76. *Some Physico-chemical Properties of Acridine Antimalarials, with Reference to their Biological Action. Part I. Basic Dissociation Constants and Reduction Potentials.*

By D. Ll. Hammick and S. F. Mason.

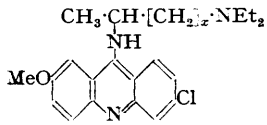
Two homologous series of acridine antimalarials analogous to Mepacrine (I) have been examined with the polarograph over a range of concentrations and pH values in order to measure reduction potentials and other quantities defining the oxidation–reduction behaviour of these compounds. Further measurements have been made of the pH values of aqueous and aqueous-alcoholic solutions of the dihydrochlorides of these derivatives in the presence of various amounts of alkali, and from the results obtained the basic dissociation constants of the compounds have been calculated. No definite correlation between antimalarial activity and either basicity or reduction potential has been observed.

Chemotherapeutic agents belonging to the same structural type appear to act on a given organism by the same or very similar mechanisms. The efficacy of their action varies widely however, and in some cases seems to depend on the values of certain physico-chemical properties, as Bell and Roblin (*J. Amer. Chem. Soc.*, 1942, **64**, 2905) have discovered for the sulphonamides, and Albert and his co-workers (*Brit. J. Exp. Path.*, 1945, **26**, 160) for the simple aminoacridines.

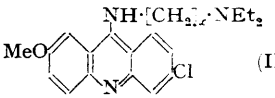
In the present work an attempt has been made to correlate the values of some physico-chemical properties of acridine antimalarials with their relative biological activities. The properties thought possibly relevant were selected by considering the nature of the action of Mepacrine on enzyme systems, bacteria, and the malaria parasite. Amongst other things, Mepacrine (I) interferes with the growth of bacteria by competing with natural bases, notably polyamines (Silverman and Evans, *J. Biol. Chem.*, 1944, **154**, 521), and also with the hydrogen carrier, riboflavin (Madinaveitia, *Biochem. J.*, 1946, **40**, 373; Haas, *J. Biol. Chem.*, 1944, **155**, 321). The competition of two bases and two oxidation–reduction systems for a common substrate may be said to be governed physico-chemically by the relative basicities and reduction potentials of the two substances. Thus it was thought that the relative values of the dissociation constants and the half-wave potentials of a structurally similar series of acridine antimalarials might possibly be related to their relative antimalarial activities.

Experimental.

Preparation of Materials.—Two homologous series of acridine antimalarials were prepared by standard methods (Magidson *et al.*, *Ber.*, 1936, **69**, 396; Breslow, *J. Amer. Chem. Soc.*, 1941, **63**, 156; 1946, **68**, 100). One, homologous with Mepacrine (I; $x = 3$), consisted of four members (I) having $x = 1$ to 4; the other possessed straight side-chains, six compounds (II) being prepared having $x = 2$ to 7.



(I.)



(II.)

2-*Chloro*-5-(1-*diethylamino*-2-*propylamino*)- (cf. I; $x = 1$), m. p. 223° (Found: C, 56·6; H, 6·4; N, 9·3; Cl, 22·4. $C_{21}H_{26}ON_3Cl,2HCl,H_2O$ requires C, 56·45; H, 6·7; N, 9·4; Cl, 23·05%), and 2-*chloro*-5-(7-*diethylaminoheptylamino*)-7-*methoxyacridine dihydrochloride* (cf. II; $x = 7$), m. p. 240—242° (Found: C, 58·4; H, 7·2; N, 8·3; Cl, 21·0. $C_{25}H_{34}ON_3Cl,2HCl$ requires C, 58·3; H, 7·5; N, 8·8; Cl, 22·1%), 5-(3-diethylaminopropylamino)acridine dihydrochloride, m. p. 230—232° (cf. Gupta, *J. Indian Chem. Soc.*, 1945, **22**, 364, who give m. p. 230°), and the compounds listed in Table I were examined.

Table I.

2-*Chloro*-5-(*R*)-7-*methoxyacridine dihydrochlorides* (cf. I and II).

R.	M. p.	Literature.
4-Diethylamino-2-butylamino *	262°	Magidson, *loc. cit.*; m. p. 265°.
6-Diethylamino-2-hexylamino	112	Kritchewski, *J. Microbiol. U.S.S.R.*, 1935, **14**, 642; m. p. 114°.
2-Diethylaminoethylamino	258—259	Breslow, *loc. cit.*; m. p. 257—259°.
3-Diethylaminopropylamino	249—251	,, ,, m. p. 254—255°.
4-Diethylaminobutylamino †	246—249	Magidson, *loc. cit.*; m. p. 246—248°.
5-Diethylaminoamylamino	266—268	Breslow, ,, m. p. 266—267°.
6-Diethylaminohexylamino	250—253	,, ,, m. p. 253—255°

* Free base, m. p. 80°. † Hydrate.

Polarography.—The above compounds were examined polarographically over a range of concentrations in supporting electrolytes buffered to various pH values. The buffers were 0·05M. and were prepared after Sörensen: pH 2 and pH 4, citrate–hydrochloric acid; pH 5·5 and pH 7·3, phosphate; pH 9, glycine–sodium hydroxide.

Breyer, Buchanan, and Duewell (*J.*, 1944, 360) found that 5-aminoacridine is reduced in two steps of approximately equal height at the dropping-mercury electrode. We find similar steps with our acridines, all of which are derivatives of 5-aminoacridine. The half-wave potentials and diffusion currents of the four Mepacrine homologues (I), at a concentration of 10^{-4}M. in various buffered supporting electrolytes, are given in Table II. The half-wave potentials of the homologues of the straight-chain series (II) vary in a similar manner, and their values for a drug concentration of 10^{-4}M. in supporting electrolytes of pH 7·3 are given in Table III.

TABLE II.

Mepacrine Homologues (I).

The diffusion currents were measured in microamps., and the half-wave potentials in volts. All the potentials are negative, and were measured relative to the saturated calomel electrode.

		pH 2.		pH 4.		pH 5·5.		pH 7·3.		pH 9.	
		1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.	1st step.	2nd step.
$x = 1$	$E_{\frac{1}{2}}$	0·74	—	0·78	1·18	0·84	1·20	0·86	1·21	0·85	1·09
	I_d	0·26	—	0·26	0·26	0·20	0·16	0·16	0·11	0·04	0·04
$x = 2$	$E_{\frac{1}{2}}$	0·74	—	0·80	1·18	0·91	1·20	0·94	1·21	0·93	1·09
	I_d	0·23	—	0·22	0·22	0·21	0·19	0·19	0·18	0·19	0·20
$x = 3$	$E_{\frac{1}{2}}$	0·74	—	0·80	1·18	0·93	1·20	0·97	1·21	0·94	1·09
	I_d	0·23	—	0·22	0·20	0·20	0·12	0·18	0·11	0·18	0·12
$x = 4$	$E_{\frac{1}{2}}$	0·74	—	0·80	1·18	0·93	1·20	0·97	1·21	0·94	1·09
	I_d	0·21	—	0·19	0·15	0·19	0·13	0·18	0·12	0·16	0·12

At pH 2 the hydrogen discharge wave overlaps the second reduction steps of these acridines in all cases.

TABLE III.

2-Chloro-5-R-7-methoxyacridines (II).

R.	50% EtOH–H_2O. pK_{b_1}.	50% EtOH–H_2O. pK_{b_2}.	H_2O. pK_{b_2}.	$E_{\frac{1}{2}}$. 1st step.	$E_{\frac{1}{2}}$. 2nd step.
$Et_2N{\cdot}CH_2{\cdot}CHMe-$	6·51	8·32	7·31	0·86	1·21
$Et_2N{\cdot}[CH_2]_2{\cdot}CHMe-$	5·90	7·65	6·80	0·94	1·21
$Et_2N{\cdot}[CH_2]_3{\cdot}CHMe-$	5·11	7·13	6·47	0·97	1·21
$Et_2N{\cdot}[CH_2]_4{\cdot}CHMe-$	4·52	6·99	6·26	0·97	1·21
$Et_2N{\cdot}[CH_2]_2-$	6·01	7·84	6·95	0·84	1·07
$Et_2N{\cdot}[CH_2]_3-$	5·50	7·00	6·41	0·93	1·19
$Et_2N{\cdot}[CH_2]_4-$	5·04	6·64	6·14	0·94	1·19
$Et_2N[CH_2]_5-$	4·90	6·47	6·00	0·94	1·19
$Et_2N{\cdot}[CH_2]_6-$	4·66	6·43	5·98	0·94	1·19
$Et_2N{\cdot}[CH_2]_7-$	4·44	6·27	5·77	0·94	1·19
5-(3-Diethylaminopropylamino)acridine	4·61	6·26	5·46	0·97	1·20

By applying Ilkovic's equation, *viz.*:

$$I_d = 605\, nD^{1/2}Cm^{2/3}t^{1/6}$$

it is found that only one electron is taken up at each step, indicating that the first product of reduction is a free radical. Thus, for Mepacrine itself, in a phosphate buffer of pH 7·3, at 18°, we have C, the concentration of Mepacrine in millimoles/l. = 0·0993, D, the diffusion coefficient = 4·11 cm.²/sec., m, the rate of flow of mercury = 1·36 mg./sec., and t, the drop time = 2·07 sec.

For the first step, I_d, the diffusion current = 0·185 μa.; hence, n = 1·065 electrons.

For the second step, I_d = 0·115 μa.; hence n = 0·705 electron.

The diffusion coefficient, D, was calculated from Nernst's equation, $D = \boldsymbol{RT}\Lambda_0/n\boldsymbol{F}^2$. An approx. value of Λ_0 for Mepacrine was obtained by measuring the equivalent conductance of 10^{-2}M-Mepacrine dihydrochloride (54·7 mhos/cm.²), and by multiplying this quantity by the ratio $\Lambda_0/\Lambda_{10^{-2}\text{M}}$. for barium nitrate, a salt of the same valency type.

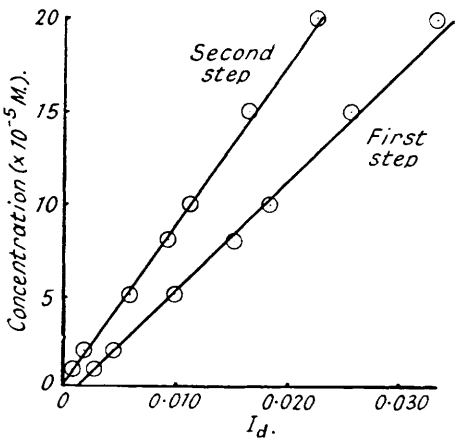
It was further found that there is a linear relation between the concentration of the acridine drug and the diffusion current to which it gives rise in the polarographic cell, for both steps of the reduction process over the concentration range 10^{-5} to 2×10^{-4}M. For the case of Mepacrine itself in buffered supporting electrolytes of pH 7·3, the results obtained are given in the table below (diffusion currents in microamps.).

Concentration, 10^{-5}M.....	1	2	5	8	10	15	20
I_d, 1st step	0·029	0·044	0·104	0·153	0·185	0·260	0·329
I_d, 2nd step	0·010	0·020	0·060	0·093	0·114	0·165	0·224

The gradient of the line connecting concentration with the diffusion current of the first step (1·750 mg. cm.²/sec.) is much closer to the theoretical value (1·705) calculated from the Ilkovic equation

than is that of the second step (1·250) (see Fig. 1). In the region where there is concentration–diffusion current proportionality, the current–voltage reduction curves obtained with the polarograph may be analysed by plotting log $I/(I_d - I)$ (where I is the current at chosen, successive points on the curve) against the applied E.M.F. Such an analysis of the waves given by Mepacrine at a concentration of 10^{-4}M., in a buffer supporting electrolyte of pH 7·3, gave a linear plot for both steps of the reduction. The plot obtained from the first wave again gave a gradient (57·6 mv.) closer to the theoretical (59·1 mv.) than that obtained by the analysis of the second wave (43·8 mv.) (see Figs. 2 and 3).

FIG. 1.



pH *Titrations.*—Mepacrine, and all the members of the two series of acridine antimalarials here dealt with, are di-acid bases. The second dissociation constant of any of these compounds can be determined by titrating an aqueous solution of the dihydrochloride with standard alkali, the pH being measured after each addition. Owing to the precipitation of free base after the addition of one equivalent of alkali, the first dissociation constant cannot be discovered in this way, as Christophers (*Ann. Trop. Med. Parasitol.*, 1937, **31**, 43) found in the case of Mepacrine. However, the free base is quite soluble in 50% aqueous-ethanolic solution and in this medium comparative values of both dissociation constants can be found by pH titrations. In this work N-sodium hydroxide was added from a microburette to 100 c.c. of M/100-solutions of the dihydrochlorides of each homologue, the pH being found by means of a glass electrode and Cambridge pH meter after each addition of alkali. No correction was applied for sodium-ion error as comparative values of the dissociation constants were all that this enquiry demanded. The results obtained are listed in Table III, together with the values of the half-wave potentials (reduction potentials), measured at a concentration of 10^{-4}M., in buffered supporting electrolytes of pH 7·3, at 18° ± 1°.

FIG. 2.

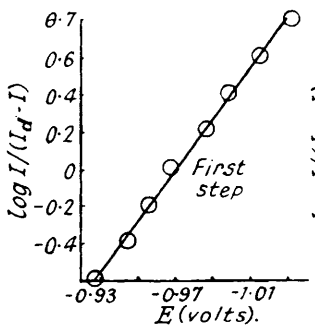
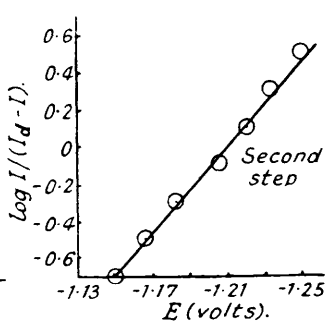


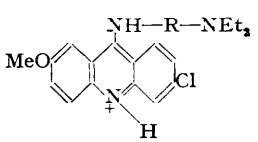
FIG. 3.



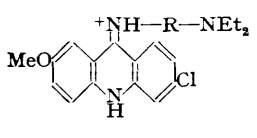
DISCUSSION.

The polarographic measurements indicate that the first step in the reduction of Mepacrine and its analogues is probably thermodynamically reversible, since it conforms fairly closely to theoretical expectations, whilst the second would appear to be irreversible, as the deviations from theory are much larger. These views are consonant with the hypothesis that a free radical is the product of the first step, and a 5 : 10-dihydroacridine derivative the product of the second, as the former would be labile and the latter more stable (Breyer *et al.*, *loc. cit.*).

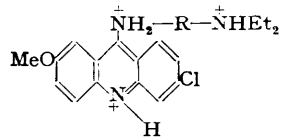
The increase in basicity with increasing length of side chain in these two series of acridine antimalarials may be ascribed to a decreasing field effect, though it is not possible to interpret this effect quantitatively in the terms used by Schwarzenbach (*Helv. Chem. Acta*, 1933, **16**, 522) for the symmetrical diamines, as the ionising powers of the two basic centres in these acridine derivatives cannot be taken as identical; further it is not possible to assign a precise location to the charge on the acridine nucleus, since the cation is probably a resonance hybrid of the forms (III) and (IV) :



(III.)



(IV.)



(V.)

In the first member of each series the tertiary nitrogen atom of the side chain is separated by only two carbon atoms from the secondary nitrogen atom at position 5 of the

acridine nucleus, and thus an inductive effect down the side chain may discourage the formation of the quinonoid structure (IV), compelling the nitrogen atom at position 10 to carry a greater share of the positive charge. The lowering of the electron density round the nuclear nitrogen atom would then account for the observed lower basicity and greater ease of reduction in the first member of the series. The inductive effects of the chloro- and methoxy-substituents also decrease the basicity and enhance the cationoid activity of these compounds, as 5-(3-diethylaminopropylamino)acridine possesses a greater basicity and lower reduction potentials than the corresponding 2-chloro-7-methoxy-derivative.

In strongly acid media there is the possibility of tri-acid salt formation, as there are three basic centres in these acridine antimalarials. Christophers (*loc. cit.*) has shown that the possible third dissociation constant in Mepacrine must be greater than pK_b 11, though it is well known that Mepacrine salts, when crystallised from acid solution, contain more than two equivalents of acid, the excess being tenaciously held. If the third dissociation constant of these 5-diaminoacridines lies between pK_b 11 and 12, the tri-acid salt will predominate in buffer solutions of pH 2. Since the resonance postulated above is impossible in the tri-acid cation (V), the electron densities round the nuclear nitrogen atom in all of the homologues should be the same; the electron densities should be smaller than at other pH values where resonance " spreads " the positive charge in the di-acid cation, which might account for the fact that the half-wave potentials of the homologues are identical at pH 2, and lower than at other pH values.

The antimalarial activity of these 5-diaminoacridines reaches a maximum in both series when four carbon atoms separate the nitrogen atoms of the side chain (Kritchevski, *loc. cit.*; Magidson, *loc. cit.*). However, neither the reduction potentials nor the basicities show a maximum or minimum at this point in the series. With antimalarial drugs a simple relation between the values of some prescribed physico-chemical property and biological efficacy is hardly to be expected. In practice the drug must traverse the cellular tissue of the host and perhaps that of the parasite in order to interfere with some enzyme system or systems within the latter organism, and quite different factors might determine transfer from those that determine efficacy at the site of action. It was decided therefore to investigate those physico-chemical properties of acridine antimalarials that might influence their transit through biological material. This work will be described and discussed in a subsequent communication.

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