Flavin Model Systems. 1. The Electrochemistry of 1-Hydroxyphenazine and Pyocyanine in Aprotic Solvents

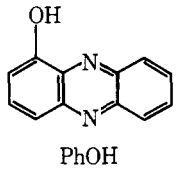
Mark M. Morrison, Eddie T. Seo, John K. Howie, and Donald T. Sawyer*

Contribution from the Department of Chemistry, University of California, Riverside, California 92521. *Received March 28, 1977*

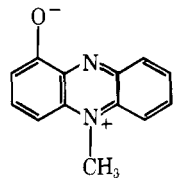
Abstract: The oxidation-reduction chemistry of 1-hydroxyphenazine (PhOH) and pyocyanine (Py) has been studied by cyclic voltammetry and controlled potential coulometry in acetonitrile and dimethyl sulfoxide solution. The reactant and product species have been monitored by UV-visible, IR, and ESR spectroscopy. In neutral solution PhOH is reduced by two irreversible one-electron steps at −0.76 V vs. SCE and −1.6 V, respectively. In acidic acetonitrile PhOH is protonated to give a species with two new reversible one-electron reductions at +0.36 and +0.08 V. Pyocyanine is reversibly reduced by two one-electron steps to the anion radical and the dianion, respectively. Proton addition to Py also results in two new reduction peaks. The protonation behavior of PhOH and Py has been confirmed by comparison with 1-methoxyphenazine and *N*-methylphenazinium ion. The results establish that the oxidation-reduction chemistry of PhOH and Py has many similarities to that for riboflavin. Pyocyanine appears to be an especially good redox model for flavin systems.

The nonaqueous electrochemistry of phenazine and substituted phenazines is of interest because of their use as dye stuffs, the biological importance of some derivatives, and the structural relationship to isoalloxazine and to flavoproteins, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD).[1-16] Because electrochemical studies of flavoproteins, FMN, and FAD are experimentally difficult, investigations often examine simpler model compounds, such as riboflavin, lumiflavin, 3-methyllumiflavin, 10-methylisoalloxazine, 8-quinolinol, and phenazine. Previous studies have noted the deficiencies of these model systems and ambiguities due to disproportionation reactions in aqueous solution.[15,16]

1-Hydroxyphenazine (PhOH) and pyocyanine (Py) are well



PhOH



Py

suited to serve as isoalloxazine models owing to their similar conjugation, ring structure, and substitution pattern. The exo substituent at the 1 position for PhOH and Py, and the methyl substituent at the 5 position for Py, make possible metal chelation similar to that for riboflavin. The ability of PhOH to chelate a variety of metals has been demonstrated.[17-27] In addition, pyocyanine is of interest due to its biosynthesis by *Pseudomonas aeruginosa*.[28-31]

Previous electrochemical studies of PhOH and Py have been limited to polarography. Muller observed a prewave for the reduction of aqueous PhOH,[32] which has been attributed to adsorption of product.[14] He concluded that at neutral pH the primary reduction mechanism involves reduction of the bridging nitrogens, followed by proton addition to form the 5,10-dihydro species.[32] Nonaqueous electrochemical studies of PhOH have been limited to dimethylformamide.[8] In this medium the first reduction is an irreversible one-electron process in contrast to the usual reversible one-electron reduction for phenazine. Previous electrochemical investigations of Py are limited to aqueous media[33] and to the use of Py as an electron mediator ($E^{0\prime} = -0.034$ V vs. NHE).[34]

This paper summarizes the results of a study of the redox reactions of Py and PhOH in acetonitrile and dimethyl sulfoxide and their redox behavior in the presence of added acid and base. Reaction mechanisms for PhOH and Py are compared to those for 1-methoxyphenazine (PhOMe) and *N*-methylphenazinium methylsulfate ($MePh^+$), and intercompared with isoalloxazine and its derivatives.

Experimental Section

1-Hydroxyphenazine (Aldrich Chemical Co.) (PhOH) was chromatographed on acidic alumina (Woelm) with benzene-ether, recrystallized from benzene-heptane, and sublimed to give a bright yellow-orange product, mp 157-158 °C (lit. 153-155 °C).[35] *N*-Methylphenazinium methylsulfate ($MePh^+$) was obtained from Aldrich and used without further purification. Pyocyanine (Py) was prepared by the photochemical oxidation of $MePh^+$ in aqueous solution.[35] Crystals were obtained from chloroform-hexanes, mp 131-132 °C (lit. 133 °C).[35] Py was stored over P_4O_{10} in vacuo prior to analysis. 1-Methoxyphenazine was prepared by condensing oxidized 3-methoxycatechol with *o*-phenylenediamine.[35] The product was chromatographed on neutral alumina (Woelm) with benzene-ether and recrystallized from benzene-hexanes to yield small, lemon-colored crystals, mp 168-169 °C (lit. 167-169 °C).[35]

Acetonitrile (AN) ("Spectroquality", MCB, or "Distilled-in-Glass", Burdick and Jackson Laboratories, Inc.) and dimethyl sulfoxide (Me_2SO) (Baker Analyzed) were used without further purification. Tetra-*n*-propylammonium perchlorate (TPAP) was synthesized by reacting stoichiometric amounts of aqueous tetra-*n*-propylammonium hydroxide with dilute perchloric acid, recrystallized several times from water, and dried in vacuo at 50 °C. For all electrochemical experiments the solvent contained 0.10 M TPAP as supporting electrolyte. Standardized tetraethylammonium hydroxide (TEAOH) (25% in methanol, Eastman Organic) and 1.00 M $HClO_4$ were used as titrants to study protonation behavior.

The electrochemical instrumentation consisted of a Princeton Applied Research Corp. Model 173A potentiostat, Model 179 digital coulometer, Model 175 Universal Programmer, and a Hewlett-Packard Model 7030A X-Y recorder. The three-electrode system included an Ag/AgCl (aqueous Me_4NCl) cracked bead reference electrode adjusted to 0.000 V vs. SCE,[36] a Pt flag auxiliary electrode separated from the bulk of the solution by a medium porosity fritted disk, and a Pt working electrode. The reference electrode was held in a Luggin capillary to minimize IR errors in the measured potential. For cyclic voltammetry experiments a Beckman Pt disk electrode (0.23 cm^2 area) was used as the working electrode. For controlled potential electrolysis and coulometry a cylindrical Pt gauze working electrode was used. The electrolysis cell consisted of either a Leeds and Northrup polyethylene cell top with a 100-mL electrolysis beaker or a Brinkman titration cell. Solutions were degassed with high-purity argon for 10 min prior to analysis and blanketed during analysis to exclude oxygen.

Electron spin resonance (ESR) spectra were obtained on a Varian V-4500 spectrometer using standard quartz cells. The field was

0002-7863/78/1500-0207$01.00/0

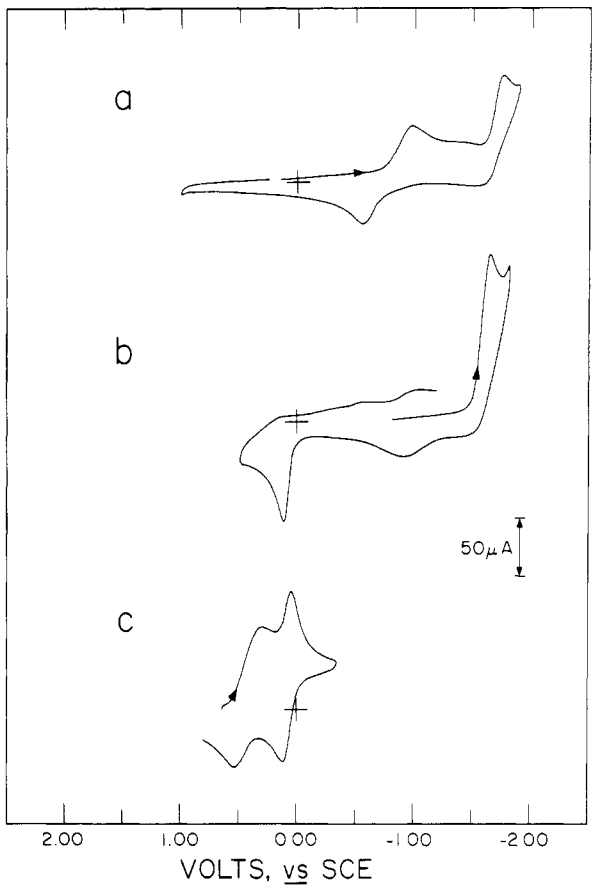


Figure 1. Cyclic voltammograms of 1 mM 1-hydroxyphenazine in 0.10 M TPAP-acetonitrile at a Pt electrode (scan rate 0.10 V s^{-1}); (a) neutral solution, (b) with 1 mM tetraethylammonium hydroxide added, and (c) with 1 mM $HClO_4$ added.

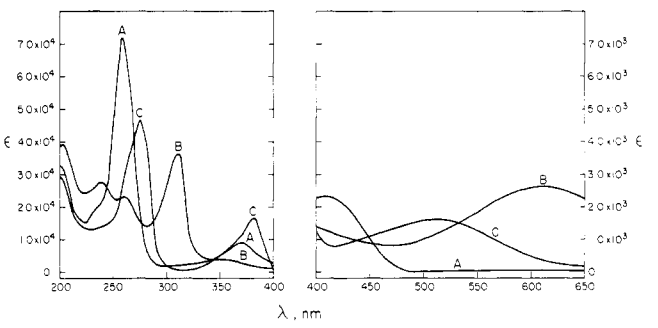


Figure 2. Ultraviolet and visible spectroscopy of 1-hydroxyphenazine in acetonitrile; (A) neutral solution, (B) with 1 equiv of tetraethylammonium hydroxide added, and (C) with 1 equiv of $HClO_4$ added.

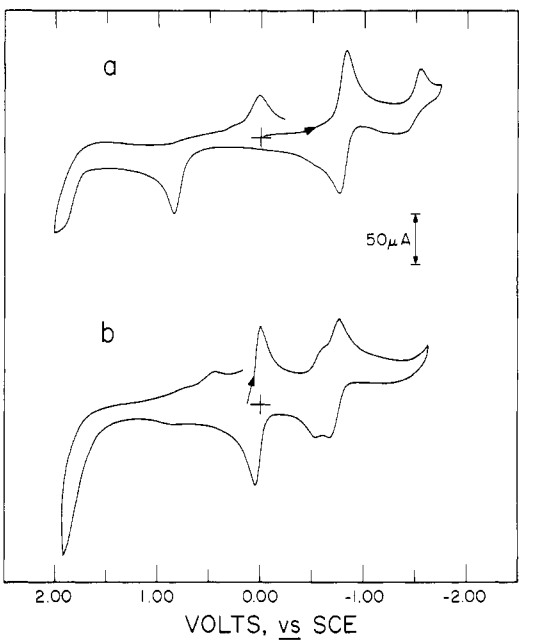


Figure 3. Cyclic voltammograms of 1 mM pyocyanine in 0.10 M TPAP-acetonitrile at a Pt electrode (scan rate 0.10 V s^{-1}); (a) neutral solution, (b) with 1 mM $HClO_4$ added.

standardized with potassium nitrosodisulfonate (g = 2.00550). Ultraviolet, visible, and near-infrared spectra were obtained with a Cary Model 14 spectrophotometer using quartz cells.

Results

1-Hydroxyphenazine. Figure 1a illustrates the cyclic voltammogram for 1-hydroxyphenazine (PhOH) in neutral acetonitrile, with an irreversible one-electron reduction peak at −0.92 V vs. SCE. The coupled anodic peak at −0.60 V is characteristic of molecular hydrogen. An ESR signal is not observed either before or after a one-electron controlled potential reduction at −1.00 V. The original yellow solution (Figure 2A) becomes blue (Figure 2B) after electrolysis at −1.00 V, and yields the cyclic voltammogram shown in Figure 1b. Addition of 1 equiv of acid after the electrolysis yields a yellow solution with electrochemical properties that are identical with those for PhOH. The second reduction wave for PhOH at −1.67 V (Figure 1a) is reversible, but the product appears to abstract protons from the solvent media. Controlled potential coulometry for this wave is not possible because of the negative potential, but on the basis of relative peak heights, this reduction also appears to be a one-electron process. Additional small peaks are observed for the cyclic voltammogram for PhOH, but they appear to be due to secondary processes and may involve adsorbed product species. In addition, a poorly defined oxidation peak occurs at +1.5 V. These small waves and the oxidation of PhOH have not been investigated further.

In acetonitrile addition of 1 equiv of tetraethylammonium hydroxide per PhOH produces a blue solution that exhibits the same redox (Figure 1b) and spectroscopic characteristics (Figure 2B) as that obtained for PhOH after reduction at −1.00 V. The irreversible oxidation peak at −0.14 V in Figure 1b is characterized by film formation on the electrode surface after electron transfer, and therefore precludes study of the electron stoichiometry. The irreversible reduction peak at −1.67 V appears to be a multielectron step because of the catalytic reduction of solvent.

With addition of protons the yellow PhOH solution turns red (Figure 2C). Two new reversible reduction peaks appear, which, for a 1:1 mol ratio of protons to PhOH, occur at +0.36 and +0.08 V. Coulometry confirms that each wave is a single electron process. The redox wave at +0.36 V shifts positively with added protons; at a 2:1 mol ratio of H^+ to PhOH, the redox process shifts to a constant +0.45 V. The one-electron reduction at +0.20 V yields a green solution with a seven-line ESR spectrum (similar in shape to that obtained for 5,10-dihydrophenazine cation radical)[37,38] that is centered at g = 2.005 ± 0.001. At 2 or more equiv of acid, the observed current for each one-electron wave is approximately twice as large as that observed for the reduction of PhOH at −0.76 V in neutral acetonitrile. The potential of the second redox process at 0.08 V does not shift with added acid; electrolysis at −0.20 V yields a virtually colorless solution.

In Me_2SO similar redox behavior for PhOH is observed in neutral and basic solution with only slight potential shifts. After reductive electrolysis, however, a new distorted oxidation

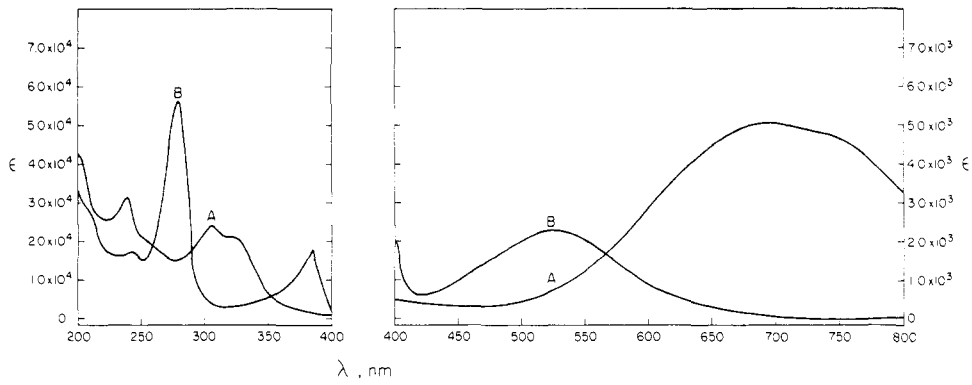


Figure 4. Ultraviolet, visible, and near-infrared spectroscopy of pyocyanine in acetonitrile: (A) neutral solution, (B) with 1 equiv of tetraethylammonium hydroxide added.

peak appears at −0.15 V. In acidic Me_2SO a single new one-electron reduction peak occurs at −0.15 V, similar in shape to that obtained after electrolysis at −1.0 V. Addition of protons to PhOH in Me_2SO does not cause any dramatic color changes. The potential of the −0.15 V peak is independent of added protons.

Pyocyanine. In neutral acetonitrile pyocyanine (Py) is reduced in two steps at −0.78 and −1.64 V (Figure 3a). The one-electron electrolysis at −1.00 V yields a green solution with a 13-line ESR spectrum (similar in shape to that of phenazine anion radical)[37,38] that is centered at $g = 2.001 \pm 0.001$. For the second reduction peak at −1.64 V, the current for the reverse peak is smaller than for the forward process owing to proton abstraction from the solvent medium. The suppressed intensity of the second reduction peak probably is due to interfering chemical processes at the electrode surface.

The cyclic voltammogram for a 1:1 mol ratio of H^+ to Py in acetonitrile exhibits two new reversible reductions at +0.02 and −0.58 V (Figure 3b). At less than a 1:1 ratio of acid to Py, the reversible reduction of Py shifts to more positive potentials with increasing proton concentration. With the addition of protons the blue color of pyocyanine (Figure 4A) changes to red (Figure 4B). Addition of a second equivalent of acid causes little change in the spectra, but shifts the reduction peaks to +0.48 and +0.09 V. Controlled potential coulometry confirms that the electron stoichiometry is one for each wave. After reductive electrolysis at +0.3 V a green solution is obtained which exhibits a nine-line ESR spectrum (generally similar in shape to that observed for dihydrophenazine cation radical)[37,38] that is centered at $g = 2.005 \pm 0.001$.

In neutral Me_2SO solution nearly identical results are obtained. In acidic Me_2SO a single new reduction peaks occurs at −0.01 V, which is independent of acid concentration.

1-Methoxyphenazine. In acetonitrile 1-methoxyphenazine (PhOMe) is reduced by a single one-electron process at −1.19 V to give a multiline ESR spectrum (similar to that obtained for phenazine anion radical)[37,38] that is centered at $g = 2.005 \pm 0.001$.

When 1 equiv of acid is added, two new reversible one-electron reduction peaks are observed. The potential of the first peak is dependent on the concentration of acid and shifts positively from +0.38 V; for high concentrations of acid it shifts back to +0.34 V. Electrolysis at the first reduction peak yields a green solution with a seven-line ESR spectrum (similar in shape to the 5,10-dihydrophenazine cation radical) at $g = 2.005 \pm 0.001$. The second reduction peak occurs at +0.09 V and is independent of acid concentration.

In neutral Me_2SO PhOMe has reduction peaks at −1.16 and −1.90 V. The second production species appears to abstract protons from the solvent medium. In acidic solution a single irreversible reduction peak occurs at −0.14 V, which is independent of the concentration of acid.

***N*-Methylphenazinium Ion.** In neutral acetonitrile *N*-methylphenazinium ion ($MePh^+$) undergoes two reversible one-electron reductions at −0.05 and −1.02 V. Electrolysis at the first peak potential yields a solution with an ESR spectrum similar to that previously identified as being due to *N*-methylphenazyl free radical.[39,40]

In acidic acetonitrile solution, two new reversible one-electron reduction peaks appear at +0.50 and +0.14 V. At less than stoichiometric H^+ concentrations, the peak for $MePh^+$ at −0.05 V shifts to more positive potentials with increasing H^+ concentrations. Electrolysis at +0.25 V of an acidified solution of $MePh^+$ yields a green solution with an ESR spectrum similar to that previously observed for 5-methyl-10-hydrophenazine cation radical.[39,40]

In Me_2SO solution almost identical behavior is observed except that for acidic conditions only a single reduction process is observed at +0.09 V.

Discussion and Conclusions

The oxidation-reduction reactions and potentials for 1-hydroxyphenazine, pyocyanine, 1-methoxyphenazine, and *N*-methylphenazinium ion are summarized in Table I for acetonitrile and dimethyl sulfoxide solutions, as well as the protonation equilibria for the various species.

Because the addition of hydroxide ion to 1-hydroxphenazine results in identical spectra and cyclic voltammograms to those obtained by electrolysis of PhOH at −1.00 V, the electrode process must involve the reduction of the hydroxyl proton on PhOH to form the ESR inactive anion, PhO^- (reaction 1). Further reduction of PhO^- (or PhOH) appears to form the ESR inactive H_2PhO^- species from reaction with hydrogen atoms that are produced either from solvent medium protons or from protons reduced in the first electrochemical step. Undoubtedly these processes are analogous to those observed for phenazine dianion.[4,15] The electrochemical steps in Table I for basic solutions are self-explanatory.

In Me_2SO solutions reductive electrolysis of PhO^- (from the first reduction process) appears to result in hydrogen addition by reaction with active hydrogen at the Pt electrode surface followed by disproportionation of the undetected radical species $PhOH^{-}\cdot$ to form PhO^- and H_2PhO^-.

When PhOH is in the presence of 1 equiv of acid two new reduction processes occur. Because only the potential of the first peak shifts with proton concentration, the reduction process must involve a follow-up proton addition[41,42] (reaction 14). After 2 or more equiv of acid are added, the potential for the first reduction remains constant, which indicates that reactions 12 and 17 occur. The second reduction process for acidified PhOH is straightforward (reaction 16); the ESR

Table I. Summary of Protonation and Oxidation-Reduction Reactions with Half-Wave Potentials in Acetonitrile (AN) and Dimethyl Sulfoxide (Me_2SO)

	Reaction no.	AN	Me_2SO
A. 1-Hydroxyphenazine			
1. Neutral Solution			
$PhOH + 1e^- \rightarrow PhO^- + \frac{1}{2}H_2$	(1)	−0.93[a]	−0.93[a]
$PhO^- + 1e^- \rightleftharpoons \cdot PhO^{2-}$	(2)	−1.67[a]	−1.69[a]
$\cdot PhO^{2-} + 2HSol \rightarrow H_2PhO\cdot + 2Sol^-$	(3)		
$\cdot PhO^{2-} + H_2PhO\cdot \rightarrow PhO^- + H_2PhO^-$	(4)		
$2PhOH^{-}\cdot \rightarrow PhO^- + H_2PhO^-$	(5)[b]		
2. Basic Solution			
$PhOH + OH^- \rightarrow H_2O + PhO^-$	(6)		
$PhO^- \rightarrow PhO\cdot + 1e^-$	(7)	+0.14[c,d]	+0.18[c,d]
$PhO\cdot \rightarrow X$ (film formation)	(8)		
$PhO^- + 1e^- \rightleftharpoons \cdot PhO^{2-}$	(9)	−1.67[a]	−1.69[a]
$\cdot PhO^{2-} + 2HSol \rightarrow H_2PhO\cdot + 2Sol^-$	(10)		
3. Acidic Solution			
$PhOH + H^+ \rightleftharpoons HPhOH^+$	(11)		
$HPhOH^+ + H^+ \rightleftharpoons H_2PhOH^{2+}$	(12)[e]		
$HPhOH^- + H^+ \rightleftharpoons H_2PhOH$	(13)[e]		
$HPhOH^+ + e^- \rightleftharpoons H_2PhO\cdot$	(14)	+0.36[f]	−0.145
$H_2PhO\cdot + H^+ \rightleftharpoons H_2PhOH^+\cdot$	(15)[e]		
$H_2PhOH^+\cdot + e^- \rightleftharpoons H_2PhOH$	(16)	+0.08	NR
$H_2PhOH^{2+} + e^- \rightleftharpoons H_2PhOH^+\cdot$	(17)	+0.45	NR
B. Pyocyanine			
1. Neutral Solution			
$Py + 1e^- \rightleftharpoons Py^-\cdot$	(18)	−0.78	−0.71
$Py^-\cdot + 1e^- \rightleftharpoons Py^{2-}$	(19)	−1.64	−1.62
$Py^{2-} + HSol \rightarrow HPy^- + Sol^-$	(20)		
2. Acidic Solution			
$Py + H^+ \rightleftharpoons HPy^+$	(21)		
$HPy^+ + H^+ \rightleftharpoons HPyH^{2+}$	(22)[e]		
$Py^-\cdot + H^+ \rightleftharpoons HPy\cdot$	(23)		
$HPy^+ + 1e^- \rightleftharpoons HPy\cdot$	(24)	+0.02[f]	−0.01[f]
$HPy\cdot + 1e^- \rightleftharpoons HPy^-$	(25)	−0.58[f]	NR
$HPy\cdot + H^+ \rightleftharpoons HPyH^+\cdot$	(26)		
$HPy^- + H^+ \rightleftharpoons H_2Py$	(27)[e]		
$H_2Py^{2+} + 1e^- \rightleftharpoons H_2Py^+\cdot$	(28)	+0.48	NR
$H_2Py^+\cdot + 1e^- \rightleftharpoons H_2Py$	(29)	+0.09	
C. 1-Methoxyphenazine			
1. Neutral Solution			
$PhOMe + 1e^- \rightleftharpoons PhOMe^-\cdot$	(30)	−1.19	−1.15
$PhOMe^-\cdot + 1e^- \rightleftharpoons PhOMe^{2-}$	(31)	NR	−1.90
$PhOMe^{2-} + HSol \rightarrow \frac{1}{2}H_2 + PhOMe^-\cdot + Sol^-$	(32)[b]		
2. Acidic Solution			
$PhOMe + H^+ \rightleftharpoons HPhOMe^+$	(33)		
$HPhOMe^+ + H^+ \rightleftharpoons H_2PhOMe^{2+}$	(34)[e]		
$HPhOMe^+ + 1e^- \rightleftharpoons HPhOMe\cdot$	(35)	+0.38[f]	−0.14
$HPhOMe\cdot + H^+ \rightleftharpoons H_2PhOMe^+\cdot$	(36)[e]		
$H_2PhOMe^+\cdot + 1e^- \rightleftharpoons H_2PhOMe$	(37)	+0.09	NR
$H_2PhOMe^{2+} + 1e^- \rightleftharpoons H_2PhOMe^+\cdot$	(38)	+0.34	NR
D. *N*-Methylphenazinium Methylsulfate			
1. Neutral Solution			
$MePh^+ + 1e^- \rightleftharpoons MePh\cdot$	(39)	−0.05	−0.04
$MePh\cdot + 1e^- \rightleftharpoons MePh^-$	(40)	−1.02	−0.96
2. Acidic Solution			
$MePh^+ + H^+ \rightleftharpoons HMePh^{2+}$	(41)		
$MePh\cdot + H^+ \rightleftharpoons HMePh^+\cdot$	(42)		
$HMePh^{2+} + 1e^- \rightleftharpoons HMePh^+\cdot$	(43)	+0.50	+0.09
$HMePh^+\cdot + 1e^- \rightleftharpoons HMePh$	(44)	+0.14	NR

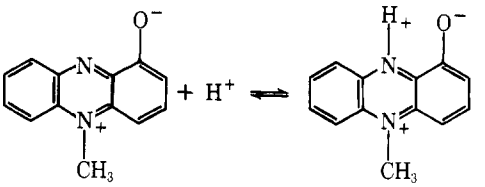
[a] Cathodic peak potential. [b] Only in Me_2SO solution. [c] Anodic peak potential. [d] Film formation on electrode after oxidation process. [e] Only in AN solution. [f] $E_{1/2}$ shifts with $[H^+]$; data for $[H^+]$:[species] equal to 1:1.

spectrum for $H_2PhOH^+\cdot$ is similar to that obtained for other dihydrophenazine cation radicals.

The electrochemistry of pyocyanine in neutral solution resembles that of other phenazines (reactions 18–20), and includes the general tendency of the doubly reduced species to abstract protons from the solvent medium (reaction 20).

When less than 1 equiv of protons is added to Py, the reduction (reaction 18) shifts to more positive potentials (reaction 23). Two new redox couples appear in the cyclic voltammogram after the addition of 1 equiv of protons. In acetonitrile, after the addition of 1 equiv of protons per Py, the potentials of both reductions (reactions 24 and 25) are proton dependent, which indicates that proton addition occurs after electron transfer (reactions 26 and 27). Addition of 2 or more equiv of protons per Py causes the potentials of the reductions to become proton independent, which is indicative of fully protonated species (reactions 22, 28, and 29).

The change in the spectrum for Py with the addition of acid (Figure 4) to one that resembles that for H_2PhOH^{2+}, along with the similarity in the potentials of reactions 28 and 29, indicates that the first proton adds to the N-5 of Py.



On the basis of the ESR signal in neutral acetonitrile, PhOMe is reduced to form the anion radical (reaction 30). A second reduction peak (reaction 31) is observed in Me_2SO solutions, which yields a species that abstracts protons from the solvent (reaction 32).

Protons add to PhOMe stepwise (reactions 33 and 34) in a manner similar to the protonation of PhOH and Py. The dependence of the potential for the first reduction on proton concentration (reaction 35) indicates that a proton adds after electron transfer (reaction 36), which causes reaction 37 to be independent of H^+ concentration. Addition of a second proton to PhOMe only occurs at high acid concentrations.

The two reduction peaks that are observed for $MePh^+$ in neutral solution represent simple electron transfer processes. When less than 1 equiv of protons per $MePh^+$ is added, the potential for the first reduction (reaction 39) shifts positively owing to proton addition after electron transfer (reaction 42). Addition of 1 or more equiv of protons causes relatively large shifts in the peak potentials for the two processes (reactions 43 and 44).

The electrochemistry of pyocyanine is similar to that observed for other phenazine species. In aprotic media a one-electron reduction process produces an anion radical; the second reduction usually (but not always)[4] is followed by a chemical reaction.[1-17]

The electrochemical behavior of PhOH in acetonitrile and Me_2SO is remarkably similar to that observed in DMF.[8] The slight change in potentials can be accounted for on the basis of different solvation of the species in the three solvents.

In general the redox reactions of PhOH in neutral solution are not similar to those of riboflavin or that ordinarily associated with phenazines. The hydroxyl proton reduction is similar to that observed in 8-quinolinol and related molecules, rather than to addition of an electron to the ring system as in riboflavin. The protonation behavior of PhOH is somewhat similar to that of riboflavin, where two closely spaced reduction waves are observed for the protonated cation radical in acetonitrile.[42]

The reduction potentials of riboflavin,[16] 3-methyllumiflavin,[16] and pyocyanine in neutral Me_2SO solution are compared

Table II. Comparison of Oxidation-Reduction Reactions of Riboflavin, 3-Methyllumiflavin, and Pyocyanine in Neutral Me_2SO Solution

Reaction	$E_{1/2}$ V vs. SCE		
	Riboflavin[a]	3-Methyllumiflavin[a]	Pyocyanine
$L + e^- \rightleftarrows L^-\cdot$	$-0.82^{b,c}$	-0.80	-0.72
$L^-\cdot + e^- \rightleftarrows L^{2-}$	-1.4^c	-1.62^c	-1.62

[a] Reference 16. [b] Anion radical is hydrogen bonded. [c] Cathodic peak potential.

in Table II. The similarity of the potentials for the first reduction process is striking. The potentials for the second reduction of riboflavin agree less well because intramolecular hydrogen bonding in the anion radical stabilizes the species.[16] This hydrogen bonded species also causes the oxidation peak that is associated with the first reduction to shift to −0.55 V. Such behavior is not observed for Py or 3-methyllumiflavin. This supports the conclusion that the hydrogen bonding involves the ribityl side chain.

Addition of protons to solutions of the three species causes protonation of the nitrogens and anodic shifts in the reduction potentials. For both 3-methyllumiflavin and riboflavin disproportionation reactions are observed in acidic solution, while such behavior is not observed for Py.

In conclusion, the redox behavior of pyocyanine mimics that of riboflavin more closely than 1-hydroxyphenazine or phenazine, and therefore serves as a useful redox model for riboflavin, FMN, FAD, and flavoproteins. In addition, Py and $Py^-\cdot$ complex a variety of metal ions; this is the subject of the next paper in this series.[43]

Acknowledgments. This work was supported by the National Science Foundation under Grant CHE 76-24555. We are grateful to Mr. James J. Houts and Mr. Gregory Pachiano for experimental assistance.

References and Notes

(1) T. Kubota, K. Nishikida, H. Miyazaki, K. Iwatani, and Y. Oishi, *J. Am. Chem. Soc.*, **90**, 5080 (1968).
(2) D. Van der Meer and D. Feil, *Recl. Trav. Chim. Pays-Bas*, **87**, 746 (1968).
(3) A. E. Brodsky, L. L. Gordienko, and L. S. Degtiarev, *Electrochim. Acta*, **13**, 1095 (1968).
(4) S. Millefiori, *J. Heterocycl. Chem.*, **7**, 145 (1970).
(5) L. Marcoux and R. N. Adams, *J. Electroanal. Chem. Interfacial Electrochem.*, **49**, 111 (1974).
(6) K. Alwair and J. Grimshaw, *J. Chem. Soc., Perkin Trans. 2*, 1811 (1973).
(7) L. L. Gordienko, *Sov. Electrochem. (Engl. Transl)*, **1**, 1351 (1965).
(8) S. Nakamura and T. Yoshida, *Denki Kagaku*, **43**, 159 (1975).
(9) J. Volke and S. Bevan, *Collect. Czech. Chem. Commun.*, **40**, 2232 (1975).
(10) R. F. Nelson, D. W. Leedy, E. T. Seo, and R. N. Adams, *Z. Anal. Chem.*, **224**, 184 (1967).
(11) A. Stuwe, M. Weber-Schafer, and H. Baumgartel, *Tetrahedron Lett.*, 427 (1972).
(12) V. A. Stuwe, M. Weber-Schafer, and H. Baumgartel, *Z. Phys. Chem. (Frankfurt am Main)*, **78**, 309 (1971).
(13) L. L. Gordienko and Y. S. Rosum, *Sov. Electrochem. (Engl. Transl.)*, **7**, 1770 (1971).
(14) D. N. Bailey, D. M. Hercules, and D. K. Roe, *J. Electrochem. Soc.*, **116**, 190 (1969).
(15) D. T. Sawyer and K. Y. Komai, *Anal. Chem.*, **44**, 715 (1972).
(16) D. T. Sawyer, R. Y. Komai, and R. L. McCreery, *Experientia, Suppl.*, **18**, 563 (1971).
(17) Y. Kidani, *Chem. Pharm. Bull.*, **6**, 556 (1958).
(18) Y. Kidani, *Chem. Pharm. Bull.*, **6**, 563 (1958).
(19) Y. Kidani, *Chem. Pharm. Bull.*, **7**, 68 (1959).
(20) Y. Kidani, *Chem. Pharm. Bull.*, **7**, 74 (1959).
(21) Y. Kidani, K. Inagaki, and H. Koike, *Bunseki Kagaku*, **20**, 1576 (1971).
(22) Y. Kidani, *Yakugaku Zasshi*, **93**, 401 (1973).
(23) Y. Kidani, K. Inagaki, and H. Koiki, *Yakugaku Zasshi*, **93**, 1089 (1973).
(24) Y. Kidani, K. Inagaki, and H. Koike, *Chem. Pharm. Bull.*, **23**, 29 (1975).
(25) Y. Kidani, K. Inagaki, and H. Koike, *Bunseki Kagaku*, **24**, 221 (1975).
(26) K. Inagaki, Y. Kidani, and H. Koike, *Chem. Pharm. Bull.*, **24**, 156 (1976).
(27) K. Inagaki and Y. Kidani, *Chem. Pharm. Bull.*, **24**, 839 (1976).
(28) C. Cattaneo and G. Sartori, *Arch. Sci. Biol.*, **24**, 361 (1941).
(29) M. E. Flood, R. B. Herbert, and F. G. Holliman, *J. Chem. Soc., Perkin Trans. 1*, 622 (1972).
(30) F. G. Holliman, *J. Chem. Soc. C*, 2514 (1969).
(31) R. B. Herbert and F. G. Holliman, *J. Chem. Soc. C*, 2517 (1969).
(32) O. H. Muller, *J. Biol. Chem.*, **145**, 425 (1942).
(33) C. Cattaneo and G. Sartori, *Gazz. Chim. Ital.*, **72**, 38 (1942).
(34) V. Massey and T. P. Singer, *J. Biol. Chem.*, **229**, 755 (1957).
(35) "Organic Syntheses", Collect. Vol. III, Wiley, New York, N.Y., 1955, p 753.
(36) D. T. Sawyer and J. L. Robetts, Jr., "Experimental Electrochemistry for Chemists", Wiley-Interscience, New York, N.Y., 1974.
(37) Y. Fellion and J. Uebersfeld, *Arch. Sci.*, **9**, Spec. No. 89 (1956).
(38) K. H. Hauser, A. Habich, and V. Franzen, *Z. Naturforsch. A*, **16**, 836 (1961).
(39) J. Dobkowski and W. Rubaszewska, *Rocz. Chem. Ann. Soc. Chim. Pol.*, **50**, 1435 (1976).
(40) W. S. Zaugg, *J. Biol. Chem.*, **239**, 3964 (1964).
(41) L. Meites, "Polarographic Techniques", 2nd ed, Wiley-Interscience, New York, N.Y., 1965.
(42) M. M. Morrison and D. T. Sawyer, unpublished results.
(43) M. M. Morrison and D. T. Sawyer, *J. Am. Chem. Soc.*, following paper in this issue.

Flavin Model Systems. 2. Pyocyanine Complexes of Divalent Manganese, Iron, Nickel, Copper, and Zinc in Dimethyl Sulfoxide

Mark M. Morrison and Donald T. Sawyer*

Contribution from the Department of Chemistry, University of California, Riverside, California 92521. *Received March 28, 1977*

Abstract: The complexation reactions of pyocyanine with metal ions have been studied as models for metal-flavin interactions. In particular, the formation constants for the complexes of pyocyanine and of pyocyanine anion radical with Mn(II), Fe(II), Ni(II), Cu(II), and Zn(II) ions have been determined by use of spectrophotometric and electrochemical techniques. The complexation behavior of pyocyanine and pyocyanine anion radical parallels that of riboflavin and its anion radical.

Investigations of metal-flavin interactions constitute an essential first step in developing an understanding of the properties and reactions of metalloflavoproteins. In recent years there has been increasing evidence that metal ions can strongly influence the reactivity of flavins.[1-9] Redox inactive metal ions displace the flavin semiquinone dismutation equilibrium toward the semiquinone state.[1-4] The oxidation-reduction reactions of riboflavin are significantly changed by

0002-7863/78/1500-0211$01.00/0