171. *Aminoacridines: Some Partition and Surface Phenomena.*

By Adrien Albert, Reginald Goldacre, and Eric Heymann.

In order to obtain data for a closer correlation of physical and biological properties among kationic antiseptics, the oil–water partition coefficients and the air–water surface activities of a number of aminoacridines have been measured. Some correlations between these physical properties and the molecular structure are discussed.

The results suggest that marked oleophilic and surface-active properties are unnecessary for, and if present in high degree are inimical to, the development of good antiseptic properties in this series.

It has been shown (Albert, Goldacre, and Rubbo, *Nature*, 1941, **147**, 709) that those monoaminoacridines which are most active against bacteria are more strongly ionised and more hydrophilic than those with weakly bacteriostatic properties. To widen the scope of this observation, determination of the distribution of a number of other aminoacridines between olive oil and water has now been made. Further, the surface activity of all these compounds at the air–water interface has been measured, since it may serve as a rough guide to the relative degrees of adsorption at non-water–water interfaces in living material. Apart from a determination of the comparative drop numbers of rivanol and euflavine at various pH values (Michaelis and Hayashi, *Z. Immunforsch.*, 1923, **36**, 518), no investigation of distribution phenomena in the acridine series has yet been made.

Col. A = pH value. Col. B = Approximate percentage ionised. Col. C = Partition coeff., olive oil/water, at 20° ± 5°. Col. D = Depression of surface tension of water at 15° ± 2° by 2·5 × 10^{-4} mol./l., in dynes/cm.

	A.	B.	C.	D.
Acridine		0·8	200	2
1-Aminoacridine		0·1	1200	7
2-Aminoacridine		93	5·2	0·2
3-Aminoacridine		5	90	1·5
4-Aminoacridine		11	55	0
5-Aminoacridine (mesocrin)		100	1·2	0
2 : 5-Diaminoacridine		100	1	0
2 : 7-Diaminoacridine		94	*	0
2 : 8-Diaminoacridine (proflavine)		100	0·7	0
2 : 5-Diamino-7-ethoxyacridine (rivanol)		100	21	12·5
2 : 8-Diaminoacridine-10-methochloride (euflavine)	7·0—7·2	100	<0·2	0
2 : 8-Bisdimethylaminoacridine (acridine-orange)		100	7·3	1·5
2-Aminoacridine-7-sulphonic acid		90	0·9	0
2-Chloro-5-(δ-diethylamino-α-methyl)butylamino-7-methoxyacridine (atebrin or mepacrine)		100	31	0·2
5-Methylaminoacridine		100	12	0
5-Butylaminoacridine		100	16	1·5
5-*cyclo*Hexylaminoacridine		100	77	2·5
5-Heptylaminoacridine		100	*ca.* 1000	18
5-Dodecyl- and 5-hexadecyl-aminoacridines		100	*	*
5-Aminoacridine	10·0	40	3	—
3-Aminoacridine	4·7	93	—	1·5
3-Aminoacridine	2·3	100	—	0
5-Butylaminoacridine	3·6	100	—	0·5
5-Heptylaminoacridine	4·0	100	—	9
5-Dodecylaminoacridine	4·3	100	—	36
5-Hexadecylaminoacridine	4·0	100	—	26
Rivanol (see above)	4·6	100	—	0
2-*Chloro-5-amino-7-methoxyacridine*	4·6	100	—	1

* Solubility difficulties made measurements impossible.

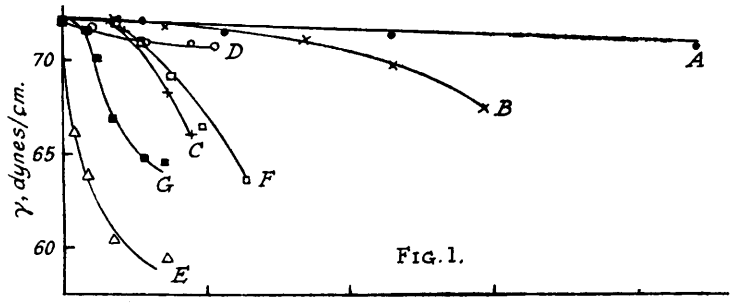
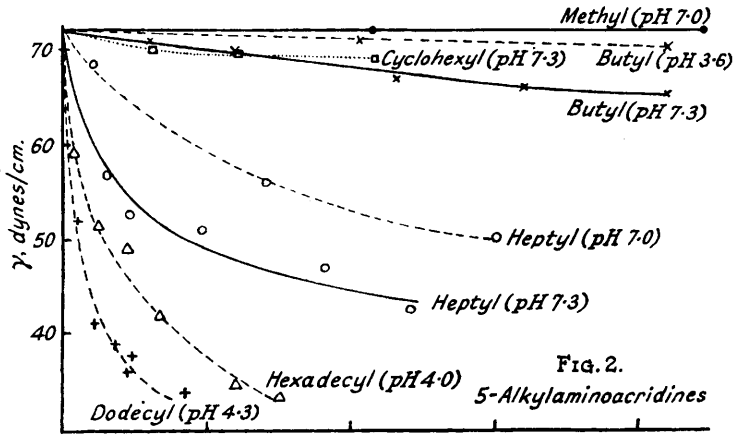


FIG. 1.

A. 2-Aminoacridine, pH 7·1.
B. Atebrin, pH 7·2.
C. Acridine, pH 7·2.
D. Acridine-orange, pH 7·0.
E. Rivanol, pH 7·0.
F. 3-Aminoacridine, pH 7·2.
G. 1-Aminoacridine, pH 7·2.



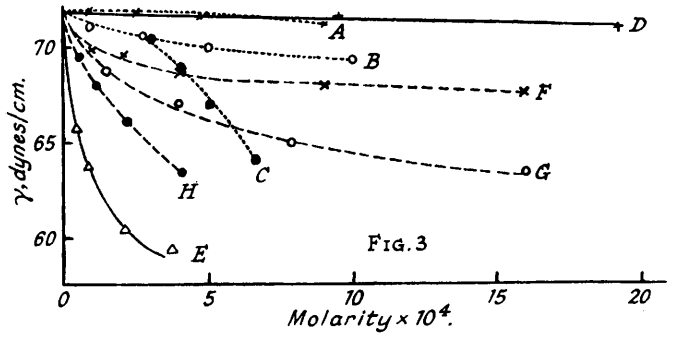


FIG. 3.

A. 3-Aminoacridine, pH 2·3.
B. 3-Aminoacridine, pH 4·7.
C. 3-Aminoacridine, pH 7·2.
D. Rivanol, pH 4·6.
E. Rivanol, pH 7·0.
F. Crystal-violet, pH 4·5.
G. Crystal-violet, pH 7·1.
H. Crystal-violet, pH 8·4.

Discussion of Partition Coefficients.—The partition coefficients oil/water at pH 7 of acridine and the five aminoacridines are seen to be roughly parallel to the amounts of un-ionised bases present. That other factors play some part is suggested by the relatively low value for 5-aminoacridine at pH 10, and it is unlikely that all the undissociated bases are equally oleophilic or all the ions equally hydrophilic. As would be expected, the partition coefficient of an aminoacridine is lowered by addition of another amino-group (*e.g.*, proflavine and 2 : 5-diaminoacridine), but only slightly, since at pH 7 no new ion is formed. Quaternisation of the ring nitrogen above (cf. euflavine) also reduces the coefficient. On the other hand, it is raised by alkylation of a primary amino-group (cf. acridine-orange) and increases with the length of the substituent group until (as with 5-dodecylaminoacridine) the salts, although completely ionised in aqueous solution, have become so oleophilic that they pass from water entirely into the oil. The heightened coefficients of rivanol (I) and the antimalarial atebrin (II) as compared with 5-aminoacridine are attributed to the ethoxy- and the chlorine substituent respectively.

Discussion of Surface Activities.—It will be seen from the table that a number of acridine derivatives do not lower the surface tension of water at pH 7, and 2-aminoacridine and atebrin lower it only slightly. The remaining substances show substantial reductions (Figs. 1 and 2), and although the orders of surface activities and partition coefficients do not coincide, these substances have high partition coefficients.

Two important factors that favour surface-activity are feeble ionisation and a relative propinquity of the main centres of hydration to one another so as to create the tendency towards a definite orientation. This concept may serve to explain the high surface activity of acridine and 1-aminoacridine, the lower degrees of activity shown by 2- and 3-aminoacridine, and the absence of this quality in the 4- and the 5-isomeride. The apparent reversal of order of 2- and 3-aminoacridines, as estimated by the propinquity factor, is accounted for

by the much greater degree of ionisation of the former (see table). Some examples are given (Fig. 2 and particularly Fig. 3) of the effect of pH on surface activity. The latter is less in acid solution on account of the increased ionisation (see table), and this accords with the general principle that ions, because of their greater hydration, are adsorbed less strongly at a water boundary than are the corresponding un-ionised molecules.

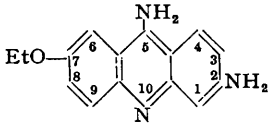
Traube's rule, that the surface tension of water is lowered more strongly as the chain length of an alkyl radical increases, is qualitatively exemplified by the series of 5-alkylaminoacridines (Fig. 2). As the higher homologues have water-insoluble hydrochlorides and phosphates, a series of acetates were measured at pH 4. The apparent reversal of positions of the dodecyl and hexadecyl homologues is connected with the formation of colloidal micelles as indicated by the increasing turbidity of their solutions. Actually, the lower members of this series pass through ultrafilters (prepared according to Bechhold and Gutlohn, *Z. angew. Chem.*, 1924, **37**, 494, from 5% collodion in acetic acid), although there is considerable adsorption on the membrane. The particles of the two higher homologues, however, are completely retained by these filters, whose pore-size can be judged from the fact that they retain most proteins but allow serum albumin to pass.

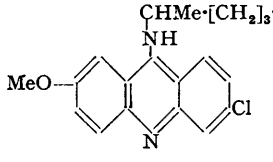
As for the connexion between constitution and surface activity among the more complicated structures, the increased activity of rivanol (I) over 2 : 5-diaminoacridine is attributed to the ethoxy-group, but atebrin (II), which is more oleophilic than (I), is only slightly surface active. Observations on 2-chloro-5-amino-7-methoxyacridine, *i.e.*, atebrin shorn of its side chain, could not be carried out at pH 7 because of solubility difficulties, but at pH 4·6 it was more surface-active than rivanol.

Crystal-violet (*tert.*-dimethylaminotriphenylcarbinol anhydride chloride), which is the most important member of the surface-active triphenylmethane antiseptics, has been included in Fig. 3 for comparison.

Dr. S. D. Rubbo, of Melbourne University, who has been assessing the antiseptic values of these substances, reports that the most antibacterial of the aminoacridines were found among the most hydrophilic members of the above list, *i.e.*, those with partition coefficients of about unity and little or no surface-activity. Rivanol, which is rather oleophilic and yet almost as antibacterial as 5-aminoacridine, was the sole exception. Further, there are indications of a correlation between surface-activity and the depression of bactericidal activity on the addition of red blood cells (cf. Rubbo, Albert, and Maxwell, *Brit. J. Exp. Path.*, 1942, **23**, 69). Phenobarbitone and butanol are instanced as other biologically active substances with an oil/water coefficient of about unity.

(I.)





(II.)

Experimental.

The percentage ionisation was calculated from both unpublished and published (Albert and Goldacre, this vol., p. 454) figures for pK_a values in dilute alcohols, the pK_a values in water being estimated as approximately 0·5 unit higher.

Neutral olive oil was prepared, following the Argentinian Pharmacopœia, by determining the free acidity of olive oil (B.P. grade) and treating it with sodium carbonate crystals (roughly equal to the weight of fatty acid present) dissolved in ten times their volume of water, the oil being added in 5 successive portions to the alkali at 40° during 2½ hours, with vigorous shaking for 5 mins. after each addition. After 15 hours, the mixture was filtered through dried paper, mixed with a third of its volume of 95% alcohol, and shaken vigorously several times during 24 hours. The oil was separated and heated at 160° for an hour.

Partition coefficients were determined by dissolving the hydrochlorides of each amine (range 0·5—5·0 mg. usually, in regular steps) in phosphate buffer (100 ml., pH 7·0). This was shaken at 25° for 2 hours with sufficient neutral olive oil to extract approximately half the amine, as determined from preliminary experiments. The emulsion was centrifuged, and the aqueous layer compared in a colorimeter with the unextracted solution, which was then diluted to approximately the value found for the extracted solution, and the two solutions were compared again to guard against deviations from Beer's law. In such cases where there was a marked change of colour on acidification, both solutions were then brought to pH 2·5 and compared again. Concordant figures were obtained from the various methods, but some substances, marked with an asterisk in the table, could not be measured because either they were too sparingly soluble in both phases or they were exclusively soluble in one of the phases. The principal difficulty encountered was the matching of so many yellow solutions, an error of up to 10% with this colour being hard to avoid. The method is illustrated by results for 4-aminoacridine :

Mg. per 100 ml.	0·9	1·0	1·2	1·5	2·5	5·0
Coefficient	54	55	56	49	58	57 (average 55)

No evidence that any compound was associated in the oily layer was found. The values for acridine and 1-aminoacridine were obtained by a slightly different method, as previously described (Albert, Goldacre, and Rubbo, *loc. cit.*), the data previously quoted having been slightly amended by averaging the results of further experiments.

Surface tensions were measured by the ring method, a Cenco–Du Nouy tensiometer being employed. The experimental precautions specified by Harkins and Brown (*J. Amer. Chem. Soc.*, 1919, **41**, 499; also Harkins, Young, and Cheng, *Science*, 1926, **64**, 333; Harkins and Jordan, *J. Amer. Chem. Soc.*, 1930, **52**, 1751) were rigidly observed and the experimental values were corrected by the aid of factors worked out by these investigators. With some compounds (*e.g.*, 2 : 7-diaminoacridine and acridine-orange) difficulties were encountered because of the comparative physical instability of the solution at the pH studied. The majority of the experiments were carried out in phosphate buffer between pH 7·0 and 7·2 as measured by a glass electrode and Cambridge valve potentiometer. The more acid solutions were buffered with citric acid and disodium phosphate according to McIlwaine.

Materials.—Acridine, m. p. 110°, was purified by steam distillation. The other substances were prepared and purified according to the following methods: 1-, 2-, 3-, and 4-aminoacridines (Albert and Ritchie, *J. Soc. Chem. Ind.*, 1941, **60**, 120); 5-amino- and 5-chloro-acridine (*idem, Organic Syntheses*, 1942, **22**, 5); proflavine (Albert, J., 1941, 121); euflavine (Hall and Powell, *Quart. J. Pharm.*, 1934, **7**, 522); 5-methylaminoacridine (Albert and Ritchie, this vol., p. 454); 2 : 7-diaminoacridine (Aust. Patent, 115,480, 1942, to University of Sydney); 2 : 5-diaminoacridine and 2 : 5-diamino-7-ethoxyacridine (Albert and Gledhill, *J. Soc. Chem. Ind.*, 1942, **61**, 159); 2 : 5-dichloro-7-methoxyacridine and mepacrine (I. G. Farb. Ind., B.P. 1930, 363,392); acridine-orange (Biehringer, *J. pr. Chem.*, 1896, **54**, 243); 2-aminoacridine-7-sulphonic acid (Aarons and Albert, J., 1942, 183); crystal-violet (Kovache, *Ann. Chim.*, 1918, **10**, 247).

New Compounds.—*2-Chloro-5-amino-7-methoxyacridine.* 2 : 5-Dichloro-7-methoxyacridine (2·8 g.) and phenol (11 g.) were heated to 70°, and effloresced ammonium carbonate (1·4 g.) added. The mixture was then heated at 120° for 15 mins., cooled, and treated with acetone (30 ml.). The precipitated hydrochloride of 2-chloro-5-amino-7-methoxyacridine was washed with acetone and recrystallised from 1 l. of boiling water; yield, 2·5 g. (85%) of brilliant yellow crystals, m. p. 340° (decomp.), soluble in about 290 parts of hot and 2,000 parts of cold water with a green fluorescence. The *base*, liberated with N-sodium hydroxide and recrystallised from 60 parts of 60% alcohol, formed orange-yellow crystals, m. p. 271° (281°, corr.), slightly soluble in chloroform, ether, and acetone, very soluble in alcohol with a green fluorescence (Found: N, 11·0. $C_{14}H_{11}ON_2Cl$ requires N, 10·8%).

5-Butylaminoacridine hydrochloride. *n*-Butylamine (3·6 g., b. p. 76—78°), 5-chloroacridine (5 g.), and phenol (15 g.) were heated under reflux, the bath being raised from 70° to 130° during an hour. *5-Butylaminoacridine hydrochloride* was precipitated from the cooled mass with ether (50 ml.). It recrystallised from alcohol (9 ml.) in yellow needles (60% yield), m. p. 189—190°, and was soluble in 5 parts of boiling water and 1·5 parts of boiling alcohol. In ultra-violet light (λ 3650—3663 A.), but not in daylight, the aqueous solution fluoresces green, changing to an intense blue on dilution. The salt is very slightly soluble in acetone, benzene, and ether (Found: C, 71·1; H, 6·8; N, 9·6. $C_{17}H_{18}N_2$,HCl requires C, 71·2; H, 6·7; N, 9·75%). The base, recrystallised from benzene–light petroleum (3 : 1), formed orange-yellow crystals, m. p. 100—102°, very soluble in alcohol with a green fluorescence.

*cyclo*Hexylamine (3·1 g., b. p. 134—135°), 5-chloroacridine (5·35 g.), and phenol (15 g.) were heated at 120° for ½ hour, cooled, and the 5-cyclo*hexylaminoacridine hydrochloride* precipitated with ether (50 ml.) and recrystallised from alcohol (60 ml.); yield, 70% of greenish-yellow crystals, m. p. 271° (280° corr.; sealed tube), soluble in 11 parts of boiling alcohol and 16 parts of boiling water without marked fluorescence, and only slightly soluble in benzene or acetone (Found: C, 72·9; H, 7·0; N, 9·1. $C_{19}H_{20}N_2$,HCl requires C, 73·0; H, 6·8; N, 9·0%). The base, recrystallised from benzene, gave yellow crystals, m. p. 170—171°, very soluble in alcohol with a green fluorescence.

5-Heptylaminoacridine hydrochloride, similarly prepared in 70% yield from *n*-heptylamine (b. p. 154—156°) and recrystallised from water (300 ml.), gave yellow crystals, m. p. 106° (138° when anhydrous), soluble in its own weight of boiling alcohol and in 600 parts of cold water with very little fluorescence; moderately soluble in boiling benzene (Found: C, 69·6; H, 7·9. $C_{20}H_{24}N_2$,HCl,H_2O requires C, 69·2; H, 7·8%).

Dodecylamine (2·4 g.; b. p. 133—135°/15 mm.), 5-chloroacridine (2·9 g.), and phenol (9 g.) were heated for ½ hour at 160°, and the phenol distilled off in a vacuum. The residue was stirred with light petroleum (30 ml.) and ether (90 ml.), kept on ice overnight, and the *5-dodecylaminoacridine hydrochloride* filtered off, washed with a little acetone, and recrystallised from acetone (130 ml.). Yield, 85% of yellow, slippery crystals, m. p. 92°, sparingly soluble in cold water with a brilliant blue fluorescence visible only in ultra-violet light. Soluble in its own weight of boiling benzene or of alcohol; soluble in about 700 parts of 30% alcohol (Found: C, 72·2; H, 9·0; N, 6·8. $C_{25}H_{34}N_2$,HCl,H_2O requires C, 72·0; H, 9·0; N, 6·7%). The base, m. p. 67°, liberated from the alcoholic solution with N-sodium hydroxide (aqueous) and recrystallised from light petroleum, was yellow and more soluble in 10% acetic acid than in mineral acids. The alcoholic solution had a green fluorescence.

5-Hexadecylaminoacridine hydrochloride, similarly prepared (75% yield) from cetylamine (m. p. 44°), formed yellow, slippery needles, m. p. 99—100°, almost insoluble in boiling water, soluble in its own weight of boiling benzene and in about 200 parts of boiling acetone (Found: C, 73·3; H, 9·7; N, 6·0. $C_{29}H_{42}N_2$,HCl,H_2O requires C, 73·6; H, 9·6; N, 5·9%). The base, crystallised from light petroleum and from 75% alcohol (green fluorescence), formed yellow crystals, m. p. 66°, readily soluble in a slight excess of 10% acetic acid.

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