926. *The Rates of Hydrolysis of Some Acridine Antimalarials.*

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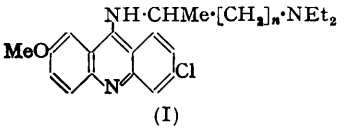
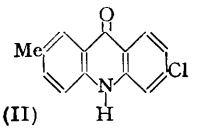
The rates of hydrolysis of mepacrine and three of its homologues (I; n = 1—4) in aqueous buffers of pH 7·3 and 9·1 at 37° have been measured. The polarograph was used to estimate the decrease in the concentrations of the compounds with time, the possible hydrolysis products (II—V) showing negligible interference under the experimental conditions. The hydrolyses are of the first order with respect to the acridine derivative, and the rate constants of the hydrolysis of mepacrine at the two pH's distinguish between the various possible mechanisms.

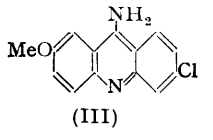
Aqueous solutions of mepacrine dihydrochloride (cf. I; n = 3) are hydrolysed to 4-amino-1-diethylaminopentane and 2-chloro-7-methoxyacridone (II), the hydrolysis being complete when a 5% solution is refluxed for 60 hours (Mietzsch and Mauss, *Indian Med. Gas.*, 1936, **71**, 521). When heated with concentrated hydrochloric acid under pressure for some hours at 120—125° mepacrine gives 5-amino-2-chloro-7-methoxy- (III) and 5-amino-2-chloro-7-hydroxy-acridine (IV) (Magidson and Grigorovsky, *Ber.*, 1936, **69**, 396). The degradation products obtained *in vitro* under acid conditions may be isolated from the urines of animals and patients ingesting mepacrine, together with 2-chloro-5-(4-diethylamino-1-methylbutylamino)-7-hydroxyacridine (V) (Scudi and Jelinek, *J. Biol. Chem.*, 1944, **152**, 27; Hammick and Firth, *Nature*, 1944, **154**, 461; Hammick and Mason, *ibid.*, 1945, **156**, 718). The acridone (II) obtained *in vitro* under milder hydrolytic conditions cannot be detected in the fresh urines of animals and patients dosed with mepacrine though it soon becomes detectable on storage.



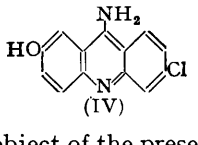
(I)



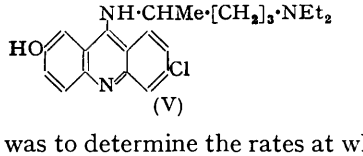
(II)



(III)



(IV)



(V)

The primary object of the present work was to determine the rates at which the acridine antimalarials (I; n = 1—4) are hydrolysed under the temperature and pH conditions of the human blood stream (37° and pH 7·3), and to characterise the compounds produced. It was hoped to throw light on the fate of mepacrine and its degradation products in the human body, and indicate further factors on which the differential biological activity of the homologous series depends (Hammick and Mason, *J.*, 1950, 345, 348, 351). For effective antimalarial action, the concentration of the acridine homologues in the blood stream must be maintained at a given value for weeks or months, and the rates at which they are hydrolysed were thought possibly to be related to their relative activities. Moreover, protein stained with acridone (II) does not take up mepacrine, while the same protein unstained does (Reid, Report to the M.R.C., M.L.A., 1944, p. 34). Thus the acridone (II) may influence the antimalarial action of mepacrine, and the rate at which (II) is produced by the homologues under physiological conditions may influence their relative activities.

As the polarographic diffusion current of mepacrine in buffered supporting electrolytes of pH 7·3 is proportional to its concentration at 10^{-5}—2×10^{-4}M (Hammick and Mason, *J.*, 1950, 345), it was thought that hydrolyses of the acridine homologues could be followed conveniently with the polarograph. An objection to the method was that the products might interfere but preliminary experiments with mixtures of mepacrine and its possible hydrolysis products (II—V) showed that (II) and (IV) were too insoluble at pH 7·3 to interfere, and that (III) and (V) gave polarograms qualitatively different from those given by mepacrine alone. In the latter case the mepacrine concentration could be estimated in

the mixtures, whilst amounts of (V) down to 4% of the total acridine concentration and of (III) down to 2% could be detected. Another possible objection was that the degradation products themselves might hydrolyse to give compounds which would interfere with the polarographic estimations; but, in solutions of pH 7·3 at 37°, (III) remained unchanged after 12 days and (II) and (IV) showed no change after a month under the same conditions, while (V) was hydrolysed to products which did not affect the polarographic estimations.

Hydrolysis of mepacrine and its homologues could be followed by analysing samples polarographically or by making the reaction vessel itself a polarographic cell. The latter course was adopted as more convenient, for, although some of the acridine homologue studied was reduced and thus lost when the polarographic determination was made, at most 0·04% of the total acridine was reduced during a single estimation. Accordingly some fifty estimations could be made before the experimental error in reading the diffusion current (approximately 1%) was exceeded.

Experimental

The homologues (I; n = 1—4) were prepared by standard methods (Magidson *et al.*, *Ber.*, 1936, **69**, 396; Breslow *et al.*, *J. Amer. Chem. Soc.*, 1941, **63**, 156; 1946, **68**, 100), and were made up to known concentrations of about 10^{-4}—2×10^{-4}M, the upper limit of the linear relation between polarographic diffusion current and concentration, in 0·05M-phosphate buffer of pH 7·3 (prepared after Sörensen). The buffer solution had been warmed previously in the thermostat at 37° ± 0·1°. A 25-c.c. sample of each freshly prepared solution was placed in a specially constructed polarograph cell which could be kept and operated in the thermostat. Concentrations were estimated by taking polarograms at 12—48-hour intervals, until 33—67% decomposition had occurred (10 days to a month). The half-life of each homologue was found to be independent of its initial concentration, so that the hydrolyses were of the first order with respect to the derivatives studied. The hydrolyses were also independent of the particular conditions prevailing in the polarograph cell—the presence of mercury and the absence of oxygen—for mepacrine in control solutions free from mercury or exposed to oxygen had the same half-life as in the experimental polarograph cells. Control solutions of mepacrine containing glass wool also had the same half-life, indicating that the hydrolysis was not a surface reaction. Light, however, appeared to enhance the rate of hydrolysis of mepacrine, so the thermostat was darkened during the runs. In the control experiments, the concentration of mepacrine was estimated polarographically in samples of the reactant solutions. In the main runs the reaction vessel itself was a polarographic cell: a typical run, starting with a mepacrine concentration of 85·2 mg./l., is given in the Table.

Time (hours)	Diffusion current (microamps.)	First-order velocity const. (hours^{-1}), $\times 10^3$	Error in velocity const. (obs. — avg.), $\times 10^3$	Time (hours)	Diffusion current (microamps.)	First-order velocity const. (hours^{-1}), $\times 10^3$	Error in velocity const. (obs. — avg.), $\times 10^3$
0	2·275	—	—	533	1·250	1·12	0·02
44	2·150	1·25	0·15	575	1·200	1·11	0·01
93	2·050	1·11	0·01	621	1·150	1·10	0
142	1·950	1·09	−0·01	669	1·100	1·09	−0·01
190	1·850	1·09	−0·01	717	1·050	1·08	−0·02
237	1·750	1·10	0	764	1·000	1·08	−0·02
285	1·650	1·20	0·10	815	0·950	1·08	−0·02
334	1·550	1·15	0·05	861	0·900	1·07	−0·03
382	1·475	1·13	0·03	909	0·850	1·07	−0·03
479	1·325	1·14	0·04				

At the end of each experimental run a precipitate was found in the reaction vessel. Polarograms given by the residual solution indicated that neither (III) nor (V) had been formed to an extent exceeding the limits of the polarographic method of detecting them (see above). After one of the mepacrine runs the precipitate formed was filtered off and washed free from adherent mepacrine and phosphate buffer. The precipitate was soluble in *iso*propyl alcohol, and a standard solution of the precipitated compound therein was made up. Polarograms given by this standard solution mixed with an equal volume of 0·1N-tetramethylammonium hydroxide showed a single reduction step of half-wave potential −1·47 v, a potential which preliminary experiments had shown to be characteristic of 2-chloro-7-methoxyacridone (II) in 50% *iso*propyl alcohol–tetramethylammonium hydroxide. Such preliminary experiments had in-

dicated also that the diffusion current given by the acridone (II) in this mixture was linearly proportional to its concentration over the range 4×10^{-5} to 2×10^{-4}M, and from this relation the amount of acridone (II) in the standard *iso*propyl alcohol solution of the precipitate left at the end of hydrolytic run was calculated. This showed that, within an error of 5%, 2-chloro-7-methoxyacridone (II) was the sole acridine hydrolysis product formed by mepacrine at 37° in buffer solutions of pH 7·3. (III), (IV), and (V) were not detected, either in the precipitate or in the residual solution.

The four acridine homologues (I; n = 1—4) were hydrolysed in aqueous buffer solutions at 37° at rates characterised by the half-lives and first-order velocity constants tabulated.

Value of n in (I)	pH at 37°	Half-life (hours)	Velocity const. (hours^{-1}, $\times 10^3$)	Dissociation consts.* pKa_1	pKa_2
1	7·3	440	1·6	8·1	6·8
2	7·3	470	1·5	8·7	7·3
3	7·3	650	1·1	9·5	7·7
3	9·1	220	3·2	—	—
4	7·3	2800	0·25	10·0	7·9

* The dissociation constants, $Ka_1 = [B] \cdot [H^+]/[BH^+]$, $Ka_2 = [BH^+] \cdot [H^+]/[BH_2^{++}]$, where (B) represents the uncharged acridine homologue (I; n = 1—4) base, BH^+ its singly charged cation, and BH_2^{++} its doubly charged cation, are quoted from Hammick and Mason (*J.*, 1950, 345). The pKa_1 values were determined in 50% ethanol–water, and here they are corrected by 0·5 of a pK unit which is the average difference between the pKa_2 values of the acridine homologues in water and 50% ethanol–water.

Discussion

The preceding Table indicates that the rates at which the acridine homologues (I; n = 1—4) are hydrolysed decrease as their basicities increase and, in the case of mepacrine, the rate increases with the pH of the buffer solvent. The rate-determining step in these hydrolyses may be the attack of any of the species H_3O^+, H_2O, or OH^-, on the base of the acridine homologue B, its singly charged cation BH^+, or its doubly charged cation BH_2^{++}, or a combination thereof. If the hydrolyses are not subject to general acid–base catalysis, and if one mode of attack largely predominates, some light may be thrown on the mechanism of the hydrolysis by the ratio of the magnitudes of the velocity constants for the hydrolysis of mepacrine at pH 7·3 and pH 9·1. In an attack by a H_3O^+ ion on B, by a H_2O molecule on BH^+ or by a OH^- ion on BH_2^{++}, the first-order velocity constant of the hydrolysis can be shown, from the relevant equilibria equations, to be determined by a factor $[H^+]/(Ka_1 \cdot Ka_2 + Ka_2 \cdot [H^+] + [H^+]^2)$. The magnitudes of this factor for the pH values of 7·3 and 9·1 stand in the ratio of 2·9, and this should be ratio of the velocity constants for the hydrolysis of mepacrine at pH 7·3 and pH 9·1 if the mechanism of the reaction is any one, or a combination, of the three modes of attack instanced above. The observed values of the velocity constants, $1·1 \times 10^{-3}$ and $3·2 \times 10^{-3}$, are in the ratio 2·9, the exact agreement perhaps being fortuitous in view of the uncertainty in the factor used to correct the Ka_1 values to aqueous solution, though the factor is not critical. For the other possible mechanisms of the hydrolytic reaction the theoretical ratios for the magnitudes of the velocity constants of the mepacrine hydrolysis at pH 7·3 and pH 9·1 range from the value of $8·9 \times 10^3$ for the attack of the OH^- ion on B, $1·6 \times 10^2$ for the attack of an OH^- ion on BH^+ or the attack of a H_2O molecule on B, $5·1 \times 10^{-2}$ for the attack of a H_3O^+ ion on BH^+ or of a H_2O molecule on BH_2^{++}, to $9·3 \times 10^{-4}$ for the attack of a H_3O^+ ion on BH_2^{++}. Thus the mechanism of the hydrolysis is likely to be either the attack of a H_3O^+ ion on B, of a H_2O molecule on BH^+, of a OH^- ion on BH_2^{++}, or a combination of such modes of attack. The methods used in the present investigation do not distinguish between these three probable modes of reaction, though in the hydrolysis of the acridine homologues (I; n = 1—4) the attack of an OH^- ion on BH_2^{++} is perhaps important since 5-amino-2-chloro-7-methoxyacridine (III), which does not form a doubly charged cation, is hydrolysed very slowly. Moreover, reasons have been given for supposing that the analogous reaction of hydrogen sulphide with 5-aminoacridines, to give thioacridones, proceeds by a similar mechanism involving an initial attack by a SH^- ion (Asquith, Hammick, and Williams, *J.*, 1948, 1181).

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