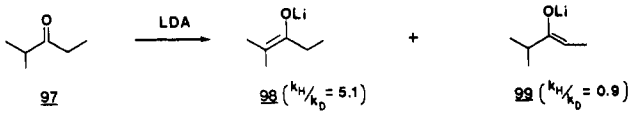
that reactions occur via small amounts of undetected species is well precedented, and more detailed studies of possible CIPE reactions are needed before the suggested mechanisms can be considered definitive.

It should also be noted also that complexes involving lithium may play a major role in reactions for which classical resonance and inductive effects have been considered dominant. For example, Saunders has observed dramatically different isotope effects for two different sites in the kinetic enolization of 2-methyl-3-pentanone **97** by lithium diisopropylamide. Lithiation



at the 2-position (**98**) has a k_H/k_D of 5.1 while substitution at the 4-position (**99**) shows a k_H/k_D of 0.9. Saunders[46] suggests that isomeric lithium complexes are involved. The normal isotope effect for the tertiary hydrogen results when deprotonation of the *syn*-isopropylcarbonyllithium complex is slow relative to reversal of complexation by lithium syn to the isopropyl. The negligible isotope effect for the secondary hydrogen reflects fast deprotonation relative to slow reversal of the complexation for the species with the lithium syn to the ethyl group. As Saunders notes, this proposal is reasonable on steric and electronic grounds. Once again, an unexpected result is understood by considering a complex-induced proximity effect.[47]

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Summary

In this Account we have drawn attention to the use of the complex-induced proximity effect (CIPE) as a rationale for a number of novel reactions of organolithium compounds. The importance of such complexation has been recognized for some time (vide supra), but recent work suggests that proximity in a transition state related to the initial complex can be dominant over classical effects in determining the course of a reaction. CIPE processes are notable in the formation and reactions of a variety of carbanionic synthetic equivalents, ranging from α-lithioamines, allyl anions, and electrophilic nitrogen to enolates. The regio- and stereocontrol provided in these reactions is a matter of continuing interest. The geometry of the relevant transition states need to be probed in more detail as does the nature of the specific reactants. Detailed understanding of these reactions is at an early stage and the CIPE proposal should be a useful guide for correlating observations, devising new reactions, and designing mechanistic probes.

We are grateful to our co-workers in Urbana and Fort Collins for their continuous efforts, to the National Science Foundation and the National Institutes of Health for support, and to Professor G. W. Klumpp for providing information from his laboratories prior to publication and to Professor Victor Snieckus for comments on the manuscript.

(47) *Note Added in Proof.* For recent cases which further illustrate CIPE processes in novel reactions see: (a) Ritter, R. H.; Cohen, T. *J. Am. Chem. Soc.* **1986**, *108*, 3718. Dickman, D. A.; Meyers, A. I. *J. Am. Chem. Soc.*, in press.

Toward Functional Models of Metalloenzyme Active Sites: Analogue Reaction Systems of the Molybdenum Oxo Transferases

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A significant portion of research in bioinorganic chemistry has been directed toward the synthesis of representations of the metal-containing sites in metallobiomolecules.[1] These are usually intended to serve as stereochemical and electronic analogues of these sites, and have the substantial advantage of being amenable to characterization at a very high level of detail. Leading examples include Fe–S complexes as related to ferredoxin sites[2] and, more recently, binuclear μ-oxo Fe(III) species,[3] which convey many of the essential

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(2) Berg, J. M.; Holm, R. H. In *Metal Ions in Biology*; Spiro, T. G., Ed.; Interscience: New York, 1982; Vol. 4, Chapter 1.
(3) (a) Armstrong, W. H.; Spool, A.; Papaefthymiou, G. C.; Frankel, R. B.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 3653. (b) Armstrong, W. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 4632. (c) Spool, A.; Williams, I. D.; Lippard, S. J. *Inorg. Chem.* **1985**, *24*, 2156. (d) Lippard, S. J. *Chem. Br.* **1986**, *22*, 222. (e) Wieghardt, K.; Pohl, K.; Gebert, W. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 727. (f) Chaudhuri, P.; Wieghardt, K.; Nuber, B.; Weiss, J. *Ibid.* **1985**, *24*, 778.

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features of the sites in hemerythrins.

Simultaneous attainment of biological structure *and* function in a synthetic system has proven more difficult, with the best examples drawn from the multifarious investigations of hemes,[4] especially ingeniously modified varieties (picket fence, strapped, capped, etc.) which promote approaches to reversibility of dioxygen binding as it is found with myoglobin and hemoglobin. The problem becomes even more demanding when catalysis is involved. Inasmuch as definition of (even minimal) coordination structure normally lags far behind knowledge of enzymatic function, reduction of the latter to a molecular basis of description founded on demonstrated chemistry cannot always await the former. Certainly, the impressive progress in developing epoxidation and alkane hydroxylation reactions[5] similar to those of the P-450 enzymes is a case in point. As will be shown here, a parallel opportunity exists with the molybdenum hydroxylases,[6-8] whose function is, in effect, the addition of an oxygen atom to, or its removal from, substrate. Without mechanistic implication, these processes are referred to as oxo-transfer reactions and the enzymes that catalyze them as oxo transferases.

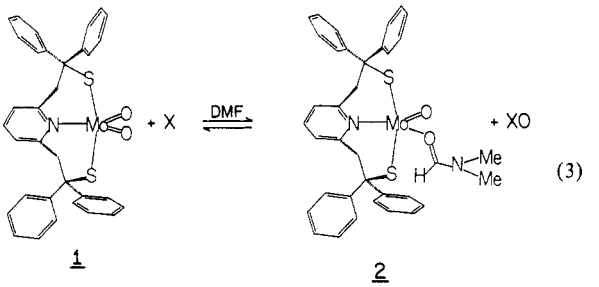
Design of an Analogue Reaction System

We have adopted as our working hypothesis that at least some enzymatic oxo transfers occur by the forward or reverse reaction 1,[9] and in certain cases no exogenous

$$Mo(VI)O_2L_n + X \rightleftharpoons Mo(IV)OL_n + XO \qquad (1)$$

reactant other than substrate is required for product formation. These reactions amount to the formal half-reaction $X + H_2O \rightleftharpoons XO + 2H^+ + 2e^-$, illustrating the two-electron nature of all processes catalyzed by these enzymes, which include, inter alia, xanthine oxidase (xanthine → uric acid), sulfite oxidase ($SO_3^{2-} \rightarrow SO_4^{2-}$), and nitrate reductase ($NO_3^- \rightarrow NO_2^-$). A functional model of the active sites of these enzymes should meet several criteria. It should approach or achieve the stereochemistry and composition of the Mo coordination environment, execute oxo-transfer reactions with biologically relevant substrates, possess Mo oxidation states interconvertible in both directions so as to allow catalysis, and not engage in the oxo "dimerization" reaction 2. The latter is pervasive in synthetic Mo sys-

$$MoO_2L_n + MoOL_n \rightleftharpoons L_nMo(=O)-O-Mo(=O)L_n \qquad (2)$$



(3)

Figure 1. Substrate oxidation/reduction reactions by oxo transfer using the sterically hindered complexes $MoO_2(L\text{-}NS_2)$ (1) and $MoO(L\text{-}NS_2)(DMF)$ (2) in DMF solution.

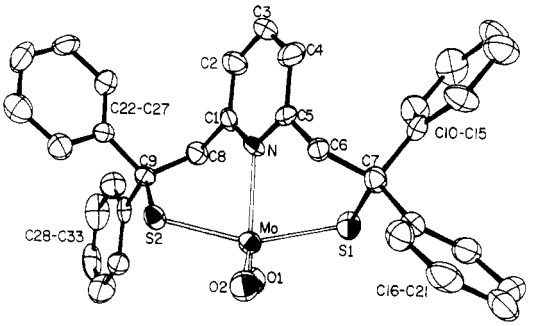


Figure 2. The structure of $MoO_2(L\text{-}NS_2)$.[12] Note that one ring of each *gem*-diphenyl group projects along one Mo=O bond, thereby shielding it from attack by Mo(IV) in the course of attempted formation of a Mo-O-Mo bridge.

tems and, if irreversible, precludes catalysis. Further, the occurrence of reactions 1 and 2 in the same system substantially complicates analysis of the kinetics of oxo transfer, although a formal solution to this problem has been obtained.[10] In enzymes, reaction 2 is obviated by protein structure.

Complexes $MoO_2(L\text{-}NS_2)$ (1) and $MoO(L\text{-}NS_2)(DMF)$ (2)[11,12] of reaction 3 in Figure 1, derived from the hindered dithiol 2,6-bis(2,2-diphenyl-2-mercaptoethyl)pyridine, were designed to meet the foregoing criteria. Reaction of the dithiol with $MoO_2(acac)_2$ affords $MoO_2(L\text{-}NS_2)$ as an orange solid. Treatment of the latter with X = Ph_3P yields purple $MoO(L\text{-}NS_2)(DMF)$. The structure of $MoO_2(L\text{-}NS_2)$ is provided in Figure 2; diffraction-quality crystals of $MoO(L\text{-}NS_2)(DMF)$ have not been obtained. The stereochemistry of $MoO_2(L\text{-}NS_2)$ is best described as distorted trigonal bipyramidal with two axial sulfur ligands and the two oxo and the nitrogen atoms in the equatorial plane. The S(1)-Mo-S(2) angle is 156.4°; other bond angles at Mo occur in the range 78-126°. The molecule approaches C_2 symmetry with the 2-fold axis coincident with the Mo-N bond. $MoO_2(L\text{-}NS_2)$ contrasts with other MoO_2(tridentate) complexes which either adopt a polymeric structure with one oxo atom acting as a bridging ligand or are mononuclear with an additional monodentate ligand completing six-coordination.[13]

The presence of $Mo(VI)O_2$ or $Mo(IV)O$ groups and two thiolate ligands in the complexes is consistent with the results of Mo EXAFS analysis of a number of enzymes.[14] Among these is sulfite oxidase, for which the

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(11) Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1984**, *106*, 3035.

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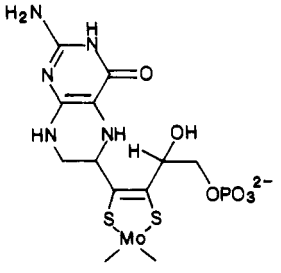
(13) Berg, J. M.; Holm, R. H. *Inorg. Chem.* **1983**, *22*, 1768, and references therein.

minimal coordination units $MoO_2(SR)_{2,3}$ and $MoO(OH/OH_2)(SR)_3$ have been deduced for the oxidized and dithionite-reduced forms, respectively, from a combination of EXAFS and EPR results.[14a] On similar evidence, the situation with xanthine oxidase/dehydrogenase differs in that a sulfide ligand is present in the oxidized coordination unit, $MoOS(SR)_2$, and hydrosulfide in the reduced form, $MoO(SH)(SR)_{2,3}$. The closely related enzyme aldehyde oxidase very likely contains these same units. In no case, however, is the ligand set fully defined, a point that should be borne in mind when evaluating the model systems which follow. The mean Mo–O (1.694 Å) and Mo–S (2.416 Å) distances in $MoO_2(L\text{–}NS_2)$ agree well with those in enzymes determined from EXAFS.[14] The Mo–S distances further ensure that the sulfur ligands in the enzyme are of the thiolate type. One of the phenyl rings in each *gem*-diphenyl group overlies a Mo=O bond in the direction of a potential Mo–O–Mo bridge. Inasmuch as bridge formation requires the joining of two $MoO(L\text{–}NS_2)$ fragments, steric encumbrance is amplified and reaction 2 is of no consequence in systems containing these complexes. A conspicuous feature of $MoO_2(L\text{–}NS_2)$ is its five-coordination, which is unusual among $Mo(VI)O_2$ complexes. The probable structure of $MoO(L\text{–}NS_2)(DMF)$ is a derivative of this arrangement, in which a labile solvent molecule replaces an oxo ligand. This aspect provides a binding site for potential substrate molecules.

A highly desirable goal of a synthetic approach to oxo transferase active sites is the dissociable Mo cofactor[7b,15,16] (Mo-co), which is obligatory to enzyme activity. Primarily by degradation studies of cofactor extracts, the Duke group, in a highly significant contribution, has proposed the Mo-co minimal structure to be **3**, con-



3

taining a pterin nucleus with a side chain at the 6-position carrying an ethylenedithiolate chelating group. In the oxidized form two oxo ligands (not shown) should be present. The remaining one or two coordination positions are very likely occupied by side chain ligands of amino acid residues, binding the cofactor to the protein. Mo-co has not yet been isolated in the intact state, and not all observations are fully reconcilable with **3**.[16] However, incubation of Mo-co extracts from a variety of enzymes with the cofactor-deficient nitrate reductase from *Neurospora crassa* nit-1 restores enzymatic activity,[16,17] thereby providing an imperative for isolation and full characterization of Mo-co. While the complexes of reaction 3 in their entirety are far removed from the cofactor, they nonetheless are promising vehicles with which to examine oxo transfer mediated by Mo(VI,IV) in a biologically credible coordination environment.

Oxo-Transfer Reactions

Prior to the inception of our investigations,[10–13,18–21] a limited set of oxo-transfer reactions had been reported for Mo(VI,IV), nearly all with *N,N*-disubstituted dithiocarbamate complexes. The earliest results of pertinence are those of Barral et al.[22] in 1972, who demonstrated catalytic aerial oxidation of tertiary phosphines in the presence of $MoO_2(S_2CNR_2)_2$. These reactants produced the phosphine oxide and $MoO(S_2CNR_2)_2$. The latter was reoxidized by dioxygen to the initial Mo(VI) complex, affording a catalytic cycle. Other contributions in the same period confirmed and extended the reduction of $MoO_2(S_2CNR_2)_2$ with phosphines,[23,24] and provided qualitative indications that $MoO(S_2CNEt_2)_2$ could be oxidized by atom transfer from Me_2SO, PhN(O)=NPh, and *t*-$BuONO_2$.[25] Additionally, thermochemical data were provided for oxo-transfer reactions of the Mo(VI,IV) dithiocarbamates with several substrates.[24] In our kinetics analysis of systems simultaneously displaying reactions 1 and 2, we showed that the second-order rate constants for reduction of $MoO_2(S_2CNEt_2)_2$ followed the basicity order of series 4 (k, M^{-1} s^{-1}) and proved that Me_2SO and

$$Ph_3P(0.071) < Ph_2EtP(0.23) < PhEt_2P(0.43) < Et_3P(0.53) \quad (4)$$

N-methylmorpholine *N*-oxide oxidized $MoO(S_2CNEt_2)_2$ to $MoO_2(S_2CNEt_2)_2$.[10] These collective results demonstrated the feasibility of oxo transfer *to* and *from* substrate in stoichiometric and catalytic systems with appropriate substrates and Mo complexes. Consequently, investigations of reaction system 3 were commenced using, for reasons already presented, the complexes $MoO_2(L\text{–}NS_2)$ and $MoO(L\text{–}NS_2)(DMF)$ in DMF solution.

(1) Substrate Oxidation. As a model oxo-transfer process, reaction 3 with X = Ph_3P was investigated. Because of the distinctive chromophores involved, the reaction is readily monitored spectrophotometrically as shown in Figure 3. It is first order in phosphine and Mo(VI) with k = 7 (1) × 10^{-3} M^{-1} s^{-1}, 10 times slower

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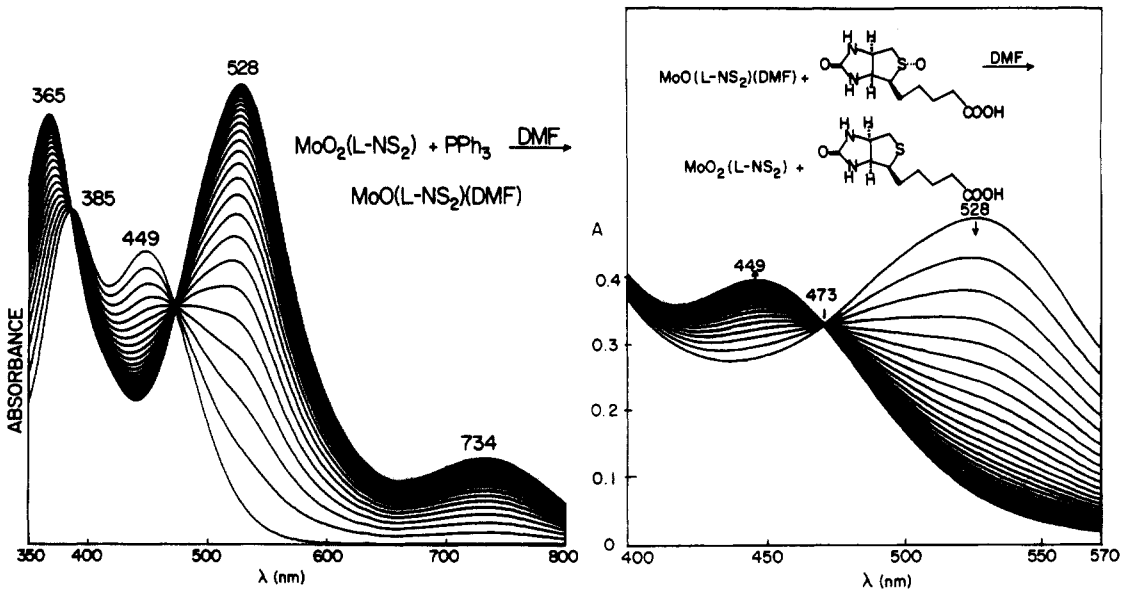
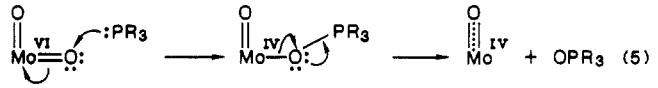


Figure 3. Spectral changes in the oxidation of Ph_3P by $MoO_2(L\text{-}NS_2)$ (λ_{max} 385, 449 nm) (left) and the reduction of *d*-biotin *S*-oxide by $MoO(L\text{-}NS_2)(DMF)$ (λ_{max} 365, 528 nm) (right) in DMF solutions.[19]

than the reaction of $MoO_2(S_2CNEt_2)_2$ with Ph_3P. The clean isosbestic points observed in this and all other reactions 3 indicate the absence of any appreciable amount of μ-oxo dimer. Mechanistic scheme 5 is



probable, wherein the phosphine initiates attack on a Mo=O group by interaction with its vacant π^* orbital. Oxygen atom removal is, of course, the equivalent of a two-electron reduction. The potential oxophiles R_3N and Ph_3As do not react with $MoO_2(L\text{-}NS_2)$. Indeed, the reverse reaction 6 occurs with the arsine oxide, as is the

$$MoO(L\text{-}NS_2)(DMF) + Ph_3AsO \rightarrow MoO_2(L\text{-}NS_2) + Ph_3As \quad (6)$$

case with $MoO(S_2CNEt_2)_2$.[26] The direction of this and reaction 3 with X = Ph_3P appears largely controlled by differences in oxo atom bond strengths: P–O, 133 (6), and As–O, 106 (6) kcal/mol, in $Ph_3EO_{(g)}$.[27]

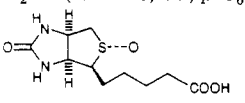
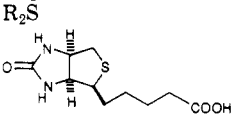
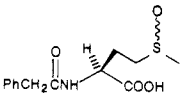
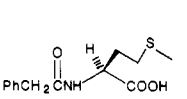
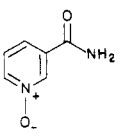
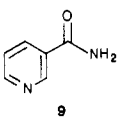
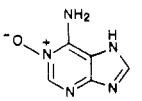
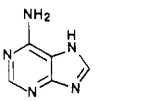
(2) Substrate Reduction. As a prototypic oxo-transfer reaction from substrate to Mo(IV), reaction 3 with XO = Me_2SO was first examined.[19] This process was observed to occur quantitatively with tight isosbestic points developed, again indicating no accumulation of a μ-oxo species during reaction. This and other substrates reduced by $MoO(L\text{-}NS_2)(DMF)$ are listed in Table I. These include *d*-biotin *S*-oxide (**4a**), which is reduced to the coenzyme *d*-biotin (**4b**), and two epimers of carbobenzoxy-*L*-methionine *S*-oxide (**5**), which are converted to **6**.[19]

The reaction of **4a**, the spectral time course for which is shown in Figure 3, is of particular significance because it is the natural substrate of the Mo-co-dependent enzyme *d*-biotin *S*-oxide reductase.[28] The rate of sulfoxide reduction is first order in the Mo complex and at sufficiently high [R_2SO] is independent of substrate concentration. The substrate saturation behavior has been successfully interpreted in terms of equilibrium reaction 7 and irreversible product formation step 8.

$$MoO(L\text{-}NS_2)(DMF) + R_2SO \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MoO(L\text{-}NS_2)(OSR_2) + DMF \quad (7)$$

$$MoO(L\text{-}NS_2)(OSR_2) \xrightarrow{k_2} MoO_2(L\text{-}NS_2) + R_2S \quad (8)$$

Table I.
Oxo-Transfer Substrates of $MoO_2(L\text{-}NS_2)$ and $MoO(L\text{-}NS_2)(DMF)$ and Products[a]

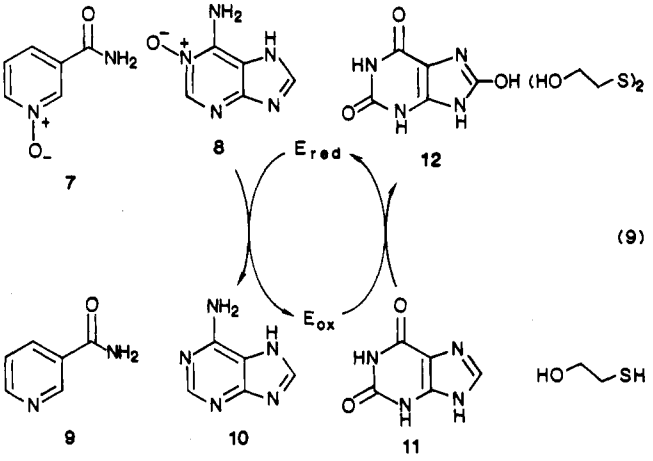
substrate, X,XO	product, XO,X
Ph_3P	Ph_3PO
R_2SO (R = Me, Ph, *p*-C_6H_4F)	R_2S
	
4a	**4b**
	
5	**6**
	
	
7	**9**
	
8	**10**
$(PhCH_2)_3NO$	$(PhCH_2)_3N$
Ph_3AsO	Ph_3As

[a] Results in DMF solutions at ambient temperature; from ref 10 and 17–20.

(26) Lu, X.; Sun, J.; Tao, X. *Synthesis* **1982**, 185.
(27) Tsvetkov, V. G.; Aleksandrov, Yu. A.; Glushakova, V. N.; Skorodumova, N. A.; Kol'yakova, G. M. *J. Gen. Chem. USSR (Engl. Transl.)* **1980**, *50*, 198.
(28) (a) del Campillo-Campbell, A.; Campbell, A. *J. Bacteriol.* **1982**, *149*, 469. (b) del Campillo-Campbell, A.; Dykhuizen, D.; Cleary, P. P. *Methods Enzymol.* **1979**, *62*, 379.

Kinetics parameters for the reaction with **4a** are $K = k_1/k_{-1} = 1.6 \times 10^4$ and $k_2 = 1.4 \times 10^{-3}$ s^{-1}. Values for other sulfoxides are quite similar. All values were obtained under the assumption that the ligand substitution reaction is fast compared to oxo transfer. To the extent that this assumption is incorrect, the values of k_2 represent lower limits for the true oxo-transfer rate constants. The quantity k_2/K_m, where $K_m = [DMF]/K$, is an effective second-order rate constant for sulfoxide reduction. For Me_2SO and **4a** these values are 0.5 and 1.8 M^{-1} s^{-1}, respectively. This illustrates a rate advantage for $MoO(L\text{-}NS_2)(DMF)$, at least with small substrates, inasmuch as the former value is over three orders of magnitude greater than the second-order rate constant for reduction of the same substrate by $MoO(S_2CNEt_2)_2$.[10]

Other substrates in Table I are *N*-oxides, and all are actual substrates or pseudosubstrates of oxo transferases. As illustrated by catalytic cycle 9, nicotinamide

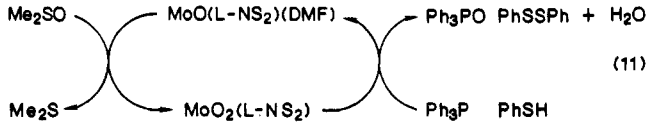


N-oxide (**7**) and adenine 1-oxide (**8**) are reduced to **9** and **10**, respectively, by rat liver or milk xanthine oxidase.[29] Oxidation of the natural substrate xanthine (**11**) to uric acid (**12**) supplies the two electrons required for reduction of the *N*-oxide. A thiol may also be used as the electron donor. Other heterocyclic *N*-oxides and tertiary amine *N*-oxides have been shown to be reduced by liver aldehyde oxidase in the presence of aldehydes or 2-hydroxypyrimidine as the electron donor.[30] All of these compounds are alternative substrates for enzymes originally recognized for another activity, but which are also *N*-oxide reductases. Evidently, $MoO(L\text{-}NS_2)(DMF)$ is capable of reducing a variety of enzymic substrates. It is entirely probable that this reactivity can be extended to a much larger group of sulfoxides and *N*-oxides than that in Table I.

(3) Catalysis. No reaction occurs between Ph_3P and Me_2SO at 189 °C for at least 1 h.[31] However, the oxidation of phosphine and the reduction of sulfoxide constitute the components of a catalytic cycle, which has been realized by using Me_2SO as a test substrate.[19] Thus, systems of $MoO_2(L\text{-}NS_2)$ in Me_2SO/DMF containing up to 1500 equiv of Ph_3P retain the characteristic orange color of the Mo(VI) complex, and spectrophotometric examination reveals no features not attributable to this complex. However, ^{31}P NMR spectra showed progressive oxidation of Ph_3P to Ph_3PO and a chemical test proved formation of Me_2S in essentially quantitative yield based on the stoichiometry of reaction 10. The first reaction in the cycle was found to

$$Ph_3P + Me_2SO \rightarrow Ph_3PO + Me_2S \qquad (10)$$

be essentially first order in $MoO_2(L\text{-}NS_2)$ with an initial second-order rate constant of 6×10^{-3} M^{-1} s^{-1}. This is in good agreement with the rate constant measured in the absence of Me_2SO, and identifies the first step in the system as the reduction of $MoO_2(L\text{-}NS_2)$ by Ph_3P. The catalytic rate is limited by the rate of oxo abstraction from the Mo(VI) by phosphine, as expected from the ratio of second-order rate constants 0.5:0.007 = 70:1 for sulfoxide reduction and phosphine oxidation. Despite a secondary reaction between $MoO(L\text{-}NS_2)(DMF)$ and Ph_3P, catalytic system 11 is quite robust



and in excess of 500 turnovers/Mo have been achieved.[19] Catalytic *N*-oxide reduction systems should also be possible that use a tertiary phosphine as the reductant.

Table II.
Enthalpy Data for the Reactions X + 1/2 $O_{2(g)}$ → XO[a]

X	XO	ΔH, kcal/mol
$S_2O_3^{2-}{}_{(aq)}$	$S_2O_4^{2-}{}_{(aq)}$	+12
$ClO_2^-{}_{(aq)}$	$ClO_3^-{}_{(aq)}$	−8
$NO_2^-{}_{(aq)}$	$NO_3^-{}_{(aq)}$	−25
$Me_2S_{(g)}$	$Me_2SO_{(g)}$	−27
$MoO(S_2CNEt_2)_2$	$MoO_2(S_2CNEt_2)_2$	−35
$MoO(L\text{-}NS_2)(DMF)$	$MoO_2(L\text{-}NS_2)$	*b*
$2PhSH_{(g)}$	$PhSSPh_{(g)} + H_2O_{(g)}$	−53
$MeCHO_{(g)}$	$MeCO_2H_{(g)}$	−64
$SO_3^{2-}{}_{(aq)}$	$SO_4^{2-}{}_{(aq)}$	−65
Ph_3P	Ph_3PO	−67

[a] Enzyme substrates in boldface; data from ref 20. [b] See text.

Thermodynamic Reaction Series

The reactions of the substrates in Table I raise the question as to the range of potential Mo-mediated oxo-transfer reactions. Of particular interest are the properties of Mo complexes required for the occurrence of these reactions. As an initial step in this rather involved problem, we have attempted to reduce substrate reactions to a common thermodynamic base by use of available data for the reaction X + $^1/_2O_{2(g)}$ → XO. Reaction enthalpies are listed in Table II, including those for some six enzyme substrates. A more extensive tabulation is available elsewhere,[20] as is a rather complete list of oxo transferases and their substrates.[32] The pioneering thermochemical work of Watt et al.[24] has provided the ΔH value for oxidation of $MoO(S_2CNEt_2)_2$ in 1,2-dichloroethane solution, and has shown that oxo-transfer reduction of nitrate by this complex and oxidation of sulfite and acetaldehyde by $MoO_2(S_2CNEt_2)_2$ are thermodynamically favored. The position of the $L\text{-}NS_2$ complexes in the series can be par-

(29) (a) Murray, K. N.; Chaykin, S. *J. Biol. Chem.* **1966**, *241*, 2029, 3468. (b) Murray, K. N.; Watson, J. G.; Chaykin, S. *Ibid.* **1966**, *241*, 4798. (c) Stohrer, G.; Brown, G. B. *Ibid.* **1969**, *244*, 2498.

(30) (a) Shimokawa, O.; Ishimoto, M. *J. Biochem.* **1979**, *86*, 1709. (b) Yoshihara, S.; Tatsumi, K. *Arch. Biochem. Biophys.* **1985**, *242*, 213. (c) Kitamura, S.; Tatsumi, K. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 602.

(31) Szmant, H. E.; Cox, O. *J. Org. Chem.* **1966**, *31*, 1595.

(32) Garner, C. D.; Bristow, S. In ref 8, Chapter 7.

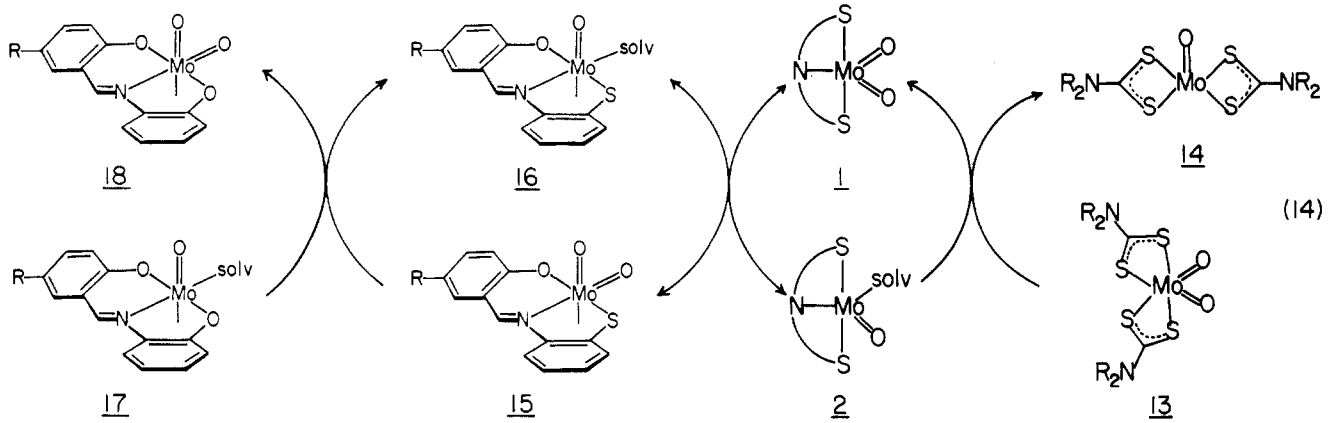


Figure 4. Schematic depiction of the relative oxo transfer abilities of Mo(IV,VI) dithiocarbamate (**13,14**), L-NS_2 (**1,2**), 2-(salicylideneamino)benzenethiolate (**15,16**), and 2-(salicylideneamino)phenolate (**17,18**) complexes.[20] Spontaneous reactions occur from left to right; the middle reaction is an equilibrium that favors **2** and **15**.

tially fixed by recalling that $MoO(L\text{-}NS_2)(DMF)$ reduces Me_2SO and observing that reactions 12 and 13

$$MoO_2(L\text{-}NS_2) + 2PhSH \rightarrow MoO(L\text{-}NS_2)(DMF) + PhSSPh + H_2O \quad (12)$$

$$MoO(L\text{-}NS_2)(DMF)\ (\mathbf{2}) + MoO_2(S_2CNEt_2)_2\ (\mathbf{13}) \rightarrow MoO_2(L\text{-}NS_2)\ (\mathbf{1}) + MoO(S_2CNEt_2)_2\ (\mathbf{14}) \quad (13)$$

are spontaneous.[20] The latter, an intermetal oxo transfer, is schematically depicted in reaction sequence 14 in Figure 4. Thus, the ΔH value for the oxidation of $MoO(L\text{-}NS_2)(DMF)$ falls within the approximate limits of −35 and −53 kcal/mol. While the data in Table II are not precisely comparable due to differences in physical state and solvent, they are considered adequate to provide a thermodynamic range of "fitness" for Mo complexes to engage in oxo transfer. Consequently, *the complexes 1/2 and 13/14 are positioned so as to oxidize or reduce spontaneously the enzymic substrates listed.* Available ΔG data do not alter this conclusion, which is based on negative reaction enthalpies for reactions such as (3). Unfortunately, ΔH values for the formation of aromatic or tertiary *N*-oxides are not available.

Clearly, there could have been no a priori basis for knowing that the foregoing complexes are so strategically positioned in the thermodynamic reaction series for reaction with biological substrates. If, for example, the pair **1/2** had been at the very bottom of the table, $MoO(L\text{-}NS_2)(DMF)$ would be able to reduce all oxidized species but $MoO_2(L\text{-}NS_2)$ would be incapable of oxidizing any reduced species; and conversely if the pair were at the very top. We next inquire into the factors rendering Mo complexes thermodynamically competent in oxo transfer.

The spontaneous intermetal oxo-transfer reactions in sequence 14 establish the oxo acceptor strength order as **17** > **16** > **2** > **14**, and the oxo donor strength order as **13** > **1** > **15** > **18**. The requirement that $\Delta H \lesssim -27$ kcal/mol for a Mo(IV)O complex to be able to reduce Me_2SO, the most reductively resistant substrate in Table II, is met by all complexes in the set. As one example, **17** rapidly reduces Me_2SO.[20] In the only other pertinent case, $[MoO(dttd)Cl]^{1-}$ is reported to reduce Me_2SO.[33] Consequently, the stoichiometric reduction of XO by some Mo(IV)O complex is a necessary but not sufficient test for a functional model inasmuch as the available sample indicates that all such complexes, even without thiolate ligands, act as oxo acceptors.

For a $Mo(VI)O_2$ complex to oxidize acetaldehyde and other substrates, $\Delta H \gtrsim -64$ kcal/mol. This feature is possessed by $MoO_2(S_2CNEt_2)_2$ on the basis of calorimetry,[24] and also by $MoO_2(L\text{-}NS_2)$ from its reactivity with Ph_3P and PhSH. In contrast, the Schiff base complexes **15** and **18** do not react with Ph_3P in DMF at temperatures up to 60 °C. The more basic Ph_2EtP is required to observe appreciable reaction rates.[34,35] These observations suggest that **15** and **18** may not be thermodynamically capable of executing the substrate oxidations, or, at least, that substantial kinetic barriers exist which do not exist for complexes with more anionic sulfur ligation. Although the complexes involved are rather different, the oxo donor reactivity series implies a greater stability of the Mo(IV)O state when anionic sulfur ligands are present. This is borne out by redox potential differences $E_S - E_O$ of species identical except for replacement of sulfur by oxygen donor atoms. Several of these are given below (ox = 8-hydroxyquinolinate, tox = 8-mercaptoquinolinate); the maximum difference occurs in the reductions of $MoO_2(L\text{-}NS_2)$ and $MoO_2(L\text{-}NO_2)(DMF)$.[12]

$$[\mathbf{15}]^{0,1-} - [\mathbf{18}]^{0,1-} \quad 0.10\ V^{36}$$

$$[MoOCl(tox)_2]^{0,1-} - [MoOCl(ox)_2]^{0,1-} \quad 0.11\ V^{37}$$

$$[MoOCl(tox)_2]^{0,1+} - [MoOCl(ox)_2]^{0,1+} \quad 0.69\ V^{37}$$

The majority of the reported potentials do not correspond to either physiological or reversible reactions. Nonetheless, that all available data (for some twelve pairs of complexes) afford positive potential differences suggests that this effect of thiolate ligation, which renders species easier to reduce, is of general consequence. Further, a similar effect extends to reaction rates. In one part of their valuable contributions to this field, Topich and Lyon[35] found a linear relationship between the (irreversible) reduction potential of **15** and the reaction rate with Ph_2EtP, as substituent R is

(33) Kaul, B. B.; Enemark, J. H.; Merbs, S. L.; Spence, J. T. *J. Am. Chem. Soc.* **1985**, *107*, 2885. dttd = 2,3:8,9-dibenzo-1,4,7,10-tetrathiadecane(2−).

(34) Boyd, I. W.; Spence, J. T. *Inorg. Chem.* **1982**, *21*, 1602.

(35) (a) Topich, J.; Lyon, J. T., III. *Polyhedron* **1984**, *3*, 61. (b) Topich, J.; Lyon, J. T., III. *Inorg. Chem.* **1984**, *23*, 3202.

(36) Rajan, O. A.; Chakravorty, A. *Inorg. Chem.* **1981**, *20*, 660.

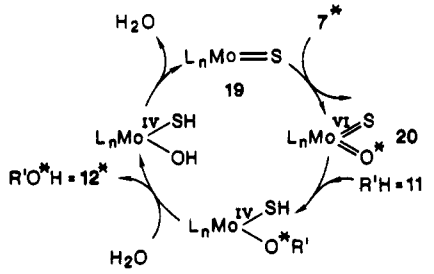
(37) Taylor, R. D.; Street, J. P.; Minelli, M.; Spence, J. T. *Inorg. Chem.* **1978**, *17*, 3207.

varied. Differences in rate constants and potentials are small but the behavior of faster rates with increasing potentials appears to be clearcut. From a comparison of $\Delta H^{\ddagger}$ values for oxo abstraction from MoO_2L_n complexes by phosphines, these workers concluded that reactivity decreases in the ligand donor set order S_4 > S_2N_2 > ONS > ONO, where the first two entries refer to L = $Et_2NCS_2^-$ and Cys·OEt$^-$. The last two entries correspond to **15** and **18**, which follow the order deduced from sequence 14 and are converted to **16** and **17**, respectively. For reasons unclear, these reaction systems do not appear to generate any μ-oxo Mo(V) dimers, although there is no evident steric or other impediment to their formation. Series 14 provides the most direct means of evaluation of relative oxo-transfer tendencies.

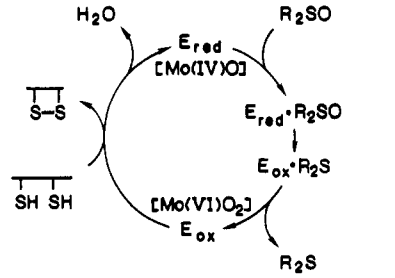
A final property that can affect the positions of all of the *dioxo*–Mo(VI) complexes within the thermodynamic oxo donor series is the presence of the second ("spectator") oxo ligand. Rappé and Goddard[38] have noted important differences in the multiple bond character between the Mo–oxo bonds in dioxo vs. monooxo complexes. In the former the Mo–O bond is essentially a double bond, whereas in the latter it has considerable triple bond character. This effect is calculated to make the Mo–O bond strength in MoO_2Cl_2 more than 20 kcal/mol less than that in $MoOCl_4$.

Biological Aspects

Are there any oxo-transfer reactions in biology that support the working hypothesis of reaction 1? Fortunately, evidence bearing on this point, while decidely scant, is encouraging. Murray et al.[29b] in 1966 demonstrated that liver xanthine oxidase supplied with xanthine (**11**) and ^{18}O-labeled nicotinamide *N*-oxide (**7***) effected incorporation of 0.67 atom of oxygen from the latter into xanthine, converting it to uric acid (**12***). A similar result was obtained with the milk enzyme. We suggest interpretation of these findings in terms of cycle 9, possibly involving the catalytic-site states in scheme 15. The oxo-transfer hypothesis is met in the oxidation



(15)



(16)

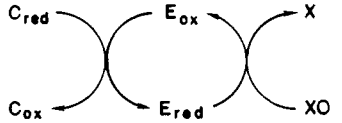
of state **19** to **20** by the *N*-oxide. The remaining steps are drawn from a recent proposal by the Sussex group.[39]

(38) Rappé, A. K.; Goddard, W. A., III *J. Am. Chem. Soc.* **1982**, *104*, 3287.

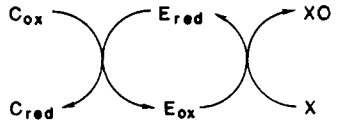
The lack of full incorporation of ^{18}O may be due to some exchange of **20** with bulk water and/or another pathway. Use of labeled water and unlabeled **7** with the liver enzyme led to ca. 25% of the labeled product found in the reciprocal case. When thiol is used as the reductant, the reaction is likely "pure" oxo transfer, under which description the product is formed directly, without another bond breaking/making step. Enzymatic sulfoxide reduction presumably proceeds this way.

On the basis of a study of system **11**, scheme 16 has been proposed for the catalytic reduction of sulfoxides.[19] The original reductant in the analogue reaction system, Ph_3P, is physiologically objectionable and has been replaced with the protein thioredoxin,[40] whose redox function is expressible as 2Cys·SH ⇌ Cys·SS·Cys + $2H^+$ + $2e^-$. This suggestion is based on the utilization of a cysteinyl-containing protein by *d*-biotin *S*-oxide reductase,[28b] and demonstrations of sulfoxide reduction by liver cytosolic enzymes and yeast methionine sulfoxide reductase in the presence of thioredoxin.[41] Recently, we have demonstrated reaction **12**[20] and have further shown (^{19}F NMR) that in the system MoO_2(L–NS_2)/*p*-FC_6H_4SH/(*p*-$FC_6H_4)_2SO$ the products (*p*-$FC_6H_4S)_2$ and (*p*-$FC_6H_4)_2S$ are formed in equal amounts.[21] Some time earlier, $MoO(S_2CNEt_2)_2$, which can reduce Me_2SO, was isolated in good yield from the reaction of $MoO_2(S_2CNEt_2)_2$ and PhSH.[42] These results improve the biological credibility of reaction system **11** and strengthen the proposed enzymatic cycle **16**.

What can be said about the factors (other than the presence of oxo ligands) that render enzymatic Mo units fit to sustain *catalytic* oxo transfer, presupposing that it is a general pathway as in reaction cycles 17 and 18?



(17)



(18)

Species C are electron carriers or other redox agents. In substrate reduction cycle 17, the nature of the reduced enzyme site would not appear to be overly restrictive in terms of ligand set inasmuch as all Mo(IV)O complexes yet examined reduce Me_2SO and those that have been tested also reduce pyridine *N*-oxide. Similarly, in oxidation cycle 18 return of the enzyme to the oxidized state should not be difficult, judging from the extreme sensitivity of the synthetic Mo(IV)O species to dioxygen and, presumably, other physiological oxidants. The lack of a suitable ligand set could, however,

(39) (a) Bray, R. C.; George, G. N. *Biochem. Soc. Trans.* **1985**, *13*, 560, and references therein. (b) For a discussion of xanthine and sulfide oxidases and their reaction mechanisms, cf. Hille, R.; Massey, V. In ref 8, Chapter 9.

(40) Holmgren, A.; Söderberg, B.-O.; Eklund, H.; Brändén, C.-I. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2305.

(41) (a) Porqué, P. G.; Baldesten, A.; Reichard, P. *J. Biol. Chem.* **1970**, *245*, 2371. (b) Anders, M. W.; Ratnayake, J. H.; Hanna, P. E.; Fuchs, J. A. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 846. Note, however, that these enzymes have not been proven to be Mo-dependent.

(42) McDonald, J. W.; Corbin, J. L.; Newton, W. E. *Inorg. Chem.* **1976**, *15*, 2056.

place the oxidized enzyme at a potential (real or effective) too negative to permit reduction to Mo(IV) under physiological circumstances, either by two single-electron transfers in the return leg of cycle 17 or by oxo abstraction in the substrate reaction step of cycle 18. Here the role of two or more thiolate ligands in modulating the reducibility of a $Mo(VI)O_2$ or Mo(VI)OS coordination unit to a physiologically attainable condition may be crucial to catalysis. A further aspect relates to the role of the pterin portion of the minimal Mo-co structure **3**. Our current view is that it may constitute part of the electron-transfer circuit in and out of the catalytic site. Thus, we have demonstrated in analogue reaction systems the reduction of dihydropterin by thiol to tetrahydropterin (consistent with earlier results[43]), and reduction of $MoO_2(L–NS_2)$ by the latter. This allows tentative inclusion of the H_2pterin/H_4pterin couple in the C_{ox}/C_{red} apparatus of cycle 17. The reverse process, oxidation of the Mo(IV)O state as in cycle 18, remains to be demonstrated.

Concluding Remarks

The results, interpretations, and speculations offered here and elsewhere[11,12,18–21] represent our initial endeavors directed toward reducing the mechanism(s) of action of the Mo-oxo transferases to a molecular description founded on proven chemistry. Much remains to be done in the immediate future before this goal can be achieved. Outstanding among required investigations are additional isotope labeling work designed to probe the operation of enzymatic oxo-transfer pathways, synthesis and characterization of Mo(VI)OS and Mo(IV)S species as pertinent to xanthine and aldehyde oxidases, further elucidation of the role of pterin in reactivity, and development of additional analogue reaction systems capable of, e.g., oxidizing sulfite, aldehydes, and xanthine and other heterocyclic substrates, and cleanly reducing nitrate to nitrite.

Another significant goal is the attainment of models that closely simulate the EPR spectra of enzymic Mo(V) states.[7a,44] Under the oxo-transfer hypothesis, these states appear mainly as intermediates in redox reactions returning the enzyme to its E_{ox} or E_{red} form. An example is thiol reduction of E_{ox} to E_{red} in cycle 16, via a Mo(V) intermediate (not shown). Encouraging results have recently been obtained with a Mo(V)O(OH) complex.[45] A variety of Mo(V) species $[MoO(L–NS_2)L']^{0,1+}$ are accessible by oxidation of $MoO(L–NS_2)(DMF)$.[12] It is probable that reductions of sulfoxides and *N*-oxides are not only among the first oxo transferase reactions to be achieved but are also the simplest of substrate transformations to perform in analogue systems. Nonetheless, these results provide a suitable beginning to the attainment of oxo transferase analogue reaction systems and a description of enzymatic action.

In a more general context, progress on systems mimicking the action of metalloenzymes such as carboxypeptidase A,[46] carbonic anhydrase,[47] urease,[48] tyrosinase,[49] catechol dioxygenases,[50] and cytochrome P-450[5] further sustain the proposition that functional models of enzymatic sites are viable objectives that ultimately will be contributory to elucidation of catalytic mechanisms.

This research was supported by National Science Foundation Grant CHE 81-06017. We thank our co-workers, John Caradonna, Ed Harlan, and Martha Reynolds, for their important contributions to this work.

Registry No. Mo, 7439-98-7; hydroxylase, 9046-59-7.

(43) (a) Kaufman, S. *J. Biol. Chem.* **1961**, *236*, 804. (b) Bublitz, C. *Biochim. Biophys. Acta* **1969**, *191*, 249.

(44) Bray, R. C. *Biol. Magn. Resonance* **1980**, *2*, 45.

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(46) (a) Breslow, R.; Chin, J.; Hilvert, D.; Trainor, G. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4585. (b) Groves, J. T.; Chambers, R. R., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 630.

(47) Slebocka-Tilk, H.; Cocho, J. L.; Frakman, Z.; Brown, R. S. *J. Am. Chem. Soc.* **1984**, *106*, 2421.

(48) Blakely, R. L.; Treston, A.; Andrews, R. K.; Zerner, B. *J. Am. Chem. Soc.* **1982**, *104*, 612.

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Mechanism of Acid-Catalyzed Hydrolysis of Ketene Dithioacetals: Reversibility of the Carbon Protonation

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It is generally accepted that acid-catalyzed hydration of olefins occurs through the initial rate-determining protonation of the carbon–carbon double bond to give carbocation intermediates.[1] The reaction of simple

$$>C{=}C< + H_3O^+ \xrightarrow{\text{slow}} R^+ + H_2O \rightarrow ROH + H^+ \quad (1)$$

alkenes is slow, and the rates are measured conveniently only in strong acids. The olefinic linkage activated by electron-releasing substituents, such as alkoxy groups,

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(1) For recent reviews, see (a) Nowlan, V. J.; Tidwell, T. T. *Acc. Chem. Res.* **1977**, *10*, 252–258. (b) Schmid, G. H.; Garratt, D. G. In *The Chemistry of Double-Bonded Functional Groups*; Patai, S., Ed.; Wiley: New York, 1977; pp 744–751.

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