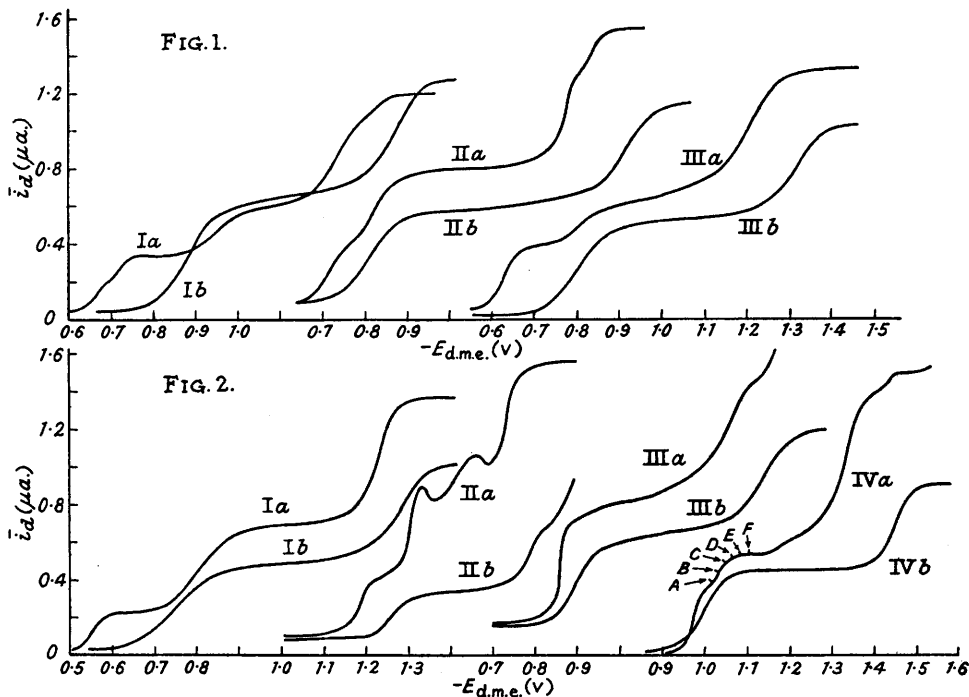

FIG. 1.

Polarograms in (a) *water and* (b) 50% *alcohol.*

I. 1-*Aminoacridine*, 4×10^{-4}M; (a) pH 7·38, (b) pH 8·29.
II. 2-*Aminoacridine*, 4×10^{-4}M; (a) pH 7·38, (b) pH 8·29.
III. 3-*Aminoacridine*, 4×10^{-4}M; (a) pH 7·38, (b) pH 8·29.

FIG. 2.

Polarograms in (a) *water and* (b) 50% *alcohol.*

I. 4-*Aminoacridine*, 4×10^{-4}M; (a) pH 7·38, (b) pH 8·29.
II. 5-*Aminoacridine*, 2×10^{-4}M; (a) pH 9·07, (b) pH 10·39.
III. 2 : 7-*Diaminoacridine*, 4×10^{-4}M; (a) pH 7·38, (b) pH 8·29.
IV. 2 : 8-*Diaminoacridine*, 2×10^{-4}M; (a) pH 7·38, (b) pH 8·29 (*current scale half that shown*).

of reducible material to the electrode surface. After reaching a maximum, the current then falls slightly, probably because adsorption has caused a temporary depletion of reducible; material in the solution near the dropping-mercury electrode. This is eventually made good by diffusion from the bulk of the solution, whereupon the current finally rises slowly. Oscillograms *C*, *G*, *H*, and *I* (Fig. 3) all refer to a fixed electrode potential ($-1·054$ v against the saturated calomel electrode), and illustrate the effects of the addition of increasing amounts of alcohol to the supporting electrolyte (current amplification is lower for *C* than for *G*, *H*, or *I*). Clearly 20% of alcohol does not prevent adsorption, 50% does not quite, and 60% does almost completely eliminate it. Since the polarogram with 50% alcohol shows no adsorption anomalies, it is evident that slight adsorption is undetectable polarographically,

8 H

and that the oscillogram provides the more reliable evidence of adsorption. The adsorption of 2 : 8-diaminoacridine on the dropping-mercury electrode is weaker than that of acridine, as shown by the less pronounced polarographic anomalies and the complete suppression of adsorption by a lower alcohol concentration. Apart from the complication due to the adsorption's being of semiquinone and not of unreduced material in the case of acridine, this is to be expected when the molecule is made more hydrophilic by the introduction of two amino-groups, and accords with the observation (Albert, Goldacre, and Heyman, *J.*, 1943, 651) that acridine and 2 : 8-diaminoacridine are respectively the most and the least hydrophobic and surface-active of a series of acridines.

2 : 7-Diaminoacridine also gives a small anomalous after-wave on the first main reduction step at all pH values in aqueous solutions; inclusion of 50% of alcohol removes the anomalies (Fig. 2, Curves III). Brdička's theory (*loc. cit.*) and the current–time oscillogram (Fig. 3, *J*) suggest that, as with the 2 : 8-isomer, principally the unreduced compound is adsorbed.

1-, 2-, 3-, and 4-aminoacridine were studied, in aqueous solution, only at pH 7·38, because of the scarcity of materials. In all cases a pronounced anomalous fore-wave occurred on the first reduction step, and for the 1- and 2-isomers, an after-wave on the second step (Figs. 1—2). The fore-wave for 1-aminoacridine was a double wave resembling that obtained with acridine in acid solutions. For all four compounds, inclusion of 50% of alcohol eliminated the anomalies. On Brdička's theory, these fore- and after-waves suggest that chiefly semiquinone is adsorbed, as with acridine (it is shown later that both reduction steps are one-electron steps). The current–time oscillogram (Fig. 3, *K*) obtained during the reduction of 4-aminoacridine supports this view, since it shows that, after detachment of the mercury drop, the current stays at the minimum value for about 0·1 second before starting to grow; this is presumably due to blocking of the mercury surface by some adsorbate which is not reducible at the particular electrode potential applied, unlike the unreduced compound, which, as we have seen in the case of 2 : 8-diaminoacridine, would upon adsorption cause a sharp increase in current.

Breyer, Buchanan, and Duewell (*J.*, 1944, 360) sought to explain the apparent 3-stage polarographic reduction of acridine and of 4-aminoacridine in aqueous solution by assuming compound formation between the unreduced material and the semiquinone product of the first reduction step. The foregoing evidence shows that for 4-aminoacridine, as in the case of acridine (*J.*, 1950, 27), this interpretation is incorrect, and that two, not three, reduction steps occur, complicated by adsorption. The lack of proportionality between step height and concentration reported by these workers is due to adsorption complications, and we find that it is eliminated for the aminoacridines, as with acridine, on the inclusion of 50% of alcohol.

For aqueous solutions of 5-aminoacridine the polarograms obtained over the pH range 0—14 were considerably distorted by maxima, in addition to an anomalous fore-wave on the first step. Inclusion of 50% of alcohol suppressed the fore-wave but only partly removed the maxima; practically complete suppression of the latter was achieved by adding 0·1% methyl cellulose to the 50% alcoholic solution, and restricting the concentration of 5-aminoacridine to 2×10^{-4}M.

(ii) *Semiquinone Formation.*—Breyer, Buchanan, and Duewell (*loc. cit.*) applied the Ilkovič equation (*Coll. Czech. Chem. Comm.*, 1934, **6**, 498) $\bar{i}_d = 605 \ nD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}c$ to show that acridine and several of its amino-derivatives were reduced at the dropping-mercury electrode in two one-electron stages (i_d = mean diffusion current in amperes, n = number of electrons

TABLE I.

Aminoacridine.	c (milli-mol./l.)	First step		Second step		$-E_1{}^{b,c}$	$-E_2{}^{b,c}$	$E_i{}^{b,c}$	$\log \sqrt{\bar{K}}{}^b$
		$\bar{i}_d$ (μa.)	n	$\bar{i}_d$ (μa.)	n				
1-	0·4	0·53	1·09	0·54	1·13	0·748	1·350	0·301	5·10
2-	0·4	0·49	1·00	0·45	0·94	0·760	1·328	0·334	5·65
3-	0·4	0·48	0·98	0·42	0·88	1·724	1·230	0·253	4·29
4-	{ 0·2	0·22	0·90	0·25	1·04 ·	—	—	—	—
	{ 0·4	0·43	0·88	0·48	1·00	0·650	1·220	0·285	4·85
5- *a*	0·2	0·24	0·99	0·28	1·17	1·210	1·607	0·198	3·36
Diaminoacridine.									
2 : 7-	{ 0·2	0·24	0·98	0·25	1·04	—	—	—	—
	{ 0·4	0·46	0·94	0·48	1·00	0·840	1·252	0·208	3·52
2 : 8-	{ 0·2	0·22	0·90	0·22	0·92	—	—	—	—
	{ 0·4	0·44	0·90	0·46	0·96	1·007	1·362	0·177	3·00

a At pH 10·39 (in 50% alcohol). *b* At pH 7·0 (in 50% alcohol).
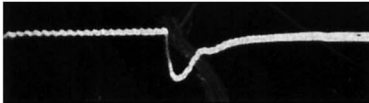*c* In volts against the Saturated Calomel Electrode.

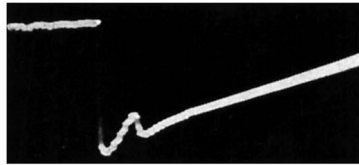Fig. 3.
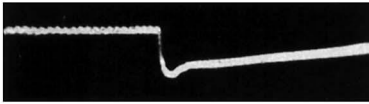
(A) — 1·022 mv

(B) — 1·037 mv

(C) — 1·054 mv

(D) — 1·068 mv
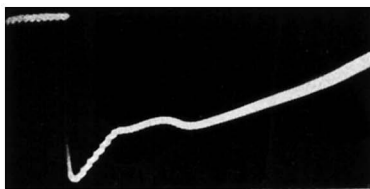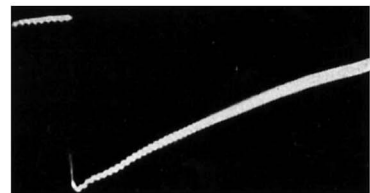
(E) — 1·080 mv

(F) — 1·107 mv

(G) — 1·054 mv
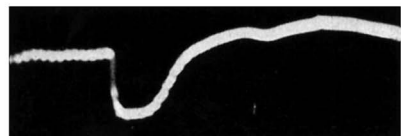
(H) — 1·050 mv

(I) — 1·054 mv

(J)

(K)

involved per molecule, D = diffusion coefficient of the reducible molecule in cm.^{2}sec.,$^{-1}$, m = mercury flow rate in mg./sec., t = drop time in sec., and c = concentration in millimol./l.). Since adsorption at the dropping-mercury electrode in aqueous solutions might invalidate the Ilkovič equation, which is derived on the assumption that diffusion alone controls the supply of electro-active material to the electrode, we have recalculated n, Ilkovič's equation being
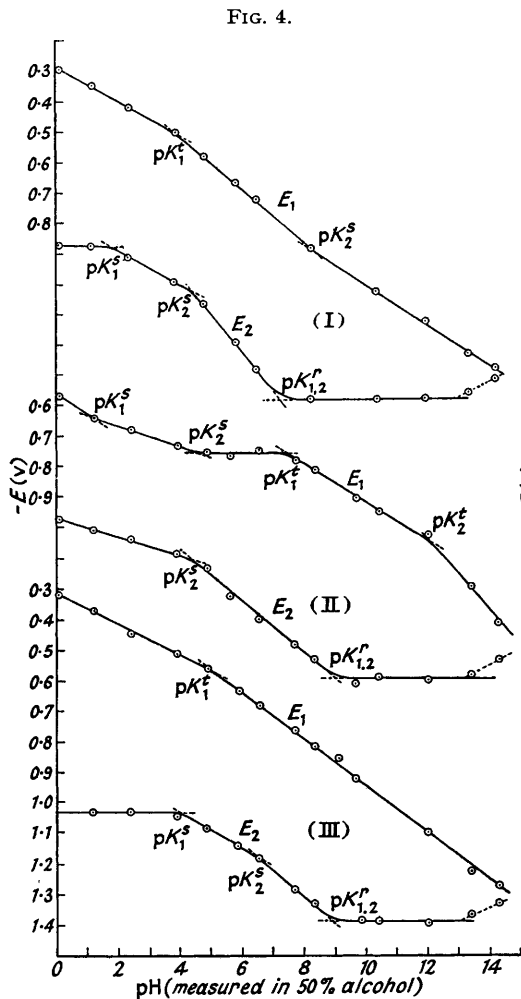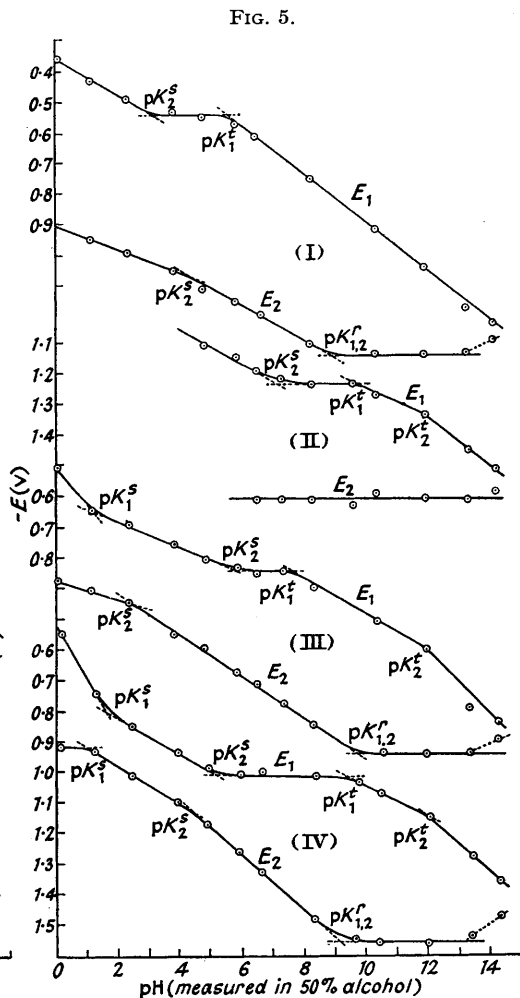

FIG. 4.

FIG. 5.

FIG. 4.

Variation with pH *of the half-wave potentials*, E$_1$ *and* E$_2$, *in 50% alcohol.*

I. 1-*Aminoacridine*, 4 × 10^{-4}M. II. 2-*Aminoacridine*, 4 × 10^{-4}M.
III. 3-*Aminoacridine*, 4 × 10^{-4}M.

FIG. 5.

Variation with pH *of the half-wave potentials*, E *and* E$_2$, *in 50% alcohol.*

I. 4-*Aminoacridine*, 4 × 10^{-4}M. II. 5-*Aminoacridine*, 2 × 10^{-4}M.
III. 2 : 7-*Diaminoacridine*, 4 × 10^{-4}M. IV. 2 : 8-*Diaminoacridine*, 4 × 10^{-4}M.

used, for 50% alcoholic solutions of the aminoacridines, in which adsorption is insufficient to distort polarograms. The values used for m and D (taken to be the same as for acridine) were as reported previously (*J.*, 1950, 27) ; t values appropriate to each half-wave potential were employed. The resulting n values, given in Table I, refer to the single pH of 8·29 (measured in 50% alcohol), but they apply over the pH range 0—14 since wave-heights were practically independent of pH within experimental error, except for 5-aminoacridine, for which incipient

maxima caused significant pH-variation of wave-height, and an overlapping hydrogen wave at pH <4·8 rendered the recording of the two waves impossible. Table I shows that the amino-acridines, like the parent compound, are reduced in two one-electron stages, with the intermediate formation of a semiquinone radical. The half-wave potentials E_1 and E_2 of the first and second steps, respectively, for pH 7·0 in 50% alcohol, the index potential, which for well-separated waves is given by $E_i = \frac{1}{2}(E_1 - E_2)$, and the value of log K (K = apparent semiquinone formation constant) are also given in Table I. Values of K were calculated from the equation (Michaelis, *Ann. N.Y. Acad. Sci.*, 1940, **40**, 39) $\sqrt{K} = 10^{E_i/0.059} - 3 \times 10^{-E_i/0.059}$, which applies only to reversible systems, whereas logarithmic analysis (Kolthoff and Lingane, "Polarography," Interscience Publishers Inc., New York, 1946, p. 154) shows that all the polarograms refer to irreversible processes. Nevertheless, the large K values suggest that the semiquinones are highly stable, and, as in the case of acridine, we shall regard K as a measure of apparent stability.

(iii) *Relationships between Half-wave Potentials and* pH.—In view of the variation with pH of the biological properties of the aminoacridines (Albert *et al.*, *Brit. J. Exp. Path.*, 1945, **26**, 160), we have determined E_1 and E_2 in 50% aqueous-alcoholic solutions at several pH values over the range 0—14 (Figs. 4—5). The similar results obtained by Breyer, Buchanan, and Duewell (*loc. cit.*) for aqueous solutions are less complete and are complicated by adsorption effects. In interpreting our results, we may note the generalisation made by Clark (*Chem. Reviews*, 1925, **2**, 127) that, as pH increases, the graph of normal redox potential against pH exhibits a fairly sharp bend corresponding to algebraic decrease (increase) of slope at a pH equal to the pK of the oxidised (reduced) form of the system concerned. This is confirmed by the equations developed below on the assumption that E_1 and E_2 may be treated as normal redox potentials.

TABLE II.

From E_1–pH curve.

Compound	pK_1^s	pK_2^s	pK_1^t	pK_2^t	$-E_1^{\circ\prime}$ (v)	Slope in mv for pH			
						$<pK_1^t$	pK_1^t—pK_2^s	pK_1^t—pK_2^t	$>pK_2^t$
1-Aminoacridine ...	—	8·2	3·8	—	0·295	57ᵃ	—	87ᶜ	67ᵈ
2- ,, ...	1·2	4·6	7·4	12	0·570	65	35	60	118
3- ,, ...	—	—	5·1	—	0·320	52ᵃ	—	78ᵇ	—
4- ,, ...	—	3·2	5·6	—	0·360	—	59	80ᵇ	—
5- ,, ...	—	7·4	9·6	12	—	—	58	44	80
2 : 7-Diaminoacridine	1·2	5·8	7·3	12	0·500	120	43	58	103
2 : 8- ,,	1·4	5·0	9·3	12	0·520	180	60	50	88

ᵃ For pH < pK_1^t.   ᵇ for pH > pK_1^t.   ᶜ For pH between pK_1^t and pK_2^s.   ᵈ For pH > pK_2^s.

From E_2–pH curve.

Compound	pK_1^s	pK_2^s	$pK_{1',2}$	Slope in mv for pH		$-E_2^{\circ\prime}$ (v)	Electrometric pK_1^t (at 20°)
				pK_1^s—pK_2^s	pK_2^s—$pK_{1',2}$		
1-Aminoacridine ...	1·7	4·5	7·3	57	127	0·887	3·59
2- ,, ...	—	4·4	9·0	30	83	0·970	7·61
3- ,, ...	4·0	6·3	8·9	59	86	1·036	5·03
4- ,, ...	—	4·7	8·9	38	60	0·904	5·50
5- ,, ...	—	—	—	—	—	—	9·45
2 : 7-Diaminoacridine	—	2·5	9·8	35	67	0·870	7·74
2 : 8- ,,	1·0	4·5	9·1	60	85	0·920	9·50

The general form of the E_1–pH curve is similar for the 2-, 4-, and 5-amino-, and 2 : 7- and 2 : 8-diamino-acridines (in the case of 5-aminoacridine, results for pH below 4·8 could not be obtained, as already explained). In general, four bends occur, two of them causing an algebraic increase of slope, and thus related to pK values of the semiquinone, and two in the opposite sense and thus related to pK values of the unreduced substance. Of the latter pair, one occurs at a lower pH value, recorded as pK_1^t in Table II, which corresponds closely with the pK of the unreduced aminoacridine (Table II, last column) determined electrometrically by Albert and Goldacre (*J.*, 1946, 704) for 50% aqueous-alcoholic solutions. The second of these two bends, at pH 12 (pK_2^t in Table II), occurs only for the 2- and 5-amino- and 2 : 7- and 2 : 8-diamino-compounds, for which Albert and Goldacre found only a single pK in each case. It may be significant that these anomalous pK_2^t-bends occur where the supporting electrolyte is changed from buffer solutions to sodium hydroxide solutions, but it has been shown (see below) that

they are not due to the increase in ionic strength associated with this change of supporting electrolyte.

At $pH > pK_1{}^t$ all the aminoacridines are in the free base form T (I). Where a $pK_2{}^t$-bend occurs, this is true up to $pH = pK_2{}^t$. At $pH < pK_1{}^t$, down to at least pH 2·5, spectrographic evidence (Craig and Short, *J.*, 1945, 419; Turnbull, *ibid.*, p. 441) indicates that they are in the form T$^+$ (II), and that addition of a second proton (to substituent amino-group) is at most

(I.) (II.) (III.)

(IV.) (V.) (VI.)

(VII.) (VIII.) (IX.)
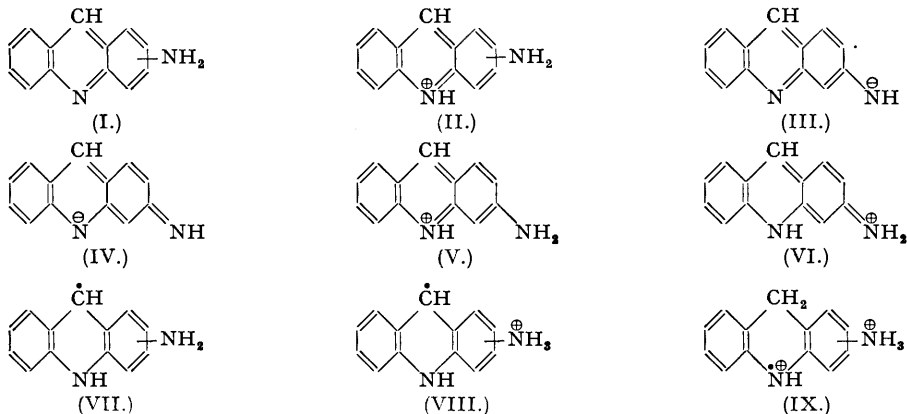
only incomplete at about pH 0. Where a $pK_2{}^t$-bend occurs, it is difficult to avoid the conclusion that for $pH > pK_2{}^t$ the aminoacridine exists in the form of an anion T$^-$. The latter might perhaps result from the loss of a proton from the amino-group, giving an ion such as (III), stabilised by resonance with (IV). This view is suggested by the fact that formation of T$^-$ occurs only with 2- or 5-amino-substituted acridines, while strong resonance between structures of the type (V) and (VI), which is precisely similar to that between (III) and (IV), occurs only for 2- and 5-aminoacridine (Albert and Goldacre, *loc. cit.*).

An important feature of the E_1–pH curve, common to all the compounds except 1- and 3-aminoacridine, is the horizontal central section. Here the pH is less than $pK_1{}'$, so the amino-acridine is mainly present as T$^+$; also the first reduction step is independent of pH and thus involves no proton uptake. The electrode process may be written
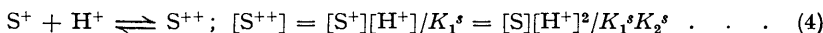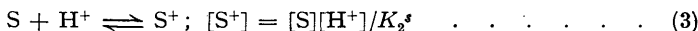
$$T^+ + e \longrightarrow S \qquad \qquad (1)$$

where S denotes the uncharged semiquinone (VII). For a reversible process corresponding to this equation, with standard redox potential $E_1{}°$, the redox potential in v at 25° is given by

$$E = E_1{}° + 0·059 \log ([T^+]/[S]) \qquad \qquad (2)$$

Since two bends associated with the acid constants of the semiquinone occur in the E_1–pH curves for 2-aminoacridine and both diaminoacridines, the semiquinones of these compounds apparently exist at three ionisation levels. Both bends occur at pH lower than where we have already assumed that the semiquinone is in the form S, and thus the remaining two ionisation levels must correspond to successive addition of protons to S, giving ions S$^+$ and S^{++}. For 4- and 5-aminoacridines, the experimentally accessible pH range did not extend low enough for the second bend and S^{++}-formation to occur. For acridine (*J.*, 1951, 27) there are no bends indicating formation of S$^+$ and S^{++} down to pH 0. This suggests that S$^+$ has the structure (VIII), formed by addition of a proton to a substituent amino-group, which is more strongly basic in S than in T$^+$ because of the additional odd electron in the molecule. Since S^{++}-formation occurs in mono- as well as in di-aminoacridines, it cannot be due to proton addition at a second amino-group; we tentatively assume that the proton is added at the 5-carbon atom, as in (IX).

The concentrations of S, S$^+$, and S^{++} are inter-related by the following ionisation equilibria :

$$S + H^+ \rightleftharpoons S^+; \quad [S^+] = [S][H^+]/K_2{}^s \qquad \qquad (3)$$

$$S^+ + H^+ \rightleftharpoons S^{++}; \quad [S^{++}] = [S^+][H^+]/K_1{}^s = [S][H^+]^2/K_1{}^s K_2{}^s \qquad (4)$$

Thus the total concentration of semiquinone in all three forms is

$$[Sem] = [S] + [S^+] + [S^{++}] = [S]\{K_1{}^s K_2{}^s + K_1{}^s[H^+] + [H^+]^2\}/K_1{}^s K_2{}^s \qquad (5)$$

Similarly, the total concentration of unreduced material in all three forms is

$$[Ox] = [T^-] + [T] + [T^+] = [T^+]\{K_1^t K_2^t + K_1^t[H^+] + [H^+]^2\}/[H^+]^2 \quad . \quad . \quad (6)$$

Substituting for $[T^+]$ in terms of $[Ox]$ from equation (6), and for $[S]$ in terms of $[Sem]$ from equation (5), equation (2) becomes, when applied to the half-wave potential (where $E = E_1$ and $[Ox] = [Sem]$),

$$E_1 = E_1^{\circ\prime} + 0.059 \log [H^+]^2\{K_1^s K_2^s + K_1^s[H^+] + [H^+]^2\}/\{K_1^t K_2^t + K_1^t[H^+] + [H^+]^2\} \quad . \quad (7)$$

where $E_1^{\circ\prime} = E_1^{\circ} - 0.059 \log K_1^s K_2^s$.

It being assumed that $K_1^s \gg K_2^s \gg K_1^t \gg K_2^t$, it follows from equation (7) that the E_1–pH graph should consist of linear portions separated by bends at pH = pK_1^s, pK_2^s, pK_1^t, and pK_2^t, with slopes -59 mv for pH between pK_1^t and pK_2^t or pK_1^s and pK_2^s, -118 mv below pK_1^s and above pK_2^t, and zero between pK_2^s and pK_1^t. This is in general agreement with the form of the experimental curves of all but the 1- and 3-aminoacridine, although of course no pK_2^t bend occurs in the 4-isomer, and the slopes (Table II) differ from the theoretical values in most cases, probably because the polarographic reduction is thermodynamically irreversible, as with acridine. The discrepancy is least for 2 : 7-diaminoacridine. The central horizontal portions of the E_1–pH curve of 1- and 3-aminoacridine are missing, indicating, according to equation (7), that pK_2^s is not considerably less than pK_1^t, as it is for the other aminoacridines. In fact, analysis of the E_2–pH curves for these two compounds (see below) yields values of pK_2^s (Table II) which are definitely greater than the corresponding pK_1^t values derived from the E_1–pH curves and confirmed by Albert and Goldacre's electrometric data (*loc. cit.*). It being assumed then that for these two compounds $pK_2^s > pK_1^t > pK_1^s$, the E_1–pH graph according to equation (7), the term in K_2^t being omitted, should consist of linear sections separated by bends at pH = pK_2^s, pK_1^t, and pK_1^s, with slopes of -59 mv for pH above pK_2^s or between pK_1^t and pK_1^s, and -118 mv below pK_1^s or between pK_2^s and pK_1^t. For both compounds, the bends actually found (Fig. 4) are not very pronounced, so that, because of the insufficient number of points on the curves, it is difficult to determine whether a pK_1^s bend is present. For the 1-isomer, apart from this difficulty, agreement between theory and experiment (Fig. 4, curve I) is reasonable, although the slopes do not have the theoretical values. For the 3-isomer, however, there is no indication of a pK_2^s bend.

The reason why semiquinone formation enhances the basic strength of a substituent amino-group relative to that of ring nitrogen (*i. e.*, makes $pK_2^s > pK_1^t$) more strongly for the 1- and 3-aminoacridine than for all the other compounds studied, may be stated as follows. For base-weakening resonance of the type :
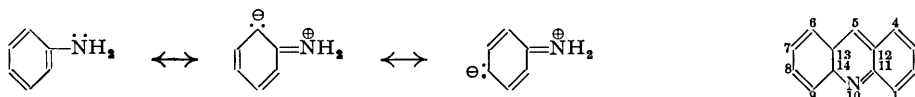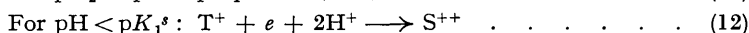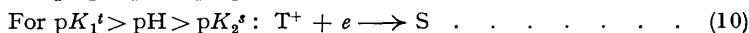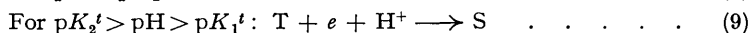

in the case of all but these two compounds, the unshared electron pair and negative formal charge may reside on the ring nitrogen or on carbon atoms 1, 3, 7, 9, 12, and 13; for 1- and 3-aminoacridines, however, only on carbon atoms 2, 4, 5, 6, 8, 11, and 14; this resonance explains the low pK values for the amino-group of about -1 to $+1$ found for most of the compounds by Albert and Goldacre (*J.*, 1943, 454), Craig and Short, and Turnbull (*locc. cit.*). Now in the semiquinones, the only ring positions occupiable by the odd electron are 2, 4, 5, 6, 8, 11, and 14; thus the base-weakening resonance is inhibited to a greater extent for 1- and 3-aminoacridines than for the other compounds.

In connexion with a theory relating the antibacterial activity of the aminoacridines to their reduction behaviour (Kaye, *J. Pharm. Pharmacol.*, 1950, **2**, 902), it is significant that the active compounds (i) form apparently highly stable semiquinones, (ii) give E_1–pH curves with a horizontal section, where semiquinone formation involves uptake of an electron only and no proton, and (iii) have this horizontal section covering the biologically important pH region around pH 7. Some inactive compounds comply with (i) and (ii), but only the more active ones comply with (iii) as well. Since (iii) necessitates that $pK_1^t > 7 > pK_2^s$, the correlation between basicity (or pK_1^t) and activity noted by Albert, Rubbo, and Goldacre (*Nature*, 1941, **147**, 332) may be viewed in a new light.
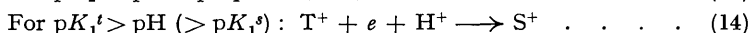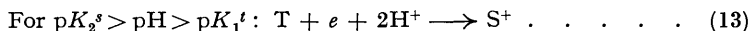
From a consideration of the slopes of the various sections of the E_1–pH curves, we may now

represent the electrode processes for the first reduction step of all but 1-, 3-, and 4-amino-acridine in different pH regions :
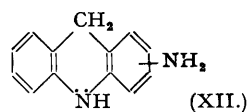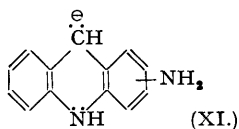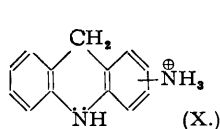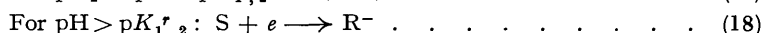
$$\text{For pH} > pK_2{}^t : \quad T^- + e + 2H^+ \longrightarrow S \quad \ldots \ldots \quad (8)$$

$$\text{For } pK_2{}^t > \text{pH} > pK_1{}^t : \quad T + e + H^+ \longrightarrow S \quad \ldots \ldots \quad (9)$$

$$\text{For } pK_1{}^t > \text{pH} > pK_2{}^s : \quad T^+ + e \longrightarrow S \quad \ldots \ldots \quad (10)$$

$$\text{For } pK_2{}^s > \text{pH} > pK_1{}^s : \quad T^+ + e + H^+ \longrightarrow S^+ \quad \ldots \ldots \quad (11)$$

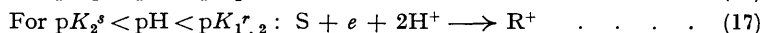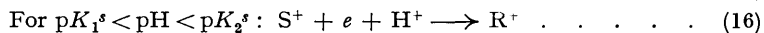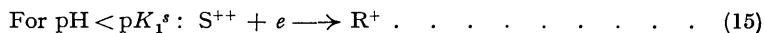$$\text{For pH} < pK_1{}^s : \quad T^+ + e + 2H^+ \longrightarrow S^{++} \quad \ldots \ldots \quad (12)$$

For 4-aminoacridine, with no $pK_2{}^t$, equation (8) is inapplicable, equation (9) applies at pH $> pK_1{}^t$, and equations (10), (11), and (12) apply unchanged. Similarly, for 1- and (possibly) 3-amino-acridine, equation (9) applies for pH $> pK_2{}^s$, equation (12) would apply for pH $< pK_1{}^s$ if a bend had occurred at $pK_1{}^s$, and instead of equations (10) and (11) we now have

$$\text{For } pK_2{}^s > \text{pH} > pK_1{}^t : \quad T + e + 2H^+ \longrightarrow S^+ \quad \ldots \ldots \quad (13)$$

$$\text{For } pK_1{}^t > \text{pH} \ (> pK_1{}^s) : \quad T^+ + e + H^+ \longrightarrow S^+ \quad \ldots \ldots \quad (14)$$

We may now consider the E_2–pH curves (Figs. 4 and 5), which are essentially similar in form for all the compounds (for 5-aminoacridine no results are available for pH $< 4·8$, as explained previously). Two bends corresponding to pK^s values for the semiquinone appear (only one for the 2-, 4-, and 2 : 7-compound), and two oppositely directed bends, apparently corre-sponding to pK^r values for the fully reduced product (aminoacridan). The pK^s bends should of course occur at the same pH values as the corresponding bends in the E_1–pH curves. This is seen to be true for the 2- and the 2 : 8-compound (Table II). In the E_1–pH curves of 1- and 3-aminoacridine, $pK_1{}^s$ bends are missing or doubtful, and the $pK_2{}^s$ bend is missing for the 3-isomer and in poor agreement with the corresponding E_2–pH bend for the 3-isomer. For the 4- and the 2 : 7-compound the correspondence is only fair, and for 5-aminoacridine E_2–pH data are lacking.

We come now to the two E_2–pH bends apparently related to pK^r values. The one at the lower pH annuls the effect on the slope of two successive pK^s bends, and may thus be regarded as due to two acid constants, $pK_1{}^r$ and $pK_2{}^r$, not greatly different in value. The remaining bend, at about pH 13, is unlikely to indicate that the aminoacridan can ionise off yet another proton, on constitutional grounds. The bend occurs just where sodium hydroxide solutions replace buffers as supporting electrolyte. Since Burstein and Davidson (*Trans. Electrochem. Soc.*, 1941, **63**, 1636) showed that the stability of uncharged semiquinone decreased considerably with rising ionic strength of the solution in the case of certain anthraquinone derivatives, it seemed likely that the increasing ionic strength of the sodium hydroxide solutions at pH 13 and 14 might result in the displacement of the reduction potential of semiquinone to increasingly less negative values. In confirmation of this view, inclusion of N-potassium chloride in 2 : 8-diaminoacridine solutions in 50 alcoholic buffers at pH 10·39 and 12·0 made E_2 about 80 mv less negative. We may thus neglect this bend as an ionic-strength effect. Incidentally, the addition of potassium chloride shifted E_1 also to somewhat less negative values. Hence the anomalous $pK_2{}^t$ bends on the E_1–pH curves towards more negative potentials with rising pH cannot be due to ionic-strength effects.

From a consideration of the slopes of the various sections of the E_2–pH curves we may write the electrode processes for the second reduction step at various pH regions :

$$\text{For pH} < pK_1{}^s : \quad S^{++} + e \longrightarrow R^+ \quad \ldots \ldots \quad (15)$$

$$\text{For } pK_1{}^s < \text{pH} < pK_2{}^s : \quad S^+ + e + H^+ \longrightarrow R^+ \quad \ldots \ldots \quad (16)$$

$$\text{For } pK_2{}^s < \text{pH} < pK_1{}^r{}_{,2} : \quad S + e + 2H^+ \longrightarrow R^+ \quad \ldots \ldots \quad (17)$$

$$\text{For pH} > pK_1{}^r{}_{,2} : \quad S + e \longrightarrow R^- \quad \ldots \ldots \quad (18)$$

Here R^+ and R^- denote structures (X) and (XI), respectively; R^-, which probably abstracts a proton from a water molecule immediately after formation, thus changing to the form R

(XII), is similar to the anionic form of reduction product previously postulated for acridine, by analogy with the views of other workers with different substances (*J.*, 1951, 27).

By writing the equation for the electrode potential of a reversible reduction corresponding to equation (18), introducing the dissociation constants $K_1^r = [R][H^+]/[R^+]$, $K_2^r = [R^-][H^+]/[R]$, and proceeding as in the derivation of equation (7), we obtain :

$$E_2 = E_2^{\circ\prime} + 0.059 \log \{K_1^r K_2^r + K_1^r[H^+] + [H^+]^2\}/\{K_1^s K_2^s + K_1^s[H^+] + [H^+]^2\} \quad . \quad (19)$$

It being assumed that $pK_1^s < pK_2^s < pK_1^r \approx pK_2^r$, this equation leads to E_2–pH curves similar to those in Figs. 4 and 5 (especially Fig. 4, curves I and III, and Fig. 5, curve I), except that the slopes of the various portions are multiples of -59 mv. Apart, then, from the discrepancy in slopes (cf. Table II), attributable to thermodynamic irreversibility of the electro-reduction, equation (19) represents satisfactorily the experimental E_2–pH curves for all the compounds.

Although equations (8)—(18) are deducible directly from the E_1–pH and E_2–pH curves, the representation of the latter by means of equations (7) and (19) is open to the possible criticism that these two equations are based on the assumption of reversible equilibria, when at least some of the processes concerned are known to be irreversible. In particular, the existence of pK_2^r appears to be peculiar to the polarographic-electroreduction technique, and it is unlikely to be encountered in electrometric determinations. However, pK_1^r should be real enough and determinable electrometrically. Appropriate comparison data are lacking, apart from the pK values of 4·72 (in 50% alcohol) and 5·2 (in water) given for 3-aminoacridan by Albert and Ritchie (*J.*, 1943, 458) and Albert, Rubbo, Goldacre, Davey, and Stone (*Brit. J. Exp. Path.*, 1945, 26, 160), respectively, which do not correspond with any bend in the E_1–pH or E_2–pH curve in the present work.

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