Figure 3. Alkylation of N,S chelated cysteine via the Pummerer rearrangement.

sulfoxide/acetic anhydride mixture yielded a single isomer of a red complex(VI) in >90% yield. This product had essentially the same analysis as the reactant but exhibited widely different ^{1}H NMR and visible spectra from Ia or Ib and from the S-bound sulfenato and sulfinato complex products derived from other oxidants.[4] Charge transfer absorption obscured the second ligand field band, suggesting S bound to Co(III). However, no sensible structural assignment could be made with all these data and a crystal of the $ZnCl_4{}^{2-}$ salt was therefore examined by x-ray crystallography. Anal. Calcd for $CoC_7H_{20}N_5SO_2ZnCl_4$: C, 16.7; H, 4.0; N, 13.9; S, 6.4; Cl, 28.1. Found: C, 16.7; H, 4.2; N, 13.4; S, 6.4; Cl, 27.8.

Crystal data: $CoZnCl_4SO_2N_5C_7H_{20}$; orthorhombic with $a = 16.455$ (2), $b = 16.646$ (2), $c = 6.315$ (7) Å; space group $P2_12_12_1$, M = 504.4 amu; $d_m = 1.9$, $d_c = 1.94$ g cm^{-3} for $Z = 4$. For 1905 independent data with $F_o{}^2 > 3(F_o{}^2)$ the R index is 0.035. Coordination about Co(III) is octahedral (normal), and there are no significant distortions in the chelates. (N2–S1–C3, 99.0 (3)°; Co–N2–C2, 107.6 (4)°; S1–C3–C4, 114.4 (5)°).

The structure (Figure 2) points to two remarkable features for the mild conditions (20 °C, ~12 h) of the synthesis. A shift from the N,S to the N,O bonded chelate has occurred and the freed S atom has then condensed with one of the N atoms of one ethylenediamine chelate to generate an unusual heterocyclic six membered (chair) ring containing a sulfenamide linkage. Overall a new quadridentate has been formed from a *tris* chelate.

The ^{1}H NMR of VI (10^{-3} M DCl and D_2O) was complex. In addition to the characteristic CH_2 (τ 7.26, m, br, 6 H) and NH_2 (τ 4.7, 4.95, 5.59, s, br, 6 H) signals of en, resonances were observed at τ 4.04 (s, br, 2 H, NH_2 of amino acid), τ 6.88–7.20 (m, br, 2 H, CH_2 of en α to N-bound sulfenamide linkage), and, as a complex pattern of sharp lines, at τ 6.48–6.95 (m), 6.26 (d), 6.13 (d), 5.96 (s), and 5.92 (d, br) (S–CH$_2$ and –CH–, ABX pattern). The NH sulfenamide proton was exchanged instantly even in 1 M DCl but was clearly observed in Me_2SO-d_6 at τ 2.48 (s, br, 1 H). The proton decoupled ^{13}C NMR (D_2O) showed all seven C atoms as separate sharp singlets, confirming the presence of a single isomer (vide infra). The first ligand field band ($\epsilon^{486}{}_{max}$ 135; 10^{-2} M HClO$_4$) in the visible spectrum is consistent with a CoN_5O chromophore. The intense charge transfer band ($\epsilon^{345}{}_{max}$ 2590) which obscures the second ligand field band is ascribed to the chromophore of the sulfenamide, although bonded through the N atom rather than the S atom. The band is reduced in intensity and shifted to lower energies relative to the S bonded complexes I. Complex VI shows a pK_a of ~10 (NH of sulfenamide), and crystals of a deep wine red deprotonated form have been isolated.

The extraordinary rearrangement (Figure 1) has a rational interpretation if the properties of the Me_2SO/acetic anhydride

mixture are exercised. Acetylation of the $(CH_3)_2SO$ oxygen[5] yields the sulfoxonium ion II and allows a nucleophilic displacement by the coordinated mercaptide ion of the cysteinato complex Ia to generate a disulfide linkage III and acetate ion. This immediately renders the coordinated cysteine S$^-$ positive and thence a good leaving group. Capture of the free carboxyl group gives the N,O bound ion IV. The alkylated disulfide residue is now susceptible to nucleophilic attack and removal of a proton from an ethylenediamine nitrogen by acetate provides this opportunity. Finally the coordinated amide ion of V cleaves the disulfide link to generate the complex product VI and $(CH_3)_2S$.

In larger scale preparations of the sulfenamide VI a small quantity (~2%) of an orange 3+ product (VIII) was isolated and characterized (anal., ^{1}H NMR, visible, and ORD spectra) as $\Delta Co(en)_2$-(R)-$(NH_2CH(CH_2SCH_2SCH_3)COOH)^{3+}$ (Figure 3). This arises from the Pummerer rearrangement of the sulfoxonium ion II to the thioether VII[5] followed by nucleophilic attack of the bound mercaptide ion of I. Similar rearrangements have been observed with the Me_2SO/$(CF_3CO)_2O$ reagent.[5,6]

The remarkable stereospecificity of the sulfenamide reaction prompts comment. The chirality of the amino acid directs the condensation to one N atom (Figure 1). However two other isomeric products are possible, one inverted about the cobalt center and the other inverted about the chiral sulfenamide N center (Figure 2). We have shown that Λ-N,S-Co(en)$_2$-(R)-cysteinato^{2+} ion undergoes the same type of oxidation to give the same Δ sulfenamide isomer described here. These stereochemical aspects and the equilibration and structures of both the Λ and Δ sulfenamide isomers will be described in a subsequent publication.

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Disproportionation at the Ligand in Nitro Complexes of Ruthenium(III)

Sir:

Complexes of Ru(II) and Ru(III) are usually substitution inert and stable, and Ru(III)/Ru(II) couples have been used extensively in the study of one-electron transfer processes.[1] This is certainly true for *cis*-bis-2,2′-bipyridine (bpy) complexes where more than 100 reversible Ru(III)/Ru(II) couples are known.[2] However, oxidation to Ru(III) can lead to chemical instability because of oxidation of a bound ligand (reactions 1, 2).
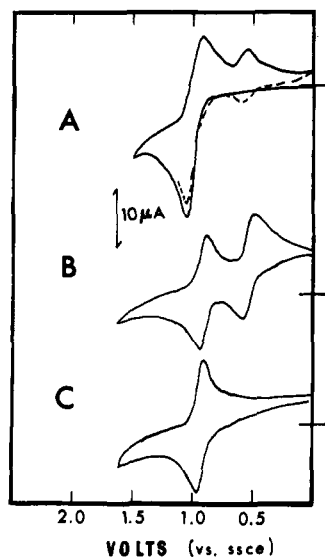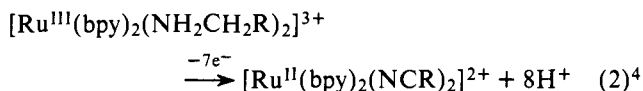
$[Ru^{III}(bpy)_2(N_3)_2]^+ + CH_3CN$

$\rightarrow [Ru^{II}(bpy)_2(CH_3CN)N_3]^+ \tfrac{3}{2}N_2 \quad (1)$[3]
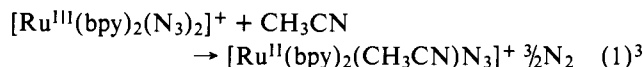
Figure 1. Cyclic voltammograms (200 mV/s) in 0.1 M [N(C$_4$H$_9$)] PF$_6$-CH$_3$CN vs. the SSCE at 22 ± 2 °C: A, [Ru(bpy)$_2$(NO$_2$)py]PF$_6$ (1.5 × 10^{-3} M); B, solution A after exhaustive electrolysis at +1.2 V (n = 1.5/Ru); C, solution A with added H$_2$O (1%) and 2,6-lutidine (1%) after exhaustive electrolysis at 1.2 V (n = 3.0/Ru).
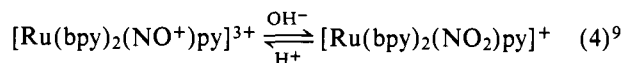

Figure 2. The 200-mV/s cyclic voltammograms in 0.2 M [N(n-C$_4$-H$_9$)$_4$]PF$_6$-propylenecarbonate following exhaustive electrolysis of [Ru(bpy)$_2$(NO$_2$)Cl] at 0.8 V: A, 1 min after completion of the electrolysis; B, 2 min; C, 4 min; D, 6 min; E, 8 min; F, 10 min. As shown in 1A, some disproportionation has occurred during the electrolysis. Rate data were obtained by monitoring the growth in peak current for the reversible reduction of [Ru(bpy)$_2$(NO)Cl]$^{2+}$ at $E_{p,c}$ = 0.20 V.

$$[Ru^{III}(bpy)_2(NH_2CH_2R)_2]^{3+}$$

$$\xrightarrow{-7e^-} [Ru^{II}(bpy)_2(NCR)_2]^{2+} + 8H^+ \quad (2)^4$$

Attempts to prepare nitroruthenium(III) complexes by electrochemical oxidation of [Ru^{II}(bpy)$_2$(NO$_2$)L]$^{n+}$ have been unsuccessful;[5] in fact, there appear to be no known examples of stable, monomeric Ru(III)-NO$_2$ complexes.[6] We find that complexes like [Ru^{III}(bpy)$_2$(NO$_2$)Cl]$^+$ do exist but as kinetic transients. Their stability is limited by an unusual reaction in which intermolecular disproportionation occurs at the nitro group. The reactions are also unusual in that they utilize Ru(III)-NO$_2$ intermediates as oxide ion donor, two-electron acceptor oxidants in which electron acceptor sites at both metal and ligand are used.

A cyclic voltammogram of [Ru(bpy)$_2$(NO$_2$)py]$^+$ in acetonitrile (0.1 M in [N(C$_4$H$_9$)$_4$](PF$_6$)) is given in Figure 1A.[7] The oxidative sweep shows the expected Ru(II) → Ru(III) oxidation ($E_{p,a}$ = 1.06 V vs. SSCE at 22 ± 2 °C). The reductive sweep shows two new waves ($E_{p,c}$ = 0.90 and 0.47 V), and a further cycle shows that the two new waves are associated with reversible couples. Exhaustive electrolysis of the solution past 1.06 V gives n = 1.5 (by coulometry), and cyclic voltammetry shows that two products are formed in equal amounts both of which have reversible redox chemistry (Figure 1B). The less anodic wave ($E_{1/2}$ = 0.53 V) corresponds to the nitrosyl complex, [Ru(bpy)$_2$(NO)py]$^{3+}$.[8] [Ru^{II}(bpy)$_2$(NO)py]$^{3+}$ and related nitrosyls are known to undergo reversible reductions localized largely at the NO group (reaction 3) in the potential range 0.2–0.6 V (at 22 ± 2 °C vs. SSCE).[8] It is also known

$$[Ru^{II}(bpy)_2(NO^+)py]^{3+} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} [Ru(bpy)_2(\dot{N}O)py]^{2+} \quad (3)^8$$
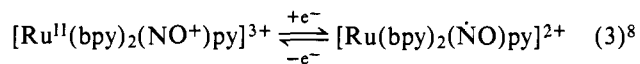
that nitrosyl and nitro groups are interconverted by simple acid–base chemistry (reaction 4).

$$[Ru(bpy)_2(NO^+)py]^{3+} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} [Ru(bpy)_2(NO_2)py]^+ \quad (4)^9$$

The second electrolysis product is apparently [Ru^{III}-(bpy)$_2$(NO$_3$)py]$^{2+}$. Electrolytic redution at a potential between the waves which appear following electrolysis (Figure 1B) gave n = 0.5 per total Ru by coulometry. Electrochemical
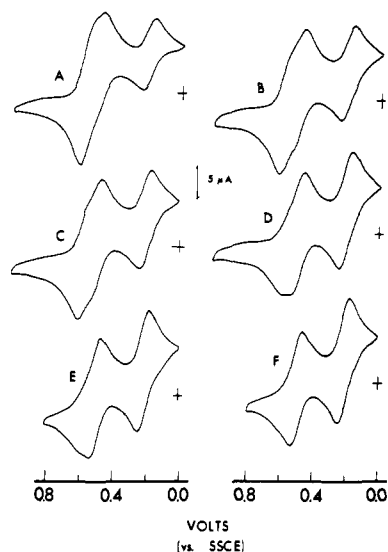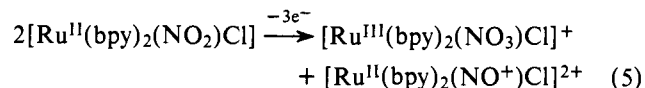
reduction gives Ru(II), presumably as [Ru^{II}(bpy)$_2$(NO$_3$)py]$^+$, which then undergoes solvolysis ($t_{1/2} \sim$ 10 min) to give [Ru(bpy)$_2$(CH$_3$CN)py]$^{2+}$ ($E_{1/2}$ = 1.36 V).[2] Evaporation of the reduced solution showed the presence of free nitrate ion by IR (ν 1050 cm^{-1}).[10] We have been unable to obtain a sample of [Ru(bpy)$_2$(NO$_3$)py]$^{2+}$ free of supporting electrolyte. However, electrochemical oxidation of [Ru^{II}(bpy)$_2$(NO$_2$)Cl] at 0.8 V also occurs with n = 1.5 and the products are clearly [Ru(bpy)$_2$(NO)Cl]$^{2+}$ [8] and [Ru(bpy)$_2$(NO$_3$)Cl]$^+$. The nitrato complex was identified by spectral and electrochemical comparisons with a known sample prepared by chemical oxidation (see below). The chloro complex differs from the pyridine complex only in that the [Ru(bpy)$_2$(NO$_2$)Cl]$^{+/0}$ couple is reversible on the cyclic voltammetry time scale, $E_{1/2}$ = 0.58 V.
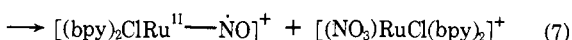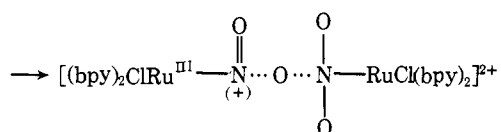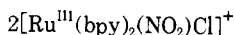
As shown in reaction 5 for the chloro complex, electrochemical oxidation leads to oxidation of nitro to nitrato and of Ru(II) to Ru(III), but both in a single complex.

$$2[Ru^{II}(bpy)_2(NO_2)Cl] \xrightarrow{-3e^-} [Ru^{III}(bpy)_2(NO_3)Cl]^+$$
$$+ [Ru^{II}(bpy)_2(NO^+)Cl]^{2+} \quad (5)$$

In the mechanism, [Ru^{II}(bpy)$_2$(NO$_2$)Cl] is first oxidized to [Ru^{III}(bpy)$_2$(NO$_2$)Cl]$^+$ (reaction 6),

$$[Ru^{II}(bpy)_2(NO_2)Cl] \xrightarrow{-e^-} [Ru^{III}(bpy)_2(NO_2)Cl]^+ \quad (6)$$
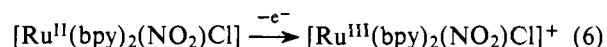
and the subsequent chemistry is sufficiently slow so that it can be followed directly by cyclic voltammetry (Figure 2). The reaction (eq 7) is second order in [Ru(III)-NO$_2$] and in propylene carbonate as solvent (I = 0.2 M, [N(n-C$_4$H$_9$)$_4$](PF$_6$)), $k(25.0$ °C) = 3.0 ± 0.3 M^{-1} s^{-1}.

$$2[Ru^{III}(bpy)_2(NO_2)Cl]^+$$

$$\longrightarrow [(bpy)_2ClRu^{III}-\overset{\overset{\displaystyle O}{\|}}{\underset{(+)}{N}}\cdots O \cdots \overset{\overset{\displaystyle O}{|}}{\underset{\displaystyle O}{N}}-RuCl(bpy)_2]^{2+}$$

$$\longrightarrow [(bpy)_2ClRu^{II}-\dot{N}O]^+ + [(NO_3)RuCl(bpy)_2]^+ \quad (7)$$

A final n value of 3 is obtained in the electrochemical experiment (eq 5) because the reduced nitrosyl complex formed in reaction 7 is oxidized to $[Ru(bpy)_2(NO^+)Cl]^{2+}$ (eq 3) at the potential used for the electrolysis (reaction 8).[11]

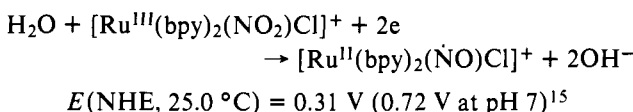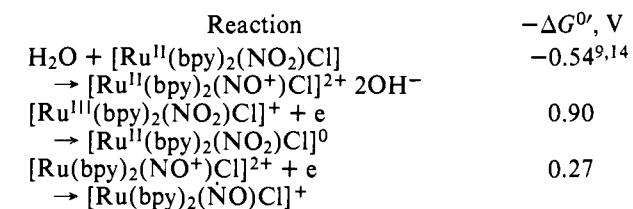$$[Ru^{II}(bpy)_2(NO)Cl]^+ \xrightarrow{-e^-} [Ru^{II}(bpy)_2(NO^+)Cl]^{2+} \quad (8)$$
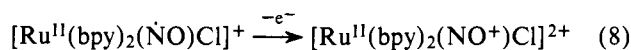
Kinetic studies using Ce(IV) as oxidant in acetonitrile show that the rate constant for disproportionation of $[Ru^{III}(bpy)_2(NO_2)py]^{2+}$ must exceed 10^7 M^{-1} s^{-1}. The reaction with Ce(IV) is first order in both Ce(IV) and $[Ru(bpy)_2(NO_2)py]^+$ ($k(25.0\ ^\circ C; I = 0.1\ M) = 1.7 \times 10^6$ M^{-1} s^{-1}), and the disproportionation products are observed immediately. Disproportionation at the nitro group is also observed following oxidation of the complexes $[Ru(bpy)_2(NO_2)NH_3]^+$ and $[Ru(bpy)_2(NO_2)CH_3CN]^+$. There appears to be at least a qualitative correlation between the potential for oxidation of Ru(II) to Ru(III) and the rate of disproportionation at the nitro group in the four complexes $[Ru^{III}(bpy)_2(NO_2)L]^{n+}$ (L = Cl⁻, NH₃, py, CH₃CN).

The key to the instability of the nitroruthenium(III) complexes is the disproportionation step (reaction 7). The reaction is unusual in that a net two-electron oxidation occurs at the nitro group of one ruthenium ($NO_2^- \rightarrow NO_3^-$) but the complementary reduction uses sites both at the metal (Ru(III) $\rightarrow$ Ru(II)) and at a ligand (Ru–NO⁺ $\rightarrow$ Ru–NO).

One inference that can be drawn from the mechanism is that complexes like $[Ru^{III}(bpy)_2(NO_2)Cl]^+$ can act as oxide-ion donor, two-electron acceptor oxidants. They could be facile oxidants since electron transfers to Ru(III) and Ru–NO⁺ acceptor sites are known to be rapid,[8,12] and, other than oxide ion transfer, there are no major structural changes involved.[13]

Formal reduction potentials for the Ru(III)–NO₂ complexes as oxide ion donor, two-electron acceptor oxidants can be calculated by a combination of electrochemical and equilibrium measurements (Scheme I). A similar scheme using $E_{p,a}$[17] for the oxidation of $[Ru^{II}(bpy)_2(NO_2)py]^+$ gives $E \geq 0.26$ V (≥ 0.67 V at pH 7)[15] for the $[Ru^{III}(bpy)_2(NO_2)py]^{2+}/[Ru^{II}(bpy)_2(NO)py]^{2+}$ couple. The reduction potential values show that the Ru(III)–NO₂ intermediates are only moderately strong as oxidants.[18]
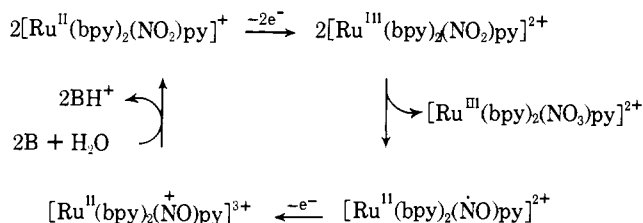
Scheme I (in 1.0 M aqueous NaCl at 25.0 °C)

Reaction	$-\Delta G^{0\prime}$, V
$H_2O + [Ru^{II}(bpy)_2(NO_2)Cl]$ $\rightarrow [Ru^{II}(bpy)_2(NO^+)Cl]^{2+} + 2OH^-$	-0.54[9,14]
$[Ru^{III}(bpy)_2(NO_2)Cl]^+ + e$ $\rightarrow [Ru^{II}(bpy)_2(NO_2)Cl]^0$	0.90
$[Ru(bpy)_2(NO^+)Cl]^{2+} + e$ $\rightarrow [Ru(bpy)_2(NO)Cl]^+$	0.27

$$H_2O + [Ru^{III}(bpy)_2(NO_2)Cl]^+ + 2e$$
$$\rightarrow [Ru^{II}(bpy)_2(NO)Cl]^+ + 2OH^-$$

$$E(NHE, 25.0\ ^\circ C) = 0.31\ V\ (0.72\ V\ at\ pH\ 7)^{15}$$

The series of reactions 6–8 describes a single oxidation cycle (reaction 5) in which one Ru(II)–NO₂ complex is oxidized and the other converted into a nitrosyl. It is possible to continue through additional cycles simply by adjusting the pH. The nitrosyl–nitro interconversion (reaction 4) can be studied quantitatively by pH and spectrophotometric measurements. For reaction 9, $K(1.0\ M\ NaCl, 25.0 \pm 0.1\ ^\circ C) = (1.0 \pm 0.3) \times 10^{20}$ M^{-2} and for the $[Ru(bpy)_2(NO_2)Cl]$–$[Ru(bpy)_2(NO)Cl]^{2+}$ equilibrium, $K(1.0\ M\ NaCl, 25.0 \pm 0.1\ ^\circ C) = 1.4 \times 10^9$.[9]

$$2OH^- + [Ru(bpy)_2(NO^+)py]^{3+}$$
$$\rightleftharpoons [Ru(bpy)_2(NO_2)py]^+ + H_2O \quad (9)$$
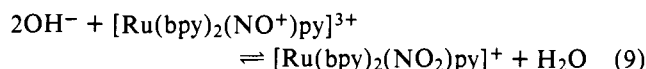
For the pyridine system, equal concentrations of nitro and nitrosyl complexes are present at pH 3.8. In an acetonitrile solution containing 1% water and 1% 2,6-lutidine, electrochemical oxidation of $[Ru(bpy)_2(NO_2)py]^+$ occurs ($n = 3.0$), $[Ru(bpy)_2(NO_3)py]^{2+}$ is the sole product, and the oxidation reaction is driven to completion (Figure 1C). Under these conditions, complete oxidation of Ru^{II}NO₂ to Ru^{III}NO₃ occurs because Ru^{II}NO⁺ once formed (eq 5) is converted into Ru^{II}NO₂ which reenters the oxidation cycle as shown below.

$$2[Ru^{II}(bpy)_2(NO_2)py]^+ \xrightarrow{-2e^-} 2[Ru^{III}(bpy)_2(NO_2)py]^{2+}$$

$$2BH^+ \quad \quad \quad \quad \rightarrow [Ru^{III}(bpy)_2(NO_3)py]^{2+}$$
$$2B + H_2O$$

$$[Ru^{II}(bpy)_2(NO)py]^{3+} \xleftarrow{-e^-} [Ru^{II}(bpy)_2(NO)py]^{2+}$$

The oxidation cycle can also be driven chemically. Using essentially the same reaction conditions but with chlorine as the oxidant and triethylamine as the base led to complete oxidation of $[Ru(bpy)_2(NO_2)Cl]$ to $[Ru(bpy)_2(NO_3)Cl]^+$. The chemical oxidation has allowed the nitrato complex to be isolated as its chloride salt which has been characterized by elemental analyses.[19]

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(5) G. M. Brown, Ph.D. Dissertation, The University of North Carolina. 1974; G. M. Brown and T. R. Weaver, unpublished results.

(6) W. P. Griffith, "The Chemistry of the Rarer Platinum Compounds", Interscience, New York, N.Y., 1967, p 159.

(7) Unless otherwise noted electrochemical results were obtained in 0.1 M [N(n-C₄H₉)₄](PF₆)–acetonitrile at 22 ± 2 °C using platinum electrodes. Platinum bead electrodes were used for cyclic voltammetry experiments and platinum gauze electrodes for electrolyses. $E_{1/2}$ values for chemically reversible couples were calculated as the mean of the E_p values for the anodic and cathodic waves in cyclic voltammetry experiments. Peak separations (ΔE_p) of ~70 mV were observed which were independent of sweep rate in the range 50–1000 mV s⁻¹. The reference electrode was the saturated sodium chloride calomel electrode (SSCE).

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(9) J. B. Godwin and T. J. Meyer, *Inorg. Chem.*, **10**, 2150 (1971).

(10) K. Nakamoto, "Infrared Spectra of Inorganic and Coordination Compounds", Wiley, New York, N.Y., 1963.

(11) Actually, given the relative potentials for the $[Ru(bpy)_2(NO)Cl]^{2+/+}$ and $[Ru(bpy)_2(NO_3)Cl]^{+/0}$ and related pyridine couples (Figures 1 and 2) and the expected rapid self-exchange rates,[6,12] reaction 7 would be followed by rapid electron transfer between $[Ru(bpy)_2(NO)Cl]^+$ and $[Ru^{III}(bpy)_2(NO_3)Cl]^+$ to give $[Ru(bpy)_2(NO^+)Cl]^{2+}$ and $[Ru^{II}(bpy)_2(NO_3)Cl]$. At the potentials used for the electrolyses, $[Ru^{II}(bpy)_2(NO_3)Cl]$ would be reoxidized to Ru(III).

(12) (a) R. W. Callahan, F. R. Keene, T. J. Meyer, and D. J. Salmon, *J. Am. Chem. Soc.*, in press; (b) R. C. Young, F. R. Keene, and T. J. Meyer, *ibid.*, in press.

(13) That they are facile oxidants is suggested by the disproportionation reactions since there is no reason to believe that a nitro group bound to Ru(III) should be especially easy to oxidize.

(14) Calculated from $-\Delta G^{0\prime} = RT$ ln K where K refers to the nitrosyl–nitro acid–base equilibrium constant (reaction 9).

(15) Electrochemical measurements were made vs. the SCE. Values vs. NHE were calculated using a value of 0.236 V for SCE vs. NHE. The values were not corrected for junction effects which are presumably small.[16]

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(17) $E_{p,a}$, the anodic peak potential for the cyclic voltammetric wave, is independent of sweep rate over the range of scan rates 50–500 mV s^{-1}.
(18) W. M. Latimer, "Oxidation Potentials", Prentice-Hall, Englewood Cliffs, N.J., 1952.
(19) Calcd for [Ru(bpy)$_2$(NO$_3$)Cl]Cl: C, 43.97; H, 2.95; N, 12.82. Found: C, 43.24; H, 3.36; N, 12.64.

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One and Two Electron Transfer Reactions of Glucose Oxidase

Sir:

The mechanisms by which the flavin adenine dinucleotide cofactor (FAD) of glucose oxidase is reduced by glucose and reoxidized by molecular oxygen have received considerable attention.[1-4] Two electron transfer reactions have been considered to be general for the mechanisms of flavoprotein oxidases, dehydrogenases, etc.,[5] because radical intermediates have not been detected.[6] However, we have recently proposed that the interconversion HC(R$_2$)OH $\rightleftarrows$ R$_2$C=O accompanied by flavin reduction (Fl$_{ox}$ $\rightleftarrows$ FlH$_2$) may well be radical in nature.[7] We report, herein, preliminary results of a study of: (i) the reduction of glucose oxidase (E-FAD) using α-hydroxy-carbonyl compounds (I-IV) as glucose analogues and (ii) the oxidation of reduced glucose oxidase (E-FADH$_2$) employing the nitroxide V as a "model" of ^{3}O$_2$ (I, dihydroxyacetone; II, glyceraldehyde; III, phenacyl alcohol; IV, furoin; V, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl). The glucose oxidase (A-niger) was obtained from Worthington Biochemical Corp. (GOP).

The α-hydroxycarbonyl compounds I-III (0.05 M) were found to reduce E-FAD (2 $\times$ 10^{-5} M) directly to E-FADH$_2$ (pH 7.3, H$_2$O, 30 °C, μ = 1.0); no semiquinone (E-FAD·) intermediate could be detected on repetitive scanning (320–600 nm) during the course of reaction. These substrates (I-III) do not reduce 3-methyllumiflavin or FAD at pH 7.0 in aqueous solution so that the enzyme is performing the role of catalyst. Compounds I-III readily reduced the electron defficient 7- and 8-cyano-3,10-dimethylisoalloxazines. In contrast, with furoin (IV) as substrate E-FAD, 7- and 8-cyano-3,10-dimethyli-soalloxazine, as well as 3-methyllumiflavin were reduced (pH 7.0). The reaction with the enzyme but not with isoalloxazine was biphasic producing the semiquinone (E-FAD·, λ_{max} 400 nm)[8] as an intermediate. The rate of formation of E-FAD· far exceeded its rate of conversion to E-FADH$_2$. Since furoin and furil comproportionate (eq 1) at high pH it was crucial to de-

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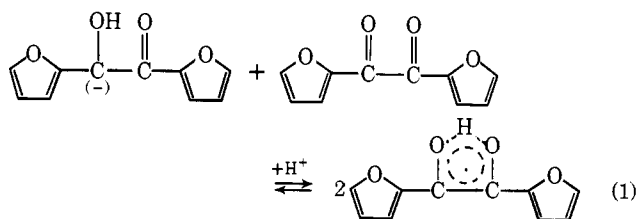
termine if the observed one electron reduction of E-FAD was merely due to trace concentrations of furil (and, therefore, of semidione radical). The *initial rates* for conversion of oxidized glucose oxidase (2 $\times$ 10^{-5} M) to its radical form in the presence of a constant concentration of furoin (3.9 $\times$ 10^{-4} M) and as a function of added furil (0 to 3.9 $\times$ 10^{-4} M) were determined (pH 7.26). Substituting the equilibrium constant of
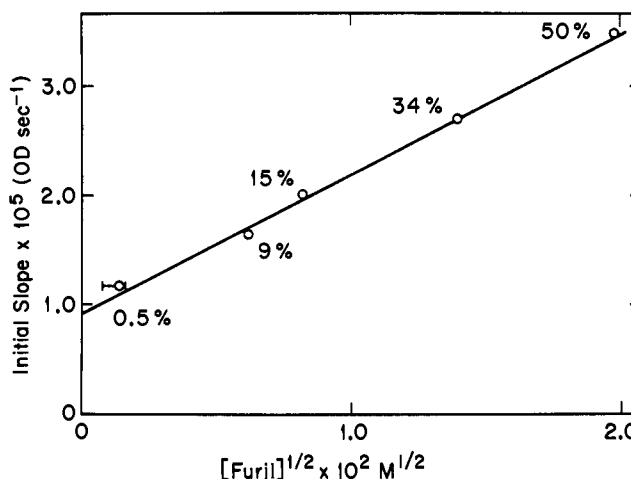

Figure 1. Plot of the initial slope vs. [furil]$^{1/2}$ for the reduction of glucose oxidase ($\sim$2 $\times$ 10^{-5} M based on FAD) with furoin (3.9 $\times$ 10^{-4} M) (pH 7.26, 0.063 M phosphate, μ = 1 with KCl, 6% CH$_3$CN, 30 °C) in the presence of added furil. The percent furil present is indicated in the figure. The horizontal bar is an estimate of the amount of furil present as an impurity.

semidione formation (K_e = [semidione]^2/[furil][furoin]) into the appropriate rate expression for one electron reduction of E-FAD by both furoin and semidione (i.e., k_{obsd} = k_1[semidione] + k_2[furoin]) provides eq 2.

$$k_{obsd} = k_1 K_e^{1/2}[\text{furil}]^{1/2}[\text{furoin}]^{1/2} + k_2[\text{furoin}] \quad (2)$$

From eq 2, at constant [furoin]:

$$k_{obsd} = k_1 K_e'[\text{furil}]^{1/2} + c \quad (3)$$

A plot of initial rate of E-FAD· formation vs. [furil]$^{1/2}$ was found to be linear with a markedly positive intercept (Figure 1). The point on the plot of initial rate vs. [furil]$^{1/2}$ corresponding to the lowest furil concentration represents the maximum concentration of furil impurity present (HPLC analysis) in the furoin sample used. The intercept of the plot of Figure 1 pertains to the rate for one electron transfer (k_2) from furoin to E-FAD. These results support a one electron transfer reaction from both furoin and semidione radical to glucose oxidase (eq 4 and 5). In model reactions of furoin (and

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$$\text{(5)}$$
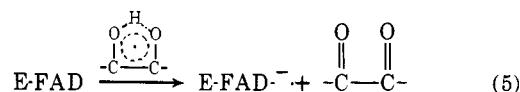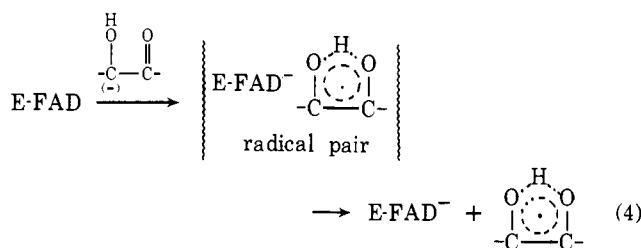
benzoin) with oxidized flavins the respective carbanions (enediolate ions) have been shown to be the reactive substrates.[9] The reactions of eq 4 and 5 are proposed. The distinction between furoin (IV) which undergoes a one electron transfer to glucose oxidase, and I, II, and III where E-FAD· intermediate cannot be detected is postulated to reside in the lessened standard free energy of formation ($\Delta G°$) of the semidione radical derived from IV as compared to the $\Delta G°$'s for the radicals formed from I, II, and III. The greater the $\Delta G°$ for -Ċ(OH)CO- formation the closer the free energy content of the radical pair (eq 4) to the $\Delta G^{\ddagger}$ for conversion of E-FAD· to E-FADH$_2$ (i.e., the lower the free energy barrier for the