

Table II. Comparison of Oxidation-Reduction Reactions of Riboflavin, 3-Methyllumiflavin, and Pyocyanine in Neutral Me₂SO Solution

Reaction	<i>E</i> _{1/2} V vs. SCE		
	Ribo-flavin ^a	3-Methyl-lumiflavin ^a	Pyocyanine
$L + e^- \rightleftharpoons L^{\cdot-}$	-0.82 ^{b,c}	-0.80	-0.72
$L^{\cdot-} + e^- \rightleftharpoons L^{2-}$	-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⁻ 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.

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

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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

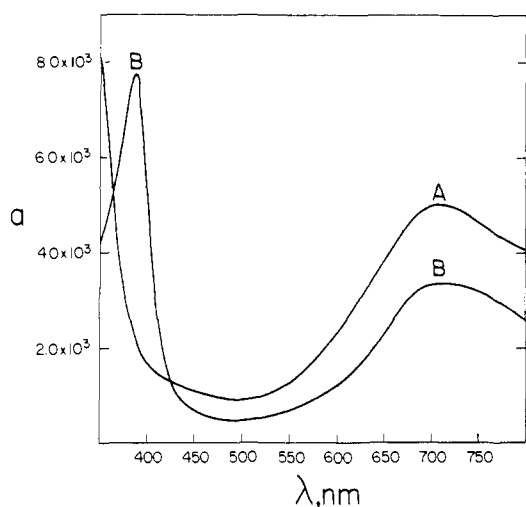
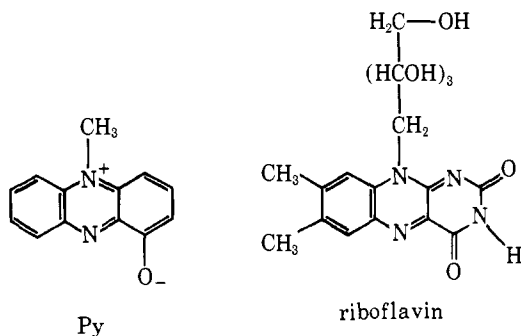


Figure 1. Absorption spectra of 1 mM solutions of (A) pyocyanine and of (B) the zinc(II)-pyocyanine complex in dimethyl sulfoxide that contains 0.10 M tetrapropylammonium perchlorate. *a* represents the molar absorptivity.

complexation with a variety of metal ions.⁵⁻⁸ The presence of metal ions also stabilizes riboflavin to decomposition in basic solution.⁹

Various studies have shown that the reactions of flavins in nonaqueous polar media closely resemble the reactions and properties of metalloflavoproteins.^{4,10} Several investigators have shown that in nonaqueous polar solvents metal ions interact with both flavins and their semiquinones.^{4,11}

Pyocyanine (Py), a blue pigment from *Pseudomonas*



aeruginosa,^{12,13} is structurally similar to riboflavin and has antibiotic activity.^{14,15} In the preceding paper¹⁶ we have shown that Py is a useful redox model for riboflavin, with similar oxidation-reduction reactions and potentials in a common solvent medium. Consideration of the structures for pyocyanine and pyocyanine anion radical indicates that they should be similar to riboflavin and its anion radical with respect to metal-ion complexation.

This paper summarizes the results of a spectroscopic and electrochemical study of the complexation by pyocyanine of Mn(II), Fe(II), Ni(II), Cu(II), and Zn(II) ions in dimethyl sulfoxide in the presence of 0.1 M tetrapropylammonium perchlorate.

Experimental Section

The electrochemical and spectroscopic measurements were made by use of conventional instrumentation that has been described elsewhere.¹⁶ Manganous, ferrous, and zinc perchlorates were obtained from G. F. Smith Chemical Co., and the CuCl₂ and NiCl₂ salts were reagent grade. The preparation of solutions and of pyocyanine has been described in the preceding paper.¹⁶

Results

Spectrophotometric Studies. Addition of metal ions to py-

ocyanine causes a shift in its absorption spectrum (Figure 1). This has been used as the basis for a series of mole-ratio plots that provides the means to determine the stoichiometries and the formation constants, K_{ox} , of the metal-pyocyanine complexes, $MePy^{2+}$. For a 1:1 complex

$$K_{ox} = \frac{[MePy^{2+}]}{[Me^{2+}][Py]} \quad (1)$$

Because the absorption maxima of pyocyanine and the metal-pyocyanine complex overlap, simultaneous equations have been used to determine the concentrations of free and complexed pyocyanine. For the Cu(II)-pyocyanine complex, corrections have been made for the absorption of the colored metal ion.

When the concentrations of Py in dimethyl sulfoxide are 10^{-4} - 10^{-3} M (with 0.1 M tetrapropylammonium perchlorate) the mole-ratio data indicate that 1:1 complexes are formed with the various divalent metal ions. The values of the formation constants are summarized in Table I.

Electrochemical Studies. The reversible one-electron reduction of Py in dimethyl sulfoxide has been characterized for a variety of solution conditions in the preceding paper.¹⁶ Scan rate studies establish that the redox reaction is free of adsorption processes.¹⁷ When divalent metal ions are added to a Py solution, a new reversible reduction peak is observed in the cyclic voltammogram at a potential more positive than that of uncomplexed pyocyanine (at -0.72 V vs. SCE). The potential of this new peak has been studied as a function of metal ion concentration for the range from 10^{-4} to 5×10^{-2} M (limit of solubility) in the presence of 10^{-3} M Py. In each case the potential of this new redox process shifts positively with increasing metal-ion concentration; for the highest concentrations the shift is 30 mV for each tenfold increase in uncomplexed metal ion concentration. Such a shift in half-wave potential is consistent with a one-electron reduction followed by a change in the stoichiometry of the metal complex from 1:1 to 3:2.



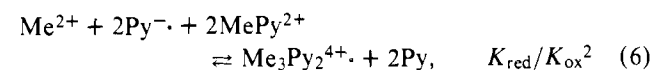
The ratio of the formation constant for $Me_3Py_2^{4+}$, K_{red} , to the square of the formation constant for $MePy^{2+}$, K_{ox} , can be evaluated by use of the relation¹⁷

$$\Delta E_{1/2} = \frac{-(p-q)0.059}{n} \log [Me^{2+}] + \frac{0.059}{n} \log \frac{K_{red}}{K_{ox}^2} \quad (4)$$

where $\Delta E_{1/2}$ is the shift in half-wave potential between the complexed pyocyanine reduction and the pyocyanine reduction; $(p-q)$ the change in the number of metal ions per ligand (Py); n the number of electrons transferred in the electrode reaction; and

$$K_{red} = \frac{[Me_3Py_2^{4+}]}{[Me^{2+}]^3[Py^-]^2} \quad (5)$$

Hence, the overall complexation reaction is



On the basis of cyclic voltammetric measurements for a series of metal ion concentrations a value for the half-wave potential at 1 M Me^{2+} can be extrapolated from a plot of $E_{1/2}$ vs. $\log [Me^{2+}]$. Because values of K_{ox} have been determined from the spectrophotometric measurements (Table I), combination of these data permits evaluation of K_{red} for the $Me_3Py_2^{4+}$ complexes. The values of K_{red} for Mn(II), Fe(II), Ni(II), and Zn(II) are summarized in Table I. This method of evaluation is analogous to the polarographic determination of formation constants and stoichiometry for complexed metal ions where

Table I. Formation Constants of Pyocyanine, K_{ox} ; Pyocyanine Anion Radical, K_{red} ; 1-Hydroxyphenazine, K_1 ; 5-Hydroxyquinoxaline, K_5 ; and 8-Quinolol, K_8 , Complexes^a

Metal ion	Log K_{ox}	Log K_{red}/K_{ox}^2	Log K_{red}	Log K_1^b	Log K_5^c	Log K_8^d
Mn ²⁺	2.2 ± 0.2	18.0 ± 0.5	22.4 ± 0.7			15.35
Fe ²⁺	3.6 ± 0.2	13.6 ± 0.5	20.8 ± 0.7			
Ni ²⁺	3.8 ± 0.2	21.6 ± 0.5	29.2 ± 0.7	13.7	14.81	21.38
Cu ²⁺	3.3 ± 0.2	<i>e</i>	<i>e</i>	17.5	18.50	26.22
Zn ²⁺	3.3 ± 0.2	23.2 ± 0.5	29.8 ± 0.7	13.6	12.78	18.86

^a $K_{ox} = [MeX^{2+}]/[Me^{2+}][X]$; K_1 , K_5 , and $K_8 = [MeX_2]/[Me^{2+}][X^-]^2$; $K_{red} = [Me_3X_2^{4+}]/[Me^{2+}]^3[X^-]^2$. ^b 50% ethanol-water, ref 19. ^c 50% dioxane-water, ref 20. ^d 50% dioxane-water, ref 21. ^e Cu(II) reduction interferes with evaluation.

the metal ion is involved in the reduction or oxidation process.¹⁷ However, in the present system the electroactive species is the ligand rather than the metal ion. The shape of the redox wave is broadened in a manner analogous to the predictions from a theoretical treatment by Shuman for a third-order reaction.¹⁸

The electrochemical method does not permit the determination of K_{red} for the Cu(II) complex with Py^- because the Cu(II)/Cu(I) redox couple occurs at +0.34 V vs. SCE in Me_2SO .

Discussion and Conclusions

The formation constants for the pyocyanine and the pyocyanine anion radical complexes of the group of divalent metal ions are summarized in Table I. For comparison the values for related constants of the 5-hydroxyquinoxaline, 1-hydroxyphenazine, and 8-quinolol complexes of the same group of metal ions are included. Reference to the data in this table indicates that the metal-pyocyanine complexes are weak. The stabilities of the complexes formed by pyocyanine anion radical are considerably greater than those of 1-hydroxyphenazine,¹⁹ 5-hydroxyquinoxaline,²⁰ and 8-quinolol.²¹ The increased stability of the Py^- complexes relative to those of 8-quinolol can be attributed to the increased basicity of the N-5 group as a result of the unpaired electron.

The activity of many antibiotics has been related to their ability to form metal complexes. The stabilization of the pyocyanine anion radical through complexation with metal ions may explain in part the antibiotic activity of pyocyanine. Similar arguments have been used to relate antibiotic activity with the ability of 8-quinolol,²² 1-hydroxyphenazine,^{23,24} and 5-hydroxyquinoxaline²⁵ to form metal complexes.

The stabilities of the metal complexes formed by pyocyanine are similar to those that are observed for flavin species. Hemmerich and Lauterwein⁴ have described three types of metal-riboflavin complexes: flavoquinone metal chelates, flavosemiquinone-metal complexes, and so-called charge-transfer chelates of flavoquinone. The flavoquinone-metal chelates appear to be similar to the pyocyanine metal complexes. The relative stability constants of the metal-flavoquinone complexes⁴ are Ni(II) > Zn(II) > Fe(II) >> Mn(II). In the case of the pyocyanine complexes the relative stabilities are Ni(II) > Fe(II) > Zn(II) >> Mn(II). Also, the relative stabilities of the pyocyanine anion radical complexes are analogous to those for riboflavin semiquinone.^{1-8,26-30}

In conclusion, pyocyanine is a useful model for riboflavin, based on both redox considerations and the ability to form similar complexes. Additionally, the metal complexation of pyocyanine anion radical may account for the antibiotic activity of pyocyanine.

Acknowledgments. This work was supported by the National Science Foundation under Grant CHE 76-24555.

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