via a $\sigma_{(O-H)}$ bond. For H_2O_2 and hydroquinone as reducing agents there are acidic protons in $\sigma_{(O-H)}$ bonds and redox orbitals which are either σ_{O-O} or π in character.

In a simultaneous electron-proton transfer, intramolecular electronic interactions between $\sigma_{(O-H)}$ and the redox orbitals lead to electronic communication between sites and the correlated transfer of both groups to the oxidant. The critical mode is a $\nu_{(O-H)}$ stretch and the motions of the electron and proton are coupled.

The utilization of a simultaneous electron/proton-transfer pathway places special demands on the oxidant as well as the reductant. The oxidant must be capable of accepting *both* an electron and a proton simultaneously. This requirement is met by either $[(bpy)_2(py)Ru^{IV}(O)]^{2+}$ or $[(bpy)_2(py)Ru^{III}(OH)]^{2+}$ as oxidants. These contain both electron and proton *acceptor* capabilities, which are also based on separate sites. The electron is accepted into a vacancy in the $d\pi$ orbitals. The proton is transferred to the O-atom of, what is initially, the oxo group of Ru(IV) or the hydroxo group of Ru(III).

The reactions in Scheme V stand in contrast to the allylic oxidation of olefins[31a] or the oxidations of HCO_2^- or of $PhCH_2OH$ by $[(bpy)_2(py)Ru^{IV}(O)]^{2+}$. In these reactions H_2O/D_2O kinetic isotope effects are negligible, but large C-H/C-D kinetic isotope effects are observed.[5,31] It has been suggested in these cases that the mechanisms involve direct attack on a C-H bond. The C-H bond serves as the source of both electrons and protons. Both are transferred in the redox step.

We propose that there are at least two distinct mechanisms for simultaneous electron/proton transfer. We also propose that one be called *proton-coupled electron transfer*. In this mechanism (Scheme V) the electron and proton donor functions are located at separate sites in the reductant but are transferred simultaneously. It can be anticipated that other examples will be found based on proton transfer from dissociable -NH or -SH bonds. Although the examples studied here involve one-electron transfer, there is also the possibility that proton-coupled two-electron transfer may occur in some reactions. In a second mechanism involving simultaneous electron-proton transfer, the transfer occurs from the same bond. We propose that the term *hydrogen atom transfer* be reserved for this direct transfer of an e^-/H^+ pair from the same bond of the reductant to the oxidant.

Acknowledgment is made to the National Science Foundation for financial assistance from Grant Nos. CHE-8601604 and CHE-8906794 and from the National Institutes of Health under Grant No. 5-RO1-GM32296-06.

Registry No. BQ, 106-51-4; $[(bpy)_2(py)Ru^{IV}(O)]^{2+}$, 67202-43-1; $[(bpy)_2(py)Ru^{III}(OH)]^{2+}$, 75495-07-7; $[(bpy)_2(py)Ru^{II}(OH_2)]^{2+}$, 67202-42-0; $[(bpy)_2(py)Ru^{II}(NCCH)]^{2+}$, 82769-09-3; $[(bpy)_2(py)Ru^{II}$-(benzoquinone)$]^{2+}$, 116374-54-0; titanium, 7440-32-6; hydroquinone, 123-31-9; 2-chlorohydroquinone, 615-67-8; 2,6-dichlorohydroquinone, 20103-10-0; 2-methylhydroquinone, 95-71-6; 2,6-dimethylhydroquinone, 608-43-5; 2-chloro-2,5-cyclohexadiene-1,4-dione, 695-99-8; 2,6-dichloro-2,5-cyclohexadiene-1,4-dione, 697-91-6; 2-methyl-2,5-cyclohexadiene-1,4-dione, 553-97-9; 2,6-dimethyl-2,5-cyclohexadiene-1,4-dione, 526-86-3.

Supplementary Material Available: Figure 1S, showing the solvolysis of the reduction product $[(bpy)_2(py)Ru^{II}(OH_2)]^{2+}$ by CH_3CN, and Figure 2S, showing the spectrum of the reaction intermediate $[(bpy)_2(py)Ru^{II}(p\text{-benzoquinone})]^{2+}$ (2 pages). Ordering information is given on any current masthead page.

(31) (a) Curry, M.; Dobson, J. C.; Stultz, L.; Meyer, T. J., manuscript in preparation. (b) Dobson, J. C.; Seok, W. K.; Meyer, T. J. *Inorg. Chem.* **1986**, *25*, 1514.

Equilibrium between 2-Oxomorpholin-3-yl Radicals and Viologen Radicals. Determination of Reduction Potentials

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Abstract: Bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (TM-3 dimer) undergoes bond homolysis to yield 3,5,5-trimethyl-2-oxomorpholin-3-yl (TM-3), which reduces propyldiquat (PDQ^{2+}) to its radical cation PDQ^+. The byproduct is 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**8**). Similarly, bi(5,5-dimethyl-4-ethyl-2-oxomorpholin-3-yl) (DEM-3 dimer) cleaves to 5,5-dimethyl-4-ethyl-2-oxomorpholin-3-yl (DEM-3), which reduces paraquat (PQ^{2+}) to its radical cation PQ^+. The byproduct, 5,5-dimethyl-4-ethyl-3-methoxy-2-oxomorpholine (**10**), results from rapid addition of methanol solvent to the transient 5,6-dihydro-4-ethyl-5,5-dimethyl-1,4-oxazin-2-onium cation (**11**). Concentration versus time data for the respective viologen radical cations together with reduction potentials for the viologens place the reduction potentials for TM-3 dimer and DEM-3 dimer at -0.56 and -0.33 V versus NHE, respectively, in Tris/Tris·H^+ buffered methanol. The kinetics of reduction are analyzed using numerical integration, and the two reducing agents are compared with dithionite.

Introduction

3,5,5-Trimethyl-2-oxomorpholin-3-yl (TM-3) is a persistent carbon radical of the captodative[1] or merostablized class,[2] which shows one-electron redox activity with easily reduced substrates.[3] It forms spontaneously upon dissolution of *meso*- or *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*meso*- and *dl*-TM-3 dimers) in solvents ranging from hydrocarbons to water.[4,5] TM-3 exists in equilibrium with *meso*- and *dl*-TM-3 dimers in the absence of

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(1) Viehe, H. G.; Merényi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 917. Viehe, H. G.; Janousek, Z.; Merényi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *12*, 148.

(2) Baldock, R. W.; Hudson, P.; Katritzky, A. R. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422.

(3) Burns, J. M.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 849.

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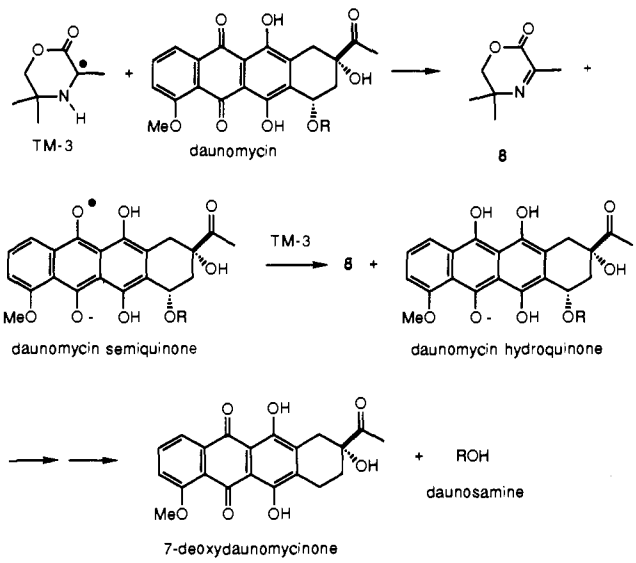
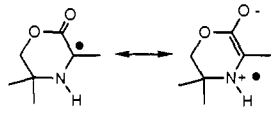
Scheme I



a reducible substrate, and the equilibrium constant for the dissociation is a function of solvent polarity, at its maximum in protic polar solvent (3×10^{-11} M in ethanol at 25 °C).[6] The solvent effects on the equilibrium and the EPR hyperfine coupling constants[5,6] are consistent with a polar structure for the radical, represented in valence bond terms as shown in below.



Redox reactions of TM-3 of particular interest are reductive glycosidic cleavage of anthracyclines to their 7-deoxyaglycons,[7,8] such as the reduction of daunomycin to 7-deoxydaunomycinone shown in Scheme I and the reduction of molecular oxygen to hydrogen peroxide.[9] The former reduction likely occurs by electron transfer with the intermediacy of a semiquinone and a hydroquinone and the latter by a covalent mechanism with the intermediacy of a peroxide. Other substrates which are quantitatively reduced, presumably by single-electron transfer,[3,10] are *N*-methylisatin (**2**) to *N*-methylisatid (**1**), benzil (**3**) to benzoin (**4**), di-*tert*-butyl nitroxide (**5**) to di-*tert*-butylhydroxylamine (**6**), silver cation to silver metal, ferric complexes to ferrous complexes, diphenylpicrylhydrazyl (DPPH) to diphenylpicrylhydrazine (**7**), and viologens to their radical cations (Schemes II–IV). The oxidation product of TM-3 is 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (**8**).[3]

Because of the redox activity of TM-3 and related radicals, establishing the reduction potential became important for predicting reactivity. This is especially true with regard to in vivo applications of 2-oxomorpholinyl radicals.[11] Initial efforts to measure the reduction potential of oxazinone **8** to TM-3 electrochemically were unsuccessful because the reduction was irreversible. The electrochemical experiments did suggest that the reduction potential was in the range of −0.5 to −0.6 V vs the normal hydrogen electrode (NHE).[12] This range indicated that the reduction potential could be established from the equilibrium position of a viologen redox reaction. We describe here the measurement of the redox potential for two oxomorpholinyl radical dimers in methanol at an apparent pH of 7 using the reduction of two viologens to their radical cations.

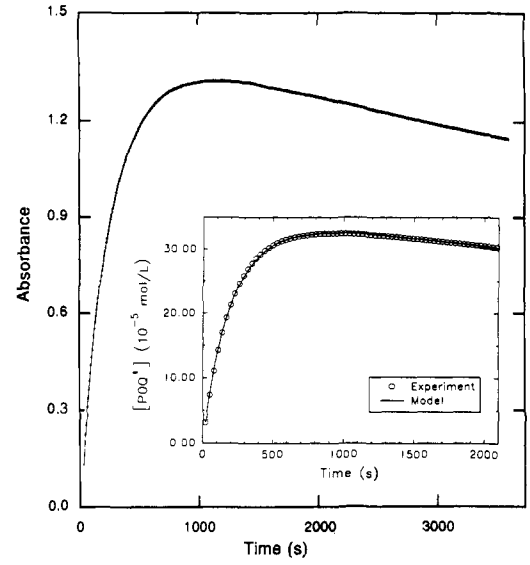
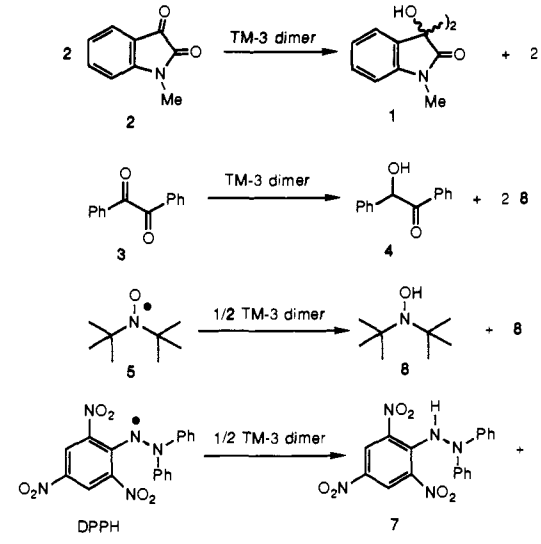


Figure 1. Absorbance at 506 nm versus time for anaerobic reaction of 2.77×10^{-4} M TM-3 dimers (*meso*/*dl* = 1.76) with 4.26×10^{-4} M propyldiquat (PDQ^{2+}) dibromide in 0.010 M Tris/Tris·HCl buffered methanol (apparent pH 7) at 25 °C. The inset shows the concentration of PDQ^+ vs time superimposed on the calculated line resulting from fitting the data to the mechanism shown in Scheme III by numerical integration.

Scheme II

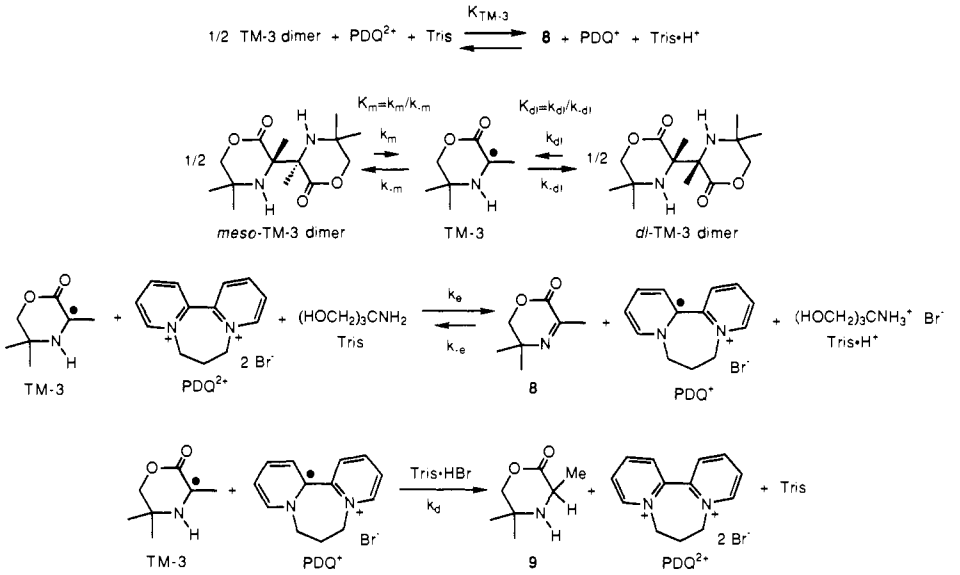


Results and Discussion

Reaction of TM-3 Dimer with Propyldiquat Dibromide. Propyldiquat dibromide ($PDQ^{2+}2Br^-$) was selected for determination of the reduction potential of TM-3 dimer in methanol because its reduction potential in water was known to be −0.55 V vs the normal hydrogen electrode (NHE),[13] within the range estimated for TM-3 dimer. A mixture of TM-3 dimers in the thermodynamic ratio of *meso*/*dl* = 1.75 was reacted with a freeze–thaw-degassed, buffered methanol solution of PDQ^{2+} at 25 °C; the apparent pH was 7, achieved with Tris/Tris·HCl buffer. Formation of the propyldiquat radical cation (PDQ^+) was monitored by visible absorption at λ_{max} 506 nm as a function of time, as shown

(4) Koch, T. H.; Olesen, J. A.; DeNiro, J. *J. Am. Chem. Soc.* **1975**, *97*, 7285.
(5) Himmelsbach, R. J.; Barone, A. D.; Kleyer, D. L.; Koch, T. H. *J. Org. Chem.* **1983**, *26*, 2989.
(6) Olson, J. B.; Koch, T. H. *J. Am. Chem. Soc.* **1986**, *108*, 756.
(7) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380.
(8) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146.
(9) Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1986**, *108*, 5014.
(10) Burns, J. M. Ph.D. Thesis, University of Colorado, Boulder, CO, 1979.
(11) Averbuch, S. D.; Gaudiano, G.; Koch, T. H.; Bachur, N. R. *Cancer Res.* **1985**, *45*, 6200. Averbuch, S. D.; Boldt, M.; Gaudiano, G.; Stern, J. B.; Koch, T. H.; Bachur, N. R. *J. Clin. Invest.* **1988**, *81*, 142.
(12) Watkins, R. L. M.S. Thesis, University of Colorado, Boulder, CO, 1983.
(13) Tsukahara, K.; Wilkins, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 2632.

Scheme III



in Figure 1. The thermodynamic mixture of TM-3 dimers was employed to simplify the kinetics of approach to equilibrium (vide infra). The complete UV–vis spectrum was also periodically monitored, and it showed the presence of a mixture of PDQ^{2+} and PDQ^{+}. The concentration of PDQ^{+} reached a maximum in approximately 1000 s and then began to fall slowly. Subsequent gas chromatographic analysis of the reaction mixture, after opening the reaction vessel and air-oxidizing residual TM-3 dimer, showed the presence of oxazinone **8** and a small amount of 3,5,5-trimethyl-2-oxomorpholine (**9**). Oxomorpholine **9** is the product of one-electron reduction of TM-3 radical[4] and is proposed to result from slow cross-disproportionation of TM-3 with PDQ^{+}, as shown in Scheme III.

The data needed to calculate the reduction potential of oxazinone **8** to form TM-3 dimer were the molar extinction coefficient of PDQ^{+} in methanol at 506 nm, the one-electron reduction potential of PDQ^{2+} in methanol, and the absorbance of the reaction solution at equilibrium in the absence of disproportionation. The molar extinction coefficient of PDQ^{+} at 506 nm in methanol was established as $(4.00 \pm 0.01) \times 10^{-3}$ M^{-1} cm^{-1} by reduction to completion with zinc, and a reduction potential = −562 mV vs NHE was determined in methanol by cyclic voltammetry. Other reducing agents such as sodium dithionite gave lower and less reproducible values for the extinction coefficient. The kinetics of approach to equilibrium for the first two steps of the mechanism shown in Scheme III are apparent first-order when the system is close to equilibrium,[14] and TM-3 dimers at time zero are present in a thermodynamic ratio. Consequently, the absorbance at equilibrium in the absence of disproportionation can be estimated using the Kezdy–Swinbourne method for determination of the absorbance at time infinity (A_∞) for a first-order reaction. The Kezdy–Swinbourne analysis using data from time 445 s to time 535 s gave a linear plot and $A_\infty = 1.306$. A plot of $\ln (A_t - A_\infty)$ versus time was also linear with a slope equal to 6.7×10^{-3} s^{-1}, the apparent first-order rate constant for approach to equilibrium in the absence of disproportionation. The correlation coefficient from the least-squares analysis was 1.000. The calculated A_∞ together with the molar extinction coefficient gave the calculated equilibrium concentrations of PDQ^{2+}, PDQ^{+}, TM-3 dimer, and oxazinone **8** in the absence of disproportionation equal to 9.90×10^{-5}, 3.27×10^{-4}, 1.14×10^{-4}, and 3.27×10^{-4} M, respectively, which established an equilibrium constant of 0.101 $M^{1/2}$. Using

$$K_{TM\text{-}3}[\text{Tris}]/[\text{Tris}\cdot H^+] = [PDQ^+]_{eq}[\text{oxazinone } \mathbf{8}]_{eq}/\{[\text{TM-3 dimer}]_{eq}^{1/2}[PDQ^{2+}]_{eq}\} \quad (1)$$

the ratio $[\text{Tris}]/[\text{Tris}\cdot H^+] = 0.083$, $K_{TM\text{-}3} = 1.22$ $M^{1/2}$. The experiment was repeated by another co-worker using fresh solutions and a $[\text{Tris}]/[\text{Tris}\cdot H^+]$ ratio of 0.075. The average of the two measurements of $K_{TM\text{-}3}$ was 1.11 $M^{1/2}$ ($\sigma_m = 0.08$). The error in reproducibility arises from the error associated with predicting the absorbance at equilibrium in the absence of disproportionation by the Kezdy–Swinbourne method. Although the error is significant, it translates into a small error (0.001 V) in the reduction potential (vide supra). The formal reduction potential ($E^{\circ\prime}$) for the half-reaction:

$$\text{oxazinone } \mathbf{8} + \text{Tris}\cdot H^+ + e^- \rightleftarrows \tfrac{1}{2}\text{TM-3 dimer} + \text{Tris} \quad (2)$$

was then calculated using the Nernst equation with the average equilibrium constant and the reduction potential of PDQ^{2+} in methanol.

$$E^{\circ\prime}(\text{TM-3 dimer}) = E^{\circ\prime}(PDQ^{2+}) - (RT/nF)\ln K_{TM\text{-}3} = -0.56 \text{ V vs NHE}^{15} \quad (3)$$

The analytical expression for the apparent first-order rate constant ($k_{TM\text{-}3}$) in terms of the parameters defined in Scheme III and the concentrations at equilibrium in the absence of disproportionation is as follows:[14]

$$k_{TM\text{-}3} = k_e\{K_mK_{dl}/(K_m + K_{dl})\}^{1/2}\{[\text{TM-3 dimer}]_{eq}^{-1/2}[PDQ^{2+}]_{eq}/4 + [\text{TM-3 dimer}]_{eq}^{1/2}\} + k_{-e}\{[PDQ^+]_{eq} + [\mathbf{8}]_{eq}\} \quad (4)$$

Rate constants defined in Scheme III were estimated by fitting of the concentration of PDQ^{+} vs time data calculated from absorbance vs time data using the numerical integration program FACSIMILE.[16] To simplify the fitting, the first process, bond homolysis of TM-3 dimer, was reduced to single, composite forward and reverse rate constants, $k_{m\text{-}dl}$ and $k_{-(m\text{-}dl)}$, respectively, with a composite equilibrium constant, $K_{m\text{-}dl}$. The quality of the fit is shown in the inset to Figure 1, and the calculated rate constants with 5–95% confidence values are reported in Table I. Confidence values were not determined for $k_{-(m\text{-}dl)}$ because the final step in the fitting procedure fixed the least sensitive rate constant, in this case $k_{-(m\text{-}dl)}$. The value of $k_{m\text{-}dl}$ is in the range of the independently measured rate constants, $k_m = 2.2 \times 10^{-3}$ s^{-1} and $k_{dl} = 3.4 \times 10^{-3}$ s^{-1}.[7,17] The self-consistency of $k_{m\text{-}dl}$, $k_{-(m\text{-}dl)}$,

(14) King, E. *J. Chem. Educ.* **1979**, *56*, 580. For the specific derivation of eqs 4 and 9 using the methodology of E. King, see: Mahoney, R. P. Ph.D. Thesis, University of Colorado, Boulder, CO, 1991.

(15) A sample calculation is as follows: $E^{\circ\prime}(\text{TM-3 dimer}) = -0.562 \text{ V} - (1.987 \times 10^{-3} \text{ kcal deg}^{-1} \text{ mol}^{-1} \times 298 \text{ deg}/(1 \times 23 \text{ kcal V}^{-1}))\ln(1.11) = -0.562 \text{ V} - 0.003 \text{ V} = -0.565 \text{ V}$.

(16) FACSIMILE: A computer program for flow and chemistry simulation and general initial value problems, Harwell Laboratory, Computer Science and System Division, Oxfordshire OX11 0RA, UK.

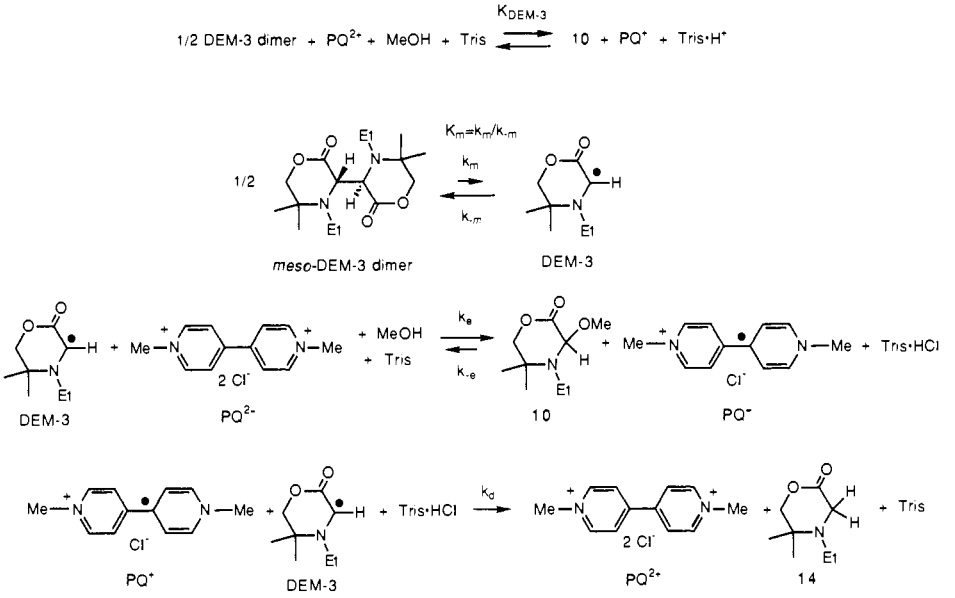
(17) Gaudiano, G.; Koch, T. H. *Chem. Res. Toxicol.* **1991**, *4*, 1.

Table I. Kinetic and Thermodynamic Parameters

radical dimer	K_{TM-3} or K_{DEM-3}[a] (σ_m)	K_{TM-3} or K_{DEM-3}[b] s^{-1} (corr coeff)	$k_{m\cdot dl}$[c] or k_m[d] s^{-1} (5–95% or σ)	$k_{-m\cdot dl}$[c] or k_{-m}[d] M^{-1} s^{-1} (5–95%)	k_e[e] M^{-1} s^{-1} (5–95%)	k_{-e}[e] M^{-1} s^{-1} (5–95%)	k_d[e] M^{-1} s^{-1} (5–95%)
TM-3	1.11 $M^{1/2}$ (0.08)	6.7×10^{-3} (1.00)	3.15×10^{-3} ($(3.00–3.30) \times 10^{-3}$)	2.44×10^{6}	2.73×10^{4} ($(1.99–3.74) \times 10^{4}$)	0.304 (0.93–0.315)	4.83×10^{3} ($(3.51–6.67) \times 10^{3}$)
DEM-3	5.2×10^{-3} $M^{-1/2}$ (0.1×10^{-3})	5.7×10^{-4} (1.00)	2.74×10^{-4} (0.004×10^{-4})	3.10×10^{6} ($(0.80–12.02) \times 10^{6}$)	6.50×10^{3} ($(3.31–12.80) \times 10^{3}$)	5.67 (5.64–5.72)	2.16×10^{2} ($(1.09–4.32) \times 10^{2}$)

[a] Defined in eqs 1 and 6. [b] Defined in eqs 4 and 9. [c] Defined in text. [d] Defined in Scheme IV. [e] Defined in Schemes III and IV.

Scheme IV



k_e, and k_{-e} were checked by using them to calculate k_{TM-3} and K_{TM-3} as defined in eqs 1 and 4, substituting $K_{m\cdot dl}$ for $K_m K_{dl}/(K_m + K_{dl})$. The calculated values are 1.3×10^{-2} s^{-1} and 3.2 $M^{1/2}$, respectively, in modest agreement with the observed values; the observed values are approximately 50% and 65% lower, respectively. A major source of error is the uncertainty in $k_{-(m\cdot dl)}$ as can be seen in Table I for the corresponding rate constant, k_{-m}, for recombination of DEM-3 radicals defined in Scheme IV (vide infra). The rate constants k_e and k_{-e} define an equilibrium constant $K_e = k_e/k_{-e} = 8.9 \times 10^4$ for the reduction of PDQ^{2+} by TM-3 radical and, correspondingly, an $E^{\circ\prime}$ of –0.85 V for the half-reaction:

$$\text{oxazinone } \mathbf{8} + \text{Tris}\cdot H^+ + e^- \rightleftarrows \text{TM-3} + \text{Tris} \qquad (5)$$

An additional self-consistency check of the rate constants from the numerical integration is a comparison of the ΔG° value for the bond homolysis of TM-3 dimer calculated from the difference in the two reduction potentials and from the two rate constants, $k_{m\cdot dl}$ and $k_{-(m\cdot dl)}$. The former is 13 and the latter is 12 kcal/mol, both at 25 °C. The value determined earlier in an ethanol solvent from absolute spin concentration measurements by EPR spectroscopy is 14 kcal/mol;[6] a value slightly less is expected in methanol because the rate constant for bond homolysis is approximately 3 times larger in methanol.

Reaction of DEM-3 Dimer with Paraquat Dichloride. 5,5-Dimethyl-4-ethyl-2-oxomorpholin-3-yl (DEM-3) exists in equilibrium with *meso*-bi(5,5-dimethyl-4-ethyl-2-oxomorpholin-3-yl) (*meso*-DEM-3 dimer).[18] DEM-3 differs structurally from TM-3 by the placement of an alkyl group at the 4-position rather than the 3-position of the morpholine ring. Consequently, DEM-3 bears a tertiary amine functionality and is a secondary captodative carbon radical. Unlike TM-3 dimer, DEM-3 dimer exists exclusively as the meso isomer in methanol solution. DEM-3 was thought to be a poorer reducing agent than TM-3 because it did not reduce daunomycin to 7-deoxydaunomycinone, even with a substantially longer reaction time and 10 °C higher reaction temperature; however, the lack of reactivity might have been for kinetic rather than thermodynamic reasons. DEM-3 dimer did reduce propyldiquat as indicated by the appearance of blue color and a characteristic EPR signal upon mixing DEM-3 dimer with PDQ^{2+} in methanol under anaerobic conditions. Initial experiments to measure the equilibrium constant indicated that the position of the equilibrium was heavily toward PDQ^{2+}; consequently, the more easily reduced viologen, paraquat dichloride ($PQ^{2+}2Cl^-$), was selected to determine the reduction potential.

Mixing DEM-3 dimer with PQ^{2+} in anaerobic methanol at 25 °C and an apparent pH of 7.3 produced the characteristic UV–vis

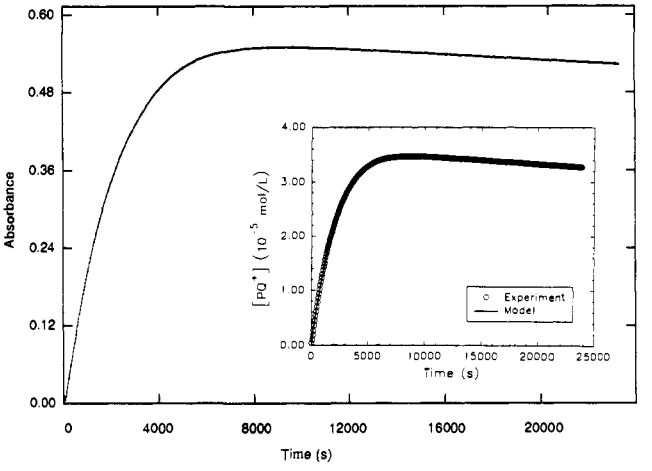
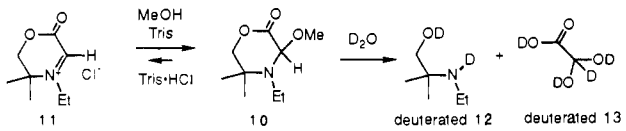


Figure 2. Absorbance at 606 nm versus time for anaerobic reaction of 4.30×10^{-5} M *meso*-DEM-3 dimer with 6.11×10^{-5} M paraquat (PQ^{2+}) dichloride in 0.010 M Tris/Tris·HCl buffered methanol (apparent pH 7) at 25 °C. The inset shows the concentration of PQ^+ vs time superimposed on the calculated line resulting from fitting the data to the mechanism shown in Scheme IV by numerical integration.

(18) Benson, O., Jr.; Demirdji, S. H.; Haltiwanger, R. C.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 8879.

spectrum of the radical cation PQ^+. The absorbance at λ_{max} 606 nm for the radical cation as a function of time is shown in Figure 2, and the reaction mechanism is shown in Scheme IV. Again, the pH was achieved with Tris/Tris·HCl buffer. The absorbance versus time plot indicated much slower cross-disproportionation of DEM-3 and PQ^+ at longer reaction times than was observed for TM-3 and PDQ^+.

The product from oxidation of DEM-3 by paraquat dichloride was assigned the structure, 5,5-dimethyl-4-ethyl-3-methoxy-2-oxomorpholine (**10**), resulting from addition of the methanol solvent to transient 5,5-dimethyl-4-ethyl-1,4-oxazin-2-onium chloride (**11**) on the basis of the following observations. The ^1H NMR spectrum of the residue from solvent evaporation dissolved in $CDCl_3$ showed a mixture of DEM-3 dimer and **10**. Although **10** could not be isolated, a clean ^1H NMR reference spectrum without interference from DEM-3 dimer signals was produced by oxidation of DEM-3 dimer with diphenylpicrylhydrazyl (DPPH) in methanol solvent. The reference spectrum was completely consistent with the assigned structure and included a methoxy singlet and an ethyl ABX_3 pattern. The superposition of this reference spectrum on the spectrum of DEM-3 dimer plus Tris accounted for greater than 90% of the material originating from DEM-3 dimer present in the paraquat reaction mixture residue. In a second experiment, the methanol solvent was rapidly rotary evaporated from a reaction mixture at equilibrium, and the residue was extracted with deuterium oxide and deuteriochloroform. ^1H NMR analysis of the extraction solutions showed the presence of DEM-3 dimer and a product of the hydrolysis of **10**, 2-(ethylamino)-2-methylpropanol (**12**), in the organic phase and paraquat dichloride and a small amount of 2-(ethylamino)-2-methylpropanol hydrochloride in the aqueous phase. The other anticipated product of the hydrolysis of **10**, glyoxal monohydrate (**13**), was not observed in the NMR spectrum of either phase, presumably because of rapid deuterium exchange of the proton at the 3-position of **10** prior to hydrolysis. A control experiment showed that glyoxal monohydrate did not undergo rapid deuterium exchange of the proton at the 2-position. Integration of the spectra indicated that the conversion of DEM-3 dimer to **10** at equilibrium was approximately 40%.



As shown in Figure 2, the apparent subsequent disproportionation of DEM-3 with PQ^+ was slower than the disproportionation of TM-3 with PDQ^+. In spite of disproportionation being relatively slow, the equilibrium point in the absence of disproportionation was still calculated using the Kezdy–Swinbourne method over the time period 2990 to 5090 s with a τ value of 1770 s. The plot of A_t vs $A_{t+\tau}$ was linear, and the slope and intercept gave an A_∞ value of 0.558 versus the observed A_{max} value of 0.545. The A_∞ value was used to calculate the concentration of PQ^+ at equilibrium in the absence of disproportionation. Again, the extinction coefficient of PQ^+ at 606 nm in methanol was determined from anaerobic reduction of a known concentration of PQ^{2+} with an excess of zinc, and the reduction potential of PQ^{2+} in methanol was measured by cyclic voltammetry. From the extinction coefficient and A_∞, the equilibrium concentrations of PQ^{2+}, DEM-3 dimer, and PQ^+, equal to the concentration of **10**, were calculated to be 2.49×10^{-5}, 2.49×10^{-5}, and 3.62×10^{-5} M, respectively. These concentrations established an equilibrium

$$K_{DEM\text{-}3}[MeOH][Tris]/[Tris{\cdot}H^+] = [PQ^+]_{eq}[\mathbf{10}]_{eq}/\{[DEM\text{-}3\ dimer]_{eq}^{1/2}[PQ^{2+}]_{eq}\} \quad (6)$$

constant of 1.05×10^{-2} $M^{1/2}$. The equilibrium concentrations showed 42% conversion of DEM-3 dimer to methoxymorpholine **10** and are consistent with the yield of 2-(ethylamino)-2-methylpropanol (**12**) from the extraction experiment (vide supra). The determination of the equilibrium constant was repeated using fresh solutions. The average of the two measurements of $K_{DEM\text{-}3}[MeOH][Tris]/[Tris{\cdot}H^+]$ was 1.08×10^{-2} $M^{1/2}$ ($\sigma_m = 0.02 \times 10^{-2}$). Reproducibility was better in this measurement because of the smaller rate constants for reaction and disproportionation and, consequently, the better prediction of the absorbance at equilibrium in the absence of disproportionation. Using a concentration of methanol equal to 25 M, determined from the density at 25 °C, the ratio of [Tris]/[Tris·H⁺] = 0.083 gives a $K_{DEM\text{-}3}$ value of 5.2×10^{-3} $M^{-1/2}$. The formal reduction potential ($E^{\circ\prime}$) for the half-reaction:

$$\mathbf{10} + Tris{\cdot}H^+ + e^- \rightleftarrows \tfrac{1}{2}DEM\text{-}3\ dimer + MeOH + Tris \quad (7)$$

was then calculated using the Nernst equation with the average equilibrium constant and the reduction potential of PQ^{2+} in methanol, −465 mV (average literature value in water −0.45 V[13]).

$$E^{\circ\prime}(DEM\text{-}3\ dimer) = E^{\circ\prime}(PQ^{2+}) - (RT/nF)\ln(K_{DEM\text{-}3}) = -0.33\ V\ vs\ NHE \quad (8)$$

The rate of approach to equilibrium in the time period from 3140 to 4400 s was first-order, and a plot of ln $(A_t - A_\infty)$ vs time gave an apparent first-order rate constant of 5.7×10^{-4} s^{-1}. The analytical expression for the apparent first-order rate constant ($k_{DEM\text{-}3}$) in terms of the parameters defined in Scheme IV and the concentrations at equilibrium is as follows:[14]

$$k_{DEM\text{-}3} = k_e K_m^{1/2}\{[DEM\text{-}3\ dimer]_{eq}^{-1/2}[PQ^{2+}]_{eq}/4 + [DEM\text{-}3\ dimer]_{eq}^{1/2}\} + k_{-e}\{[PQ^+]_{eq} + [\mathbf{10}]_{eq}\} \quad (9)$$

The acid–base solvolysis step is presumed to occur rapidly. Of the terms in eq 9, the concentrations at equilibrium in the absence of disproportionation were established as described above. The k_m portion of K_m was measured independently by trapping the DEM-3 radical as it was formed with DPPH to produce diphenylpicrylhydrazine, measuring the change in the optical density of the DPPH at 516 to 520 nm. Regression analysis[19] of A_t versus time gave $k_m = 2.74 \times 10^{-4}$ s^{-1} ($\sigma = 0.004 \times 10^{-4}$). The other rate constants defined in Scheme IV were determined by a fitting of the concentration of PQ^+ vs time data calculated from absorbance vs time data using the numerical integration program FACSIMILE.[15] The quality of the fit is shown in the inset to Figure 2, and the calculated rate constants together with the 5–95% confidence values are reported in Table I. The self-consistency of k_{-m}, k_e, and k_{-e} were checked by using them together with the measured value of k_m to calculate $k_{DEM\text{-}3}$ and $K_{DEM\text{-}3}$, defined in eqs 6 and 9. The calculated values are 7.9×10^{-4} s^{-1} and 1.08×10^{-2} $M^{1/2}$, respectively, and agree with the observed values well within the 5–95% confidence range; the observed values are 30% and 50% lower, respectively. The rate constants k_e and k_{-e} define an equilibrium constant $K_e = k_e/k_{-e} = 1.15 \times 10^3$ for reduction of PQ^{2+} by DEM-3 radical and, correspondingly, an $E^{\circ\prime}$ of −0.65 V for the half-reaction:

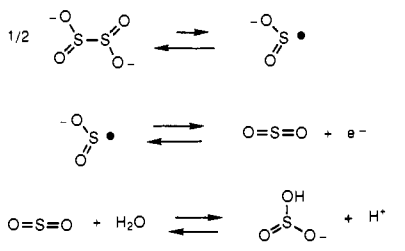
$$\mathbf{10} + Tris{\cdot}H^+ + e^- \rightleftarrows DEM\text{-}3 + MeOH + Tris \quad (10)$$

Again, an additional self-consistency check of the rate constants from the numerical integration is a comparison of the ΔG° value for the bond homolysis of DEM-3 dimer calculated from the difference in the two reduction potentials and from the two rate constants, k_m and k_{-m}. The former is 15 and the latter is 14 kcal/mol, both at 25 °C.

Discussion of the Reduction Potentials. The $E^{\circ\prime}$ values for TM-3 and DEM-3 dimers differ by 0.23 V, with TM-3 dimer being the stronger reducing agent. The origin of the difference resides significantly in differences in the thermodynamics of final product formation, oxazinone **8** versus methoxymorpholine **10**, including solvation effects. Some effect, 1–2 kcal/mol, also resides in differences in the free energy for the initial bond homolysis. For both reductions, the overall thermodynamics will be a function of the pH since a proton is released. The solvent will also be an important factor. In reductions with DEM-3 dimer, the solvent

(19) *Regression*, Blackwell Scientific Software, Blackwell Scientific Publications, Osney Mead, Oxford OX2 0EL, UK.

Scheme V



is incorporated into the final product, and for both reducing agents solvent significantly influences bond homolysis. For TM-3 dimer, $\Delta G°$ for bond homolysis is 14 kcal/mol in ethanol, 18 kcal/mol in 1,2-dimethoxyethane, and 21 kcal/mol in benzene at 25 °C.[6]

Even though the redox potentials are dependent upon the presence of the buffer and the nature of the solvent, they can be used to explain the successful reduction of Tris/Tris·HCl buffered methanol solutions of daunomycin with TM-3 dimer and unsuccessful reduction with DEM-3 dimer. The value in the literature for the reduction potential of daunomycin varies widely, −0.31 to −0.46 V vs NHE, depending on solvent and method.[20-23] The overall two-electron reduction to the hydroquinone state can occur in one-electron steps (Scheme I); electrochemical measurements in water at pH 7.1 give a value for the peak potential of −0.42 V and indicate that the first step is more difficult than the second.[23] Assuming that methanol versus water has little effect on the reduction potential of daunomycin, the reduction potentials for the radical dimers determined here are consistent with exergonic reduction by TM-3 dimer and endergonic reduction by DEM-3 dimer. Because the hydroquinone of daunomycin undergoes irreversible glycosidic cleavage as shown in Scheme I, reductive cleavage should still be possible with the weaker reducing agent DEM-3 dimer, although the reaction should be slower. In fact, reaction of daunomycin for 60 h at 60 °C with excess reducing agent yielded 52% 7-deoxydaunomycinone and 9% recovered daunomycin along with a number of anthracycline-type byproducts; disproportionation of the DEM-3 dimer also occurred as indicated by the formation of 4-ethyl-5,5-dimethyl-2-oxomorpholine (**14**). Oxomorpholine **14** was characterized earlier as an intermediate in the synthesis of DEM-3 dimer.[18] Hence, disproportionation of DEM-3 competed with reduction of daunomycin. This result contrasts with the reduction of daunomycin by TM-3 dimer, which is quantitative and complete in 1 h at 25 °C.[7]

The values for the reduction potentials of TM-3 and DEM-3 dimers determined here suggest that these reducing agents should be able to reduce the flavin electrophore in one-electron steps[24] but not the NAD^+ electrophore in one-electron steps.[25]

A close inorganic analogue to the TM-3 and DEM-3 dimers in terms of redox activity is dithionite. Dithionite also exists in equilibrium with a radical, $SO_2^{\bullet-}$, which is a one-electron reducing agent, and the product, SO_2, hydrates to bisulfite as shown in Scheme V. Dithionite is a reducing agent with a reduction potential in the region of pH 7 similar to that of TM-3 dimer. However, in 1 M hydroxide solution it is a substantially stronger reducing agent: $E^{0\prime} = -1.12$ V.[26] At pH 7 the midpoint potential, E_m, is concentration dependent; with 1×10^{-5} M dithionite, E_m is calculated to be −0.54 V, and with 1×10^{-3} M dithionite, −0.48 V vs NHE.[27] The effect of the bond homolysis and the solvolysis steps are apparent from the potentials for portions of the overall process: the potential for the couple SO_2/SO_2^- is estimated to be −0.26 V,[28] and for the couple HSO_3^-/SO_2^-, −0.66 V.[27] Obviously, the bond homolysis step makes the potential more positive and the solvolysis more negative relative to the SO_2/SO_2^- couple. These effects with regard to the reducting agents TM-3 and DEM-3 are similar. In 1 M proton solution, dithionite is a substantially weaker reducing agent, $E^{0\prime} = -0.082$ V,[26] resulting from the protonation of $S_2O_4^{2-}$, which inhibits the bond homolysis step. Similarly, protonation of TM-3 and DEM-3 dimers on one nitrogen substantially inhibits the bond homolysis[29] because the protonated incipient radical is no longer stabilized by its nitrogen lone pair of electrons. The effect of pH was evident from the observation that reduction of PQ^{2+} with DEM-3 dimer proceeded only about 5% in the absence of buffer in contrast to 40% in the presence of pH 7 buffer.

Reductions with the TM-3 and DEM-3 dimers differ from reductions by dithionite in terms of the rate of reductions. The observed rate constant is either the rate constant for bond homolysis or the rate constant for reduction times the equilibrium constant for bond homolysis, depending upon the rate of radical recombination versus the rate of reduction. TM-3 dimer can be semiquantitatively compared with dithionite. The respective equilibrium constants for the bond homolyses are 3.2×10^{-11} M for TM-3 dimer in ethanol at 25 °C[6] and 1.4×10^{-9} M for $S_2O_4^{2-}$ in water at 25 °C.[30] The equilibrium constant for TM-3 dimer in water is probably at least 1 order of magnitude larger because the rate constant for bond homolysis is 1 order of magnitude larger.[29] Then, assuming equal rate constants for reduction of the substrate by the respective radical and reversible bond homolysis, reductions with TM-3 dimer in water will be about 5 times slower than reductions with dithionite. The equilibrium constant for the bond homolysis of DEM-3 dimer has not been measured directly in any solvent, but it appears to be smaller than that for TM-3 dimer on the basis of the relative EPR signal intensity of the DEM-3 radical in ethanol solutions of DEM-3 dimer[18] and the rate constant for bond homolysis, which is 1 order of magnitude smaller. The calculations described above place it about 15 times smaller in methanol solvent. Dithionite differs from the 2-oxomorpholinyl radical dimers in that dithionite is a reducing agent itself for some substrates without prior bond homolysis.[30,31]

Experimental Section

General Remarks. UV and visible absorption spectra were recorded with a Hewlett-Packard 8450A or 8452A diode array spectrophotometer using 10-mm square quartz cuvettes unless otherwise specified. ^1H NMR spectra were obtained using a Varian VXR-300 spectrometer; chemical shifts are reported in ppm on the δ scale using tetramethylsilane as a reference, and coupling constants are reported in hertz. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All pH measurements were performed with a Fisher Accumet Model 825MP pH meter. Capillary gas chromatography (GC) was performed with a Hewlett-Packard 5790A chromatograph equipped with a 25-m Hewlett-Packard Ultra 2 column, 0.32-mm i.d. with a 0.17-μm thickness of 5% cross-linked phenyl methyl silicone stationary phase. The temperature program was 70 °C for 1 min followed by a ramp of 5 °C/min to 115 °C, which was held for 2.8 min.

Methanol and dichloromethane were spectrophotometric grade, and the water was HPLC grade. Prepurified argon was used to oxygen-degas solutions. 1,1′-Propylene-2,2′-bipyridylium dibromide (propyldiquat dibromide, $PDQ^{2+}2BR^-$) was prepared as described by Homer and Tomlinson.[32] Elemental analysis showed the material to be in the monohydrate form with a formula weight of 376.08 g/mol. 1,1′-Dimethyl-4,4′-bipyridylium dichloride (paraquat, $PQ^{2+}2Cl^-$) was obtained from Aldrich, and elemental analysis showed it to be hydrated, with the formula $C_{12}H_{14}N_2Cl_2 \cdot 2.7H_2O$ and having a formula weight of 305.81 g/mol. *meso*- and *dl*-bi(3,5,5-trimethyl-2-oxomorpholin-3-yl) (*meso*- and *dl*-TM-3 dimer) were prepared and separated as described earlier.[33]

(20) Berg, H.; Horn, G.; Jacob, H.-E.; Fiedler, U.; Luthardt, U.; Tresselt, D. *Bioelectrochem. Bioenerg.* **1986**, *16*, 135.
(21) Anne, A.; Moiroux, J. *Nouv. J. Chim.* **1985**, *9*, 259.
(22) Svingen, B. A.; Powis, G. *Arch. Biochem. Biophys.* **1981**, *209*, 119.
(23) Rao, G. M.; Lown, J. W.; Plambeck, J. A. *J. Electrochem. Soc.* **1978**, *125*, 534.
(24) Edmondson, D. E.; Singer, T. P. *J. Biol. Chem.* **1973**, *248*, 8144.
(25) Bresnahan, W. T.; Elving, P. J. *J. Am. Chem. Soc.* **1981**, *103*, 2379.
(26) Huheey, J. E. *Inorganic Chemistry: Principles of Structure and Reactivity*; Harper & Row: New York, 1972; pp 257–266.
(27) Mayhew, S. G. *Eur. J. Biochem.* **1978**, *85*, 535.
(28) Stanbury, D. M.; Lednicky, L. A. *J. Am. Chem. Soc.* **1984**, *106*, 2847.
(29) Gaudiano, G.; Koch, T. H. Unpublished work.
(30) Lambeth, D. O.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 6095.
(31) Mehrotra, R. N.; Wilkins, R. G. *Inorg. Chem.* **1980**, *19*, 2177. Scaife, C. W. J.; Wilkins, R. G. *Inorg. Chem.* **1983**, *22*, 3990. Bradic, Z.; Wilkins, R. G. *J. Am. Chem. Soc.* **1984**, *106*, 2236.
(32) Homer, R. F.; Tomlinson, T. E. *J. Chem. Soc.* **1960**, 2498.

meso-Bi(5,5-dimethyl-4-ethyl-2-oxomorpholin-3-yl) (*meso*-DEM-3 dimer) and 2-(ethylamino)-2-methylpropanol were prepared as described elsewhere.[18] Ferrocene was sublimed before use. All other chemicals were used as received from Aldrich, Mallinkcrodt, J. T. Baker, or Fisher Scientific.

Kinetic and thermodynamic measurements were performed in two-compartment cells with one compartment a cuvette; the two compartments were used to separate the reagents during degassing. The cells were equipped with a 9-mm tube for attachment to a vacuum line with an Ultra-torr union. Reaction solutions were freeze–pump (6×10^{-6} Torr or less)–thaw–sonicate degassed and sealed with a torch. The reagents were equilibrated to 25 °C before mixing.

The ratio (0.0833) of tris(hydroxymethyl)aminomethane (Tris) to tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) that would make a pH 7.0 buffer solution in water was used uncorrected in methanol solvent. A 0.100 M buffer solution consisted of 3.64 g of Tris·HCl and 0.23 g of Tris in 250 mL of methanol. The resulting apparent pH was 7.3 as determined with a saturated KCl glass electrode.

Kinetics of Reaction of DEM-3 Dimer with DPPH. The measurement was performed as described in detail earlier for DEM-3 dimer in ethanol solvent.[18] A solution of diphenylpicrylhydrazyl (DPPH) in 3.0 mL of spectral grade methanol was prepared such that the optical density at 750 nm was 1.4. This solution was placed in the side arm of a two-compartment cell. Solid DEM-3 dimer, approximately 0.1 mg, was introduced into the cuvette chamber. The methanolic solution was freeze–thaw–degassed through four cycles and sealed with a torch. The solution and cell holder were equilibrated at 25 °C, and then the contents of the two compartments were vigorously mixed. The average absorbance from 740 to 760 nm was monitored as a function of time. Less than half of the DPPH was destroyed during the reaction. Nonlinear regression analysis of the data gave the first-order rate constant, $k_m = (2.74 \pm 0.004) \times 10^{-4}$ s^{-1}.

Reductions of PDQ^{2+} and PQ^{2+} with Zinc. The buffer solution previously described was diluted to 10% of its original concentration with methanol, resulting in a 0.010 M Tris/Tris·HCl pH 7 buffered methanol solution. A propyldiquat (PDQ^{2+}) stock solution was prepared by addition of 11.79 mg of PDQ^{2+} to the buffered methanol in a 100-mL volumetric flask. The optical chamber of a large two-compartment cell was charged with 1.10 mg of zinc dust (−325 mesh). The second chamber was filled with 20 mL of the 3.13×10^{-4} M PDQ^{2+} solution. The cell was sealed with a rubber serum stopper and the solution oxygen-degassed by purging with argon for at least 10 min. The reagents were then mixed, allowing the reduction to occur. The optical density at 506 nm was monitored as a function of time while the cell was periodically removed and shaken to promote the heterogeneous reaction. The maximum absorbance (1.255) gave an extinction coefficient for PDQ^+ of $\epsilon_{506} = 4.01 \times 10^3$ M^{-1} cm^{-1}. Two additional measurements of ϵ_{506} with fresh solutions gave values of 3.99×10^3 and 4.01×10^3 M^{-1} cm^{-1}.

This method was similarly used to determine the extinction coefficient for paraquat radical cation, PQ^+, at 606 nm in methanol. Three separate measurements gave the values: $\epsilon_{606} = 1.59 \times 10^4$, 1.55×10^4, and 1.56×10^4 M^{-1} cm^{-1}.

Cyclic Voltammetry (CV) of Propyldiquat and Paraquat in Methanol. A BAS-100 electrochemical analyzer was used for all CV measurements. The Ag/Ag^+ reference electrode consisted of a small Pyrex tube with a frit at the bottom filled with silver nitrate reference solution with a silver wire immersed in it. The reference solution was 49.0 mM $AgNO_3$ and 101 mM LiCl in methanol. The auxiliary electrode was a coiled platinum wire, and the working electrode was the glassy carbon electrode supplied with the BAS-100 analyzer. The analytical samples were 101 mM LiCl as the supporting electrolyte, 4.9 mM ferrocene as an internal reference, and either 5.1 mM propyldiquat dibromide or 5 mM paraquat dichloride in methanol solvent. The samples, 10 mL in 5-dram vials wrapped loosely with Parafilm around the electrode wires, were oxygen-degassed with nitrogen for 5 min prior to analysis. The nitrogen was shut off 30 s before starting a scan to prevent unwanted mixing in the sample. The first sweep segment was +100 to −1300 mV, and then the second segment returned from −1300 to +100 mV, all at a speed of 100 mV/s. Both propyldiquat and paraquat were reversibly reduced with peak separations comparable to those of the internal ferrocene signals. This allowed the determination of $E°'(PDQ^{2+}/PDQ^+) = -962$ mV vs fer^+/fer and $E°'(PQ^{2+}/PQ^+) = -865$ mV vs fer^+/fer in methanol. These $E°'$ values referenced internally to ferrocene were then converted to the normal hydrogen electrode (NHE) scale by adding +400 mV to the ferrocene scale value.[34] The values were $E°'(PDQ^{2+}/PDQ^+) = -562$ mV vs NHE and $E°'(PQ^{2+}/PQ^+) = -465$ mV vs NHE.

Reaction of TM-3 Dimers with Propyldiquat. A methanolic solution of *meso* and *dl*-TM-3 dimers in the thermodynamic ratio, *meso*/*dl* = 1.75, was prepared to eliminate complications in the kinetics from equilibration during the reaction. The thermodynamic ratio was determined by integration of the ^{1}H NMR spectrum of an oxygen-degassed solution in methanol-d_4 at equilibrium. A solution, 8.30×10^{-4} M in TM-3 dimer, was prepared by dissolving 7.53 mg of *meso*- and 4.27 mg of *dl*-TM-3 dimer in 50 mL of dichloromethane (*meso*/*dl* = 1.76). The buffer solution previously described was diluted to 10% of its original concentration with methanol, giving a pH 7, 0.010 M Tris/Tris·HCl buffered methanol solution. A propyldiquat stock solution was made by adding 16.02 mg of propyldiquat dibromide monohydrate to the buffer solution in a 100-mL volumetric flask. A two-component cell was charged by evaporation of 1.0 mL of the TM-3 dimer stock solution onto the walls of the optical cell with a stream of nitrogen, followed by addition of 3.0 mL of the PDQ^{2+} stock solution to the second compartment. The solution was freeze–thaw-degassed through five cycles, sealed, and equilibrated to 25 °C. The contents of the compartments were mixed as the spectral data accumulation was started. The optical density at 506 nm of the solution at 25 °C was measured every 5 s for 3000 s as shown in Figure 1. Capillary GC analysis of the reaction mixture allowed to react past the equilibrium point showed the presence of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one and 3,5,5-trimethyl-2-oxomorpholine by coinjection with authentic samples.[4]

Reaction of *meso*-DEM-3 Dimer with Paraquat. The DEM-3 dimer exists predominantly in the meso form; equilibration with the *dl* isomer was inconsequential. A radical dimer solution was made by dissolving 2.02 mg of *meso*-DEM-3 dimer in 50 mL of dichloromethane in a volumetric flask. A stock paraquat solution was prepared by adding 1.87 mg of paraquat dichloride hydrate to 10 mL of the stock Tris-buffered methanol solution and diluting to 100 mL with methanol in a volumetric flask. The two-compartment cell was prepared as described for the reaction of TM-3 dimers with PDQ^{2+}. The optical density at 606 nm of the reaction mixture at 25 °C was measured every 30 s for 23 000 s as shown in Figure 2.

Determination of the Product Structure from Oxidation of DEM-3 Dimer with Paraquat. A solution of 0.0406 g (1.33×10^{-4} mol) of paraquat dichloride hydrate and 0.0322 g (2.66×10^{-4} mol) of tris(hydroxymethyl)aminomethane in 16.0 mL of spectral grade methanol was introduced into one compartment of a two-compartment apparatus. Into the second compartment was placed 0.0207 g (6.63×10^{-5} mol) of solid DEM-3 dimer. The methanol solution was freeze–thaw-degassed through three cycles, and the apparatus was sealed with a torch. The reagents were mixed and the reaction mixture was left at ambient temperature in the dark for 2 h; during this time the reaction mixture turned dark blue. The seal was broken and the solvent was removed by rotary evaporation. The ^{1}H NMR spectrum of the residue in $CDCl_3$ showed 40% *meso*-DEM-3 dimer and 60% 5,5-dimethyl-4-ethyl-3-methoxy-2-oxomorpholine (**10**). The presence of **10** was established by comparison of the NMR spectrum of the mixture with individual spectra of *meso*-DEM-3 dimer and of a sample of **10** produced by oxidation of DEM-3 dimer with diphenylpicrylhydrazyl (DPPH).[17] The ^{1}H NMR spectra in $CDCl_3$ were as follows: **10**, δ 1.09 (t, X part of an ABX_3 pattern, $J_{AX} = J_{BX} = 7$), 1.13 (s, 6 H), 2.82 (d of q, B part of ABX_3 pattern, $J_{BX} = 7$, $J_{AB} = 15$), 2.92 (d of q, A part of ABX_3 pattern, $J_{AX} = 7$, $J_{AB} = 15$), 3.32 (s, 3 H), 3.87 (d, $J = 11$, 1 H), 4.41 (s, 1 H), 4.49 (d, $J = 7$, 1 H); *meso*-DEM-3 dimer, δ 1.09 (s, 3 H), 1.10 (s, 3 H), 1.13 (t, X part of an ABX_3 pattern, $J_{AX} = J_{BX} = 7$, 3 H), 2.84 (A part of an ABX_3 pattern, $J_{AB} = 15$, $J_{AX} = 7$, 1 H), 3.75 (d, $J = 11$, 1 H), 3.93 (s, 1 H), 4.30 (d, $J = 11$, 1 H).

A similar reaction was performed with no tris(hydroxymethyl)-aminomethane present and with nitrogen degassing. During the 2-h reaction period, the solution turned light blue; ^{1}H NMR analysis of the residue from solvent evaporation showed only 6% formation of **10**.

2-(Ethylamino)-2-methylpropanol Hydrochloride. A 20-mg sample of 2-(ethylamino)-2-methylpropanol[17] was dissolved in 5 mL of 1 N aqueous hydrochloric acid; the solvent was then evaporated on a rotary evaporator equipped with a mechanical pump. The residue was identified as 2-(ethylamino)-2-methylpropanol hydrochloride from its ^{1}H NMR spectrum: (D_2O) δ 1.13 (t, $J = 7.5$, 3 H), 1.14 (s, 6 H), 2.90 (q, $J = 7.5$, 2 H), 3.44 (s, 2 H).

Reaction of DEM-3 Dimer with Paraquat Followed by Extraction with $CDCl_3/D_2O$. A. Unbuffered Methanol Medium. An argon-degassed solution of 17.33 mg of paraquat in 10.0 mL of methanol was prepared, and 8.85 mg of DEM-3 dimer was added while argon was flushed out of the flask. The solution turned light blue, and after 112 min of reaction time the vial was opened. The solvent was rotary evaporated, and the residue was dissolved in a two-layer solvent system consisting of 1 mL of D_2O and 1 mL of $CDCl_3$. The solute in the organic phase was iden-

(33) Bennett, R. W.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1980**, *102*, 2345.

(34) Gagné, R. R.; Koval, C. A.; Lisensky, G. C. *Inorg. Chem.* **1980**, *19*, 2855.

tified as ca. 99% unreacted DEM-3 dimer by ^{1}H NMR (vide supra). The components of the aqueous phase were identified by ^{1}H NMR as paraquat (δ 4.35 (s, 3 H), 8.37 (d, J = 7, 2 H), 8.90 (d, J = 7, 2 H)) and 2-(ethylamino)-2-methylpropanol hydrochloride (spectrum identical to that described above). Integrals of the NMR signals for paraquat and the amino alcohol indicated a 2.5% conversion of DEM-3 dimer to the amino alcohol.

B. Buffered Methanol Medium. The reaction was performed in pH 7, Tris-buffered methanol. This time the reaction mixture turned dark blue. Again, the residue from solvent evaporation was extracted into 1 mL of D_2O and 1 mL of $CDCl_3$. The components of the organic phase were identified by ^{1}H NMR as DEM-3 dimer and 2-(ethylamino)-2-methylpropanol (δ 1.06 (s, 6 H), 1.07 (t, J = 7.2, 3 H), 2.44 (q, J = 7.2, 2 H), 3.33 (s, 2 H)). The components of the aqueous phase were identified as paraquat and a small amount of 2-(ethylamino)-2-methylpropanol hydrochloride, also from the ^{1}H NMR spectrum. Integrals of the NMR signals indicated a 41% conversion of DEM-3 dimer to amino alcohol.

Attempted Reduction of Daunomycin with DEM-3 Dimer. The reaction vessel was a 9 mm × 20 cm Pyrex tube equipped with a 2.5-cm side arm. The side arm was charged with 2.65×10^{-6} mol of DEM-3 dimer dissolved in methylene chloride, and the methylene chloride was evaporated with a stream of nitrogen. The main tube was charged with 2 mL of 2×10^{-3} M 1:1 Tris/Tris·HCl buffered methanol containing 2.66×10^{-6} mol of daunomycin. The methanol solution was freeze–thaw-degassed, and the tube was sealed with a torch. After mixing the reagents, the solution was heated at 36 °C for 18 h. C-18 reverse-phase HPLC analysis as described earlier[35] showed no formation of 7-deoxydaunomycinone.

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(35) Bird, D. M.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1991**, *113*, 308.

Molecular Meccano. 1. [2]Rotaxanes and a [2]Catenane Made to Order

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Abstract: A new synthetic strategy for the elaboration of supramolecular species and molecular compounds containing noncovalently interacting components is described, with the long-term objective of constructing highly ordered, wholly synthetic assemblies from readily available starting materials. These could serve as a basis for the future development of mechanoelectrical and photoelectrical communication systems and devices capable of storing and processing information. The approach was conceived against a background of a quarter of a century's experience in supramolecular, alias host–guest, chemistry. It is based on the use of irreversibly interlocked molecular systems that take the form of catenanes and rotaxanes. Such compounds are seen to be the ideal vehicles through which to transfer from supramolecular and host–guest chemistry the knowledge and experience gained from studying complexes between small chemical entities to very much larger molecular assemblies. Once we know how to interlock molecular components irreversibly and efficiently, we shall have a very much clearer idea on how to intertwine related polymer chains reversibly. A number of template-directed syntheses of [2]rotaxanes and a [2]catenane is discussed. They illustrate that *there are inherently simple ways of making apparently complex unnatural products from appropriate substrates without the need for reagent control or catalysis.* The noncovalent bonding interactions that are used to self-assemble the 1:1 complexes, which serve as precursors to the rotaxanes and the catenane, as well as to the [2]rotaxanes and the [2]catenane themselves, "live on" in their structures and superstructures after the self-assembly process is complete. A variety of methods, including X-ray crystallography, fast atom bombardment mass spectrometry, ultra violet–visible, luminescence, nuclear magnetic resonance, and electron spin resonance spectroscopies, and electrochemistry, demonstrate the high structural order that is incorporated into these new molecular assemblies in both the solid and solution states.

The living world is made up of molecular compounds that interact physically and react chemically with each other, often in rather specific and selective ways. The phenomenon of molecular recognition has evolved around the principles of self-organization, self-assembly, and self-synthesis to the extent that participating molecules must not only be blessed with a reasonably precise chemical form but they also usually perform a particular biological function. One of the most exciting and potentially rewarding challenges the chemist faces today is to devise and realize wholly synthetic systems[1] that function like their biological

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(1) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89–112. *J. Inclusion Phenom.* **1988**, *6*, 351–396. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304–1319. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J.; Dürr, H., Eds.; VCH: Weinheim, Germany, 1991; pp 1–28.

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