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Coenzyme Models. 34. Synthesis and Oxidation Ability of a Metal-coordinative Flavin

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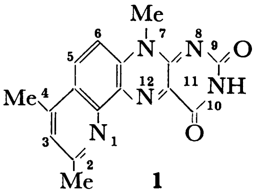
A new metal-coordinative flavin(2,4,7-trimethylquino[8,7-*g*]pteridine-9,11(7*H*,10*H*)-dione: (**1**)) which has a phenanthroline-like skeleton within the molecular structure was synthesized. Being different from conventinal flavins, the new flavin showed a significant affinity toward metal ions and the absorption spectra were sensitively affected by added metal ions. The spectral patterns were classified into three types, which were assigned respectively to the different coordination structures. It was demonstrated through oxidation of dihydropyridine derivatives, phenylhydrazine, and L-ascorbic acid that complexed metal ions are able to "activate" **1** as oxidizing agent. The complexes with Zr^{4+} and Fe^{3+} even oxidized alcohols at ambient temperature. Unexpectedly, it was found that tetrahedral coordination metals such as Cd^{2+}, Zn^{2+}, and Co^{2+} frequently give the stable semiquinone radical of **1**. The remarkable stabilization effect of these metals was attributed to a bent, butterflywing structure of the semiquinone radical. These findings suggest that the **1**–metal complexes are useful not only as a model system of metalloflavoproteins but also as an *in vitro* oxidation catalyst.

Flavin coenzymes are versatile redox catalysts in many biological systems and it is now known that more than 100 proteins require flavin coenzymes.[1–3] In contrast to the ability of flavin coenzymes to oxidize a variety of substrates *in vivo*, however, flavin molecules *in vitro* exhibit a rather weak oxidation ability. We have been aiming at "activating" flavin molecules in nonenzymatic systems with the intention of utilizing flavins as an organic turnover oxidation catalyst. There are now recognized two main methods to facilitate flavin-mediated oxidation reactions: That is, (i) to convert substrates into more "specific" ones with the aid of second cofactors or environmental effects and (ii) to shift the redox potential of flavins to more positive values. Method (i) has been achieved by employing cyanide ion,[4–7] thiazolium ions,[8–13] and thiols[14,15] as second cofactors or by conducting flavin-mediated reactions in aqueous micelles and water-soluble polymers.[16–22] Method (ii) is precedented by Cl-, CN-, pyridino-, and nitro-substituted flavins.[19,23–27] To the best of our knowledge, however, no precedent for the metal-activation of flavin catalyses exists, which would be classified as an example of method (ii). The difficulty of the method is solely ascribable to the lack of the metal affinity of flavins, except a few metal ions such as Ag^+ and Ru^{2+}.[28,29]

It thus occurred to us that it would be of value to synthesize a new flavin which has a metal-coordinative function. With the object in view, we synthesized 2,4,7-trimethylquino[8, 7-*g*]pteridine-9, 11 (7*H*, 10*H*)-dione (**1**)† which has a phenanthroline-like skeleton in the molecular structure and examined the influence of complexed metal ions on the oxidation ability of **1**. As control experiments, we used 10-ethylisoalloxazine (**7**). Since flavin coenzymes mediate electron transfers to and from ion-sulfur, molybdenum, and heme in proteins, the investigation would be also of value as a model study of metalloflavoproteins to gain an insight into metal–flavin interactions.



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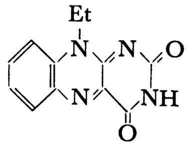

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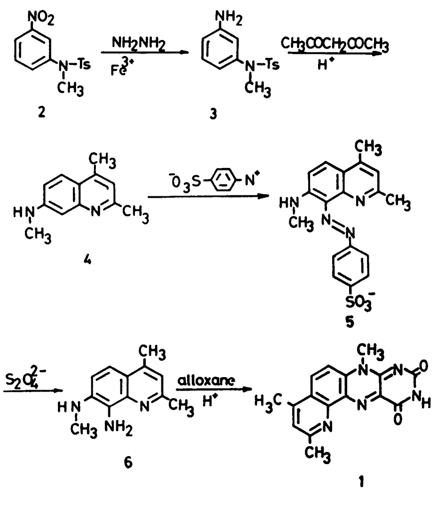
Experimental

Materials. Compound **1** was synthesized according to a following reaction sequence (Ts = tosylate).



m-Nitro-N-methyl-N-tosylaniline (**2**). *m*-Nitro-*N*-tosylaniline (100 g; 0.34 mol) was dissolved in 800 ml of 1 M NaOH (1 M = 1 mol dm^{-3}) aqueous solution and dimethyl

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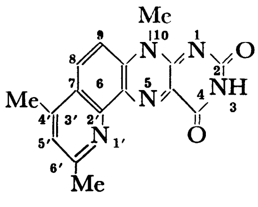


† When the isoalloxazine ring is taken as a basic skeleton, **1** may be named 4′,6′,10-trimethyl-6,7-(2′,3′-pyrido)-isoalloxazine. For the sake of the analogy to conventional isoalloxazines, we will use this naming when we point to the atoms in **1**.

sulfate (45.5 g; 0.34 mol) was added dropwise with stirring at room temperature. After 1 h, 50 ml of 1 M NaOH aqueous solution was added and then dimethyl sulfate (10 g; 0.075 mol) was added dropwise again. The same operation was repeated two more times. The white precipitate was collected by suction, washed with aqueous NaOH, dilute HCl, and water, and then dried *in vacuo*; mp 109.5—110.5 °C, yield 95%. The row product was used for the following synthesis without further purification.

N-*Methyl*-N-*tosyl*-m-*phenylenediamine (3)*. The nitro group was reduced by Fe^{3+}–hydrazine.[30] **2** (100 g; 0.33 mol) was dissolved in 100 ml of methanol containing 10 g of active carbon and 1.5 g (5.5×10^{-3} mol) of $FeCl_3\cdot 6H_2O$, and the solution was refluxed for 15 min. Hydrazine hydrate (90%) (35 g; 0.495 mol) was added dropwise and then the reaction mixture was heated under reflux for 2.5 h. We here confirmed that the spot of **2** in TLC had disappered. The solution was filtered while it was hot, the solvent being evaporated *in vacuo*. The residue (mp 100—102 °C, yield 94%) was subjected to the following analyses. Found: C, 60.76; H, 5.84; N, 10.17%. Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14%. NMR($CDCl_3$): Ar–CH_3, 2.39 ppm, 3H; *N*-CH_3, 3.09 ppm, 3H; NH_2, 3.69 ppm, 2H; aromatic protons, 6.34 ppm (1H), 6.52 ppm (2H), 7.01 ppm (1H), 7.22 ppm (2H), 7.48 ppm (2H).

2,4-Dimethyl-7-(methylamino)quinoline (4). In 120 ml of acetic acid containing 20 ml of concd sulfuric acid were added **3** (80 g; 0.29 mol) and acetylacetone (34 g; 0.319 mol), and the reaction mixture was refluxed. The progress of the reaction was followed by the TLC method (silica gel–methanol). In order to accelerate the reaction, 3.4 g (0.032 mol) of acetylacetone was added several times so that the total amount of added acetylacetone became 0.49 mol. After 3 d, the solution was concentrated *in vacuo* to one-third and poured into 200 ml of water. The precipitate was collected by suction and washed with hot water. The solid was once dissolved in hot NaOH solution. On cooling, the yellow precipitate was formed, which was recovered by filtration and washed with water: mp 140.0—140.5 °C, yield 28.7%. Found: C, 76.79; H, 7.52; N, 14.91%. Calcd for $C_{12}H_{14}N_2$: C, 77.38; H, 7.58; N, 15.04%. IR(KBr): ν_{NH} 3220 cm^{-1}, ν_{Ar-H} 842, 830, 801 cm^{-1}. NMR ($CDCl_3$): 4-CH_3, 2.56 ppm, 3H; 2-CH_3, 2.64 ppm 3H; *N*-CH_3, 2.94 ppm, 3H; NH, 4.16 ppm 1H; 6-H, 6.82 ppm, 1H(quartet); 3-H, 6.84 ppm, 1H(singlet); 8-H, 7.02 ppm, 1H(doublet); 5-H, 7.88 ppm, 1H(doublet). IR and NMR spectral data support that the product is 2,4-dimethyl-7-(methylamino)quinoline (**4**) but not 2,4-dimethyl-5-(methylamino)quinoline.

4-(2,4-Dimethyl-7-methylamino-8-quinolylazo) benzenesulfonate (5). Compound **4** (11.2 g; 0.06 mol) was dissolved in 60 ml of hot 3.5% HCl solution, and after cooling, the solution was poured into 500 ml of aqueous solution containing the diazonium salt of sulfanilic acid (10.4 g; 0.06 mol). 120 g of urea was added in order to accelerate the coupling reaction. The solution was adjusted to pH 4.5±0.5 with sodium acetate and the reaction was continued for 90 h at 0—10 °C. The precipitate was recovered by filtration. The product solid was once dissolved in aqueous NaOH solution and reprecipitated by adding NaCl. The precipitate was then dissolved in aqueous solution and reprecipitated again by adding concd HCl solution; mp 271—273 °C (red powder), yield 82.4%. IR(KBr): ν_{NH} 3400 cm^{-1}, $\nu_{\text{quinoline ring}}$ 1620 cm^{-1}, SO_3H, 1219 and 1025 cm^{-1}. Found: C, 55.70; H, 4.80; N, 14.25%. Calcd for $C_{18}H_{18}N_4O_3S$: C, 58.30; H, 4.90; N, 15.13%.

2,4-Dimethyl-7-methylamino-8-quinolinamine (6). Compound **5** (0.5 g; 1.4 mmol) was dissolved in 40 ml of 3% NaOH aqueous solution. 0.7 g (4.0 mmol) of sodium dithionite (practical grade) was added, and the reaction mixture was refluxed for 1 h. The product was extracted with 1-butanol (100 ml×2). Since the product **6** was relatively sensitive to air oxidation, the 1-butanol extract was immediated used for the following reaction. Meanwhile, the aliquot was withdrawn from the solution and cooled in a nitrogen atmosphere. The precipitate was recovered by filtration and subjected to the NMR measurement. NMR($CDCl_3$): 4-CH_3, 2.55 ppm, 3H; 2-CH_3, 2.60 ppm, 3H; *N*-CH_3, 2.96 ppm 3H; 7-NH and 8-NH_2, 3.98 ppm, 3H; 3-H, 6.84 ppm 1H(singlet); 6-H, 7.02 ppm, 1H(doublet); 5-H, 7.37 ppm, 1H(doublet). The NMR spectral data show that diazocoupling occurs at 8-position of **4**.

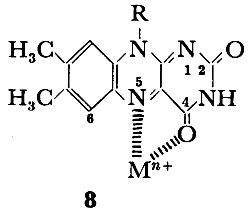
To the 1-butanol solution containing **6** were added 0.21 g (1.4 mmol) of alloxane and 0.24 g (1.4 mmol) of *p*-toluenesulfonic acid, and the reaction mixture was refluxed for 6 h. The precipitate formed on the addition of several drops of pyridine was collected by suction and washed with water. The product solid **1** was recrystallized from acetic acid with active carbon: mp>293 °C, yield from **5**, 40%. Mass spectrum: M^+ 307. Found: C, 58.39; H, 4.61; N, 20.95%. Calcd for $C_{16}H_{13}N_5O_2\cdot H_2O$: C, 59.07; H, 4.65; N, 21.53%.

Preparations of 10-ethylisoalloxazine (**7**) and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (BCQH) were described previously.[31,32]

Kinetics. The kinetic measurements of the flavin oxidation of phenylhydrazine, L-ascorbic acid, and alcohols were carried out at 30 °C under anaerobic conditions. The rates were determined by following each absorption maximum of the **1**–metal complexes. The reactions with the dihydro compounds were carried out at 30 °C aerobically under the recycle conditions.[17] The rates were determined by following each absorption maximum of BCQH (340 nm) and 10-methyl-9,10-dihydroacridine (284 nm). The further detailed reaction conditions are recorded in footnotes to each table.

Results and Discussion

Influence of Metal Ions on the Absorption Spectrum of 1. Early spectral studies of model flavin–metal interactions reveal that oxidized flavins exhibit no singificant affinity for most metals, whereas Ag(I), Cu(I), Mo(V), Ru(II), and Fe(II) were found to complex oxidized flavins probably owing to a metal–flavin charge transfer stabilization.[28,29,33–35] The spectral studies suggested that the metal binds to *O*(4) or *N*(5), or both as **8**.[28,29,35] Crystallographic studies of Ag(I) complexes later verified *O*(4)–*N*(5) coordination and in addition revealed second chelate site consisting of *N*(1)–*O*(2).[33,34]



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We measured absorption spectra of **1** and **7** in methanol at 30 °C. The absorption spectrum of **1**, which features the shift of the S2 band (317 nm) to shorter wavelength and the weakened S1 band (437 nm) relative to the S2 band, is closely akin to that of some 6-substituted flavins[36] rather than to that of conven-

Table 1. Influence of added metal ions on the absorption spectra of **1** in methanol at 30 °C

Metal salt ($[M^{n+}]/[\mathbf{1}]$)	λ_{max}/nm (ε_{max})	
None	317(14300)	437(8910)
$ZrCl_4$(2)	328(27800)	434(13800)
$FeCl_3$(12)	328(22800)	434(10300)
$SbCl_3$(11)	328(23000)	434(11000)
(104)	328(20400)	434(9800)
(218)	328(23500)	434(11200)
$FeCl_2$(5)	327(29800)	434(13800)
(10)	327(31600)	434(15200)
(60)	327(34900)	434(16100)
$HgCl_2$(6)	318(11300)	450(6870)
(12)	320(17500)	452(10500)
(120)	322(21300)	453(12200)
$ZnCl_2$(19)	318(33600)	462(8900)
$CuCl_2$(40)	322(7900)	455(6440)
$AgNO_3$(19)	319(5900)	460(5000)
$Mg(ClO_4)_2$(19)	317(10300)	437(7060)

a) $[\mathbf{1}]=2.00\times10^{-5}$ M. In the spectral measurements, the sample cell contains **1** and metal salt while the reference cell contains metal salt only.

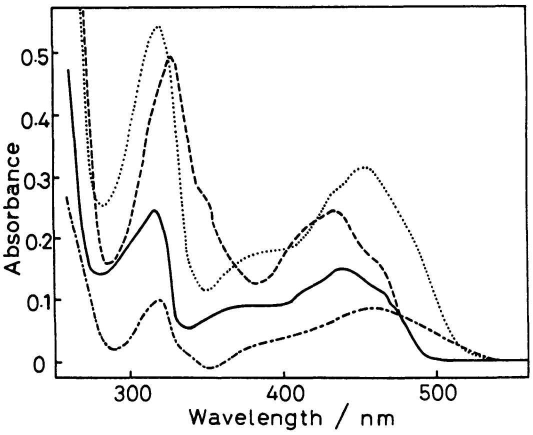


Fig. 1. Influence of added metal ions on the difference absorption spectra of **1** (1.72×10^{-5} M) in methanol at 30 °C. The reference cell contains metal ion equal to that in the sample cell. ——, No metal ion; ------, $[Zr^{4+}]=3.30\times10^{-5}$ M (type A); ······, $[Hg^{2+}]=3.30\times10^{-4}$ M (type B); ·—·—·, $[Ag^{+}]=3.30\times10^{-4}$ M (type C).

tional flavins. As reported previously,[28,35] no significant spectral change of **7** occurred on the addition of metals listed in Table 1 (expect Ag(I)). On the other hand, the absorption spectrum of **1** was sensitively affected by various kinds of metal ions (Table 1 and Fig. 1). The influence of metal ions on the absorption spectra would be classified into three types (Table 2). In type A, the absorption bands are enhanced in whole wavelength region of **1**. In type B, a new absorption band spreading to longer wavelength region appears in addition to type A. Type C features the reduction of the flavin bands and the enhancement of the longer wavelength band. On the other hand, the absorption spectrum changed only slightly on the addition of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Ni^{2+}, and Mn^{2+}.

Table 2. Classification of metal ions from spectral patterns

Type	ε_{max} at 317 nm	ε_{max} at 437 nm	Longer wavelength	Metal
A	Increase	Increase	No	Zr^{4+}, Sb^{3+}, Fe^{3+}, Mo^{5+}
B	Increase	Increase	Increase	Hg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}
C	Decrease	Decrease	Increase	Cu^{2+}, Ag^{+}

Two facts established in the early spectral studies[28,29,35] are very helpful to speculate the coordination structure: That is, (i) conventional flavins do not form complexes with most metals, indicating that the complexation with **1**, when it occurs, must involve the coordination of *N*(1′)-position to metal and (ii) the interaction of metal with *O*(4) is the origin of the longer-wavelength absorption band. Metal ions of type A have not the longer-wavelength absorption band. Thus, the coordination structure possibly involves the interaction at *N*(5) and *N*(1′) but not at *O*(4).

Type C metals are Cu^{2+} and Ag^{+}. As described above, Ag^{+} is capable of forming the stable complex with conventional flavins through the *O*(4)–*N*(5) coordination. Also in the present system, Ag^{+} would form the complex through the interaction with *O*(4) and *N*(5) and would not interact with *N*(1′), because the three-point *O*(4)–*N*(5)–*N*(1′) coordination requires a planar structure while the typical coordination structure of Ag^{+} is tetrahedral. In contrast, Cu^{2+} would reasonably interact with **1** at *O*(4), *N*(5), and *N*(1′), because it usually adopts a planar coordination structure.

To rationalize the coordination structure of type B metals is very difficult. Since these metal ions do not form the complex with conventional flavins, one must take the interaction with *N*(1′) into account. Since the absorption band at longer wavelength region exists, one must also take the intraction with *O*(4) into account. Thus, the three-point coordination at *O*(4)–*N*(5)–*N*(1′) becomes a candidate, but this is basically incompatible with the fact that these metal ions favorably adopt a tetrahedral coordination structure. Another reasonable candidate that satisfies the above requirements is the *O*(4)–*N*(1′) coordination. In fact, the basicity of *N*(5) is very low($pK_a=0$).[37] It is more unlikely, however, that the complex is formed by the *O*(4)–*N*(1′) coordination because the metal complex has an eight-membered ring. It thus seems too complicated to elucidate the coordination structure of type B metals only from the absorption spectroscopy. As a separate attempt, we inquired whether the spectral classification is associated with the ion radius of metal ions but could not find a good correlation between them.

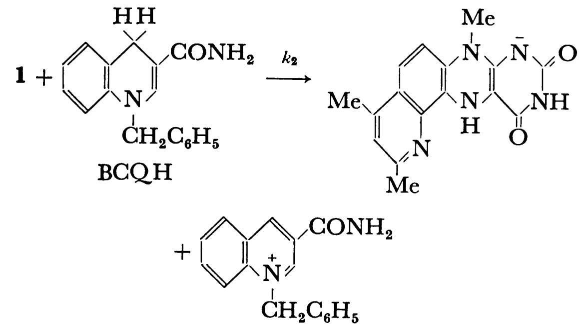
Oxidation of Dihydrophyridine Derivatives. It is interesting to know the co-catalytic effect of complexed metals on the oxidation ability of **1**. The determination of the redox potential of metal–**1** complexes without isolation may be difficult, but one may easily evaluate it through the reaction with NADH model compounds. The reaction is a typical inter-coenzyme reaction and the logarithm of the rate constants is linearly correlated with polarographic half-wave potentials.[38] We first used 1-benzyl-1,4-dihydronicotinamide as a NADH model compound. We found, however, that most of

TABLE 3. EFFECT OF ADDED METAL IONS ON THE OXIDATION OF BCQH, AND 10-METHYL-9,10-DIHYDROACRIDINE[a)]

Substrate	Metal (concn/mM)	$k' \times 10^4/s^{-1}$		k_2[c)] $/M^{-1}\,s^{-1}$
		Metal	Metal+**1**	
BCQH	None	——	0	0
	NaCl(1.00)	0	0	0
	$HgCl_2$(0.334)	0	0.489	1.76
	$SbCl_3$(0.337)	0	0.057	0.19
	$CuCl_2$(0.660)[b)]	1.8	3.2	4.76
	$FeCl_3$(0.330)[b)]	24.5	9.7	——
	$ZrCl_4$(0.033)[b)]	0.5	0.5	0
N-Methylacridan	None	——	0	0
	$SbCl_3$(0.330)	0.003	0.111	0.44
	$CuCl_2$(0.066)	0.106	0.125	0.06
	$FeCl_3$(0.066)[b)]	18.6	8.9	——

a) 30 °C, methanol, O_2, [dihydro compound]$=5.00 \times 10^{-5}$ M, [**1**]$=(2.5—3.3)\times 10^{-5}$ M. b) 2,6- Lutidine was added: [2,6-lutidine]/[metal]=5.0. c) $k_2 = (k'_{\mathrm{metal+1}} - k'_{\mathrm{metal}})/[\mathbf{1}]$.

metal ions are capable of oxidizing (or decomposing) the dihydronicotinamide at ambient conditions in methanol. We thus employed more "deactivated" and "acid-resistant" NADH model compounds, 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (BCQH)[32)] (Eq. 1) and 10-methyl-9,10-dihydroacridine. The kinetic results are summarized in Table 3.



(1)

The reaction rates were monitored spectrophotometrically by following the disappearance of the absorption band of BCQH. The examination of Table 3 reveals that (i) the reaction does not occur in methanol in the absence of metal, (ii) Hg^{2+} ad Sb^{3+}, which do not or scarcely oxidize the dihydro compounds by themselves, serve as efficient co-catalysts for the oxidation by **1**, and (iii) Cu^{2+} is able to oxidize the dihydro compounds by itself but the Cu^{2+}–**1** complex exhibits a markedly higher oxidation ability. As the addition of NaCl provides no catalytic effect on the reaction rate, the co-catalytic behavior of the heavy metals cannot be attributed to a simple salt effect. Strangely, the rate for Fe^{3+} was rather suppressed by the addition of **1**. Compound **1** may weaken the oxidation power of Fe^{3+} through the complex formation. On the other hand, the reaction with **7** which was carried out as a control experiment was not facilitated at all by metal ions listed in Table 3.

Oxidation of Phenylhydrazine. It is known that conventional flavins oxidize phenylhydrazine in the presence of a strong base such as 1,8-diazabicyclo[5.4.0]undec-7-ene, the product from phenylhydrazine being benzene.[39)] The reaction takes place, although slowly, even in the presence of a basic component. The second-order rate constants (k_2) in the absence of metal (Table 4) show that **1** is 18.9 times more reactive than **7**. We found that the oxidation of phenylhydrazine by **1** in ethanol is strongly catalyzed by Cu^{2+} and Fe^{3+}. The examination of Table 4 manifests, however, that the oxidation by **7** is also catalyzed by Co^{2+}, Mg^{2+}, and Fe^{3+}. Hence, the metal catalysis may not be rationalized in terms of the preequilibrium complexation between **1** and metals but rather in terms of the stabilization of the transition state by metal ions.

More interesting is the finding that in the oxidation of

TABLE 4. EFFECT OF ADDED METAL IONS ON THE OXIDATION OF PHENYLHYDRAZINE[a)]

Flavin	Metal	$k_2 \times 10^3/M^{-1}\,s^{-1}$ [b)]	Radical species
1	None	17.3	−
	Cu^{2+}	72.6	−
	Co^{2+}	c)	+
	Cd^{2+}	c)	+
	Zn^{2+}	2.84	+
	Mg^{2+}[d)]	8.56	−
	Fe^{3+}	66.2	+
	Sb^{3+}	15.9	−
	Zr^{4+}	14.2	−
7	None	0.917	−
	Cu^{2+}	c)	−
	Co^{2+}	2.23	−
	Zn^{2+}	0.23	−
	Mg^{2+}[d)]	16.3	−
	Fe^{3+}	9.45	−

a) 30 °C, ethanol, N_2. [**1** or **7**]$=1.50\times 10^{-5}$ M, [phenylhydrazine]=0.510 M, [metal ion (as chloride salt)] $=2.25\times 10^{-4}$M. b) $k_2=k_1'$ (pseudo-first-order rate constant)/[phenylhydrazine]. c) The increase in the absorption band continued and the absorbance did not satisfy the first-order equation. d) $Mg(ClO_4)_2$.

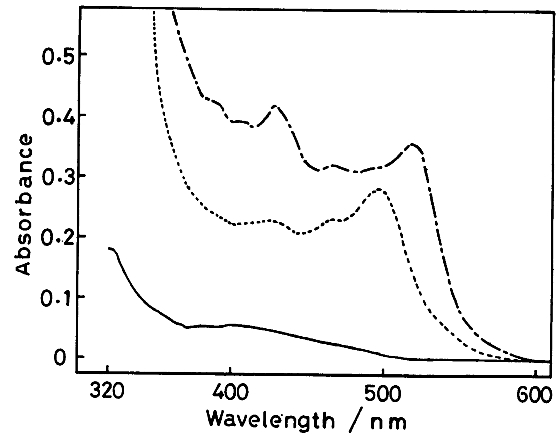
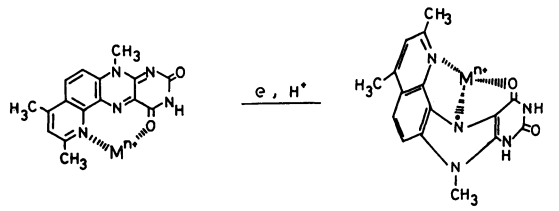


Fig. 2. Absorption spectra of reduced and semiquinone species. ——, Reduced by EDTA and light; —·—, semiquinone in phenylhydrazine+Cd^{2+}; ------, semiquinone in phenylhydrazine+Zn^{2+}. 30 °C, [**1**]$=2.80\times 10^{-5}$ M, [metal chloride]$=4.20\times 10^{-4}$ M, [phenylhydrazine]$=9.67\times 10^{-1}$ M, ethanol containing 0.8 vol% *N*,*N*-dimethylformamide.

phenylhydrazine by **1** a new absorption maximum assignable to the semiquinone radical species[28,40] appears at around 510 nm in the presence of Co^{2+}, Cd^{2+}, Zn^{2+}, and Fe^{3+} (Fig. 2). The absorption band immediately disappeared when oxygen was introduced, and the oxidized **1** was regenerated quantitatively. In other cases, the spectrum of oxidized **1** changed to that of reduced **1** without accumulation of any intermediary absorption spectrum. The authentic spectrum of reduced **1** was obtained by photoreduction with disodium salt of ethylenediaminetetraacetic acid([EDTA]/[**1**]=60). It warrants considerable attention that these metal ions (except Fe^{3+}) are classified as type B in Table 1 and usually adopt tetrahedral coordination. It is now unequivocally established that the steric shape of reduced flavins is folded along the *N*(5)–*N*(10) axis like a butterfly wing.[41,42] It is very conceivable that the semiquinone radical species have a similar bent structure. If so, the tetrahedral coordination metals would be able to specifically stabilize the semiquinone radical species of **1** through a three-point coordination (Scheme 1). Probably, the tetrahedral metals of type B interact with semiquinone **1** at *O*(4)–N(5)–*N*(1′).



$1\text{-}M^{n+}$(type B) complex(?)

Semiquinone–M^{n+} complex

Scheme 1.

Table 5. Effect of added metal ions on the oxidation of L-ascorbic acid[a)]

Flavin	Metal	$k_{decomp} \times 10^4/s^{-1}$ [b)]	$k_1' \times 10^4/s^{-1}$ [c)]	Radical species
1	None	0.11	89	–
	Mg^{2+}[e)]	0.20	164	–
	Ca^{2+}	5.5	308	–
	Zr^{4+}	0.23	184	–
	Zn^{2+}	1.5	59	+
	Cd^{2+}	92	86	+
	Co^{2+}	39	d)	–
7	None	0	19	–
	Mg^{2+}[e)]	0	d)	–
	Ca^{2+}	0	d)	–
	Zr^{4+}	0	0.63	–
	Zn^{2+}	0	d)	–
	Cd^{2+}	0	9.3	–
	Co^{2+}	18	52	–

a) 30 °C, 95% ethanol, N_2. [**1** or **7**]=1.40×10^{-5} M, [DBU] = 0.14 M, [ascorbic acid] = 1.43×10^{-2} M, [metal ion (as chloride salt)]=2.15×10^{-4}M. b) Rate constant for the first-order decrease in the absence of ascorbic acid. c) Rate constant for the first-order decrease in the presence of ascorbic acid. d) The increase in the absorption band continued and the absorbance did not satisfy the first-order equation. e) $Mg(ClO_4)_2$.

Oxidation of L-*Ascorbic Acid.* The oxidation of L-ascorbic acid to dehydroascorbic acid is another diagnostic reaction to estimate the oxidation ability of flavins. In the absence of base the reaction did not occur in 95% ethanol, so that we used excess DBU. The addition of DBU caused not only the oxidation (k_1') but the decomposition (probably hydrolytic) of the isoalloxazines (k_{decomp}) which was observed in the absence of L-ascorbic acid. In particular, Cd^{2+} and Co^{2+} (type B metals) caused a remarkably fast decomposition of **1**. It is known that the base-mediated decomposition of isoalloxazines is usually facilitated by electron-withdrawing substituents.[24] Therefore, the k_{decomp} values in Table 5 suggest that the metal ions complexed with **1** make the isoalloxazine ring more electron-deficient. However, the fact that type B metals efficiently catalyze the (hydrolytic) decomposition may imply that these metals have some specific catalytic mechanism (*e.g.*, nucleophilic attack of metal-coordinated hydroxide). On the other hand, Mg^{2+}, Ca^{2+}, and Zr^{4+} are good catalyst for the oxidation of ascorbic acid by **1**, but other metals play no or little cocatalytic role. We have found that the **1**–Zr^{4+} complex acts as a strong oxidizing agent.[47] On the other hand, Mg^{2+} and Ca^{2+} would stabilize the transition state through the interaction with ascorbate anion. Interestingly, the complex with Zn^{2+} and Cd^{2+}, which are tetrahedral coordination metals, gave a radical peak at around 510 nm. The finding supports again that the type B metals are able to stabilize the semiquinone radical of **1**.

Oxidation of Alcohols. It is known that many electron-deficient organic molecules (*e.g.*, azodicarboxylic diesters, 5-deazaisoalloxazines, 2,3-dichloro-5,6-dicyanobenzene, *etc.*) serve as oxidizing reagents for alcohols.[43–46] However, few of them are able to catalyze the oxidation in a recycle manner because of the insensitivity of the reduced forms to molecular oxygen. If the **1**–metal complexes are able to oxidize alcohols, a new possibility arises that alcohols are oxidized by molecular oxygen with the aid of the ping-pong-type

Table 6. Effect of added metal ions on the oxidation of alcohols and hydroquinone[a)]

Alcohol (concn/M)	Metal	$k_2 \times 10^4/M^{-1}\,s^{-1}$ [b)]
Benzyl alcohol (0.29)	None	0
	Cu^{2+}	0
	Co^{2+}	0
	Cd^{2+}	0
	Sb^{3+}	0
	Fe^{3+}	0.1
	Zr^{4+}	1.6
Cyclohexanol(0.57)	Nonc	0
	Zr^{4+}	1.9
Ethanol(1.1)[c)]	None	0
	Zr^{4+}	0.5
Hydroquinone(0.067)[c)]	None	0
	Zr^{4+}	2.7

a) 30 °C, acetonitrile, N_2. [**1**]=1.50×10^{-5} M, [metal ion (as chloride salt)]=2.30×10^{-4} M. b) k_2(apparent second-order rate constant)= $v_{initial}$/[**1**][alcohol]. c) Acetonitrile containing 0.8 vol% *N*,*N*-dimethylformamide.

flavin catalysis. We tested the oxidation of several alcohols by the **1**–metal complexes in various solvents and found out that in acetonitrile the alcohols are in fact oxidized to corresponding aldehydes and ketone (Table 6).[47] The effective metal ions are Zr^{4+} and Fe^{3+}. The result indicates that the complexes with these metal cations are so electron-deficient as to abstract electron(s) from alcohols. The finding suggests that the present **1**–metal complexes are useful not only as a model system of metalloflavoproteins but also as an oxidation catalyst for alcohols and others.

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