# Acid-catalysed Reduction of Flavin Analogues by an NADH Model Compound, 10-Methyl-9,10-dihydroacridine and *cis*-Dialkylcobalt(III) Complexes

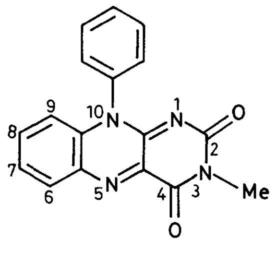
**Shunichi Fukuzumi,* Sadaki Kuroda, Tatsushi Goto, Kunio Ishikawa, and Toshio Tanaka**
*Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan*

An acid-stable NADH model compound, 10-methyl-9,10-dihydroacridine ($AcrH_2$) (**3**), and *cis*-dialkylcobalt(III) complexes, *cis*-$[R_2Co(bipy)_2]^+$ (R = Me, Et; bipy = 2,2′-bipyridine), can reduce flavin analogues {Fl: 3-methyl-10-phenylbenzo[*g*]pteridine-2(1*H*),4(3*H*)-dione (**1**) and riboflavin (**2**)}, efficiently in the presence of perchloric acid ($HClO_4$) in acetonitrile (MeCN) at 298 K to yield the corresponding dihydroflavin radical cations ($FlH_2^{+\bullet}$). Essentially, no reaction occurs in the absence of $HClO_4$ under the same conditions. The radical cations ($FlH_2^{+\bullet}$) formed are very stable to oxygen in the presence of $HClO_4$ in MeCN. Large primary kinetic isotope effects [$k_H/k_D$ 9.6 ± 0.8 and 9.9 ± 0.8 for (**1**) and (**2**), respectively] have been observed for the formation of $FlH_2^{+\bullet}$, indicating that hydride transfer from $AcrH_2$ to the protonated flavins ($FlH^+$) to give the dihydroflavins ($FlH_2$) is followed by fast comproportionation between $FlH_2$ and $FlH^+$ to yield $FlH_2^{+\bullet}$ in the presence of $HClO_4$ in MeCN. The reaction mechanisms of hydride transfer from $AcrH_2$ to $FlH^+$ are compared with the acid-catalysed electron-transfer reactions from *cis*-$[R_2Co(bipy)_2]^+$ to $FlH^+$.

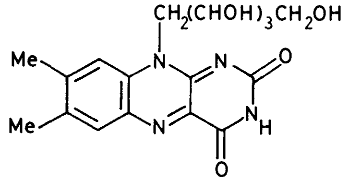
The reduced and oxidized forms of nicotinamide adenine dinucleotide (NADH and $NAD^+$, respectively) and flavins are typical coenzymes which play essential roles in biological redox reactions, especially in the primary step for the reduction of dioxygen through the respiratory chain.[1] Since Singer and Kearney[2] discovered the non-enzymatic oxidation of NADH by riboflavin extensive studies on the reactions between various NADH model compounds and flavin analogues have been reported, which aid the understanding of the mechanisms of these fundamental reactions.[3–8] Most studies on the reactions, so far reported, have been limited to those in aqueous solution,[3–8] since flavin analogues are more reactive in aqueous solution than in aprotic solvents.[9]

As previously reported the oxidizing ability of flavin analogues in MeCN is significantly improved by complexation with $Mg^{2+}$ and $Zn^{2+}$ ion.[10] In fact, a typical NADH model compound, 1-benzyl-1,4-dihydronicotinamide (BNAH), is readily oxidized by flavin analogue–$Zn^{2+}$ complexes in MeCN, although essentially no reaction occurs in the absence of the metal ion.[9] Since the metal ion which can activate a flavin analogue may act as hard acid, more efficient activation may be expected if a stronger acid such as perchloric acid ($HClO_4$) is used. However, NADH and the ordinary model compounds are known to be subject to acid-catalysed hydration,[11,12] and so cannot be used as the reductants in the presence of strong acids.

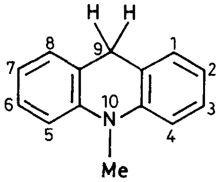
It was the aim of the present work to use an acid-stable NADH model compound, 10-methyl-9,10-dihydroacridine ($AcrH_2$) (**3**),[13] as a reductant in order to investigate the acid-catalysed reduction of flavin analogues in the presence of $HClO_4$, (in MeCN). The acid-catalysed electron-transfer reactions from *cis*-dialkylcobalt(III) complexes, *cis*-$[R_2Co(bipy)_2]^+$, to flavin analogues in the presence of $HClO_4$ in MeCN, which can be compared with the acid-catalysed reduction of flavin analogues by $AcrH_2$, is also reported.



(**1**)



(**2**)



(**3**)

## Experimental

*Materials.*—Preparation of 3-methyl-10-phenylbenzo[*g*]-pteridine-2(1*H*),4(3*H*)-dione (**1**)[10,14] and $AcrH_2$[15] is described elsewhere. Riboflavin (**2**) was obtained commercially and purified by standard procedures. The 9,10-dideuteriated analogue of $AcrH_2$, $AcrD_2$, was prepared by reduction of 10-methyl-9-acridone with $LiAlD_4$.[16,17] 10-Methylacridinium perchlorate ($AcrH^+ClO_4^-$) was prepared by the addition of $Mg(ClO_4)_2$ to 10-methylacridinium iodide in water. *cis*-Dialkylcobalt(III) complexes, *cis*-$[R_2Co(bipy)_2]ClO_4$ (R = Me, Et) were prepared by the reaction of $CoCl_2{\cdot}6H_2O$ with an excess of $NaBH_4$ in the presence of the corresponding alkyl halide.[18,19] They were isolated as the perchlorate salts and recrystallized from methanol–water. Perchloric acid (70%) was obtained from Wako Pure Chemicals. Reagent grade acetonitrile was purified by a standard procedure.[20]

*Analytical Procedures.*—Electronic absorption spectra were-measured using a Union SM-401 spectrophotometer with a quartz cuvette which was placed in a thermostatted compartment at 298 K. The protonation equilibria of (**1**) were examined from the change in the electronic spectra of (**1**) by addition of $HClO_4$ to MeCN solutions of (**1**) with a range of water concentration. Spectral titrations were carried out by adding known quantitites of a deoxygenated stock solution of $AcrH_2$ or *cis*-$[R_2Co(bipy)_2]^+$ in MeCN to a quartz cuvette

**Table 1.** Protonation equilibrium constants ($K_1$) of (**1**) in the presence of $HClO_4$ in MeCN containing $H_2O$ at 298 K.

| $H_2O$/mol dm$^{-3}$ | $K_1$/dm$^3$ mol$^{-1}$ |
|---|---|
| $<10^{-3}$ | $2.7 \times 10^6$ |
| $5.4 \times 10^{-1}$ | $4.3 \times 10^3$ |
| 27 | $9.2 \times 10^{-1}$ |

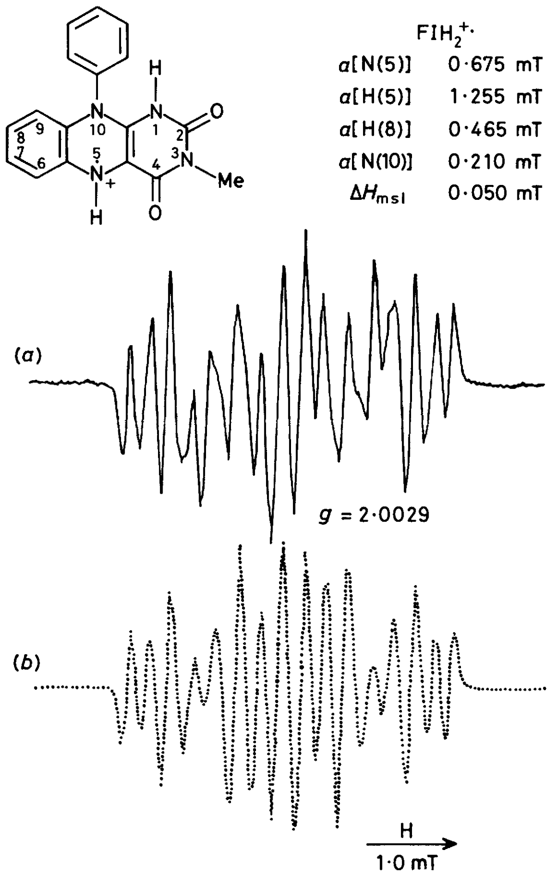


**Figure 1.** (*a*) E.s.r. spectrum of $(\mathbf{1})H_2^{+\cdot}$ formed by the reduction of $(\mathbf{1})H^+$ ($5.0 \times 10^{-4}$ mol dm$^{-3}$) by $AcrH_2$ ($5.0 \times 10^{-4}$ mol dm$^{-3}$) in the presence of $HClO_4$ ($1.0 \times 10^{-2}$ mol dm$^{-3}$) in degassed MeCN; (*b*) the computer-simulated spectrum of $(\mathbf{1})H_2^{+\cdot}$.

containing a known aliqout of the flavin analogue (Fl) in the presence of $HClO_4$ in MeCN. The amounts of the dihydroflavin radical cations ($FlH_2^{+\cdot}$) and 10-methylacridinium ion ($AcrH^+$) formed were determined from the absorbances at $\lambda_{max}$ [470 and 500 nm for $(\mathbf{1})H_2^{+\cdot}$ and $(\mathbf{2})H_2^{+\cdot}$, respectively, and 358 nm for $AcrH^+$]. The gaseous products formed in the reactions of *cis*-$[R_2Co(bipy)_2]^+$ with the flavin analogue in the presence of $HClO_4$ in MeCN were analysed by gas chromatography using a 2 m Unibeads 1S column.

The e.s.r. spectroscopic measurements were carried out using a JEOL-X band spectrometer (JES-ME-LX) at room temperature. After the completion of the reaction of (**1**) or (**2**) ($8.0 \times 10^{-4}$ mol dm$^{-3}$) with $AcrH_2$ or *cis*-$[Et_2Co(bipy)_2]^+$ ($1.1 \times 10^{-3}$ mol dm$^{-3}$) in the presence of $HClO_4$ ($2.0 \times 10^{-3}$ mol dm$^{-3}$) in degassed MeCN, an aliquot of the resulting solution was transferred to a 1 mm quartz tube under nitrogen, and it was thoroughly degassed by successive freeze–pump–thaw cycles. The e.s.r. spectra were recorded with a non-saturating microwave power (modulation amplitude $8.0 \times 10^{-2}$ mT). Further reduction of modulation amplitude did not improve the hyperfine coupling resolution. The *g*-value and the hyperfine coupling constants (h.f.c.) of the e.s.r. spectra were calibrated using an $Mn^{2+}$ e.s.r. marker. The e.s.r. spectra were simulated using a NEC 9801 $VM_2$ microcomputer.

*Kinetic Measurements.*—Kinetic measurements were carried out using a Union RA-103 stopped-flow spectrophotometer for reactions of half-life less than 10 s and a conventional spectrophotometer for the anaerobic reactions (half-lives greater than 100 s). Rates were monitored by the rise in the absorbances at $\lambda_{max}$ of $FlH_2^{+\cdot}$ (see above) under pseudo-first-order conditions in the presence of more than tenfold excess Fl and $HClO_4$ at 298 K. Pseudo-first-order rate constants were determined by least-squares curve fitting using a Union System 77 microcomputer.

## Results

*Protonation of Flavin Analogues.*—Flavin analogues (Fl) are known to be protonated at the N-1 position in strongly acidic aqueous solution (p$K_a$ *ca.* 0).[21] In MeCN, the protonation of (**1**) [equation (1)] occurs much more readily than in $H_2O$,

$$\mathrm{Fl} + \mathrm{H}^+ \overset{K_1}{\rightleftharpoons} \mathrm{FlH}^+ \tag{1}$$

and the absorption band due to (**1**) ($\lambda_{max}$ 439 nm) in the absence of $HClO_4$ in MeCN undergoes a hypsochromic (blue) shift in the presence of $HClO_4$ even in slight excess ($\lambda_{max}$ 366 nm). Such a blue-shift due to protonation is also observed for $(\mathbf{2})H^+$ in MeCN ($\lambda_{max}$ 388 nm). The protonation equilibrium constant $K_1$ ($K_1 = K_a^{-1}$) can be determined from the spectral change using equation (2), [$A_0$ is the absorbance at 439 nm due

$$A_0/A - 1 = K_1\{[\mathrm{HClO_4}]_0 + (A/A_0 - 1)[\mathrm{Fl}]_0\} \tag{2}$$

to (**1**) in the absence of $HClO_4$, $A$ is the absorbance in the presence of $HClO_4$, and the subscript 0 denotes the initial concentration. The $K_1$ values for different concentrations of $H_2O$ in MeCN, determined from the slopes of the linear plots of $A_0/A - 1$ *vs.* $[HClO_4]_0 + (A/A_0 - 1)[Fl]_0$, are listed in Table 1. The $k_1$ value in MeCN, which is much larger than that in $H_2O$, decreases significantly with an increase in the $H_2O$ concentration in MeCN (Table 1).

*Formation of Dihydroflavin Radical Cations.*—On mixing $AcrH_2$ with the protonated flavin analogue $FlH^+$ [$(\mathbf{1})H^+$ or $(\mathbf{2})H^+$] in the presence of $HClO_4$ in MeCN, an absorption band, characteristic of $AcrH^+$ ($\lambda_{max}$ 358 nm), was observed together with new absorption bands at $\lambda_{max}$ = 470 and 500 nm for $(\mathbf{1})H^+$ and $(\mathbf{2})H^+$, respectively. The absorption band at 500 nm derived from $(\mathbf{2})H^+$ can be assigned to the dihydroriboflavin radical cation, $(\mathbf{2})H_2^{+\cdot}$.[22,23] Thus, the new absorption band at $\lambda_{max}$ 470 nm derived from (**1**) may also be assigned to the corresponding dihydroflavin radical cation, $(\mathbf{1})H_2^{+\cdot}$. This assignment was confirmed by the e.s.r. spectrum (*a*) of the resulting solution (see Figure 1). A computer-simulated spectrum of $(\mathbf{1})H_2^{+\cdot}$ is also shown (*b*) with the best-fit parameters, the hyperfine coupling constant (h.f.c.), $a_5$(N) 0.675, $a_5$(H) 1.255, $a_8$(H) 0.465, $a_{10}$(N) 0.210 mT, and the maximum slope line width, $\Delta H_{msl}$ = 0.050 mT. The simulated spectrum agrees well with the observed spectrum, except for some line intensities, which probably disagree due to disregarding the h.f.c. for the 10-phenyl group. The large h.f.c. value due to N-5 proton and the lack of appreciable h.f.c. due to C-6 and C-9 protons are characteristic of a dihydroflavin radical cation.[24] The lack of appreciable h.f.c. due to the C(7) proton, N-1, or N-3 is also seen for flavin radical species [flavosemiquinone radial

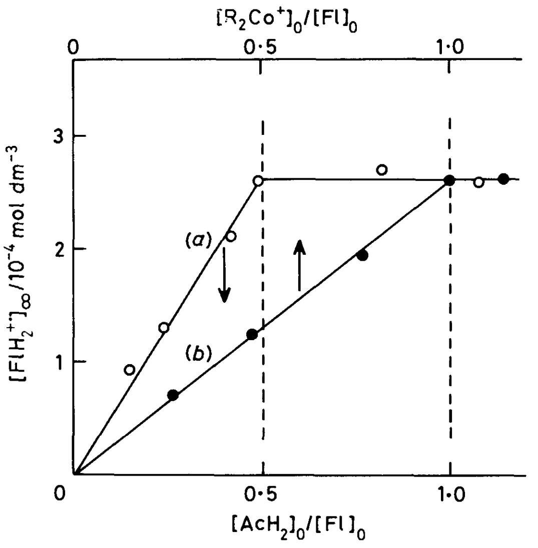


**Figure 2.** Plots of the concentration of $FlH_2^{+\cdot}$ formed in the reduction of $FlH^+$ [(2)$H^+$] by (*a*) $AcrH_2$ (○) and (*b*) *cis*-$[Et_2Co(bipy)_2]^+$ (●) in the presence of excess $HClO_4$ in MeCN *vs.* $[AcrH_2]_0/[(2)H^+]_0$ and $[cis\text{-}Et_2Co^+]_0/[(2)H^+]_0$.

anion ($Fl^{-\cdot}$), neutral flavosemiquinone ($FlH^\cdot$), and dihydro flavin radical cation ($FlH_2^{+\cdot}$)],[24] as confirmed by the MO calculation.[25] Essentially the same electronic and e.s.r. spectra were observed in the reactions of (1)$H^+$ with *cis*-$[R_2Co(bipy)_2]^+$ in the presence of $HClO_4$ in MeCN.

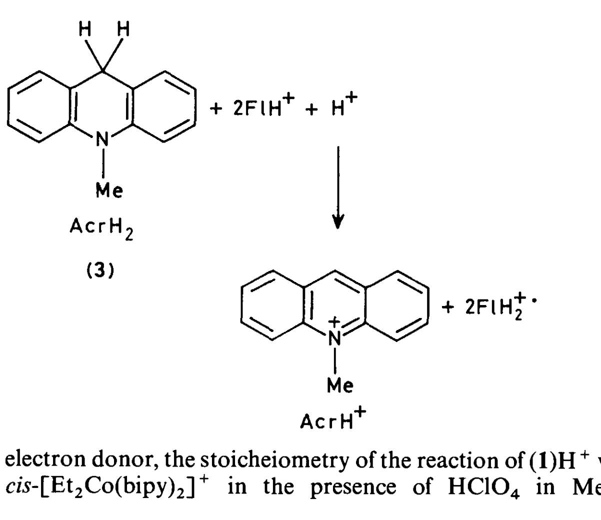
The dihydroflavin radical cations ($FlH_2^{+\cdot}$) are stable to oxygen in the presence of $HClO_4$ in MeCN, and it takes *ca.* 10 h to be completely oxidized by oxygen. The stability of $FlH_2^{+\cdot}$ to oxygen may be due to the protonation of $FlH^\cdot$, equation (3). In

$$FlH^\cdot + H^+ \overset{K_2}{\rightleftharpoons} FlH_2^{+\cdot} \qquad (3)$$

fact, the deprotonated flavosemiquinone $Fl^{-\cdot}$ is known to be most reactive towards oxygen (rate constant of $2.5 \times 10^8$ dm$^3$ mol$^{-1}$ s$^{-1}$), but the direct oxidation of the protonated species ($FlH^\cdot$) by oxygen becomes so slow that the oxidation of $FlH^\cdot$ may occur only *via* $Fl^{-\cdot}$ which is in equilibrium with $FlH^\cdot$.[26] Thus, further protonation of $FlH^\cdot$ to $FlH^{+\cdot}$ may decrease reducing ability significantly.

The stoicheiometry of the formation of $FlH_2^{+\cdot}$, determined from the electronic spectra in the reaction of (1)$H^+$ with $AcrH_2$ in the presence of $HClO_4$ in MeCN [Figure 2 (*a*)], is given by equation (4). In contrast with the case of $AcrH_2$ which is a two-electron donor, the stoicheiometry of the reaction of (1)$H^+$ with *cis*-$[Et_2Co(bipy)_2]^+$ in the presence of $HClO_4$ in MeCN [Figure 2(*b*)] indicates that *cis*-$[Et_2Co(bipy)_2]^+$ acts as a one-electron donor. Quantitative amounts of ethane and butane were obtained in the reactions of (1) with *cis*-$[R_2Co(bipy)_2]^+$ (R = Me and Et, respectively) in the presence of $HClO_4$ in MeCN (see the Experimental section), as observed in the one-electron oxidation of *cis*-$[R_2Co(bipy)_2]^+$ by organic and inorganic one-electron oxidants.[19,27] Thus, the stoicheiometry is given by equation (5).

$$cis\text{-}[R_2Co(bipy)_2]^+ + FlH^+ + H^+ \longrightarrow R\text{–}R + [Co(bipy)_2]^{2+} + FlH_2^{+\cdot} \qquad (5)$$



(4)

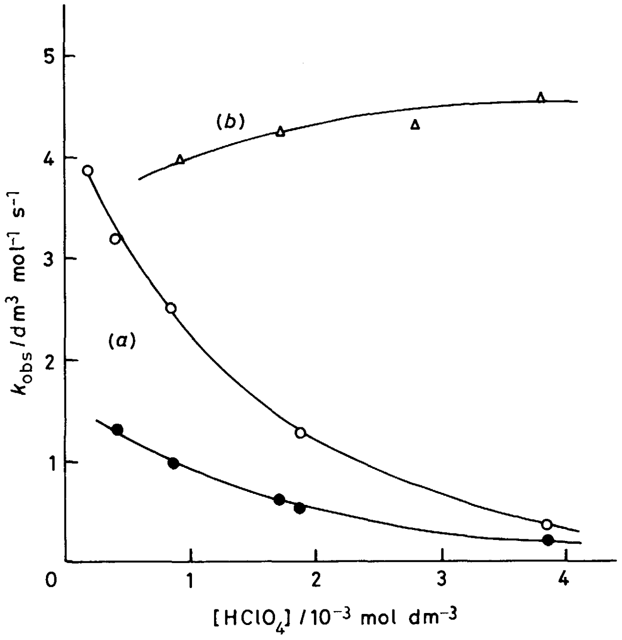


**Figure 3.** (*a*) Dependence of the observed second-order rate constant $k_{obs}$ on $[HClO_4]$ for the reduction of $FlH^+$, (1)$H^+$ (○) and (2)$H^+$ (●), in the presence of $HClO_4$ in MeCN at 298 K, and (*b*) the $[HClO_4]$ dependence of $k_{obs}$ of (1)$H^+$ in the presence of 0.54 mol dm$^{-3}$ $H_2O$ in MeCN (Δ).

*Kinetics.*—The rate of formation of $FlH_2^{+\cdot}$ in the reduction of $FlH^+$ by $AcrH_2$ obeyed pseudo-first-order kinetics in the presence of excess Fl and $HClO_4$, and the pseudo-first-order rate constant was proportional to the $FlH^+$ concentration, as given by equation (6). The dependence of the observed second-

$$d[FlH_2^{+\cdot}]/dt = k_{obs}[AcrH_2][FlH^+] \qquad (6)$$

order rate constant $k_{obs}$ of both (1)$H^+$ and (2)$H^+$ on the $HClO_4$ concentration in MeCN is shown in Figure 3(*a*). In the absence of $HClO_4$, essentially no reaction occurs, but the $k_{obs}$ value decreases with an increase in the $HClO_4$ concentration [Figure 3(*a*)]. When $H_2O$ (0.54 mol dm$^{-3}$) is added to the $AcrH_2$–(1)$H^+$ system, the decrease of $k_{obs}$ with an increase in the $HClO_4$ concentration [Figure 3(*a*)] is suppressed and $k_{obs}$ becomes independent of the $HClO_4$ concentration [Figure 3(*b*)]. The primary kinetic isotope effects were also examined by replacing $AcrH_2$ with the 9,9′-dideuteriated analogue ($AcrD_2$) and the $k_H/k_D$ values for the reduction of (1)$H^+$ and (2)$H^+$ by $AcrH_2$ and $AcrD_2$ in the presence of various concentrations of $HClO_4$ in MeCN at 298 K are listed in Table 2.

The $k_H/k_D$ values are constant with the change in the $HClO_4$ concentration and large primary kinetic isotope effects are observed for both (1)$H^+$ and (2)$H^+$, $k_H/k_D = 9.6 \pm 0.8$ and $9.9 \pm 0.8$, respectively (Table 2).

**Table 2.** Primary kinetic isotope effects ($k_H/k_D$) for the reduction of $FlH^+$ [(1)$H^+$ and (2)$H^+$] by $AcrH_2$ and $AcrD_2$ in the presence of $HClO_4$ in MeCN at 298 K.

| $[HClO_4]$/ mol $dm^{-3}$ | $k_H/k_D$ [a] (1)$H^+$ | (2)$H^+$ |
|---|---|---|
| $1.9 \times 10^{-4}$ | 9.3 | — |
| $4.2 \times 10^{-4}$ | 9.6 | 10.2 |
| $8.7 \times 10^{-4}$ | 8.8 | 10.6 |
| $1.9 \times 10^{-3}$ | 10.4 | 9.1 |
| $3.9 \times 10^{-3}$ | 9.8 | 9.9 |

[a] The experimental errors are within ±10%.

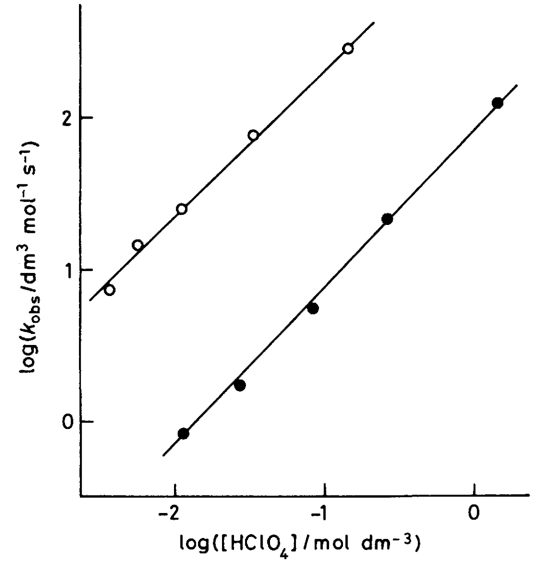


**Figure 4.** Plots of $\log(k_{obs}/dm^3\ mol^{-1}\ s^{-1})$ vs. $\log([HClO_4]/mol\ dm^{-3})$ for the reduction of (2)$H^+$ by *cis*-$[R_2Co(bipy)_2]^+$ [R = Me (●) and Et (○)] in the presence of $HClO_4$ in MeCN at 298 K.

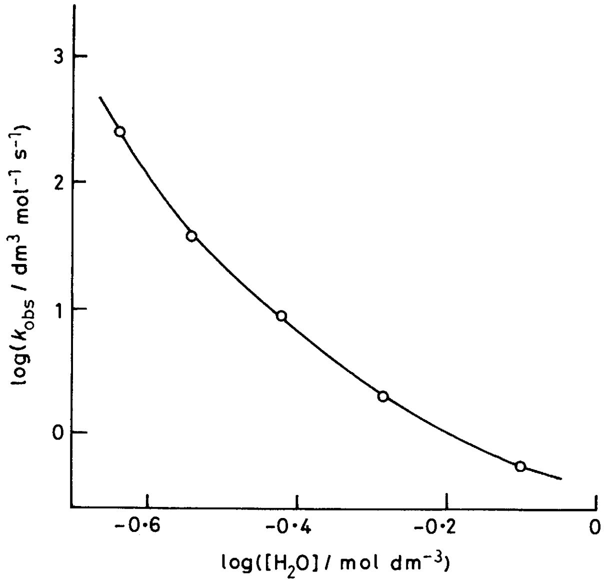


**Figure 5.** Dependence of $k_{obs}$ on the $H_2O$ concentration for the reduction of (2)$H^+$ by *cis*-$[Et_2Co(bipy)_2]^+$ in the presence of $HClO_4$ in MeCN containing $H_2O$ at 298 K.

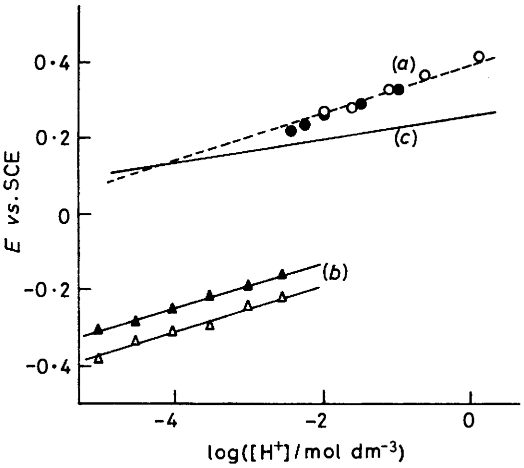


**Figure 6.** Plots of the reduction potentials of Fl *vs.* $\log([H^+]/mol\ dm^{-3})$; (*a*) one-electron reduction potential of (2)$H^+$ [$E(FlH^+/FlH_2^{+\bullet})$] in the presence of $HClO_4$ in MeCN at 298 K, determined from the one-electron reduction of (2)$H^+$ by *cis*-$[R_2Co(bipy)_2]^+$ [R = Me (●) and Et (○)] using equation (8); (*b*) the reported one-electron reduction potential of (2) [$E(Fl/FlH^{\bullet})$] (△) and (2)$H^{\bullet}$ [$E(FlH^{\bullet}/FlH_2)$] (▲) in $H_2O$;[29] and (*c*) the two-electron oxidation potential of $AcrH_2$ [$E(AcrH^+/AcrH_2)$] in the presence of $HClO_4$ in MeCN.[15]

The rate of electron transfer from *cis*-$[R_2Co(bipy)_2]^+$ to (2)$H^+$ in the presence of $HClO_4$ also obeyed the second-order kinetics, equation (7). In Figure 4, the log $k_{obs}$ values for

$$d[FlH_2^{+\bullet}]/dt = k_{obs}[R_2Co^+][FlH^+] \qquad (7)$$

electron transfer from *cis*-$[R_2Co(bipy)_2]^+$ (R = Me and Et) to (2)$H^+$ in the presence of $HClO_4$ in MeCN at 298 K are plotted against log $[H^+]$. The dependence of $k_{obs}$ on the concentration of $H_2O$, which is added to the *cis*-$[Et_2Co(bipy)_2]^+$–(2)$H^+$ system in the presence of 0.10 mol $dm^{-3}$ $HClO_4$ in MeCN, is shown in Figure 5. In contrast with the case of $AcrH_2$ (Figure 3), the $k_{obs}$ value increases linearly with an increase in the $HClO_4$ concentration but decreases with an increase in the $H_2O$ concentration (Figures 4 and 5, respectively).

## Discussion

*Redox Potentials of Fl in the Presence of* $HClO_4$ *in* MeCN.— The observed second-order rate constants $k_{obs}$ of electron transfer from *cis*-$[R_2Co(bipy)_2]^+$ to organic one-electron oxidants in MeCN at 298 K have been correlated with the one-electron reduction potential of the oxidant ($E^0_{red}$) by equation (8),[19] where the $E^0_{ox}$ is the one-electron oxidation potential

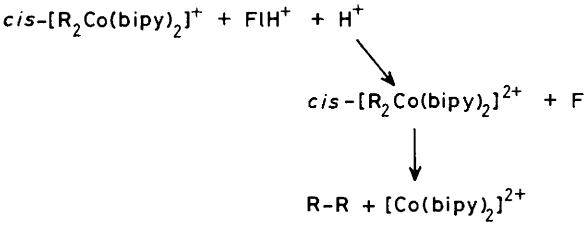
$$\log k_{obs} = -15.3(E^0_{ox} - E^0_{red}) + 6.2 \qquad (8)$$

of *cis*-$[R_2Co(bipy)_2]^+$ (0.63 and 0.57 V *vs.* SCE, for R = Me and Et, respectively).[19] Then, the one-electron reduction potential of $FlH^+$ in the presence of $HClO_4$ in MeCN at 298 K, $E(FlH^+/FlH_2^{+\bullet})$, can be evaluated from the $k_{obs}$ value using equation (8). The dependence of $E(FlH^+/FlH_2^{+\bullet})$ on the $HClO_4$ concentration, thus obtained, is shown in Figure 6, where the $E(FlH^+/FlH_2^{+\bullet})$ values derived from the rate constants of *cis*-$[Me_2Co(bipy)_2]^+$ agree well with those derived from the rate constants of *cis*-$[Et_2Co(bipy)_2]^+$ [Figure 6(*a*)]. The $E(FlH^+/FlH_2^{+\bullet})$ values are much more positive than the reported value of the one-electron reduction potential of (2) in the absence of $HClO_4$ in an aprotic solvent (−0.69 V *vs.* SCE),[28] and shifted to the positive direction with an increase in the $HClO_4$ concentration [Figure 6 (*a*)]. Such a positive shift of

$E(\mathrm{FlH^+/FlH_2^{+\cdot}})$ may be casued by the protonation of FlH$^\cdot$ [equation (3)]. Under the conditions that the protonation equilibrium constant $K_2 \gg [\mathrm{HClO_4}]^{-1}$, the Nernst equation of $E(\mathrm{FlH^+/FlH^{+\cdot}})$ may be given by equation (9) where $F$ is the

$$E(\mathrm{FlH^+/FlH_2^{+\cdot}}) = E^0(\mathrm{FlH^+/FlH^\cdot}) + (2.3RT/F)(\log[\mathrm{H^+}] + \log K_2) \quad (9)$$

Faraday constant ($2.3RT/F = 0.059$ at 298 K). According to equation (9), the $E(\mathrm{FlH^+/FlH_2^{+\cdot}})$ value is expected to be shifted to the positive direction by 0.059 V/log[H$^+$], in accordance with the results in Figure 6(*a*) [the dotted line is drawn with the slope according to equation (9)]. Thus, the electron transfer from *cis*-[$\mathrm{R_2Co(bipy)_2}$]$^+$ to FlH$^+$ becomes energetically more favourable with increased in HClO$_4$ concentration in MeCN and the rate is proportional to [HClO$_4$] (Figure 4). The *cis*-[$\mathrm{R_2Co(bipy)_2}$]$^{2+}$ formed in the electron transfer may be subject to the facile reductive coupling of the alkyl groups to yield R–R (Scheme 1).[19,27]



**Scheme 1.**

The reported values of the one-electron reduction potential of Fl [$E(\mathrm{Fl/FlH^\cdot})$] and FlH$^\cdot$ [$E(\mathrm{FlH^\cdot/FlH_2})$] in aqueous solutions[29] are also plotted against log[H$^+$] in Figure 6(*b*). The positive shifts of $E(\mathrm{Fl/FlH^\cdot})$ and $E(\mathrm{FlH^\cdot/FlH_2})$ with a decrease in pH ($= -\log[\mathrm{H^+}]$) are known to be caused by the protonation of Fl$^{-\cdot}$ and FlH$^-$ [equations (10) and (11)], respectively.[29]

$$\mathrm{Fl^{-\cdot} + H^+ \rightleftharpoons FlH^\cdot} \quad (10)$$

$$\mathrm{FlH^- + H^+ \rightleftharpoons FlH_2} \quad (11)$$

respectively.[29] In an aqueous solution, the further protonation of FlH$^\cdot$ [equation (3)] occurs only in a strongly acidic solution, since the protonation equilibrium constant $K_2$ [equation (3)] in H$_2$O ($K_2 = 1$)[21] is much smaller than that in MeCN. Thus, the one-electron reduction potential in the presence of HClO$_4$ in MeCN is shifted to the positive direction relative to that in H$_2$O, due to the further protonation of FlH$^\cdot$ in MeCN [equation (3)], as shown in Figure 6. This may be the reason why the rate constant of electron transfer from *cis*-[$\mathrm{Et_2Co(bipy)_2}$]$^+$ to (**2**)H$^+$ in the presence of a fixed concentration of HClO$_4$ decreases significantly with an increase in the H$_2$O concentration in MeCN (Figure 5). According to equation (9), the $E(\mathrm{FlH^+/FlH_2^{+\cdot}})$ value may be shifted to the negative direction with an increase in the H$_2$O concentration, since the $K_2$ value may decrease with an increase in the H$_2$O concentration, when the electron transfer becomes energetically more unfavourable.

The other important difference between the one-electron reduction potentials in H$_2$O and MeCN is that the $E(\mathrm{Fl/FlH^\cdot})$ value in H$_2$O is always more negative than the $E(\mathrm{FlH^\cdot/FlH_2})$ value, whereas the $E(\mathrm{FlH^+/FlH_2^{+\cdot}})$ value in MeCN is always more positive than the $E(\mathrm{FlH_2^{+\cdot}/FlH_2})$ value ($-0.10$ V *vs.* SCE).[30] Thus, the disproportionation equilibrium in H$_2$O [equation (12)] favours the formation of FlH$_2$, while the

$$\mathrm{2FlH^\cdot \rightleftharpoons Fl + FlH_2} \quad (12)$$

comproportionation equilibrium [equation (13)] in MeCN

$$\mathrm{FlH_2 + FlH^+ + H^+ \rightleftharpoons 2FlH_2^{+\cdot}} \quad (13)$$

favours the formation of FlH$_2^{+\cdot}$. This may be the reason why FlH$_2^{+\cdot}$ is formed in the reduction of FlH$^+$ by AcrH$_2$ [equation (4)] and *cis*-[$\mathrm{R_2Co(bipy)_2}$]$^+$ [equation (5)] in the presence of HClO$_4$ in MeCN.

*Mechanisms of the Reduction of* FlH$^+$ by AcrH$_2$ *in the Presence of* HClO$_4$ *in* MeCN.—The Gibbs energy change $\Delta G$ of the reduction of FlH$^+$ by AcrH$_2$ in the presence of HClO$_4$ in MeCN [equation (1)] is obtained from equation (14), where

$$\Delta G = 2[E(\mathrm{AcrH^+/AcrH_2}) - E(\mathrm{FlH^+/FlH_2^{+\cdot}})] \quad (14)$$

the dependence of $E(\mathrm{FlH^+/FlH_2^{+\cdot}})$ on [HClO$_4$] is shown in Figure 6(*a*) and the two-electron oxidation potential of AcrH$_2$ [$E(\mathrm{AcrH^+/AcrH_2})$] is given by equation (15).

$$E(\mathrm{AcrH^+/AcrH_2}) = [E(\mathrm{AcrH^\cdot/AcrH_2}) + E(\mathrm{AcrH^+/AcrH^\cdot})]/2 \quad (15)$$

The dependence of $E(\mathrm{AcrH^+/AcrH_2})$ on [HClO$_4$], obtained from the reported values of $E^0(\mathrm{AcrH_2^{+\cdot}/AcrH_2})$ (0.80 V *vs.* SCE), $E^0(\mathrm{AcrH^+/AcrH^\cdot})$, and p$K_a$ of AcrH$_2^{+\cdot}$ (2.0) in MeCN[15] using equation (15), is also shown in Figure 6(*c*) where the $\Delta G$ value in equation (14) becomes negative in the region [HClO$_4$] > 5 × 10$^{-5}$ mol dm$^{-3}$. On the other hand, the Gibbs energy change of hydride transfer from AcrH$_2$ to FlH$^+$ [equation (16)], $\Delta G_h$ obtained from the two-electron

$$\mathrm{AcrH_2 + FlH^+ \xrightarrow{k_h} AcrH^+ + FlH_2} \quad (16)$$

oxidation potential [$E(\mathrm{AcrH^+/AcrH_2})$] and the two-electron reduction potential [$E(\mathrm{FlH^+/FlH_2})$] using equation (17), is

$$\Delta G_h = 2\mathrm{F}[E(\mathrm{AcrH^+/AcrH_2}) - E(\mathrm{FlH^+/FlH_2})] \quad (17)$$

positive, 11 kJ mol$^{-1}$, which is independent of [HClO$_4$], and so the hydride transfer is always endothermic. Thus, the overall reaction [equation (1)] becomes exothermic by the combination of the hydride transfer [equation (16)] with the comproportionation reaction [equation (13)].

Extensive studies on the hydride transfer reactions between NADH and NAD$^+$ analogues have been reported by Kreevoy *et al.*[16,31] The rate constants of the hydride transfer reactions have been correlated with the $\Delta G_h$ values and the self-exchange rate constants between the same pair of NADH/NAD$^+$ analogues.[31] By applying the Kreevoy's relation[31] to the present case, the rate constant of the hydride transfer from AcrH$_2$ to FlH$^+$ [equation (16)] is calculated as 5.1 × 10$^{-3}$ dm$^3$ mol$^{-1}$ s$^{-1}$ from the self-exchange rate constant of AcrH$^+$/AcrH$_2$ (4.3 × 10$^{-2}$ dm$^3$ mol$^{-1}$ s$^{-1}$) and the $\Delta G_h$ value (described above). In order to compare the calculated rate constant with the observed rate constant, the protonation of AcrH$_2$ [equation (18)] should be taken into account, since the protonated species

$$\mathrm{AcrH_2 + H^+ \overset{K_3}{\rightleftharpoons} AcrH_3^+} \quad (18)$$

(AcrH$_3^+$) is known to be a much weaker reducing agent than free AcrH$_2$.[13] In such a case, the observed second-order rate constant ($k_{obs}$) is related to the rate constant of hydride transfer from unprotonated AcrH$_2$ to FlH$^+$ [$k_h$, equation (16)] as given by equation (19), which agrees with the result in Figure 3(*a*),

$$k_{obs} = k_h/(1 + K_3[\mathrm{HClO_4}]) \quad (19)$$

**Table 3.** Comparison of the observed rate constant ($k_{obs}$) for the reduction of (**2**)H$^+$ by AcrH$_2$ in the presence of HClO$_4$ in MeCN at 298 K with the calculated rate constant ($k_{calc}$) for the hydrogen transfer from AcrH$_2$ to (**2**)H$^+$.

| [HClO$_4$]/ mol dm$^{-3}$ | $k_{obs}$/ dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{calc}$$^a$/ dm$^3$ mol$^{-1}$ s$^{-1}$ |
|---|---|---|
| $4.2 \times 10^{-4}$ | 1.3 | 1.9 |
| $8.7 \times 10^{-4}$ | 1.0 | 1.0 |
| $1.7 \times 10^{-3}$ | 0.60 | 0.53 |
| $3.9 \times 10^{-3}$ | 0.20 | 0.25 |

$^a$ Calculated based on equations (21) and (22), see the text.

where $k_{obs}$ decreases with an increase in the HClO$_4$ concentration. Equation (19) can also account for the effect of H$_2$O shown in Figure 3(*b*) where $k_{obs}$ is independent of [HClO$_4$] in the presence of H$_2$O (0.54 mol dm$^{-3}$), since the $K_3$ value is known to be decreased significantly in the presence of H$_2$O (0.54 mol dm$^{-3}$) in MeCN.[13] Thus $k_{obs}$ becomes independent of [HClO$_4$] provided $K_3[\mathrm{HClO_4}] \ll 1$ [equation (19)]. According to equation (19), the calculated rate constant based on Kreevoy's relation should be divided by $(1 + K_3[\mathrm{HClO_4}])$ in order to compare the calculated rate constant with the observed rate constant in Figure 3. The calculated value using the $K_3$ value ($1.1 \times 10^4$ mol$^{-1}$ dm$^3$)[13] in MeCN in the region $[\mathrm{H_2O}] \ll 10^{-3}$ mol dm$^{-3}$, however, is $2 \times 10^3$ times smaller than the corresponding observed rate constant of (**2**)H$^+$ in Figure 3(*a*).

Kreevoy *et al.* also reported the correlation of the kinetic isotope effects ($k_H/k_D$) for hydride transfer reactions between NADH and NAD$^+$ analogues including a flavin analogue with the $\Delta G_h$ value.[16] According to the correlation,[16] the $k_H/k_D$ value is estimated as 5.4, which is much less than the observed value, $9.9 \pm 0.8$ (Table 2). Thus, neither the rate constant nor the kinetic isotope effect can be correlated with the hydride transfer reactions between NADH and NAD$^+$ analogues, which are claimed to occur *via* the one-step hydride transfer.[16,30]

An alternative mechanism for hydride transfer from AcrH$_2$ to FlH$^+$ is shown in Scheme 2, where the hydrogen-atom

$$\mathrm{AcrH_2} + \mathrm{FlH^+} \xrightarrow{k_H} (\mathrm{AcrH^{\bullet}}\ \mathrm{FlH_2}^{+\bullet}) \xrightarrow{\text{fast}} \mathrm{AcrH^+} + \mathrm{FlH_2}$$

**Scheme 2.**

(electron plus proton) transfer from AcrH$_2$ to FlH$^+$ occurs to give the radical pair (AcrH$^\bullet$ FlH$_2$$^{+\bullet}$), followed by the facile electron transfer from AcrH$^\bullet$ to FlH$_2$$^{+\bullet}$, yielding AcrH$^+$ and FlH$_2$, the latter of which is converted into FlH$_2$$^{+\bullet}$ by the fast comproportionation reaction [equation (13)]. The Gibbs energy change of electron transfer from AcrH$^\bullet$ to FlH$_2$$^{+\bullet}$ is highly exothermic ($-32$ kJ mol$^{-1}$) based on the one-electron oxidation potential of AcrH$^\bullet$ ($-0.43$ V *vs.* SCE)[15] and the one-electron reduction potential of FlH$_2$$^{+\bullet}$ ($-0.10$ V *vs.* SCE)[31] in MeCN. Thus, hydrogen transfer from AcrH$_2$ to FlH$^+$ in Scheme 2 may be the rate-determining step. The Gibbs energy change of the hydrogen transfer from AcrH$_2$ to FlH$^+$ ($\Delta G_H$) is given by equation (20), where $\Delta G_H$ is independent of

$$\Delta G_H = \mathrm{F}[E(\mathrm{AcrH^{\bullet}/AcrH_2}) - E(\mathrm{FlH^+/FlH_2}^{+\bullet})] \quad (20)$$

the HClO$_4$ concentration, since both $E(\mathrm{AcrH^{\bullet}/AcrH_2})$ and $E(\mathrm{FlH^+/FlH_2}^{+\bullet})$ are shifted to the positive direction by 0.059 mV/log[H$^+$].

The Marcus theory of atom-transfer reactions[32] can be applied to calculate the rate constant of the hydrogen-transfer reaction, which is expressed by equation (21), where $Z$ is the

$$k_H = Z\exp(-\Delta G^{\ddagger}_{H}/RT) \quad (21)$$

frequency factor, taken as $1 \times 10^{11}$ dm$^3$ mol$^{-1}$ s$^{-1}$, and the activation Gibbs energy ($\Delta G^{\ddagger}_{H}$) is given as a function of $\Delta G_H$ by equation (22).[32] The $\Delta G^{\ddagger}_{0}$ value corresponds to the activation

$$\Delta G^{\ddagger}_{H} = \Delta G^{\ddagger}_{0}(1 + \Delta G_H/4\Delta G^{\ddagger}_{0})^2 \quad (22)$$

Gibbs energy at $\Delta G_H = 0$. The $\Delta G^{\ddagger}_{0}$ value may be taken as 22.6 kJ mol$^{-1}$ which is the sum of the $\Delta G^{\ddagger}_{0}$ values of the self-exchange of AcrH$_2$$^{+\bullet}$/AcrH$_2$ and AcrH$^\bullet$/AcrH$_2$$^{+\bullet}$.[15] Then, the $k_H$ value can be calculated from the $\Delta G^{\ddagger}_{0}$ and $\Delta G_H$ values using equations (21) and (22). According to equation (19), the calculated $k_H$ value should be divided by $(1 + K_3[\mathrm{HClO_4}])$ in order to compare it with the observed rate constants. The calculated values $k_{calc}$ thus obtained in the presence of various concentrations of HClO$_4$ in MeCN at 298 K agree well with the observed values as shown in Table 3. Thus, it may be concluded that the reduction of FlH$^+$ by AcrH$_2$ in the presence of HClO$_4$ in MeCN proceeds *via* a hydrogen (or electron plus proton) transfer from AcrH$_2$ to FlH$^+$ (Scheme 2). At present, however, it is not clear whether the hydrogen transfer occurs *via* a direct transfer of a hydrogen atom or *via* the combination of electron and proton transfers. In the latter case, the acid-catalysed electron transfer from AcrH$_2$ to FlH$^+$ to yield AcrH$_2$$^{+\bullet}$ and FlH$_2$$^{+\bullet}$ is unlikely to occur since the subsequent proton transfer from AcrH$_2$$^{+\bullet}$ to FlH$_2$$^{+\bullet}$ may be energetically unfavourable. Thus, the electron transfer from AcrH$_2$ to FlH$^+$ may be followed by proton transfer from AcrH$_2$$^{+\bullet}$ to FlH$^\bullet$ yielding the radical pair (AcrH$^\bullet$ FlH$_2$$^{+\bullet}$) which is removed by the subsequent, fast electron transfer from AcrH$^\bullet$ to FlH$_2$$^{+\bullet}$ without separation by diffusion to give the products, AcrH$^+$ and FlH$_2$. The FlH$_2$ is converted into the stable radical cation (FlH$_2$$^{+\bullet}$) by the facile comproportionation reaction [equation (13)]. In such a case, it may be difficult to distinguish clearly between the one-electron process (Scheme 2) and the direct one-step two-electron process (direct transfer of a hydride ion), although the former process can account for the magnitude of the observed rate constant better than the latter (see above). It should be emphasized that both one-electron and two-electron processes are rather extreme approaches to visualizing the actual transition state; the former from the energetics of formation of the high energy species (AcrH$^\bullet$ FlH$_2$$^{+\bullet}$) used as a model of the transition state and the latter from the end of the energy surface of the reaction based on the overall Gibbs energy change. In any case, determining the actual approach should be based on the need to provide a better description of the energetics, as we have recently shown in the hydride-transfer reactions from NADH model compounds to a series of *p*-benzoquinone derivatives.[15,33]

## Acknowledgements

This work was supported in part by a grant-in-aid (to S. F.) for scientific research from the Ministry of Education, Science and Culture, Japan.

## References

1 R. C. Bohinski, 'Modern Concepts in Biochemistry,' 3rd edn., Allyn & Bacon, Boston, 1979, p. 457; L. Stryer, 'Biochemistry,' 3rd edn., Freeman, New York, 1988, ch. 17.
2 T. P. Singer and E. B. Kearney, *J. Biol. Chem.*, 1950, **183**, 409.
3 C. H. Suelter and D. E. Metzler, *Biochim. Biophys. Acta*, 1960, **44**, 22; I. M. Gascoigne and G. K. Radda, *ibid.*, 1967, **131**, 502; G. K. Radda and M. Calvin, *Biochemistry*, 1964, **3**, 384; J. L. Fox and G. Tollin,

*ibid.*, 1966, **5**, 3873; E. Y. Wu, R. E. MacKenzie, and D. B. McCormick, *ibid.*, 1970, **9**, 2219.

4 G. A. Hamilton, *Prog. Bioorg. Chem.*, 1971, **1**, 83; V. Massey and P. Hemmerich, *Enzymes*, 3rd edn., 1976, **12**, 191; C. Walsh, *Acc. Chem. Res.*, 1980, **13**, 148.

5 T. C. Bruice, L. Main, S. Smith, and P. Y. Bruice, *J. Am. Chem. Soc.*, 1971, **93**, 7327; M. Brüstlein and T. C. Bruice, *ibid.*, 1972, **94**, 6548.

6 S. Shinkai, N. Honda, Y. Ishikawa, and O. Manabe, *Chem. Lett.*, 1984, 327; *J. Am. Chem. Soc.*, 1985, **107**, 6286; S. Shinkai, Y. Ishikawa, H. Shinkai, T. Tsuno, and O. Manabe, *Tetrahedron Lett.*, 1983, **24**, 1539; Y. Yano, M. Nakazato, and E. Ohya *J. Chem. Soc., Perkin Trans. 2*, 1985, 77; Y. Yano and E. Ohya, *ibid.*, 1984, 1227; S. Shinkai, A. Harada, Y. Ishikawa, and O. Manabe, *ibid.*, 1982, 125.

7 E. T. Kaiser, H. L. Levin, T. Otsuki, H. E. Fried, and R. M. Dupeyre, *Adv. Chem. Ser.*, 1980, **191**, 35; C. Radziejewski, D. P. Ballou, and E. T. Kaiser, *J. Am. Chem. Soc.*, 1985, **107**, 3352.

8 M. F. Powell and T. C. Bruice, *J. Am. Chem. Soc.*, 1983, **105**, 1014.

9 S. Shinkai, N. Honda, Y. Ishikawa, F. Müller, and O. Manabe, *Chem. Lett.*, 1985, 543.

10 S. Fukuzumi, S. Kuroda, and T. Tanaka, *Chem. Lett.*, 1984, 417; *ibid.*, 1984, 1375; *J. Am. Chem. Soc.*, 1985, **107**, 3020; *J. Chem. Soc., Perkin Trans. 2*, 1986, 25.

11 C. C. Johnston, J. L. Gardner, C. H. Suelter, and D. E. Metzler, *Biochemistry*, 1963, **2**, 689; C. S. Y. Kim and S. Chaykin, *ibid.*, 1968, **7**, 2339.

12 P. van Eikeren, D. L. Grier, and J. Eliason, *J. Am. Chem. Soc.*, 1979, **101**, 7406; E. B. Skibo and T. C. Bruice, *ibid.*, 1983, **105**, 3316.

13 S. Fukuzumi, M. Ishikawa, and T. Tanaka, *J. Chem. Soc., Chem. Commun.*, 1985, 1069; *Tetrahedron*, 1986, **42**, 1021.

14 F. Yoneda, K. Shinozuka, K. Tsukudo, and A. Koshiro, *J. Heterocyl. Chem.*, 1979, **16**, 1365; F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.*, 1976, **98**, 830.

15 S. Fukuzumi, S. Koumitsu, K. Hironaka, and T. Tanaka, *J. Am. Chem. Soc.*, 1987, **109**, 305.

16 D. Ostović, R. M. G. Roberts, and M. M. Kreevoy, *J. Am. Chem. Soc.*, 1983, **105**, 7629.

17 P. Karrer, L. Szabo, H. J. V. Krishna, and R. Schwyzer, *Helv. Chim. Acta*, 1950, **33**, 294.

18 G. Mestroni, A. Camus, and E. Mestroni, *J. Organomet. Chem.*, 1970, **24**, 775.

19 S. Fukuzumi, K. Ishikawa, and T. Tanaka, *J. Chem. Soc., Dalton Trans.*, 1985, 899.

20 D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, 'Purification of Laboratory Chemicals,' Pergamon Press, New York, 1966.

21 P. Hemmerich, C. Veeger, and H. C. S. Wood, *Angew. Chem.*, 1965, **77**, 699; K. H. Dudley, A. Ehrenberg, P. Hemmerich, and F. Müller, *Helv. Chim. Acta*, 1964, **47**, 1354; P. F. Heelis, *Chem. Soc. Rev.*, 1982, **11**, 15.

22 W. R. Weimar, A. H. Neim, 'Riboflavin,' ed. R. S. Rivlin, Plenum Press, New York, 1975, p. 7.

23 A. N. Singh, E. Gelerinter, and E. S. Gould, *Inorg. Chem.*, 1982, **21**, 1232; E. J. Land and A. J. Swallow, *Biochemistry*, 1969, **8**, 2117.

24 F. Müller, P. Hemmerich, and A. Ehrenberg, 'Flavins and Flavoproteins,' ed. H. Kamin, University Park Press, Baltimore, 1971, p. 107.

25 A. V. Guzzo and G. Tollin, *Arch. Biochem. Biophys.*, 1963, **103**, 231.

26 M. Faraggi, P. Hemmerich, and I. Pecht, *FEBS Lett.*, 1975, **51**, 47.

27 S. Fukuzumi, K. Ishikawa, K. Hironaka, and T. Tanaka, *J. Chem. Soc., Perkin Trans. 2*, 1987, 751.

28 D. T. Sawyer and R. L. McCreery, *Inorg. Chem.*, 1972, **11**, 779.

29 R. D. Draper and L. L. Ingraham, *Arch. Biochem. Biophys.*, 1968, **125**, 802; R. F. Anderson, *Biochim. Biophys. Acta*, 1983, **722**, 158.

30 M. M. Kreevoy, D. Ostović, I.-S. H. Lee, D. A. Binder, and G. W. King, *J. Am. Chem. Soc.*, 1988, **110**, 524.

31 M. G. Dowling and M. J. Clarke, *Inorg. Chim. Acta*, 1983, **78**, 153.

32 R. A. Marcus, *J. Phys. Chem.*, 1968, **72**, 891.

33 S. Fukuzumi, N. Nishizawa, and T. Tanaka, *J. Org. Chem.*, 1984, **49**, 3571; *J. Chem. Soc., Perkin Trans. 2*, 1985, 371.

*Received* 8*th November* 1988; *Paper* 8/03427C