122. *The Nature of the Amino-group in Aminoacridines. Part I. Evidence from Electrometric Studies.*

By Adrien Albert and Reginald Goldacre.

The striking differences in antiseptic powers among the five monoaminoacridines (Rubbo, Albert, and Maxwell, *Brit. J. Exp. Path.*, 1942, **23**, 69) prompted this search for such evidence of individuality as would account for the biological variations encountered.

In this Part, the relative basicities of these and related compounds are examined, and it is found that the structure of 2- and 5-aminoacridines permits of a greater degree of resonance in the ion than occurs in the un-ionised base. Hence, these isomerides show an abnormally high degree of ionisation, an effect that parallels their high biological activity. The properties of the other isomerides suggest that they are fairly normal amino-derivatives of acridine.

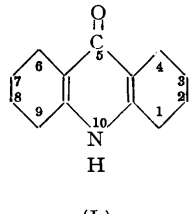
Since little is known about the structure of amino-derivatives of nitrogenous heterocyclic nuclei, the present work was undertaken to gather such information in respect of a series of isomerides, the five aminoacridines, the members of which show marked biological individuality (Rubbo, Albert, and Maxwell, *loc. cit.*), since, whilst 1-amino-acridine has less antiseptic activity than acridine, the 3- and the 4-isomeride are more, and the 2- and the 5-isomeride outstandingly more, bactericidal than acridine (Table I). This part deals with the relative basicities of the five aminoacridines, their acetyl derivatives, methobromides, and hydroxy-analogues, and of some acids derived from 2-aminoacridine.

The data in Table I were obtained by potentiometric titration with the glass electrode. Every endeavour was made to use water as the solvent because of its biological importance, but in many cases the curves showed irregularities which were attributed to a series of long-delayed equilibria due to hydrochloride being carried down by the poorly soluble (and often glutinous) base on liberation. They were reminiscent of the calcium phosphate curves obtained by Wendt and Clark (*J. Amer. Chem. Soc.*, 1923, **45**, 882). As Bennett and Glasstone (J., 1935, 1821) believe that a series of bases preserves the same order of basicity in dilute alcohols, recourse was had when necessary to 67% methanol and 50% ethanol, which, by keeping all the components in solution throughout the titration, achieved good equilibria (Fig. 1). In our experience the pK values obtained with these two solvents are practically identical but average 0·5 unit lower than the corresponding values in water (when derived from regular curves; see also Mizutani, *Z. physikal. Chem.*, 1925, **118**, 327).

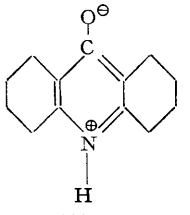
Acridine was found to be a weak base, similar in strength to aniline ($2{\cdot}7 \times 10^{-10}$) and rather weaker than pyridine ($1{\cdot}1 \times 10^{-9}$), these data being due to Britton and Williams (J., 1935, 796) (glass electrode, in water at 18°). The closeness of the values for pyridine and acridine would suggest that the benzene rings of the latter exert little influence on the central ring.

In the hydroxyacridines, too, the influence of the benzene and the pyridine rings on one another appears small. The 1-, 2-, 3-, and 4-hydroxyacridines do not differ greatly in basicity from one another or from acridine, whereas *p*-aminophenol is much more basic than its *m*-isomeride or aniline (Kuhn, *Helv. Chim. Acta*, 1928, **11**, 7). Again, the acidic strengths of 2-, 3-, and 4-hydroxyacridines are similar to one another and to that of phenol (contrast *p*-aminophenol, Kuhn, *loc. cit.*). 1-Hydroxyacridine is somewhat weaker as an acid, possibly because of an " ortho "-effect (see below).

In 5-hydroxyacridine (" acridone "), where the hydroxyl group is attached to the ring containing the nitrogen atom, a considerable interaction between them is shown by the relative absence of basic and acidic properties. This substance has a high m. p. (354°) and lacks marked phenolic or ketonic properties. Its similarities to 4-hydroxypyridine (Arndt and Kalischek, *Ber.*, 1930, **63**, 587, 2963) show it to be a resonance hybrid of acridone (I) and the otherwise unstable internal salt (II) of 5-hydroxyacridine.



(I.)



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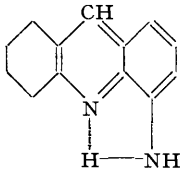

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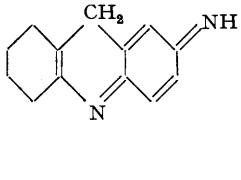
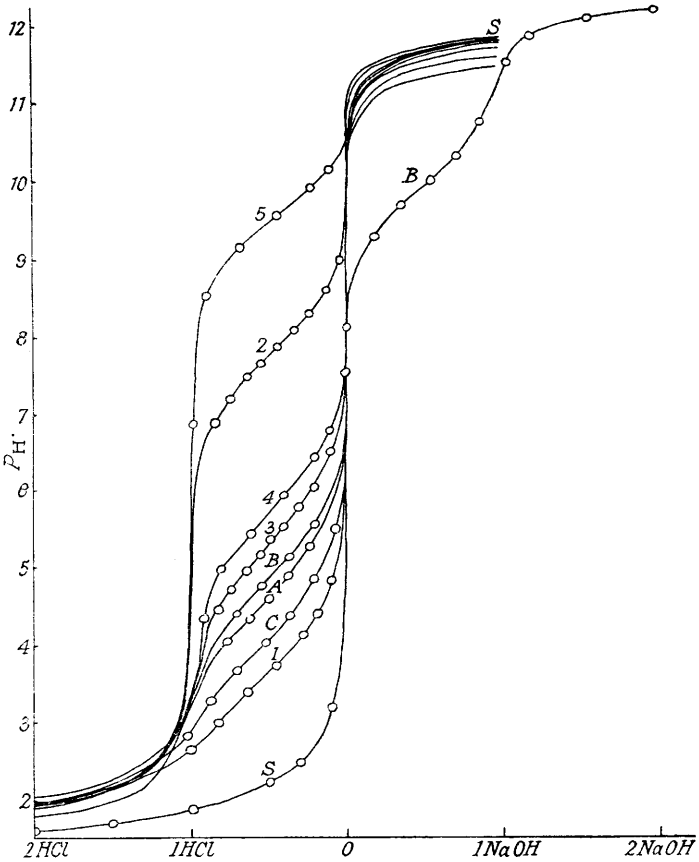


(IV.)

FIG. 1.

Potentiometric Titration Curves [in dilute alcohols as in Table I].



1 = 1-*Aminoacridine*.
2 = 2- ,,
3 = 3- ,,
4 = 4- ,,
5 = 5- ,,

A = *Acridine*.
B = 2-*Hydroxyacridine*.
C = 3-*Acetamidoacridine*.
S = *Blank*; 50% C_2H_5OH (*by vol.*).

Concerning the aminoacridines, we are indebted to our colleagues, Messrs. Mellor and Craig (Sydney) and Smith and Turnbull (Melbourne) for spectroscopic evidence that it is the *ring* nitrogen that becomes quaternary when these substances form monoacid salts, so the well-known weakness (as a base) of $-NH_2$ attached to a highly conjugated system (*e.g.*, anthracene) is seen also in the acridine series. From analogy with the hydroxyacridines, an amino-group in the benzene rings should have little influence on the basicity of the ring nitrogen, and Table I shows that this is the case with 1-, 3-, and 4-aminoacridines which, on chemical evidence (Part II) must be considered typical amino-derivatives of acridine. Although 3- and 4-aminoacridines are slightly stronger than acridine, yet 1-aminoacridine is seven times weaker as a base, and chemically somewhat less reactive (Part II). These data are consistent with the assumption that the two nitrogens are linked by hydrogen (as in III; cf. 8-acetamidoquinoline, Chaplin and Hunter, J., 1938, 375), but so little is known of the N–H–N bond and so many 1 : 2-diamines have a lower basicity than the corresponding monoamines (cf. ethylenediamine, Britton and Williams, *loc. cit.*; *o*-phenylenediamine and 2 : 3-diaminonaphthalene, Kuhn, *loc. cit.*) that it is sufficient to say that 1-aminoacridine shows a decided " ortho "-effect. It should be noted that 3-aminoacridine cannot have the imine structure (IV) because the 5 : 5-dimethyl derivative of (IV) is a typical imine, readily hydrolysed by water (Goldstein and Kopp, *Helv. Chim. Acta*, 1928, **11**, 478).

The remaining aminoacridines, the 2- and the 5-isomeride, are distinguished by their remarkable strength as bases. This appears to be due to a heightened resonance effect in the ion which, in the case of 5-aminoacridine, would be a hybrid to which the structures (V*a*), derived from the normal form of the base, and (V*b*), derived from a tautomeric form (V*c*), contribute. Similar structures (VI*a*, *b*, *c*) may be written for 2-aminoacridine. These are examples of a phenomenon, discussed by Branch and Calvin (" The Theory of Organic

TABLE I.

Dissociation constants of acridine and derivatives.

Substance (0·0005 g.-mol. in 20—30 ml.).	Solvent.*	pK_a at 20°.†	K_b.‡	p$K_{a'}$ at 20°.	$K_{a'}$.§	*B*.‖
Acridine	67% MeOH	4·54	3×10^{-10}	—	—	1·2
1-Hydroxyacridine	50% EtOH	4·18	1×10^{-10}	10·70	2×10^{-11}	—
2-Hydroxyacridine	,,	4·86	6×10^{-10}	9·86	1×10^{-10}	—
3-Hydroxyacridine	,,	4·21	1×10^{-10}	9·68	2×10^{-10}	—
4-Hydroxyacridine	,,	4·45	2×10^{-10}	9·40	3×10^{-10}	—
5-Hydroxyacridine	,,	<2	—	>12	—	—
1-Aminoacridine	67% MeOH	3·67	4×10^{-11}	—	—	0·8
2-Aminoacridine	,,	7·60	3×10^{-7}	—	—	4·2
3-Aminoacridine	,,	5·30	2×10^{-9}	—	—	1·6
4-Aminoacridine	,,	5·74	5×10^{-9}	—	—	1·8
5-Aminoacridine	,,	9·34	2×10^{-5}	—	—	4·6
2-Aminoacridine	50% EtOH	7·67	4×10^{-7}	—	—	—
5-Aminoacridine	,,	9·38	2×10^{-5}	—	—	—
1-Acetamidoacridine	67% MeOH	2·5	3×10^{-12}	—	—	0·6
2-Acetamidoacridine	,,	5·12	1×10^{-9}	—	—	0·8
3-Acetamidoacridine	,,	4·18	1×10^{-10}	—	—	1·2
4-Acetamidoacridine	,,	3·87	6×10^{-11}	—	—	0
5-*Acetamidoacridine*	,,	3·83	6×10^{-11}	11·1	8×10^{-12}	0
2-Amino-10-methylacridinium hydroxide	H_2O	>12	—	—	—	3·8
3-Amino-10-methylacridinium hydroxide	,,	9·8	—	—	—	—
4-Amino-10-methylacridinium hydroxide	,,	10·0	—	—	—	—
5-Amino-10-methylacridinium hydroxide	,,	10·1	—	—	—	4·8
2-*Aminoacridine-7-carboxylic acid*	,,	2·3	2×10^{-12}	8·0	1×10^{-8}	0
2-Aminoacridine-7-sulphonic acid	,,	<2	—	7·7	2×10^{-8}	0
2-Aminoacridine-7-sulphonamide	50% EtOH	6·7	5×10^{-8}	10·9	1×10^{-11}	1·6

* The correction for the effect of alcohols on the glass electrode has not been subtracted but is of the order of only 0·04 unit (Dole, "The Glass Electrode," New York, 1941, p. 141).

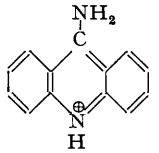
† K_a stands for the acidity constant, $(B)(H^+)/(BH^+)$. pK_a was taken as the pH of half-neutralisation and was also calculated from five other points of the curves. Where necessary it has been corrected for hydrolysis of the salt formed. In water, on account of delayed equilibria (see text) apparent pK_a values were obtained for acridine and 1-, 2-, 3-, 4-, and 5-aminoacridines of 4·3, 4·0, 8·1, 5·1, 5·5, and 9·5, respectively. 5-Acetamidoacridine has been corrected for sodium-ion error (+0·1 unit at pH 11; Dole, *op. cit.*, p. 129).

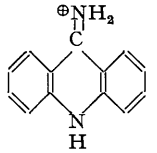
‡ K_b = First basic dissociation constant.

§ $K_{a'}$ = Acid dissociation constant.

‖ *B* is the averaged bacteriostatic index, *i.e.*, the average value of the inhibition of growth of five organisms (*Cl. Welchii, Strept. Hæm.* A, *Staph. aureus, B. coli*, and *Proteus vulgaris*) after incubation at 37° for 48 hours, in 10% serum-broth adjusted to pH 7·2 (Rubbo, Albert, and Maxwell, *loc. cit.*). As this index is expressed in powers of 2, 4-aminoacridine is twice and 5-aminoacridine is 14 ($= 2^{3\cdot8}$) times as effective as 1-aminoacridine. A biological paper shortly to be published with Dr. Rubbo shows that, in a series of forty acridines, an index higher than 2·5 is given only by those compounds which have a p*K* higher than 7 and, hence, are at least 50% ionised at the biologically important value, pH 7·2.

Chemistry," Prentice-Hall, 1941, p. 194), wherein a base is stronger than would otherwise have been predicted if its structural pecularities permit the acquisition of extra resonance forms on passing into the ion. This phenomenon is seen in its purest form in the case of 1 : 5-diaminopentamethine (VII) and its alkyl derivatives which may reach a p*K* value of 12 (Schwarzenbach and Lutz, *Helv. Chim. Acta*, 1940, **23**, 1162). A less intense exaltation of basicity is to be expected in aromatic structures because the extra ionic resonance is usually offset by the loss of nuclear resonance in one of the ionic structures. This balancing of resonance effects leaves a net gain for 5-aminoacridine ion, the symmetrical structure of the central ring being the determining factor (cf. the analogously constituted 4-aminopyridine, p*K* 9·1). A smaller net gain is shown by the 2-aminoacridine ion, whereas the low basicity of 4-aminoacridine indicates that there would be no increase in resonance energy in passing from the base to an ion of which the *o*-quinonoid structure (VIII) was a component. These concepts are proving useful in the prediction of basicity for other heterocyclic amines.


(V*a*.)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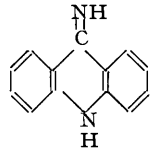
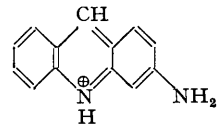
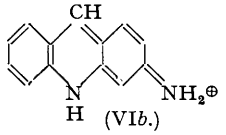
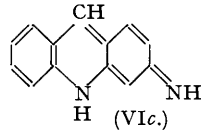
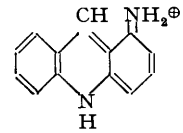

(V*c*.)


(VI*a*.)


(VI*b*.)


(VI*c*.)


(VIII.)

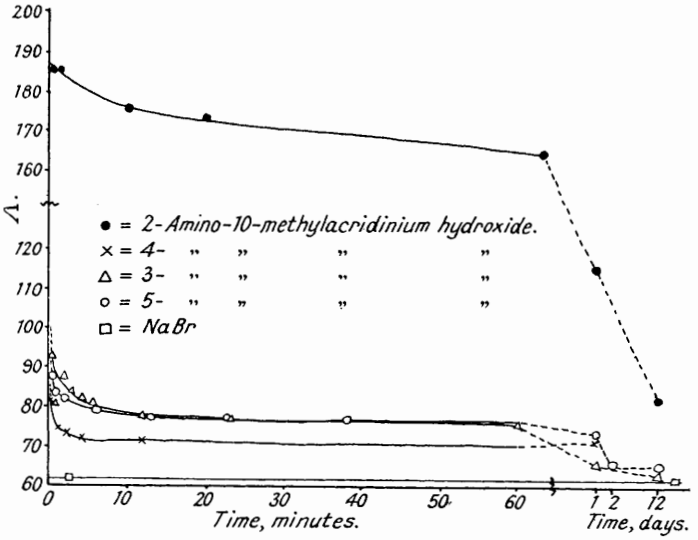
(VII.) $NH_2 \cdot CH{:}CH \cdot CH{:}CH \cdot CH{:}\overset{\oplus}{N}H_2$

Spectrometric investigation in highly acid solution (see Experimental) showed that the pK_a values corresponding to second basic dissociation constants for the 1-, 3-, and 4-aminoacridines and the 3- and 4-amino-10-methylacridinium hydroxides lay between 0·5 and 1·2 approximately. On the other hand, the monoacid salts of 2- and 5-aminoacridines and of their methochlorides gave no colour change in this range. This supports the theory of extra ionic resonance for these substances, since in passing from mono- to di-acid salts, the pairs of resonant ionic structures (V*a* and *b*; VI*a* and *b*) would be destroyed.

The values obtained for the acetamidoacridines (Table I) show that acetylation of the amino-group effects a decrease in basicity in every case. This is most marked in the 2- and the 5-isomeride, since the imino-form (V*b* and VI*b*) of the ions becomes too weak a base on acetylation to contribute much to the base-strengthening resonance. These substances have slight acidic properties also, particularly the 5-isomeride.

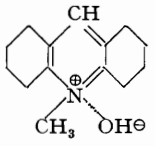
The behaviour of quaternary heterocyclic amines on alkalisation is unpredictable, and no adequate explanation can be advanced for the diverse behaviour of the methobromides of the aminoacridines when made alkaline (Fig. 2). Table I shows 2-amino-10-methylacridinium hydroxide to be a quite strong base, and conductivity measurements at 0° (see Experimental) show that it is ionised to the extent of approximately 90% at M/256 and so is almost as strong a base as sodium hydroxide. This isomeride, in fact, is almost as stable as 2 : 8-diamino-10-methylacridinium hydroxide which resists boiling for a few minutes. Fig. 2 shows that the other isomerides undergo a rapid transformation to pseudo-bases as found by Hantzsch and Kalb for 10-methylacridinium chloride (*Ber.*, 1899, **32**, 3109), which undergoes the change (IX) ⟶ (X). Hence the pK_a values given for the 3-, 4-, and 5-isomerides in Table I are only nominal, representing equilibrium values for this reversible reaction. Aston (*J. Amer. Chem. Soc.*, 1931, **53**, 1448) questions Hantzsch's interpretation of the drop in conductivity found when methylacridinium salts are treated with alkalis and attributes it to the precipitation of a quaternary ammonium hydroxide. Hantzsch's explanation, however, fits our compounds quite well, especially the 4-isomeride, whose violet solution gives a yellow precipitate with alkali. The 3- and the 4-isomeride on shaking with phosphate buffer at pH 7·3 in the Warburg apparatus rapidly took up oxygen to form, presumably, 3- and 4-amino-10-methylacridones. The 2- and the 5-isomeride were stable under these conditions. All attempts to prepare 1-amino-10-methylacridinium salts were unsuccessful.

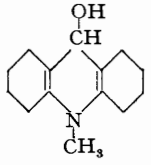
FIG. 2.



Conductivities of mixtures of amino-10-methylacridinium bromides and sodium hydroxide at 0° in M/256-solution. The 5-isomeride gives a precipitate at once, the 3-isomeride after some minutes, the others after several hours.

(IX.)





(X.)

The introduction of a carboxylic or sulphonic acid group into 2-aminoacridine has produced a mutual weakening of the manifest acid and basic properties to a degree intermediate between that seen in glycine and *p*-aminobenzoic acid on the one hand and taurine and sulphanilic acid on the other (cf. Sidgwick, " Organic Chemistry of Nitrogen," Oxford, 1937, p. 108).

EXPERIMENTAL.

Materials.—The acridine was purified by steam-distillation and had m. p. 110°. The aminoacridines were prepared according to Albert and Ritchie (*J. Soc. Chem. Ind.*, 1941, **60**, 120), and the 2-aminoacridine-7-sulphonic acid and -7-sulphonamide according to Aarons and Albert (J., 1942, 183). The acetamidoacridines were prepared by heating the aminoacridines (3 g.) in acetic anhydride (6 ml.) for 30 mins. at 105°, pouring the mixture into benzene, filtering off the solid, and treating it with aqueous ammonia. After recrystallisation from dilute alcohol, white or yellow bases were obtained which gave yellow or brown salts. The m. p. found for 4-acetamidoacridine (230°; 237°, corr.) is higher than that previously recorded (225—226°).

5-Acetamidoacridine, white crystals from 40 parts of absolute alcohol, m. p. 266° (276°, corr.), did not fluoresce in solution (Found : N, 11·7. $C_{15}H_{12}ON_2$ requires N, 11·85%). The acetyl group is not in the 10-position since methyl toluene-*p*-sulphonate gives a product which yields 5-amino-10-methylacridinium bromide (Part II) on hydrolysis.

The preparation of the hydroxyacridines and quaternary salts is given in Part II.

2-Aminoacridine-7-carboxylic acid. 2-Chloro-4-nitrobenzoic acid (20 g.; 0·1 mol.), *p*-aminobenzoic acid (20·1 g.; 0·15 mol.), sodium acetate (16·4 g., 0·2 mol.), catalytic copper (1 g.), and butanol (200 ml.) were refluxed for 4 hours, treated with excess sodium carbonate and steam-distilled. The contents of the flask were acidified, filtered at the boil, and the cake washed with boiling water then recrystallised from alcohol (150 ml.). *5-Nitrodiphenylamine-2 : 4'-dicarboxylic acid* was thus obtained (15% yield) as reddish-brown crystals, m. p. 281° (Found : N, 9·1. $C_{14}H_{10}O_6N_2$ requires N, 9·25%). This acid was refluxed for 2 hours with phosphorus oxychloride (15 ml.), poured into water, made strongly alkaline, and boiled for 15 mins. *2-Nitroacridone-7-carboxylic acid* was obtained (95% yield) on acidification as an orange powder, not melting below 360° (Found : N, 9·8. $C_{14}H_8O_5N_2$ requires N, 9·9%). To this acid (3 g.), dissolved in water (150 ml.) with sodium hydroxide (0·66 g.; 1·5 equiv.), was added freshly amalgamated aluminium foil (1·5 g.; 2 equivs.) during 45 mins. at 100°. After filtration, the cake was boiled with water (75 ml.), and the bulked filtrates acidified with concentrated hydrochloric acid (2·7 ml.). The 2-aminoacridane-7-carboxylic acid was oxidised in this solution by boiling with the theoretical amount of ferric chloride. After filtering and cooling, the hydrochloride of 2-aminoacridine-7-carboxylic acid separated as orange needles which were recrystallised from boiling water with a trace of acid. Treatment with sodium acetate solution gave the *acid* in 45% yield; scarlet solid, retaining water of crystallisation when dried at 110°, losing it at a higher temperature and decomposing at about 200° without melting (Found : N, 10·7. $C_{14}H_{10}O_2N_2,H_2O$ requires N, 10·9%). It was sparingly soluble in alcohol with a green fluorescence, and dissolved in alkalis with a brown colour and green fluorescence.

Potentiometric Titrations.—These were carried out by running N-hydrochloric acid into a solution of the base (0·0005 g.-mol. in 20—30 ml.), followed, when necessary, by N-sodium hydroxide. The solution was contained in a beaker fitted with an efficient stirrer, glass electrode, and a potassium chloride–agar bridge connected to a calomel half-cell and a Leeds and Northrup universal pH potentiometer set. The latter was checked against buffer solutions at pH 2·20, 4·00, 7·00, 8·00, 9·00, and 10·00.

Conductances.—These were measured in an ordinary Arrhenius cell placed in a Wheatstone bridge circuit (using A.C. supplied by a valve-oscillator) similar to that of Grinnell Jones and Josephs (*J. Amer. Chem. Soc.*, 1928, **50**, 1049). The cell-constant of the platinised platinum electrodes was determined by means of N/10-potassium chloride at 0°. Each amino-10-methylacridinium bromide (20 ml. of N/32-solution) was placed successively in the cell, and further dilutions obtained by withdrawing 10 ml. portions with a calibrated pipette and adding 10 ml. of water. This was repeated until the molecular conductivity at 0° became approximately constant, which occurred in the range M/256—M/1024. The following values were obtained for the 2-, 3-, 4-, and 5-isomerides respectively: 57, 56, 56, 51.

The curves shown in Fig. 2 were obtained by adding N/128-sodium hydroxide as quickly as possible to the same volume of M/128-solution of the above bromides, the cell and all solutions being maintained at 0°, and the mixture gently stirred by raising and lowering the electrodes. Care was taken to exclude carbon dioxide throughout. The percentage dissociation of 2-amino-10-methylacridinium bromide was calculated as follows, after Hantzsch and Kalb (*loc. cit.*). The Λ_{256} of the methobromide was 57, which is equivalent by Onsager's equation to $\Lambda_0 = 60$. Subtracting 41 for the Λ_0 of Br^- at 0°, the Λ_0 of the 2-amino-10-methylacridinium ion becomes 19, whilst by adding 118 for the Λ_0 of OH^-, the value of $\Lambda_0 = 137$ is obtained for the methohydroxide at 0°. The observed value of Δ_{256} for the mixture of methobromide and sodium hydroxide was 185, and taking from this 62 for the Λ_{256} of sodium bromide, the observed Λ_{256} for the methohydroxide becomes 123, and it is hence 123/137, or 90%, dissociated. The values for Br^-, OH^- and Na^+ were obtained from International Critical Tables.

An approximate value for the *second basic dissociation constant* was obtained with the help of the Hilger–Nutting Visible Spectrophotometer. Each substance (0·01 g.) was dissolved in N/50-acid (5 ml.) and, by means of a micropipette, 0·2 ml. of each solution was added in turn to a range of buffers (Clark's potassium chloride–hydrochloric acid solutions) of pH 0·1, 0·4, 0·7, 1·0, 1·2, 1·4, and 2·0. By plotting the extinction coefficients at pH 2·0 and at pH 0·1 against wave-lengths, it became possible to choose a wave-length for which the difference in absorption between these two solutions was maximal. The intensity of this wave-length at pH 2·0 was compared with its intensity in other buffers. The pH of half-change is given in Table II, from which it is deduced that the second basic dissociation constant in these cases approximates to 1×10^{-13}.

TABLE II.

Substance.	Wave-length chosen, A.	pH of mid-point in colour-change between mono- and di-acid salts.
1-Aminoacridine	4700	0·5
3-Aminoacridine	4800	1·1
3-Amino-10-methylacridinium hydroxide	5300	1·2
4-Aminoacridine	5250	0·6
4-Amino-10-methylacridinium hydroxide	5500	0·7

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