

618. *The Polarographic Reduction of Crystal-violet, Brilliant-green, Malachite-green, and Auramine.*

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All four dyes are reducible in aqueous solution at the dropping mercury electrode, giving polarograms distorted by dye adsorption on the mercury. When adsorption effects are eliminated by addition of ethanol, normal polarograms result, exhibiting two well-separated one-electron steps which indicate the formation at the first step of a semiquinone free radical of apparently very great stability. For all but malachite-green, which was not studied in sufficient detail to establish the point, the half-wave potential of the first reduction step is independent of pH in the biologically important region around pH 7, so that, as with the more strongly antibacterial aminoacridines (*J.*, 1951, 2638), semiquinone formation involves the uptake of one electron and no proton. Equations are derived which represent satisfactorily the observed variation of half-wave potential with pH.

PREVIOUS work on the polarographic reduction of acridine (*J.*, 1951, 27) and several aminoacridines (*J.*, 1951, 2638) has shown that, while all these compounds give two widely separated one-electron reduction steps, indicating the formation of a semiquinone free radical of exceptionally great apparent stability, the more strongly antibacterial of them alone have a horizontal portion of the E_1 -pH curve (E_1 = half-wave or effective standard redox potential of first step) extending over the biologically important pH region around 7. This horizontal section indicates that the formation of semiquinone involves the uptake of a single electron but no proton, a matter of significance in connection with a theory of the mechanism of these antibacterials (Kaye, *J. Pharm. Pharmacol.*, 1950, 2, 902).

In order to find whether the antibacterial triphenylmethane dyes crystal-violet, brilliant-green, and malachite-green, and the bacteriostatic diphenylmethane dye auramine behave similarly to the active aminoacridines, we have investigated their polarographic reduction. We are not aware of any previous comprehensive polarographic studies of these dyes, although Linnell and Stenlake (*J. Pharm. Pharmacol.*, 1949, 1, 314) confirmed polarographically the identity of a derivative of brilliant-green.

EXPERIMENTAL

Crystal-violet and brilliant-green of medicinal purity, and commercial malachite-green and auramine, were purified by recrystallisation from water; for the first two, prolonged standing of the solutions was necessary. All other materials, apparatus, and technique were as previously described (*J.*, 1951, 27). The temperature of all experiments was 25°, all polarograms were corrected for residual current, and electrode potentials are relative to the saturated calomel electrode standard.

RESULTS AND DISCUSSION

Crystal-violet.—In aqueous solution this dye is electro-reducible at the dropping mercury electrode, giving two-step reduction waves (Fig. 1) which are distorted, especially at the second step. The distortions were associated with anomalous galvanometer

oscillations, as with acridines, and are thus ascribed to adsorption of electroactive matter on the surface of the dropping mercury electrode. This is confirmed by current-time oscillograms taken during a single drop life (cf. *J.*, 1951, 27, 2638). Abnormal oscillograms were obtained only at potentials corresponding to the fore-wave of the second step, and their shape suggests that current growth is prevented by fully reduced material blocking the mercury surface. The inclusion of 50% by volume of ethanol in the base (supporting electrolyte) solution eliminated the adsorption effects in the polarogram (Fig. 1), the waves becoming normal apart from a tendency at certain pH values to maximum formation. This was suppressed by adding 0.01–0.02% of methyl cellulose, without affecting wave height. The presence of 50% of ethanol did not, however, completely remove the oscillogram anomalies, showing that, as with the aminoacridines, the oscillogram provides a more sensitive test for adsorption on the mercury drop than does the polarogram.

The dye was examined polarographically in 50% aqueous ethanol, at a concentration of $4 \times 10^{-4}M$, over the pH range 0–14. Between pH 1 and pH 14, the two-step reduction waves were normal and of constant height (for fixed capillary characteristics), the diffusion current constant being very nearly equal to that of the acridines in the same solvent, but only two-thirds of the value for the dye in aqueous solutions. Application of the Ilkovič

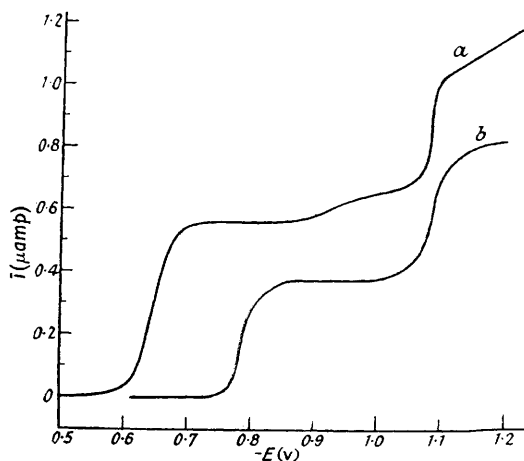


FIG. 1. Polarograms for crystal-violet, $4 \times 10^{-4}M$.
a, pH 7.38, in water; b, pH 8.29, in 50% ethanol.

equation $\bar{i}_d = 605ncD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{2}}$ (*Coll. Czech. Chem. Comm.*, 1934, 6, 498) to the results at pH 8.29 in 50% ethanol, with the values $D^{\frac{1}{2}}$ (D = diffusion coefficient of reducible molecule) = 1.52×10^{-3} cm. sec. $^{-\frac{1}{2}}$, m (mercury flow rate) = 0.916 mg. sec. $^{-1}$, c (concentration) = 0.4 millimol./l., t (drop time) = 4.1 sec. (first step) and 4.0 sec. (second step), \bar{i}_d (mean diffusion current) = 0.44×10^{-3} amp (first step) and 0.46×10^{-3} amp (second step), gave the results n (number of electrons involved per molecule) = 1.002 (first step) and 1.06 (second step). The foregoing value of $D^{\frac{1}{2}}$ was obtained from the value $D = 5.2 \times 10^{-6}$ cm. 2 sec. $^{-1}$ for the phenolphthalein molecule (of similar size to that of crystal-violet) in water at 25° (Kolthoff and Lehmicke, *J. Amer. Chem. Soc.*, 1948, 70, 1879) by multiplying its square root by $\frac{2}{3}$ to allow for the effect of the alcohol on wave height, it being assumed (cf. Shreve and Markham, *ibid.*, 1949, 71, 2993) that the presence of alcohol does not affect the value of $m^{\frac{2}{3}}t^{\frac{1}{2}}$. Thus the two steps are single-electron reductions, and the product of the first stage is a semiquinone free radical, the stability of which is apparently very great, as indicated by the wide separation of the half-wave potentials $E_{\frac{1}{2}}$ and $E_{\frac{2}{2}}$, which may be taken as equal to the standard redox potentials E_1 and E_2 of the two steps. An alternative indication is the large value ($10^{3.75}$) at pH 7 of the semiquinone formation constant

$$K = [\text{semiquinone}]^2/[\text{unreduced dye}][\text{fully reduced dye}] \quad (1)$$

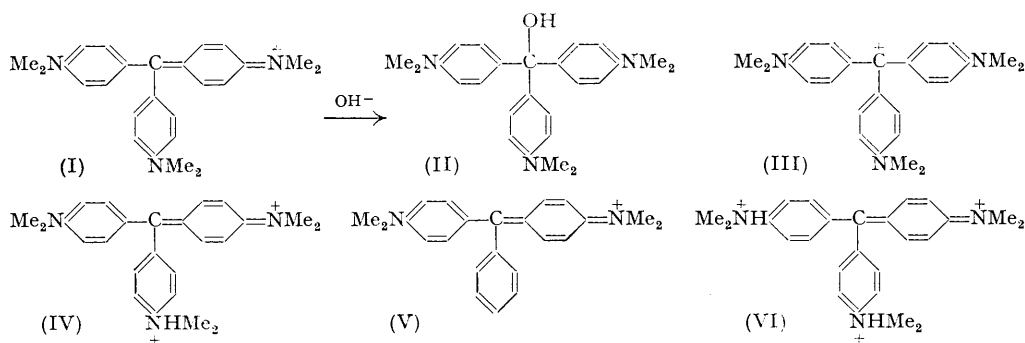
calculated from the equation (Michaelis, *Ann. N.Y. Acad. Sci.*, 1940, 40, 39)

$$K^{\frac{1}{2}} = 10^{E_1/0.059} - 3 \times 10^{-E_1/0.059} \quad (2)$$

where E_1 is the index potential defined in equation (17). Although these equations apply strictly only to reversible systems, the values of K obtained from them still retain a qualitative significance even for irreversible systems, as, *e.g.*, the aminoacridines.

At $\text{pH} \gg 11$, the wave-heights of both steps diminished at a measurable rate when the solution was kept, the dye colour meanwhile fading. The rate of the change to a colourless, non-reducible form of the dye increased with rise of pH . Thus at $\text{pH} 13$ the conversion was so rapid that it was impossible to prepare a solution for polarography before it had changed completely to irreducible material. A similar but faster time-decrease of wave-height occurred at $\text{pH} \ll 1$, rapidly yielding an equilibrium in which the extent of conversion to non-reducible matter, measured by the decrease in wave-height, increased as pH decreased and was almost 100% at $\text{pH} 0$. This conversion was also accompanied by change of colour, to green at $\text{pH} 1-0$, greenish-yellow at $\text{pH} 0$, and yellow at greater acidities.

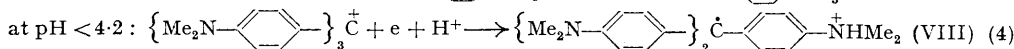
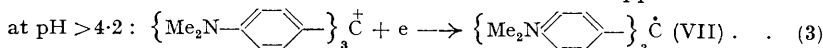
The change at $\text{pH} \gg 11$ is due to conversion of the violet form into the colourless carbinol (II) or, in the presence of ethanol, into the corresponding ether (Goldacre and Phillips, *J.*, 1949, 1724; Fischer, *Ber.*, 1900, 33, 3356). Neither carbinol nor ether has a carbonium-



ion resonance contribution. This suggests that the emergence of electroreducibility and violet colour below $\text{pH} 11$ may be ascribed to resonance between the carbonium-ion structure (III) and either three equivalent structures (I) or, if the three rings are not all coplanar (Lewis, Magel, and Lipkin, *J. Amer. Chem. Soc.*, 1942, 64, 1774), two such structures. The carbonium-ion structure would seem to confer electroreducibility because of the electron-deficient central carbon atom. At about $\text{pH} 1$, the colour change to green is probably due to acquisition of a proton by one nitrogen atom, as in (IV), which resembles the structure of malachite-green (V) in having only two nitrogen atoms and their associated rings conjugated; resonance with a carbonium-ion structure is still possible, however, and electroreducibility is still observed. At about $\text{pH} 0$, a second proton is added (*cf.* Adams and Rosenstein, *ibid.*, 1914, 36, 1452), giving structure (VI), which presumably accounts for the yellow colour. Since no further addition beyond the second proton occurs even in 100% sulphuric acid (Newman and Deno, *ibid.*, 1951, 73, 3644; Branch and Tolbert, *ibid.*, 1949, 71, 781), the quinonoid ring in (VI) is very resistant to conversion into a benzenoid ring by accession of a proton to the nitrogen atom with consequent change to carbonium-ion structure. Thus, (VI) is apparently unable to resonate with a carbonium-ion structure (*cf.* Newman and Deno, *loc. cit.*), perhaps because of repulsion of like charges, and therefore is not electroreducible.

Over the pH range 1—11, where crystal-violet exhibits the violet colour and normal electroreducibility, the variation of E_1 and E_2 with pH is as shown in Fig. 2. The E_1 - pH curve has a bend at $\text{pH} 4.2$, the direction of which indicates (Clark, *Chem. Reviews*, 1925, 2, 127) that the semiquinone has an acid dissociation constant K^s such that $\text{p}K^s = 4.2$. No bend appears in the E_1 - pH curve corresponding to the acid exponent $\text{p}K = 9.36$ found by Goldacre and Phillips (*loc. cit.*), for the carbinol form of crystal-violet in water at 25° , probably because in 50% ethanol the change to carbinol is too slow at $\text{pH} < 11$ and would in

any case yield the ether rather than the carbinol itself. Since the E_1 -pH slope is -103 mv below, and zero above, pH 4.2, the first stage of the reduction would appear to be



The last equation would indicate an E_1 -pH slope of -59 mv if the process concerned were reversible. However, a plot of $\log \{(\bar{i}_d - \bar{i})/\bar{i}\}$ against E (\bar{i} = average polarographic current at dropping mercury electrode potential E ; \bar{i}_d = average diffusion current) gave curves of varying slope for both reduction steps, indicating thermodynamic irreversibility. If it were assumed that the observed -103 mv slope corresponded to uptake of two protons,

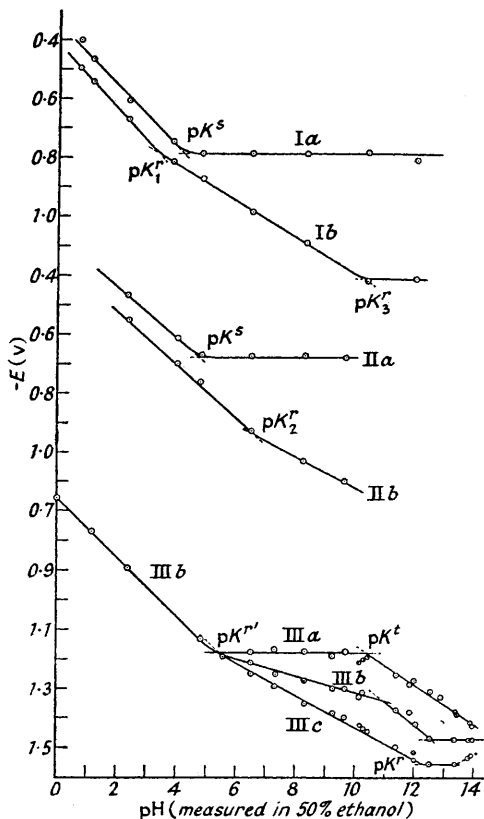


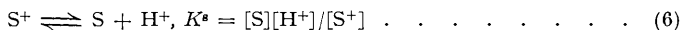
FIG. 2. Variation with pH of E_1 , E_2 , and E_m in 50% ethanol.
 I, Crystal-violet, $4 \times 10^{-4}\text{M}$: a, E_1 ; b, E_2 .
 II, Brilliant-green, $4 \times 10^{-4}\text{M}$: a, E_1 ; b, E_2 .
 III, Auramine, $4 \times 10^{-4}\text{M}$: a, E_1 ; b, E_m ; c, E_2 .

it would then be necessary to suppose that the semiquinone had two pK^s values close to each other and in the neighbourhood of 4.2.

If reaction (3) is treated as if reversible, with standard redox potential E_1^0 , the corresponding electrode potential in v at 25° is

$$E = E_1^0 + 0.059 \log [\text{Ox}]/[\text{S}] \quad (5)$$

where Ox denotes the sole ionic form ($\text{III} \leftrightarrow \text{I}$) of unreduced dye existing over pH range 1—11, and S denotes the semiquinone radical (VII). If (VIII) is denoted by S^+ , we have the ionisation equilibrium



whence the total concentration of semiquinone in both forms is

$$[\text{Sem}] = [\text{S}] + [\text{S}^+] = [\text{S}]\{1 + [\text{H}^+]/K^s\} \quad (7)$$

By substitution for $[\text{S}]$ in terms of $[\text{Sem}]$ from equation (7), and reference to the half-wave potential, where $E = E_1$ and $[\text{Ox}] = [\text{Sem}]$, equation (5) becomes

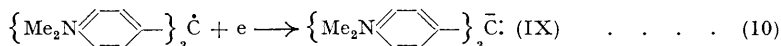
$$E_1 = E_1^0 - 0.059 \log K^s + 0.059 \log \{K^s + [\text{H}^+]\} \quad (8)$$

According to this equation, the E_1 -pH curve should have zero slope for $\text{pH} > \text{p}K^s$, and a slope of -59 mv for $\text{pH} < \text{p}K^s$. Apart from the numerical discrepancy in the latter slope, ascribed to irreversibility, the experimental curve is in conformity with this. A similar treatment, based on the assumption that S^+ (acid constant K_2^s) may add a further proton to give S^{++} (with acid constant K_1^s), leads to

$$E_1 = E_1^0 - 0.059 \log K_1^s K_2^s + 0.059 \log \{K_1^s K_2^s + K_1^s [H^+] + [H^+]^2\} \quad (9)$$

If $K_1^s \approx K_2^s$, a possibility suggested above, equation (9) indicates an E_1 -pH slope of zero for $\text{pH} > \text{p}K_1^s$, and -118 mv for $\text{pH} < \text{p}K_1^s$. The experimental slope of -103 mv is certainly nearer to the requirements of equation (9) than those of equation (8), but there is good reason (see below) to prefer equation (8).

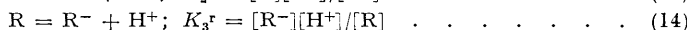
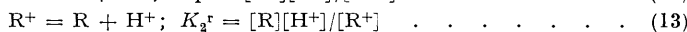
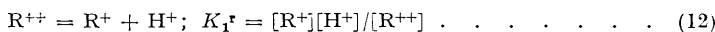
The zero slope of the E_2 -pH curve, at $\text{pH} > 10.3$ indicates that the second reduction step does not involve uptake of a proton. It is therefore represented by



leading to the formation of an anion $R^- = (\text{IX})$ similar to that postulated by us and by other workers in other comparable reductions (*J.*, 1951, 27, 2638). The electrode potential corresponding to reaction (10), treated as if reversible, is

$$E = E_2^0 + 0.059 \log [S]/[R^-] \quad (11)$$

We assume that the semiquinone exists in the forms S and S^+ as before, while fully reduced dye exists in the forms R^{++} , R^+ , R , and R^- , corresponding to successive removal of protons, according to the following processes, the last of which is almost certainly not reversible:



Then the total concentration of fully reduced dye in all four forms is

$$[\text{Red}] = [R^-] + [R] + [R^+] + [R^{++}] \\ = [R^-] \{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+] + K_1^r [H^+]^2 + [H^+]^3\} / \{K_1^r K_2^r K_3^r\} \quad (15)$$

By substitution for $[R^-]$ in terms of $[\text{Red}]$ from equation (15), and for $[S]$ in terms of $[\text{Sem}]$ from equation (7), and reference to the half-wave potential where $E = E_2$ and $[\text{Sem}] = [\text{Red}]$, equation (11) becomes

$$E_2 = E_2^0 + 0.059 \log K^s / K_1^r K_2^r K_3^r + 0.059 \log \{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+] \\ + K_1^r [H^+]^2 + [H^+]^3\} / \{K^s + [H^+]\} \quad (16)$$

With $K_1^r > K_2^r > K_3^r$, equation (16) would indicate, in the absence of the denominator $K^s + [H^+]$ of the last term, an E_2 -pH graph with linear sections of slopes -177 mv for $\text{pH} < \text{p}K_1^r$, -118 mv for pH between $\text{p}K_1^r$ and $\text{p}K_2^r$, -59 mv for pH between $\text{p}K_2^r$ and $\text{p}K_3^r$, and zero for $\text{pH} > \text{p}K_3^r$. Thus, with increasing pH , at each $\text{p}K^r$ value the slope should decrease numerically by 59 mv. Similarly the denominator $K^s + [H^+]$ should cause a numerical increase of 59 mv in the slope at $\text{pH} = \text{p}K^s$. If now it were assumed that $\text{p}K^s \approx \text{p}K_2^r$, the opposed effects on the slope of the bends at these two $\text{p}K$ values would annul each other, leaving a deceptively simple graph with slope -118 mv for $\text{pH} < \text{p}K_1^r$, -59 mv for pH between $\text{p}K_1^r$ and $\text{p}K_3^r$, and zero for $\text{pH} > \text{p}K_3^r$. A plot of equation (16) with the experimental values $\text{p}K_1^r = 3.4$, $\text{p}K_2^r = \text{p}K^s = 4.2$, $\text{p}K_3^r = 10.3$, and $E_2^0 + 0.059 \log K^s / K_1^r K_2^r K_3^r$ (extrapolated value of E_2 at $\text{pH} = 0) = -0.400$ v reproduces the experimental graph very well except for the discrepancy in slopes (-62 and -107 instead of -59 and -118 mv) due to irreversibility. If it were assumed, as suggested above in connexion with the E_1 -pH curve, that the semiquinone could exist in the form S^{++} as well as S and S^+ , a similar treatment would lead to the equation

$$E_2 = E_2^0 + 0.059 \log K_1^s K_2^s / K_1^r K_2^r K_3^r \\ + 0.059 \log \{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+] + K_1^r [H^+]^2 + [H^+]^3\} / \{K_1^s K_2^s + K_2^s [H^+] + [H^+]^2\}$$

According to this equation, the E_2 -pH slope could not exceed numerically -59 mv between $\text{pH} 1$ and $\text{pH} 11$, if $K_1^s \gg K_1^r \approx K_2^s \approx K_2^r \gg K_3^r$.

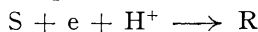
The facts that the E_1 -pH graph is horizontal in the region of pH 7, and that the semiquinone is apparently highly stable in this region ($E_1 \approx E_1 - E_2 = 0.111$ v, $K = 10^{3.75}$ at pH 7), show that this dye has redox properties similar to those of the more actively antibacterial aminoacridines.

Brilliant-green.—This dye also gave two-step polarograms in water, distorted by adsorption, which was eliminated by inclusion of 50% of ethanol in the solvent. 4×10^{-4} M-solutions of the dye in 50% ethanol were examined polarographically over the pH range 0–14. At pH >9, the dye underwent progressive fading and conversion into irreducible form as alkalinity increased, the solutions becoming turbid; the conversion rate increased with pH. These effects are ascribed to carbinol formation, as with crystal-violet. Conversion into irreducible form also occurred at pH below about 2, and was almost complete at pH 1, but little change of colour was observed until pH 0 was reached, the solution then becoming pale green-yellow. These changes may be explained similarly to the corresponding effects with crystal-violet.

In the intermediate pH range 2–9, the diffusion current constants of the two steps were independent of pH and slightly smaller than for crystal-violet. By applying the Ilkovič equation with the same values of D , m , t , and c as were used for crystal-violet, and with $\bar{i}_d = 0.35$ μ amp (first step), 0.38 μ amp (second step), the values $n = 0.79$ (first step) and 0.86 (second step) are obtained, confirming that each step involves one electron. The product of the first reduction step is thus a semiquinone radical, the apparent stability of which, at pH >5, is considerable, to judge from the wide separation of the two steps.

The E_1 -pH curve (Fig. 2) is of the same form as that for crystal-violet, and the same interpretation (equation 8) is applicable, with $pK^s = 4.8$, a somewhat greater value than for crystal-violet, in conformity with the superior electron-repelleny of ethyl compared with methyl, which renders the nitrogen atom more strongly basic in (*p*-Me₂N·C₆H₄)₂CPh than in (VII). The slope of the graph is zero in the region of pH 7, but at pH < pK^s it is -87 mv instead of -59 mv required by equation (8), presumably because of the irreversibility of electroreduction. No bend appears in the E_1 -pH graph corresponding to the pK value of 7.90 reported for pure aqueous solutions of brilliant-green at 25° by Goldacre and Phillips (*loc. cit.*), probably for the same reasons as in the case of crystal-violet.

The E_2 -pH graph (Fig. 2) is similar to that for crystal-violet, but lacks the horizontal section at extreme alkalinities. This section, starting from a bend at pH = $pK_3^r \approx 10$ –11, would probably have been obtained, had not carbinol formation prevented polarographic investigation at pH >9.5. In the pH range 6.5–10, the graph has a -52 mv slope, corresponding to the equation



for the electrode reaction. By applying the method used in deriving equation (16) for crystal-violet, but omitting R^- and K_3^r , an equation is obtained which is similar to equation (16) except for the omission of the term $K_1^r K_2^r K_3^r$ wherever it occurs. With the assumptions that $pK_2^r = 6.5$ (corresponding to the E_2 -pH bend at pH 6.5), and that $pK^s \approx pK_1^r$ (so that the opposing bends associated with these acid constants mutually cancel), the equation indicates that the E_2 -pH graph should have slopes -118 mv for pH < pK_2^r , and -59 mv for $pK_2^r > \text{pH} > pK_1^r$. The experimental slopes of -90 and -52 mv are in approximate agreement with theory, the discrepancy again being ascribed to irreversibility of the reduction. That pK_1^r , pK_2^r , and perhaps also pK_3^r are all greater for this dye than for crystal-violet is to be expected because of the greater electron repelleny of ethyl than that of methyl.

Brilliant-green, like crystal-violet, has a horizontal E_1 -pH plot and a high semiquinone apparent stability at pH 7 ($E_1 \approx E_1 - E_2 = 0.141$ v, $K = 10^{4.75}$), thus resembling the more actively antibacterial aminoacridines.

Malachite-green.—This was examined at two pH values only. Its behaviour was practically indistinguishable from that of brilliant-green, and therefore no further work was carried out on it.

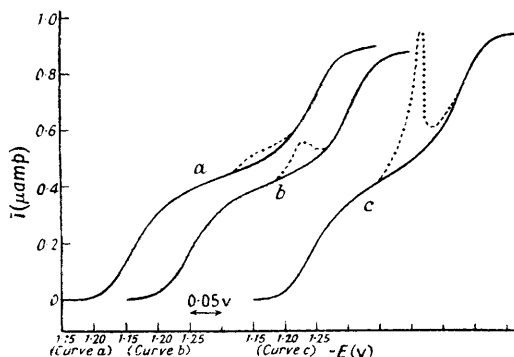
Auramine.—This dye gave grossly distorted polarograms in aqueous solution, indicating strong adsorption effects, confirmed by erratic galvanometer oscillations during a drop life,

as with acridine. The inclusion of 50% of ethanol in the supporting electrolyte again removed adsorption irregularities, but the polarograms showed a tendency to exhibit maxima, especially at high concentrations. The addition of 0.01% of methyl cellulose suppressed all maxima, including one at about -1.45 v which appeared as a hump in the pH range 11–13.5 approx. and was progressively exalted on incorporation of increasing amounts of potassium chloride in the solution (Fig. 3). Neither methyl cellulose nor potassium chloride affected the wave-height appreciably. The maxima are probably caused by adsorption of auramine or its reduction product on the mercury drops; this is enhanced by the salting-out effect of potassium chloride, or prevented by the preferential adsorption of methyl cellulose. An alternative view is that the maxima are due to non-uniform polarisation of the mercury-drop surface, with consequent tangential flow of mercury at the surface instead of the radial flow assumed in the derivation of the Ilkovič equation. This view is, however, unlikely since it would lead to the expectation that addition of potassium chloride, by increasing the conductance of the solution, should render the mercury-drop surface more uniformly polarised and thus tend to suppress the maxima.

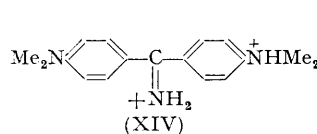
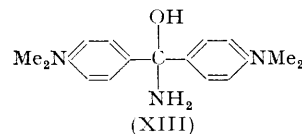
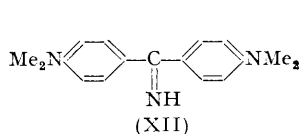
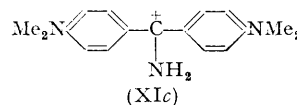
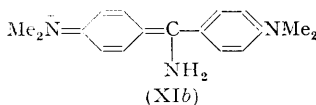
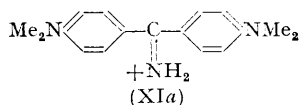
If attention is restricted to solutions in 50% ethanol, at pH >5.3 the polarograms exhibited two steps, with maximum separation at pH 10, and with diffusion current constants independent of pH. The wave-heights of the first and second steps for 4×10^{-4} M-auramine at pH 12 were 0.439 and 0.449 μ amp respectively, which are not very

FIG. 3. Polarograms for auramine, 4×10^{-4} M in 50% ethanol, pH 12.0.

- a, No potassium chloride.
b, 0.1N-Potassium chloride.
c, N-Potassium chloride.



different from the values given for crystal-violet in the same solvent with the same values of c , m , and t . If D is assumed to have roughly the same value for both dyes, the Ilkovič equation indicates that $n = 1$ for each step in the auramine reduction. At pH below about 5.3, the two steps merged into a single two-electron step. Between pH 5 and pH 7 the auramine reduction wave was followed immediately by a catalytic hydrogen wave, which rendered the polarograms ill-defined and unreliable. At pH above 7, this catalytic wave shifted to potentials sufficiently different from those of the second auramine step (about 0.7 v more negative) to preclude any interference with the latter. At the same time the catalytic wave height decreased with rise of pH, reaching zero at about pH 12.5.



Auramine (XIa), unlike the other dyes examined, is electroreducible over the entire pH range 0–14. Its yellow colour becomes greener near pH 0, and at pH above about 12 it is colourless, as also at pH well below 0. Goldacre and Phillips (*loc. cit.*) state that, upon

treatment with alkali, auramine loses a proton to give the imine (XII) with a pK of 10.71 in water at 25°; the imine takes up water only very slowly to give the colourless carbinol (XIII), which by analogy with crystal-violet carbinol is probably irreducible. We assume that the colour of auramine is due to resonance with structures such as (XI*b*), and its electroreducibility to resonance with the carbonium-ion structure (XI*c*), as with crystal-violet. Addition of a proton gives (XIV), in which reducibility persists and colour is only modified to greener shades, because both types of resonance required are still possible, although one is decreased in extent by the nitrogen atom's acquiring a proton. Further addition of protons would inhibit completely the resonance associated with the colour, and thus does not occur unless the pH is well below 0. In the imine, both types of resonance are inhibited because they would necessitate a separation of opposite charges. Thus, as in Michler's ketone ($p\text{-Me}_2\text{N}\cdot\text{C}_6\text{H}_4)_2\text{CO}$, colour disappears, but electroreducibility remains because there is still a centre of positive charge at the central carbon atom owing to the considerable polarisation of the double bond $\overset{\delta+}{\text{C}}=\overset{\delta-}{\text{N}}\text{H}$.

In connexion with the E_1 -pH and E_2 -pH graphs for auramine (Fig. 2), it is necessary to distinguish between the standard redox potentials E_1, E_2 (at a given pH) for two successive one-electron reduction steps, and the dropping mercury electrode potentials $E_{\frac{1}{2}}, E_{\frac{3}{4}}$ at $\frac{1}{2}$ and $\frac{3}{4}$ of the total reduction wave height or extent of reduction. For a reversible system buffered to constant pH, it is readily shown that, in v at 25°,

$$E_i = E_{\frac{1}{2}} - E_m = E_m - E_{\frac{3}{4}} = 0.059 \log K^{\ddagger} + 0.059 \log \left(\frac{1}{2} + \sqrt{\frac{1}{4} + 3/K} \right) \quad (17)$$

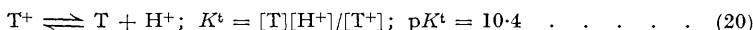
$$E_1 - E_m = E_m - E_2 = 0.059 \log K^{\ddagger} \quad (18)$$

where E_m is the potential at half the total wave height or extent of reduction. It follows that

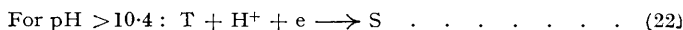
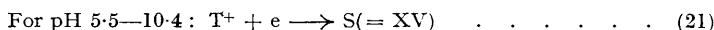
$$E_{\frac{1}{2}} - E_1 = E_2 - E_{\frac{3}{4}} = 0.059 \log \left(\frac{1}{2} + \sqrt{\frac{1}{4} + 3/K} \right) = \delta \quad (19)$$

When the two one-electron steps are well separated, so that E_1 and K are large, δ is negligible, and thus $E_{\frac{1}{2}} = E_1, E_{\frac{3}{4}} = E_2$. For $\delta = 1, 2,$ and 5 mv (the last two values being of the order of the experimental error in potential measurements), $E_i = 56, 47,$ and 37 mv, respectively (equations 17, 19). In the measurements with the triphenylmethane dyes E_i is never less than about 37 mv, so δ is negligible. For auramine the same is true for pH down to about 6.5, where E_i is 40 mv. At lower pH, however, E_i falls below 37 mv, and thus E_1 and E_2 must not be assumed equal to $E_{\frac{1}{2}}$ and $E_{\frac{3}{4}}$, but may be calculated from E_i and E_m by means of equations 2 and 18. At pH 5.5 approx., E_i for auramine is 21 mv, corresponding to the point where $E_1 = E_2 = E_m$ and the E_1 -pH and E_2 -pH graphs cross. Below about pH 5.3, E_i is constant at 14 mv, corresponding to $K = 0$ or absence of semiquinone formation; this contrasts with the behaviour of the triphenylmethane dyes, which exhibited semiquinone formation over the whole pH range examined, except where they became irreducible at extreme acidity or alkalinity.

The E_1 -pH graph (Fig. 2) consists of two linear portions with slopes 0 (pH 5.5—10.4) and -73 mv (pH >10.4). If it is assumed that auramine exists in the two forms T^+ (= XI) and T (= XII) related by the equilibrium



(cf. $pK^{\ddagger} = 10.71$ for aqueous solutions; Goldacre and Phillips, *loc. cit.*), the slopes suggest that the first reduction step is



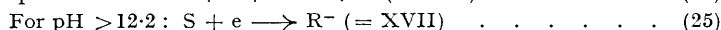
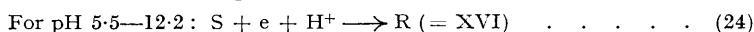
By the method described for crystal-violet, we derive the equation

$$E_1 = E_1^0 + 0.059 \log \{ [H^+]/([H^+] + K^{\ddagger}) \} \quad (23)$$

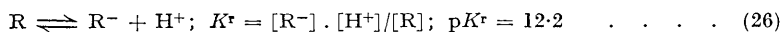
in which E_1^0 is the standard redox potential of process (21). The experimental E_1 -pH graph accords with equation (23), apart from the discrepancy between the observed and theoretical (-59 mv) slope for pH >10.4 , probably due mainly to irreversibility.

The E_2 -pH graph (Fig. 2) consists of two linear sections with slopes -53 mv (pH 5.5—

12.2) and 0 (pH 12.2—13.4), followed by an upward bend at pH 13.4. This bend will be ignored, as it is probably due to the change of supporting electrolyte from a weak-acid buffer to sodium hydroxide solution, with consequent increase in ionic strength. This increase is known to make E_2 and also E_1 more positive; thus, at pH 12.0, the inclusion of 0.1N- and 1.0N-potassium chloride in the supporting electrolyte raised E_2 from -1.544 to -1.510 and -1.480 v, and E_1 from -1.272 to -1.263 and -1.252 v, respectively. The slopes suggest that the second reduction step is



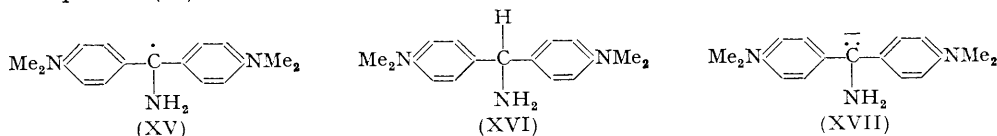
The anionic product R^- , which probably abstracts a proton from a water molecule immediately after formation, thus changing to the form R, is analogous to the anionic reduction product postulated for crystal-violet. Assuming that R and R^- are related by the equilibrium



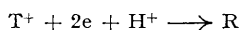
we derive by a familiar method the equation

$$E_2 = E_2^0 + 0.059 \log \{([\text{H}^+] + K^r) / K^r\} \quad (27)$$

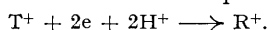
in which E_2^0 is the standard redox potential of process (25). Apart from a small discrepancy in slope for pH 5.5—12.2, the experimental E_2 -pH graph is in conformity with equation (27).



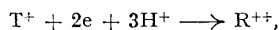
The E_m -pH graph (Fig. 2) comprises linear sections with slopes -98 mv (pH <5.3) and -29 mv (pH $5.3\text{--}10$ approx.). At pH $>ca.$ 10, the second reduction step was considerably steeper than the first, so that E_m , which was always taken as the potential at the point of inflexion between the two steps in order to allow for small differences in the wave-heights, was far from midway between E_1 and E_2 ; little theoretical significance could then be attached to E_m . For pH $5.3\text{--}10$ approx., the slope is in accordance with the overall two-electron reduction process



which is the sum of processes (21) and (24). The bend in the E_m -pH graph at pH 5.3 suggests that for pH <5.3 the reduction product exists mainly in the form R^+ resulting from addition of a proton to R, with acid exponent $pK^r = 5.3$ for the associated ionisation. For pH <5.3 , the one-stage two-electron reduction process would then be



This, however, corresponds to a slope of -59 mv. A closer approximation to the observed slope of -98 mv would be obtained by supposing that in this pH range R^+ has added a proton at one of the nitrogen atoms to give R^{++} , with acid exponent $pK^{r'}$ for the associated ionisation equilibrium only slightly smaller than pK^r . The reduction process would then be



corresponding to a slope of -87 mv.

We note, in conclusion, that auramine resembles the triphenylmethane dyes studied in having a horizontal E_1 -pH plot around pH 7 and a high apparent semiquinone stability in this region ($E_1 = 0.049$ v, $K = 38.5$ at pH 7).

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