619. *The Polarographic Reduction of Pyridine, Quinoline, and Phenazine.*

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Pyridine, quinoline, and phenazine, unlike certain bacteriostatic aminoacridines and di- and tri-phenylmethane dyes, do not form apparently highly stable semiquinones on polarographic reduction at pH $\approx$ 7. This is possibly related to their comparatively low bacteriostatic activity. Quinoline and phenazine form relatively unstable semiquinones at pH $\approx$ 7. The pH-dependence of the half-wave potentials of the two one-electron reduction steps for phenazine is similar to that of 1-hydroxyphenazine and of pyocyanine.

We have shown (*J.*, 1951, 27, 2638; and preceding paper) that several bacteriostatic aminoacridines and di- and tri-phenylmethane dyes yield, during polarographic reduction, apparently highly stable semiquinone radicals, which, for the more active of these compounds, are produced at the biologically important pH $\approx$ 7 by uptake of one electron and no proton. In order to substantiate the view that these properties are associated with bacteriostatic activity, we have examined the polarographic reduction of phenazine and (briefly) pyridine and quinoline, three non-bacteriostatic compounds structurally related to acridine.

Shikata and Tachi (*Mem. Coll. Agric. Kyoto*, 1927, **4**, 19) obtained two waves (−1·5 and −1·7 v against the saturated calomel electrode) for pyridine in 0·001N-hydrochloric acid plus 0·1N-potassium chloride, attributed to 6-electron reduction of the ionised and the un-ionised base. Later workers (Adkins and Cox, *J. Amer. Chem. Soc.*, 1938, **60**, 1151; Tompkins and Schmidt, *J. Biol. Chem.*, 1942, **143**, 643; Knobloch, *Coll. Czech. Chem. Comm.*, 1947, **12**, 407; Shchennikova and Korshunov, *J. Phys. Chem. U.S.S.R.*, 1948, **22**, 503; *Chem. Abstr.*, 1948, **42**, 7169) generally agree that one of these waves is a catalytic hydrogen wave, also obtained with pyridine derivatives, and that in buffers no wave is obtained except at pH *ca.* 6—9. For quinoline in alkaline or neutral salt solutions, Pech (*Coll. Czech. Chem. Comm.*, 1934, **6**, 126) obtained two waves of equal height, attributed to successive 2-electron reductions; inadequate buffering and polarogram distortion by adsorption on the dropping mercury electrode probably vitiate these results.

Tachi and Kabai (*J. Electrochem. Assoc. Japan*, 1935, **3**, 250; *Chem. Abstr.*, 1936, **30**, 2500), Adkins and Cox, Knobloch, and Shchennikova and Korshunov (*locc. cit.*) obtained two or more waves for quinoline in various buffered and unbuffered acid, neutral, and alkaline media; some of these waves were ascribed to catalytic hydrogen deposition, and others to adsorption effects. So far as we are aware, no polarographic investigation of phenazine has previously been published, but the reduction of 1-hydroxyphenazine has been studied polarographically by Müller (*Cold Spring Harbor Symp.*, 1939, **7**, 59), and potentiometrically by Michaelis, Hill, and Schubert (*Biochem. Z.*, 1932, **255**, 66).

Experimental

"AnalaR" pyridine was used without further purification. Quinoline was fractionally distilled, and the fraction of b. p. 236° employed. Phenazine was repeatedly recrystallised from aqueous ethanol; the m. p. of the final product was 169°. All other materials, apparatus, and technique were as previously described (*J.*, 1951, 27). All measurements were at 25°.

Results and Discussion

Pyridine.—For 4×10^{-4}M-solutions, no pyridine reduction wave was detected in aqueous buffers at pH 1·0, 7·38, and 11·0. In the unbuffered supporting electrolyte used by Shikata and Tachi (*loc. cit.*), one reduction wave (Fig. 1*b*) was obtained, with half-wave potential −1·490 v against the saturated calomel electrode. Applying the Ilkovič equation $\bar{i}_d = 605nD^{\frac{1}{2}}cm^{\frac{2}{3}}t^{\frac{1}{6}}$ (*Coll. Czech. Chem. Comm.*, 1934, **6**, 498), with $\bar{i}_d$ (mean diffusion current) = 3·83 μamp, c (concentration) = 0·4 millimole/l., m (mercury flow rate) = 0·902 mg./sec., t (mercury drop time) = 3·2 sec., D (diffusion coefficient) = $8{\cdot}6 \times 10^{-6}$ cm.^{2}/sec. (the value given for the geometrically similar molecule benzoquinone by Kolthoff and Orlemann, *J. Amer. Chem. Soc.*, 1941, **63**, 664), we obtain n (number of electrons involved

per molecule) = 4·8. This, together with the value of about 14 mv found for the index potential E_i (potential difference between the 25% and 50% reduction points), indicates that pyridine forms no semiquinone on reduction.

Quinoline.—Well-buffered 4×10^{-4}M-solutions of pH 2·0—7·4 gave polarograms badly distorted by adsorption effects, which also caused erratic galvanometer oscillations during a single drop life, as with acridine (*J.*, 1951, 27). Inclusion of 50% (by volume) of ethanol in the supporting electrolytes eliminated these effects, as shown by current–time oscillograms (Butler and Kaye, unpublished) similar to those for acridine. However, maxima still remained; these were suppressed by adding 0·04% of gelatin, normal polarograms, typified by Fig. 1*a*, being then obtained.

Applying the Ilkovič equation with $\bar{i}_d = 1{\cdot}13$ μamp, $c = 0{\cdot}4$ millimole/l., $m = 0{\cdot}902$ mg./sec., $t = 3{\cdot}2$ sec., $D = 3{\cdot}08 \times 10^{-6}$ cm.^{2}/sec., we obtain $n = 2{\cdot}35$. The value of D was obtained from the value $8{\cdot}0 \times 10^{-6}$ for quinaldinic acid in water at 25° (Stock, *J.*, 1944, 427), by deducting 38% from its square root to allow for the change from water to 50% ethanol, by analogy with the results of Gill (Thesis, London, 1947) and of Shreve and Markham (*J. Amer. Chem. Soc.*, 1949, **71**, 2993), as discussed elsewhere (*J.*, 1951, 27). Despite some uncertainty in D, it seems that the polarogram is a two-electron wave.

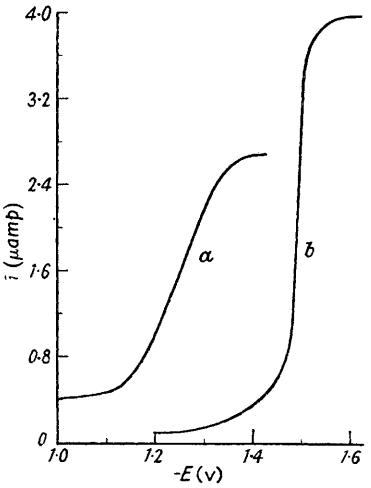


FIG. 1. *Polarograms for*: *a*, *quinoline*, 4×10^{-4}M *in* 50% *ethanol*, pH 6·51 (*current scale half of that shown*), *and b*, *pyridine*, 4×10^{-4}M *in aqueous* 0·001N-*hydrochloric acid plus* 0·1N-*potassium chloride*.

From the value $E_i = 40$ mv at pH 6·51, the semiquinone formation constant K was evaluated by Michaelis's equation $K^{\frac{1}{2}} = 10^{E_i/0{\cdot}059} - 3 \times 10^{-E_i/0{\cdot}059}$ (*Ann. N.Y. Acad. Sci.*, 1940, **40**, 39) as 16. Thus the wave consists of two overlapping one-electron steps, involving the intermediate formation of a semiquinone. The latter is apparently much less stable than the semiquinones of acridine and some bacteriostatic aminoacridines and di- and triphenylmethane dyes, for which K ranges from 43·7 to 10^{10} in the same solvent and pH region.

It is of interest to compare the above results with those obtained in aqueous media at 25° for quinaldinic acid, 8-hydroxyquinoline, quinoline-8-carboxylic acid (Stock, *J.*, 1944, 427; 1949, 586, 763), and cinchoninic acid (Casimir and Lyons, *J.*, 1950, 783). In all cases, severe distortion of polarograms due to adsorption effects and maxima render the results uncertain, but there is distinct evidence of two single-electron reduction steps at pH $\approx$ 7, the half-wave potential of the first step being independent of pH for only 8-hydroxyquinoline and possibly quinoline-8-carboxylic acid. The index potentials at pH $\approx$ 7 for these four compounds are 0·23, 0·56, 0·80, and 0·60 v respectively, corresponding to K values $7{\cdot}7 \times 10^3$, $2{\cdot}9 \times 10^9$, $3{\cdot}2 \times 10^{13}$, and $1{\cdot}4 \times 10^{10}$ respectively. It is noteworthy that 8-hydroxyquinoline, plasmoquin, and other 8-substituted quinolines display antibacterial activity.

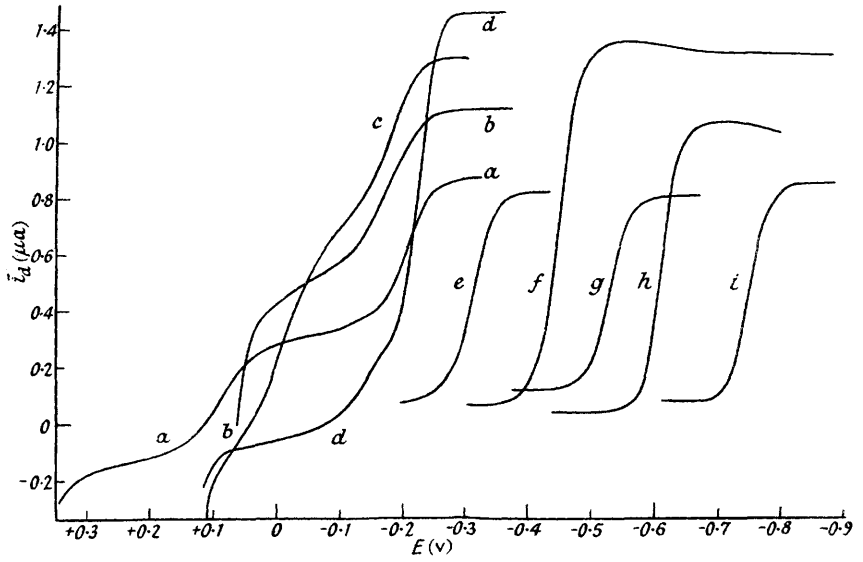
Phenazine.—4×10^{-4}M-Solutions in aqueous buffers were examined; at pH >2, it was necessary to include 10% by volume of ethanol in the supporting electrolyte because of the low aqueous solubility of the free base.

In strongly acid solutions, reduction occurred in two well-separated steps (Fig. 2), each corresponding to uptake of one electron since the $i_d/cm^{\frac{2}{3}}t^{\frac{1}{6}}$ values approximate to those of the closely similar acridine molecule. As the pH increased, these steps moved closer together, and at pH>2 they coalesced into a single two-electron step. In Fig. 3 are plotted against pH (determined for non-alcoholic buffers) values of $K^{\frac{1}{2}}$ calculated from E_i and Michaelis's equation, and also the half-wave potentials E_1 and E_2 of the first and second steps, calculated from E_m (d.m.e. potential at 50% reduction) by means of the equation

$$E_1 - E_m = E_m - E_2 = 0{\cdot}059 \log K^{\frac{1}{2}}$$

Figs. 2 and 3 show that in acid solutions a stable semiquinone is formed in the first reduction step, but its stability rapidly decreases as pH rises. At pH 3·7, where the E_1–pH and E_2–pH graphs cross, $K = 1$; at pH $\approx$ 7, $E_i = 17$ mv and $K = 0{\cdot}153$.

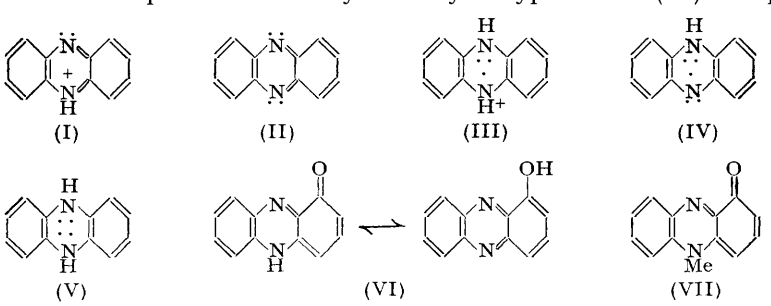
FIG. 2. *Polarograms for phenazine in* 10% *ethanol at* 25°.



10^4 Concn., M		pH	10^4 Concn., M		pH
a,	4 †	0 (N-H_2SO_4)	*f*,	4	7·38
b,*	4	1·0 (0·1N-HCl)	*g*,	2	9·07
c,	4	1·925	*h*,	4	11·0
d,	4	3·95	*i*,	2	13·0
e,	2	5·58			

* Anodic wave due to Cl^- swamps first step. † Solvent, H_2O; current twice that shown.

Fig. 3 is of practically the same form (at least up to pH 9 or 10) as the E_1–pH and E_2–pH graphs obtained potentiometrically for 1-hydroxyphenazine (VI) and pyocyanine



(VII) by Michaelis, Hill, and Schubert (*loc. cit.*), who showed unequivocally that semiquinone formation occurred with these substances. Thus a similar phenomenon for

phenazine is confirmed. Furthermore, polarographic values of E_1 and E_2 for 1-hydroxyphenazine obtained by Müller (*loc. cit.*) fall on the potentiometric E_1–pH and E_2–pH curves for this compound, when all potentials are referred to the same standard; this shows that polarography is a suitable alternative to potentiometry for investigating redox behaviour.

A familiar procedure (*J.*, 1951, 2638) shows that the two bends in the E_1–pH curve (Fig. 3) indicate acid exponents $pK_t = 3{\cdot}2$ for phenazine and $pK_s = 3{\cdot}8$ for its semiquinone. The pK_s value is confirmed approximately by the pH at the sole bend in the E_2–pH curve. We assume that phenazine exists in the ionic form T^+ (I) at $pH<pK_t$, and as neutral base T (II) at $pH>pK_t$. The slopes of the three linear sections of the E_1–pH curve indicate that the numbers of protons involved per molecule reduced in the first step accord with the equations $T^+ + e + H^+ \longrightarrow S^+ (pH<pK_t)$, $T + e + 2H^+ \longrightarrow S^+ (pK_t< pH <pK_s)$ and $T + e + H^+ \longrightarrow S (pH>pK_s)$. Thus the semiquinone exists in the forms S^+ (III) and S (IV) at pH respectively below and above pK_s. Similarly, for the second

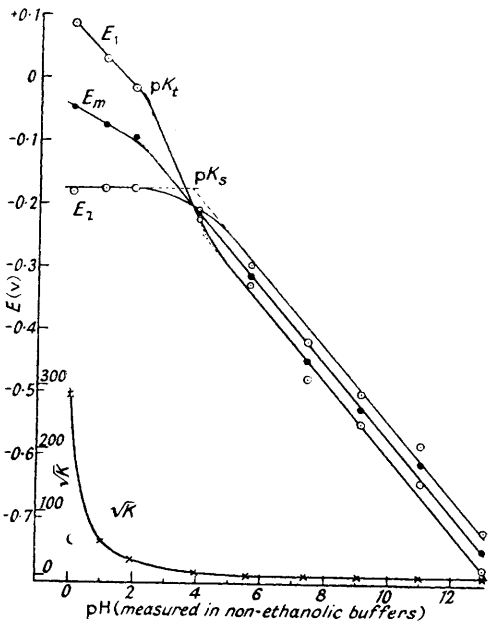


FIG. 3. *Variation with* pH *of* E_1, E_2, E_m, *and* $\sqrt{K}$ *for phenazine,* 4 × 10^{-4}M *in* 10% *ethanol.*

step we may write $S^+ + e \longrightarrow R(= V)$ for $pH<pK_s$, and $S + e + H^+ \longrightarrow R$ for $pH>pK_s$. By a well-known method (*J.*, 1951, 2638) we then deduce the following equations for the E_1–pH and E_2–pH curves :

$$E_1 = E_1^0 + 0{\cdot}059 \log \frac{K_s[H^+] + [H^+]^2}{K_t + [H^+]}$$

$$E_2 = E_2^0 + 0{\cdot}059 \log \{[H^+]/([H^+] + K_s)\}$$

in which E_1^0 and E_2^0 are the values of E_1 and E_2 respectively at pH = 0.

Species S^+ is stabilised by equivalent resonance, in which, *e.g.*, the odd electron may reside on either nitrogen atom. No equivalent resonance is possible for S, which explains why semiquinone stability becomes very small at $pH>pK_s$. The semiquinone obtained by oxidising *p*-phenylenediamine behaves similarly (Michaelis, Schubert, and Granick, *J. Amer. Chem. Soc.*, 1939, **61**, 1981).

The present results show that non-bacteriostatic pyridine, quinoline and phenazine, unlike several antibacterial aminoacridines and di- and tri-phenylmethane dyes, do not form apparently highly stable semiquinones during reduction at pH ≈ 7.

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