146. *The Ionisation of Acridine Bases.*

By Adrien Albert and Reginald Goldacre.

It has recently been claimed that antibacterial activity in the acridine series is associated with those members that exhibit more than 60% of cationic ionization, at pH 7·3 and 37° (Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exper. Path.*, 1945, **26**, 160). The present paper supplies the chemical part of the evidence for this claim (i) by reporting 95 new ionisation constants, (ii) by discussing the effects of alcohol and of temperature on the ionisation of sparingly soluble bases, and (iii) by demonstrating new correlations between basic strength and structure in the acridine series.

In an earlier paper (Albert and Goldacre, *J.*, 1943, 454), the ionisation of acridine and 22 of its derivatives was discussed, as part of a programme to correlate structure, basicity, and antibacterial activity in this series. The present paper seeks, first, to give a critical interpretation of the figures already reported, and secondly, to report and discuss 95 new values.

The Effect of Alcohol on Ionisation.—For biological purposes it is very important to know the exact pK_a values in water of bases, particularly those which lie in the range pK_a 6—8, as their degree of ionisation at pH 7 varies greatly with a small change in pK_a. Because of their poor solubility, very few acridine compounds can be titrated potentiometrically in water, and hence it is desirable to know to what extent values obtained by titration in 50% ethanol fall short of the value in water. This comparison has been carried out by determining the pK_a values of acridine and the five isomeric aminoacridines both in 50% ethanol (potentiometric titration) and in water (absorptiometry in aqueous buffers; see Experimental Section). It will be seen from Table I that the depression of basic strength by the alcohol (ΔpK_a) ranges from 0·43 to 1·46 units (average 0·77). These figures are somewhat larger than those obtained on pyridine and simple aliphatic and aromatic amines by Mizutani (*Z. physikal. Chem.*, 1925, **118**, 327), who examined 10 bases in 60% methanol, and Hall and Sprinkle (*J. Amer. Chem. Soc.*, 1932, **54**, 3469), who examined 18 bases in 50% ethanol. These authors obtained maximum values of 0·89 and 0·88, and minimum values of 0·30 and 0·26 (average 0·55 and 0·54 respectively).

The ΔpK_a for acridine is the highest ΔpK_a on record and three times as high as the average found by Mizutani and by Hall and Sprinkle, whose bases had moderately high solubilities in both water and alcohol. That this is not an unreasonably high value follows from the work of Kolthoff, Lingane, and Larson (*J. Amer. Chem. Soc.*, 1938, **60**, 2512), who deduced thermodynamically that the ΔpK_a is a function of the distribution coefficients of the different molecular and ionic species involved in the acid–base equilibrium $\Delta pK_a = \log D_{H^+} + \log D_B - \log D_{BH^+}$, where D_X is the distribution coefficient of X between the two solvents.

The acridine bases now examined are much more soluble in alcohol than in water, in contrast with their hydrochlorides which are fairly readily soluble in both solvents. It does not follow that a still more hydrophobic substance than acridine will exhibit a greater depression, for we obtained a depression of only 0·54 for 6 : 7-benzoacridine (unpublished work), but here the extra hydrocarbon ring has developed hydrophobic properties in the hydrochloride, making it much more soluble in benzene than is acridine hydrochloride.

The reality of the large depression obtained for acridine was confirmed by measuring the pH of equimolecular amounts of acridine and its hydrochloride at a total concentration of M/240, using as solvents 50, 40, 30, 20 and 10% ethanol (by volume), which gave pK_a values of 4·14, 4·35, 4·69, 4·98, 5·20, respectively, at 20°. By extrapolation to 0% alcohol, the pK_a value of 5·55 for water is obtained (cf. 5·60 in Table I), a depression of 1·41 units.

Table III provides six other examples (Nos. 10, 14, 45, 46, 47, and 61) for the calculation of ΔpK_a, which varies between 0·41 and 0·9, averaging 0·58 unit.

Table I.

Effect of alcohol on the ionisation of acridines.

No.	Substance.	pK_a * in 50% EtOH at 20° (±0·5°) (potentiometric titration).	Dilution (1/M).	pK_a in H_2O at 20° (±5°) (absorptiometry).	Dilution (1/M).	ΔpK_a.
—	Acridine	4·11	120	5·60	2500	1·49
1	1-Aminoacridine	3·59	120	4·40	3000	0·81
2	2-Aminoacridine	7·61 †	120	8·04	5000	0·43
3	3-Aminoacridine	5·03	120	5·88	6000	0·85
4	4-Aminoacridine	5·50	120	6·04	6000	0·54
5	5-Aminoacridine	9·45 †	120	9·99	2500	0·54

* pK_a is the negative logarithm of the acidity constant $[B][H^+]/[BH^+]$.

† Previously reported (Albert and Goldacre, *loc. cit.*) as pH of half-neutralisation, but now averaged from ten points on the titration curve, as has been done for the other members of this table which have not previously been reported in ethanol.

It is sometimes assumed (Dippy, *Chem. Reviews*, 1939, **25**, 151; Bennett and Glasstone, *J.*, 1935, 1821) that a series preserves the same order of pK_a in dilute alcohols as in water. Although the data of Table I do not contradict this assumption, they do show that the depression of basic strength by alcohol can vary considerably from member to member of a series of isomerides. However, this may be an exceptional series since three different types of amino-group are involved (Albert and Goldacre, *loc. cit.*) and it is unlikely that such

large differences will be encountered where the nature of the amino-group remains unaltered (*e.g.*, the isomeric methoxy-derivatives of 5-aminoacridine, Table III). Some instances where the order of basic strength of fairly closely related amines is reversed by alcohol can be gleaned from curves published by Hall and Sprinkle (*loc. cit.*).

Effect of Temperature on Ionisation.—Hall and Sprinkle (*loc. cit.*), working between 19° and 32°, have shown that, in water, bases become weaker as the temperature rises and that an almost linear relation exists between pK_a and temperature and between pK_a and dpK_a/dT. No coefficients appear to have been determined for bases in 50% ethanol. As these figures were required for the calculation of some of the values of Table III, the pK_a values of a weak and a strong base, *viz.*, acridine and 5-aminoacridine, have been determined at various temperatures in the range 5—40° and are given in Table II. These two bases give temperature coefficients of −0·0083 (−0·0125) and −0·031 (−0·020) pK_a per unit per degree respectively, the values in parentheses being those for bases of the same pK_a determined in water by Hall and Sprinkle. It would seem that temperature coefficients in 50% alcohol are of the same order as those in water.

TABLE II.

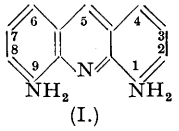
Relation between temperature and pK_a of a weak and a strong acridine base in 50% ethanol (dilution = 120).

Temp.	5°.	10°	15°.	20°.	25°.	30°.	35°.	37°.	40°.	Temp. coeff.
pK_a, acridine	4·30	4·24	4·14	4·11	4·05	4·01	3·98	3·97	3·93	−0·008
pK_a, 5-aminoacridine ...	9·93	9·75	9·65	9·47	9·29	9·14	8·95	8·90	8·81	−0·031

Effect of Substituents on Ionisation.—The effect of a second amino-group on the ionisation of an aminoacridine should vary according to the position in which it is inserted, because it was shown (Albert and Goldacre, *loc. cit.*) that (i) the primary amino-group in 3- and 4-aminoacridine had a normal aromatic character, (ii) in 1-aminoacridine exerted a base-weakening ortho-effect, and (iii) those in the 2- and the 5-isomeride entered into base-strengthening resonance with the ionised ring nitrogen. That the ring nitrogen is the first to accept a proton in all the monoaminoacridines was shown spectrographically by Craig and Short (*J.*, 1945, 419) and by Turnbull (*ibid.*, p. 441).

It is seen from Table III that the introduction of a second amino-group in the 3- or the 4-position increases the basic strength by a small amount corresponding to the effect that this operation has on acridine itself (cf. Nos. 3, 4, 9, 12, 13, 14). It does not seem to matter whether the primary amino-group originally present was also a normal aromatic group (No. 14) or one resonating with the ionised ring nitrogen (Nos. 9, 12, 13). When the second amino-group is placed in the 2- or the 5-position where it can resonate with the ionised ring nitrogen, a considerably greater increase in basic strength is seen, just as when this operation is carried out on acridine itself (cf. Nos. 2. 5, 9, 10, 11, 12, 13). This increase is very great when the first amino-group is normal (Nos. 9, 12, 13), but even where it is resonant (Nos. 10, 11, 44) the opportunities afforded for extra resonance by the contribution of alternative forms to the resonance hybrid have increased the basic strength substantially more than was effected by a normal amino-group. Just as 2- and 5-aminoacridines are vinylogous amidines, so 2 : 5- and 2 : 8-diaminoacridines are vinylogous guanidines and hence proportionately stronger as bases.

Finally, the base-weakening character of an amino-group inserted in the 1-position is maintained when an amino-group is already present (Nos. 1, 6, 7, 8). In the case of 1 : 9-diaminoacridine (I), the presence of two primary amino-groups ortho to the ring nitrogen have had the effect of excluding the proton from the latter,



(I.)

and Craig (this vol., p. 534) has shown spectrographically that the mono- and di-ions have the protons on the amino-groups and that the low pH of 18N-sulphuric acid is necessary to force a proton on to the ring nitrogen. Actually, the first and second pK_a values of this substance are closer together than those of the other diaminoacridines. The highly weakening effect of the 1-amino-group on the basic strength of 2- and 5-aminoacridine (cf. Nos. 7 and 8) cannot be explained in terms of mere steric hindrance, because the slightly larger methyl group, as in 5-amino-1-methylacridine (No. 23), is base-strengthening. The figures for these two diamines lend additional support to a hypothesis advanced for 1-aminoacridine (Albert and Goldacre, *loc. cit.*) and the analogous 8-aminoquinoline (*idem*, *Nature*, 1944, **153**, 467), *viz.*, that an ortho-effect, akin to hydrogen bonding between the two nitrogen atoms, is interfering with the approach of protons to the ring nitrogen. In all these cases, however, the ring nitrogen is still the first to accept a proton, as the bathochromic nature of the change from base to mono-ion proves (cf. Craig and Short, *loc. cit.*).

The effect of alkyl substituents in the acridine nucleus should be to increase the basic strength by a small amount, for they are electron-repelling. Reference to Nos. 15 to 32 (Table III) shows that this effect may vary from 0·09 to 0·59 unit per methyl group, excluding cases where there is a methyl group in the 1-position. In the latter cases, an increase in basic strength is still seen where there is only one methyl group ortho to a ring nitrogen of a highly basic character (5-amino-1-methylacridine, No. 23). However, when both ortho-positions are substituted with methyl groups, or when the ring nitrogen is not highly basic (Nos. 28, 29; 15, 18, 19, 20, and 22), a significant depression of basic strength is seen. No (static) steric hindrance to the approach of protons could explain the depression and elevation of basic strength by the insertion of a methyl group in the 1-position of acridine and 5-aminoacridine respectively, since the access and escape of hydrogen ions would be prevented equally.

1-Amino-9-methylacridine (No. 20) differs from its isomerides in that there are two mono-ions: the normal ion involving the ring nitrogen is red, but it exists in various solvents in equilibrium with different amounts of a colourless form involving the amino-group (see Craig, *loc. cit.*; also Experimental Section).

The phenyl-substituted acridines (Nos. 33—35) exert a base-weakening effect consonant with the electron-attracting nature of this group. In 5-amino-1-phenylacridine a steric effect is probably present also.

The effect of alkoxy-substituents attached to an aromatic structure is not entirely predictable. As with amino-substituents, there is an electrophilic effect (inductive) and an electron-repelling effect (resonant), but with alkoxy-substituents the relative magnitude of the latter effect is not so large. Thus, *m*-, *o*-, and *p*-anisidines (and phenetidines) are weaker than, equal to, and stronger than aniline, respectively, as bases. Likewise, substitution of an alkoxy-group into acridines (Nos. 36—44) causes a small but variable effect on the pK ranging from −0·35 to +0·24 unit.

TABLE III.

Ionisation of acridines at 20° (± 0·5°).

(Determined by potentiometry with glass electrode in 50% ethanol unless otherwise stated. Values marked "H_2O" were determined by absorptiometry in water.)

No.	Substance.	Source.	pK_a.*	ΔpK_a.†	Diln.
	Acridine	A	4·11		120
Diaminoacridines.				Acridines.	
6	1 : 9-	B	3·55	−0·04; 1-Amino-	300
7	2 : 9-(= 1 : 8)	C	6·74	−0·87; 2-Amino- +3·15; 1-Amino-	200
8	1 : 5-	B	8·78	−0·67; 5-Amino- +5·19; 1-Amino-	160
9	3 : 5-	D	9·91	+0·46; 5-Amino- +4·88; 3-Amino-	40
10	2 : 5-	E	11·01	+3·40; 2-Amino- +1·56; 5-Amino-	40
	2 : 5- (potentiometry in H_2O)		11·49	+1·50; 5-Amino- (H_2O)	20
11	2 : 8-	F	9·50	+1·89; 2-Amino-	40
12	2 : 7- (= 3 : 8-)	C, G	7·74	+0·13; 2-Amino- +2·71; 3-Amino-	80
13	2 : 6- (= 4 : 8-)	H	8·55	+0·94; 2-Amino- +3·05; 4-Amino-	40
14	3 : 7-	I	5·64 6·18 (H_2O)	+0·61; 3-Amino- +0·30; 3-Amino- (H_2O)	600 100,000
C-Alkyl-substituted acridines.					
15	1-Methyl-	J	3·95	−0·16; Acridine	80
16	3-Methyl-	J	4·60	+0·49; Acridine	60
17	5-Methyl-	K	4·70	+0·59; Acridine	60
18	1 : 9-Dimethyl-	A	2·88 } Absorptiometry	−1·23; Acridine	5,000
19	1 : 3 : 4 : 6 : 7 : 9-Hexamethyl-	L	3·0 } in 50% ethanol	−1·1; Acridine	30,000
20	1-*Amino-9-methyl-*	M	3·22	−0·37; 1-Amino-	160
21	3-*Amino-5-methyl-*	M	5·59	+0·57; 3-Amino-	120
22	4-Amino-1-methyl-	G	4·79	−0·71; 4-Amino-	160
23	5-Amino-1-methyl-	N	9·73	+0·28; 5-Amino-	60
24	5-Amino-2-methyl-	N	9·71	+0·26; 5-Amino-	240
25	5-Amino-3-methyl-	N	9·54	+0·09; 5-Amino-	60
26	5-Amino-4-methyl-	N	9·60	+0·15; 5-Amino-	120
27	5-Amino-1 : 3-dimethyl-	N	9·99	+0·54; 5-Amino-	60
28	5-Amino-1 : 9-dimethyl-	N	8·82	−0·63; 5-Amino-	120
29	2 : 8-Diamino-1 : 9-dimethyl-	O	8·51	−0·99; 2 : 8-Diamino-	120
30	2 : 8-Diamino-3 : 7-dimethyl (acridine-yellow)	P	9·7	+0·2; 2 : 8-Diamino-	120
31	2 : 8-Diamino-4 : 6-dimethyl-	G	10·08	+0·58; 2 : 8-Diamino-	40
32	5-Amino-1-ethyl	N	9·66	+0·21; 5-Amino-	120
33	5-Amino-1-phenyl-	N	8·67	−0·78; 5-Amino-	300
34	5-Amino-3-phenyl-	N	9·24	−0·21; 5-Amino-	120
35	2 : 8-Diamino-5-phenyl-3 : 7-dimethyl- (Benzoflavine)	Q	9·11	−0·39; 2 : 8-Diamino-	300
Alkoxy-substituted acridines.					
36	1-Methoxy- (m. p. 134°; lit. gives 130—131°)	K	4·19	+0·08; Acridine	120
37	3-Methoxy-	R	4·27	+0·16; Acridine	120
38	2 : 8-Dimethoxy-	S	5·7	+0·1; Acridine	120
39	5-Amino-1-methoxy-	N	9·38	−0·07; 5-Amino-	120
40	5-Amino-2-methoxy-	N	9·57	+0·12; 5-Amino-	120
41	5-Amino-3-methoxy-	N	9·10	−0·35; 5-Amino-	240
42	5-Amino-4-methoxy-	N	9·69	+0·24; 5-Amino-	240
43	2 : 8-Diamino-3 : 7-dimethoxy	E	9·17	−0·33; 2 : 8-Diamino-	320
44	2 : 5-Diamino-7-ethoxy- (Rivanol)	E	11·04	+0·10; 2 : 5-Diamino-	160

* For definition, see Table I. † That is, the amount by which pK_a exceeds that of the parent substance named.

No.	Substance.	Source.	pK_a.*	ΔpK_a.†	Diln.
Chloro-, cyano-, and nitro-substituted acridines.				Acridines.	
45	5-Chloro-2-amino-	T	5·97	−1·64; 2-Amino-	200
	5-Chloro-2-amino-		6·74 H_2O	−1·30; 2-Amino (H_2O)	100,000
46	7-Chloro-2-amino-	U	6·53	−1·08; 2-Amino-	120
	7-Chloro-2-amino-		6·95 H_2O	−1·09; 2-Amino- (H_2O)	100,000
47	8-Chloro-2-amino-	U	6·85	−0·76; 2-Amino	120
	8-Chloro-2-amino-		7·26 H_2O	−0·78; 2-Amino- (H_2O)	100,000
48	8-Chloro-3-amino-	U	3·98	−1·05; 3-Amino-	240
49	1-Chloro-5-amino-	N	7·82	−1·63; 5-Amino-	120
50	2-Chloro-5-amino-	N	8·33	−1·12; 5-Amino-	80
51	3-Chloro-5-amino-	N	8·31	−1·02; 5-Amino-	120
52	4-Chloro-5-amino-	N	7·89	−1·56; 5-Amino-	200
53	3 : 7-Dichloro-2 : 8-diamino-	G	7·64	−1·86; 2 : 8-Diamino-	400
54	2-Chloro-5-amino-7-methoxy-	V	8·01	−1·44; 5-Amino-	1,000
55	5-Amino-3-cyano-	N	7·46	−1·99; 5-Amino-	240
56	1-Nitro-	D	3·0	−1·1; Acridine	375
57	1-Nitro-5-amino-	N	7·34	−2·11; 5-Amino-	240
58	2-Nitro-5-amino-	E	7·30	−2·15; 5-Amino-	300
59	3-Nitro-5-amino-	D	7·3	−2·1; 5-Amino-	60
60	4-Nitro-5-amino-	N	6·95	−2·50; 5-Amino-	150
61	2-Nitro-5-amino-7-ethoxy-	E	6·6	−2·9; 5-Amino-	1,000
	2-Nitro-5-amino-7-ethoxy-		7·5 H_2O	−2·5; 5-Amino- (H_2O)	50,000
N-Alkyl-substituted acridines.					
62	2-Dimethylamino-	G	7·80	+0·19; 2-Amino-	120
63	2 : 8-Bisdimethylamino (Acridine orange)	W	10·04	+0·54; 2 : 8-Diamino-	160
64	7-Amino-2-dimethylamino-	G	8·02	+0·28; 2 : 7-Diamino-	80
65	5-Dimethylamino-	D	7·53	−1·92; 5-Amino-	40
66	5-Methylamino-	D	9·77	+0·27; 5-Amino-	40
67	5-Butylamino-	V	9·35	−0·10; 5-Amino-	40
68	5-Heptylamino-	V	9·35	−0·10; 5-Amino-	40
69	5-Dodecylamino-	V	9·1	−0·4; 5-Amino-	40
70	5-Hexadecylamino-	V	8·7	−0·8; 5-Amino-	500
71	5-*cyclo*Hexylamino-	V	8·78	−0·67; 5-Amino-	40
72	5-Phenylamino-	D	7·01	−2·44; 5-Amino-	240
73	5-(β-Hydroxyethyl)amino-	N	8·77	−0·68; 5-Amino-	200
74	5-(β-5′-Acridylamino)ethylamino- (*i.e.*, bisacridylethylenediamine)	N	8·52; $pK_2 = 6·92$	−1·0; 5-Amino-	1,200
75	Benzylideneamino-	D	3·37	−0·22; 1-Amino-	400
Miscellaneous acridines.					
76	3-*Aminomethyl*-	M	8·74; $pK_2 = 2·1$	+3·71; 3-Amino-	100
77	5-β-Aminoethyl-	X	8·57; $pK_2 = 3·95$	−0·88; 5-Amino-	300
78	5-*p*-Dimethylaminophenyl-	Y	4·4		3,000
79	2-Amino-5-*p*-aminophenyl- ("Phosphine")	Z	7·71	+0·10; 2-Amino-	120
80	5-*p*-Aminostyryl-	AA	5·10		1,000
81	5-*m*-Aminostyryl-	AA	4·43		600
82	5-*p*-Dimethylaminostyryl-	AA	3·07		1,200
83	1 : 2 : 3 : 4-Tetrahydro-	BB	5·07	+0·96; Acridine	120
84	5-Amino-1 : 2 : 3 : 4-tetrahydro-	N	9·40	−0·05; 5-Amino-	60
85	3-Aminoacridan	D	4·72	−0·31; 3-Amino-	80

* For definition, see Table I. † That is, the amount by which pK_a exceeds that of the parent substance named.

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The effect of chloro-, cyano-, and nitro-substituents, because of their strong electron-attracting character, is to diminish the basic strength of acridines very considerably. Among the monochloro-derivatives (Nos. 45—54) a depression of −0·76 to −1·64 units is observed, among the nitro-derivatives (Nos. 56—61) from −1·1—−2·9, and the cyano-derivative (No. 55) has a depression of −1·99 units. It is noteworthy that the marked differences (*e.g.*, +2·8 units) between the basic strengths of isomeric nitroanilines do not occur here because the peculiar resonance forms of *o*- and *p*-nitroanilines have no close representatives in the present compounds.

The effect of *N*-methyl substitution on the basic strength of aminoacridines is in general similar to its effect on that of aromatic amines which Hall and Sprinkle found (*loc. cit.*) to increase by 0·2 unit for each methyl group inserted (see Nos. 62, 63, 64, 66). As the amines under consideration are all derivatives of 2- and 5-aminoacridine, which owe their high basic strength to ionic resonance, it seemed likely that alkylation might so change the equivalence of the resonance forms that the basic strength would be seriously altered (cf. guanidine; Pauling, " Nature of the Chemical Bond," New York, 1942, p. 213). The results suggest that equivalence has not been seriously disturbed.

5-Dimethylaminoacridine (No. 65) shows a large depression of basic strength, indicating that ionic resonance has been diminished by steric interference between a methyl group and a hydrogen atom in the 4- or 6-position. This overlap of covalent radii is critical because a very small decrease in the distance, as in 5-amino-4-methylacridine (No. 26) or (through bending of the $N{-}C^5$ bond) in 5-methylaminoacridine (No. 66), prevents this fall in pK_a.

Nos. 72—75 represent types of electron-attracting *N*-alkyl substitution which are always base-weakening, as here.

Miscellaneous substituents (Nos. 76—85). The insulating effect of one or two methylene groups is seen in Nos. 76 and 77, where the first proton goes on to the side chain and the first pK_a value approximates to that of benzylamine (9·4) because the interaction between the amino-group and the conjugated system is largely eliminated. There is no colour or fluorescence change on adding the first proton, but addition of the second proton causes a change in colour from cream to yellow and in fluorescence from violet to green, all characteristic of the ionisation of the ring nitrogen in acridine itself and at a similar pK_a. That 5-β-aminoethylacridine can form a di-ion at pH 4 stands in contrast to 5-aminoacridine, which, because of the intense resonance in the mono-ion, gives no (spectrographic) evidence of the formation of a di-ion even in concentrated sulphuric acid (Craig, private communication). It is evident that insulating methylene groups provide a further method for converting weak heterocyclic bases into bases fully ionized at pH 7 without recourse to quaternisation or to ionic resonance.

Nos. 78—82 form a group of acridines with highly conjugated substituents in the 5-position. The fact that certain of these with *p*-amino-substituents (Nos. 78, 80, 82) formed purple to blue mono-ions whilst an isomeride with a *m*-amino-substituent (No. 81) did not, suggested that a base-strengthening resonance might be present, involving both nitrogen atoms. However, the low basic strength shows that this is not the case, and that the separate resonances of the acridine and the phenylene portion of the molecule must be too unrelated for this to occur.

A few partly hydrogenated acridines (Nos. 83—85) are seen not to differ greatly from their acridine analogues, and may be contrasted with the behaviour of pyridine (pK_a 5·0) and its hydrogenation product piperidine (pK_a 11·5).

The effect of acidic substituents is recorded in Table IV. Only derivatives of monoacidic bases (acridine and 2- and 5-aminoacridine) were examined, so that only one of each pair of pK_a values can refer to a cationic ionisation. The correct allocation of the values was simplified by consideration of the ester (Nos. 89, 92, and 97) in which the carbomethoxy-group, as expected, has caused a fall in basic strength (−1·22 to −2·15 pK_a units) intermediate between that shown by the nitro- and the chloro-derivatives. The amide group (in No. 96) behaved similarly.

It is known that the carbomethoxy- and the carboxy-group have an equal effect on the ionisation of another group in the same molecule (Edsall and Blanchard, *J. Amer. Chem. Soc.*, 1933, **55**, 2337). Hence, any differences seen in the present series between the pK_a values of the esters and the corresponding acids must be attributed to the usual interaction between the ionised (acidic and basic) groups of the latter, as in glycine. Such interaction increases the basic strength relative to that of the ester, in the present examples from +1·4 to +1·6 pK_a units (cf. Gane and Ingold, *J.*, 1931, 2153).

The effect of 50% alcohol on the basic constants (as determined in water) provides another diagnostic test for " zwitterion " structure in amino-carboxylic acids, Jukes and Schmidt (*J. Biol. Chem.*, 1934, **105**, 359) having shown that the usual depression of basic strength in bases (see above) is often lacking and a slight elevation may even occur. Although these authors worked only with aliphatic acids, this effect is seen in Nos. 86 and 87. (The pK_a of the acid group is not of diagnostic value, as acid strength is decreased by alcohol whether the compound is a zwitterion or not.)

The three acridine monocarboxylic acids showed only one marked colour change, corresponding to the basic pK_a.

Acridine-1-carboxylic acid and its 5-amino-derivative (Nos. 86 and 94) exhibit an enormous increase in basic strength over their 3-isomerides (ΔpK_a = + 2·56 and +3·7 respectively). This is attributable to Coulombic interaction between the charged groups (Gane and Ingold, *loc. cit.*), which are very close here, and to

TABLE IV.

Ionisation of amphoteric acridines and some derivatives at 20°.

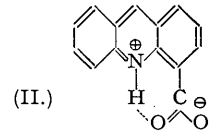
(Determined potentiometrically in 50% ethanol unless otherwise stated. Values marked " H_2O " were determined by absorptiometry in water.)

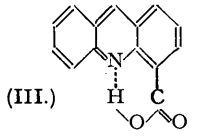
No.	Substance.	Source.	pK_a (basic).	ΔpK_a (basic).	pK_a (acidic).	Dilution.
86	*Acridine-1-carboxylic acid*	M	7·98	+3·87; Acridine	3·1	600
	Acridine-1-carboxylic acid		7·72 (H_2O)	+2·12; Acridine (H_2O)		5,000
87	*Acridine-3-carboxylic acid*	M	5·42	+1·31; Acridine	2·9	1,000
	Acridine-3-carboxylic acid		5·22 (H_2O)	−0·38; Acridine (H_2O)		13,000
88	Acridine-5-carboxylic acid	CC	5·0 (H_2O)	−0·6; Acridine (H_2O)	3	50,000
89	Methyl ester of No. 88	K	3·45 (H_2O)	−2·15; Acridine (H_2O)	None	14,000
90	Acridine-3-sulphonic acid	DD	3·96	−0·15; Acridine	2·5	60
	Acridine-3-sulphonic acid		4·74 (H_2O)	−0·86; Acridine (H_2O)		600
*91	2-Aminoacridine-7-carboxylic acid	EE	8·0 (H_2O)	−0·04; 2-Aminoacridine (H_2O)	2·3	100,000
92	*Methyl* ester of No. 91	M	6·39	−1·22; 2-Aminoacridine	None	300
	Methyl ester of No. 91		7·1 (H_2O)	−0·9; 2-Aminoacridine (H_2O)	None	100,000
*93	2-Aminoacridine-7-sulphonic acid	FF	7·32	−0·29; 2-Aminoacridine		600
	2-Aminoacridine-7-sulphonic acid		7·6 (H_2O)	−0·4; 2-Aminoacridine (H_2O)	<2	21,000
94	5-Aminoacridine-1-carboxylic acid	N	13	+3·6; 5-Aminoacridine	2·7	300
†95	5-Aminoacridine-3-carboxylic acid	N	9·35	−0·1; 5-Aminoacridine	4·06	1,100
96	Amide of No. 95	N	8·20	−1·25; 5-Aminoacridine	None	240
†97	Methyl ester of No. 95	N	7·96	−1·49; 5-Aminoacridine	None	300
*98	5-Amino-1-hydroxyacridine	N	>12	>+2·5; 5-Aminoacridine	7·79	120
	5-Amino-1-hydroxyacridine				7·05 (H_2O)	5,000
*99	5-Amino-2-hydroxyacridine	N	>12	>+2·5; 5-Aminoacridine	7·18	120
	5-Amino-2-hydroxyacridine				6·63 (H_2O)	5,000
*100	5-Amino-3-hydroxyacridine	N	>12	>+2·5; 5-Aminoacridine	8·55	120
	5-Amino-3-hydroxyacridine				7·71 (H_2O)	5,000
*101	5-Amino-4-hydroxyacridine	N	12·3	+2·8; 5-Aminoacridine	6·00	120
	5-Amino-4-hydroxyacridine			+2·8; 5-Aminoacridine	5·61 (H_2O)	5,000

* Some values for these compounds have been previously reported with the basic and acidic pK_a values reversed.
† From these figures the zwitterionic ratio (K_z) in No. 95 is seen to be 7900 (pK_z = 3·90).

K, M, and N; as in Table III.
CC. Homberger and Jensen, *J. Amer. Chem. Soc.*, 1926, **48**, 800.
DD. Matsumura, *ibid.*, 1935, **57**, 1533.
EE. Albert and Goldacre, *J.*, 1943, 454.
FF. Aarons and Albert, *J.*, 1942, 183.

hydrogen bonding which causes the proton to be more firmly attached to the molecule, as in (II), (III) or a resonance hybrid of both. Consequently, the change from $\gtrless NH^{\oplus}$ to $\gtrless N$ takes place at a higher pH value.





Though zwitterionic nature has not previously been attributed to phenolic derivatives of moderately strong bases (pK_a 8—12), the hydroxy-derivatives of 5-aminoacridine (Nos. 98—100; Table IV) must ionise in this way because the lower pK_a (*e.g.*, 7·71 for 5-amino-3-hydroxyacridine in water) increases when alcohol is added. As this behaviour is not that of a normal basic group, and as the basic group of a zwitterion would have to be the higher of the two values, the lower pK_a is assigned to the acidic group. Further, the bathochromic change at pH 7 on adding alkali (from yellow to orange) is typical of the ionisation of a phenolic group, whereas the hypsochromic change (from orange to yellow) at pH 12 is not.

This increase in acid strength above the values found for the relevant hydroxyacridines (pK_a *ca.* 10) is heightened by resonances involving keto-forms in the 2- and 4-isomerides. Hydrogen bonding between the primary amino-group and the negatively charged oxygen atom (forming a 6-membered ring) still further heightens the acid strength of the 4-isomeride by hindering the approach of hydrogen ions to the oxygen. As there is evidence (see above) for hydrogen bonding in 1-aminoacridine and its derivatives, it is surprising that it is not more discernible in 1-hydroxyacridine (Albert and Goldacre, *loc. cit.*) and 5-amino-1-hydroxyacridine.

In a collateral publication (Albert, Rubbo, Goldacre, Davey, and Stone, *loc. cit.*) these hydroxy-derivatives of 5-aminoacridine were described as anomalous in that they did not exert an antibacterial action proportional to the amount of cation believed to be present (at pH 7·3), the pK_a values being computed by adding 0·5 to the values obtained in 50% alcohol. Direct determination of the pK_a values in water has now revealed the (partly) zwitterionic nature of these compounds, which renders the computation inapplicable. It is now seen that the 3-isomeride (No. 100) contains only 76% cation at pH 7·3 (20°), and the other isomerides still less.

The pK_a values of the two sulphonic acids were assigned by rough analogy to the carboxylic acids although no elevation of the basic pK_a value by alcohol was observed.

Experimental.

Potentiometric Titrations.—These were carried out by running N/20-hydrochloric acid in 50% aqueous alcohol into a mechanically stirred solution of the base (usually 0·00025 g.-mol. in 40 ml.), followed by N/20-potassium hydroxide if acid groups were present. The solution was contained in a thermostatically jacketed beaker containing the glass and the calomel electrode supplied with the Leeds and Northrup Universal pH potentiometer set, to which they were connected with insulated and shielded leads. The set was checked with buffer solutions throughout its pH range. The values obtained were corrected, where necessary, for hydrolysis of the salt formed, and above pH 10, for potassium-ion error. No correction was made for any effect of alcohol on the glass electrode or on the saturated potassium chloride salt junction. Running alkali into a solution of the hydrochloride of a base was avoided, particularly when the base had a high pK_a, to prevent potassium-ion error in the glass electrode. No such error arises with organic cations. A typical result is as follows:

No. 16. 3-*Methylacridine* (50% ethanol; M/60-solution).

% Neutralized	10	21	32	42	53	63	74	84
pK_a	4·61	4·62	4·59	4·58	4·56	4·58	4·62	4·61

With 5-aminoacridine (and with many of its derivatives of pK_a above 9), a slight but definite apparent decline in basic strength is seen after 60% neutralisation and is attributed to association, *e.g.*, one where a resonance association between ion and base causes a proportion of the latter to combine with its own ion. A striking example of this effect is the following:

No. 35. *Benzoflavine* (50% ethanol; M/300-solution).

% Neutralized	27	43	59	75	91
pK_a	9·15	9·14	9·03	8·62	8·2

The tendency to micelle formation of benzoflavine is illustrated by the gelling of a M/50-aqueous solution of its hydrochloride. Gelling does not occur in 50% alcohol, but the titration figures indicate that the micelles are not completely broken up in spite of the lower dielectric constant of the solvent.

Absorptiometry.—A solution of the hydrochloride of the base was added to a series of M/100-buffers of different pH so that the final concentration of the base was about M/10,000. Some substances, notably zwitterions, were so insoluble that they had to be measured at M/50,000. Where precipitation prevented a satisfactory reading being obtained, 50% alcoholic buffers were used. These usually had a pH of about 1 unit higher than the aqueous buffers from which they were prepared (effect of alcohol on the pK_a of the buffer acid). The approximate wave-length of maximum difference in extinction coefficient (ϵ) between the solution of the ion and the base was noted on a Pulfrich photometer, and the extinction measured at intervals of about 0·5 pH unit on a sensitive model Hilger Spekker absorptiometer fitted with Ilford gelatin filters. From the degree of ionisation found at various pH values, the pK_a was calculated and averaged. The difficult determination of the pK_a of 5-aminoacridine, where a suitable difference in ϵ extends over only 40 A., was accomplished by using the 4360 A. mercury line (isolated with a filter).

1-*Amino*-9-*methylacridine.*—3-Methylanthranilic acid, *o*-bromonitrobenzene, and dehydrated potassium carbonate (10 g. of each), catalytic copper (0·1 g.), and *cyclo*hexanol (20 ml.) were refluxed for two hours in a bath at 175°. After steam-distillation, the residual solution was filtered from copper, acidified, and filtered at the boil, giving 12 g. of brown crystals, m. p. 180°. Recrystallisation from 60% alcohol and from 80 parts of benzene gave bright yellow crystals of 2′-*nitro*-6-*methyldiphenylamine*-2-*carboxylic acid*, m. p. 188—189° (Found: C, 62·2; H, 4.4; N, 10·3. $C_{14}H_{12}O_4N_2$ requires C, 61·7; H, 4·45; N, 10·3%). This acid (10 g.) was heated in a boiling water-bath for 15 minutes with sulphuric acid (70 ml.) cautiously poured into boiling water (500 ml.), and the mixture heated for ½ hour on the boiling water-bath. The 1-*nitro*-9-*methylacridone* was filtered off, washed, digested at 80° with dilute ammonia, washed with boiling water, and dried at 120°; yield, 70%, m. p. 226°. After recrystallisation from 50 parts of pyridine trihydrate and then from 150 parts of boiling alcohol, yellow-orange needles, m. p. 228°, were obtained, moderately soluble in glacial acetic acid or toluene (Found: N, 11·0. $C_{14}H_{10}O_3N_2$ requires N, 11·0%). Alcoholic sodium hydroxide gives a scarlet solution of the sodium derivative.

1-Nitro-9-methylacridone (5·3 g.), sodium bicarbonate (26 g.), and alcohol (150 ml. of 90%) were placed in a flask immersed in a bath maintained at 70°, stirred vigorously whilst a rapid stream of carbon dioxide was passed through the suspension, and sodium amalgam (270 g. of 2½%) added during 1½ hours. The contents were then refluxed for an hour, cooled, and filtered. The filtrate was taken to dryness and added to the precipitate, which was then treated with dilute acetic acid until effervescence ceased. The precipitate was freed from mercury and suspended in concentrated hydrochloric acid (50 ml.). Ferric chloride (140 ml. of 10%) was added, and the solution boiled for 15 minutes and filtered. The filtrate was concentrated to 50 ml. and refrigerated, yielding yellow crystals which were powdered, suspended in boiling sodium hydroxide solution, filtered, and recrystallised from light petroleum (50 parts). Yield 50% of 1-*amino*-9-*methylacridine*, yellow-orange needles, m. p. 111°, very soluble in methanol, alcohol, and benzene, all without fluorescence (Found: C, 80·7; H, 5·9; N, 13·4. $C_{14}H_{12}N_2$ requires C, 80·7; 5·9; N, 13·5%). The deep red solution in boiling 0·5N-hydrochloric acid turns pale yellow on cooling and deposits white crystals; this effect is reversible by re-heating. Unlike 1-aminoacridine, it does not give a red colour in 10% acetic acid. The yellow dihydrochloride crystallizes out of concentrated hydrochloric acid on cooling, as happens with 1-aminoacridine. The hydrochlorides do not diazotize normally, but give a white precipitate of the azoimide with nitrous acid, just as 1-aminoacridine does (cf. similar behaviour of 8-aminoquinoline under special conditions, Boehringer, D.R.P., 613,627).

3-*Amino*-5-*methylacridine.* 3-Nitro-5-methylacridine (2·5 g.; Jensen and Rethwisch, *J. Amer. Chem. Soc.*, 1928, **50**, 1144), stannous chloride crystals (11·25 g.), and concentrated hydrochloric acid (15 ml.) were heated in a boiling water-bath for an hour, and treated with sufficient sodium hydroxide solution to redden Orange-II paper. On recrystallisation of the precipitate from alcohol, bright yellow crystals were obtained (80% yield), m. p. 210—211°, unaltered by further recrystallisation (Found: N, 13·3. $C_{14}H_{12}N_2$ requires N, 13·45%). This *substance* was obtained in an impure state by Sharp, Sutherland, and Wilson (*J.*, 1943, 344). The base (1·4 g.) and acetic anhydride (3 ml.) were heated at 105° for ½ hour and washed with light petroleum–benzene (2 : 1). On recrystallisation from alcohol–benzene, pale yellow crystals of 3-acetamido-5-methylacridine, m. p. 273° (sealed tube), were obtained (90% yield) (Found: N, 11·2. Calc. for $C_{16}H_{14}ON_2$: N, 11·2%). The above authors reported m. p. 260° (decomp.), but the late Prof. F. J. Wilson confirmed our sharp m. p. later in 1943, using a sealed tube.

3-*Amino*-5 : 10-*dimethylacridinium bromide* was prepared as 3-amino-10-methylacridinium bromide (Albert and Ritchie, *J.*, 1943, 458) and purified by recrystallisation from 7 parts of water; deep red crystals, remaining hydrated at 110°, m. p. 259—260° (sealed tube) (Found: N, 8·8. $C_{15}H_{15}N_2Br,H_2O$ requires N, 8·7%). The red aqueous solution does not fluoresce. It gives no precipitate with sodium carbonate. Sodium hydroxide gives an immediate pale yellow precipitate of the pseudo-base, soluble in ether, and converted in turn into the red methobromide, and the yellow hydrobromide of the latter, by hydrobromic acid.

3-Aminomethylacridine. 4′-Cyanodiphenylamine-2-carboxylic acid (2·38 g.; Albert and Gledhill, *J. Soc. Chem. Ind.*, 1945, **64**, 169), aqueous ammonia (60 ml. of 10%), and Raney nickel (*ca.* 0·5 g.) were hydrogenated at room temperature and pressure. The mixture was filtered, and the filtrate boiled until free from ammonia, acidified slightly with acetic acid, and refiltered. The precipitate was dried at 120°, extracted with alcohol (30 ml.), taken up in alkali, and the solution stirred with decolorizing carbon, filtered, and acidified with acetic acid, affording *4′-aminomethyldiphenylamine-2-carboxylic acid* (70%) as a yellow-orange solid decomposing at about 200° (sealed tube), practically insoluble in common organic solvents and in water (Found: C, 69·3; H, 6·0; N, 11·5. $C_{14}H_{14}N_2O_2$ requires C, 69·4; H, 5·8; N, 11·6%). The hydrochloride is almost insoluble in water but dissolves readily in alcohol.

The above acid (2 g.) and sulphuric acid (6 ml.) were heated for ½ hour in a boiling water-bath, cooled, poured into water, and made just alkaline to phenolphthalein. The precipitate was dissolved in alcoholic sodium hydroxide, treated with charcoal, filtered, and the *3-aminomethylacridone* precipitated by adding water; bright yellow solid, m. p. 340° (sealed), almost insoluble in all common solvents (Found: C, 74·9; H, 5·5. $C_{14}H_{12}ON_2$ requires C, 75·0; H, 5·4%). It dissolves in concentrated hydrochloric acid and is precipitated on the addition of 2 vols. of water; on further dilution a violet fluorescence appears.

3-Aminomethylacridone (3·4 g.) and alcohol (67 ml.) were stirred for 2 hours at 80° whilst a brisk stream of carbon dioxide was passed through the mixture and sodium amalgam (54 g. of 2½%) added. Stirring and heating were continued for 2 hours more and the mixture was then filtered. The filtrate was taken to dryness, and the residue was dissolved in dilute hydrochloric acid, treated with ferric chloride crystals (8 g.), and boiled for ½ hour. Sodium carbonate was then added to faint alkalinity to litmus, and the solution filtered from iron carbonates. The precipitate was boiled once with water, filtered, and the combined filtrates concentrated to 40 ml. and chilled. The crystals were filtered off, dissolved in water (10 ml.), made faintly alkaline to litmus (precipitate rejected) and then chilled and made alkaline to orange-II paper. The oily precipitate solidified after scratching and refrigeration. It was filtered off and dried in a desiccator. From this crude base (1·6 g.; 50% yield) *3-aminomethylacridine hydrochloride* was prepared by dissolution in water (7 ml.) with 1 equiv. of concentrated hydrochloric acid. After dilution with acetone (7·5 ml.) and refrigeration, the hydrochloride was filtered off, washed with acetone, and dried at 120°, forming a creamy-white powder very soluble in water with violet fluorescence (Found: N, 11·6. $C_{14}H_{12}N_2$,HCl requires N, 11·5%). The dihydrochloride is yellow with a slight orange fluorescence. The free *base* forms buff crystals from 33% methanol and decomposes above 130° without melting sharply. It is volatile in steam and sternutatory. It is soluble in about 5 parts of boiling methanol (with a violet fluorescence) and rather soluble in cold water, but precipitated by excess of sodium hydroxide (Found: C, 80·7; H, 6·0; N, 13·3. $C_{14}H_{12}N_2$ requires C, 80·7; H, 5·8; N, 13·45%). It is decomposed by hot alkaline solutions, and is considerably less stable than 5-aminoethylacridine.

Acridine-1-carboxylic acid. Acridone-1-carboxylic acid (2·75 g.) was stirred with 0·5N-aqueous sodium hydroxide (25 ml.) for 2 hours at 80° whilst sodium amalgam (21 g.; 2½%) was added. The mixture was then boiled for 2 hours, filtered, and the filtrate made acid to methyl-orange with hydrochloric acid. The solution was boiled with a 3-fold excess of ferric chloride, made alkaline with solid sodium carbonate, and filtered whilst hot. The cake was extracted with sodium carbonate solution and the combined filtrates were treated with hydrochloric acid until methyl-orange paper was reddened. The precipitate (2·1 g.) was extracted with alcohol (200 ml.), and the extract concentrated to 15 ml. and diluted with water (60 ml.). *Acridine-1-carboxylic acid* (yield 60%) formed yellow crystals, m. p. 189—190° (Found: C, 75·1; H, 4·1; N, 6·3. $C_{14}H_9O_2N$ requires C, 75·3; H, 4·1; N, 6·3%), moderately soluble in hot water (with gradient) and in alcohol (almost colourless solution with very faint green fluorescence). It is sparingly soluble in benzene, very sparingly soluble in dilute acetic acid, and gives a bright yellow solution in dilute hydrochloric acid with a bright green fluorescence and a pale yellow solution in dilute sodium hydroxide with a moderate green fluorescence.

Acridine-3-carboxylic acid. Acridone-3-carboxylic acid (4 g.) was reduced with aluminium amalgam in water (method of Aarons and Albert, *J.*, 1942, 183). The combined filtrates and washings were treated with ferric chloride at the boil until the green colour of the acridan–acridine meriquinonoid complex disappeared. A great excess of ferric chloride was required because the complex, like the violet complex arising in the reduction of acridone-3-sulphonic acid, is stabilised by the electron-attracting substituent and the usual potassium ferrocyanide test for complete oxidation is invalid.

The precipitate was taken up in sodium carbonate solution, filtered, made neutral to litmus, and the *acridine-3-carboxylic acid* filtered off and recrystallised from aqueous cellosolve, giving buff, sternutatory crystals, decomposing without melting at 270° (Found: C, 75·8; H, 3·9; N, 6·5. $C_{14}H_9O_2N$ requires C, 75·3; H, 4·1; N, 6·3%). It is slightly soluble in water with a green fluorescence that becomes violet on dilution, practically insoluble in all common organic solvents, slightly soluble in dilute hydrochloric acid with a yellow colour and green fluorescence, and very soluble in dilute ammonia with a brilliant blue fluorescence that becomes violet on dilution.

Methyl 2-aminoacridine-7-carboxylate. 2-Aminoacridine-7-carboxylic acid (1 g.; Albert and Goldacre, *J.*, 1943, 454), methanol (10 ml.), and concentrated sulphuric acid (0·6 ml.) were refluxed for 6 hours, poured into ice and water, and made alkaline with sodium carbonate. The precipitate was taken up in cold, dilute acetic acid, the solution filtered, and the filtrate precipitated with sodium carbonate. After recrystallisation from 250 parts of 50% acetone, 0·4 g. of the *methyl* ester was obtained as orange crystals, m. p. 280° (sealed tube) (Found: C, 71·2; H, 4·7; N, 11·2. $C_{15}H_{12}O_2N_2$ requires C, 71·4; H, 4·8; N, 11·1%). It is somewhat soluble in methanol with an orange colour and intense green fluorescence, but almost insoluble in benzene and toluene. The hydrochloride is scarlet and dissolves in water with a green fluorescence. It diazotizes and couples normally.

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