platinum(III) complex $[Pt_2(\mu\text{-}P_2O_5H_2)_4X_2]^{4-}$. This complex is soluble in aqueous solution, but in contrast to $[Pt_2(PO_4H)_4Cl_2]^{4-}$, there is no observable halide (X = Cl, Br, I) aquation even after several days. Anation of $[Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2]^{4-}$ occurs by a combination of $[Pt_2(\mu\text{-}P_2O_5H_2)_4]^{4-}$ catalyzed and REOA pathways, as well as by a slow dissociative pathway (eq 12).[19] We

$$Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2^{4-} \xrightarrow{k(H_2O)} Pt_2(\mu\text{-}P_2O_5H_2)_4Cl(H_2O)^{3-} + Cl^- \quad (12)$$

have made a full kinetic analysis of this system, and the slow dissociative step is the aquation reaction.[19] This aquation reaction has a first-order rate constant of 9 (2) $\times 10^{-6}$ s^{-1}. This rate is slightly faster than that found for $[PtCl_6]^{2-}$ but *considerably* slower than that found for the phosphato complex $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$. In these μ-pyrophosphito-*P,P* complexes the Pt–Pt bond distance is considerably longer than that found in $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$.[3] For example the Pt–Cl distance in $[Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2]$ is 2.407 (2) Å, which is intermediate between that of $[PtCl_6]^2$ (2.323 (1) Å) and $[Pt_2(\mu\text{-}PO_4H)_2(\mu\text{-}PO_4H_2)_2Cl_2]^{4-}$ (2.448 (4) Å). In earlier papers we and others[3,2] have proposed that the differences in Pt(III)–Pt(III) distances in $[Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2]^{4-}$ and $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$ are primarily due to differences in preferred bridge angles in the two ligands and do not necessarily reflect changes in intermetallic bonding. We believe that this conclusion remains valid, but that a 4 orders of magnitude increase in the rate constants for aquation in $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$ over $[Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2]^{4-}$ reflects a major difference in reactivity, which may be due to changes in equatorial ligands or intermetallic bonding.

(19) Bryan, S. A. Ph.D. Thesis, Washington State University, 1985.

Estimation of the strength of a metal–metal bond in a bridged bimetallic complex is not achieved simply. Variations in intermetallic separation may give a useful first approximation, but it is certainly not a definitive method.[20] Mulliken has shown that the oscillator strength in an electronic transition is approximately proportional to the square of the overlap integral.[21] We find that the oscillator strength of the 296-nm band in $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$ is 0.46, which is actually smaller than that of 0.70 found for the 282-nm band in $[Pt_2(\mu\text{-}P_2O_5H_2)_4Cl_2]^{4-}$.[22] If these 296- and 282-nm absorptions are accurately assigned to a pure $d\sigma \rightarrow d\sigma^*$ transition between platinums, these oscillator strength values would suggest that $[Pt_2(\mu\text{-}PO_4H)_4Cl_2]^{4-}$ has the weaker intermetallic bond. This spectral assignment, however, remains speculative, and until a better assessment of the Pt–Pt bonding in these complexes becomes available we cannot draw definitive conclusions about its effect on kinetic reactivity.

Obviously much more work needs to be done before we can make strong correlations between chemical reactivity and intermetallic bonding, nevertheless these results show that large variations in kinetic reactivity can be induced by changes in equatorial ligands or by differences in intermetallic bonding.

Acknowledgment. We thank the American Cancer Society for support (Grant No. IN133E). We thank the Libyan Government for financial support of R.E.M. We thank L. Byers for helpful discussions.

(20) Reference 1, p 339–341. Meyer, T. J. *Prog. Inorg. Chem.* **1975**, *19*, 1–50. Chisholm, M. H.; Rothwell, I. P. *Prog. Inorg. Chem.* **1982**, *29*, 1–72.
(21) Mulliken, R. A. *J. Chem. Phys.* **1939**, *7*, 20–34.
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Coenzyme Models. 41. On the Unusual Reactivities of *N*(5)-Hydrogen-Bonded Flavin. An Approach to Regiospecific Flavin Activation through Hydrogen Bonding[1]

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Abstract: A new flavin with hydrogen-bonded N(5) (OHFl: sodium 1-hydroxy-7-methylnaphtho[8,7-*g*]pteridine-9,11-(7*H*,10*H*)-dione-3-sulfonate) was synthesized. The second-order rate constants (k_2) for the oxidation of NADH model compounds by OHFl ($E_{1/2}$ = −0.579 V) were similar to those for the oxidation by 3-methyllumiflavin (MeLFl, $E_{1/2}$ = −0.538 V), and the plot for OHFl was included in a linear relation between log k_2 vs. $E_{1/2}$ ($E_{1/2}$: polarographic half-wave potential of flavins). Hence, OHFl acts as a "normal" flavin in this reaction. On the other hand, the pseudo-first-order rate constants (k_1') for the oxidation of thiols by OHFl were greater by 33–645-fold than those for the oxidation by MeLFl, and log k_1' for OHFl deviated to the upper area from the linear log k_1' vs. $E_{1/2}$ relationship by more than two log units. The pseudo-first-order rate constant ($k_{obsd(1)}$) for the adduct formation of OHFl with SO_3^{2-} was further enhanced (1830-fold relative to that of MeLFl!). The upper deviation from the linear log $k_{obsd(1)}$ vs. $E_{1/2}$ relationship corresponded to 3.61 log units. The OHFl–SO_3^{2-} adduct (λ_{max} 458 nm) further reacted with SO_3^{2-} and finally yielded 8-sulfonated 1,5-dihydro-OHFl. It was suggested on the basis of several experimental data that the intermediate absorbing at 458 nm is the 4a adduct. It was concluded, therefore, that OHFl is "regiospecifically" activated toward reactions involving 4a intermediates such as oxidation of thiols and adduct formation with SO_3^{2-}. This novel finding was ascribed to activation of the 4a position through hydrogen bonding with N(5). Thus, the present study is the first example to support a hypothesis proposed by Massey and Hemmerich that the relative reactivity of C(4a) to N(5) in flavin coenzymes is regulated by the position of hydrogen bonding with flavoapoproteins.

Flavin coenzymes serve as versatile redox catalysts in many biological systems, and it is now known that more than 100 proteins require flavin coenzymes as their prosthetic groups.[2-5] Recently, Massey and Hemmerich[6] suggested that a large number

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(2) Hemmerich, P.; Nagelschneider, G.; Veeger, C. *FEBS Lett.* **1970**, *8*, 69.

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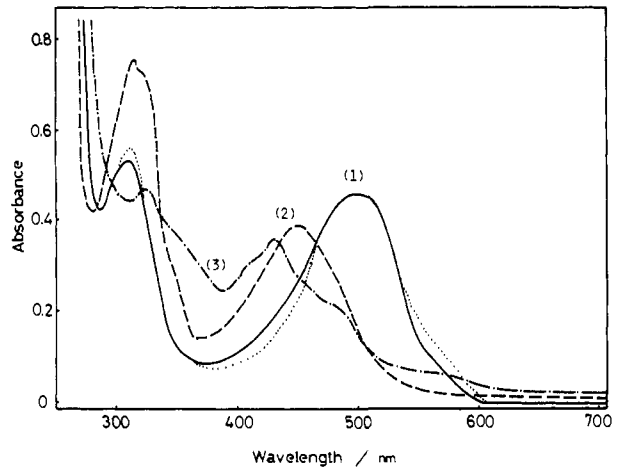
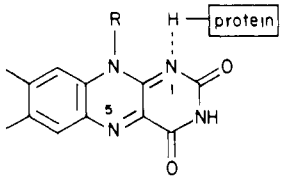


Figure 1. Absorption spectra of (1) OHFl (—), (2) SO_3^{2-} adduct (---), and (3) 1,5-dihydro-**2** (-·-): 30 °C, pH 9.50, [flavin] = 3.00×10^{-5} M, [K_2SO_3(when added)] = 0.300 M. The dotted line partially overlapping with spectrum 1 is the spectrum of **2**.

of flavoproteins may be classified mainly into two classes from a viewpoint of their regiospecific reactivities: the first group (dehydrogenases/oxidases) is characterized by red semiquinone radical, high sulfite affinity, and bent structure of reduced form, whereas the second group (electron-transferases or dehydrogenases/electron-transferases) is characterized by blue semiquinone radical, low sulfite affinity, and almost planar structure of reduced form. They proposed that the essential difference between these two groups stems from regiospecific hydrogen bonding between flavin coenzymes and apoproteins: that is, the first group has a hydrogen bond with N(1) (I) leading to activation of the N(5) position while the second group has a hydrogen bond with N(5) (II) leading to activation of the C(4a) position. This proposal reminds us of the role of the 3-hydroxyl group in pyridoxal coenzymes activating the neighboring aldehyde and imino groups through hydrogen bonding.[7]



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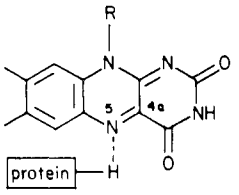
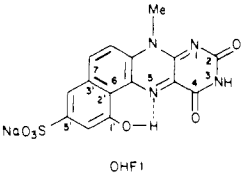
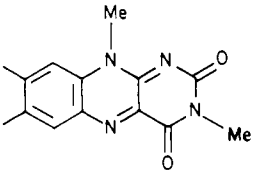


II

In order to obtain an insight into the latent capability of the hydrogen bonding to control the reactivities of flavin coenzymes, we synthesized a new flavin with hydrogen-bonded N(5) (OHFl: sodium 1-hydroxy-7-methylnaphtho[8,7-*g*]pteridine-9,11-(7*H*,10*H*)-dione-3-sulfonate).[8] OHFl contains within a molecule both an isoalloxazine skeleton and a proton sponge-like (1,8-bis(dimethylamino)naphthalene) structure. The 3-sulfonate group was introduced to make this compound water soluble. We have found that the reactivities of OHFl are remarkably different from those of 3-methyllumiflavin (MeLFl) used as a reference flavin and that the abnormal properties are rationalized in terms of C(4a) activation by intramolecular hydrogen bonding with N(5).



OHFl



MeLFl

Table I. Rate Constants for the Reactions with OHFl and MeLFl (30 °C)

reactant	pH		rate constant: OHFl	rate constant: MeLFl	k_{OHFl}/k_{MeLFl}
BNAH	8.81	k_2 (M^{-1} s^{-1})	11.1	12.1	0.917
BCQH	5.69	k_2 (M^{-1} s^{-1})	0.402	0.311	1.29
$HS(CH_2)_4SH$[a]	9.80	k_1' (s^{-1})	7.10×10^{-3}	2.13×10^{-4}	33.5
$HO(CH_2)_2SH$[b]	9.20	k_1' (s^{-1})	4.43×10^{-3}	6.87×10^{-6}	645
K_2SO_3[c]	7.21	k_1' (s^{-1})	3.05×10^{-2}	1.67×10^{-5}	1830

[a] [1,4-Butanedithiol] = 2.40×10^{-3} M, 10 vol % ethanol. [b] [2-Mercaptoethanol] = 0.0180 M. [c] $[SO_3^{2-}] + [HSO_3^{2-}] = 1.56 \times 10^{-3}$ M.

Table II. Comparison of Rate Constants for OHFl and CNFl (30 °C, pH 8.30)

reactant		rate constant: OHFl	rate constant: CNFl	k_{CNFl}/k_{OHFl}
BNAH	k_2 (M^{-1} s^{-1})	11.1[a]	256	23.1
$HO(CH_2)_2SH$[b]	k_1' (s^{-1})	8.90×10^{-4}	1.26×10^{-3}	1.42

[a] pH 8.81. [b] $[HO(CH_2)_2SH] = 2.00 \times 10^{-2}$ M.

Results and Discussion

Comparison of Reactivities between OHFl and MeLFl. The absorption spectra of OHFl and reduced OHFl are recorded in Figure 1. The aqueous solution of OHFl was red. This color is due to an absorption maximum at 502 nm (ϵ_{max} 14 900). The absorption spectra of OHFl and reduced OHFl were pH dependent because of dissociation of the 1'-OH group. We have determined the pK_a of the 1'-OH of OHFl to be 10.7 from the pH-dependent change in an absorption band at 580 nm which newly appeared at high pH.[9] Examination of past literatures reveals that pK_a's of 1-naphthol derivatives are much lower than 10.7: for example, pK_a = 9.6 for 1-naphthol and 8.7 for 1-naphthol-3-sulfonate.[10,11] Furthermore, one should note that the isoalloxazine moiety acts as an electron-withdrawing "substituent" as strong as a nitro group.[12] The pK_a = 10.7 for OHFl therefore supports that 1'-OH forms a hydrogen bond with N(5).

It is well established that logarithms of rate constants for flavin-mediated reactions are correlated linearly with polarographic half-wave potentials ($E_{1/2}$) or with redox potentials of flavins.[13-17] For example, the logarithms of the second-order rate constants (k_2) for flavin oxidation of NADH model compounds is linearly correlated with $E_{1/2}$ with a gradient of 15.1 V^{-1}.[13] We estimated

(3) (a) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256. (b) Bruice, T. C. In "Biomimetic Chemistry"; American Chemical Society: Washington, D.C., 1980; p 89.
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(7) Bruice, T. C.; Benkovic, S. In "Bioorganic Mechanisms"; Benjamin: New York, 1966; Vol. 2, Chapter 8.
(8) This compound may be named as an isoalloxazine derivative sodium 1'-hydroxybenzo[2',3'-*h*]-10-methylisoalloxazine-5'-sulfonate. For the sake of simplicity we use this nonseptematic nomenclature when we point the atom in OHFl in the text.

OHFl

(9) Shinkai, S.; Honda, N.; Ishikawa, Y.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1985**, 565.
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(11) Baker, K.; Friez-David, H. E. *Helv. Chim. Acta* **1950**, *33*, 2011.
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(13) Gascoigne, I. M.; Radda, G. K. *Biochim. Biophys. Acta* **1967**, *131*, 498.
(14) Bruice, T. C.; Main, L.; Smith, S.; Bruice, P. Y. *J. Am. Chem. Soc.* **1971**, *93*, 7327.
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(16) Shinkai, S.; Yamada, S.; Kunitake, T. *Macromolecules* **1978**, *11*, 65.
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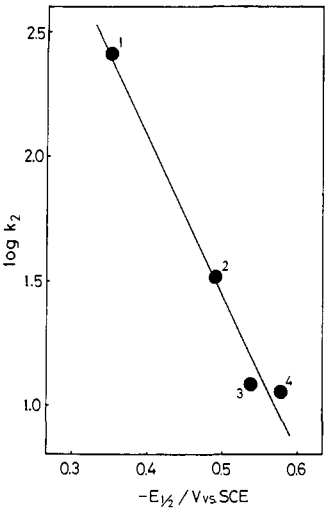
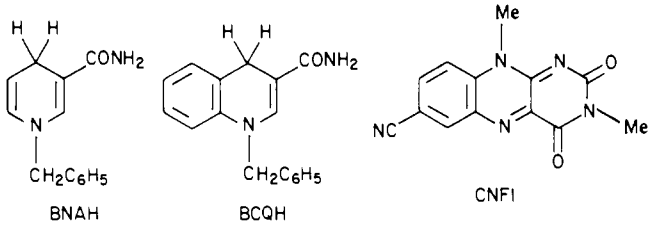


Figure 2. Correlation of $E_{1/2}$ with log k_2 (M^{-1} s^{-1}) for the oxidation of BNAH: pH 8.70, [flavin] = 2.50×10^{-5} M, [BNAH] = 5.00×10^{-5} M. Flavins ($E_{1/2}$ (V) vs. SCE) used are the following: (1) CNFl (−0.353), (2) 3-methyltetra-*O*-acetylriboflavin (−0.492), (3) MeLFl (−0.538), and (4) OHFl (−0.579).

the $E_{1/2}$ of OHFl to be −0.579 V vs. SCE (30 °C, pH 6.9 with 0.010 M phosphate, μ = 0.10 with $NaNO_3$). This value is quite comparable with that of MeLFl (−0.538 V vs. SCE).[18]

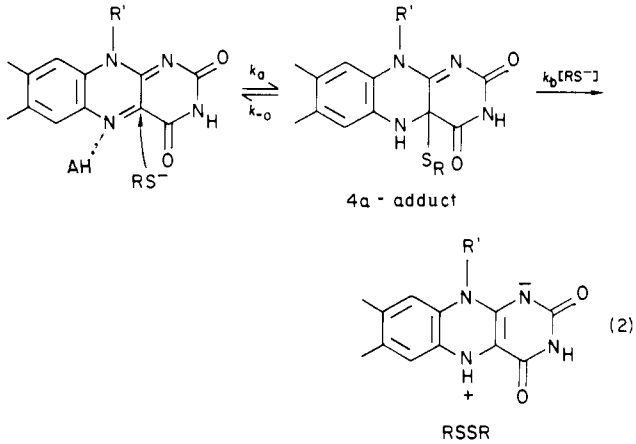
We determined the k_2 for the oxidation of NADH model compounds such as 1-benzyl-1,4-dihydronicotinamide (BNAH) and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (BCQH) at 30 °C. The reactions were first order in flavins and NADH models under the employed reaction conditions. The rate constants for OHFl decreased significantly at pH >10 because of dissociation of the 1′-OH, so that we carried out the reactions at pH <10 where the k_2 values were almost constant. The results (Table I) indicated that the k_2's for OHFl are very close to those for MeLFl.



3,10-Dimethyl-7-cyanoisoalloxazine (CNFl) synthesized by Bruice et al.[18] is an electron-deficient flavin with $E_{1/2}$ = −0.353 V vs. SCE and serves as a strong oxidizing agent. As shown in Table II, CNFl can oxidize BNAH 23.1 times more efficiently than OHFl. In Figure 2, the logarithm of k_2 for the oxidation of BNAH is plotted against $E_{1/2}$ of several flavins including OHFl. The plot provided a good linear relationship (r = 0.99) expressed by eq 1. The slope (6.38 V^{-1}) for BNAH may be comparable with 15.1 V^{-1} for NADH.[13]

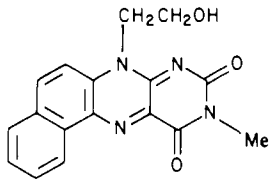
$$\log k_2 = 6.38E_{1/2} + 4.64 \quad (1)$$

The foregoing data ($E_{1/2}$, k_2, and LFER) all support that OHFl acts apparently as a "normal" flavin in the oxidation of NADH model compounds. We noticed, however, that the rate constants for the oxidation of thiols are remarkably different between OHFl and MeLFl (Table I): in the oxidation of mono- and dithiols, the pseudo-first-order rate constants (k_1') for OHFl are greater by 33–645-fold than those for MeLFl. Furthermore, the k_1' for the adduct formation between OHFl and SO_3^{2-} is enhanced by 1830-fold relative to that between MeLFl and SO_3^{2-}. In Table II, the k_1' for the reaction of 2-mercaptoethanol with OHFl is compared with that with CNFl. Despite the positive shift of the $E_{1/2}$, CNFl was only 1.4 times more reactive than OHFl. The result clearly indicates that OHFl is activated toward 2-mercaptoethanol much more than expected from the $E_{1/2}$. It is firmly established that flavin oxidation of mono- and dithiols proceeds via 4a adducts: that is, nucleophilic attack of the first thiolate on the 4a position followed by breakdown reaction with the second thiolate (eq 2).[19-21] The foregoing findings imply, therefore, that OHFl is "regiospecifically" activated toward reactions involving 4a intermediates.



(2)

The unusual reactivity cannot be rationalized by a benzo group fused at the 6,7 position with the basic isoalloxazine skeleton, because Gumbley and Main[15] have found that the rate constant for the oxidation of 2-hydroxy-1,3-propanedithiol by benzo[*h*]-3-methyl-10-(β-hydroxyethyl)isoalloxazine (**1**) well satisfies a linear free-energy relationship between log k_1' vs. $E_{1/2}$. One may conclude, therefore, that the unusual reactivities of OHFl should be ascribed to activation of C(4a) through hydrogen bonding with N(5). Subsequently, we consider the origin of the unusually high reactivities of OHFl in detail.



1

Reactions with Mono- and Dithiols. OHFl could rapidly oxidize various kinds of thiols, and the absorption spectrum of OHFl changed directly to that of reduced OHFl with several isosbestic points. The result establishes that OHFl oxidizes thiols according to a simple A → B process without the buildup of absorbing intermediates or the intervention of side reactions. Hence, one can consider that the 4a intermediate of OHFl exists only in a level of the steady-state concentration. Introduction of O_2 into the reaction solution regenerated the absorption spectrum of OHFl quantitatively. The TLC analysis of the reoxidized solution with a high-speed chromato-scanner idicated that OHFl was recovered quantitatively without contamination by byproducts.

The flavin oxidation of dithiols is subjected to general-acid catalysis.[13,20,21] For example, the reaction of riboflavin and dihydrolipoic acid (25 °C, pH 7.8 with Tris–HCl buffer) has a buffer rate term of the form k_{ga}[riboflavin][dihydrolipoic acid][buffer].[13] We obtained k_{ga} = 0.36 M^{-2} s^{-1} by computing the data in ref 13 according to the least-squares procedure (r = 0.98). We carried out the buffer dilution for the reaction of OHFl and 1,4-butanedithiol under similar reaction conditions (30 °C, pH 7.8 with Tris–HCl buffer) and obtained $k_{ga} = 8.6 \times 10^{-3}$ M^{-2} s^{-1} (r = 0.99). Thus, the buffer-catalyzed term for OHFl +

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(20) Loechler, E. L.; Hollocher, T. C. *J. Am. Chem. Soc.* **1975**, *97*, 3235.
(21) Loechler, E. L.; Hollocher, T. C. *J. Am. Chem. Soc.* **1980**, *102*, 7312, 7322, and 7328.

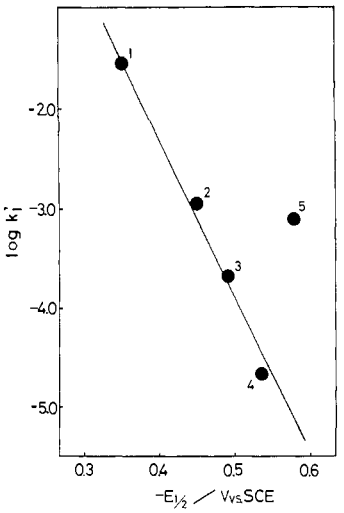


Figure 3. Correlation of $E_{1/2}$ with log k_1' (s^{-1}) for the oxidation of 1,4-butanedithiol: pH 9.80, 20 vol % ethanol, [flavin] = 3.00×10^{-5} M [1,4-butanedithiol] = 2.40×10^{-4} M. Flavins used are the following: (1) CNFl, (2) 10-phenylisoalloxazine ($E_{1/2}$ = −0.450 V vs. SCE), (3) 3-methyltetra-*O*-acetylriboflavin, (4) MeLFl, and (5) OHFl.

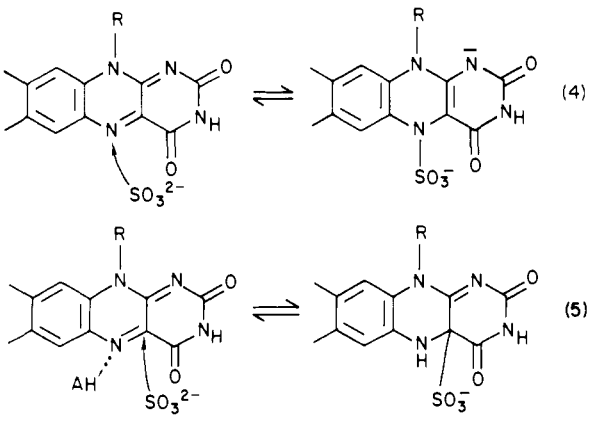
1,4-butanedithiol is smaller by a factor of 42 than that for riboflavin + dihydrolipoic acid. The smaller buffer-catalyzed term in the oxidation by OHFl is rationalized in terms of 1′-OH acting as an intramolecular general acid.

It is known that the logarithm of the rate constants for flavin oxidation of thiols is linearly correlated with $E_{1/2}$ and the higher is the $E_{1/2}$ value the faster is the reaction rate.[13-15] In Figure 3, we plotted log k_1' (s^{-1}) for the oxidation of 1,4-butanedithiol (pH 9.80, 30 °C) against $E_{1/2}$. As expected, four flavins gave a very good straight line (r = 0.995) expressed by eq 3. The slope (16.5

$$\log k_1' = 16.5E_{1/2} + 4.32 \quad (3)$$

V^{-1}) is comparable with those reported by Bruice et al.[14] (32 V^{-1}) for 1,4-butanedithiol (pH 8.98, 29.9 °C) and by Gumbley and Main[13] (25 V^{-1}) for 2-hydroxy-1,3-propanedithiol (pH 9.20, 30 °C). Importantly, a plot for OHFl deviated to the upper area from the linear relation by 2.12 (in log units). This suggests that OHFl is activated by more than two orders of magnitude than that expected from $E_{1/2}$.

Reaction with Sulfite Ion. Sulfite ion (SO_3^{2-}) is one of a few nucleophiles which can form covalent adducts with flavins.[3-6,14,22-25] Bruice et al.[22,23] have demonstrated that SO_3^{2-} usually attacks N(5) to give the 5 adduct (eq 4), but the 4a adduct also results when an electron-withdrawing substituent is introduced at the 8 position (eq 5). In the latter case, the reaction is assisted by general-acid catalysis.



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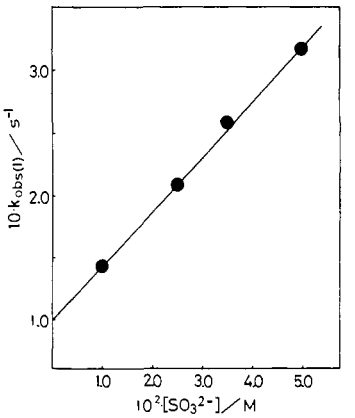
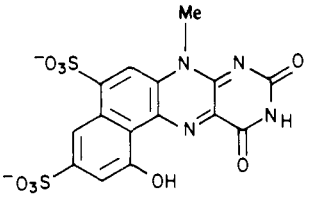


Figure 4. $k_{obsd(1)}$ plotted against sulfite concentration. [OHFl] = 3.00×10^{-5} M.

As recorded in Table I, SO_3^{2-} rapidly reacts with OHFl (red) to give a yellow adduct absorbing at λ_{max} 458 nm (spectrum 2 in Figure 1). Interestingly, we found that the OHFl–SO_3^{2-} adduct slowly decays to spectrum 3 (λ_{max}, 431 nm, slightly yellow) under anaerobic conditions. Spectrum 3 was similar to but a little different from that of 1,5-dihydro-OHFl (λ_{max} 427 nm; prepared by the reaction with 1,4-butanedithiol). Introduction of O_2 into this solution regenerated instantaneously a red solution (λ_{max} 502 nm), the spectrum of which was characterized by a stronger absorption band at 500–600 nm than that of OHFl. The product analysis (see Experimental Section) indicated that this product is an 8-sulfonated derivative of OHFl (**2**:1-hydroxy-7-methylnaphtho[8,7-*g*]pteridine-9,11(7*H*,10*H*)-dione-3,5-disulfonate or 1′-hydroxybenzo[2′,3′-*h*]-10-methylisoalloxazine-5′,8-disulfonate). The reduction of **2** with 1,4-butanedithiol (3.00×10^{-3} M) gave the final spectrum which was completely in accord with spectrum 3 in Figure 1. The k_1' for **2** determined at pH 9.80 was 338 times greater than that for OHFl. The rate enhancement is ascribed to the electron-withdrawing nature of the 8-sulfonate group. Thus, the reaction of OHFl and SO_3^{2-} proceeds according to the scheme A → B → C.



2

In Figure 4 the pseudo-first-order rate constants for the first step ($k_{obsd(1)}$) are plotted as a function of the sulfite concentration (no buffer, pH 9.50). The existence of the intercept, which corresponds to the rate constant for the reverse reaction,[22,23] indicates the first step to be an equilibrium reaction. Thus, the $k_{obsd(1)}$ can be expressed by eq 6,[23-25] where k_f and k_r are the rate

$$k_{obsd(1)} = k_f[SO_3^{2-}] + k_r = k_f[SO_3^{2-}] + (k_f/K) \quad (6)$$

constants for the forward and the reverse reaction, respectively, and $K = k_f/k_r$. We determined the slope (k_f) and the intercept (k_f/K) by the least-squares computation: k_f = 4.36 M^{-1} s^{-1}, k_r = 0.100 s^{-1}, and K = 43.6 M^{-1}. The corresponding terms for conventional flavins (e.g., 3-methyltetra-*O*-acetylriboflavin and 3-methyl-10-ethylisoalloxazine) have been determined (30 °C, pH 9.50):[25] k_f = 0.01–0.02 M^{-1} s^{-1}, k_r = 0.01–0.015 s^{-1}, and K = 0.8–1.3 M^{-1}. Comparison of these terms indicates that both k_f and k_r for OHFl are enhanced by several orders of magnitude.

Bruice et al.[14] reported previously that a plot of log $k_{obsd(1)}$ vs. $E_{1/2}$ also gives a linear relation. We determined the $k_{obsd(1)}$ values at pH 7.21 for the five flavins including OHFl and plotted them against their $E_{1/2}$ (Figure 5). Four plots (except for OHFl) provided a good straight line (r = 0.998) expressed by eq 7. On

$$\log k_{obsd(1)} = 6.78E_{1/2} + 1.02 \quad (7)$$

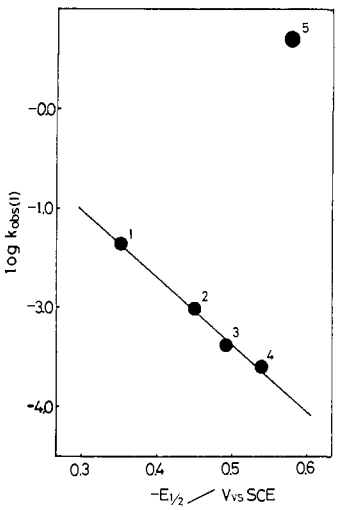


Figure 5. Correlation of $E_{1/2}$ with log $k_{obsd(1)}$ (s^{-1}) for the adduct formation with SO_3^{2-}: pH 7.21, [flavin] = 3.00×10^{-5} M, [K_2SO_3] = 0.100 M. Flavins used are the following: (1) CNFl, (2) 10-phenylisoalloxazine, (3) 3-methyltetra-*O*-acetylriboflavin, (4) MeLFl, and (5) OHFl.

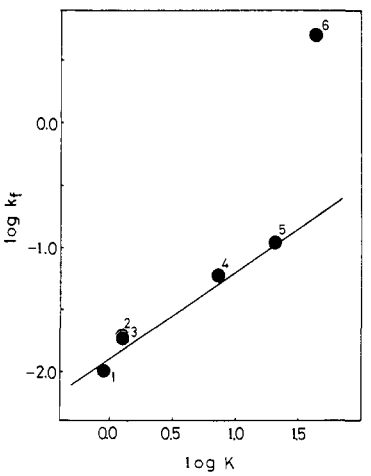


Figure 6. Correlation between log K–log k_f for the adduct formation with SO_3^{2-}. Flavins used are the following: (1) 3-methyl-10-ethylisoalloxazine, (2) 3-methyl-10-butylisoalloxazine, (3) 3-methyltetra-*O*-acetylriboflavin, (4) polymer-bound flavin (see ref 25), (5) 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine-6,8-disulfonate, and (6) OHFl.

the other hand, log $k_{obsd(1)}$ for OHFl deviated from the linearity to the upper area by 3.61 log units, indicating that the affinity of OHFl with SO_3^{2-} is remarkably enhanced. Previously, Lindquist and Cordes[26] noticed that log k_f and log K for the addition of cyanide ion to NAD^+ and its model compounds hold a good linear relationship. This implies that the increase in K is mainly caused by the increase in k_f. We found that a plot of log k_f and log K for the addition of sulfite ion to flavins also holds a good linear relationship expressed by eq 8.[25] As shown in Figure 6,

$$\log k_f = 0.69 \log K - 1.89 \quad (8)$$

log k_f for OHFl deviated by 1.41 log units to the upper area. The upper deviation suggests that k_f for the reaction of OHFl and SO_3^{2-} is specifically enhanced. Thus, the deviation from the linear free-energy relationships suggests again that OHFl adopts a mechanism quite different from conventional flavins in the SO_3^{2-} adduct formation.

Here, a question arises as to the position of the SO_3^{2-} attack. In conventional flavins, the nucleophilic attack of SO_3^{2-} occurs at 4a or 5 (eq 5 and 6). These adducts can be distinguished either by absorption spectroscopy[22] or by ^{13}C NMR.[27] According to

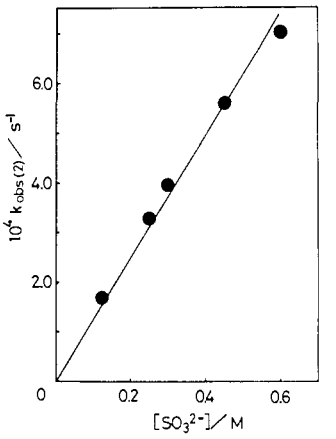


Figure 7. $k_{obsd(2)}$ plotted against sulfite concentration. [OHFl] = 3.00×10^{-5} M.

Hevesi and Bruice,[22] λ_{max} for 5 adducts characteristically appears between 296 and 330 nm (about 110-nm blue shift from the S1 band of oxidized flavin) and λ_{max} for 4a adducts invariably appears between 360 and 370 nm (about 60-nm blue shift). This spectroscopic method could not be applied to the present system because of the red shift of the S1 band of OHFl. Also, it was difficult to measure ^1H and ^{13}C NMR of the OHFl–SO_3^{2-} adduct because of its sparing solubility.[28a] However, we favor the 4a adduct on the basis of several lines of evidence: (i) as shown in the oxidation of thiols, the 4a position is activated while the 5 position is sterically protected by 1′-OH,[28b] (ii) the 5 adduct usually gives an absorption spectrum similar to that of 1,5-dihydroflavin, but the absorption spectrum of the OHFl–SO_3^{2-} adduct (spectrum 2 in Figure 1) is quite different from that of 1,5-dihydro-OHFl (λ_{max} 427 nm), (iii) λ_{max} of the intermediate (458 nm) corresponds to the 44-nm blue shift from the S1 band of OHFl (502 nm), and (iv) the solvent isotope effect ($k_H/k_D = 1.9 \pm 0.1$) was observed for the initial step of the reaction between OHFl and SO_3^{2-}, whereas that for the reaction of MeLFl and SO_3^{2-} which forms the 5 adduct[22-25] was equal to unity ($k_H/k_D = 1.0 \pm 0.1$). (iv) supports that the formation of the OHFl–SO_3^{2-} adduct is general-acid catalyzed. These findings suggest that most probably, the reaction of OHFl and SO_3^{2-} proceeds via the 4a intermediate.

The rate of the second step (OHFl–SO_3^{2-} → 1,5-dihydro-**2**) was monitored by following the disappearance of the absorption band of the intermediate (458 nm). It satisfied the first-order rate equation for up to 3 half-lives. The pseudo-first-order rate constants ($k_{obsd(2)}$) were plotted against [SO_3^{2-}] in Figure 7. A good linear relation between $k_{obsd(2)}$ and [SO_3^{2-}] supports that the second step is first order in SO_3^{2-}. On the basis of the least-squares computation, we estimated the slope ($=k_d$) to be 1.12×10^{-3} M^{-1} s^{-1}. As a summary of the foregoing findings, we offer eq 9 which consists of a fast adduct formation at the 4a position followed by a slow conversion to the 8-sulfonated reduced form.[29]

$$\underset{\text{(red)}}{\text{OHFl}} + SO_3^{2-} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \underset{\text{(yellow)}}{\text{OHFl-(4a)-}SO_3^{2-}} \xrightarrow{k_d[SO_3^{2-}]} \underset{\text{(slightly yellow)}}{\text{1,5-dihydro-}\mathbf{2}} \quad (9)$$

Now, we discuss a reaction route from OHFl to 1,5-dihydro-**2** occurring according to eq 9. Hevesi and Bruice[22] found that in the presence of excess SO_3^{2-} 3-methyl-10-(2′,6′-dimethyl-

(26) Lindquist, R. N.; Cordes, E. H. *J. Am. Chem. Soc.* **1968**, *90*, 1269.

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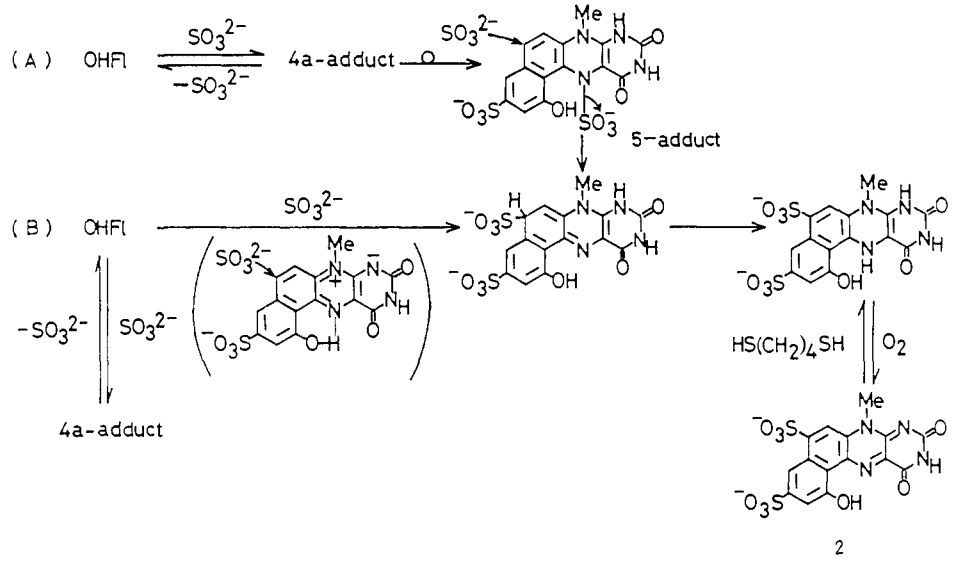
(28) (a) The OHFl–SO_3^{2-} adduct was not soluble in D_2O in the presence of excess Na_2SO_3 enough to measure the NMR spectra. (b) Examination of CPK models suggests that the nucleophilic attack of SO_3^{2-} on N(5) is sterically difficult and the 5-adduct has some steric hindrance unless it adopts the bent structure along the N(5)–N(10) axis.

(29) We first considered the final product to be 1,5-dihydro-OHFl because of the spectral similarity.[1] The later product analysis verified this compound to be 1,5-dihydro-**2**, however.

Scheme I



phenyl)isoalloxazine is converted to its 6,8-disulfonated derivative. The reaction scheme involves the nucleophilic attack of SO_3^{2-} upon the 8 position of the 5 adduct as a key step: that is, the nucleophilic attack displaces SO_3^{2-} from the 5 position of the adduct. If this mechanism is applicable to the present system, one can write route A which consists of (i) 4a addition, (ii) rearrangement to the 5 adduct, (iii) nucleophilic attack at the 8 position, and (iv) prototropy to 1,5-dihydro-**2** (Scheme I). In route A the steps from iii to iv are quite reasonable, but the feasibility of the rearrangement from the 4a to the 5 adduct is not yet established firmly. Furthermore, the 5 adduct of OHFl should be highly destabilized by the steric crowding.[28b]

An alternative route is B, in which neither the 4a-to-5 rearrangement nor the formation of the 5 adduct is involved along the obligatory path: that is, the 4a adduct is a nonproductive intermediate and SO_3^{2-} directly attacks the 8 position of OHFl. This route is kinetically equivalent to route A if OHFl exists only in the steady-state concentration in the second step. Since the K value for the first equilibrium step is known (43.6 M^{-1}), one can estimate the equilibrium concentration of OHFl under the spectroscopic conditions ($[OHFl]_0 = 3.00 \times 10^{-5}$ M, $[SO_3^{2-}] =$ 0.300 M) to be 2.1×10^{-6} M. This value is fully smaller than $[OHFl]_0$. The structure in parentheses is one of the resonance forms, the contribution of which should become more significant with the aid of hydrogen bonding with 1′-OH. Hence, the formation of the 8 adduct from OHFl may be regarded as a well-known nucleophilic attack of SO_3^{2-} on *p*-benzoquinone or 1,2-naphthoquinone.[30,31] At the present time, we consider that route B is more likely because one cannot find any unreasonable steps.

Comments on the Regiospecific Reactivities of OHFl. The mechanism by which NADH and its model compounds transfer a "hydride equivalent" to flavins has been a controversial problem.[2-6,32] Although the existence of some preequilibrium association between NADH and flavin has been proposed,[14,33,34] there is no useful information to specify which position in the isoalloxazine skeleton accepts the "hydride equivalent" from NADH. The fact that the plot for the reaction of OHFl and BNAH does not deviate from LFER suggests that the "hydride equivalent" is *not* transferred to the 4a position. If the 4a position accepts the "hydride equivalent", the plot should largely deviate from LFER as seen for the reactions with thiols and SO_3^{2-}.

The regiospecific reactivities of OHFl are primarily ascribed to polarization of the N(5)=C(4a) bond due to the 1′-OH···N(5) interaction. This hydrogen-bonding effect is very similar to the role of the 3-hydroxy group in pyridoxal coenzymes which activates the 4-CHO in the initial state and facilitates transamination in the transition state.[8] Similarly, certain aromatic C=C and C=N double bonds with the *o*-hydroxyl group are reducible nonenzymatically by NADH model compounds.[35-38] This is also ascribed to the polarization of these double bonds through hydrogen bonding.

Here, a question related to the reactivities of OHFl is raised: why 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine gives the disulfonated derivative[22] while OHFl gives the monosulfonated **2**. The 8 adduct from 3-methyl-10-(2′,6′-dimethylphenyl)isoalloxazine newly provides a reactive C=C double bond at the 6,7 position. It can accept a second SO_3^{2-} ion to yield the 6,8-disulfonated derivative.[22] Even though the prototropy from 8 to N(5) occurs in preference to the second SO_3^{2-} attack, it would afford the more electrophilic 8-sulfonated derivative, which would react readily with the second SO_3^{2-} ion. In the 8 adduct from OHFl, on the other hand, the C=C bond is protected from the nucleophilic attack as a part of the aromatic ring. Hence, the prototropy to 1,5-dihydro-**2** should occur in preference to the nucleophilic attack of the second SO_3^{2-} ion.

Conclusion. The foregoing considerations, mainly based on LFER methods, support that OHFl is activated toward the reactions involving 4a intermediates. The molecular design of "activated" flavins can be readily achieved by introducing electron-withdrawing substituent(s) into the isoalloxazine skeleton, whereas the "regiospecifically activated" flavins are designed, at the present time, only by the specific hydrogen bonding. The regiospecific reactivities of OHFl are solely ascribed to the hydrogen bonding formed between 1′-OH and N(5). Thus, the present paper is the first example to demonstrate the latent role of the hydrogen bonding in the regiospecificity in the flavin-dependent reactions. To obtain further insight into the flavin activation through hydrogen bonding, we are now synthesizing an *N*(1)-hydrogen-bonded flavin which would act as a model for the first-group flavoproteins.

Experimental Section

Materials. The synthesis of OHFl was described previously.[9] Preparations of BNAH and BCQH were also described.[37,39] CNFl is a gift from Professor T. C. Bruice. MeLFl was synthesized according to the

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method of Hemmerich.[40] Thiols were distilled under a N_2 stream before use.

Kinetic Measurements. The kinetic measurements for the oxidation of NADH model compounds by flavins were carried out aerobically under the recycle conditions by following the decrease in the absorption maximum of NADH model compounds: λ_{max} 357 nm for BNAH and 345 nm for BCQH. The decrease obeyed the first-order rate equation for up to 3 half-lives. The kinetic measurements for the oxidation of thiols and the adduct formation with SO_3^{2-} were carried out anaerobically by following the decrease in the absorption maximum of flavins: 502 nm for OHFl, 428 nm for CNFl, and 453 nm for MeLFl. The anaerobic reaction mixtures were prepared by using a Thunberg cuvette. The reactions also obeyed the first-order rate equation. All kinetic measurements were conducted at 30 °C.

Product Analysis. An attempt to obtain **2** on a preparative scale was the following: Na_2SO_3 (172.5 g, 1.30 mol) was dissolved in 1050 mL of deaerated water, and after a N_2 substitution OHFl (60.0 mg, 1.02 mmol) was added to the solution; the anaerobic reaction was continued at 30 °C for 30 h in the dark. The reaction mixture (red) turned immediately yellow and then faded slowly to a slightly yellow color. After 30 h we confirmed that the absorption spectrum of the reaction mixture resembles very closely spectrum 3 in Figure 1. In order to remove excess Na_2SO_3, concentrated HCl (650 mL) was added and N_2 was passed vigorously into the solution. By this means Na_2SO_3 was converted to SO_2 which was swept from the reaction mixture.[22,41] The completion of the SO_2 removal was confirmed with the wet pH-test paper. From this point on, no care was taken to exclude air from the system. The solution was concentrated in vacuo to about 50 mL and mixed with methanol (150 mL) to precipitate NaCl. The precipitated NaCl was filtered and washed with methanol. The combined solution was concentrated in vacuo to precipitate NaCl again. This operation was repeated three times. Finally, the filtrate was evaporated to dryness in vacuo: red crystals, yield 5.47 g. The product was purified by means of paper chromatography (PC) (developing solvent, ethanol:water = 1:1 v/v, R_f 0.87): yield 44.0 mg. Under the identical PC conditions OHFl gave R_f 0.73. ^{1}H NMR (Me_2SO-d_6) δ 4.16 (s, N-CH_3), 7.66 (s, 6'-H), 8.60 (s, 4'-H), 8.99 (s, 9-H). The ^{1}H NMR spectrum indicates that there are three aromatic protons and each proton gives a singlet peak. The titration after treatment with ion-exchange resin gave the reasonable neutralization value to assume disulfonated OHFl. These data support the final product isolated from the reaction mixture of OHFl and SO_3^{2-} to be **2**.

The OHFl solutions after the reactions with BNAH, 1,4-butanedithiol, and 2-mercaptoethanol were analyzed under aerobic conditions by using a high-speed TLC scanner (Shimadzu CS-920). The result indicated that OHFl is the sole colored material present in the product mixtures.

Miscellaneous. The polarographic half-wave potentials ($E_{1/2}$) were determined at 30 °C in a thermostated cell with Yanagimoto P8 polarographic equipment: [flavin] = (2-5) × 10^{-4} M, pH 6.90 with 0.010 M phosphate, μ = 0.10 with $NaNO_3$.

Acknowledgment. The authors wish to thank Professor V. Massey for fundamental discussions on the reactivities of *N*(5)-hydrogen-bonded flavins. They also thank Professor E. L. Loechler and T. C. Hollocher for stimulating discussions. This research was supported by a grant from the Ministry of Education of Japan.

Registry No. 2, 98170-66-2; 1,5-H_2-**2**, 98193-88-5; OHFl, 91077-96-2; BNAH, 952-92-1; BCQH, 17260-79-6; CNFl, 51595-98-3; MeLFl, 18636-32-3; K_2SO_3, 10117-38-1; $HS(CH_2)_4SH$, 1191-08-8; $HO(CH_2)_2SH$, 60-24-2; Na_2SO_3, 7757-83-7; 3-methyltetra-*O*-acetylriboflavin, 21066-33-1; 10-phenylisoalloxazine, 6851-14-5.

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Synthesis, Structure, and Electronic Properties of $(\eta$-$C_5Me_5)_2V(\mu$-OC)$V(CO)_5$. A Complex with a Linear V-O-C-V Bond

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Abstract: The reaction between $V(CO)_6$ and $(\eta$-$C_5Me_5)_2V$ yields the μ-isocarbonyl complex, I $(\eta$-$C_5Me_5)_2V(\mu$-OC)$V(CO)_5$. Crystals of I belong to the space group $C2/c$ with Z = 4, a = 14.140 (5) Å, b = 14.303 (3) Å, c = 13.212 (3) Å, β = 94.72 (2)°, and V = 2663.0 Å^3. For the 1802 reflections that had $F_o > 2.50F_o$ solution by direct methods led to a final R of 0.0461 and R_w of 0.0568. An important aspect of the structure is the linear V-O-C-V moiety with V-O = 2.075 (4) Å, C-O = 1.167 (6) Å, and V-C = 1.899 (5) Å. Complex I is paramagnetic, contains two unpaired electrons, and obeys the Curie law between 5 and 298 K. SCC-Xα-DV calculations of $(\eta$-$C_5H_5)_2V(\mu$-OC)$V(CO)_5$ show that nearly degenerate frontier orbitals localized on the $(\eta$-$C_5H_5)_2V^+$ fragment lead to a high-spin $^3B_1$ ground state. The V-O bond arises mainly from an electrostatic interaction between $(\eta$-$C_5Me_5)_2V^+$ and $V(CO)_6^-$; however, a small covalent π back-donation from a $d(t_{2g})$ orbital on $V(CO)_6^-$ into a partly occupied b_1 π orbital on the $(\eta$-$C_5H_5)V^+$ fragment is observed. Photolysis of I as well as its thermal reaction with carbon monoxide in solution yields $[(\eta$-$C_5Me_5)_2V(CO)_2][V(CO)_6]$.

Few compounds have been synthesized[2-8] for which carbon monoxide, bound at both carbon and oxygen, bridges two metal centers (hereafter denoted μ-isocarbonyl). Weakening of the carbon-oxygen bond and strengthening of the metal-carbon bond, as evidenced by IR stretching frequencies and bond lengths, raise the question of μ-isocarbonyl reactivity. In this context it is desirable to better understand the bridging bond.

Complexes that have been structurally characterized exhibit bending about the M-O-C bond, with the angle varying from 135.35 (46)° in $Cp_2Ti(THF)(\mu$-OC)$MoCp(CO)_2$[3] to 167.7 (9)° in $(OC)_5V(\mu$-CO)$V(THF)_4(\mu$-OC)$V(CO)_5$.[7] While packing forces may account for the slight bending in the latter compound, the other known examples must bend either to relieve steric inter-

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