77. *Some Physico-chemical Properties of Acridine Antimalarials, with Reference to their Biological Action. Part II. Lipoid Partition Coefficients, Surface Activities, and Protein Affinities.*

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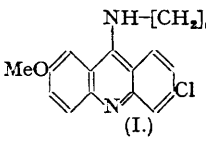
The partition coefficients of a homologous series of acridine antimalarials between vegetable oil and aqueous buffers have been measured, and have been found to be related to the basic dissociation constants of the compounds. In aqueous solution these acridine derivatives are surface-active, and the lowering of the surface tension that they produce has been determined over a range of concentrations. The relative affinities of these compounds for the protein of egg albumin have also been determined.

Attempts have been made (preceding paper) to correlate the relative antimalarial activities of six members ($n = 2$—7) of a homologous series of acridine drugs (I) with their basicities and reduction potentials, but no definite relation has been observed. These physico-chemical properties were selected as a result of general considerations as to the nature of the action of acridine antimalarials on isolated enzyme systems, and on cultures of bacteria and the malaria parasite. In these cases the drug acts on biological systems isolated from their environment or host, whereas *in vivo* a drug must be able to permeate through the cellular material of the host and perhaps that of the parasite in order to be effective at some site of action within the parasite. Thus the relative permeabilities of a structurally similar series of antimalarial drugs may affect the antimalarial activities as determined in practice. The permeability of a drug through organic material is presumably dependent on physico-chemical factors, such as the solubility of the drug, its surface activity, lipoid partition coefficient, affinity for various

proteins, and it was thought that the permeability of a given type of drug might possibly be optimum for certain values of these properties.



(I.)

The partition coefficients of six acridine antimalarials (I; $n = 2$—7) between vegetable oil and aqueous buffer solutions of pH 7·3 have been measured, as well as their surface activities and their relative affinities for the protein of egg albumin, both in M/20-phosphate buffers of pH 7·3 (the pH of blood).

Experimental.

Partition Coefficients.—Five standard solutions of the dihydrochloride of compound, covering the concentration range 10^{-4} to 10^{-3} M., were made up in M/20-phosphate buffer of pH 7·3. Each solution was divided into two parts: one was retained as a colorimetric comparator, whilst the other was shaken mechanically for 4 hours with such a volume of acid-free castor oil * as would extract between one-third and two-thirds of the acridine derivative (as indicated by preliminary experiments). The emulsion obtained was centrifuged, and the aqueous layer was then compared colorimetrically with the control solution. The latter was diluted to approx. the concentration of the extracted solution, and the comparison was repeated to guard against deviations from Beer's law. No deviations were detected, though the yellow acridine solutions did not permit of great accuracy in colorimetric comparison. Consequently the results are accurate only to within ±10%. Within these limits it was found that the ratio of the concentration of the compound in the lipoid phase to the concentration in the extracted aqueous phase

Fig. 1.

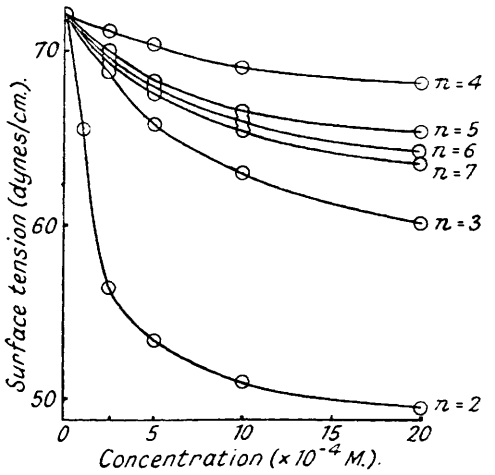
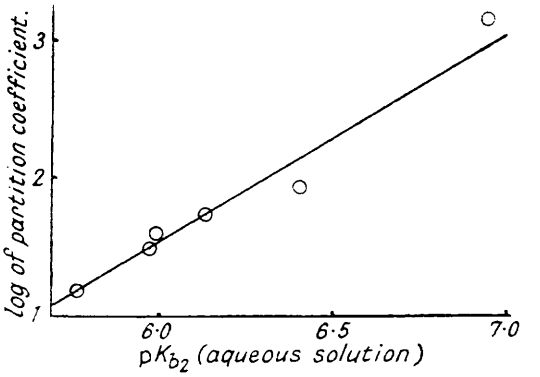


Fig. 2.



was constant for each derivative over the concentration range studied. This ratio was then taken to be the partition coefficient of the compound between vegetable oil and aqueous buffer solution of pH 7·3, the values obtained being listed in the table below.

Surface Activities.—The surface tensions of solutions of each of the prepared acridine derivatives in M/20-phosphate buffer of pH 7·3 were measured by the drop-weight method, using a stalagmometer, over the concentration range 5×10^{-5} to 2×10^{-3} M. The stalagmometer was standardised with distilled water and with the phosphate buffer, by weighing in each case ten drops that had been allowed to fall from the orifice during about a minute. The weighings were repeated several times for reproducibility, the rate of flow of liquid being maintained at a constant value throughout the measurements by means of a capillary air leak. Measurements were then carried out with the solutions of various concentrations prepared from each of the derivatives, the weight of ten drops being determined several times. The surface tension of such solutions is proportional to the weight of the drops they form at a clean orifice, and thus the tensions may be evaluated from the known surface tension of a standard, such as distilled water. The results obtained are considered to be accurate to within about ±1% and are listed in the table and Fig. 1. They are perhaps less relevant to the purposes of this enquiry than the lipoid partition coefficients and protein affinites of the compounds, as there are not likely to be many air–water interfaces in the host–parasite system, and surface activities at such interfaces bear no necessary relation to activities at other interphase boundaries, such as those between lipoid and water, which are biologically more significant.

Protein Affinities.—Perhaps the most accurate and significant measure of drug–protein affinity is afforded by dialysis experiments (Davis, *Science*, 1942, **95**, 78). A membrane, such as Cellophane, separating isotonic solutions of protein and drug, permits the diffusion of a crystalloid drug, but not of a colloidal protein. At equilibrium there is a greater concentration of drug in the protein solution than

* This work was carried out during the war when castor oil was the only vegetable oil available to the authors.

in the aqueous solution, the excess affording a measure of the drug–protein affinity. A more convenient method is the precipitation of protein from an aqueous buffer solution with alcohol in the presence of the drug (Wormall and Dewey, *Biochem. J.*, 1946, **40**, 119). The relative amount of the drug carried down with the protein may then be taken to be a measure of the drug-protein affinity.

Both of these methods have been tried with one of the acridine homologues ($n = 4$) at six different concentrations over the range 5×10^{-5} to 2×10^{-3} M. in M/20-phosphate buffers of pH 7·3. In the dialysis experiments 20 c.c. of the buffered acridine solution were placed in a short boiling-tube fitted with a cork through which passed a piece of glass tubing closed at the lower end by a Cellophane membrane and at the upper end by a rubber bung. Into this tubing were pipetted 5 c.c. of an isotonic solution containing 5% by weight of crystalline egg albumin in a buffer of pH 7·3. The dialysis tubes were then kept in a refrigerator for two weeks, after which the experimental acridine drug solutions were compared colorimetrically with control solutions.

In the alcohol-precipitation experiments 5 c.c. of the same protein solution were added to 5 c.c. of a standard acridine drug solution in phosphate buffer. After some 30 minutes, 10 c.c. of ethyl alcohol were added, and the precipitated protein was centrifuged. The supernatant aqueous-alcoholic solution was then compared colorimetrically with a control that had been appropriately diluted with phosphate buffer and alcohol.

By both methods it was found that the amount of drug taken up by the protein was directly proportional to the drug concentration in the buffer solution up to concentrations of 5×10^{-4} M. under the conditions described above. The proportionality factors were very nearly the same in both cases, and were taken as measures of the drug–protein affinity. The protein affinities of the other members of the homologous series were then determined by the alcohol-precipitation method, as this was the more convenient. The partition of each derivative between protein and aqueous buffer was measured under comparable conditions by the means described above, at four concentrations over the range 5×10^{-5} to 5×10^{-4} M. where direct proportionality between the amount of drug taken up by the protein and the drug concentration in the residual solution had been observed. The ratio of these two quantities was then taken as the protein affinity of the compound under the given experimental conditions, and the values obtained were referred to the protein affinity of the homologue ($n = 2$) taken as unity. The results obtained are listed in the table. They are accurate only to within $\pm 10\%$, the limiting factor being the sensitivity of the eye in the comparison of yellow acridine solutions.

2-Chloro-5-(ω-diethylaminoalkylamino)-7-methoxyacridine (I).

n.	Partition coeffs. between vegetable oil and buffer of pH 7·3.	Relative distribution factors between albumin and buffer of pH 7·3.	Lowering of the surface tension of buffer of pH 7·3 in dynes/cm. at drug concn. 10^{-3} M.
2	1600	1·0	21·0
3	82	2·1	9·0
4	54	2·7	3·0
5	43	6·8	5·5
6	31	14	6·0
7	15	17	6·6
5-(3-Diethylaminopropylamino)acridine	22	15	6·4

Discussion.

In general the lyophobic character of organic compounds tends to increase with chain length in a homologous series, but in the case of the acridine homologues studied in the present work this relation did not hold. This may possibly be ascribed to the fact that under the experimental conditions at which measurements of oil–water partition coefficients were made, *i.e.*, at pH 7·3, these 5-diaminoacridines exist in the form of univalent and bivalent cations, the ratio of the two forms being determined by the value of pK_{b_2} for aqueous solutions. A plot of the logarithms of the partition coefficients for the series against the values of pK_{b_2} ($-\log K_2$) for aqueous solutions (preceding paper) gives a straight line with a slope of 1·6 (Fig. 2), indicating an inverse proportionality of this power between the vegetable-oil partition coefficient and the second dissociation constant of this particular type of compound at pH 7·3.

The surface activities and protein affinities of the acridine homologues seem not to be related in any definite way with other physico-chemical properties or structural features of the series. However, the relative values of these properties do seem to be related to the biological activities of the compounds, and in a subsequent communication these possible relations are discussed.

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