on cis–trans photostationary ratios[10a] is diagnostic for the presence of ^{3}t. Our previous[11b] observation of the absence of an azulene effect for β-methylstyrene (**9**) required either a very short triplet lifetime or a negligible fraction of ^{3}t present. The lifetime we now report for triplet **9** would be quite sufficient to permit observation of an azulene effect if even 5% of the triplets are transoid, and the absence of the effect now requires that ^{3}p be the only species present in significant amount. This conclusion finds theoretical support.[3e]

The values for k_{PQ} we report nicely reinforce our conclusion of a twisted structure. If the triplets were planar, increasing alkyl substitution would increase k_{PQ}, since the olefin triplet would thereby be a better electron donor. Although k_{PQ} increases (see Table I) on going from *p*-methoxystyrene (**1**) to 1-(*p*-methoxyphenyl)propene (**2**), it decreases for the dimethyl and trimethyl analogues **7** and **8**, in contrast to the prediction for a series of planar triplets. That k_{PQ} attains a maximum for an intermediate degree of substitution can be rationalized easily for twisted triplets as resulting from a balance of two effects. The increased electron-donating ability of the olefin will tend to increase k_{PQ}, and the increased steric hindrance to reaching planarity will tend to decrease k_{PQ}, as the degree of alkylation increases. We believe that the planar geometry is the preferred one for electron transfer, both from the observed high values of k_{PQ} for the near-planar arylcyclopentenes and from the expectation of a planar geometry for the styrene radical cation produced.

The effect of alkyl substitution may relate fundamentally to the mechanism of T_1–S_0 intersystem crossing. We suggest that the effect of alkylation upon the lifetimes of the twisted triplets arises in substantial part from the contribution of vinyl C–H vibrational modes to triplet decay. Saltiel et al.[15] have reported a substantial effect of vinyl deuterium on the stilbene triplet lifetime. We find a value of 1.11 ± 0.07 (two standard deviations), in the same direction, for anethole triplet vs. anethole-d_2 triplet. Rigorous statistical analysis[16] shows that this (admittedly modest) effect is significant at the 99% confidence level. Our isotope effect thus reinforces the conclusion from the analogous stilbene one[15] that there is at least some contribution from vinyl C–H bonds in inducing the decay of these twisted triplets. Our alkylation effect is nicely convergent with the isotope effects, in that replacement of H by alkyl should increase the lifetime analogously to the replacement of H by D. Both decrease the number of vinyl C–H bonds, the motions of which contribute to the decay.

An alternate explanation could be that alkylation alters the electronic structure of the triplet, which in turn affects the lifetime. Note that the effect of *p*-methoxy in **2** vs. **9** apparently requires such a contribution. Alkylation, through hyperconjugative delocalization, could increase the average distance between the unpaired electrons, thus[14] decreasing spin–orbit coupling and increasing the lifetime. However, on the basis of alkyl radical models, EPR spectra of which show that hyperconjugation is rather modest,[17] we expect such an effect to be small. It is in any case difficult to see how this mechanism per se could predict the observed isotope effects. We conclude that variation of electronic structure with alkyl substitution is at best an incomplete explanation.

The involvement of nuclear motions in decay of triplet biradicaloids follows the suggestion of Shaik and Epiotis[18] that such effects should be important. Further confirmation of this conclusion could have profound consequences for the mechanisms and dynamics of triplet-state photoreactions.

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Fast Oxidants for NADH and Electrochemical Discrimination between Ascorbic Acid and NADH

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The ubiquity of NADH and ascorbate makes their oxidation chemistry of special significance. We report here on chemically catalyzed, electrochemical oxidations. The study has demonstrated that the oxidized forms of aromatic diamines, which are flavin analogues, are very active NADH and ascorbate oxidants and revealed a crucial aspect of the NADH oxidation mechanism. This mechanistic knowledge led to the discovery of a catalyst which gave separate electrochemical responses for NADH and ascorbate. A unique method for the quantitative analysis of either compound using differential pulse voltammetry was then demonstrated.

NADH is electrochemically oxidized[1] in a process which has a large activation energy (overpotential). The electrochemical response is difficult to reproduce and highly dependent on the nature of the electrode surface and its history. As shown by Kuwana and co-workers,[2] this slow oxidation at the electrode can be catalyzed by certain other redox couples in the solution. *o*-Hydroquinones are, for example, electrochemically oxidized with a low activation energy and the resulting *o*-quinones will in turn rapidly oxidize NADH in the solution near the electrode. This approach has been further developed by binding a hydroquinone to an electrode surface and using the resulting electrode to oxidize NADH by an analogous two-stage process.[2,3]

We have studied several diamine redox couples in solution as catalysts. These were chosen for study because they are readily available and the oxidized forms are related in structure to oxidized flavins and natural quinones which are known to be NADH oxidants.[4] Consider first the electrochemical behavior of 1,4-diaminobenzene (**1a**). Cyclic voltammograms were measured in pH 7.0 aqueous solution (phosphate buffer) on a polished vitreous carbon disk electrode of area 7.07 mm^2. An SCE reference was employed. A voltammogram showing the quasi-reversible couple (ΔE_p = 40 mV at ν = 50 mV s^{-1}) for oxidation of **1a** is shown in Figure 1a. This voltammogram is exactly what one expects from many previous studies on this redox system.[5] At 0.20 V, **1a** produces a quinone diimine **2a**, which is stable on this time scale.[6]

(1) Moiroux, J.; Elving, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 6533 and references therein to earlier studies of Elving and co-workers. Underwood, A. L.; Burnett, R. W. In "Electroanalytical Chemistry"; Bard, A. J., Ed., Marcel Dekker: New York, 1972; Vol. 6. Dryhurst, G. "Electrochemistry of Biological Molecules"; Academic Press: New York, 1977. Blaedel, W. J.; Jenkins, R. A. *Anal. Chem.* **1975**, *47*, 1337. Leduc, P.; Thevenot, D. *Bioelectrochem. Bioenerg.* **1974**, *1*, 96. *J. Electroanal. Chem.* **1973**, *47*, 543. Jaegfeldt, J. *Ibid.* **1980**, *110*, 295.
(2) (a) Tse, D. C.-S.; Kuwana, T. *Anal. Chem.* **1978**, *50*, 1315. (b) Electroanalytical schemes involving NADH, NAD$^+$, enzymes, and redox reagents have also been discussed. See references in: Cheng, F. S.; Christian, G. D. *Ibid.* **1977**, *49*, 1785.
(3) Degrand, C.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102*, 5728.
(4) Creighton, D. J.; Hadjic, J.; Sigman, D. S. *Bioorg. Chem.* **1978**, *4*, Chapter 14.
(5) See references in: Adams, R. N. "Electrochemistry at Solid Electrodes"; Marcel Dekker: New York, 1969.
(6) The pK_a of **1a** is 6.2. The pK_a of **2a** is unknown.

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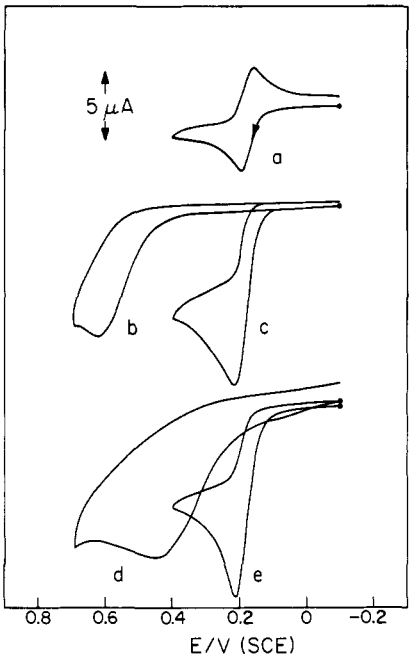
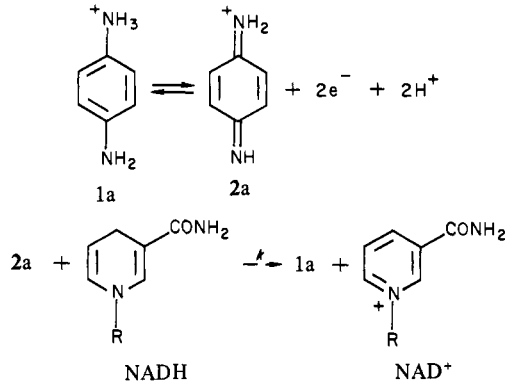


Figure 1. Cyclic voltammograms; ν = 20 mV s^{-1}, pH 7.0 phosphate buffer (0.05 M), 0.1 M $NaClO_4$. (a) 0.25 mM 1,4-diaminobenzene (**1a**); (b) 1 mM NADH; (c) 0.25 mM **1a**, 1 mM NADH; (d) 1 mM ascorbic acid; (e) 0.25 mM **1a**, 1 mM ascorbic acid (2nd sweep).

NADH is oxidized on glassy carbon at pH 7.0 with E_p near 0.6 V.[1] The curve is quite irreproducible, but a typical example is shown in Figure 1b. The cyclic voltammogram of NADH in the presence of **1a** is shown as Figure 1c. The dramatically enhanced anodic peak current at 0.2 V and diminished cathodic peak current demonstrate that an oxidized product from **1a** oxidizes NADH. NADH oxidation is thereby catalyzed and takes place at 0.20 V, not 0.6 V. One of the four species which might be responsible for oxidizing NADH is **2a**. It is shown below to demonstrate the two-stage scheme for catalysis. The rate con-



stant, k, for the oxidation of NADH in the solution phase can be estimated[7] from the peak current ratio for voltammograms measured for **1a** and **1a** plus NADH. The $k = 2.1 \times 10^3$ L mol^{-1} s^{-1} was shown to be independent of ν and NADH concentration. It will be realized that this electrochemical method is extremely useful for kinetics, since the rates are fast and the diimine oxidant is hydrolytically unstable.

Catalysis has been observed by using a variety of aromatic diamines and other compounds, and second-order rate constants as high as 10^5 L mol^{-1} s^{-1} have been measured for NAD^+ formation.[8] The rates are much faster than those measured for flavin

Table I. Relative Rates for NADH Oxidations[a]

	reduced catalyst[b]					
	R_1	R_2	R_3	X	E_0[c]	k_{rel}[d]
1a	H	H	H	H	0.16	1.0
1b	CH_3	H	H	H	0.18	18
1c	CH_3	CH_3	H	H	0.19	10
1d	CH_3	CH_3	CH_3	H	0.06	<0.02[e]
1e	H	H	H	Cl	0.22	0.8
1f	H	H	H	OCH_3	0.11	0.8
3	*p*-aminophenol				0.11	<0.02[e]
4	*p*-hydroquinone				0.15	<0.02[e]
5	hydroxymethylferrocene				0.23	<0.02[e]

[a] 0.05 M phosphate buffer, 0.1 M $NaClO_4$, 1.0 mM NADH, 0.1 mM catalyst, ν = 0.01–0.5 V s^{-1}. k's were calculated from the working curves of Figure 3, ref 7, and were nearly independent of ν in each case. [b] See above structural formula for R_1, R_2, R_3, and X positions. [c] $E_0' = (E_p^a + E_p^c)/2$. All couples were quasi-reversible with ΔE_p = 40–60 mV at 0.02 V s^{-1} and larger ΔE_p at larger ν except **5** and 1d, which gave reversible couples with ΔE_p = 60 mV, and **4** gave ΔE_p > 300 mV at 0.05 V s^{-1}. [d] k_{rel} for catalysis by 1/2 is taken to be 1.0. [e] No measurable enhancement of i_p at 0.01 V s^{-1}.

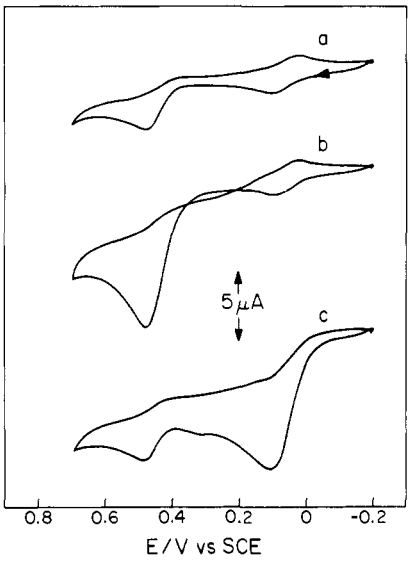


Figure 2. Cyclic voltammograms; ν = 10 mV s^{-1}, first sweep. (a) 0.2 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (**1d**); (b) 0.2 mM **1d**, 1 mM NADH; (c) 0.2 mM **1d**, 1 mM ascorbic acid.

or flavin analogue oxidations of NADH.[4] The rates relative to that for the **1a** system are shown in Table I as k_{rel}. The results demonstrate that (a) diamines **1a–c,e,f** provide catalytic systems which are superior to the corresponding *p*-aminophenol **3** or *p*-hydroquinone **4**, (b) there is a general lack of correlation between k_{rel} and the E_0' of the catalyst, (c) methylation on nitrogen leads to $k_{rel} > 1$ for the catalyst systems from **1b** and **1c**, but (d) *N,N,N',N'*-tetramethylphenylenediamine (**1d**) and the ferrocene **5**, which are one-electron redox systems at the first peak, do not give observable catalysis on this time scale.

These data are not consistent with mechanisms involving electron transfer from NADH (forming $NADH^{+}\cdot$), followed by proton loss from $NADH^{+}\cdot$ to water and a second electron transfer. In such mechanisms the oxidant is simply an electron-transfer agent, and a strong dependence of rate on E_0' and a small dependence on the structure of the oxidant would be observed. The data are consistent with mechanisms in which *the NADH hydrogen is transferred to the oxidant*. Consider one specific and attractive mechanism in which a hydride transfer or its two-step

(7) The rate constants were estimated by using the theory developed by: Andrieux, C. P.; Blocman, C.; Dumas-Bouchiat, J. M.; M'Halla, F.; Saveant, J. M. *J. Electroanal. Chem.* **1980**, *113*, 19. Although this theory is adequate to handle the high rates involved here, where previous treatments were not, the theory does not take into account the difference in diffusion coefficients for the catalyst species and NADH. This will introduce a small error in the absolute value of the rate constant.

(8) Preparative electrolysis at 0.2 V of diamines and NADH shows that NAD^+ is formed in quantitative yield and n = 2. The pulse polarograms of **1a,b,e,f** show wave heights twice that for **1d**. Compound **1c** shows two closely spaced peaks. The quoted value of 0.19 is the second peak, and k_{rel} was measured at this peak.

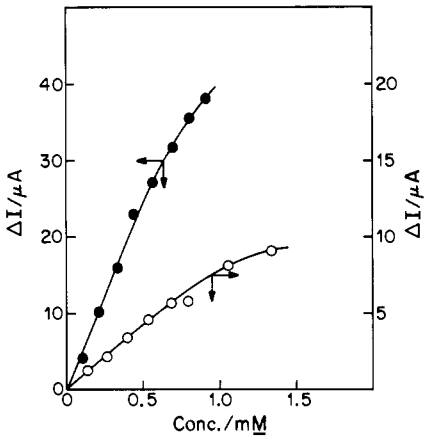
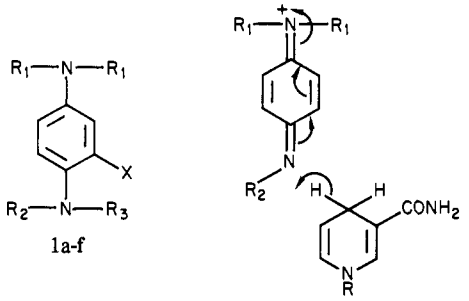


Figure 3. Increase of peak current from **1d** due to NADH or ascorbate using differential pulse voltammetry. [**1d**] = 7 × 10^{-5} M, ν = 10 mV s^{-1}, modulation amplitude = 50 mV. (○) Increase of first peak current due to addition of ascorbate. (●) Increase of second peak current due to addition of NADH.

equivalent occurs from NADH to **2**[9] (see the transition state shown below). This mechanism accounts for the high reactivity of



oxidized diamines in that iminium ions are known to be better hydride acceptors than ketones, and the mechanism has analogy in flavin oxidation of NADH. Most importantly, it accounts for the lack of catalysis from **1d**. **1d** has electrochemistry different from the other diamines discussed here, because it cannot lose an amino proton upon oxidation. At the first oxidation peak (see Figure 2a), only one electron is transferred forming $\mathbf{1d}^{+\cdot}$. Because hydride addition on nitrogen would require that nitrogen expand its octet, $\mathbf{1d}^{+\cdot}$ is not a good hydride acceptor. It will be noted that $\mathbf{1d}^{2+}$ could accept hydride, and, indeed, it should be an excellent NADH oxidant.

Ascorbic acid oxidation on carbon is very similar to NADH oxidation in that it has a large activation energy.[10] The cyclic voltammogram shows a difficult to reproduce, anodic peak at 0.4 V (Figure 1d). Several reports of the catalysis of this process have appeared,[2,11] and catalysis can also be achieved by using diamine **1a** (Figure 1e).[12] More interestingly, however, **1d** also gives good catalysis (Figure 2c).

These observations lead us to propose that an electroanalytical discrimination between NADH and ascorbate could be made by using **1d** or a similar catalyst. Because these two biological reductants often coexist, the analytical problem is of some interest. We have discovered that **1d** can, indeed, provide separate electrochemical responses for NADH and ascorbate. The voltammogram of **1d** at pH 7.0 shows two anodic peaks, the first reversible and the second irreversible. *Ascorbic acid is catalyzed at the first peak and NADH at the second oxidation peak.*

In Figure 3 are shown the results of differential pulse voltammetry studies on NADH and ascorbate[13] by using 0.07 mM **1d** as a catalyst. For analyte concentrations less than 0.7 mM the first peak current responds linearly to ascorbate concentration and the second peak current responds linearly to NADH concentration. The mechanistic concept underlying these results should provide a guide, and more extensive studies with **1d** and other catalysts, therefore, may reveal a useful analytical method. Although differential pulse voltammetry is widely appreciated as a technique for mixture analysis, it has not, to our knowledge, been used in the presence of catalysts. The combination seems attractive and worthy of further exploration. The unexpected observation of catalysis of NADH oxidation from a chemically irreversible couple also deserves, and will receive, further study.

Acknowledgment. This work was supported by the National Science Foundation. The assistance of Dr. Y. H. So is gratefully acknowledged.

(9) In addition to iminium ions, e.g., **2**, there are three other oxidants which could be involved in the NADH reaction. They are diamine cation radicals, diamine dications, e.g., **1**, minus two electrons, and diamine, e.g., **2a**, minus a proton. The latter is improbable on the basis of the k_{rel} of **1a–c**. The cation radical and dication are more probable, but the k_{rel} of **1a–c** compared to **1d** supports iminium ions as the key oxidants. This problem will be resolved in a future publication.

(10) The electrochemistry of ascorbic acid has received continued attention, using various techniques and materials. See, for example: Pachla, L. A.; Kissinger, P. T. *Anal. Chem.* **1976**, *48*, 364. Perone, S.; Kretlow, W. J. *Ibid.* **1966**, *38*, 1763.

(11) Cheng, H.-Y.; Strope, E.; Adams, R. N. *Anal. Chem.* **1979**, *51*, 2243. Dautartis, M.; Evans, J. F. *J. Electroanal. Chem.* **1980**, *109*, 301. Evans, J. F.; Kuwana, T.; Henne, M. T.; Royer, G. P. *Ibid.* **1977**, *80*, 409.

(12) Ascorbic acid shows very large and irreproducible catalytic waves on the first sweep. Data were, therefore, taken on later cycles.

(13) Differential pulse polarograms of nicotinamide and ascorbic acid have been published: Lindquist, J.; Farroha, S. M. *Analyst (London)* **1975**, *100*, 377.

Reactivity of the $[(\eta^5\text{-}C_5H_5)Ti(Mo_5O_{18})]^{3-}$ Anion: Synthesis and Structure of MoO_2Cl^+ and $Mn(CO)_3^+$ Adducts

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The $[(\eta^5\text{-}C_5H_5)Ti(Mo_5O_{18})]^{3-}$ anion[1,2] (**1**) (see Figure 1) is surprisingly unstable relative to the $VMo_5O_{19}^{3-}$ anion[2,3] which is closely related structurally by replacement of its $[O^{V}]^{3+}$ vanadyl group by a $[(\eta^5\text{-}C_5H_5)Ti^{IV}]^{3+}$ unit. The $VMo_5O_{19}^{3-}$ anion is stable toward moisture and elevated temperature (80 °C) in CH_3CN whereas anion **1** decomposes rapidly under the same conditions. We have therefore examined the structure of anion **1** and its reactivity toward electrophiles in order to better understand the origin of this instability and utilize its reactivity to synthesize new types of polyoxoanion supported organometallic complexes. We report here preliminary results of these studies, which include (a) the first X-ray crystallographic structure determinations of polyoxoanion supported organometallic complexes to be reported in the

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(1) Unless otherwise specified, the $(n\text{-}C_4H_9)_4N^+$ ion was employed as a countercation for all anions discussed in this communication.

(2) Klemperer, W. G.; Shum, W. *J. Chem. Soc., Chem. Commun.* **1979**, 60.

(3) Filowitz, M.; Ho, R. K. C.; Klemperer, W. G.; Shum, W. *Inorg. Chem.* **1979**, *18*, 93.

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