LETTERS TO THE EDITORS

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Further Correlation of Physical and Biological Properties in an Acridine Series

THE partition coefficients between olive oil and water of the five monoaminoacridines[1] have been investigated, and the results, set out in the accompanying table, include acridine and proflavine (2 : 8-diaminoacridine) for comparison.

The method followed was to dissolve the amine hydrochloride in a large excess of phosphate buffer solution (*p*H 7·0) and to shake this for two hours at 25° C. with sufficient acid free olive oil[2] to extract approximately half the amine. The emulsion was centrifuged and the aqueous layer compared in a colorimeter with the unextracted solution. The latter was then diluted to the concentration found for the extracted solution, and the two were again compared in order to compensate for any deviation from Beer's Law. Finally where, as in the cases of 3- and 4-aminoacridines, there is a profound change of colour on acidification, both solutions were brought to *p*H 2·5 and again compared. Concordant readings were given by all methods. The concentrations of each amine at equilibrium were arranged to vary in different experiments, the aqueous layer containing 0·5–3·0 mgm. per 100 ml. in regular steps. In spite of this sixfold variation, no change in the coefficient was noted, indicating that association does not take place.

On account of the sparing solubility in water of acridine and 1-aminoacridine, it was necessary to devise a modified technique by reversing Farmer's procedure[3] for calculating the dissociation constant of an amine from its partition coefficient. Accordingly 1-aminoacridine was extracted by oil from its solutions in *N*/100 sulphuric acid and the aqueous layer (*p*H 2·1) compared with unextracted solution brought to the same *p*H value The amount of amine in the oil was found by subtraction and was divided by the amount of free base existing in the aqueous layer at equilibrium as found from the equation :

$$\log \frac{[B]}{[BH]} = p\mathrm{H} - 14 - \log K \quad . \quad . \quad . \quad (i)$$

which is derived from $\frac{[B][H]}{[BH]} = \frac{10^{-14}}{K}$,

$[B]$ and $[BH]$ representing concentrations of free base and its salt respectively.

Since salt-formation has been taken into consideration, partition coefficients calculated as above are comparable[3] with those found at *p*H 7, at which value 1-aminoacridine and acridine are almost entirely hydrolysed. Acridine, being almost colourless, was estimated by precipitation with excess picrolonic acid, filtration through fritted glass, and titration of the uncombined picrolonic acid with methylene blue[4].

It will be seen from the table that there is a correlation of physical and biological properties in this series in so far as the members most active against bacteria are the most basic and the most hydrophilic,

	Partition coefficient oil/water	Dissociation constant, water, 25° C. × 10^{-10}	Bacteriostatic index[1] (Sum total of inhibitory dilutions)	Average lethal dose (mouse)	Bactericidal value[5]
				gm./kgm.	
5-Aminoacridine	1	300,000	43	0·08	96
Proflavine	0·5	120,000	41	0·14	92
2-Aminoacridine	5	12,000	41	0·17	82
4-Aminoacridine	55	30	17	0·33	61
3-Aminoacridine	90	12	17	0·33	56
Acridine	250	2	12	0·30	Not tried
1-Aminoacridine	1200	1	8	0·91	49

whereas the least basic and most hydrophobic members have only feeble antiseptic properties.

The bacteriostatic index is quoted from a previous communication[1]. The average lethal dose (*L.D.* 50) was found by subcutaneous injection of mice using 6–12 mice for each dose and interpolating for a 50 per cent kill after plotting the logarithm of each dose against the percentage killed. It will be noted that the mammalian toxicity rises with the bacteriostatic index, but as the latter figure represents powers of 2, it can be shown that the mammalian toxicity does not tend to rise on so steep a gradient as the toxicity to bacteria. The bactericidal values quoted in the table represent the percentage reduction of hæmolytic streptococci effected by a 1 : 10,000 concentration of the derivative in 10 per cent serum broth after two hours at 37° C.

The causes underlying the correlations are not yet clear. The hydrophilic properties of the strong bases are not due to the fact that they exist mainly as salts at *p*H 7. For example, 5-aminoacridine, which at *p*H 7 contains only 0·3 per cent of free base (from equation (1)), has its partition coefficient of 1 raised only to 3 at *p*H 10 where it contains 80 per cent of free base. Moreover the biological properties may be found to depend not so much on the basicity as on the various types[1] of amino groups which the basicities indicate.

The biological results quoted in this paper will appear elsewhere in greater detail[5]. Meanwhile 5-aminoacridine is undergoing clinical trial as a non-staining antiseptic with the general properties of proflavine.

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