ADVANCES IN INORGANIC CHEMISTRY, VOL. 40

BIOINORGANIC CHEMISTRY OF PTERIN-CONTAINING MOLYBDENUM AND TUNGSTEN ENZYMES*

JOHN H. ENEMARK† and CHARLES G. YOUNG‡

†Department of Chemistry, University of Arizona, Tucson, Arizona 85721; and ‡School of Chemistry, University of Melbourne, Parkville, Victoria 3052, Australia

I. Scope and Overview
II. The Molybdenum Cofactor (Mo-co)
  A. Molybdopterin and Mo-co
  B. Synthetic Approaches to Mo-co, Molybdopterin, and Related Species
III. Coordination about the Molybdenum Atom
  A. Probes of Molybdenum Centers
  B. Sulfite Oxidase
  C. Xanthine Oxidase
IV. Synthetic Models of the Molybdenum Centers
  A. Background
  B. Models of Enzymes Containing $[MoO_2]^{2+}$ Oxidized Centers
  C. Models of Enzymes Containing $[MoOS]^{2+}$ Oxidized Centers
V. Reactions of Oxo-Molybdenum Centers
  A. Oxygen Atom Transfer (OAT) Reactions
  B. One-Electron Reactions
  C. Coupled Electron–Proton Transfer (CEPT) Reactions
  D. Cyanolysis Reactions
  E. A Model for Enzymes with $[MoO_2]^{2+}$ Oxidized Centers
VI. The Xanthine Oxidase Cycle
VII. Intramolecular Electron Transfer in Molybdenum Enzymes
  A. Xanthine Oxidase
  B. Sulfite Oxidase
  C. Model Systems
VIII. Pterin-Containing Tungsten Enzymes
  A. Introduction
  B. *Clostridium thermoaceticum* Formate Dehydrogenase
  C. *Pyrococcus furiosus* Aldehyde:Ferredoxin Oxidoreductase
  D. *Methanobacterium wolfei* Formylmethanofuran Dehydrogenase

* Dedicated to Professor R. C. Bray on the occasion of his retirement and in recognition of his elegant and stimulating research on molybdenum enzymes, particularly his EPR studies of their molybdenum centers.

E. Other Enzymes
F. Tungsten Chemistry and Model Studies
IX. Future Directions
X. Abbreviations
References
Note Added in Proof

I. Scope and Overview

Molybdenum is the only metal of the second and third transition series that is absolutely essential for all forms of life—microbial, plant, and animal (*1*). Molybdenum-containing enzymes are involved in the metabolism of carbon, nitrogen, and sulfur and are important in the natural cycles involving these elements. In plants and microorganisms molybdenum enzymes are essential for the fixing and uptake of inorganic nitrogen. The nitrogenases, which catalyze the reduction of dinitrogen to ammonia, and the assimilatory and dissimilatory (respiratory) nitrate reductases that catalyze the reduction of nitrate to nitrite are molybdenum-containing enzymes. In animals, molybdenum enzymes are especially important for the oxidation of sulfite to sulfate (sulfite oxidase) and aspects of purine catabolism (xanthine hydroxylases).

The molybdenum centers of enzymes appear to belong to two general classes. In one class, the nitrogenases, the molybdenum atom is part of an iron–molybdenum–sulfur cluster (the iron–molybdenum cofactor, FeMo-co) (*1–5*). All the remaining molybdenum enzymes appear to possess a similar molybdenum cofactor (Mo-co) that contains a single molybdenum atom and a 6-substituted pterin ring system. Table I lists the properties of and reactions catalyzed by representative enzymes of this latter class. The general form of these reactions is given by Eq. (1).

$$X + H_2O \rightleftharpoons XO + 2H^+ 2e^- \quad (1)$$

In the forward direction, (1) is a two-electron oxidation that adds an oxygen atom to the substrate, X; the reverse reaction is a two-electron reduction that removes an oxygen atom from XO. In Table I the enzymes are arranged according to the atom (reaction locant) to or from which oxygen atom transfer takes place. Enzymes containing dioxo-Mo(VI) oxidized active sites effect reactions in which sulfur or nitrogen is the reaction locant. Enzymes containing oxo-thio-Mo(VI) oxidized active sites effect reactions in which oxygen is inserted into a C—H bond; these latter enzymes are also deactivated upon reaction with

TABLE I

PTERIN-CONTAINING MOLYBDENUM ENZYMES

Reaction locant[a]	Enzyme	Source	MW (kDa), subunits[b]	Composition	Substrate/ product[c]	Reference
S	Sulfite oxidase	Mammalian livers	110, α_2	2Mo, 2 cyt_b	SO_3^{2-}/SO_4^{2-}	(*257, 306, 307*)
S	Sulfite oxidase	*T. novellus*	40, M	Mo, heme Fe	SO_3^{2-}/SO_4^{2-}	(*308*)
S	DMSO reductase	*R. sphaeroides* *R. capsulatus*	82, M	1Mo	Me_2SO/Me_2S	(*71, 72, 309*)
S	Biotin-*S*-oxide reductase	Bacteria				(*310*)
N	Nitrate reductase	Plants, fungi, algae, bacteria	228 (fungal)	2Mo, 2 cyt_b, 2 FAD	NO_3^-/NO_2^-	(*311*)
N	Trimethylamine *N*-oxide reductase	*E. coli*	200, M	2Mo, 1Fe, 1.5Zn	Me_3NO/Me_3N	(*312*)
C	Xanthine oxidase[d]	Cow's milk, mammalian livers, kidney	275, α_2	2Mo, $4Fe_2S_2$, 2 FAD	Xn/XnO[e]	(*10, 17, 313, 314*)
C	Xanthine dehydrogenase[d]	Chicken liver, bacteria	300, α_2		Xn/XnO[e]	(*313*)
C	Aldehyde oxidase (pyrimidine oxidase)[d]	Rabbit liver	280, α_2	2Mo, $4Fe_2S_2$, 2 FAD	RH/RHO	(*314, 315*)
C	Formate dehydrogenase[d]	Fungi, yeast, bacteria, plants	105–263	Mo, Se, Fe_nS_n	HCO_2^-/HCO_3^-	(*315, 316*)
C	Carbon monoxide dehydrogenase	Bacteria	230–300	2Mo, $4Fe_2S_2$, 2 FAD, 2Se	CO/CO_2	(*317*)

[a] Enzymes are grouped according to the type of atom in the substrate that undergoes oxidation/reduction.
[b] M, monomer.
[c] The products and reactants of general Eq. (1) are quoted.
[d] These enzymes are deactivated upon reaction with cyanide. Reactivation requires a source of sulfur, typically sulfide.
[e] Xn, xanthine or other purine substrates; XnO, uric acid or other purine products.

cyanide.[1] Pterin-containing tungsten enzymes are also known to catalyze Eq. (1) in hyperthermophilic Archaea and are thereby related to the molybdenum enzymes.

This chapter is restricted to the bioinorganic chemistry of pterin-containing molybdenum and tungsten enzymes. Primary emphasis is given to recent results and to the interplay of model and enzyme chemis-

[1] The molybdenum-containing oxidoreductases that catalyze Eq. (1) have been variously termed "molybdenum hydroxylases" (*6*), "oxotransferases" (*7*), and "oxo-type" molybdenum enzymes (*8*). Molybdenum hydroxylase aptly describes the conversion of xanthine to uric acid, but the name seems less appropriate for the reactions catalyzed by sulfite oxidase and nitrate reductase; oxotransferase implies that the function of these enzymes is to transfer oxo groups, even though relatively little is known about their actual mechanism of action; and the name oxo-type molybdenum enzyme recognizes both the apparent oxo transfer chemistry of Eq. (1) and the fact that the molybdenum atom in each of these enzymes contains at least one terminal oxo group. In this chapter, we shall refer to these enzymes as "pterin-containing molybdenum enzymes" because a 6-substituted pterin appears to be a common chemical feature of all of the enzymes.

try. Particular attention is directed to sulfite oxidase and xanthine oxidase, the archetypal examples of molybdenum enzymes containing, respectively, dioxo-Mo(VI) and oxo-thio-Mo(VI) oxidized centers. The literature is examined selectively, not exhaustively, in an effort to reflect, within the scope and space limitations, the important developments in this area. Our apologies to any authors whose work could not be included or that was inadvertently overlooked. For reviews of earlier research in this area the reader is referred to several volumes (*1–3, 5*) and reviews (*6, 7, 9–19*) dedicated to molybdenum enzymes and related molybdenum chemistry. Tungsten enzymes have not been previously reviewed.

The chapter consists of nine sections. Sections II through VII deal with the pterin-containing molybdenum enzymes. Biochemical and model studies of molybdopterin, Mo-co, and related species are described in Section II. In Section III, we briefly survey physical and spectroscopic techniques employed in the study of the enzymes, and consider their impact upon the current understanding of the coordination about the molybdenum atom in sulfite oxidase and xanthine oxidase. Model studies are described in Sections IV and V. Section IV concentrates on structural and spectroscopic models, whereas Section V considers aspects of the reactivity of model and enzyme systems. The xanthine oxidase cycle (Section VI) and facets of intramolecular electron transfer in molybdenum enzymes (Section VII) are then treated. Section VIII describes the pterin-containing tungsten enzymes and the evolving model chemistry thereof. Future directions are addressed in Section IX.

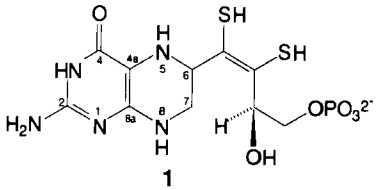
II. The Molybdenum Cofactor (Mo-co)

A. Molybdopterin and Mo-co

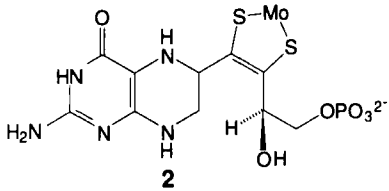
The review by Rajagopalan (*19*) provides a detailed analysis of the genetics and biochemistry of the molybdenum cofactor, as well as an excellent historical account of the experiments that led to the present view of this entity. Here we briefly outline this history and the current status of chemical approaches to understanding Mo-co.

In the early 1970s Nason and co-workers showed that extracts of *nit-1* mutants of *Neurospora crassa* exhibited nitrate reductase activity when mixed with solutions of other molybdenum enzymes that had been subjected to denaturing conditions (*20–23*). The isolation and

characterization of the common molybdenum cofactor was vigorously pursued by Rajagopalan and co-workers (*19, 24–26*). They found that the molybdenum cofactor is extremely unstable and that the molybdenum atom is readily lost upon release of the cofactor from the protein. On the basis of a series of degradative and spectroscopic studies of *molybdenum-free* derivatives of the released cofactor, Rajagopalan, Johnson, and co-workers proposed that the molybdenum cofactor contains a reduced pterin derivative (molybdopterin[2], **1**) that can bind molybdenum through the sulfur atoms of a dithiolene side chain (**2**)



1



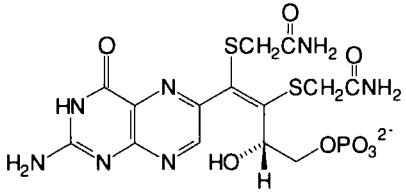
2

(*24–26*). Metal dithiolenes are well-known coordination compounds that often exhibit facile redox reactions (*16, 27, 28*). Free dithiolenes, on the other hand, are generally unstable, and there appear to be no other examples of naturally occurring dithiolenes.

Deistung and Bray (*29*) have described a procedure for anaerobic isolation of active intact molybdenum cofactor from xanthine oxidase. The molecular mass by gel filtration was about 610. Hawkes and Bray (*30*) have reported that Mo-co from xanthine oxidase and sulfite oxidase can be stabilized under anaerobic conditions in the presence of dithionite and that oxidation in the presence of thiophenol results in EPR signals characteristic of Mo(V) and little loss of cofactor activity (*31*). However, to date intact Mo-co has not been structurally characterized. The possible coordination about the molybdenum atom in Mo-co in enzymes is discussed in Section III.

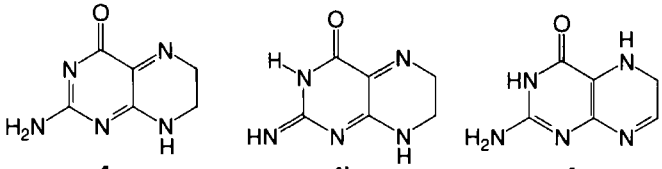
Molybdopterin itself is also extremely unstable when released from a protein and has never been structurally characterized in its native state (*32, 33*). Mass spectral and NMR studies of the di(carboxamidomethyl) derivative of the *oxidized* form of molybdopterin have provided convincing evidence that this derivative is a 6-substituted pterin that possesses structure **3** (*34*). A 6-substituted pterin moiety now appears to be a common feature of all of the molybdenum enzymes of Table I. There is still some question about the oxidation state of the pterin ring

[2] Note that molybdopterin is the organic component of the molybdenum cofactor (Mo-co) and contains *no* molybdenum. Frequently molybdopterin is incorrectly equated to Mo-co.

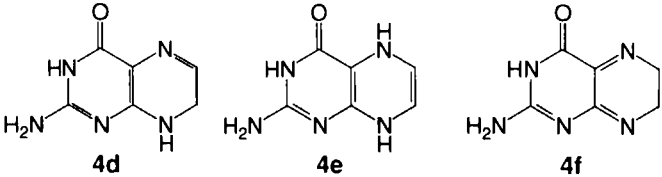


3

system in the native enzymes. Present data favor a dihydropterin (*35*, *36*) rather than a tetrahydropterin as previously postulated and as shown in **1**. Several isomers are possible for a dihydropterin (**4**). Gardlik



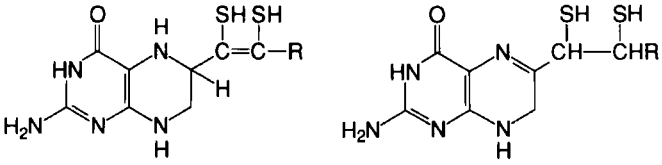
4a **4b** **4c**



4d **4e** **4f**

and Rajagopalan (*35*) postulate that xanthine oxidase contains a quinoid dihydropterin (**4a**), but that native sulfite oxidase contains a different dihydro isomer. Model molybdenum–pterin complexes are discussed in Section II.B.

The proposed dithiolene structure in the side chain of **1–3** is unusual for a naturally occurring compound and is still the subject of some controversy. Curtius *et al.* (*37*) have pointed out that a tetrahydropterin ring system possessing an unsaturated (dithiolene) side chain in the 6-position (e.g., **5a**) is a redox tautomer of **5b**, a structure with a satu-



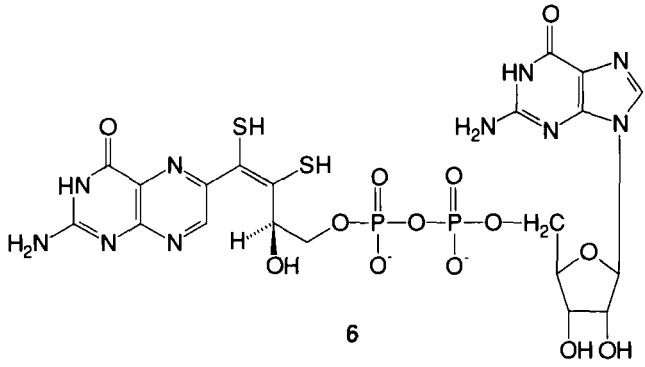
5a **5b**

rated (dithiol) side chain and a dihydropterin ring. The exact structure of the side chain at the 6-position may depend upon the form of the

enzyme and the conditions used for isolation and purification. Edmondson and D'Ardenne (*38*) interpret the electron-nuclear double resonance (ENDOR) spectra of the desulfo-inhibited form of xanthine oxidase as favoring a dithiol structure **5b**. However, Howes *et al.* (*39*) claim that their more extensive ENDOR spectra of several forms of xanthine oxidase provide no evidence for a saturated side chain (**5b**) in this enzyme.

Consistent with the dithiolene structure proposed for the *oxidized* derivative of molybdopterin (**3**), no nonexchangeable ^1H resonances are observed in the region expected for the CH protons of a dithiolate structure (**5b**). The mass spectrum of **3** is also consistent with the proposed dithiolene structure (*19, 34*). Resonance Raman spectra of DMSO reductase show bands at 1575 cm^{-1} (oxidized form) and 1568 cm^{-1} (reduced form) that are assigned to the C=C stretch of the dithiolene unit of **2** (*40*). However, the delocalized electronic structure of dithiolene ligands makes it difficult to assign the C=C stretch with certainty. As Rajagopalan notes (*19*), "ultimate proof of the structure [of molybdopterin] will have to await either X-ray studies on a molybdoenzyme or unequivocal chemical synthesis of the molecule."

In recent years several bacterial proteins have been isolated that contain a pterin that is similar but not identical to molybdopterin from eucaryotic organisms (**1**) (*33, 41–43*). These "bactopterins" (*44*) have been shown to be molybdopterin dinucleotides (e.g., **6** is the oxidized



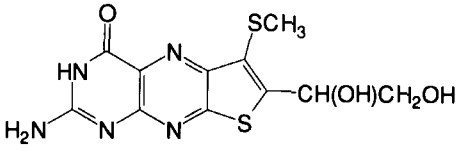
6

form of molybdopterin guanine dinucleotide) (*33, 41*). The analogous adenine (*43*), cytosine (*42*), and hypoxanthine (*43*) molybdopterin dinucleotides are also known, and the di(caboxamidomethyl) derivative of oxidized molybdopterin cytosine dinucleotide has been studied by ^1H and ^{13}C NMR spectroscopy (*44*). All of the pterin-containing molybdenum cofactors give a positive response in the *nit-1* assay because the crude extracts also contain the enzymes that cleave off the dinucleotide fragment to produce **1** (*45*).

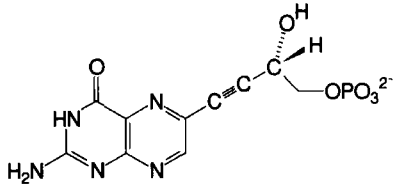
In summary, a 6-substituted pterin was first identified as a structural component of the molybdenum cofactor from sulfite oxidase, xanthine oxidase and nitrate reductase in 1980 (*24*). Subsequent studies provided good evidence that these enzymes possessed the same unstable molybdopterin (**1**), and it seemed likely that **1** was a constituent of all of the enzymes of Table I. It now appears that there is a family of closely related 6-substituted pterins that may differ in the oxidation state of the pterin ring, the stereochemistry of the dihydropterin ring, the tautomeric form of the side chain, and the presence and nature of a dinucleotide in the side chain. In some ways the variations that are being discovered for the pterin units of molybdenum enzymes are beginning to parallel the known complexity of naturally occurring porphyrins, which may have several possible side chains, various isomers of such side chains, and a partially reduced porphyrin skeleton (*46*).

B. Synthetic Approaches to Mo-co, Molybdopterin, and Related Species

The modeling and ultimate total synthesis of molybdopterin and Mo-co have been hampered by difficulties associated with the pterin chemistry involved. However, synthetic assaults on molybdopterin, the molybdenum cofactor, and the degradation products of the molybdenum cofactor are now well underway. The total chemical synthesis of urothione (**7**), the postulated metabolic excretory product of Mo-co (*25*), has recently been reported (*47*). Compound **7** is a naturally occurring substituted thiophene that is found in the urine of normal humans. It is absent (*25*) from the urine of children who lack the molybdenum cofactor due to a genetic defect and who are unable to metabolize sulfite (*48*). The absolute configuration of Form A (**8**), another degradation product of



7



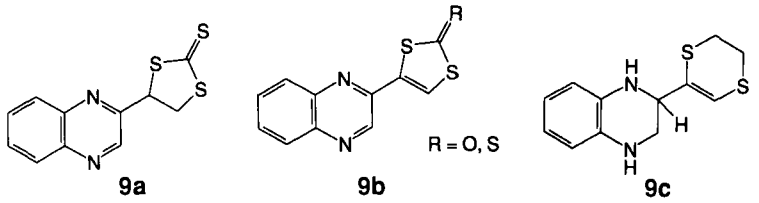
8

the oxidation of Mo-co, has been shown to be *S* by synthesis of the dephospho analogue of **8** and comparison of its circular dichroism (CD) spectrum to that of dephospho Form A from natural sources (*49*).

In view of the difficulties associated with pterin chemistry, substituted quinoxalines (**9**) have served as the initial synthetic models for molybdopterin (**1**). Garner, Joule, and co-workers (*50–53*) have pre-

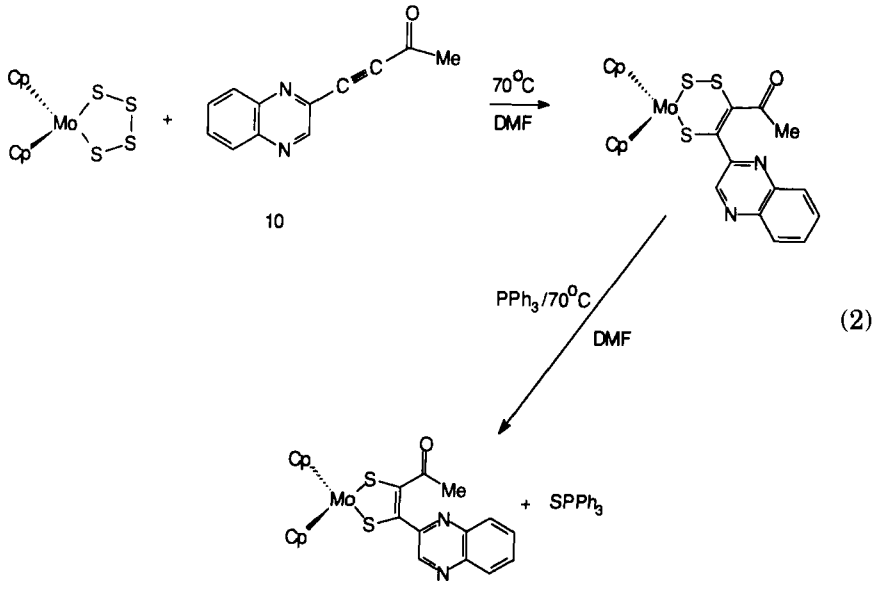
pared molecules containing both saturated (**9a**) and unsaturated (**9b**) side chains, as well as a tetrahydroquinoxaline (**9c**) with a 1,2-dithiolene side chain.

Alkyne-substituted quinoxalines (**10**) and pterins have been converted to molybdenum–dithiolenes via Eq. (2) (*54*) and Eq. (3) (*55*) by exploiting the reaction of activated acetylenes with molybdenum polysulfide complexes (*9, 27, 28*). The Mo–S vibration of **11**, the final



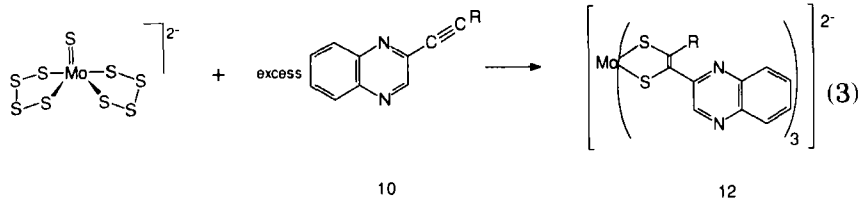
9a **9b** **9c**

product of Eq. (2), has been unambiguously assigned as 349 cm^{-1} using Raman spectroscopy and isotopic labeling with ^{34}S (*54*). For DMSO reductase Mo—S vibrations are observed in the 350–390 cm^{-1} range (*40*) (Section III.A.5).



10 11 (2)

Oxidation of the tris(dithiolene) complex **12** yields **13**, a molecule containing a substituted thiophene ring (*55*). One of the products of the oxidation of the molybdenum cofactor is Form B (**14**) (*26*), a substituted



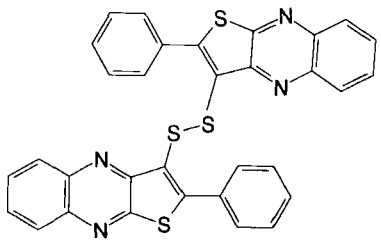
10 12

thiophene. Urothione (**7**) also contains a substituted thiophene ring. The formation of **13** from **12** (*55*) provided the first experimental evidence that molybdenum–dithiolene complexes yield substituted thiophenes upon oxidation, and further supports the proposal that molybdopterin (**1**) possesses a dithiolene group.

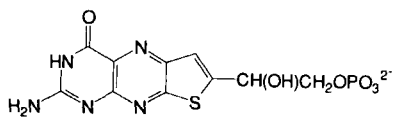
The heterocyclic pterin ring system itself has several potential metal binding sites (*13*), and a variety of metal complexes containing O- and N-bound pterins have been prepared and characterized (*56–62*). In the dinuclear dioxo-Mo(VI) complex, $[Mo_2O_5(xanthopterinate)_2]^{2-}$ (**15**, Fig. 1), the pterin ring system functions as a tridentate bridging ligand with atoms O3, O4, and N5 coordinated to the Mo atoms (*57*). The complexes formed from the reaction of tetrahydropterins and dioxo-Mo(VI) compounds are especially pertinent to the previous discussion of the oxidation state and valence tautomerism of the pterin ring system (Section II.A). Burgmayer *et al.* (*60*) have demonstrated that 6,7-dimethyl-5,6,7,8-tetrahydropterin (H_4dmp) is oxidized to a quinoid dihydropterin (**4a**) by $MoO_2(S_2CNEt_2)_2$ (Eq. (4)). They claim that "this normally reactive dihydropterin is significantly stabilized when generated by $MoO_2(S_2CNEt_2)_2$ oxidation" (*60*).

$$MoO_2(S_2CNEt_2)_2 + H_4dmp \longrightarrow MoO(S_2CNEt_2)_2 + H_2dmp + H_2O \qquad (4)$$

Fischer *et al.* (*62*) have shown that tetrahydro-*L*-biopterin reacts with $Mo^{VI}O_2Cl_2$ to give **16** (Fig. 2), which is formulated as an oxo-Mo(IV) complex of protonated 1,5-quinoid-7,8-dihydro-6*H*-*L*-biopterin. The short N(5)—C(4a) bond distance (1.334(8) Å), the long N(5)—C(6) dis-



13



14

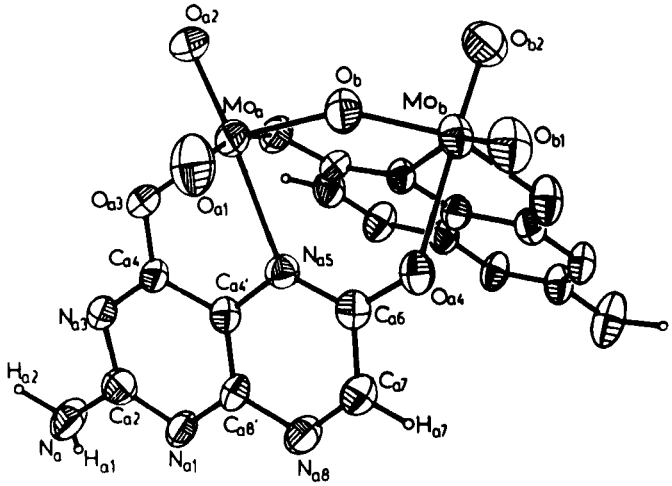


FIG. 1. Structure of the $[Mo_2O_5(xanthopterinate)_2]^{2-}$ ion (**15**). Reproduced with permission from Burgmayer and Stiefel (*57*). Copyright 1986, American Chemical Society.

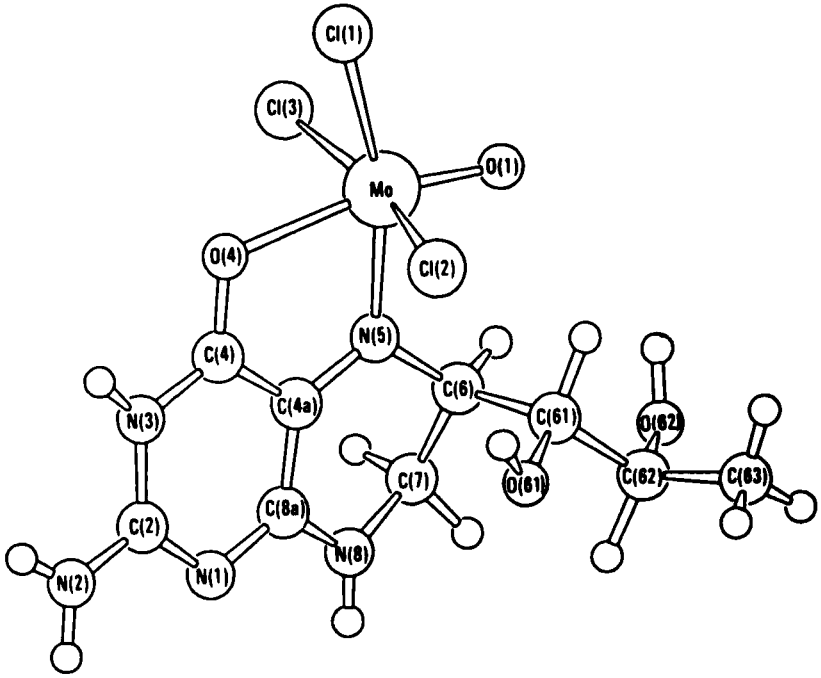
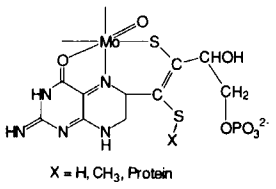


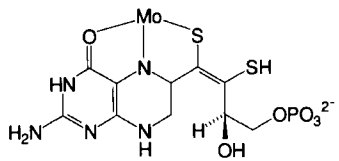
FIG. 2. Structure of **16**; adapted from Fischer *et al.* (*62*) with permission.

tance (1.484(8) Å), and the short C_{4a}—C_{8a} distance (1.426(7) Å) support this tautomeric description of the pterin ring system. Other structural features of interest are the unusually short Mo—N(5) distance (2.017(4) Å) and the weak coordination of the ketonic oxygen atom (C(4)—O(4) = 1.274(7) Å; Mo—O(4) = 2.229(4) Å). However, Burgmayer *et al.* (*63*) have suggested that complexes such as **16** can also be formulated as Mo(VI) complexes of a deprotonated tetrahydropterin ligand. This debate over the formal oxidation state description of **16** underscores the redox noninnocence of pterin ligands. For Mo-co the combination of the pterin ring system and the well-recognized noninnocent redox behavior of dithiolene ligands (*27, 28*) may play a subtle role in molybdenum enzyme catalysis.

The structure of **16** led to the proposals of an alternative structure (**17**) (*62, 63*) in which the dithiolene moiety adopts *trans* stereochemistry with only one of the sulfur atoms coordinated to the Mo atom. It is interesting to note that this particular coordination mode is perfectly set up to permit ligand degradation to thiophenes such as urothione (**7**) and might exist in transient form during oxidative degradation of pterin dithiolene complexes (*55*) and Mo-co. Structure **18** is an alternative to **2** that has *cis*-dithiolene geometry and a mode of coordination about the Mo atom similar to **17**. In both **17** and **18** the noncoordinated sulfur atom would presumably be stabilized by interactions with other groups in the protein or active site.



17



18

We conclude this section by noting that substantial progress has been made toward synthesizing Mo-co in recent years, but that the ultimate objective still appears some distance away. In the following sections we turn our attention to the molybdenum atom of Mo-co and address the following questions: (1) What methods can be used to directly probe the molybdenum centers of enzymes (Section III.A)? (2) What are the coordinating atoms and coordination geometry (Sections III.B and C)? (3) What oxidation states are involved in catalysis (Sections IV and V)? (4) How does substrate oxidation (reduction) occur (Sections IV and V)? (5) What insight concerning the structures and

catalytic reactions of pterin-containing molybdenum enzymes can be obtained from model molybdenum chemistry (Sections IV–VI)?

III. Coordination about the Molybdenum Atom

A. Probes of Molybdenum Centers

1. General

As yet, no X-ray crystal structures are available for any of the molybdenum enzymes in Table I. Therefore, *present descriptions of the coordination environment of the molybdenum centers of the enzymes rest primarily upon comparisons of the spectra of the enzymes with the spectra of well-characterized molybdenum complexes.* The two most powerful techniques for directly probing the molybdenum centers of enzymes are electron paramagnetic resonance (EPR) spectroscopy and X-ray absorption spectroscopy (XAS), especially the extended X-ray absorption fine structure (EXAFS) from experiments at the Mo K-absorption edge. Brief summaries of techniques are presented in this section, followed by specific results for sulfite oxidase (Section III.B), xanthine oxidase (Section III.C), and model compounds (Section IV).

2. EPR and ENDOR Spectroscopies

These techniques are applicable only to paramagnetic Mo(V) centers, but the EPR parameters are extremely sensitive to coordination changes at the molybdenum center (*17, 64*). The molybdenum and ligand hyperfine splittings can provide additional information about the coordination environment of the molybdenum(V) species and the chemical reactions at the molybdenum center. EPR spectra from xanthine oxidase were first reported in 1959 by Bray *et al.* (*65*), and Bray and co-workers have continued to develop the application of EPR spectroscopy to molybdenum enzymes (*17, 64*). In 1966 it was shown (*66*) that mixing $[MoO_4]^{2-}$ with dithiols produced EPR signals with $\langle g \rangle$ and $\langle A(^{95,97}Mo) \rangle$ values similar to those of xanthine oxidase. Only recently, however, have the structures of such thiolate complexes been determined (see Section IV.B.2.b). ^{1}H (*39*) and ^{31}P (*67*) ENDOR spectroscopies have been applied to xanthine oxidase and sulfite oxidase.

3. X-Ray Absorption Spectroscopy

EXAFS at the Mo K-edge is usually applied to enzymes in their fully oxidized (Mo(VI)) or fully reduced (Mo(IV)) states (*68*), but studies of enzymes poised in the Mo(V) oxidation state have also been described

(*69*). EXAFS data from frozen solutions and polycrystalline samples can provide information about the number, type, and distances of atoms coordinated to the molybdenum atom, but not angular information that defines stereochemistry. Mo L-edge spectroscopy has been used to investigate the $2p \rightarrow 4d$ electronic transitions of the molybdenum atom in sulfite oxidase and model compounds in order to obtain information about the symmetry and electronic structure of the molybdenum center (*70*).

4. Electronic Spectroscopy

Conventional electronic spectroscopy has been of little help in characterizing the molybdenum site because most of the pterin-containing molybdenum enzymes (Table I) possess other prosthetic groups (Fe—S, flavin, heme) whose electronic transitions dominate the electronic spectra of the enzymes. However, the recent isolation and purification of DMSO reductase from *Rhodobacter sphaeroides* forma specialis *denitrificans* (*71*) and *Rhodobacter capsulatus* (*72*) may finally permit detailed spectroscopic study of the molybdenum center. DMSO reductase from *R. sphaeroides* contains *only* the molybdopterin guanine dinucleotide cofactor (**6**) and a single polypeptide chain (*33*). Therefore, for this protein it is possible to use electronic spectroscopy to probe the molybdenum center without interference from other highly absorbing prosthetic groups. The unique absorption spectra of this enzyme are shown in Fig. 3.

5. Raman Spectroscopy

Resonance Raman spectroscopy has been used to probe the metal coordination in a variety of metalloproteins (*73*). For most pterin-containing molybdenum enzymes, however, the other strongly absorbing prosthetic groups (Table I) dominate the electronic and resonance Raman spectra, and little is known about molybdenum–ligand vibrations. An additional complication is the large number of naturally occurring molybdenum isotopes (mass range, 92–100) whose abundances are sufficiently great to broaden weak low-frequency vibrational modes.

To date only DMSO reductase from *R. sphaeroides* forma specialis *denitrificans* (*71*) (Section III.A.4) has been studied by resonance Raman spectroscopy (*40*). The oxidized and reduced forms of DMSO reductase show vibrations in the 335- to 385-cm^{-1} region that shift upon enrichment of the enzyme with ^{34}S and that have been assigned to Mo—S vibrations. The most prominent feature is the band at 350 cm^{-1} in oxidized DMSO reductase, which shifts to 341 cm^{-1} upon ^{34}S enrichment. For the *oxidized* state of the enzyme (presumably Mo(VI)) the 350-cm^{-1} band has been assigned to a Mo—S(dithiolene) vibration

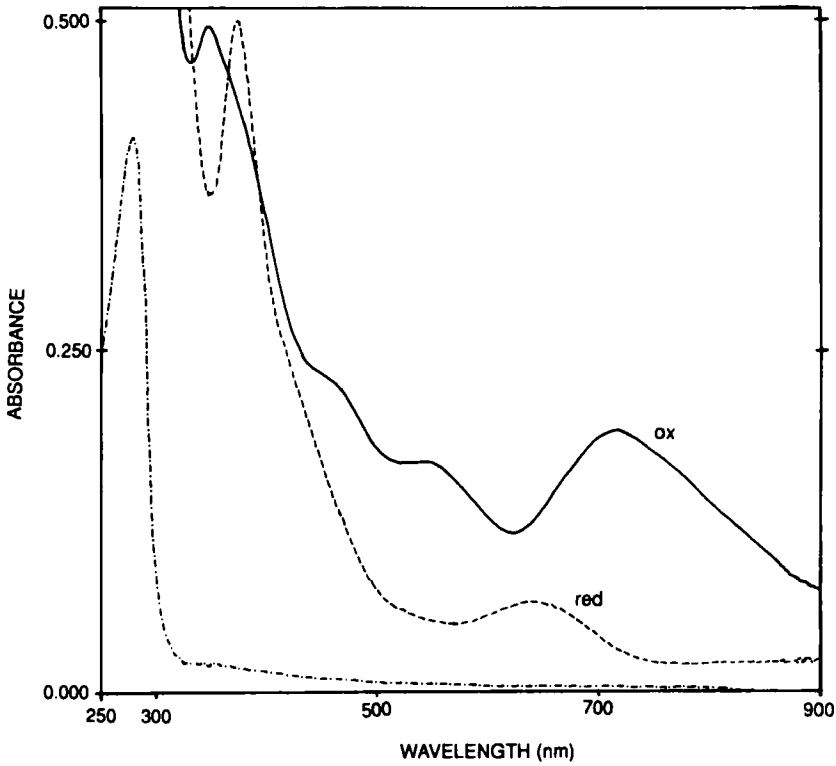


FIG. 3. Absorption spectra of DMSO reductase in 50 m*M* Tris·HCl, pH 7.5. (—) Oxidized enzyme at 7.9 mg/ml; (—·—·) oxidized enzyme at 0.3 mg/ml; (---) enzyme anaerobically reduced with dithionite at 7.8 mg/ml. Reproduced from Bastian *et al.* (*71*) with permission.

from comparison with the 349-cm^{-1} band in the *formally Mo(IV)* model compound 11 (Section II.B) (*54*). However, the possibility that the Mo—S bands in DMSO reductase arise from other Mo—S(thiolate) vibrations cannot be eliminated.

Resonance Raman studies on model complexes containing Mo=O and Mo=S groups have shown that the Mo=O group is a very weak Raman scatterer and is unlikely to be detectable in an enzyme (*74*). The Mo=S group showed maximum Raman enhancements of about 33 and may be detectable in oxidized xanthine oxidase and other enzymes possessing $[MoOS]^{2+}$ oxidized centers.

6. *Fluorescence Spectroscopy*

The characteristic fluorescence spectra of oxidized pterins observed in solutions of denatured molybdenum enzymes provided some of the first clues that the molybdenum cofactor contains a pterin unit (*24*).

The absence of such fluorescence in intact enzymes led to the original proposal that molybdopterin (1) is a tetrahydropterin (*25*). However, fluorescence from an oxidized pterin ring is also effectively quenched (>95%) in model complexes that contain a metallodithiolate on the C(6) side chain (*54*). Urothione (7), the proposed metabolic excretory product of molybdopterin, is also nonfluorescent (*25*). In general, the fluorescence behavior of model molybdenum complexes has received little attention (*75*).

7. *Electrochemistry*

The postulated catalytic cycles for pterin-containing molybdenum enzymes involve a two-electron change at the molybdenum atom (Mo(VI) ↔ Mo(IV)). Microcoulometric titrations of nitrate reductase (*Chlorella vulgaris*) (*76*), milk xanthine oxidase (*77*), and sulfite oxidase (*78*) show that their molybdenum centers are reduced by two electrons. The reduction potentials for the molybdenum center of chicken liver sulfite oxidase are strongly dependent upon pH and upon anion concentration (*78*).

Electrochemical methods have been extensively used to characterize model oxo-molybdenum compounds (Sections IV and V). Electrochemistry provides a convenient method for generating reactive molybdenum complexes *in situ* (see Sections V.B and C) and for investigating the reaction rates and possible reaction mechanisms of transient molybdenum complexes.

8. *Other Possible Probes*

a. ^{31}P NMR. Several modifications of xanthine oxidase have been investigated by ^{31}P NMR (*79*). The intensity of the ^{31}P resonance assigned to the phosphorous atom of Mo-co diminishes significantly in the Mo(V) state of the glycol-inhibited desulfo form of the enzyme, consistent with this phosphorus group being close to the paramagnetic Mo(V) center. ^{31}P NMR studies of model paramagnetic oxo-Mo(V) complexes with pendant organophosphate groups show that the ^{31}P linewidth is strongly dependent upon the Mo···P distance (Section IV.B.2.b) (*80, 81*).

b. ^{95}Mo NMR. This technique has proven useful for the study of molybdenum compounds, but to date no one has successfully observed the ^{95}Mo NMR spectrum of an enzyme (*82*). It seems likely that the linewidths will be too broad and the intensities too weak for direct observation of ^{95}Mo NMR spectra from enzymes.

c. Magnetic Circular Dichroism (MCD). In principle it should be possible to use MCD spectroscopy to probe the paramagnetic Mo(V) centers of enzymes in the presence of other diamagnetic chromophores because the intensities of MCD transitions from paramagnetic species increase dramatically at low temperature, whereas the MCD spectra from diamagnetic centers are temperature independent. For the desulfo inhibited form of xanthine oxidase (Section III.C), the difference MCD spectrum shows evidence for a Mo(V) contribution, but the spectral changes constitute only a small fraction of the total signal (*83*). The recent discovery that DMSO reductases from *R. sphaeroides* (*71*) and *R. capsulatus* (*72*) contain only a molybdenum cofactor and no other prosthetic groups has opened the way to direct investigation of the Mo(V) states of enzymes by MCD spectroscopy (*84, 85*). The Mo(V) states of these enzymes show rich MCD spectra between 300 and 800 nm that have been assigned to π-dithiolene-to-Mo(V) charge transfer transitions arising from structure **2**. For the native enzymes only a small fraction (ca. 6–25%) of the Mo(V) state of the enzyme can be present because of the small separation between the Mo(VI)/Mo(V) and the Mo(V)/Mo(IV) redox couples (*71*). However, glycerol-inhibited *R. sphaeroides* DMSO reductase forms a stable Mo(V) state that is similar to the glycerol inhibited state of desulfo xanthine oxidase (*86*) and that contains about 80% Mo(V). Figure 4 shows the electronic, EPR, and MCD spectra of this glycerol-inhibited Mo(V) state (*85*). Detailed interpretation of the MCD results from enzymes awaits elucidation of the MCD spectra of model oxo-molybdenum(V) complexes (*87, 88*).

B. Sulfite Oxidase

Early Mo K-edge EXAFS data for sulfite oxidase showed that the oxidized resting state contains a $[Mo^{VI}O_2]^{2+}$ unit, whereas the fully reduced enzyme possesses a $[Mo^{IV}O]^{2+}$ unit (*8*). Both units are ligated by two or three sulfur atoms and possibly additional N or O ligands. Two of the coordinated sulfur atoms are presumably provided by molybdopterin, as shown in **2**. However, similar EXAFS results would be expected if the molybdenum atom were bound to thiolate groups of the protein itself.

The transient molybdenum(V) states of sulfite oxidase have been probed by both EXAFS and EPR spectroscopy. The EPR spectral parameters are sensitive to pH (*89*) and to anions in the medium (*90*), as shown in Fig. 5 (*69*). Comparison of the enzyme EPR parameters to those of known Mo(V) complexes (Section IV.B.2) shows that the large

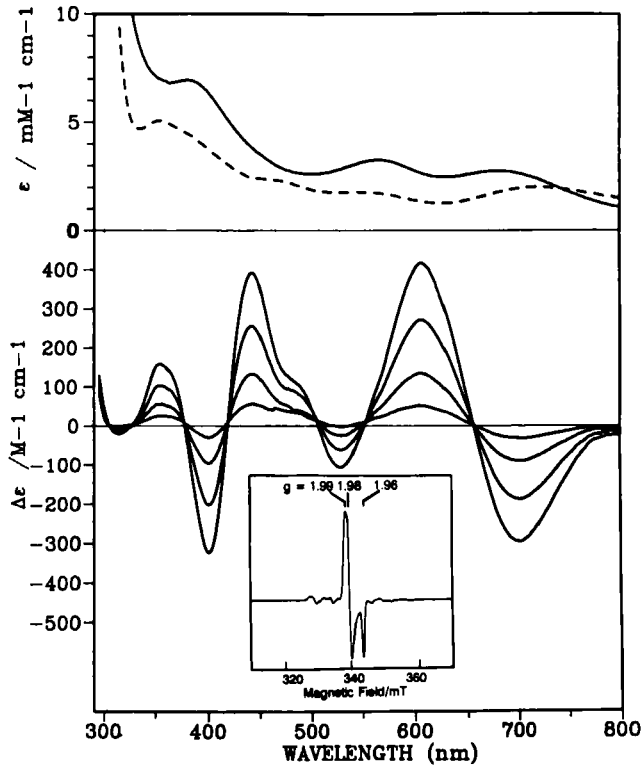


FIG. 4. UV-visible absorption, MCD, and EPR spectra of the glycerol-inhibited Mo(V) form of DMSO reductase from *R. sphaeroides*. Upper panel: (---) room temperature absorption spectrum of the native Mo(VI) form; (—) glycerol-inhibited Mo(V) form. Lower panel: MCD spectra of the glycerol-inhibited Mo(V) form at a magnetic field of 4.5 T at 1.61, 4.22, 9.6, and 27.2 K, showing that all bands increase in intensity with decreasing temperature. Inset shows the X-band EPR spectrum of the MCD sample recorded at 150 K. Reproduced with permission from Finnegan *et al.* (*85*).

g_1 (>2.00) for the low-pH/high-Cl^- form of sulfite oxidase is consistent with an $[Mo^VO]^{3+}$ unit ligated by at least two thiolate ligands (*91*). The low-pH/high-Cl^- spectrum (Fig. 5, top) also shows well-resolved coupling to a single exchangeable proton that is absent in the high-pH/low-chloride spectrum (Fig. 5, bottom) (*69*). However, the high-pH spectrum does show distinct shoulders on both sides of the g_1 feature (arrows), which George (*92*) has shown are due to spin–flip transitions from an anisotropically coupled exchangeable proton. George (*92*) has proposed that an Mo–OH group is responsible for the observed ^1H

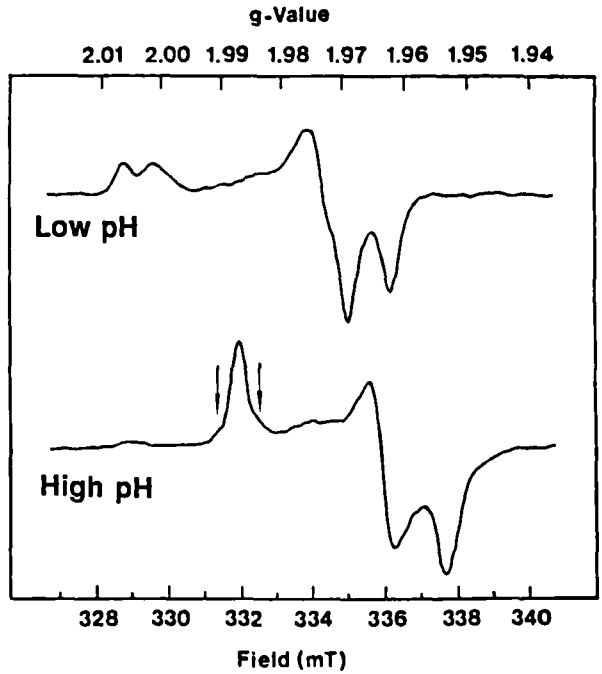


FIG. 5. EPR spectra of the low-pH/Cl^- (top) and high-pH (bottom) forms of sulfite oxidase. Adapted with permission from George *et al.* (*69*). Copyright 1989, American Chemical Society.

coupling in both spectra of Fig. 5. The two species can be interconverted by varying the pH and chloride concentration.

Recently, Mo K-edge EXAFS data have been obtained at low pH/high Cl^- and high pH/low Cl^- for each of the three oxidation states of the molybdenum center of sulfite oxidase (*69*) by poising the potential with redox dyes (*78*). This latter EXAFS study provides strong evidence that one chloride ion binds to the Mo(IV) and Mo(V) states of the enzyme at low pH/high Cl^-, but that Cl^- does not appear to bind to the Mo(VI) state of the enzyme. Combination of the EXAFS and EPR data for sulfite oxidase yields the minimal structures for the molybdenum center shown in Fig. 6.

Phosphate is an effective inhibitor of sulfite oxidase (*90*), and the phosphate inhibited enzyme exhibits a characteristic Mo(V) EPR spectrum (*93*). George *et al.* (*94*) have reinvestigated the EPR spectra of the Mo(V) phosphate complex and interpreted the ^{31}P hyperfine coupling as arising from *two* independent ^{31}P atoms, implying that two phosphate

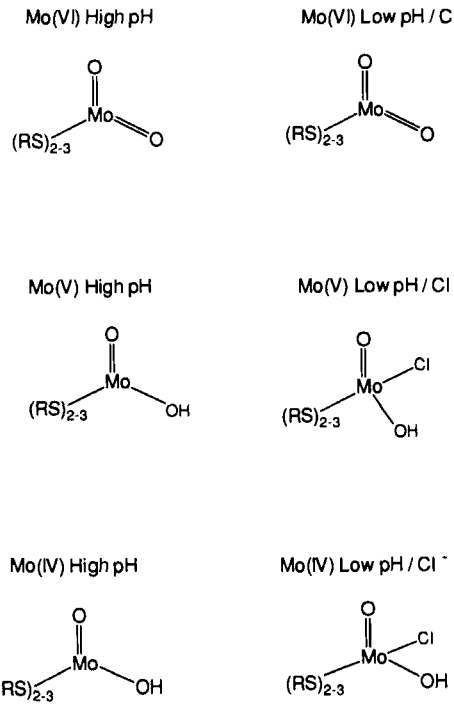
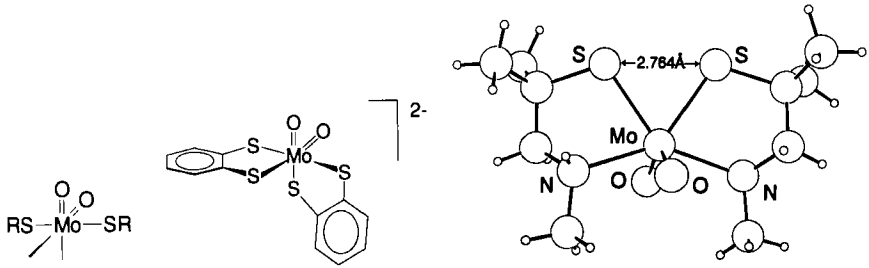


FIG. 6. Minimal structures for the high- and low-pH species in the Mo(VI), Mo(V), and Mo(IV) oxidation states of sulfite oxidase, based upon EXAFS and EPR results. Adapted with permission from George *et al.* (*69*). Copyright 1989, American Chemical Society.

groups, rather than one, bind to Mo(V) in the inhibited complex. The spectra also provide evidence for hyperfine coupling to at least one exchangeable proton.

In the absence of an X-ray crystal structure of sulfite oxidase, what more can be deduced about the coordination environment about the Mo atom from the EXAFS and EPR data described above? Let us consider the geometry of the $[Mo^{VI}O_2]^{2+}$ unit that is present in the oxidized resting state of the enzyme (see Section IV.B.1 for model studies) (*8*). For most known dioxo-molybdenum(VI) complexes, the O—Mo—O angle is about 108°, close to the tetrahedral angle of the $[MoO_4]^{2-}$ ion. Numerous six-coordinate compounds containing the *cis*-$[MoO_2]^{2+}$ unit and two thiolate ligands have been characterized by X-ray crystallography. Such compounds usually adopt distorted octahedral stereochemistry with the two thiolate ligands *cis to both oxo groups and trans to one another* (**19**) (*15, 95*). This stereochemistry is *not*



19 20 21

possible for *cis*-dithiolene coordination by molybdopterin (**2**) because **19** would require that at least one of the sulfur atoms of **2** be *trans* to an oxo group. Only one compound with a thiolate sulfur atom *trans* to a terminal oxo group has been structurally characterized (**20**) (*96*). Skew trapezoid bipyramidal geometry, displayed by **21**, is another stereochemistry found for some six-coordinate $[MoO_2]^{2+}$ derivatives of N-alkylated cysteamines (*97, 98*). This stereochemistry is compatible with *cis*-dithiolene coordination by molybdopterin (**2**). There are no known examples of molybdenum(VI) complexes with *trans* oxo groups, another conceivable geometry. The possibility that only one of the sulfur atoms of molybdopterin is coordinated to the Mo atom has been discussed in Section II.B.

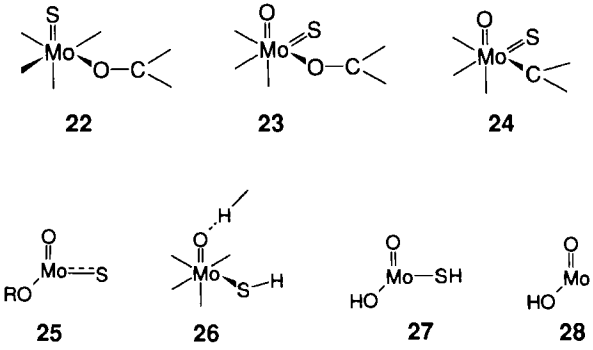
C. Xanthine Oxidase

The molybdenum center in xanthine oxidase has been much more extensively probed than that of sulfite oxidase. However, the presence of sulfo (active) and desulfo (inactive) forms of the enzyme (*10, 17, 64*) in most preparations can make spectroscopic data from xanthine oxidase difficult to interpret unambiguously. Nevertheless, EXAFS studies from several laboratories (*8, 68, 99–102*) now point to an $[Mo^{VI}OS]^{2+}$ unit in the oxidized state and a $[Mo^{IV}O(SH)]^{+}$ unit in the reduced state for the active forms of the enzyme, as first suggested by Williams and Wentworth (*103*). Both states are ligated by at least two thiolate groups. Unambiguous interpretation of the EXAFS data is hindered somewhat by the lack of model compounds containing the fundamental $[Mo^{VI}OS]^{2+}$ and $[Mo^{IV}O(SH)]^{+}$ units (Section IV.C). Eagle *et al.* have demonstrated that the thio ligand in $[Mo^{VI}OS]^{2+}$ centers may be stabilized by S···S interactions without lengthening the Mo=S distance beyond the range established by EXAFS for the enzyme center (Section IV.C.1) (*104*). The coordination about the Mo atom in the inactive desulfo form of xanthine oxidase appears to be similar to that

in sulfite oxidase, with an $[Mo^{VI}O_2]^{2+}$ unit in the oxidized form and an $[Mo^{IV}O]^{2+}$ unit in the reduced form.

Bray and co-workers have extensively investigated the transient Mo(V) states of xanthine oxidase by EPR spectroscopy using Rapid-freeze methods and isotopic labeling techniques (*17, 64*). The Very Rapid EPR signal ($t_{1/2}$, 10 msec) appears under substrate limiting conditions and is accompanied by the Rapid signal ($t_{1/2}$, 25 msec) with excess xanthine. Other distinctive EPR signals observed for the Mo(V) state of xanthine oxidase include the Slow signal, the Inhibited signal, and the Desulfo Inhibited signal. Several different Mo(V) coordination environments have been proposed over the years to account for the diverse and distinctive EPR parameters for the various forms of xanthine oxidase (*10, 17, 64*), but well-characteized model Mo(V) compounds to test the proposed structures have only recently started to become available (Section IV.C.2).

Gutteridge and Bray originally proposed (*105*) that the Very Rapid signal arose from a Mo(V) center with oxygen-bound substrate in which the Mo=S functionality had been retained, but from which the Mo=O functionality had been lost (**22**). Subsequent EPR studies of xanthine oxidase by George and Bray (*106*) using isotopic enrichment with ^{95}Mo, ^{97}Mo, ^{33}S, and ^{17}O resulted in two additional models (**23** and **24**) for the Very Rapid signal center of xanthine oxidase; these contained both terminal oxo and terminal thio groups, and either oxygen- (**23**) or carbon-bound (**24**) substrate. From multiple-frequency EPR studies of isotopically labeled model compounds and enzyme, Spence, Wedd, and their co-workers (*107–109*) have proposed that the Very Rapid signal arises from an oxo-thio-Mo(V) species with a reduced Mo—S bond order and oxygen-bound substrate (**25**). The Rapid EPR signals are proposed to arise from oxo-hydrosulfido Mo(V) centers (**26** (*17*) and **27** (*109*)) and the Slow signal is assigned to an oxo-hydroxo species (**28**) (*17, 109*).



22 **23** **24**

25 **26** **27** **28**

The possible role of these structures in the intimate mechanism for xanthine oxidase is discussed in Section VI.

Further support for an Mo=O group in the reduced states of xanthine oxidase was provided by Mo K-edge EXAFS studies of the Mo(V) state of xanthine oxidase inhibited with pyridine-3-carboxaldehyde and of the Mo(IV) state of alloxanthine-inhibited enzyme (*102*). Both of these inhibited species showed clear evidence for an Mo=O group (1.70 Å), but neither exhibited an Mo=S distance of 2.15 Å, as is observed for the oxidized state of functional xanthine oxidase (8, 68, 100–102). Comparison of the EPR parameters for model compounds containing the $[Mo^{V}=O]^{3+}$ and $[Mo^{V}=S]^{3+}$ groups also favors the presence of an Mo=O group in the Mo(V) states of xanthine oxidase (Section IV.C.2) (*110*). Section VI presents a molecular mechanism for xanthine oxidase that combines the wealth of EPR and EXAFS data available for the various forms of the enzyme (*17, 64, 107–109*) with recent developments in model molybdenum chemistry (*107–109, 111–113*).

IV. Synthetic Models of the Molybdenum Centers

A. Background

The synthetic analogue or model approach (*114*) can provide insights into complex biomolecules through the design, synthesis, and study of small molecules that mimic a component, typically an active site or prosthetic group, of the biomolecule. The approach is particularly valid for metal active sites that have not been unambiguously characterized by other methods or where key chemical or spectroscopic information is required for the interpretation of the properties of the biomolecule.

The biological role of molybdenum has greatly stimulated development of the coordination and bioinorganic chemistry of this element over the past 30 years (*7, 9, 14, 16, 115*). Much of this chemistry relates to enzyme behavior in only a broad or peripheral sense; very few "model" systems accurately target the structure, spectroscopy, and reactivity of the enzymes as they are now perceived. In this section we concentrate on models that have a direct bearing on the structural and spectroscopic properties of the Mo centers. Section IV.B considers models for enzymes possessing oxidized $[Mo^{II}O_2]^{2+}$ centers, e.g., sulfite oxidase; Section IV.C considers models for enzymes that possess oxidized $[Mo^{VI}OS]^{2+}$ centers, e.g., the xanthine hydroxylases. Rather than treat individual models separately, we have chosen to discuss the state of modeling in each of the above classes according to molybdenum

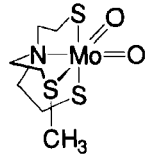
oxidation state. We defer the consideration of reactivity models for these enzymes until Section V.

B. Models of Enzymes Containing $[MoO_2]^{2+}$ Oxidized Centers

1. Mo(VI) Complexes

The chemistry of Mo(VI) is dominated by complexes containing the *cis*-$[MoO_2]^{2+}$ fragment (*9, 15, 115*), and the incorporation of this unit into model complexes is generally straightforward. Rather, it is the construction of an appropriate co-ligand environment for the $[MoO_2]^{2+}$ center that presents the greatest modeling problem. This is primarily due to an inadequate knowledge of the coordination environment of the $[MoO_2]^{2+}$ group. Sulfur donor ligands are an essential (Section III) but not sufficient feature for such co-ligands. The most valuable models are those which mimic enzyme reactions or, by virtue of the existence of Mo(V) and/or Mo(IV) counterparts, serve as broader models of the enzymes. Exploration of the chemistry of the Mo(V) and/or Mo(IV) counterparts requires sterically demanding ligands in order to prevent dinucleation of the Mo(V) components (Sections IV.B.2 and V). Accordingly, the discussion below concentrates on such complexes, which may be broadly classified according to ligand type (Fig. 7).

a. Tetradentate N-, S-, and O-Donor Ligand Complexes. The complex $MoO_2\{N(CH_2CH_2S)_2(CH_2CH_2SMe)\}$ (**29**), reported in 1979 by Stiefel *et al.* (*116*), exhibits an EXAFS spectrum that closely matches that of sulfite oxidase (*8, 117*). Compound **29** possesses distorted octahedral stereochemistry (**19**) with a *cis*-$[MoO_2]^{2+}$ unit (Mo—O = 1.69 Å), two mutually *trans* thiolate groups (Mo—SR = 2.40 Å), and a thioether group (Mo—SR_2 = 2.80 Å) *trans* to one of the Mo=O groups. A distinctly different EXAFS spectrum is produced by the complex in which the SMe group is replaced by an NMe_2 group. The close match of the EXAFS spectra of **29** and of oxidized sulfite oxidase has played a major role in the development of proposals for the coordination environment about the Mo atom in the enzyme (Section III.B). Unfortunately, the chemistry of **29** and related compounds has not been extended to the Mo(V) or Mo(IV) oxidation states.



29

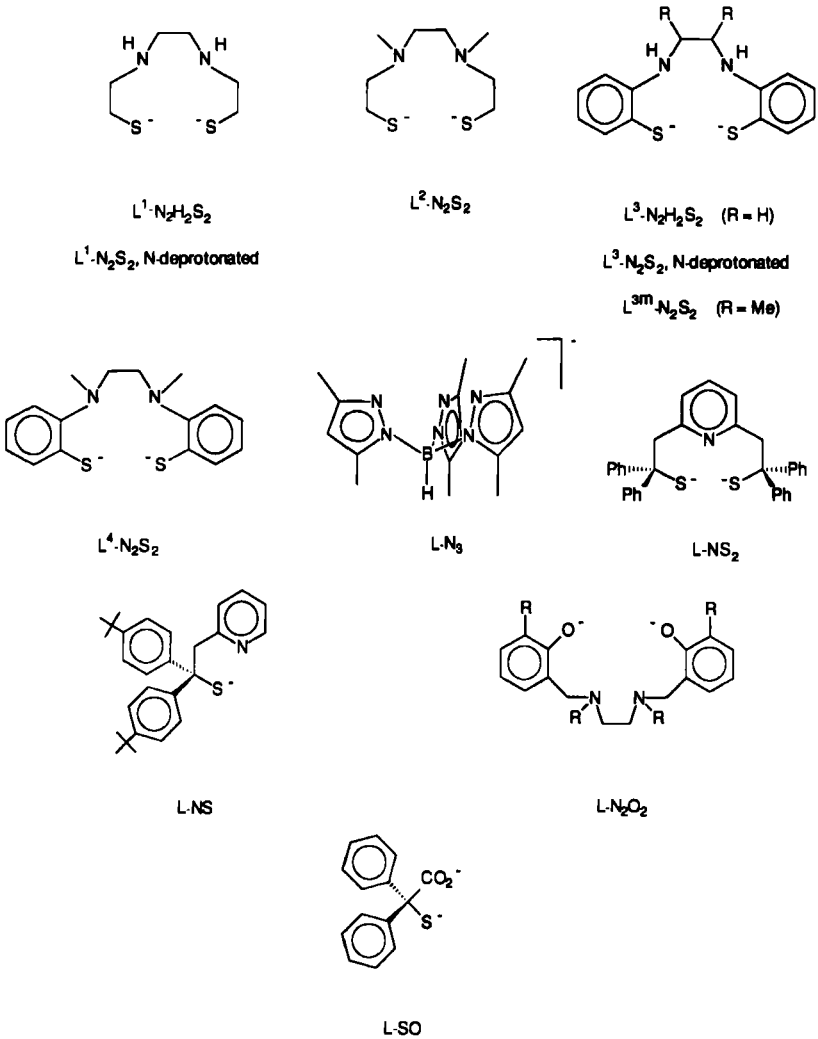


FIG. 7. Polydentate ligands and their abbreviations.

A wide variety of N_2S_2-ligand complexes are easily prepared by reaction of $MoO_2(acac)_2$ or $(NBu_4)_4[Mo_8O_{26}]$ with the appropriate free acid LH_2 (*118–122*). Many of these diamagnetic yellow to red-brown materials have been fully characterized by X-ray structure determinations (*95, 122*). All possess closely related distorted octahedral structures (**19**) in which the *cis*-$[MoO_2]^{2+}$ group is ligated by two thiolate sulfur atoms that are *trans* to one another. The two N atoms are *cis* to one

another and *trans* to the oxo ligands. The O—Mo—O angles (≈108°), Mo—O distances (≈1.70 Å), and Mo—SR distances (2.40–2.46 Å) are remarkably similar. The Mo—N bond distances vary with geometry and are longer (>2.30 Å) in the case of tetradentate ligands in which N is bound *trans* to the oxo groups (*95*). The compounds that contain sterically bulky N-alkylated ligands, for example $(L^4\text{-}N_2S_2)MoO_2$ (**30**, Fig. 8a) (*11, 95, 108*), are also precursors to important Mo(V) model compounds (Section IV.B.2). The chemical and electrochemical features of these compounds are discussed in Section V.

Reaction of $(NBu_4)_4[Mo_8O_{26}]$ with $L\text{-}S_4H_2$ produces $(L\text{-}S_4)MoO_2$ (**31**). This compound also adopts structure **19** with the thioether sulfur atoms *trans* to the oxo ligands (Fig. 8b). The Mo—SR_2 distance is 2.687(6) Å; the Mo—SR distance is 2.402(7) Å (*123, 124*). The electrochemistry of **31** is discussed in Section V.

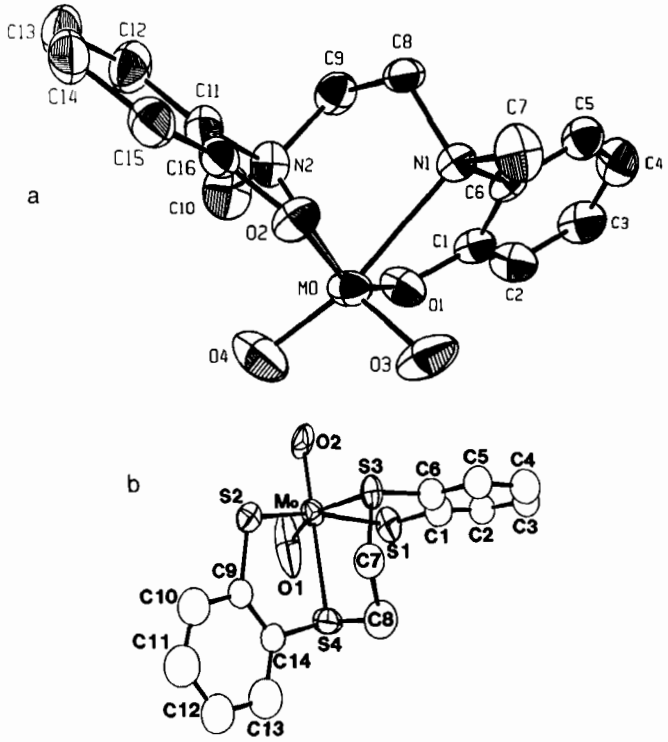
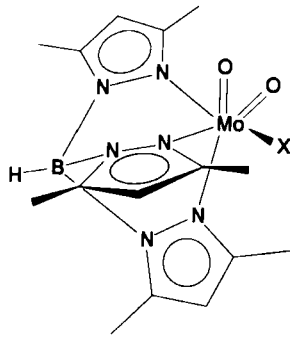


FIG. 8. (a) Structure of $(L^4\text{-}N_2S_2)MoO_2$ (**30**); (b) structure of $(L\text{-}S_4)MoO_2$ (**31**). Reproduced with permission from Hinshaw *et al.* (*95*) and Kaul *et al.* (*124*), respectively. Copyrights, American Chemical Society.

b. Trispyrazolylborate Complexes. The facial tridentate hydrotris(3,5-dimethylpyrazolyl)borate ligand (L-N_3) (*125*) inhibits dinucleation and restricts the chemistry of six-coordinate oxo-Mo complexes to the remaining facial sites (*126–128*). These restrictions facilitate the exploration of the fundamental reactions that oxo-molybdenum centers are postulated to undergo in enzymes. The (L-N_3)MoO_2X complexes (**32**) that are the cornerstones of this model chemistry may be prepared



32

by a number of synthetic methods, most of which involve ligand substitution on (L-N_3)MoO_2Cl, which is readily prepared by the reaction of MoO_2Cl_2 and K(L-N_3) in dmf (*127*). Efficient syntheses for (L-N_3)MoO_2(SR) (e.g., R = Ph, Fig. 9) complexes have permitted important recent developments in model chemistry. These (L-N_3)MoO_2(SR) complexes undergo oxygen atom transfer (OAT) reactions and are cleanly electrochemically or chemically (e.g., with SH^-) reduced to stable EPR-active $[(L\text{-}N_3)MoOEX]^-$ and (L-N_3)MoO(EH)X (E = O or S) complexes, some of which are isolable in substance (*128*). In contrast to most other systems, especially those involving tetradentate ligands, ready variation of the X group in these trispyrazolylborate complexes permits modulation of the chemical, structural, spectroscopic, and redox properties of these complexes. As such, these trispyrazolylborate complexes constitute a very important model system for the molybdenum enzymes.

c. Tridentate NS_2-Donor Ligand Complexes. Berg and Holm (*129*) have prepared the novel five-coordinate trigonal bipyramidal compound (L-NS_2)MoO_2 (**33**, Fig. 10a) by the reaction of 2,6-bis(2,2-diphenyl-2-mercaptoethyl)pyridine with $MoO_2(acac)_2$. The sulfur atoms occupy the two *trans* apical positions (mean Mo—S = 2.416 Å) and the equatorial plane contains the two oxo groups (mean Mo—O = 1.694 Å; O—Mo—O = 110.5°) and the nitrogen donor atom (Mo—N = 2.244 Å).

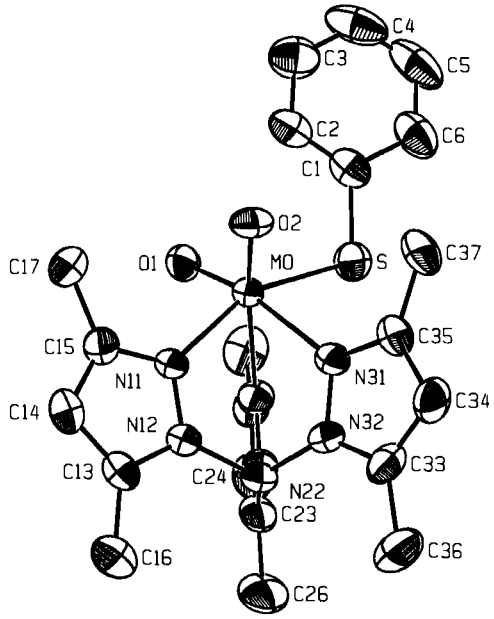


FIG. 9. Structure of $(L\text{-}N_3)MoO_2(SPh)$.

Compound **33** participates in a variety of OAT reactions (*130*) (Section V.A), but the structure of the reduced Mo(IV) product is not known. In contrast to systems containing N_2S_2- and trispyrazolylborate ligands, there have been no reports of the isolation of an oxo-Mo(V) complex containing $L\text{-}NS_2$, despite the reported reversible electrochemical oxidation of solutions of $(L\text{-}NS_2)Mo^{IV}O(dmf)$ at -0.27 V vs SCE (*129*).

d. Bis(bidentate) NS-Donor Ligand Complexes. Recently Holm and co-workers (*131*) have prepared $(L\text{-}NS)_2MoO_2$ (**34**, Fig. 10b). The compound adopts familiar stereochemistry **19**, and the bulky *p-tert*-butylphenyl groups of the L-NS ligand provide steric hindrance to the formation of dinuclear complexes. Compound **34** exhibits OAT chemistry (Section V.A) and the oxo-Mo(IV) product $(L\text{-}NS)_2MoO$ has been characterized by an X-ray structure determination (Section IV.B.3.b).

Stiefel *et al.* (*97, 98*) have shown that modified cysteamine ligands give $[Mo^{VI}O_2]^{2+}$ complexes with the unusual skew trapezoid bipyramidal stereochemistry (**21**). They suggest that structure **19** would result in unfavorable steric interactions between the methyl groups on the N atoms of the cysteamine ligands. A noteworthy feature of **21** is the

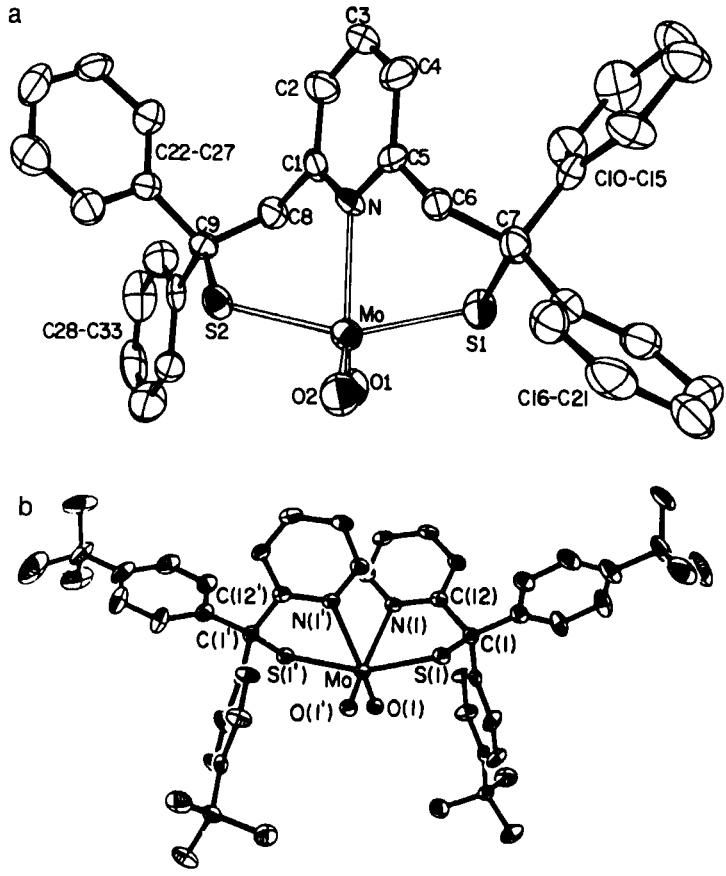
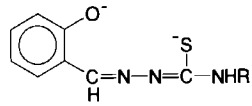


FIG. 10. (a) Structure of (L-NS_2)MoO_2 (**33**); (b) structure of (L-NS)$_2MoO_2$ (**34**). Reproduced with permission from Berg and Holm (*129*) and Gheller *et al.* (*131*), respectively. Copyrights, American Chemical Society.

short S···S distance of 2.764 Å. No oxo-Mo(V) or oxo-Mo(IV) chemistry has been reported for complexes with structure **21**.

e. Other Systems. The sterically hindered 2,2-diphenyl-2-mercaptoacetate ligand (L-SO) reacts with $(NH_4)_2[MoO_4]$ to yield $(NH_4)_2[MoO_2(L\text{-}SO)_2]$, which possesses structure **19** with Mo=O and Mo—S bond lengths averaging 1.712 and 2.44 Å, respectively. The $[MoO_2(L\text{-}SO)_2]^{2-}$ anion may be transformed into Mo(V) and Mo(IV) complexes of the type $[MoO(L\text{-}SO)_2]^{n-}$ (Section IV.B.2.b) (*132–134*).

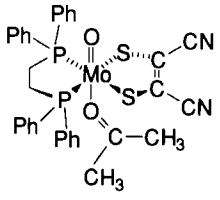
Purohit *et al.* (*135*) have described $LMoO_2$ and $LMoO_2L'$ complexes of SNO-donor thiosemicarbazone ligands (L = salicylaldehyde thiosemicarbazone or salicylaldehyde 4-phenylthiosemicarbazone **35**, L' = γ-picoline or imidazole). The thiosemicarbazone ligand is coordinated in the dianionic tridentate enolate form according to infrared evidence; the $LMoO_2$ complexes appear to be polymeric while the $LMoO_2L'$ complexes are monomeric. The complexes are irreversibly reduced electrochemically but undergo clean OAT chemistry (Section V.A.5).



35a (R =H)
35b (R =Ph)

A number of Schiff base complexes containing the dioxo-Mo(VI) fragment have been prepared by various workers (*136–141*), and two have been structurally characterized (*141*). We pass briefly over these complexes because their relevance to enzymes has been diminished by recent evidence for facile formation of dinuclear Mo(V) complexes upon reduction (*141*).

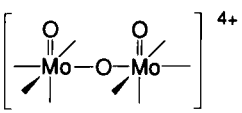
The only well-characterized $[MoO_2]^{2+}$ complex containing dithiolene ligands is $(NEt_4)_2[Mo^{VI}O_2(C_6H_4S_2)_2]$ (**20**, Section III.B). Reactions involving oxo-Mo(VI) complexes and dithiolene salts usually lead to the formation of deoxygenated ML_3 or reduced $[MoOL_2]^{2-}$ complexes (*16*). A significant observation is that MoO_2Cl_2 reacts with $Na_2[S_2C_2(CN)_2]$ in tetrahydrofuran to give an orange species that is stable below −45°C and that reacts with tmeda, PPh_3, to produce isolable derivatives such as **36** (*142*). These complexes are also reported to react with oxygen atom donors, but the nature of the products has not yet been described. The synthesis of $[MoO_2]^{2+}$ complexes possessing a single dithiolene ligand remains an important aim for model studies.



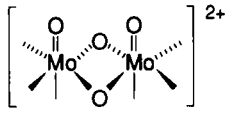
36

2. *Mo(V) Complexes*

A major obstacle to the isolation of model mononuclear molybdenum(V) compounds is the tendency of such species to form dinuclear diamagnetic complexes containing the $[Mo_2O_3]^{4+}$ (**37**) or $[Mo_2O_4]^{2+}$ (**38**)



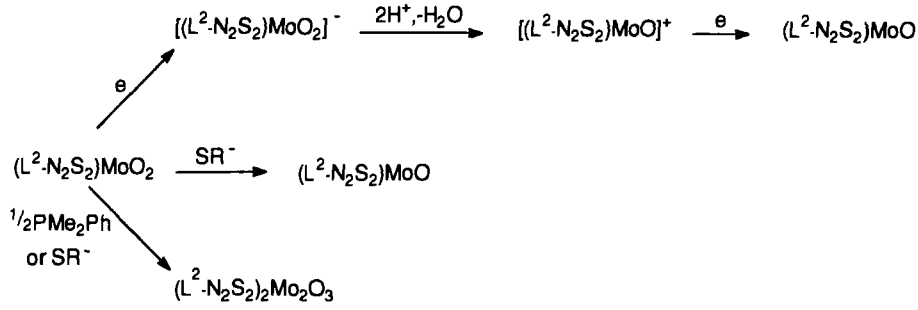
37



38

units in the presence of water. In order to isolate pure mononuclear oxo-Mo(V) complexes it is usually necessary to employ rigorously anhydrous reaction conditions (*143–147*) and/or bulky co-ligands that inhibit the formation of dinuclear complexes (see below). Although difficulties have attended the synthesis of mononuclear Mo(V) complexes, their chemistry has steadily developed, and powerful models for Mo(V) enzyme centers are now available.

a. $[MoO_2]^+$ and Related Complexes. (1) Tetradentate N-, S-, and O-donor ligand complexes. Several groups, notably Spence, Wedd, and their co-workers, have been involved in the development of successful spectrocopic models based on tetradentate N_2S_2-ligands. The first complex of this type, $(L^3\text{-}N_2H_2S_2)MoO_2$, was prepared by Gardner *et al.* (*120*). The participation of various of these ligands in *N*-deprotonation reactions was noted in the formation of non-oxo complexes upon attempted ligand exchange reactions with dioxo-Mo starting materials. Subsequently, Spence *et al.* (*148*), in an investigation of the chemistry of $L^3\text{-}N_2H_2S_2$, prepared $[(L^3\text{-}N_2S_2)MoO]^-$ by ligand exchange on $[MoO(SPh)_4]^-$. Deprotonation was postulated to enforce the ligand to occupy equatorial sites, thereby preventing dinucleation. A detailed study of the $(L^2\text{-}N_2S_2)MoO_2$ complex was reported by Zubieta *et al.* (*121, 122*), who demonstrated the chemistry shown in Scheme 1. These workers also first reported the electrochemical generation of a mononuclear dioxo-Mo(V) complex. Green $[(L^2\text{-}N_2S_2)MoO_2]^-$, produced only under strictly anhydrous conditions, is stable for several seconds at room temperature and exhibits the following frozen EPR parameters: $g_\perp$, 1.954; $g_\parallel$, 2.015; $A_\parallel$, 56×10^{-4} cm^{-1}. Coulometry at room temperature in wet tetrahydrofuran generates $(L^2\text{-}N_2S_2)MoO$ and H_2O via $[(L^2\text{-}N_2S_2)MoO]^+$, which may be detected by EPR spectroscopy. A signal due to $(L^2\text{-}N_2S_2)MoO(OH)$ was not detected at this relatively high temperature. Spence provided another indication that mononuclear Mo(V) species as well as dinuclear Mo(V) complexes could be isolated with a

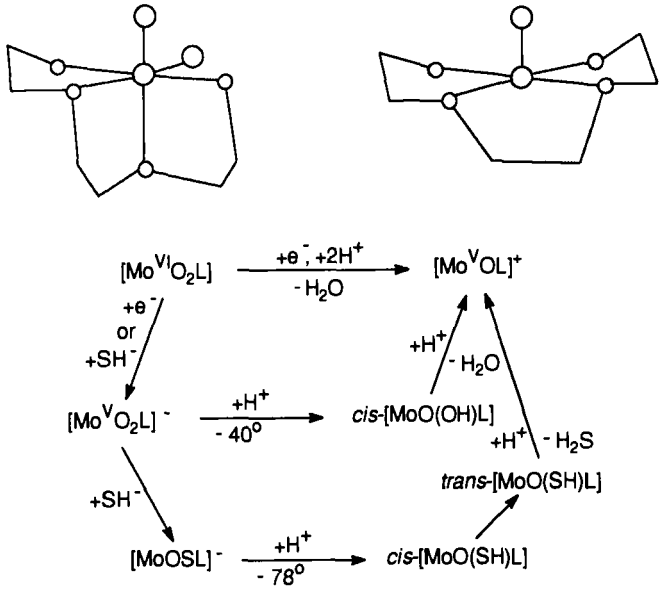


SCHEME 1

range of N_2S_2 and N_2O_2 ligands (*123*). Interestingly, Mo(V) monomers formed upon electrochemical reduction of $LMoO_2$, whereas dinuclear species formed upon mixing Mo(IV) and Mo(VI) complexes.

In 1986, independent reports provided evidence for the generation of $[(L^2\text{-}N_2S_2)MoO_2]^-$, $(L^2\text{-}N_2S_2)MoO(OH)$ (*111*), $[(L\text{-}N_2O_2)MoOS]^-$, and $(L\text{-}N_2O_2)MoO(SH)$ (*112*). Electrolysis of $(L^2\text{-}N_2S_2)MoO_2$ in thf/0.1 *M* H_2O at −42°C produced $(L^2\text{-}N_2S_2)MoO(OH)$ with $A(^1H) = 13.6 \times 10^{-4}$ cm^{-1} and $A(^{17}O) = 7(2) \times 10^{-4}$ cm^{-1}; in dry solvents $[(L^2\text{-}N_2S_2)MoO_2]^-$ is produced. Hinshaw and Spence (*112*) generated related oxo-Mo(V) complexes by electrochemical reduction of $(L\text{-}N_2O_2)MoO_2$ complexes. Reaction of the reduced species with SH^- provided evidence for the formation of the novel $[(L\text{-}N_2O_2)MoOS]$ and $(L\text{-}N_2O_2)MoO(SH)$ complexes.

Subsequent collaborative efforts by Spence, Wedd, and co-workers (*107–109, 113, 149*) developed a successful and very significant model of Mo(V) enzyme centers. Their elegant synthetic, electrochemical, and EPR studies of isotopically labeled complexes are summarized in Scheme 2. In MeCN, dmf, or thf, electrochemical reduction of $(L^4\text{-}N_2S_2)MoO_2$ is reversible at all temperatures investigated. The complex formed under anhydrous conditions exhibits a rhombic and highly anisotropic EPR signal with small g ($\langle g \rangle = 1.9007$) and large A values, especially g_3 and A_3, and has been characterized as $[(L^4\text{-}N_2S_2)MoO_2]^-$. At room temperature, the presence of acid, water, or chloride induces the formation of oxo-Mo(V) species, but at −42°C $(L^4\text{-}N_2S_2)MoO(OH)$, with $\langle g \rangle = 1.957$ and $A(^1H) = 15.1 \times 10^{-4}$ cm^{-1}, is stabilized. When labeled with ^{17}O, this species exhibits two quite different $A(^{17}O)$ values. Neither $(L^4\text{-}N_2S_2)MoO_2$ or $[(L^4\text{-}N_2S_2)MoO_2]^-$ exchanges oxygen with H_2O. Reaction of $(L^4\text{-}N_2S_2)MoO_2$ with excess SH^- leads to reduction to $[(L^4\text{-}N_2S_2)MoO_2]^-$, which subsequently undergoes ligand exchange to



L = L^4-N_2S_2

SCHEME 2

form (L^4-N_2S_2)MoO(SH) and [(L^4-N_2S_2)MoOS]$^-$. Isolation and full characterization of (L^2-N_2S_2)MoO(OSiMe$_3$) (Eq. (5)), along with EPR comparison of (L^2-N_2S_2)MoO(OR) (R = H, SiMe$_3$) complexes, strongly supports the (L^n-N_2S_2)MoO(OH) formulations (*113*).

$$(L^2\text{-}N_2S_2)MoO_2 + (Me_3Si)_2S \longrightarrow (L^2\text{-}N_2S_2)MoO(OSiMe_3) + 0.5(Me_3Si)_2S_2 \quad (5)$$

(2) Trispyrazolylborate complexes. A number of very recent developments indicate that trispyrazolylborate complexes exhibit chemistry similar to that of the above L-N_2S_2 complexes and in some cases permit the generation and isolation of important Mo(V) complexes. The (L-N_3)MoO_2(SPh) complex (Fig. 9) is the cornerstone of a single model displaying all of the centers and processes involved in catalysis by enzymes containing $[Mo^{VI}O_2]^{2+}$ oxidized active sites (Section V.E) (*128*). Some of the Mo(V) components of this model are discussed below. Coordinately unsaturated (L-N_3)Mo^{IV}O(SPh) or weakly solvated (L-N_3)Mo^{IV}O(SPh)(solvent), generated by the reaction of (L-N_3)MoO_2(SPh) with phosphines in wet tetrahydrofuran or toluene, reacts with water

and oxidant to produce $(L\text{-}N_3)Mo^{V}O(OH)(SPh)$ (g, 1.953; $A(^{95,97}Mo)$, 43.3×10^{-4} cm^{-1}; $A(^{1}H)$, 13.1×10^{-4} cm^{-1} in toluene), whose formation would appear to involve an intermediate aquo-Mo(IV) complex (Eq. (6)).

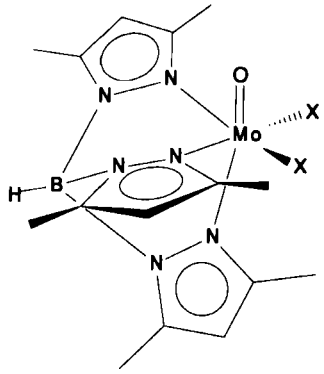
$$(L\text{-}N_3)Mo^{IV}O(OH_2)(SPh) + (L\text{-}N_3)Mo^{VI}O_2(SPh) \longrightarrow 2(L\text{-}N_3)Mo^{V}O(OH)(SPh) \quad (6)$$

Certainly, $(L\text{-}N_3)Mo^{V}O(OH)(SPh)$ can be trapped oxidatively as $(L\text{-}N_3)Mo^{VI}O_2(SPh)$ with O_2 in 85% isolated yield. Water, not dioxygen, is the source of the oxo ligand: use of $H_2{}^{18}O$ (95 atom% ^{18}O) under anaerobic conditions with a prolonged incubation at the Mo(V) level followed by admission of $^{16}O_2$ provides material enriched with 80 atom% ^{18}O. Use of $H_2{}^{17}O$ (51.5 atom% ^{17}O) leads to a clearly resolved ^{17}O structure in the EPR spectrum of the $[LN_3Mo^{V}O(OH)(SPh)]$ intermediate ($A(^{17}O)$, ca. 7×10^{-4} cm^{-1}) (cf. Ref. (*113*)). The first isolation of a dioxo-Mo(V) complex results from the one-electron reduction of $(L\text{-}N_3)MoO_2(SPh)$ by $CoCp_2$ (Cp = η^5-$C_5H_5^-$) (Eq. (7)).

$$(L\text{-}N_3)Mo^{VI}O_2(SPh) + CoCp_2 \longrightarrow [CoCp_2][(L\text{-}N_3)Mo^{V}O_2(SPh)] \quad K, \text{ ca. } 10^3 \quad (7)$$

In acetonitrile, the product of (7) is rapidly precipitated as air-sensitive green microcrystals. The ν(MoO) bands of the anionic product are extremely low in energy, consistent with the population of a π^* component in the *cis*-MoO_2 bonding scheme. Dissolution of $[CoCp_2][(L\text{-}N_3)Mo^{V}O_2(SPh)]$ in CH_2Cl_2 at room temperature initially produces a broad EPR signal (g, 1.920; $A(^{95,97}Mo)$, 41×10^{-4} cm^{-1}; $W_{1/2}$, 1 mT), a characteristic fingerprint of $[Mo^{V}O_2]^+$ centers (*107, 108*). Over time, the signal is replaced by that of the conjugate acid $(L\text{-}N_3)Mo^{V}O(OH)(SPh)$ via reaction with trace H_2O. Upon freezing this solution at 77 K, the highly anisotropic spectrum of $[(L\text{-}N_3)Mo^{V}O_2(SPh)]^-$ (g values, 1.991, 1.931, 1.843) appears, presumably due to the equilibrium being shifted toward the conjugate base by freezing out of H_2O. Dioxygen rapidly and quantitatively oxidizes $[(L\text{-}N_3)Mo^{V}O_2(SPh)]^-$ to $(L\text{-}N_3)Mo^{VI}O_2(SPh)$, and $(L\text{-}N_3)Mo^{V}O(OSiMe_3)(SPh)$ is produced upon reaction with Me_3SiCl. When these reactions are performed on ^{18}O-labeled anion *there is no significant loss of the label in the products.*

b. $[MoO]^{3+}$ Complexes. (1) Trispyrazolylborate complexes. An extensive series of mononuclear, EPR-active $(L\text{-}N_3)MoOX_2$ complexes (**39**) has been prepared, primarily by ligand exchange reactions on $(L\text{-}N_3)MoOCl_2$. Several of the complexes have been structurally characterized (*91*). The influence of ligand variations on the electronic structure of $(L\text{-}N_3)MoOX_2$ complexes has been probed by electronic and EPR



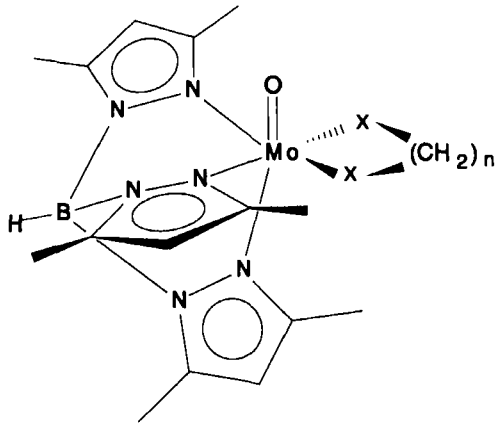
39

spectroscopies. The complex of aminobenzenethiolate possesses an exchangeable proton on the N atom and exhibits a ^1H hyperfine interaction of $\sim 8 \times 10^{-4}$ cm^{-1}. Complexes with X = SR have g and $A(^{95,97}Mo)$ values similar to those observed in the Mo(V) states of enzymes, and g_1 is often greater than the free electron value (2.0023). Mabbs (*91, 110, 150*) has pointed out that admixture of charge-transfer states involving significant contributions from appropriate metal d orbitals can lead to g values ≥ 2.0023 because such excited charge transfer states mix into the ground state under metal spin–orbit coupling to give contributions with the sign opposite those involving simple d–d excitations (Eq. (8)).[3]

$$g_i = 2.0023 - \sum \left(\frac{\xi_{Mo} F}{\Delta E^*} \right) + \sum \left(\frac{\xi_{Mo} G}{\Delta E} \right) \tag{8}$$

Complexes **39** exhibit a quasi-reversible one-electron reduction, and the potential of the Mo(V/IV) couple varies by 1.2 V as a function of donor atom; complexes with X = SR are more easily reduced than those with X = OR. Variation of the ligand backbone in (L-N_3)MoO{X-$(CH)_n$-X} (X = O, S; n = 2–4) complexes (**40**) produces a dramatic

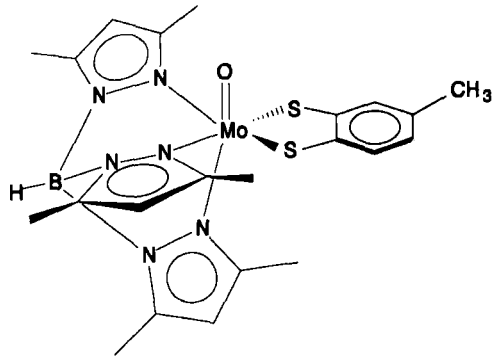
[3] Each summation of Eq. (8) contains all appropriate excited-state wave functions. ξ_{Mo} is the one-electron spin–orbit coupling constant for an unpaired electron in a d orbital of the molybdenum atom; F and G are terms containing spin–orbit coupling contributions from the ligands and mixing of ground and appropriate excited states and may be expected to be relatively insensitive to delocalization of unpaired electrons; ΔE^* is the energy associated with the ligand-field transition; and ΔE is the energy associated with a charge-transfer transition from the filled ligand orbitals to the half-filled HOMO (highest occupied molecular orbital).



40

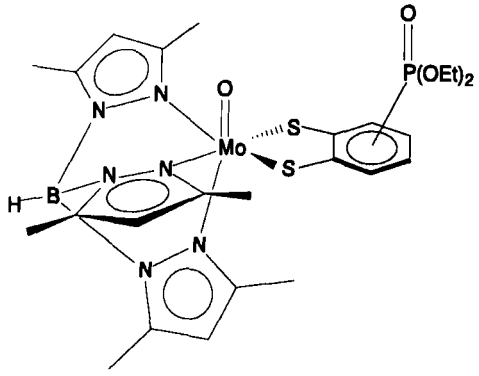
effect on spectroscopic properties and reduction potentials. Upon increasing the chelate ring size, hypsochromic shifts are observed for the lowest energy d–d transitions of the diolato (**40**, X = O) complexes and bathochromic shifts are observed for the lowest energy charge transfer bands of the dithiolato (**40**, X = S) complexes. The value of $\langle g \rangle$ becomes smaller with increasing ring size in the diolato complexes. The dithiolato complexes are more easily reduced than the diolato complexes, and both types of complex exhibit a decrease in reduction potential with increasing chelate ring size; the magnitude of this effect is 0.12 to 0.22 V per additional methylene group (*151*). The dependence of the redox potentials of the diolato complexes upon chelate ring size persists in the gas phase, as evidenced by He I PES spectroscopy which revealed a shift to higher energy of the HOMO (d_{xy}) upon increasing ring size (*152*). In contrast, only minor perturbations in electronic structure result from variation of the R substituents on the five-membered chelated ring in the series (L-N_3)MoO{X-CHR′-CHR″-Y} (X/Y = O/S, R = H, Me) and in (L-N_3)MoOCl(XR) (X = O/S, R = alkyl) complexes, where no chelate ring is present (*153*).

For (L-N_3)MoO$(SR)_2$ complexes the two thiolate groups must be *cis* to one another, as is required by the proposed structure for molybdopterin (**1**). Several (L-N_3)MoO$(SR)_2$ complexes containing both monodentate and chelating thiolate ligands have been prepared (*91, 151, 153*). Their EPR parameters are similar to those for the Mo(V) states of enzymes. To date, (L-N_3)MoO(tdt) (tdt, toluenedithiolate) (**41**) is the only example of a model compound that contains *both* an $[Mo^{V}O]^{3+}$ group and a single dithiolene ligand, a structural feature postulated for the molybdenum(V) state of Mo-co (**2**).



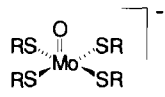
41

The L-N_3 ligand has also been used to stabilize mononuclear $[Mo^{V}O]^{3+}$ complexes possessing pendant phosphate ester groups (**42**) and Mo···P distances of 4–8 Å (*80, 81*). The ^{31}P NMR linewidths are strongly dependent upon the Mo···P distance, and reasonable Mo···P distances were derived from longitudinal relaxation times using the Solomon equation (*154*). Molecular modeling studies of Mo-co indicate that phosphate coordination to the Mo atom is stereochemically feasible, but the ^{31}P ENDOR studies of the Mo(V) states of xanthine oxidase (*67*) estimate the Mo···P distance as 7–12 Å and seem to rule out such a structure.



42

(2) $[MoO(SR)_4]^-$ complexes. Sulfur coordination has been held important since early studies by Meriwether *et al.* revealed the generation of enzyme-like EPR signals upon reaction of molybdate with various thiols (*66*). The blue $[MoO(SPh)_4]^-$ complex (**43**) was among the first mononuclear oxo-Mo(V) complexes containing S-donor ligands to be



43

isolated; it was prepared (*155*) by reaction of Mo(VI) and Mo(V) precursors with benzene thiol and structurally characterized as the $AsPh_4^+$ salt (*156*). Related complexes (*121, 148, 157*) may be prepared by thiol exchange reactions, and the structural and spectroscopic properties of these EPR-active, square–pyramidal complexes have been well documented (*14, 115*). In recent years Garner, Nakamura, and their coworkers have been major contributors to this area (*158–164*). Electrochemical studies (*161*) reveal a correlation of the Mo(VI)/Mo(V) and Mo(IV)/Mo(V) potentials with the Hammett σ function in substituted aryl thiolates, but no effect is observed on the EPR spectra. The Mo—SR distances and S—Mo—S angles in $[MoO(S_2C_6H_4)_2]^{n-}$ (*159*) and $[MoO(S\text{-}o\text{-}CH_3CONHC_6H_4)_4]^{n-}$ (*164*) (Mo(V), $n = 1$; Mo(IV), $n = 2$) are essentially independent of the oxidation state of the Mo atom, consistent with the electrons occupying a nonbonding d_{xy} orbital. Incorporation of sterically bulky 2,4,6-trialkylbenzenethiolate (R = Me, Pr^i) produced complexes that are capable of reversible oxidation to Mo(VI) as well as reversible reduction to Mo(IV) species (*160, 165*).

A number of complexes containing peptide dithiolate ligands have been reported (*160, 162*). Early work employed the reaction of $MoOCl_3(thf)_2$ with thiols to produce $[MoOL_2]^-$ complexes (L = Z–Cys–Val–OMe or Z–Cys–Pro–Leu–Cys–OMe (Z = benzyloxycarbonyl), but more recently ligand exchange reactions involving $[MoO(SPh)_4]^-$ have been employed in the synthesis of the latter compound (*162*), which exists as two isomers defined by the parallel and antiparallel coordination of the peptide ligands. These isomers exhibit quite distinct infrared, Raman, electronic, and EPR spectra and reduction potentials. Spectral and electrochemical features were assigned by comparison with the properties of an analogue wherein the two peptide chains were constrained to be parallel by linking of their amino termini. These workers concluded that the reduction potential of oxo-Mo(V) complexes is largely controlled by variations in the O—Mo—S—C torsion angle.

Recently Nakamura and co-workers (*164*) have prepared a series of $[Mo^VO(S\text{-}o\text{-}RCONHC_6H_4)_4]^-$ complexes. The structure of the complex with R = Me has all four acylamino groups located on the Mo=O side of the anion (Fig. 11) in spite of the steric congestion among the aryl groups. All four NH groups are involved in intraligand NH···S hydrogen

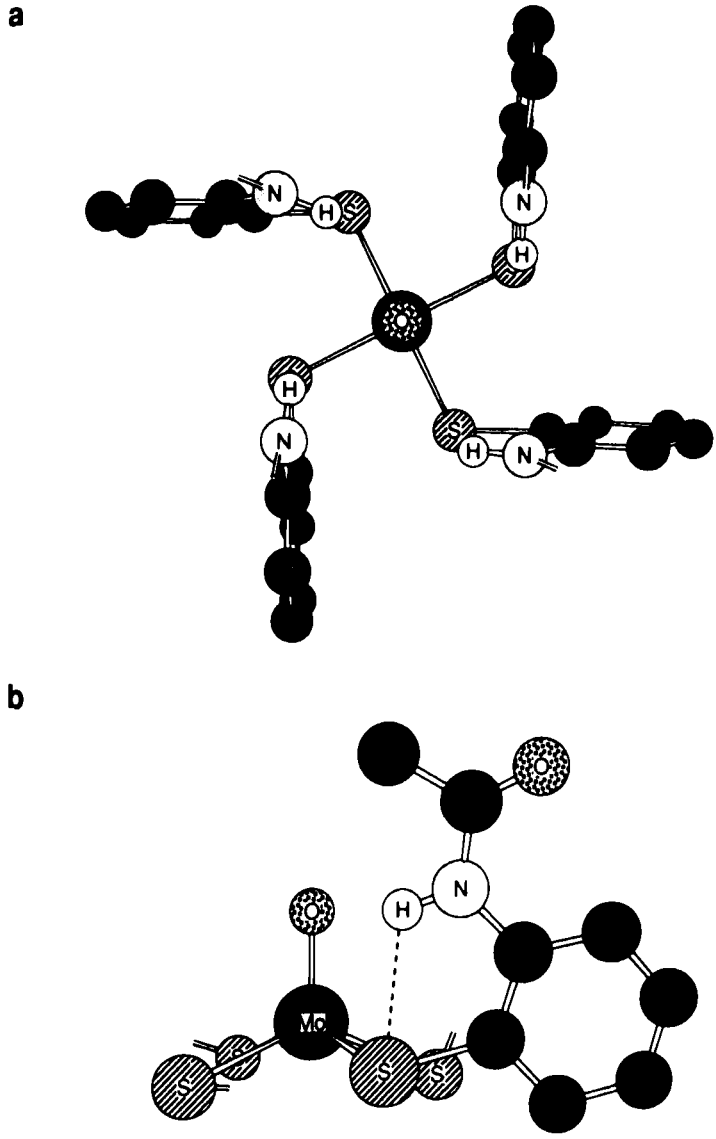


FIG. 11. Intraligand NH···S interaction in the $[Mo^{V}O(S\text{-}o\text{-}CH_3CONHC_6H_4)_4]^-$ anion. Acetyl groups (a) or three aryl groups (b) are omitted for clarity. Reproduced with permission from Ueyama *et al.* (*164*). Copyright 1992, American Chemical Society.

bonds that are thought to contribute to the large positive shift in the Mo(V)/Mo(IV) reduction potential (+450 mV relative to the Mo(V)/Mo(IV) potential in $[MoO(SPh)_4]^-$). Such NH···S hydrogen bonding may play a major role in modulating the redox potentials of oxo-molybdenum centers in enzymes.

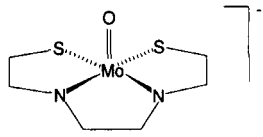
(3) $[MoO(L\text{-}SO)_2]^{2-}$ complexes. While undergoing a two-electron electrochemical reduction (*132*) in aqueous solution, $(NH_4)_2[MoO_2(L\text{-}SO)_2]$ reacts with one equivalent of thiol ligand to form the Mo(V) complex $[MoO(L\text{-}SO)_2]^-$ (*133, 134*). The EPR-active complex exhibits $\langle g \rangle$ and $\langle A \rangle$ values of 1.978 and 36×10^{-4} cm^{-1}, respectively, and a square–pyramidal structure with an apical Mo=O ligand when isolated as the NBu_4^{n+} salt. The Mo=O and Mo—S distances are 1.633 (8) and 2.342 (3) Å, respectively. The presence of protonic carboxylate groups favors the Mo(VI) to Mo(V) process, which is proposed to proceed via initial formation of $[MoO(L\text{-}SO)_2]^{2-}$. Ultimate formation of a Mo(V) complex parallels the isolation of amido-Mo(V) complexes upon one-electron reduction of dioxo-Mo(VI) complexes containing coordinated amino thiol ligands (*148, 166*). The Mo(V) complex is reversibly electrochemically reduced to the analogous Mo(IV) dianion (*134*).

The EPR properties of the above complexes provide good evidence for the formation of $[MoO\{O_2CC(S)H_2\}_2]^-$ and $[MoO(SCH_2CH_2S)_2]^-$, respectively, in the $[MoO_4]^{2-}$/thioglycolic acid (*66, 167*) and $[MoO_4]^{2-}$/ethanedithiol systems (*66*). The $[MoO(SCH_2CH_2S)_2]^-$ complex is also produced when oxidized Mo-co (*31*) and xanthine oxidase are treated with ethanedithiol (*158*).

(4) $[MoO(L\text{-}S_2N_2)]^-$ complexes. Six-coordinate *cis*-$[MoO_2]^{2+}$ complexes having tetradentate N_2S_2-ligands with secondary amine groups undergo facile irreversible one-electron electrochemical reduction to give monomeric oxo-Mo(V) complexes whose EPR spectra show hyperfine coupling to two equivalent ^{14}N nuclei (Section V.C) (*148, 166, 168*). The compounds are proposed to have square–pyramidal geometry with trigonal N atoms (**44**) but no crystal structures are available.

3. *Mo(IV) Complexes*

Oxo-Mo(IV) complexes are generally produced by removal of an oxygen atom from dioxo-Mo(VI) complexes or by suitable modification of



44

an oxo-Mo(IV) precursor. In the presence of oxidants or Mo(VI) complexes, they are generally unstable with respect to dinuclear Mo(V) complexes; only in a few model systems has the isolation of oxo-Mo(IV) species been convincingly demonstrated.

a. Trispyrazolylborate Complexes. Six-coordinate oxo-Mo(IV) complexes of these ligands were first reported in 1987 (*169*). The first complexes of this type, $(L\text{-}N_3)MoOL$, where L is a 1,1-dithio ligand were prepared according to Eq. (9).

$$Mo^{IV}OL_2 + K(L\text{-}N_3) \longrightarrow (L\text{-}N_3)Mo^{IV}OL + KL \qquad (9)$$

These green ($L = S_2CNR_2^-$, $S_2PR_2^-$) or blue ($L = S_2P(OR)_2^-$), air-stable, diamagnetic complexes can be electrochemically or chemically oxidized to analogous Mo(V) or dioxo-Mo(VI) complexes. Oxo-Mo(IV) complexes of $L\text{-}N_3$ have also been accessed via reduction of Mo(VI) complexes (*126–128*). Reaction of $(L\text{-}N_3)MoO_2X$ complexes with phosphines in coordinating solvents such as pyridine or acetonitrile results in the formation of $(L\text{-}N_3)MoOX$(solvent) complexes (*126–128*); $(L\text{-}N_3)MoOCl$(pyridine) (*127*) and $(L\text{-}N_3)MoO$(SPh)(pyridine) (*170*) have been structurally characterized. These and other reactions demonstrate the initial formation of a coordinatively unsaturated or weakly solvated "$(L\text{-}N_3)MoOX$" fragment upon oxygen atom abstraction by phosphine. A particularly significant model for the oxo-Mo(IV) enzyme center, but one which has not been isolated in substance, is the putative aquo or hydroxo complex formed upon reaction of $(L\text{-}N_3)MoO_2$(SPh) with PR_3 in the presence of water. It has been demonstrated that ^{18}O from water is incorporated into Mo(V) and Mo(VI) complexes formed upon the $^{16}O_2$ oxidation of "$(L\text{-}N_3)MoO(SPh)(H_2{}^{18}O)$," providing good evidence for the binding of water at the vacant site in the initially formed oxo-Mo(IV) complex (*128*). The participation of these oxo-Mo(IV) complexes in model reaction cycles is discussed in more detail in Section V.E.

b. Other Ligand Systems. Reaction of *cis*-$(L\text{-}NS)_2Mo^{VI}O_2$ (Section IV.B.1.d, Fig. 10b) with phosphines yields $(L\text{-}NS)_2Mo^{IV}O$, which has a distorted trigonal bipyramidal structure (Fig. 12) with the O and N atoms in the equatorial plane and apical thiolate groups (*131*). Earlier claims that mononuclear oxo-Mo(IV) complexes were isolated from reactions involving phosphines and Schiff-base MoO_2L complexes (*136–140*) have been disproved by recent work by Holm and coworkers (*141*).

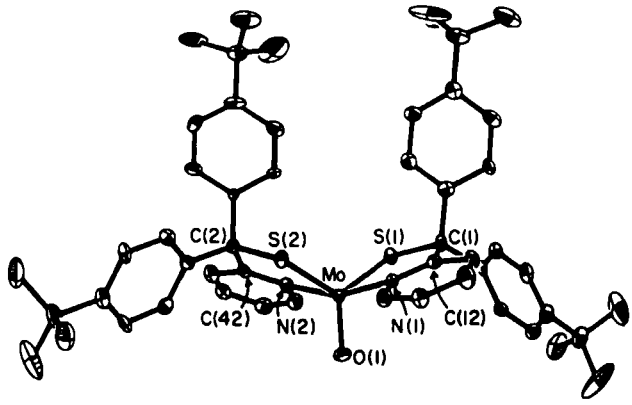


FIG. 12. Structure of $(L\text{-}NS)_2MoO$. Reproduced with permission from Gheller *et al.* (*131*). Copyright 1992, American Chemical Society.

C. MODELS OF ENZYMES CONTAINING $[MoOS]^{2+}$ OXIDIZED CENTERS

1. Mo(VI) Complexes

a. Background and Synthetic Strategies. The oxo-thio-Mo(VI) active site proposed for oxidized xanthine oxidase and related enzymes (Section III.C) is supported by EXAFS studies, which reveal the presence of an oxo ligand (Mo=O = 1.67–1.74 Å) and a sulfur-donor ligand, presumed to be a terminal thio ligand on the basis of its short Mo—S distance (2.15–2.25 Å) (*68*). An $[Mo^{VI}OS]^{2+}$ site is also indirectly supported by strong EPR evidence for $[Mo^{V}OS]^{+}$ and $[Mo^{V}O(SH)]^{2+}$ centers in xanthine oxidase and model systems (*17, 108*). However, $[MoOS]^{2+}$ complexes are extremely rare and are generally quite unstable. Thus, the synthesis of models for the $[MoOS]^{2+}$ center remains a significant challenge. The reasons for this are twofold. First, mononuclearity must be maintained despite the fact that sulfur is a promiscuous participant in polynucleation processes, especially when Mo(V) is generated. Second, due to a close energy matching of the sulfur $3p$ and molybdenum $4d$ orbitals, facile intramolecular electron transfer almost invariably results in the reduction of Mo and the formation of polysulfido or otherwise oxidized-sulfur-containing ligands. The redox interplay of Mo and S has been highlighted by a number of workers (*171–173*).

A number of feasible synthetic approaches to $[MoOS]^{2+}$ complexes may be envisaged (Eqs. (10–12)), but success depends on deliberately or serendipitously preventing the Mo=S unit from undergoing further reactions.

$$[MoO_2]^{2+} + [S^{2-}] \longrightarrow [MoOS]^{2+} + [O^{2-}] \quad (10)$$

$$[MoO]^{2+} + [S] \longrightarrow [MoOS]^{2+} \quad (11)$$

$$[MoS]^{2+} + [O] \longrightarrow [MoOS]^{2+} \quad (12)$$

b. Reactions of $[Mo^{VI}O_2]^{2+}$ Centers with Sulfiding Agents. The reaction of dioxo-Mo(VI) centers with sulfiding agents such as H_2S, B_2S_3, and $(Me_3Si)_2S$ (Eq. (10)) has been the most frequently exploited strategy for the synthesis of oxo-thio-Mo(VI) complexes.

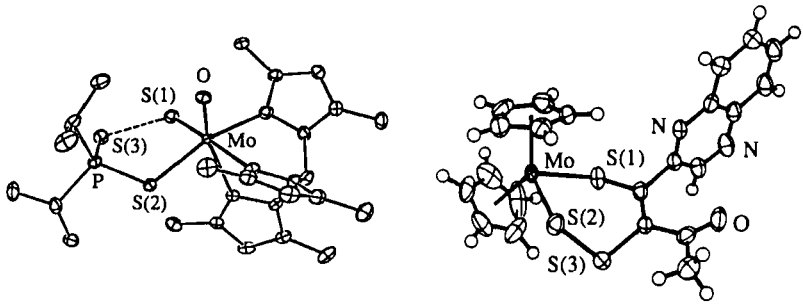
(1) Oxo-thiomolybdates, $[MoO_{4-n}S_n]^{2-}$ (n = 1–3), are prepared by the reaction of molybdate solutions with H_2S under strictly controlled conditions of pH, temperature, and counterion. Excellent reviews of the syntheses and properties of these historically important four-coordinate "tetrahedral" species are available (*174, 175*).

(2) Hydroxylamido complexes, $MoOS(R_2NO)_2$, were first reported in 1981 by Wieghardt and co-workers (*176, 177*). Since then a number of reports describing alternative syntheses, and chemical, spectroscopic, electrochemical, and structural properties have appeared (*178–181*).

(3) Organometallic $Cp^*MoOS(CH_2SiMe_3)$ was prepared in 1989 by Faller and Ma (*182*) by the reaction of the dioxo analogue with H_2S. Under similar conditions the methyl derivative produced $Cp^*MoO(S_2)(Me)$, which was readily oxidized to stable $Cp^*MoO(S_2O)(Me)$. The formation of the disulfido complex highlights the reactivity of nascent Mo=S bonds. Satisfactory structural characterization of the oxo-thio-complex has not been achieved.

For a variety of reasons (*7*) none of the $[MoOS]^{2+}$ complexes described above constitute realistic enzyme models. The complexes in 2 and 3 do, however, demonstrate the feasibility of producing oxo-thio- and dithio-Mo(VI) complexes in addition to the simple thiomolybdates in 1.

c. $[(L\text{-}N_3)Mo^{VI}OS]^+$ Complexes. The two strategies represented by Eqs. (11) and (12) have great potential but have been exploited successfully in only one class of compound (*104*). Sulfur atom transfer to $(L\text{-}N_3)MoO(\eta^2\text{-}S_2PPr_2^i)$, effected by reaction with propylene sulfide, results in the formation of the oxo-thio-Mo(VI) complex $(L\text{-}N_3)MoOS(\eta^1\text{-}S_2PPr_2^i)$; related $S_2PR_2^-$ and $S_2P(OR)_2^-$ complexes are also accessible. The $(L\text{-}N_3)MoOS(\eta^1\text{-}S_2PPr_2^i)$ complex possesses a distorted octahedral structure (Fig. 13a) composed of the tridentate $L\text{-}N_3$ ligand, a terminal oxo ligand (Mo—O(1) = 1.702 (4) Å) and a novel fragment formed by association of the thio and $\eta^1\text{-}S_2PPr_2^{i-}$ ligands. The short Mo—S(1) bond distance of 2.227(2) Å is consistent with significant π bonding between these atoms, and the S(1)—S(3) distance of 2.396 (3) Å is indicative of a partial S···S bond. These structural features are consis-



(a)	Mo-S(1)	2.227 (2) Å	(b)	Mo-S(1)	2.510 (2) Å
	Mo-S(2)	2.431 (2) Å		Mo-S(2)	2.451 (2) Å
	S(1)-S(3)	2.396 (3) Å		S(2)-S(3)	2.074 (2) Å

FIG. 13. Molecular structures and parameters for (a) (L-N_3)MoOS(η^1-$S_2PPr_2^i$) and (b) Cp_2Mo[(2-quinoxalyl)C(S)=C(S_2)C(O)Me]. Adapted with permission from Eagle *et al.* (*104*) and Pilato *et al.* (*54*), respectively. Copyrights, American Chemical Society.

tent with an oxo-thio-Mo(VI) formulation; a dative interaction involving the donor S(3) $p_\perp$ orbital and an acceptor $\pi^*_\perp$ orbital of the $[MoOS]^{2+}$ fragment may contribute to the weak S(1)···S(3) bond. Indeed, reactions involving the Mo=S π^* orbital may dominate the chemistry of the $[MoOS]^{2+}$ fragment; solution species (e.g., "S", which forms S_x^{2-} ligands) (*183*) or co-ligands (e.g., $S_2PR_2^-$) (*104*) have been identified in such reactions. Thus, although sulfur atom transfer to oxo-Mo(IV) complexes is an attractive strategy for the synthesis of $[MoOS]^{2+}$ species, such chemistry may be expected to yield alternative products when reaction of the nascent $[MoOS]^{2+}$ center is possible. Stabilization of enzymatic $[MoOS]^{2+}$ centers through an active site interaction, possibly with cysteine sulfur or molybdopterin, provides an attractive reconciliation of the extreme reactivity of such groups and their apparent presence in natural systems. It is significant that the stabilization of the $[MoOS]^{2+}$ fragment in (L-N_3)MoOS(η^1-$S_2PPr_2^i$) by a sulfur–sulfur interaction only slightly perturbs the Mo=S bond, which falls within the range of Mo=S distances found by EXAFS for molybdenum enzymes (Fig. 13a) (*68*). The quite different set of Mo—S and S—S bond distances observed in Cp_2Mo[(quinoxalin-2-yl)C(S)=C(S_2)C(O)Me] (Fig. 13b) is consistent with its formulation as an ene-1-perthiolate-2-thiolate-Mo(IV) complex (*54*). Subtle modulation of electron density (and consequently, Mo and S formal oxidation states) is clearly evident in the structures shown in Fig. 13. Theoretical studies of the bonding in such complexes may provide considerable insight into the enzyme problem.

Stiefel has previously postulated that the variation in interligand S···S interactions may be a mechanism by which the electron density at Mo may be finely tuned in enzymes (*97, 98*). Finally, (L-N_3)MoOS(η^1-$S_2PPr_2^i$) reacts quantitatively with cyanide (Section V.D) in a manner that mimics the deactivation of xanthine oxidase upon cyanolysis (*181, 184*).

d. Other Complexes. Electrochemical oxidation of [(L^4-N_2S_2)Mo^VOS]$^-$ is postulated to form (L^4-N_2S_2)Mo^{VI}OS, but no firm structural evidence for the compound's existence is available (*185*).

2. Mo(V) Complexes

a. Tetradentate N-, S-, and O-Donor Ligand Complexes. Complexes containing the [Mo^VOS]$^+$ center have been generated in a number of systems (*107, 112*). The first compounds, reported by Hinshaw and Spence in 1986 (*112*), were formed when solutions of [(L-N_2O_2)MoO_2]$^-$ complexes were reacted with excess NBu_4^nSH. They exhibit broad room-temperature EPR signals with $\langle g \rangle$ higher than their oxo precursors (1.904 vs 1.885) and anisotropic frozen spectra with less rhombicity than the oxo species. Reaction with CF_3COOH/H_2O produced the conjugate acid (L-N_2O_2)MoO(SH) with $A(^1H)$ of 8.9×10^{-4} cm^{-1}. Subsequently, it was reported that reaction of (L^4-N_2S_2)MoO_2 with excess NBu_4^nSH produced [(L^4-N_2S_2)MoOS]$^-$, which could be protonated to form (L^4-N_2S_2)MoO(SH) (*107*) (Scheme 2). These complexes exhibit very small coupling to ^{17}O ($<6.7 \times 10^{-4}$ cm^{-1}) and $\langle g \rangle$ values greater than those of their oxo analogues, consistent with protonation at sulfur, not oxygen. Definitive evidence for coordinated sulfur atoms was obtained from elegant multiple frequency EPR studies using a compound doubly isotopically labeled with ^{98}Mo ($I = 0$) and ^{33}S ($I = \frac{3}{2}$). The isotopomer [(L^4-N_2S_2)^{98}MoO^{33}S]$^-$ shows a remarkably large and anisotropic coupling ($A(^{33}S) = 10.8 \times 10^{-4}$ cm^{-1}) (Fig. 14 (*108*)), whereas (L^4-N_2S_2)^{98}MoO(^{33}SH) has a greatly reduced coupling ($A(^{33}S) = 1.4 \times 10^{-4}$ cm^{-1}) (Fig. 15 (*108*)). Scheme 2 summarizes the various EPR active species resulting from chemical and electrochemical reduction of (L^4-N_2S_2)MoO_2. Detailed ^{17}O studies using multiple frequency EPR are also consistent with Scheme 2 (*109*). The implications of these EPR parameters for the mechanism of action of xanthine oxidase are discussed in Section VI.

Singh *et al.* (*185*) have precipitated the postulated *cis*-[(L^4-N_2S_2)-MoOS]$^-$ complex (*107*) from solution as the PPh_4^+ salt, following the treatment of (L^4-N_2S_2)MoO_2 with excess hydrosulfide in the absence of acid. The product has been characterized by EXAFS, which reveals a

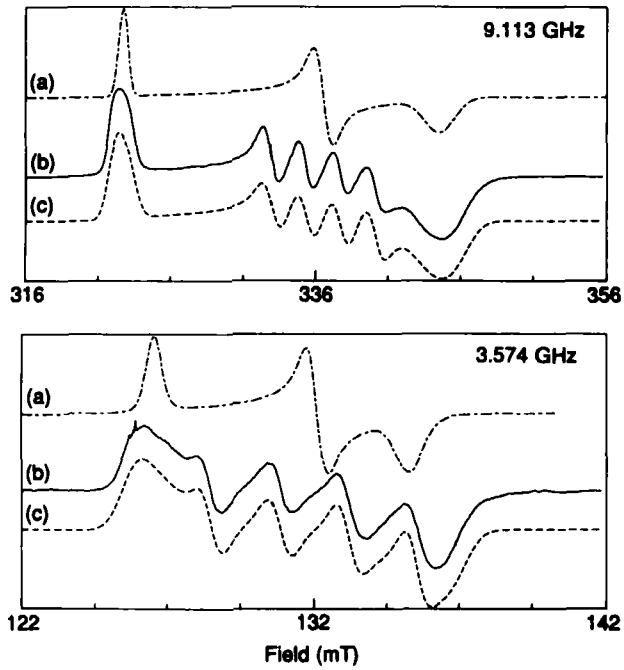


FIG. 14. EPR spectra of $[(L^4\text{-}N_2S_2)MoOS]^-$ (^{98}Mo, 98.72 atom %): (a) S isotopes at natural abundance (I = 0, 99.22 atom %), (b) ^{33}S (I = 3/2; 99.25 atom %), (c) simulation of b. Reproduced with permission from Wilson *et al.* (*108*). Copyright 1991, American Chemical Society.

short Mo=O distance of 1.68 Å and the presence of two to three sulfur atoms at 2.36 Å. No evidence for a short Mo=S bond (≈2.15 Å) was found, and it was suggested that the Mo(V)=S bond is considerably longer than those of Mo(VI) and Mo(IV) analogues. In the presence of acid, the apparently water stable *trans*-$(L^4\text{-}N_2S_2)MoO(SH)$ is reportedly isolable in substance (*185*).

b. Trispyrazolylborate Complexes. Reaction of $(L\text{-}N_3)MoO_2X$ compounds (Section IV.B.1.b) with SH^- produces *cis*-$[MoOS]^+$ and *cis*-$[MoO(SH)]^{2+}$ complexes analogous to *cis*-$[(L^4\text{-}N_2S_2)MoOS]^-$ and *cis*-$(L^4\text{-}N_2S_2)MoO(SH)$, as evidenced by the similarity of their respective EPR parameters (*186*).

Mononuclear $[Mo^VS]^{3+}$ complexes are extremely rare; orange-brown $(L\text{-}N_3)MoSCl_2$, produced upon reaction of $(L\text{-}N_3)MoOCl_2$ with boron sulfide, remains the only known example (*110*). The g values for $(L\text{-}N_3)MoSCl_2$ are all *smaller* than those for the corresponding $[Mo^VO]^{3+}$

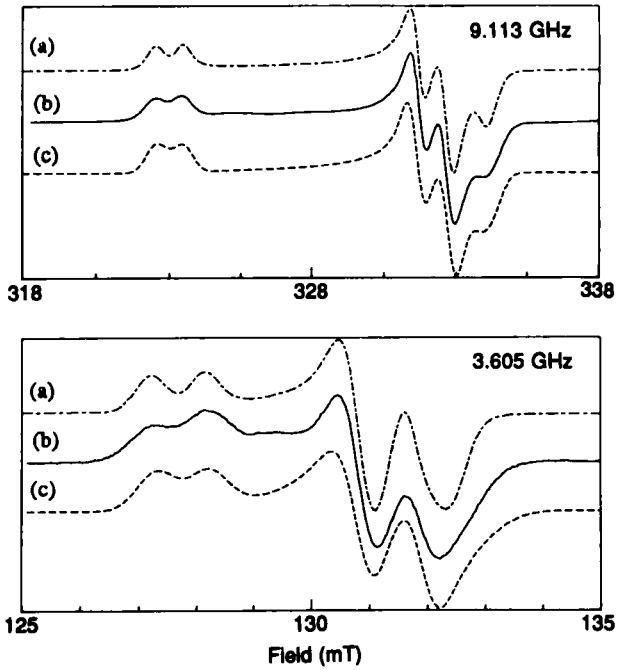


FIG. 15. EPR spectra of $(L^4\text{-}N_2S_2)MoO(SH)$ (^{98}Mo, 98.75 atom %); (a) S isotopes at natural abundance (I = 0, 99.22 atom %), (b) ^{33}S (I = 3/2; 99.25 atom %), (c) simulation of b. Reproduced with permission from Wilson *et al.* (*108*). Copyright 1991, American Chemical Society.

complex, whereas the $A(^{95,97}Mo)$ hyperfine parameters are very similar for the two species. The EPR data for these $[Mo^VS]^{3+}$ and $[Mo^VO]^{3+}$ complexes disfavor the earlier proposal (*105*) that the relatively high g values for the Very Rapid EPR signal in xanthine oxidase arise from an $[Mo^VS]^{3+}$ center. The thio complex has not been crystallographically characterized, but resonance Raman (*74*) (Section III.A.5) and EXAFS (*185*) studies of the $(L\text{-}N_3)MoECl_2$ (E = O, S) complexes have been reported.

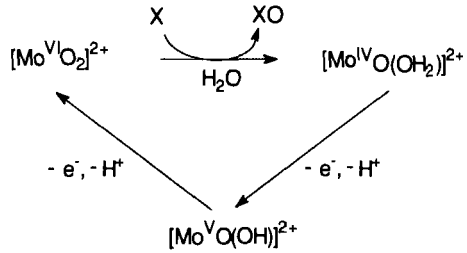
Very recently, the intriguing six-coordinate $[MoO(SH)_3]$ complex, *mer*-$[MoO(SH)_3(tdpm)]^-$, (tdpm, thiodipivaloylmethanide anion) was isolated and structurally characterized (*187*). This complex possesses distorted octahedral stereochemistry with equatorial sulfur atoms, a mean Mo—SH distance of 2.378(2) Å, and a Mo=O distance of 1.670(5) Å. Unfortunately this interesting complex is not readily accessible synthetically.

3. *Mo(IV) Complexes*

At present there are no adequate models of the Mo(IV) form of active xanthine oxidase. Only one $[MoO(SH)]^+$ complex is known; the $[MoO(SH)(16\text{-ane}[S_4])]^+$ ion exhibits a *trans* structure with Mo=O and Mo—SH distances of 1.667(3) and 2.486(1) Å, respectively (*188*).

V. Reactions of Oxo-Molybdenum Centers

Physical studies on oxidized and reduced enzymes show that all of the enzymes studied to date possess an oxo-molybdenum center that cycles between the Mo(VI) and the Mo(IV) states during catalysis and that the Mo(V) state can be detected as a transient species by EPR spectroscopy. Scheme 3 shows a simple cycle of reactions that describes the oxidation (or in reverse, reduction) of a substrate at an oxo-molybdenum center, such as that present in sulfite oxidase.



SCHEME 3

The intimate mechanisms of the molybdenum enzymes have yet to be fully elucidated. Current evidence supports the transfer of an oxygen atom between Mo(IV/VI) and substrate (*189*) and the regeneration of the active site by two one-electron processes, the first of which generates transient Mo(V) centers (*17*). In view of the reactions taking place at the Mo center (Eq. (13)) and in an overall sense (Eq. (14)), chemical modeling has naturally concentrated on:

(i) OAT reactions.
(ii) Coupled electron–proton transfer (CEPT) reactions.

$$[Mo^{VI}O_2]^{2+} + X \rightleftharpoons [Mo^{IV}O]^{2+} + XO \quad (13)$$

$$X + H_2O \rightleftharpoons XO + 2H^+ + 2e^- \quad (14)$$

It appears likely that the mechanism adopted by the enzymes may depend on the conditions of catalysis and that under physiological

conditions substrates are transformed by an elegant combination of formal OAT and CEPT reactions (see Section VI). Very recently, a single model exhibiting both oxygen atom and electron–proton transfer chemistry has been described (Section V.E). Before treating this system we explore the two categories of reactions introduced above, along with simple one-electron processes.

A. Oxygen Atom Transfer (OAT) Reactions

The possible role of oxygen atom transfer in molybdenum enzyme catalysis was recognized in the early 1970s (*190–194*). In the ensuing years, a wealth of chemistry has established molybdenum as the premier exponent of such reactions (*7, 195*). Importantly, related dioxo-Mo(VI) and oxo-Mo(IV) complexes are interconverted by oxygen atom transfer reactions (Eq. (13)). These reactions are effected by reductants (X) such as tertiary alkyl and aryl compounds of the group 15 elements (especially phosphines) and oxidants (XO) such as *S*- and *N*-oxides. In many cases, however, the Mo(VI) and Mo(IV) compounds participate in a comproportionation reaction yielding dinuclear Mo(V) complexes (Eq. (15)).

$$[Mo^{VI}O_2]^{2+} + [Mo^{IV}O]^{2+} \rightleftharpoons [Mo^{V}_2O_2(\mu\text{-}O)]^{4+} \qquad (15)$$

There is overwhelming evidence that oxo-Mo(IV) complexes are initially formed upon removal of an oxygen atom from a dioxo-Mo(VI) complex. Detection and isolation of the Mo(IV) species depend on the rate of dinucleation and the reversibility of the process. Where dinucleation is rapid and irreversible with respect to oxygen atom transfer, only the Mo(V) complex is accessible; systems exhibiting this behavior cannot effect catalytic oxygen atom transfer reactions. In the historically important dithiocarbamate system, first described by Barral *et al.* (*196, 197*), $MoO_2(S_2CNR_2)_2$ and $MoO(S_2CNR_2)_2$ exist in a reversible equilibrium with $Mo_2O_3(S_2CNR_2)_4$; therefore the Mo(IV) complex is isolable and any of the three complexes can serve as a catalyst-precursor for the aerial oxidation of PPh_3 (*198–203*). A general kinetics treatment for systems complicated by Eq. (15) has been developed by Reynolds *et al.* (*204*).

The comproportionation reaction (15) is biologically unimportant and must be prevented in meaningful enzyme models. Accordingly, much effort has been invested in ligand design aimed to sterically inhibit the formation of dinuclear species; several successful and significant

TABLE II

RATE CONSTANTS FOR THE REACTION OF Mo(VI) COMPLEXES WITH PPh_3

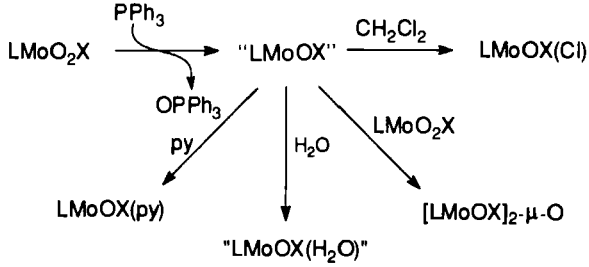
Mo(VI) complex (°C, solvent)	$10^3\ k$ (M^{-1} sec^{-1})	$\Delta H‡$ (kJ mol^{-1})	$\Delta S‡$ (J K^{-1} mol^{-1})	Reference
$MoO_2(S_2CNEt_2)_2$ (25, 1,2-$C_2H_4Cl_2$)	71 (3)	—	—	(*204*)
(L-NS_2)MoO_2 (23, dmf)	7 (1)	—	—	(*130*)
(L-NS_2)MoO_2 (23, dmf)[a]	9.7 (4)	49 (3)	−119 (7)	(*209*)
MoO_2(L-Cys-OEt)$_2$ (35, C_6H_6)	0.29	46	−153	(*318*)
MoO_2{$OC_6H_4CHNNC(S)SCH_2Ph$} (25, dmf) (L-N_3)MoO_2L'	8.9 (2)	55 (1)	−102 (3)	(*215*)
L′ = OPh^- (25, dmf)	0.002 (1)	—	—	(*127*)
L′ = SPh^- (25, dmf)	0.59 (2)	—	—	(*127*)
L′ = $S_2P(OEt)_2^-$ (25, C_6H_5Me)	3.4 (1)	64 (1)	−121 (8)	(126)
L′ = $S_2PPr_2^{i-}$ (30, C_6H_5Me)	0.25 (3)	—	—	(*205*)

[a] These data were obtained using P(*p*-$C_6H_5F)_3$ as the reductant.

models have now evolved. Available kinetics data are summarized in Tables II and III.

1. *Trispyrazolylborate Complexes*

OAT reactions interconvert dioxo-Mo(VI) and oxo-Mo(IV) complexes of tridentate trispyrazolylborate ligands such as L-N_3. Depending upon the co-ligands and reaction conditions, particularly solvent, a number of events follow OAT from (L-N_3)MoO_2X (X = Cl, Br, NCS, OPh, SPh) to PPh_3 (Scheme 4); these events dictate the final product of the reaction (*127, 128*). In dry noncoordinating solvents such as toluene, the initially



L = L-N_3

SCHEME 4

TABLE III

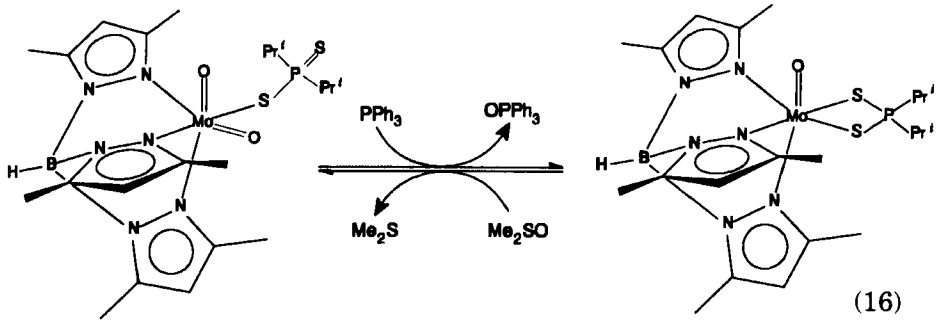
REDUCTION OF SUBSTRATES BY OXO-Mo(IV) COMPLEXES

Complex/substrate (°C, solvent)	10^3 k (sec^{-1})	$\Delta H‡$ (kJ mol^{-1})	$\Delta S‡$ (J K^{-1} mol^{-1})	Reference
$MoO(S_2CNEt_2)_2$				
Me_2SO (25, 1,2-$C_2H_4Cl_2$)	0.16 (1)[a]	—	—	(*204*)
(L-NS_2)MoO				
Me_2SO (23, dmf)	1.50 (3)	—	—	(*130*)
Ph_2SO (23, dmf)	1.43 (3)	—	—	(*130*)
(p-$FC_6H_4)_2SO$ (25, dmf)	1.40 (7)	98 (6)	30 (8)	(*209*)
D-Biotin *S*-oxide (23, dmf)	1.36 (3)	—	—	(*130*)
Cbz-(*S*)-Met *l*-(S-O) (23, dmf)	1.63 (3)	—	—	(*130*)
Cbz-(*S*)-Met *d*-(S-O) (23, dmf)	1.70 (3)	—	—	(*130*)
3-FC_6H_4NO (25, dmf)	1.60 (8)	92 (5)	11 (7)	(*209*)
NO_3^- (23, dmf)	1.49 (5)	99 (3)	33 (8)	(*210*)
(L-N_3)MoO{$S_2P(OEt)_2$}				
Me_2SO (25, MeC_6H_5)	0.0151 (6)[a]	64 (2)	−121 (2)	(*126*)
(L-N_3)MoO{$S_2PPr^i_2$}				
Me_2SO (25, MeC_6H_5)	0.055 (3)[a]	64 (1)	−111 (1)	(*205*)

[a] Second-order rate constants (M^{-1} sec^{-1}).

formed "(L-N_3)MoOX" complexes react with starting material to yield dinuclear species (L-$N_3)_2Mo_2O_3X_2$; in this solvent only 0.5 mol of $OPPh_3$ is produced per mole of complex. In dichloromethane, 1 mol of $OPPh_3$ is produced per mole of complex, but oxidation of the putative (L-N_3)MoOX intermediate to the corresponding Mo(V) (L-N_3)MoOX(Cl) complex takes place upon abstraction of a chlorine atom from the solvent. In coordinating solvents, such as dmf, pyridine, and MeCN, 1 mol of $OPPh_3$ is produced per mole of complex and oxo-Mo(IV) complexes of the type (L-N_3)MoOX(solvent) are formed (*127, 128*); (L-N_3)MoO(Cl)(py) has been structurally characterized (*127*). The rate of OAT is easily modified by changing the nature of X. The incorporation of sulfur donor ligands clearly enhances the rate of OAT (Tables II and III). In wet solvents the reactions take interesting and subtle courses, leading to Mo(V) species (see Section IV.B.2.a).

A number of the (L-N_3)MoO_2X complexes catalyze the oxidation of PPh_3 by Me_2SO (*126–128*). In a rather nice catalytic system, OAT interconverts complexes of the type (L-N_3)$MoO_2(\eta^1$-$S_2PR_2)$ and (L-N_3)$MoO(\eta^2$-$S_2PR_2)$ (R = alkoxy, alkyl, aryl) while effecting the oxidation of PPh_3 by Me_2SO (*126, 205*); an example is shown in Eq. (16). Kinetics studies reveal that the forward and reverse reactions are

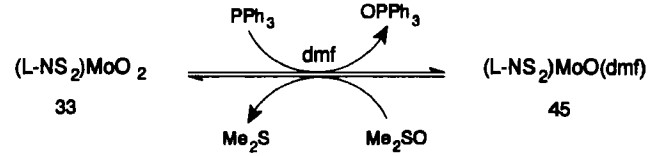


(16)

first order with respect to complex and substrate, consistent with a bimolecular reaction in both cases. Oxygen atom transfer is obviously facilitated by the ambidentate character of the 1,1-dithio ligands. These models were the first for which isolation and complete structural characterization of both the Mo(VI) and the Mo(IV) complexes were achieved (*126*).

2. Tridentate NS_2-Donor Ligand Complexes

Holm and co-workers have developed a significant OAT model based on complexes of L-NS_2; it has been extensively documented in the literature (*129, 130, 206–210*) and has been the subject of a number of reviews (*7, 211, 212*). We therefore limit our discussion to the key features. Five-coordinate (L-NS_2)MoO_2 (**33**, Fig. 10a) reacts with tertiary phosphines to form a purple compound (**45**), formulated as (L-NS_2)MoO(dmf) (Eq. (17)); this reaction is bimolecular but slower



(17)

than the corresponding reduction of $MoO_2(S_2CNEt_2)_2$. Characterization data for **45** are limited, and an X-ray diffraction study has not been possible. Reaction of **45** with a variety of oxygen atom donors, including the biological substrates Me_2SO, *D*-biotin-*S*-oxide and nicotinamide *N*-oxide (Eq. (17)), regenerates **33**. Considerable kinetics data are available for these reactions (Tables II and III). Interestingly, substrate reduction follows saturation kinetics, which are interpreted in terms of initial binding of the substrate to complex **45**, followed by slow oxygen atom transfer. Surprisingly, there is little variation in the rates of

substrate reduction, and the entropy changes appear anomalously high. The transition states relevant to these and other oxygen atom transfer reactions have been discussed (*7*). Thiols, which are physiologically relevant reducing agents, can be substituted for PPh_3 in a number of catalytic reactions described by Eq. (18) (*208, 209*).

$$XO + 2RSH \longrightarrow X + RSSR + H_2O \qquad (18)$$

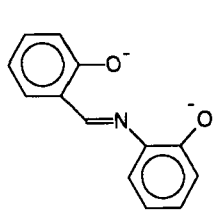
A simple thermodynamic scale of OAT reactivities has been developed, and an assessment of the thermodynamic fitness of molybdenum as an enzyme active site has been made. Complex **45** is reversibly oxidized by one electron but details concerning the expected mononuclear Mo(V) complex have not been reported.

3. Bis(bidentate) NS-Donor Ligand Complexes

Recently Holm and co-workers (*131*) have described a new OAT reaction system that cycles between $(L\text{-}NS)_2Mo^{VI}O_2$ (Fig. 10b) and $(L\text{-}NS)_2Mo^{IV}O$ (Fig. 12). The steric hindrance of the two bulky *p-tert*-butylphenyl groups prevents comproportionation [Eq. (15)] to form dinuclear $[Mo_2{}^{V}O_3]^{4+}$ centers (**37**). This reaction system is thermodynamically competent to oxidize or reduce all enzymatic substrates except those requiring the $[Mo^{VI}OS]^{2+}$ center as oxidant. The system is stable in the presence of strong oxo donors, such as Me_3NO and IO_4^-. The kinetics of substrate oxidation are second order and sensitive to substrate, indicating a different mechanism than the previously studied system based upon $(L\text{-}NS_2)MoO_2$ (*130*).

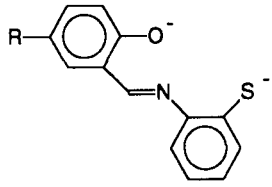
4. Schiff Base Complexes

In 1982, Boyd and Spence (*213*) reported the synthesis of $Mo^{IV}OL$ (dmf) and $Mo^{IV}OL$(bpy) (L = sap (**46**) or ssp (**47**)) by reaction of MoO_2L (dmf) (*136*) with PPh_2Et in the absence and presence, respectively, of 2,2′-bipyridine (bpy). The synthesis of the same compounds from two



sap

46



ssp R = H

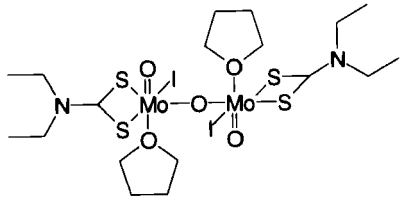
5-X-ssp R = X

47

Mo(IV) starting materials provided the best evidence for the proposed Mo(IV) formulations. Subsequently, Topich and Lyon (*137–140*) described kinetics studies of these and closely related systems; reported rates and activation parameters for the reduction of MoO_2L (L = 5-X-ssp (**47**)) complexes with PPh_2Et correlated with the Mo(VI) irreversible reduction potentials and Hammett σ_p functions of the X groups. At 30°C in dmf, k for the second-order reactions varied from 19.6(6) × $10^{-4}\,M^{-1}\,sec^{-1}$ (X = Br) to 8.4(4) × $10^{-4}\,M^{-1}\,sec^{-1}$ (X = OMe), with $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the reaction involving MoO_2(ssp) being 65.2 kJ mol^{-1} and -86.5 J K^{-1} mol^{-1}, respectively. It was concluded, on the basis of tight isosbestic points, UV-visible spectroscopy, and the previous results of Boyd and Spence (*213*), that oxo-Mo(IV) complexes were the final products of these reactions. However, Craig *et al.* (*141*) demonstrated that MoO_2L(solvent) complexes react with phosphines to ultimately produce dinuclear Mo(V) complexes, $Mo_2O_3L_2(solvent)_2$. This was proven by a combination of stoichiometric, mass spectrometric, and X-ray crystallographic studies. Indeed, the complex $Mo_2O_3(ssp)_2(H_2O)_2$ was reported as early as 1981 by Rajan and Chakravorty (*136*); it formed upon hydrazine reduction of MoO_2(ssp) and was oxidized back to starting material by dioxygen. Craig *et al.* have also shown (*141*) that some of the $Mo_2O_3L_2(solvent)_2$ complexes react with sulfoxides and NO_3^- to produce MoO_2L(solvent); the generalized reaction sequence (19) was postulated.

$$Mo_2O_3L_2(solvent)_2 + XO \rightleftharpoons Mo_2O_3L_2(solvent)(XO) + solvent$$
$$Mo_2O_3L_2(solvent)(XO) + solvent \longrightarrow 2MoO_2L(solvent) + X \qquad (19)$$

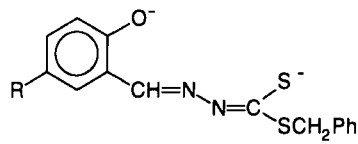
A second example of such a reaction, also involving a complex with labile solvent ligands, was reported by Baird *et al.* in 1989 (*214*). Reaction of $Mo_2O_3(S_2CNEt_2)_2I_2(thf)_2$ (**48**) with a wide variety of *N*- and *S*-oxides results in the formation of an unidentified dioxo-Mo(VI) complex, which is capable of catalyzing OAT from pyridine *N*-oxide or Me_2SO to PPh_3.



48

5. *Other Systems*

Kaul *et al.* (*124*) have reported that complex **31** participates in OAT reactions with PPh_2Et and Me_2SO, but details of the reactions are not available. Aspects of this model suggest that a spectro (EPR) electrochemical study would be rewarding. In a new system involving complexes of the thiosemicarbazone ligands (**35**), Purohit *et al.* (*135*) report the OAT reactions of MoO_2L and MoOL with phosphines and sulfoxides, respectively. It is not certain whether the MoOL complexes are true mononuclear Mo(IV) complexes. Bhattacharjee and Bhattacharyya (*215*) have reported kinetics studies of the PPh_3 reduction of MoO_2L complexes incorporating related ligands **49** (see Table II) and the use of such complexes in OAT catalysis. Finally, the complex $[MoO_2(L\text{-}SO)_2]^{2-}$ catalyzes the aerial oxidation of PPh_3 (*132*).



R = H, Me, Cl, Br

49

Kinetics data from the reaction of $MoOCl_3(OPPh_3)_2$ with NO_3^-, which led to the eventual formation of an unidentified Mo(VI) complex and NO_2, have been interpreted in terms of a three-step process (*216, 217*). The first step involves the coordination of NO_3^- to Mo via an S_N1 mechanism involving the initial loss of $OPPh_3$. The second step is proposed to involve chelation and/or isomerization of coordinated NO_3^- into a position *cis* to the oxo group. This permits rapid electron transfer to produce products. The third step involves binding of NO_3^- to the Mo(VI) complex and subsequent chemistry. In related reactions involving chloro-Mo(V) complexes containing bidentate co-ligands, kinetics studies support a similar mechanism involving loss of chloride, NO_3^- coordination, and isomerization, followed by product formation (*218*). Reduction of nitrate by the dinuclear Mo(III) complex $[L_2Mo_2(\mu\text{-}OH)_2(H_2O)_2]^{4+}$ (Eq. (20)) has been investigated by Wieghardt *et al.* (*219*).

$$[L_2Mo_2(\mu\text{-}OH)_2(H_2O)_2]^{4+} + NO_3^- \longrightarrow [L_2Mo_2O_2(\mu\text{-}O)_2]^{2+} + NO_2^- + 2H_3O^+ \quad (20)$$

The reaction is first order with respect to both reactants with $k = 0.1$ M^{-1} sec^{-1} (25°C). Experiments employing $N^{18}O_3^-$ provided infrared

evidence for the incorporation of label as a terminal oxo ligand and not as a bridging oxo ligand, consistent with an associative mechanism and the substitution of coordinated aquo ligands by NO_3^- in the rate determining step. Reduction of NO_3^- to NO_2^- is accompanied by a redox change from Mo(IV) to Mo(VI) when the reaction is effected using **45** (*210*). As for related reductions employing **45**, saturation kinetics are observed, and the postulated OAT in the initially formed adduct complex is first order with $k = 1.49 \times 10^{-3}$ sec^{-1}. The $[MoO_2(L\text{-}SO)_2]^{2-}$ complex is reportedly formed when $[MoO(L\text{-}SO)_2]^-$ is reacted with nitrate, but only scant details were given (*134*).

In the cycle shown in Scheme 3 the oxygen atom that is incorporated into the oxidized substrate was previously attached to the molybdenum atom. Clearly, model oxomolybdenum complexes can oxidize molecules in organic solvents by direct oxygen atom transfer. Does transfer of an oxygen atom from the oxomolybdenum center to the substrate also occur in enzymes? The mechanism of action of xanthine oxidase has been investigated using single-turnover experiments (excess enzyme) (*189*). Reaction of xanthine with ^{16}O-labeled enzyme in $H_2{}^{18}O$ produced ^{16}O-labeled ureate; conversely, oxidation of xanthine with ^{18}O-labeled enzyme in $H_2{}^{16}O$ led to ^{18}O-labeled ureate. In the light of the OAT reactions discussed in this section, these results strongly suggest that the oxygen atom of the $[Mo^{VI}OS]^{2+}$ unit is transferred to product during catalysis (Eq. (21)).

$$\text{R-H} + \text{H}_2\text{O} + \text{—Mo}^{\text{VI}}(\text{O}^*)\text{=S} \longrightarrow \text{R-O}^*\text{H} + \text{—Mo}^{\text{IV}}(\text{O})\text{—SH} + \text{H}^+ \qquad (21)$$

After the substrate oxidation oxygen atoms from water are incorporated into the molybdenum center. Catalysis in the presence of excess xanthine (multiple-turnover conditions) incorporates the oxygen label from water into the ureate products (*220*). The chemical mechanisms of xanthine oxidase catalysis are discussed in more detail in Section VI.

B. One-Electron Reactions

Cyclic voltammetry has shown that the Mo(VI)/Mo(V) couple is usually electrochemically irreversible (*136, 148, 166, 168, 221, 222*); however, *reversible* one-electron reductions are observed in aprotic solvents for six-coordinate $[Mo^{VI}O_2]^{2+}$ complexes of *N*-alkylated tetradentate N_2S_2-ligands (*107, 121, 223*) or tetradentate S_4-ligands (*124*) and for $(L\text{-}N_3)MoO_2X$ complexes. The reversibility of the electrochemical reduc-

tion of $(L\text{-}N_3)MoO_2X$ complexes depends on the nature of X (*127*). When X is a halogen, reduction is irreversible, but for X = NCS^-, OMe^-, OPh^-, and SPh^- a reversible one-electron reduction is observed. Substitution of a sulfur donor ligand for an oxygen donor ligand leads to a decrease in the reduction potential for the complex. Reversible one-electron reductions of six-coordinate *mono*-oxo molybdenum complexes have been observed (*151, 224*), but electrochemical reduction of seven-coordinate $[Mo^{VI}O]^{4+}$ complexes is irreversible (*225*). The Mo(V)/Mo(IV) couple usually exhibits reversible or nearly reversible electrochemical behavior. This couple has been extensively studied for $(L\text{-}N_3)MoO(X, Y)$ compounds (*91, 151, 153*) and exhibits a potential range of 1.2 V as a function of the X and Y donor atoms. We have previously noted (Section IV.B.2.b) that the Mo(V)/Mo(IV) couple for $[MoO(SR)_4]^{n-}$ complexes is extremely sensitive to intramolecular NH···S interactions (*164*). Recently it has been shown that the one-electron reduction of $(L^{3m}\text{-}N_2S_2)MoO_2$ is strongly pH dependent (*226*). For sulfite oxidase, both the Mo(VI)/Mo(V) and the Mo(V)/Mo(IV) couples show a marked pH dependence (*78*). Clearly, the one-electron redox couples of oxomolybdenum centers are sensitive to both the nature of the donor atoms and the availability of protons on nearby groups. This leads us to a consideration of coupled electron–proton transfer reactions.

C. Coupled Electron–Proton Transfer (CEPT) Reactions

An interpretation of enzyme behavior in terms of CEPT reactions was provided by Stiefel in 1973 (*190*). π-Bonding in Mo=E (E = O, S) systems permits dramatic modulation of the pK_a of the E atom in Mo=E and Mo—EH units (and possibly other atoms in nearby ligands) when redox events occur at the Mo center. Thus, formal reduction of the metal center increases the basicity of the Mo=E ligands through population of π^* orbitals with significant ligand character. A number of facets of enzyme behavior, notably the generation of Mo(V) centers, observed proton coupling to these centers, and the role of water in the regeneration of the active site are neatly accounted for by CEPT reactions.

A very early example of this reaction was provided by Meriwether *et al.*, who reacted molybdate with various thiols, including 1,2-ethanedithiol and thioglycolic acid, to produce EPR active complexes (*66*). These reactions have now been shown, through spectral comparisons with authentic materials, to yield complexes such as $[Mo^VO(SCH_2CH_2S)_2]^-$ (*158*) and $[Mo^VO(L\text{-}SO)_2]^-$ (Section IV.B.2.b) (*133*). The reactions with molybdate may be written as Eq. (22).

$$[MoO_4]^{2-} + 5RSH \longrightarrow [MoO(SR)_4]^- + 2H_2O + OH^- + \tfrac{1}{2}RS{-}SR \quad (22)$$

In a related reaction $[Mo^{VI}O_2(L\text{-}SO)_2]^{2-}$ is reduced by excess thiol ligand to monomeric $[Mo^{V}O(L\text{-}SO)_2]^-$ (*133, 134*) according to Eq. (23).

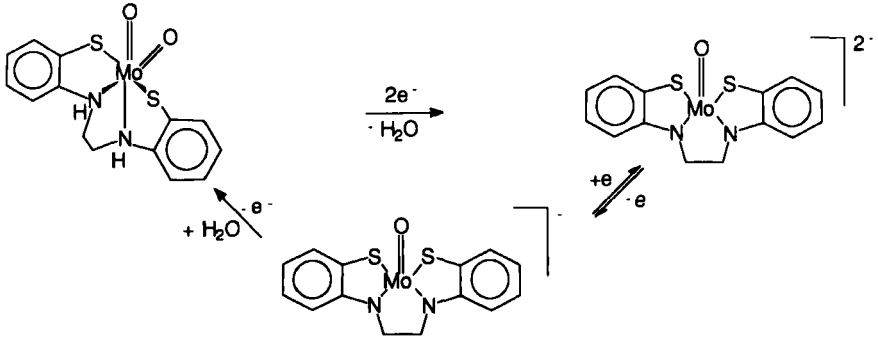
$$[MoO_2(L\text{-}SO)_2]^{2-} + RSH + H^+ \longrightarrow [MoO(L\text{-}SO)_2]^- + \tfrac{1}{2}RS{-}SR + H_2O \quad (23)$$

The first step of the reaction is postulated to be reduction to $[Mo^{IV}O(L\text{-}SO)_2]^{2-}$ followed by its comproportionation with starting material with loss of water. Reaction of $[Mo^{VI}O_2(L\text{-}SO)_2]^{2-}$ with butanethiol leads to apparent reduction to a Mo(IV) complex and subsequent decomposition; a proton source appears to be a corequisite for clean comproportionation to the Mo(V) complex.

Two-electron electrochemical reduction of $[Mo^{VI}O_2(L\text{-}SO)_2]^{2-}$ to $[Mo^{IV}O(L\text{-}SO)_2]^{2-}$ is also observed, followed by comproportionation with starting material to form $[Mo^{V}O(L\text{-}SO)_2]^-$. In general, complexes of this type are reversibly reduced to corresponding Mo(IV) complexes but irreversibly oxidized. Several are capable of participating in the redox scheme of Eq. (24) (*160, 165*).

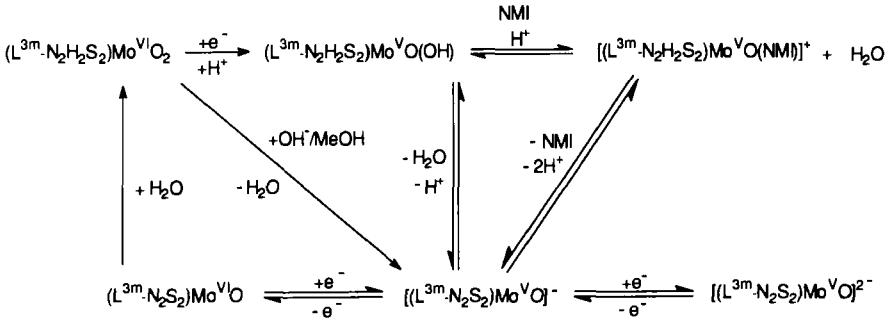
$$Mo^{VI} \underset{-e^-}{\overset{+e}{\rightleftharpoons}} Mo^{V} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} Mo^{IV} \quad (24)$$

Another classic example of a CEPT reaction is the electrochemical interconversion of the amino- and amido-thiol complexes $(L^3\text{-}N_2H_2S_2)Mo^{VI}O_2$ and $[(L^3\text{-}N_2S_2)Mo^{V}O]^-$ (Scheme 5). This reaction is proposed to proceed via two-electron reduction of the Mo(VI) complex to produce an unidentified Mo(IV) complex, which then comproportionates to produce the Mo(V) species. Removal of an oxo group from $(L^3\text{-}N_2H_2S_2)MoO_2$



SCHEME 5

is accompanied by deprotonation of the amino nitrogen atoms and the amido ligand is believed to then impose a coordination geometry that prevents dinucleation of $[(L^3\text{-}N_2S_2)Mo^{V}O]^-$ (*148, 168*). Electrochemical interconversion of the Mo(V) and Mo(IV) complexes is reversible. Related phenoxy complexes were subsequently studied (*166, 168*). When sterically bulky *tert*-butyl groups were built into the ligands, one-electron reduction of the Mo(VI) complex produced EPR active mononuclear Mo(V) complexes in high yield. Deprotonation of the amino nitrogens was again postulated to accompany loss of the oxo group. A recent electrochemical study of the related $(L^{3m}\text{-}N_2H_2S_2)Mo^{VI}O_2$ complex as a function of pH provided evidence for two separate one-electron processes. The overall chemistry involves irreversible reduction of $(L^{3m}\text{-}N_2H_2S_2)Mo^{VI}O_2$, followed by loss of water from the Mo(V) complex, and then reversible reduction to Mo(IV) (Scheme 6) (*226*).



SCHEME 6

D. CYANOLYSIS REACTIONS

Deactivation of oxidized xanthine oxidase by cyanide produces thiocyanate and an inactive or desulfo form of the enzyme. The oxidized desulfo enzyme contains a dioxo-Mo(VI) center. Since it is generally believed that a terminal thio ligand on Mo is removed in the cyanolysis reaction, the interaction of oxo-thio-Mo(VI) complexes with CN^- is worthy of investigation. In general, reactions of thio-Mo(VI) complexes (typically thiomolybdates) with cyanide leads to the formation of SCN^- by formal sulfur atom transfer and reduction of the metal, e.g., Eq. (25).

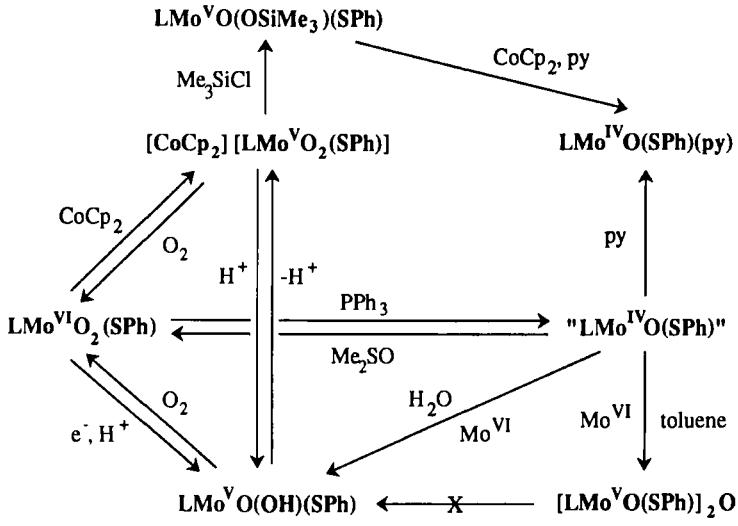
$$Mo^{VI}S + CN^- \longrightarrow Mo^{IV} + SCN^- \qquad (25)$$

An early example involved mononuclear thiomolybdates and excess cyanide (*227*). In related studies, Müller and co-workers (*175, 228–230*) have isolated and characterized a variety of polynuclear cyano anions of Mo(IV) and Mo(III). These complexes feature μ_2- and μ_3-thio ligands, which are generally quite resistant to nucleophilic attack by cyanide (*231, 232*). Only two examples of the conversion of an oxo-thio-Mo(VI) complex to the corresponding dioxo complex have been reported. First, the complex *cis*-$MoOS(C_5H_{10}NO)_2$ reacts with CN^- in chloroform to produce *cis*-$MoO_2(C_5H_{10}NO)_2$ (50–60%) and SCN^- (65–80%), but the reaction is complicated by the sacrifice of piperidine *N*-oxide in the oxidation of the initially formed oxo-Mo(IV) species (*181*). The second example closely mimics the deactivation of enzymes by cyanide (*104*). Reaction of (L-N_3)$MoOS(S_2PR_2)$ with CN^- in the absence of air and water results in rapid quantitative formation of (L-N_3)$MoO(S_2PR_2)$ and SCN^-, clearly demonstrating the formal reduction of the Mo atom upon sulfur atom transfer to CN^-. In the presence of water and oxygen, (L-N_3)$MoO_2(S_2PR_2)$ is produced in a process that may involve the oxidation of an intermediate aquo-Mo(IV) complex. A thorough study of the mechanism of this important reaction is warranted.

E. A Model for Enzymes With $[MoO_2]^{2+}$ Oxidized Centers

In sulfite oxidase and related enzymes, transfer of the oxygen atom from Mo to substrate and its replacement from H_2O both appear to occur in the Mo(VI) to Mo(IV) transformation; regeneration of the Mo(VI) state is achieved via two one-electron oxidations which are mediated by the internal electron transfer chain and which generate transient Mo(V) states (Scheme 3) (*7, 233, 234*). A number of model catalytic systems have been reported in which O_2 and/or H_2O are key participants, but the role of water has not been clarified (*196, 198, 201, 202, 235, 236*).

A new system (Scheme 7) involving the facially tridentate L-N_3 ligand displays all the important centers and processes involved in catalysis by such enzymes (cf. Scheme 3) (*128*). Reaction of (L-N_3)MoO_2(SPh) with PPh_3 produces coordinatively unsaturated "(L-N_3)$Mo^{IV}O$(SPh)," which can be trapped and structurally characterized in a number of ways, depending upon ligand availability (Section V.A.1). In the presence of water, one-electron oxidation of "(L-N_3)MoO(SPh)(H_2O)" to the EPR active (L-N_3)$Mo^{V}O$(OH)(SPh) occurs in high yield. This complex can be oxidized by O_2 to (L-N_3)$Mo^{VI}O_2$(SPh) to complete the cycle shown in Scheme 7. The two-electron Mo(VI) to Mo(IV) step is slower than



SCHEME 7

the one-electron Mo(IV) to Mo(V) step, preventing direct access to the proposed (L-N_3)$Mo^{IV}O$(SPh)(H_2O) intermediate.

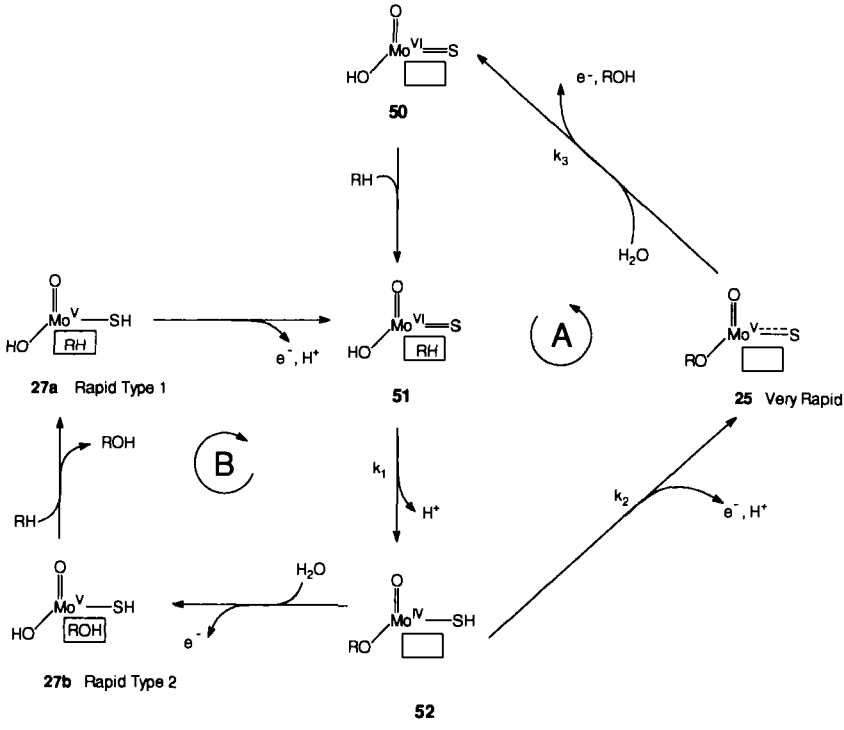
Oxygen isotope tracing showed that H_2O, not O_2, is the source of the oxo ligand regenerated in the cycle, which is capable of sustained catalysis of the oxidation of PPh_3 by H_2O and O_2. Note that the Mo oxidation state controls the level of protonation of the water-based ligand as previously outlined. The system is capable of the catalysis of coupled two-electron OAT reactions involving the usual substrates, PPh_3 and Me_2SO.

The system of Scheme 7 has also enabled a $[Mo^VO_2]^+$ center to be isolated in substance for the first time as the cobaltocenium salt, [$CoCp_2$][(L-N_3)MoO_2(SPh)]. No oxygen atom transfer chemistry has been observed to date for this species. This may be the reason that a $[Mo^VO_2]^+$ center has never been detected (via its characteristic EPR signal) (*107*) during turnover of any enzyme.

VI. The Xanthine Oxidase Cycle

Xanthine oxidase and related hydroxylase enzymes exhibit broad substrate specificity and an apparently complex catalytic cycle (*109, 237*). The important centers identified by EXAFS and EPR studies have

been presented in Section III.C. Kinetics studies have also provided important results (*189, 238*). In a particularly noteworthy study by Hille and Sprecher (*189*), single turnover experiments under substrate-limiting conditions confirmed that the oxygen atom that was transferred to the substrate originated from the Mo center and not from solvent water. For OAT from a simple *cis*-$[Mo^{VI}OS]^{2+}$ center, the transient existence of a deoxo-Mo(IV) species is implied, prior to replacement of the oxo ligand from H_2O [Eq. (21)]. However, a detailed comparison (*109*) of the enzyme EPR signals derived from active enzyme with the synthetic *cis* species $[(L^4\text{-}N_2S_2)Mo^{V}OE]^-$ and $(L^4\text{-}N_2S_2)Mo^{V}O(EH)$ (E = O, S) allows three ligands to be defined in each of the Mo(V) enzyme centers: $[Mo^{V}OS(OR)]^+$ (Very Rapid) or $[Mo^{V}O(SH)(OH)]$ (Rapid 1 or 2). The presence of bound product OR^- in the Mo(IV) center has been implied from EXAFS and resonance Raman studies (*102, 239*). The catalytic cycle given in Scheme 8 accounts for the observed behavior of the enzyme under substrate-limiting (cycle A) and substrate-excess (cycle B) conditions (*109*).



SCHEME 8

Formulation of the resting active site as a *fac*-$[Mo^{VI}OS(OH)]^+$ center (**50**) not only is consistent with available evidence but also permits a Mo=O group to be maintained at all stages of catalysis. Stabilization of the thio ligand via an active site interaction has been suggested (*104*), but as yet there is no direct evidence for this; the severing of this interaction would take place upon substrate activation and would not persist in reduced species. The *cis*-$[Mo^{VI}O(OH)]$ fragment is the equivalent of the *cis*-$[Mo^{VI}O_2]$ unit in sulfite oxidase. Substrate activation takes place at **50**, with the eventual formation of the Mo(IV) center, **52**, which contains bound oxidized substrate and an SH^- ligand. There is kinetic evidence that both substrate binding to and product dissociation from the enzyme active site are two-step processes (*240, 241*). One interpretation (*109*) is that initial substrate binding produces a noncovalent Michaelis complex, **51**, and that catalysis leads to occupation of the anion-binding site by bound product OR^-, as in species **52**. Product dissociation follows a reverse sequence, which has implications for aspects of cycle B. While details of the electronic rearrangements accompanying C(8)—H activation remain uncertain, it is likely that concerted changes in the acidity/basicity of the Mo—OH and Mo=S groups assist substrate activation and the formation of **52**. Oxidation may be initiated by interaction of the C—H bond with a Mo=S π^* orbital of **50**, which induces formal transfer of hydride and formation of the Mo—OR link in **52**. Conversely, interaction of C(8) with the hydroxy oxygen could lead to population of a Mo=S π^* orbital, followed by deprotonation of the substrate by the basic thio ligand. The role of molybdopterin moieties or nearby amino acid sites in coupled electron–proton transfer reactions remains open for speculation. The Very Rapid center, **25**, is derived from the Mo(IV) form, **52**, by a one-electron oxidation (*234*). The proton on the SH^- ligand is also lost in this process. Dissociation of the substrate and a second one-electron oxidation regenerates active site **50** and yields product.

The complications that arise when substrate is in excess (observation of the rapid signal) can be attributed to the effects of substrate binding prior to product release during the Mo(IV) to Mo(VI) one-electron transformations (*109, 234*). Under these conditions cycle B, involving the Rapid Type 2 center **27b** and the Rapid Type 1 center **27a**, may pertain. The Rapid centers, whose EPR spectra exhibit two strongly coupled protons, are proposed to differ (primarily) according to the purine bound at the noncovalent binding site.

Consistent with McWhirter and Hille's suggestion (*234*), **27a** is catalytically incompetent until oxidized to **51**, the Michaelis complex. The present proposal accommodates a number of other aspects of xanthine

oxidase activity including its role as an *N*-oxide reductase (*7, 220*) and the fact that warming the Rapid Type 2 signal (**27b**) leads to the very rapid signal (**25**) (*242*). The mechanism of Scheme 7 is similar to Bray's previously proposed mechanism (*10*) for xanthine oxidase, but includes more detail about the chemical identity of the individual species.

VII. Intramolecular Electron Transfer in Molybdenum Enzymes

With the exception of the recently reported DMSO reductases from bacteria (*71, 72*), all of the enzymes of Table I contain additional redox active prosthetic groups besides Mo-co. Substrate oxidation (reduction) occurs at the molybdenum center, and electrons are removed (added) via one of the other prosthetic groups. These two processes are coupled by intramolecular electron transfer between the molybdenum center and the other redox centers of the enzyme. Results for xanthine oxidase and sulfite oxidase and approaches to modeling the coupling in sulfite oxidase are summarized below.

A. Xanthine Oxidase

Xanthine is converted to uric acid at the molybdenum center of the enzyme, and the electrons are removed from the enzyme by oxidation of the flavin center. From early reductive titrations of xanthine oxidase with sodium dithionite, it was proposed that reducing equivalents were equilibrated among the four redox-active centers (Mo-co, two separate Fe_2S_2 centers, flavin) at a rate that was rapid relative to the overall catalytic rate of substrate turnover (*243*). Under such conditions, the flux of reducing equivalents through the enzyme should be influenced by the relative reduction potentials of the redox centers involved (*244*). Any effects of pH and temperature on the reduction potentials of individual redox components would affect the apparent rates of intramolecular transfer of the enzyme.

The potentials of the redox centers of xanthine oxidase have been investigated by titrations in the presence of redox mediator dyes. An early study (*245*) used dithionite to generate reducing equivalents and quantified the reduced species by EPR measurements at low temperature. Subsequent studies as a function of pH showed that the potential of the molybdenum center was sensitive to pH (*246*). Concern over the effect of temperature on the observed potentials led to redox titrations monitored by room temperature CD and EPR spectroscopy (*247*). These experiments indicated that the redox potentials of all of the prosthetic

groups of xanthine oxidase were sensitive to pH and that, at intermediate levels of reduction of the enzyme, the flavin was mostly reduced and the molybdenum center was mostly oxidized (*247*). Microcoulometric determination of the stoichiometry of electron uptake of xanthine oxidase at 25°C showed that the active oxidized enzyme accepted a total of six electrons (two for the Mo center, one for each of the two Fe_2S_2 centers, two for the FAD) (*77*). The derived midpoint potentials confirmed the pH dependence observed previously by room-temperature optical and EPR methods (*247*). The Mo(V)/Mo(IV) potential was 25–30 mV more negative than those determined from EPR analysis of frozen enzyme solutions that had been poised at defined potentials at 25°C (*77*).

The pathway(s) and rates of intramolecular electron transfer have been probed by several techniques. Coupling between the Mo(V) and the Fe/S I center in the EPR spectrum at low temperature gave an estimate of the Mo···Fe/S I distance of 11 ± 3 Å. There was no evidence for coupling of the molybdenum center to any of the other prosthetic groups, but coupling between the two iron centers and between the flavin and each iron center was detected (*248*). The reaction of xanthine oxidase with the 5-deazaflavin radical generated by laser flash photolysis, is biphasic and the observed rate constants were initially interpreted (*249*) as inconsistent with the previous hypothesis (*244*) that intramolecular electron equilibration was more rapid than enzyme turnover. However, stopped-flow pH jump (*250*), pulse radiolysis (*251, 252*), and steady-state experiments on xanthine oxidase containing modified flavins (*253*) are all consistent with the original proposal of "thermodynamic control" of intramolecular electron transfer in xanthine oxidase (*244*). Recent pulse radiolysis studies have provided the first unambiguous demonstration of the role of the iron–sulfur centers in mediating electron transfer between the molybdenum and flavin centers (*252*).

B. Sulfite Oxidase

Sulfite oxidase contains an oxo-molybdenum center and a *b*-type cytochrome. The proposed catalytic sequence (*254–256*) for the enzyme is shown in Fig. 16. Oxidation of sulfite to sulfate, a two-electron process, occurs at the molybdenum center with concomitant reduction of the molybdenum from VI to IV. Electrons are removed from the enzyme by interactions of the heme of the *b*-type cytochrome with exogenous cytochrome *c*, a one-electron process. Thus, the proposed mechanism of Fig. 16 involves two separate intramolecular electron transfers be-

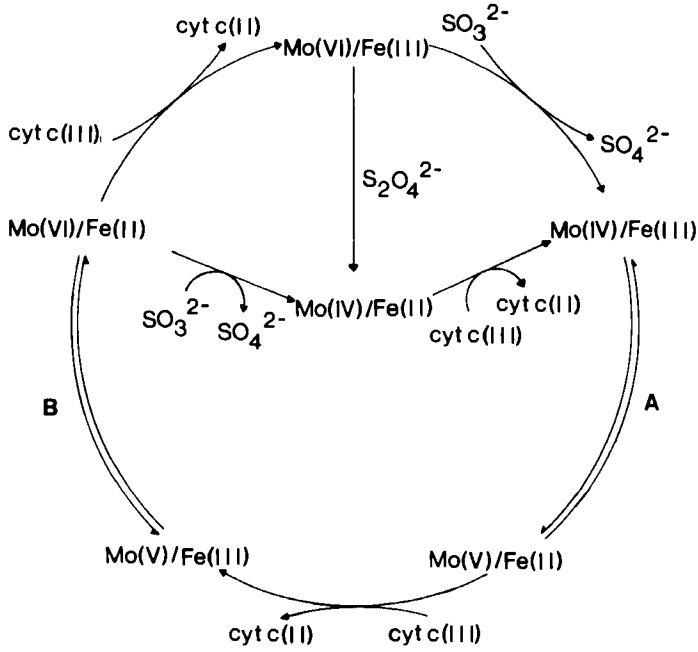


FIG. 16. Proposed catalytic cycle of sulfite oxidase, showing the two postulated intramolecular electron transfer processes, **A** and **B**. Process **B** has been studied by laser flash photolysis. Adapted with permission from Kipke *et al.* (*256*). Copyright 1988, American Chemical Society.

tween the two metal sites. Transfer **A** is from Mo(IV) to Fe(III); transfer **B** is from Mo(V) to Fe(III). After each of these intramolecular electron transfer steps, exogenous cytochrome *c* oxidizes the *b*-type iron center by one electron.

Steady-state kinetics experiments have shown that anions such as SO_4^{2-}, Cl^-, and HPO_4^{2-} are inhibitors of the flow of electrons from sulfite to cytochrome *c* but not to O_2 (*90, 257*). In 1971 Cohen and Fridovich proposed (regarding inhibition by sulfate) that "the sulfate sensitive step was not the reduction of the enzyme by sulfite, but was rather the egress of electrons from the enzyme to the 1-electron acceptors" (*257*).

Microcoulometric titrations of sulfite oxidase suggest that both the magnitude and the direction of the driving force for intramolecular electron transfer between the molybdenum and the iron centers of sulfite oxidase can be modulated by pH and anion concentration (*78*). At low pH and/or high chloride the Mo(VI) center appears to be reduced

more easily than the Fe(III) center of the *b*-type heme; whereas at high pH and/or low chloride the Fe(III) center is calculated to be more easily reduced (*78, 258*).

Intramolecular electron transfer between the molybdenum center and the *b*-type heme center of sulfite oxidase has been investigated by laser flash photolysis (*256, 258*). At pH 9 the Fe(III) center is preferentially reduced and there is little reoxidation on a millisecond time scale. However, at pH 6 there is significant intramolecular electron transfer from the reduced Fe(II) center to the molybdenum(VI) center (*258*) in accord with the predictions from microcoulometry (*78*). The *rate* of intramolecular electron transfer at pH 6 is strongly inhibited by anions, but the position of equilibrium appears unchanged. These observations have been interpreted in terms of a "gating" mechanism in which anion binding inhibits intramolecular electron transfer (*258*). The structural basis for the effect is currently unknown because there are as yet no X-ray structural data available for sulfite oxidase (or any other pterin-containing molybdenum enzyme). It is well known from EPR (*90*) and EXAFS (*69*) experiments that anions bind to the Mo(V) state of sulfite oxidase (Section II.B). However, whereas coordination of anions to Mo(V) would be expected to change the potential of the Mo(VI/V) couple (*78, 151*), the position of equilibrium of the intramolecular electron transfer reaction is essentially unchanged as a function of anion concentration. It should be noted that the flash photolysis experiments were carried out on a much faster time scale than that of the EPR and EXAFS studies of frozen equilibrated solutions. There are very few studies to date of the kinetics of anion binding to mononuclear oxo-Mo(V) centers (*259*). The marked decrease in the rate of intramolecular electron transfer in sulfite oxidase in the presence of anions could also result from an increase in the Mo···Fe distance due to general anion binding to the protein. Moser *et al.* (*260*) have recently discussed the effects of distance on intramolecular electron transfer rates. The inhibition of sulfite oxidase turnover by anions under steady-state conditions may also be due to limitations on intramolecular electron transfer because similar anion binding constants are obtained for both the steady-state (*90*) and the flash photolysis experiments (*258*).

C. Model Systems

There is very little information concerning one-electron reactions at oxo-molybdenum centers because of the tendency for oxo-molybdenum complexes to form dinuclear systems (**37** and **38**). To our knowledge, $(L\text{-}N_3)MoO_xX_{3-x}$ complexes constitute the only family of mononuclear

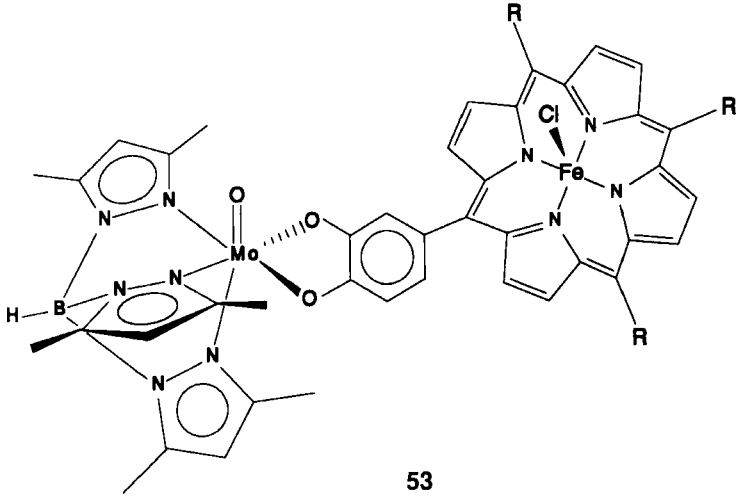
oxo-molybdenum complexes for which X-ray structures and spectroscopic data confirm that all three oxidation states of Scheme 3 (Mo(IV, V, VI)) exist as mononuclear species. The redox potentials and spectroscopic properties of these species are strongly dependent upon the nature of the X group (Sections IV and V), and reaction conditions for the chemical transformations among all of the oxidation states of Scheme 3 have been described for this family of complexes (Section V.E) (*128*).

Studies of the electron transfer reactions of oxomolybdenum complexes of trispyrazolylborate ligands with Fe(III) porphyrins show that the reduction of $(TPP)Fe^{III}Cl$ by $(L\text{-}N_3)Mo^{IV}OCl(dmf)$ (Eq. (26)) proceeds cleanly and quantitatively in dmf and is first order in Mo and *zero* order in Fe (*261*).

$$(TPP)FE^{III}Cl + (L\text{-}N_3)Mo^{IV}OCl(dmf) \longrightarrow (TPP)Fe^{II} + (L\text{-}N_3)Mo^{V}OCl_2 + dmf \quad (26)$$

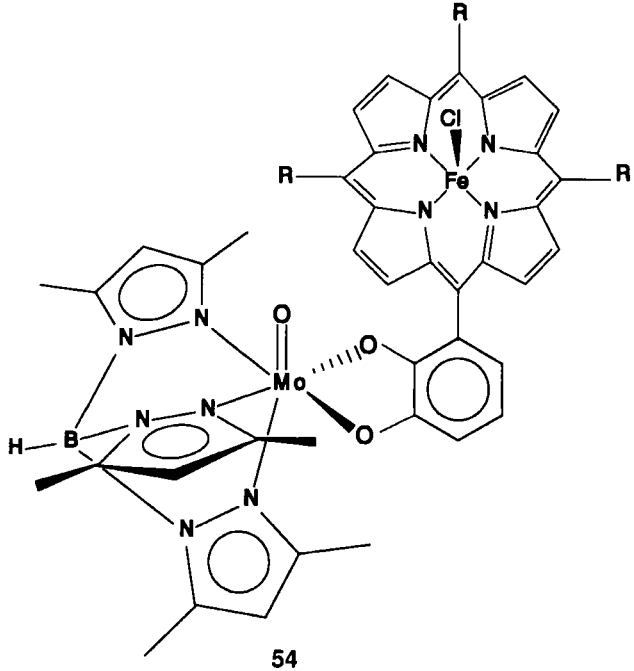
These kinetics data are consistent with a preequilibrium dissociation of dmf from the molybdenum center to form a reactive five-coordinate species that rapidly reduces the Fe(III) center via an inner sphere (halogen transfer) reaction. Other one-electron atom transfer reactions are known in oxo-molybdenum chemistry (*262*). An innersphere (atom transfer) mechanism is not a viable model for intramolecular transfer in sulfite oxidase because in the enzyme the Mo and Fe centers are almost certainly held too far apart by the protein framework. Moreover, the b_5-type heme center of sulfite oxidase is six-coordinate with axial histidine ligands from the protein and hence cannot participate in atom transfer reactions.

Covalent coupling of an $[(L\text{-}N_3)Mo^{V}O]^{2+}$ fragment to a catechol-substituted (tetraarylporphyrin) $Fe^{III}Cl$ unit gives stable dinuclear complexes with controlled Mo(V)···Fe(III) distances of ≈9.4 Å (**53**) and ≈7.4 Å (**54**) due to the steric requirements of the respective ligand systems (*261, 263*). Addition of excess *N*-methylimidazole to **53** and **54** produces a low-spin Fe(III) center whose EPR spectra are similar to those reported previously for $[(TPP)Fe(imidazole)_2]^+$ complexes (*264*). At 77 K the EPR spectra show coupling between the Mo(V) and the low-spin Fe(III) centers that is anisotropic and for which dipolar and exchange terms both appear to be important (*265*). Cyclic voltammograms for **53** and **54** show that distinct one-electron redox processes are maintained for their respective Mo and Fe centers. The electronic spectra of **53** and **54** are very similar to the parent (TTP)FeCl complex, consistent with little electron delocalization between the Mo and the Fe centers (*261*). Variation of the Mo···Fe distance in related complexes will provide benchmarks for interpreting the EPR spectra of the Mo(V)/



53

Fe(III) states of sulfite oxidase itself. Complexes **53** and **54** are the primitive precursors to incorporating the chemistry of Scheme 7 (Section V.E) with electron transfer to an Fe(III) porphyrin center as a model for the overall reaction of sulfite oxidase shown in Fig. 16.



54

VIII. Pterin-Containing Tungsten Enzymes

A. Introduction

In early studies probing the structure and function of molybdenum enzymes, the substitution of tungsten for molybdenum was found to produce "tungstoenzymes" of significantly reduced or zero activity (*266*). Consequently, it was long held that tungsten was unlikely to have a biological function. However, the isolation and characterization of a number of pterin-containing tungsten enzymes, primarily from hyperthermophilic Archaea (Gk primitive) (*267*), have established a bonafide biological role for this element. These robust enzymes are able to catalyze Eq. (1) (Section I) for extremely inert substrates (e.g., CO_2) at temperatures near 100°C.

Complete characterization of the tungsten centers is likely to require an appreciation of the complementarity as well as the similarity in the chemistries of tungsten and molybdenum, viz., known molybdenum chemistry, currently more extensive than that of tungsten, may not constitute a proper basis for understanding the structure and function of the tungsten enzymes. Like molybdenum, the ability of tungsten to support oxygen atom transfer chemistry is important to enzyme function, but the nature of Mo and W active sites may differ. The inertness of tungsten relative to molybdenum and the quite different redox potentials of the two metals extend the range of substrates and enhance the stability of the tungsten enzymes under the extreme physiological conditions in which they operate.

Tungsten enzymes were first discovered in hyperthermophilic bacteria that populate the high-temperature, hydrothermal vents (hot springs) along submarine ridge crests. The hydrothermal vents jet water rich in sulfide precipitates and support a range of exotic organisms (*268, 269*). It is believed that the first life from which bacteria and Archaea developed were thermophilic, strictly anaerobic organisms such as those found in the vent areas. Indeed it has been proposed that tungsten enzymes evolved with early life and were replaced by molybdenum enzymes after oxygen appeared in the biosphere. It may be expected that tungsten enzymes will be characterized for other hyperthermophilic organisms. The recent discovery of the tungsten enzymes and the very limited pertinent tungsten chemistry permits a simplified discussion of these systems; the enzymes are discussed first followed by a brief description of modeling studies.

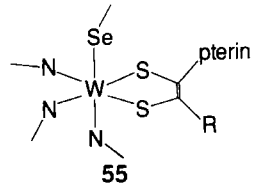
B. *Clostridium thermoaceticum* Formate Dehydrogenase

In 1973, Andreesen and Ljungdahl (*270*) reported that tungstate and selenite stimulated the growth of the acetogenic anaerobe *C. thermoaceticum* (*271*) and enhanced its formate dehydrogenase activity. The enzyme catalyzes the reversible net reaction shown in Eq. (27). The reverse reaction is the first step in the conversion of CO_2 to acetate; it is probable that HCO_3^- is the actual enzyme substrate that undergoes oxygen atom abstraction according to Eq. (1).

$$HCO_2^- \rightleftharpoons CO_2 + H^+ + 2e^- \qquad (27)$$

A decade later, Yamamoto *et al.* (*272*) isolated and purified *C. thermoaceticum* formate dehydrogenase and demonstrated that it was indeed a tungsten/selenium enzyme. The enzyme is a 340-kDa protein with an $\alpha_2\beta_2$ subunit structure. It contains two tungsten atoms, two selenium atoms, and 20–40 iron atoms per holoenzyme; the iron is present in iron–sulfur centers (*273*). The selenium is present as selenocysteine, and its requirement is not overcome by sulfide or sulfate (*274*); the selenium resides in the α subunits (*272*). Circumstantial evidence from EXAFS (see below) suggests that tungsten is also present in the α subunit. Selenocysteine is also present in molybdenum formate dehydrogenases, e.g., those from *Methanococcus vannielii* (*275*) and *Escherichia coli* (*276*). The presence of a pterin-containing cofactor in this tungsten enzyme is supported by fluoresence studies of the denatured enzyme (*272*) and the fact that, in the presence of molybdate, acidified samples of the enzyme activated the *nit-1* nitrate reductase (*277*).

EXAFS studies (*278*) of dithionite reduced enzyme suggest that the tungsten coordination sphere is composed of two or more oxygen or nitrogen donor ligands (W—O/N, 2.13 Å av) and two or more sulfur-donor ligands (W—S, 2.39 Å av). Inclusion of a W—Se interaction of 2.6 Å improves the EXAFS fit and reduces the number of sulfur atoms required. Selenium EXAFS has not yet been reported. Terminal oxo ligation was not revealed, in contrast to molybdenum formate dehydrogenase from *C. pasteurianum*. The results of low-temperature EPR studies of the enzyme poised at −400 mV vs SHE are consistent with the presence of two Fe_4S_4 and two Fe_2S_2 centers and two catalytic sites per tetrameric molecule. An EPR signal (g, 2.101, 1.980, and 1.950), produced when the enzyme is poised at −450 mV vs SHE, was tentatively assigned to a W(V) center (*279*), but detailed EPR studies have not been reported. A possible active site structure is represented in **55**.



55

C. *Pyrococcus furiosus* ALDEHYDE : FERREDOXIN OXIDOREDUCTASE

In 1989, Bryant and Adams reported that tungsten stimulated the growth of the strict anaerobe *Pyrococcus furiosus* (*280*). This Archaeon (*281*) consumes complex carbohydrates in a fermentative-type process producing H_2 and CO_2 (*282*). It converts sulfur to H_2S in a reaction thought to provide a means of removing the inhibitor dihydrogen. It oxidizes a range of aliphatic aldehydes and reduced *P. furiosus* ferredoxin at an optimal operating temperature above 90°C; the enzyme is extremely oxygen sensitive. Two forms of the enzyme have now been characterized. An inactive form, the so called "red tungsten protein" (RTP), was first reported by Mukund and Adams in 1990 (*283*). Before its inactivity was appreciated, it was subject to electrochemical, EPR, and EXAFS study; accordingly, these results must be treated with caution. The thermostable RTP has been characterized as a monomer (ca. 85 kDa) containing one tungsten, seven irons, and five acid-labile sulfides. Selenium, detected at a level of only 0.05 g-atom by plasma emission spectroscopy, does not appear to be a component of the enzyme. The presence of a WFe_3S_4 core was postulated on the basis of electrochemical and EPR results. This postulate was not supported by a later X-ray absorption study; a bound state transition indicative of terminal oxo ligation was observed on the W-L_{III} edge, whereas the EXAFS was consistent with the presence of two terminal oxo groups (W=O = 1.74 Å), three sulfur-donor ligands (W—S = 2.41 Å) and a single oxygen or nitrogen-donor ligand (W—O/N = 2.16 Å) (*284*).

As stated, the RTP is the inactive form of aldehyde : ferredoxin oxidoreductase (AOR). The active AOR enzyme may be isolated by rapid purification under anaerobic conditions using buffer containing dithiothreitol and glycerol (*282*). AOR catalyzes Eq. (28) and is postulated to contain a W—SH group not present in the RTP.

$$RCHO + H_2O \rightleftharpoons RCO_2H + 2H^+ + 2e^- \qquad (28)$$

Evidence for the presence of a pterin cofactor in this enzyme was obtained by Mukund and Adams (*282*); the fluorescence spectrum of the

denatured protein was typical of the oxidized desulfurized derivative (Form A (**8**)) of Mo-co. The chemistry of this protein appears to be closely related to that of the molybdenum hydroxylases and similar active sites may exist in these enzymes.

D. *Methanobacterium wolfei* Formylmethanofuran Dehydrogenase

The growth of *Methanobacterium wolfei* is dependent on the presence of molybdenum or tungsten, and one of the two formylmethanofuran dehydrogenases from this bacterium is a tungsten enzyme (*285*). The reversible reaction catalyzed by these enzymes (Eq. (29)) is the first step in methane formation from CO_2 in all methanogenic Archaea (*286*).

$$CO_2 + \text{methanofuran (MFR)} \rightleftharpoons \text{formyl-MFR} \qquad (29)$$

The extremely oxygen-sensitive enzyme has a molecular mass of 130 kDa, is composed of three subunits, and contains 0.3 to 0.4 mol of tungsten per mole of enzyme. A pterin cofactor, identified as molybdopterin guanine dinucleotide, is present and the enzyme is not deactivated by cyanide.

E. Other Enzymes

Clostridium thermoaceticum carboxylic acid reductase (aldehyde dehydrogenase) (*287*) catalyzes the reversible reaction shown in Eq. (28). The yield and activity of this enzyme are dependent upon sulfur (as, for example, dithionite) and tungsten in the growth medium, and the enzyme appears to exist in two forms. One has a molecular mass of 240 kDa and appears to be a tetramer that is easily dissociated into monomers. The other is the monomer (α or β monomer units). Neither enzyme has been completely characterized. The tungsten is easily dissociated and the enzyme is very oxygen sensitive. Finally, tungsten plays a role in the regulation of the activity of an iron hydrogenase in *Thermotoga maritima* (*288*).

F. Tungsten Chemistry and Model Studies

The chemistry of tungsten generally parallels that of molybdenum, but is considerably less developed (*12, 289, 290*). The recent recognition of the biological role of tungsten is now stimulating the exploration of high-valent oxo- and thio-tungsten chemistry, but it is not clear at this

stage just what tungsten chemistry, in a narrow sense, will prove relevant to the enzyme problem. Certainly considerable developments in tungsten chemistry are necessary to match, in the case of the tungsten enzymes, the state of modeling currently achieved for molybdenum enzymes. As a result, models for the tungsten enzymes are extremely rare.

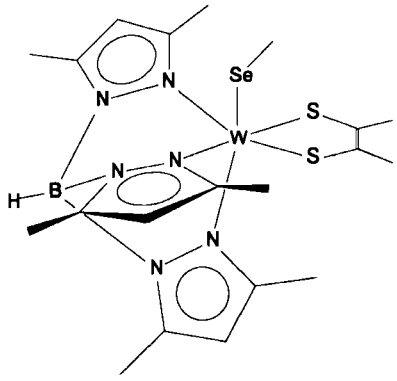
A study of W(VI), W(V), and W(IV) complexes containing benzenedithiolate ligands has focused on modeling proposed oxo-tungsten active sites (*291*). Reaction of $[WO(SPh)_4]^-$ with benzenedithiolate produces $[W^VO(S_2C_6H_4)_2]^-$, which may be easily reduced to the corresponding W(IV) complex $[WO(S_2C_6H_4)_2]^{2-}$. The W(IV) complex is readily oxidized to stable *cis*-$[WO_2(S_2C_6H_4)_2]^{2-}$, which is capable of reacting with benzoin to regenerate $[WO(S_2C_6H_4)_2]^{2-}$. All three complexes have been structurally characterized. The involvement of W(IV) and W(VI) centers in oxygen atom transfer reactions appears to be enhanced by the "dithiolene-like" benzenedithiolate ligands. Indeed, in a recent study Sarkar and Das (*292*) have demonstrated that the dithiolene complex $[W^{IV}O\{S_2C_2(CN)_2\}_2]^{2-}$ reduces CO_2/HCO_3^- to formate with generation of $[W^{VI}O_2\{S_2C_2(CN)_2\}_2]^{2-}$; the substrate reaction is identical to that shown in Eq. (27). Other oxygen atom transfer reactions involving tungsten have been reported (*7, 293, 294*).

Several considerations suggest that thio- and hydrosulfido-tungsten centers may play an important role in the enzyme systems. First, the organisms that exploit these enzymes live in a sulfur-rich environment under anaerobic and high-temperature conditions; these are conducive to thio-tungsten rather than oxo-tungsten chemistry. The presence of oxo groups in enzyme preparations may be a consequence of workup procedures, and the *in vivo* active site may not be accurately reflected in current data. Interestingly, active aldehyde : ferredoxin oxidoreductase is isolated only when rapid isolation in the presence of a thiol is employed, and EXAFS studies suggest the presence of a WO_2 center in the inactive RTP form of this enzyme (Section VIII.C) Second, there is an important complementarity in the chemistry of oxo- and thio-molybdenum complexes on one hand and oxo- and thio-tungsten complexes on the other; this may also be true of molybdenum and tungsten enzymes, which appear to operate under quite different physiological conditions. There is a need, therefore, to explore enzyme activity and, if appropriate, the physical and spectroscopic properties of enzymes under sulfiding conditions. We shall now concentrate our discussion of tungsten chemistry on thio-complexes and new models that target active sites such as **55**.

Solid-state halides constitute a large proportion of known thio-tungsten compounds (*295*), and only recently has the chemistry of molecular thio-tungsten species expanded. A number of complexes containing hydroxylamido and dithiocarbamate ligands have been known for some time; these include $WOS(C_5H_{10}NO)_2$, $WS_2(R_2NO)_2$ (R = Et, Bz) (*296*), and $WS(S_2)(S_2CNR_2)_2$ (*297*). More recently, $(PPh_4)_2[WOS(NCS)_4]$ (*298*) and a number of interesting phosphine complexes, such as *trans*-$WS_2(PMe_3)_4$ and $WS_2(PMe_3)_2(\eta^2\text{-OCHR})$, have been reported (*299*). Organometallic thio-tungsten complexes include species such as $NEt_4[WS_3(CH_2CMe_3)]$ (*300*) and Cp*WOSR, Cp*$WS(S_2)$R, and Cp*WS_2R (R = Me, CH_2SiMe_3) (*182, 301*). Few of these are directly relevant to enzyme systems, but they may one day provide important spectroscopic benchmarks for enzyme studies.

Dinucleation is also common in tungsten chemistry and must be prevented by the use of mononucleating ligands (cf. Sections IV, V). In this regard and with the aim of accurately modeling proposed active sites such as **55**, the tungsten chemistry of L-N_3 has been explored. A series of (L-N_3)$W^{VI}O_2X$, (L-N_3)$W^{V}OXY$, and (L-N_3)$W^{IV}OXY$ (X = halide, SR, SeR) complexes have been synthesized and characterized by Eagle and Young (*302, 303*). Reaction of (L-N_3)WO_2X complexes with boron sulfide produces (L-N_3)WOSX, (L-N_3)WS_2X, and various thio-W(V) complexes, (L-N_3)WSXY (depending upon solvent, etc.). The thio complexes are remarkably stable in contrast to thio-Mo complexes. The thorough investigation of these complexes should provide important spectroscopic and electrochemical data for comparison with enzyme results. Development of the chemistry of these complexes is important in view of the proposed —SH coordination in a tungsten enzyme (Section VIII.C) and the relationship of C-locant OAT reactions (Eq. (28) and (29)) to the chemistry of the molybdenum hydroxylases (which contain oxo-thio-Mo centers).

Significantly, molecules that accurately model structure **55** are accessible and should prove to be valuable spectroscopic and perhaps chemical models. Reaction of (L-N_3)WS_2Cl with SeR^- leads to the formation of (L-N_3)WS_2(SeR) complexes, which undergo reaction with substituted acetylenes to form the dithiolene complexes shown in **56** (*303*). A number of quinoxylyl acetylenes have been employed in these reactions. Interestingly, the complexes are highly air sensitive and decompose to give (L-N_3)WO_2(SeR) and **13**, a compound observed by Burgmayer (*55*) to form upon aerial oxidation of quinoxylyl dithiolene complexes of molybdenum. This compound is closely related to urothione (**7**). The modeling of the tungsten enzymes and the exploration of related tung-



56

sten chemistry promises to be a new and exciting area of bioinorganic chemistry.

IX. Future Directions

The biological importance of molybdenum was a major stimulus in the rapid development of the coordination and bioinorganic chemistry of molybdenum in the 1980s (*7*). Spectroscopic and reactivity models for pterin-containing enzymes that catalyze Eq. (1) have been reviewed above, but the detailed structure of Mo-co and its relationship to the surrounding protein and to other prosthetic groups remain an enigma because there is as yet no crystal structure available for *any* pterin-containing molybdenum or tungsten enzyme. Both sulfite oxidase (*304*) and xanthine oxidase (*305*) have been crystallized, but in neither case are the crystals yet suitable for X-ray structure determination. Hyperthermophilic organisms possess unusually stable proteins, and it is possible that the novel tungsten enzymes from such organisms may provide the first definitive X-ray structural information about the metal sites of pterin-containing enzymes that catalyze Eq. (1). An X-ray crystal structure of a pterin-containing enzyme will immediately provide new challenges to the synthetic bioinorganic chemist to replicate the chemical and structural features of Mo-co. A crystal structure will also be valuable for interpreting the spectroscopic data presently available for the enzymes.

Increasing numbers of pterin-containing molybdenum and tungsten enzymes are being discovered in bacteria. Many are not yet fully characterized, and it is often not known whether additional prosthetic groups

are also present. The discovery that DMSO reductase from *R. sphaeroides* forma specialis *denitrificans* (*71*) and from *R. capsulatus* (*72*) contains only a molybdopterin cofactor has opened up new possibilities for investigation of the molybdenum centers of enzymes without interference from other prosthetic groups. Bacterial enzymes also offer the hope of using molecular biological techniques to produce the enzymes in quantity, to probe and modify the molybdenum center and its surroundings, and to isotopically label the molybdenum center.

The unexpected discovery that several hyperthermophilic organisms possess pterin-containing tungsten enzymes promises to stimulate the development of oxo-thio-tungsten chemistry in the 1990s, as occurred for molybdenum chemistry in the 1980s. The coming years should be exciting and fruitful ones for the bioinorganic chemistry of pterin-containing molybdenum and tungsten enzymes and their models.

X. Abbreviations

16-ane[S_4]	1,5,9,13-Tetrathiacyclohexadecane
acac	2,4-Pentanedionate (acetylacetonate)
CD	Circular dichroism
CEPT	Coupled electron–proton transfer
Cp	η^5-Cyclopentadienyl
Cp*	η^5-Pentamethylcyclopentadienyl
dmf	*N, N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dppe	1,2-Bis(diphenylphosphino)ethane
ENDOR	Electron nuclear double resonance
EPR	Electron paramagnetic resonance
EXAFS	Extended X-ray absorption fine structure
FAD	Flavin adenine dinucleotide
H_2dmp	6,7-Dimethyldihydropterin
H_4dmp	6,7-Dimethyl-5,6,7,8-tetrahydropterin
HOMO	Highest occupied molecular orbital
L-N_2O_2	See Fig. 7
L-N_3	See Fig. 7
L-NS	See Fig. 7
L-NS_2	See Fig. 7
L-SO	See Fig. 7
L^1-N_2S_2	See Fig. 7
L^2-N_2S_2	See Fig. 7
L^3-$N_2H_2S_2$	See Fig. 7
L^3-N_2S_2	See Fig. 7
L^{3m}-N_2S_2	See Fig. 7

L^4-N_2S_2	See Fig. 7
MCD	Magnetic circular dichroism
Mo-co	Molybdenum cofactor
NMI	*N*-Methylimidazole
NMR	Nuclear magnetic resonance
OAT	Oxyen atom transfer
PES	Photoelectron spectroscopy
py	Pyridine
SHE	Standard hydrogen electrode
tdpm	Thiodipivaloylmethanide anion
tdt	Toluene-3,4-dithiolate
thf	Tetrahydrofuran
tmeda	Tetramethylethylenediamine
TPP	Tetraphenylporphyrin
TTP	Tetratolylporphyrin
XAS	X-ray absorption spectroscopy

ACKNOWLEDGMENTS

We acknowledge many years of stimulating collaborative research interactions with Professors J. T. Spence and A. G. Wedd, and we thank them for numerous helpful discussions during the preparation of this chapter. We thank Drs. D. M. Baird, R. C. Bray, S. J. N. Burgmayer, C. D. Garner, A. Gewirth, R. Hille, R. H. Holm, M. Johnson, U. Küsthardt, O. Meyer, A. Nakamura, R. S. Pilato, K. V. Rajagopalan, D. M. Sabel, F. A. Schultz, E. I. Stiefel, and R. K. Thauer for supplying reprints and preprints of research from their laboratories. We are greatly indebted to D. Myers for the drawings and for the preparation of the manuscript, and we thank Dr. M. A. Bruck for technical assistance with the computer graphics. JHE thanks the National Institutes of Health (Grant GM-37773) for continuing support of his research program on molybdenum enzymes. He gratefully acknowledges a Fulbright Senior Scholar Award, a Senior Alexander von Humboldt Award, and the generous hospitality of Professor W. A. Herrmann and Dr. U. Küsthardt of the Technical University of Munich, where portions of this manuscript were written. CGY thanks the Australian Research Council for support of his research in this area and acknowledges a DEET Collaborative Scientific Exchange Program Grant, which assisted in the preparation of this manuscript.

REFERENCES

1. Coughlan, M. P., ed., "Molybdenum and Molybdenum-Containing Enzymes." Pergamon, Oxford, 1980.
2. Newton, W. E., and Otsuka, S., eds., "Molybdenum Chemistry of Biological Significance." Plenum, New York, 1980.
3. Spiro, T. G., ed., "Molybdenum Enzymes." Wiley, New York, 1985.
4. Kim, J., and Rees, D. C., *Science* **257,** 1677 (1992).
5. Stiefel, E. I., Coucouvanis, D., and Newton, W. E., eds., "Molybdenum Enzymes, Cofactors and Model Systems," ACS Symp. Ser. 535. Am. Chem. Soc. Washington, D.C., 1993.

6. Bray, R. C., *in* "The Enzymes" (P. D. Boyer, ed.), 3rd ed., Vol. 12, Part B, p. 299. Academic Press, New York, 1975.
7. Holm, R. H., *Coord. Chem. Rev.* **100,** 183 (1990).
8. Cramer, S. P., Wahl, R., and Rajagopalan, K. V., *J. Am. Chem. Soc.* **103,** 7721 (1981).
9. Steifel, E. I., *Prog. Inorg. Chem.* **22,** 1 (1977).
10. Bray, R. C., *Adv. Enzymol. Relat. Areas Mol. Biol.* **51,** 107 (1980).
11. Spence, J. T., *Coord. Chem. Rev.* **48,** 59 (1983).
12. Colton, R., *Coord. Chem. Rev.* **62,** 145 (1985).
13. Burgmayer, S. J. N., and Stiefel, E. I., *J. Chem. Educ.* **62,** 943 (1985).
14. Garner, C. D., and Charnock, J. M., *Compre. Coord. Chem.* **3,** 1329 (1987).
15. Stiefel, E. I., *Compre. Coord. Chem.* **3,** 1375 (1987).
16. Garner, C. D., *Compre. Coord. Chem.* **3,** 1421 (1987).
17. Bray, R. C., *Q. Rev. Biophys.* **21,** 299 (1988).
18. Wootton, J. C., Nicolson, R. E., Cock, J. M., Walters, D. E., Burke, J. F., Doyle, W. A., and Bray, R. C., *Biochim. Biophys. Acta* **1057,** 157 (1991).
19. Rajagopalan, K. V., *Adv. Enzymol. Relat. Areas Mol. Biol.* **64,** 215 (1991).
20. Nason, A., Antoine, A. D., Ketchum, P. A., Frazier, W. A., III, and Lee, D. K., *Proc. Natl. Acad. Sci. U.S.A.* **65,** 137 (1970).
21. Ketchum, P. A., Cambier, H. Y., Frazier, W. A., III, Madansky, C. H., and Nason, A., *Proc. Natl. Acad. Sci. U.S.A.* **66,** 1016 (1970).
22. Nason, A., Lee, K.-Y., Pan, S.-S. Ketchum, P. A., Lamberti, A., and DeVries, J., *Proc. Natl. Acad. Sci. U.S.A.* **68,** 3242 (1971).
23. Nason, A., Lee, K.-Y., Pan, S.-S., and Ericson, R. H., *J. Less-Common Met.* **36,** 449 (1974).
24. Johnson, J. L., Hainline, B. E., and Rajagopalan, K. V., *J. Biol. Chem.* **255,** 1783 (1980).
25. Johnson, J. L., and Rajagopalan, K. V., *Proc. Natl. Acad. Sci. U.S.A.* **79,** 6856 (1982).
26. Johnson, J. L., Hainline, B. E., Rajagopalan, K. V., and Arison, B. H., *J. Biol. Chem.* **259,** 5414 (1984).
27. McCleverty, J. A., *Prog. Inorg. Chem.* **10,** 49 (1968).
28. Eisenberg, R., *Prog. Inorg. Chem.* **12,** 295 (1970).
29. Deistung, J., and Bray, R. C., *Biochem. J.* **263,** 477 (1989).
30. Hawkes, T. R., and Bray, R. C., *Biochem. J.* **219,** 481 (1984).
31. Hawkes, T. R., and Bray, R. C., *Biochem. J.* **222,** 587 (1984).
32. van Spanning, R. J. M., Wansell-Bettenhaussen, C. W., Oltmann, L. F., and Stouthamer, A. H., *Eur. J. Biochem.* **169,** 349 (1987).
33. Johnson, J. L., Bastian, N. R., and Rajagopalan, K. V., *Proc. Natl. Acad. Sci. U.S.A.* **87,** 3190 (1990).
34. Kramer, S. P., Johnson, J. L., Ribeiro, A. A., Millington, D. S., and Rajagopalan, K. V., *J. Biol. Chem.* **262,** 16357 (1987).
35. Gardlik, S., and Rajagopalan, K. V., *J. Biol. Chem.* **265,** 13047 (1990).
36. Gardlik, S., and Rajagopalan, K. V., *J. Biol. Chem.* **266,** 4889 (1991).
37. Curtius, H.-C., Heintel, D., Ghisla, S., Kuster, T., Leimbacher, W., and Niederwieser, A., *J. Inherited Metab. Dis.* **8,** Suppl. 1, 28 (1985).
38. Edmonson, D. E., and D'Ardenne, S. C., *Biochemistry* **28,** 5924 (1989).
39. Howes, B. D., Pinhal, N. M., Turner, N. A., Bray, R. C., Anger, G., Ehrenberg, A., Raynor, J. B., and Lowe, D. J., *Biochemistry* **29,** 6120 (1990).
40. Gruber, S., Kilpatrick, L., Bastian, N. R., Rajagopalan, K. V., and Spiro, T. G., *J. Am. Chem. Soc.* **112,** 8179 (1990).
41. Karrasch, M., Börner, G., Enssle, M., and Thauer, R. K., *Eur. J. Biochem.* **194,** 367 (1990).

42. Johnson, J. L., Rajagopalan, K. V., and Meyer, O., *Arch. Biochem. Biophys.* **283,** 542 (1990).
43. Börner, G., Karrasch, M., and Thauer, R. K., *FEBS Lett.* **290,** 31 (1991).
44. Krüger, B., and Meyer, O., *Eur. J. Biochem.* **157,** 121 (1986); Meyer, O., Frunzke, K., Tachil, J., and Volk, M., in Ref. 5, p 50.
45. Rajagopalan, K. V., private communication.
46. Kräutler, B., *Chimia* **41,** 277 (1987).
47. Taylor, E. C., and Reiter, L. A., *J. Am. Chem. Soc.* **111,** 285 (1989).
48. Wadman, S. K., Duran, M., Beemer, F. A., Cats, B. P., Johnson, J. L., Rajagopalan, K. V., Saudubray, J. M., Ogier, H., Charpentier, C., Berger, R., Smit, G. P. A., Wilson, J., and Krywawych, S., *J. Inherited Metab. Dis.* **6,** Suppl. 1, 78 (1983).
49. Taylor, E. C., Ray, P. S., Darwish, I. S., Johnson, J. L., and Rajagopalan, K. V., *J. Am. Chem. Soc.* **111,** 7664 (1989).
50. Larsen, L., Rowe, D. J., Garner, C. D., and Joule, J. A., *Tetrahedron Lett.* **29,** 1453 (1988).
51. Larsen, L., Rowe, D. J., Garner, C. D., and Joule, J. A., *J. Chem. Soc., Perkin Trans. 1,* p. 2317 (1989).
52. Russell, J. R., Garner, C. D., and Joule, J. A., *Tetrahedron Lett.* **33,** 3371 (1992).
53. Russell, J. R., Garner, C. D., and Joule, J. A., *J. Chem. Soc., Perkin Trans. 1* p. 1245 (1992).
54. Pilato, R. S., Eriksen, K. A., Greaney, M. A., Stiefel, E. I., Goswami, S., Kilpatrick, L., Spiro, T. G., Taylor, E. C., and Rheingold, A. L., *J. Am. Chem. Soc.* **113,** 9372 (1991).
55. Soricelli, C. L., Szalai, V. A., and Burgmayer, S. J. N., *J. Am. Chem. Soc.* **113,** 9877 (1991).
56. Goodgame, M., and Schmidt, M. A., *Inorg. Chim. Acta* **36,** 151 (1979).
57. Burgmayer, S. J. N., and Stiefel, E. I., *J. Am. Chem. Soc.* **108,** 8310 (1986).
58. Burgmayer, S. J. N., and Stiefel, E. I., *Inorg. Chem.* **27,** 4059 (1988).
59. Kohzuma, T., Masuda, H., and Yamauchi, O., *J. Am. Chem. Soc.* **111,** 3431 (1989).
60. Burgmayer, S. J. N., Baruch, A., Kerr, K., and Yoon, K., *J. Am. Chem. Soc.* **111,** 4982 (1989).
61. Abelleira, A., Galang, R. D., and Clarke, M. J., *Inorg. Chem.* **29,** 633 (1990).
62. Fischer, B., Strähle, J., and Viscontini, M., *Helv. Chim. Acta* **74,** 1544 (1991).
63. Burgmayer, S. J. N., Everett, K., and Bostick, L., in Ref. 5, p 114.
64. Bray, R. C., *Biol. Magn. Reson.* **2,** 45 (1980).
65. Bray, R. C., Malmström, B. G., and Vänngård, T., *Biochem. J.* **73,** 193 (1959).
66. Meriwether, L. S., Marzluff, W. F., and Hodgson, W. G., *Nature (London)* **212,** 465 (1966).
67. Howes, B. D., Bennett, B., Koppenhöfer, A., Lowe, D. J., and Bray, R. C., *Biochemistry* **30,** 3969 (1991).
68. Cramer, S. P., *Chem. Anal. (N.Y.)* **92,** 257 (1988); Cramer, S. P., *Adv. Inorg. Bioinorg. Mech.* **2,** 259 (1983).
69. George, G. N., Kipke, C. A., Prince, R. C., Sunde, R. A., Enemark, J. H., and Cramer, S. P., *Biochemistry* **28,** 5075 (1989).
70. George, G. N., Cleland, W. E., Jr., Enemark, J. H., Smith, B. E., Kipke, C. A., Roberts, S. A., and Cramer, S. P., *J. Am. Chem. Soc.* **112,** 2541 (1990).
71. Bastian, N. R., Kay, C. J., Barber, M. J., and Rajagopalan, K. V., *J. Biol. Chem.* **266,** 45 (1991).
72. McEwan, A. G., Ferguson, S. J., and Jackson, J. B., *Biochem. J.* **274,** 305 (1991).
73. Spiro, T. G., ed., "Biological Applications of Raman Spectroscopy," Vol. 3. Wiley, New York, 1988.
74. Backes, G., Enemark, J. H., and Loehr, T. M., *Inorg. Chem.* **30,** 1839 (1991).

75. Mohammed, A. K., and Maverick, A. W., *Inorg. Chem.* **31,** 4441 (1992).
76. Spence, J. T., Barber, M. J., and Solomonson, L. P., *Biochem. J.* **250,** 921 (1988).
77. Spence, J. T., Barber, M. J., and Siegel, L. M., *Biochemistry* **21,** 1656 (1982).
78. Spence, J. T., Kipke, C. A., Enemark, J. H., and Sunde, R. H., *Inorg. Chem.* **30,** 3011 (1991).
79. Davis, M. D., Edmondson, D. E., and Müller, F., *Eur. J. Biochem.* **145,** 237 (1984).
80. Küsthardt, U., and Enemark, J. H., *J. Am. Chem. Soc.* **109,** 7926 (1987).
81. Küsthardt, U., LaBarre, M. J., and Enemark, J. H., *Inorg. Chem.* **29,** 3182 (1990).
82. Minelli, M., Enemark, J. H., Brownlee, R. T. C., O'Connor, M. J., and Wedd, A. G., *Coord. Chem. Rev.* **68,** 169 (1985).
83. Peterson, J., Godfrey, C., Thomson, A. J., George, G. N., and Bray, R. C., *Biochem. J.* **233,** 107 (1986).
84. Benson, N., Farrar, J. A., McEwan, A. G., and Thomson, A. J., *FEBS Lett.* **307,** 169 (1992).
85. Finnegan, M. G., Hilton, J., Rajagopalan, K. V., and Johnson, M. K., *Inorg. Chem.* **32,** 2616 (1993).
86. Lowe, D. J., Barber, M. J., Pawlik, R. T., and Bray, R. C., *Biochem. J.* **155,** 81 (1976).
87. Sabel, D. M., and Gewirth, A. A., *Inorg. Chem.* (submitted for publication).
88. Carducci, M. D., Enemark, J. H., and Solomon, E. I., *Abstr. 203rd Nat. Meet., Am. Chem. Soc.*, San Francisco, *1992,* INOR-513 (1992).
89. Kessler, D. L., and Rajagopalan, K. V., *J. Biol. Chem.* **247,** 6566 (1972).
90. Kessler, D. L., and Rajagopalan, K. V., *Biochim. Biophys. Acta* **370,** 389 (1974).
91. Cleland, W. E., Jr., Barnhart, K. M., Yamanouchi, K., Collison, D., Mabbs, F. E., Ortega, R. B., and Enemark, J. H., *Inorg. Chem.* **26,** 1017 (1987).
92. George, G. N., *J. Magn. Reson.* **64,** 384 (1985).
93. Lamy, M. T., Gutteridge, S., and Bray, R. C., *Biochem. J.* **185,** 397 (1980).
94. George, G. N., Prince, R. C., Kipke, C. A., Sunde, R. A., and Enemark, J. H., *Biochem. J.* **256,** 307 (1988).
95. Hinshaw, C. J., Peng, G., Singh, R., Spence, J. T., Enemark, J. H., Bruck, M., Kristofzski, J., Merbs, S. L., Ortega, R. B., and Wexler, P. A., *Inorg. Chem.* **28,** 4483, and references therein (1989).
96. Yoshinaga, N., Ueyama, N., Okamura, T., and Nakamura, A., *Chem. Lett.* p. 1655 (1990).
97. Stiefel, E. I., Miller, K. F., Bruce, A. E., Corbin, J. L., Berg, J. M., and Hodgson, K. O., *J. Am. Chem. Soc.* **102,** 3624 (1980).
98. Berg, J. M., Spira, D. J., Hodgson, K. O., Bruce, A. E., Miller, K. F., Corbin, J. L., and Stiefel, E. I., *Inorg. Chem.* **23,** 3412 (1984).
99. Bordas, J., Bray, R. C., Garner, C. D., Gutteridge, S., and Hasnain, S. S., *J. Inorg. Biochem.* **11,** 181 (1979).
100. Bordas, J., Bray, R. C., Garner, C. D., Gutteridge, S., and Hasnain, S. S., *Biochem. J.* **191,** 499 (1980).
101. Cramer, S. P., and Hille, R., *J. Am. Chem. Soc.* **107,** 8164 (1985).
102. Turner, N. A., Bray, R. C., and Diakun, G. P., *Biochem. J.* **260,** 563 (1989).
103. Williams, R. J. P., and Wentworth, R. A. D., *Chem. Uses Molybdenum, Proc. Int. Conf., 1st, 1973,* p. 212 (1973).
104. Eagle, A. A., Laughlin, L. J., Young, C. G., and Tiekink, E. R. T., *J. Am. Chem. Soc.* **114,** 9195 (1992).
105. Gutteridge, S., and Bray, R. C., *Biochem. J.* **189,** 615 (1980).
106. George, G. N., and Bray, R. C., *Biochemistry* **27,** 3603 (1988).
107. Dowerah, D., Spence, J. T., Singh, R., Wedd, A. G., Wilson, G. L., Farchione, F., Enemark, J. H., Kristofzski, J. G., and Bruck, M., *J. Am. Chem. Soc.* **109,** 5655 (1987).

108. Wilson, G. L., Greenwood, R. J., Pilbrow, J. R., Spence, J. T., and Wedd, A. G., *J. Am. Chem. Soc.* **113,** 6803 (1991).
109. Greenwood, R. J., Wilson, G. J., Pilbrow, J. R., and Wedd, A. G., *J. Am. Chem. Soc.* **115,** 5385 (1993).
110. Young, C. G., Enemark, J. H., Collison, D., and Mabbs, F. E., *Inorg. Chem.* **26,** 2925 (1987).
111. Farchione, F., Hanson, G. R., Rodrigues, C. G., Bailey, T. D., Bagchi, R. N., Bond, A. M., Pilbrow, J. R., and Wedd, A. G., *J. Am. Chem. Soc.* **108,** 831 (1986).
112. Hinshaw, C. J., and Spence, J. T., *Inorg. Chem. Acta* **125,** L17 (1986).
113. Wilson, G. L., Kony, M., Tiekink, E. R. T., Pilbrow, J. R., Spence, J. T., and Wedd, A. G., *J. Am. Chem. Soc.* **110,** 6923 (1988).
114. Ibers, J. A., and Holm, R. H., *Science* **209,** 223 (1980).
115. Garner, C. D., and Bristow, S., *in* "Molybdenum Enzymes" (T. G. Spiro, ed.), p. 343. Wiley, New York, 1985.
116. Berg, J. M., Hodgson, K. O., Cramer, S. P., Corbin, J. L., Elsberry, A., Pariyadath, N., and Stiefel, E. I., *J. Am. Chem. Soc.* **101,** 2774 (1979).
117. Cramer, S. P., Gray, H. B., and Rajagopalan, K. V., *J. Am. Chem. Soc.* **101,** 2772 (1979).
118. Corbin, J. L., and Work, D. E., *Can. J. Chem.* **52,** 1054 (1974).
119. Kim, N., Kim, S., Vella, P. A., and Zubieta, J., *Inorg. Nucl. Chem. Lett.* **14,** 457 (1978).
120. Gardner, J. K., Pariyadath, N., Corbin, J. L., and Stiefel, E. I., *Inorg. Chem.* **17,** 897 (1978).
121. Pickett, C., Kumar, S., Vella, P. A., and Zubieta, J., *Inorg. Chem.* **21,** 908 (1982).
122. Bruce, A., Corbin, J. L., Dahlstrom, P. L., Hyde, J. R., Minelli, M., Stiefel, E. I., Spence, J. T., and Zubieta, J., *Inorg. Chem.* **21,** 917 (1982).
123. Subramanian, P., Kaul, B., and Spence, J. T., *J. Mol. Catal.* **23,** 163 (1984).
124. Kaul, B. B., Enemark, J. H., Merbs, S. L., and Spence, J. T., *J. Am. Chem. Soc.* **107,** 2885 (1985).
125. Trofimenko, S., *J. Am. Chem. Soc.* **89,** 6288 (1967).
126. Roberts, S. A., Young, C. G., Cleland, W. E., Jr., Ortega, R. B., and Enemark, J. H., *Inorg. Chem.* **27,** 3044 (1988).
127. Roberts, S. A., Young, C. G., Kipke, C. A., Cleland, W. E., Jr., Yamanouchi, K., Carducci, M. D., and Enemark, J. H., *Inorg. Chem.* **29,** 3650 (1990).
128. Xiao, Z., Young, C. G., Enemark, J. H., and Wedd, A. G., *J. Am. Chem. Soc.* **114,** 9194 (1992).
129. Berg, J. M., and Holm, R. H., *J. Am. Chem. Soc.* **107,** 917 (1985).
130. Berg, J. M., and Holm, R. H., *J. Am. Chem. Soc.* **107,** 925 (1985).
131. Gheller, S. F., Schultz, B. E., Scott, M. J., and Holm, R. H., *J. Am. Chem. Soc.* **114,** 6934 (1992); Schultz, B. E., Gheller, S. F., Muetterties, M. C., Scott, M. J., and Holm, R. H., *J. Am. Chem. Soc.* **115,** 2714 (1993).
132. Palanca, P., Picher, T., Sanz, V., Gómez-Romero, P., Llopis, E., Domenech, A., and Cervilla, A., *J. Chem. Soc., Chem. Commun.*, p. 531 (1990).
133. Sanz, V., Picher, T., Palanca, P., Gómez-Romero, P., Llopis, E., Ramirez, J. A., Beltrán, D., and Cervilla, A., *Inorg. Chem.* **30,** 3113 (1991).
134. Llopis, E., Doménech, A., Ramirez, J. A., Cervilla, A., Palanca, P., Picher, T., and Sanz, V., *Inorg. Chim. Acta* **189,** 29 (1991).
135. Purohit, S., Koley, A. P., Prasad, L. S., Manoharan, P. T., and Ghosh, S., *Inorg. Chem.* **28,** 3735 (1989).
136. Rajan, O. A., and Chakravorty, A., *Inorg. Chem.* **20,** 660 (1981).
137. Topich, J., and Lyon, J. T., III, *Inorg. Chim. Acta* **80,** L41 (1983).
138. Topich, J., and Lyon, J. T., III, *Polyhedron* **3,** 55 (1984).

139. Topich, J., and Lyon, J. T., III, *Polyhedron* **3,** 61 (1984).
140. Topich, J., and Lyon, J. T., III, *Inorg. Chem.* **23,** 3202 (1984).
141. Craig, J. A., Harlan, E. W., Snyder, B. S., Whitener, M. A., and Holm, R. H., *Inorg. Chem.* **28,** 2082 (1989).
142. Nicholas, K. M., and Khan, M. A., *Inorg. Chem.* **26,** 1633 (1987).
143. Boorman, P. M., Garner, C. D., Mabbs, F. E., and King, T. J., *J. Chem. Soc., Chem. Commun.*, p. 663 (1974).
144. Boorman, P. M., Garner, C. D., and Mabbs, F. E., *J. Chem. Soc., Dalton Trans.* 1299 (1974).
145. Yamanouchi, K., and Enemark, J. H., *Inorg. Chem.* **18,** 1626 (1979).
146. Yamanouchi, K., Huneke, J. T., Enemark, J. H., Taylor, R. D., and Spence, J. T., *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **B35,** 2326 (1979).
147. Yamanouchi, K., Yamada, S., and Enemark, J. H., *Inorg. Chim. Acta* **85,** 129 (1984).
148. Spence, J. T., Minelli, M., and Kroneck, P., *J. Am. Chem. Soc.* **102,** 4538 (1980).
149. Wedd, A. G., and Spence, J. T., *Pure Appl. Chem.* **62,** 1055 (1990).
150. Garner, C. D., and Mabbs, F. E., *J. Inorg. Nucl. Chem.* **41,** 1125 (1979).
151. Chang, C. S. J., Collison, D., Mabbs, F. E., and Enemark, J. H., *Inorg. Chem.* **29,** 2261 (1990).
152. Chang, C. S. J., Rai-Chaudhuri, A., Lichtenberger, D. L., and Enemark, J. H., *Polyhedron* **9,** 1965 (1990).
153. Chang, C. S. J., and Enemark, J. H., *Inorg. Chem.* **30,** 683 (1991).
154. Solomon, I., *Phys. Rev.* **99,** 559 (1955).
155. Boyd, I. W., Dance, I. G., Murray, K. S., and Wedd, A. G., *Aust. J. Chem.* **31,** 279 (1978).
156. Bradbury, J. R., Mackay, M. F., and Wedd, A. G., *Aust. J. Chem.* **31,** 2423 (1978).
157. Burt, R. J., Dilworth, J. R., Leigh, G. J., and Zubieta, J. A., *J. Chem. Soc., Dalton Trans.*, p. 2295 (1982).
158. Ellis, S. R., Collison, D., Garner, C. D., and Clegg, W., *J. Chem. Soc., Chem. Commun.*, p. 1483 (1986).
159. Boyde, S., Ellis, S. R., Garner, C. D., and Clegg, W., *J. Chem. Soc., Chem. Commun.*, p. 1541 (1986).
160. Ueyama, N., Zaima, H., and Nakamura, A., *Chem. Lett.* p. 1099 (1986).
161. Ellis, S. R., Collison, D., and Garner, C. D., *J. Chem. Soc., Dalton Trans.*, p. 413 (1989).
162. Ueyama, N., Yoshinaga, N., Kajiwara, A., and Nakamura, A., *Chem. Lett.*, p. 1781 (1990).
163. Ueyama, N., Yoshinaga, N., Kajiwara, A., Nakamura, A., and Kusunoki, M., *Bull. Chem. Soc. Jpn.* **64,** 2458 (1991).
164. Ueyama, N., Okamura, T., and Nakamura, A., *J. Am. Chem. Soc.* **114,** 8129 (1992).
165. Soong, S.-L., Chebolu, V., Koch, S. A., O'Sullivan, T., and Millar, M., *Inorg. Chem.* **25,** 4067 (1986).
166. Subramanian, P., Spence, J. T., Ortega, R., and Enemark, J. H., *Inorg. Chem.* **23,** 2564 (1984).
167. Martin, J. F., and Spence, J. T., *J. Phys. Chem.* **74,** 3589 (1970).
168. Rajan, O. A., Spence, J. T., Leman, C., Minelli, M., Sato, M., Enemark, J. H., Kroneck, P. M. H., and Sulgar, K., *Inorg. Chem.* **22,** 3065 (1983).
169. Young, C. G., Roberts, S. A., Ortega, R. B., and Enemark, J. H., *J. Am. Chem. Soc.* **109,** 2938 (1987).
170. Bruck, M. A., and Enemark, J. H., unpublished results.
171. Wedd, A. G., *in* "Sulfur, its Significance for Chemistry, for the Geo-, Bio- and Cosmosphere and Technology" (A. Müller and B. Krebs, eds.), p. 181. Elsevier, Amsterdam, 1984.

172. Pan, W.-H., Harmer, M. A., Halbert, T. R., and Stiefel, E. I., *J. Am. Chem. Soc.* **106,** 459 (1984).
173. Coucouvanis, D., Hadjikyriacou, A., Toupadakis, A., Koo, S.-M., Ileperuma, O., Draganjac, M., and Salifoglou, A., *Inorg. Chem.* **30,** 754, and references therein (1991).
174. Diemann, E., and Müller, A., *Coord. Chem. Rev.* **10,** 79 (1973).
175. Müller, A., Diemann, E., Jostes, R., and Bögge, H., *Angew. Chem., Int. Ed. Engl.* **20,** 934 (1981).
176. Hofer, E., Holzbach, W., and Wieghardt, K., *Angew. Chem., Int. Ed. Engl.* **20,** 282 (1981).
177. Wieghardt, K., Hahn, M., Weiss, J., and Swiridoff, W., *Z. Anorg. Allg. Chem.* **492,** 164 (1982).
178. Bristow, S., Garner, C. D., and Clegg, W., *Inorg. Chim. Acta* **76,** L261 (1983).
179. Bristow, S., Collison, D., Garner, C. D., and Clegg, W., *J. Chem. Soc., Dalton Trans.*, p. 2495 (1983).
180. Bristow, S., Garner, C. D., and Pickett, C. J., *J. Chem. Soc., Dalton Trans.*, p. 1617 (1984).
181. Traill, P. R., Tiekink, E. R. T., O'Connor, M. J., Snow, M. R., and Wedd, A. G., *Aust. J. Chem.* **39,** 1287, and references therein (1986).
182. Faller, J. W., and Ma, Y., *Organometallics* **8,** 609 (1989).
183. Yan, X. Y., and Young, C. G., *Aust. J. Chem.* **44,** 361 (1991).
184. Massey, V., and Edmondson, D., *J. Biol. Chem.* **245,** 6595 (1970).
185. Singh, R., Spence, J. T., George, G. N., and Cramer, S. P., *Inorg. Chem.* **28,** 8 (1989).
186. Xiao, Z., Enemark, J. H., Wedd, A. G., and Young, C. G., unpublished work.
187. Kamenar, B., Korpar-Čolig, B., Cindrić, M., Penavić, M., and Strukan, N., *J. Chem. Soc., Dalton Trans.*, p. 2093 (1992).
188. DeSimone, R. E., and Glick, M. D., *Inorg. Chem.* **17,** 3574 (1978).
189. Hille, R., and Sprecher, H., *J. Biol. Chem.* **262,** 10914 (1987).
190. Stiefel, E. I., *Proc. Natl. Acad. Sci. U.S.A.* **70,** 988 (1973).
191. Williams, R. J. P., *Biochem. Soc. Trans.* 1, 1 (1973).
192. Garner, C. D., Hyde, M. R., Mabbs, F. E., and Routledge, V. I., *Nature (London)* **252,** 579 (1974).
193. Wentworth, R. A. D., *Coord. Chem. Rev.* **18,** 1 (1976).
194. Hewitt, E. J., Notton, B. A., and Garner, C. D., *Biochem. Soc. Trans.* **7,** 629 (1979).
195. Holm, R. H., *Chem. Rev.* **87,** 1401 (1987).
196. Barral, R., Bocard, I., Sérée de Roch, I., and Sajus, L., *Tetrahedron Lett.* p. 1693 (1972).
197. Barral, R., Bocard, I., Sérée de Roch, I., and Sajus, L., *Kinet. Catal. (Engl. Trans.)* **14,** 130 (1973).
198. Newton, W. E., Corbin, J. L., Bravard, D. C., Searles, J. E., and McDonald, J. W., *Inorg. Chem.* **13,** 1100 (1974).
199. Ricard, L., Estienne, J., Karagiannidis, P., Toledano, P., Fischer, J., Mitschler, A., and Weiss, R., *J. Coord. Chem.* **3,** 277 (1974).
200. Mitchell, P. C. H., and Scarle, R. D., *J. Chem. Soc., Dalton Trans.*, p. 2552 (1975).
201. Chen, G. J.-J., McDonald, J. W., and Newton, W. E., *Inorg. Chem.* **15,** 2612 (1976).
202. Durant, R., Garner, C. D., Hyde, M. R., and Mabbs, F. E., *J. Chem. Soc., Dalton Trans.*, p. 955 (1977).
203. Berg, J. M., and Hodgson, K. O., *Inorg. Chem.* **19,** 2180 (1980).
204. Reynolds, M. S., Berg, J. M., and Holm, R. H., *Inorg. Chem.* **23,** 3057 (1984).
205. Laughlin, L. J., and Young, C. G., unpublished results.
206. Berg, J. M., and Holm, R. H., *J. Am. Chem. Soc.* **106,** 3035 (1984).
207. Harlen, E. W., Berg, J. M., and Holm, R. H., *J. Am. Chem. Soc.* **108,** 6992 (1986).

208. Caradonna, J. P., Harlan, E. W., and Holm, R. H., *J. Am. Chem. Soc.* **108,** 7856 (1986).
209. Caradonna, J. P., Reddy, P. R., and Holm, R. H., *J. Am. Chem. Soc.* **110,** 2139 (1988).
210. Craig, J. A., and Holm, R. H., *J. Am. Chem. Soc.* **111,** 2111 (1989).
211. Holm, R. H., and Berg, J. M., *Pure Appl. Chem.* **56,** 1645 (1984).
212. Holm, R. H., and Berg, J. M., *Acc. Chem. Res.* **19,** 363 (1986).
213. Boyd, I. W., and Spence, J. T., *Inorg. Chem.* **21,** 1602 (1982).
214. Baird, D. M., Falzone, S., and Haky, J. E., *Inorg. Chem.* **28,** 4561 (1989).
215. Bhattacharjee, S., and Bhattacharyya, R., *J. Chem. Soc., Dalton Trans.*, p. 1357 (1992).
216. Garner, C. D., Hyde, M. R., Mabbs, F. E., and Routledge, V. I., *J. Chem. Soc., Dalton Trans.*, p. 1180 (1975).
217. Hyde, M. R., and Garner, C. D., *J. Chem. Soc., Dalton Trans.*, p. 1186 (1975).
218. Taylor, R. D., Todd, P. G., Chasteen, N. D., and Spence, J. T., *Inorg. Chem.* **18,** 44 (1979).
219. Wieghardt, K., Woeste, M., Roy, P. S., and Chaudhuri, P., *J. Am. Chem. Soc.* **107,** 8276 (1985).
220. Murray, K. N., Watson, J. G., and Chaykin, S., *J. Biol. Chem.* **241,** 4798 (1966).
221. Taylor, R. D., Street, J. P., Minelli, M., and Spence, J. T., *Inorg. Chem.* **17,** 3207 (1978).
222. Topich, J., *Inorg. Chem.* **20,** 3704 (1981).
223. Spence, J. T., Hinshaw, C. C., Enemark, J. H., Merbs, S. L., and Ortega, R. B., *in* "Frontiers in Bioinorganic Chemistry" (A. V. Xavier, ed.), p. 54. VCH Verlagsges., Weinheim, 1986.
224. Mondal, J. U., Schultz, F. A., Brennan, T. D., and Scheidt, W. R., *Inorg. Chem.* **27,** 3950 (1988).
225. Mondal, J. U., and Schultz, F. A., *Inorg. Chim. Acta* **157,** 5 (1989).
226. Küsthardt, U., Albach, R. W., and Kiprof, P., *Inorg. Chem.* **32,** 1838 (1993).
227. Drew, M. G. B., Mitchell, P. C. H., and Pygall, C. F., *Angew. Chem., Int. Ed. Engl.* **15,** 784 (1976).
228. Wieghardt, K., Herrmann, W., Müller, A., Eltzner, W., and Zimmerman, M., *Z. Naturforsch. B: Anorg. Chem., Org. Chem.* **39B,** 876 (1984).
229. Müller, A., Jostes, R., Eltzner, W., Nie, C.-S., Diemann, E., Bögge, H., Zimmermann, M., Dartmann, M., Reinsch-Vogell, U., Che, S., Cyvin, S. J., and Cyvin, B. N., *Inorg. Chem.* **24,** 2872 (1985).
230. Müller, A., *Polyhedron* **5,** 323 (1986).
231. Newton, W. E., McDonald, J. W., Yamanouchi, K., and Enemark, J. H., *Inorg. Chem.* **18,** 1621 (1979).
232. Halbert, T. R., McGauley, K., Pan, W.-H., Czernuszewicz, R. S., and Stiefel, E. I., *J. Am. Chem. Soc.* **106,** 1849 (1984).
233. Rajagopalan, K. V., *Biochem. Elem.* **3,** 149 (1984).
234. McWhirter, R. B., and Hille, R., *J. Biol. Chem.* **266,** 23724 (1991).
235. Speier, G., *Inorg. Chim. Acta* **32,** 139 (1979).
236. Ueyama, N., Yano, M., Miyashita, H., Nakamura, A., Kamachi, M., and Nozakura, S., *J. Chem. Soc., Dalton Trans.*, p. 1447 (1984).
237. Young, C. G., and Wedd, A. G., *in* "Molybdenum Enzymes, Cofactors and Model Systems" (E. I. Stiefel, D. Coucouvanis, and W. E. Newton, eds.), ACS Symp. Ser., 535, p. 70. Am. Chem. Soc., Washington, D.C., 1993.
238. Bray, R. C., and George, G. N., *Biochem. Soc. Trans.* **13,** 560 (1985).
239. Oertling, W. A., and Hille, R., *J. Biol. Chem.* **265,** 17446 (1990).
240. Hille, R., and Stewart, R. C., *J. Biol. Chem.* **259,** 1570 (1984).
241. Davis, M. D., Olson, J. S., and Palmer, G., *J. Biol. Chem.* **259,** 3526 (1984).

242. Bray, R. C., Barber, M. J., and Lowe, D. J., *Biochem. J.* **171,** 653 (1978).
243. Olson, J. S., Ballou, D. P., Palmer, G., and Massey, V., *J. Biol. Chem.* **249,** 4363 (1974).
244. Palmer, G., and Olson, J. S., *in* "Molybdenum and Molybdenum-Containing Enzymes" (M. P. Coughlan, ed.), p. 187. Pergamon, Oxford, 1980.
245. Cammack, R., Barber, M. J., and Bray, R. C., *Biochem. J.* **157,** 469 (1976).
246. Barber, M. J., and Siegel, L. M., *Biochemistry* **21,** 1638 (1982).
247. Porras, A. G., and Palmer, G., *J. Biol. Chem.* **257,** 11617 (1982).
248. Barber, M. J., Salerno, J. C., and Siegel, L. M., *Biochemistry* **21,** 1648 (1982).
249. Bhattacharyya, A., Tollin, G., Davis, M., and Edmondson, D. E., *Biochemistry* **22,** 5270 (1983).
250. Hille, R., and Massey, V., *J. Biol. Chem.* **261,** 1241 (1986).
251. Anderson, R. F., Hille, R., and Massey, V., *J. Biol. Chem.* **261,** 15870 (1986).
252. Hille, R., and Anderson, R., *J. Biol. Chem.* **266,** 5608 (1991).
253. Hille, R., and Massey, V., *J. Biol. Chem.* **266,** 17401 (1991).
254. Garner, C. D., Buchanan, I., Collison, D., Mabbs, F. E., Porter, T. G., and Wynn, C. H., *Chem. Uses Molybdenum, Proc. Int. Conf., 4th, 1982,* p. 163 (1982).
255. Rajagopalan, K. V., *in* "Molybdenum and Molybdenum-Containing Enzymes' (M. P. Coughlan, ed.), p. 241. Pergamon, Oxford, 1980.
256. Kipke, C. A., Cusanovich, M. A., Tollin, G., Sunde, R. A., and Enemark, J. H., *Biochemistry* **27,** 2918 (1988).
257. Cohen, H. J., and Fridovich, I., *J. Biol. Chem.* **246,** 359 (1971).
258. Sullivan, E. P., Jr., Hazzard, J. T., Tollin, G., and Enemark, J. H., *J. Am. Chem. Soc.* **114,** 9662 (1992).
259. Saito, K., and Sasaki, Y., *Adv. Inorg. Bioinorg. Mech.* **1,** 179 (1982).
260. Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., and Dutton, P. L., *Nature (London)* **355,** 796 (1992).
261. LaBarre, M. J., Ph.D. Dissertation, University of Arizona, Tucson (1992).
262. Over, D. E., Critchlow, S. C., and Mayer, J. M., *Inorg. Chem.* **31,** 4643 (1992).
263. LaBarre, M. J., Raitsimring, A., and Enemark, J. H., *in* "Molybdenum Enzymes, Cofactors and Model Systems" (E. I. Stiefel, D. Coucouvanis, and W. E. Newton, eds.), ACS Symp. Ser., 535, p. 130. Am. Chem. Soc., Washington, D.C., 1993.
264. Walker, F. A., Reis, D., and Balke, V. L., *J. Am. Chem. Soc.* **106,** 6888 (1984).
265. Basu, P., Raitsimiring, A., and Enemark, J. H., unpublished results.
266. Fráusto da Silva, J. R. R., and Williams, R. J. P., "The Biological Chemistry of the Elements," p. 411. Oxford Univ. Press (Clarendon), London and New York, 1991.
267. Woese, C. R., Kandler, O., and Wheelis, M. L., *Proc. Natl. Acad. Sci. U.S.A.* **87,** 4576 (1990).
268. Spiess, F. N., Macdonald, K. C., Atwater, T., Ballard, R., Carranza, A., Cordoba, D., Cox, C., Diaz Garcia, V. M., Francheteau, J., Guerrero, J., Hawkins, J., Haymon, R., Hessler, R., Juteau, T., Kastner, M., Larson, R., Luyendyk, B., Macdougall, J. D., Miller, S., Normark, W., Orcutt, J., and Rangin, C., *Science* **207,** 1421 (1980).
269. Hekinian, R., Fevrier, M., Bischoff, J. L., Picot, P., and Shanks, W. C., *Science* **207,** 1433 (1980).
270. Andreesen, J. R., and Ljungdahl, L. G., *J. Bacteriol.* **116,** 867 (1973).
271. Fontaine, F. E., Peterson, W. H., McCoy, E., Johnson, M. J., and Ritter, G. R., *J. Bacteriol.* **43,** 701 (1942).
272. Yamamoto, I., Saiki, T., Liu, S.-M., and Ljungdahl, L. G., *J. Biol. Chem.* **258,** 1826 (1983).
273. Durfor, C. N., Wetherbee, P. J., Deaton, J. C., and Solomon, E. I., *Biochem. Biophys. Res. Commun.* **115,** 61 (1983).

274. Andreesen, J. R., Schaupp, A., Neurauter, C., Brown, A., and Ljungdahl, L. G., *J. Bacteriol.* **114,** 743 (1973).
275. Jones, J. B., and Stadtman, T. C., *J. Biol. Chem.* **256,** 656 (1981).
276. Enoch, H. G., and Lester, R. L., *J. Biol. Chem.* **250,** 6693 (1975).
277. Deaton, J. C., Solomon, E. I., Dunfor, C. N., Wetherbee, P. J., Burgess, B. K., and Jacobs, D. B., *Biochem. Biophys. Res. Commun.* **121,** 1042 (1984).
278. Cramer, S. P., Liu, C.-L., Mortenson, L. E., Spence, J. T., Liu, S-M., Yamamoto, I., and Ljungdahl, L. G., *J. Inorg. Biochem.* **23,** 119 (1985).
279. Deaton, J. C., Solomon, E. I., Watt, G. D., Wetherbee, P. J., and Durfor, C. N., *Biochem. Biophys. Res. Commun.* **149,** 424 (1987).
280. Bryant, F. O., and Adams, M. W. W., *J. Biol. Chem.* **264,** 5070 (1989).
281. Fiala, G., and Stetter, K. O., *Arch. Microbiol.* **145,** 56 (1986).
282. Mukund, S., and Adams, M. W. W., *J. Biol. Chem.* **266,** 14208 (1991).
283. Mukund, S., and Adams, M. W. W., *J. Biol. Chem.* **265,** 11508 (1990).
284. George, G. N., Prince, R. C., Mukund, S., and Adams, M. W. W., *J. Am. Chem. Soc.* **114,** 3521 (1992).
285. Schmitz, R. A., Richter, M., Linder, D., and Thauer, R. K., *Eur. J. Biochem.* **207,** 559 (1992).
286. DiMarco, A. A., Bobik, T. A., and Wolfe, R. S., *Annu. Rev. Biochem.* **59,** 355 (1990).
287. White, H., Strobl, G., Feicht, R., and Simon, H., *Eur. J. Biochem.* **184,** 89 (1989).
288. Juszczak, A., Aono, S., and Adams, M. W. W., *J. Biol. Chem.* **266,** 13834 (1991).
289. Dori, Z., *Prog. Inorg. Chem.* **28,** 239 (1981).
290. Dori, Z., *Compr. Coord. Chem.* **3,** 973 (1987).
291. Ueyama, N., Oku, H., and Nakamura, A., *J. Am. Chem. Soc.* **114,** 7310 (1992).
292. Sarkar, S., and Das, S. K., *Proc. Indian Acad. Sci.* **104,** 533 (1992).
293. Yu, S., and Holm, R. H., *Inorg. Chem.* **28,** 4385 (1989).
294. Lee, S., Staley, D. L., Rheingold, A. L., and Cooper, N. J., *Inorg. Chem.* **29,** 4391 (1990).
295. Atherton, M. J., and Holloway, J. H., *Adv. Inorg. Chem. Radiochem.* **22,** 171 (1979).
296. McDonell, A. C., Vasudevan, S. G., O'Connor, M. J., and Wedd, A. G., *Aust. J. Chem.* **38,** 1017 (1985).
297. Pan, W.-H., Halbert, T. R., Hutchings, L. L., and Stiefel, E. I., *J. Chem. Soc., Chem. Commun.*, p. 927 (1985).
298. Potvin, C., Manoli, J. M., Marzak, S., and Secheresse, F., *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **C44,** 369 (1988).
299. Rabinovich, D., and Parkin, G., *J. Am. Chem. Soc.* **113,** 5904 (1991).
300. Feinstein-Jaffe, I., Dewan, J. C., and Schrock, R. R., *Organometallics* **4,** 1189 (1985).
301. Faller, J. W., Kucharczyk, R. R., and Ma, Y., *Inorg. Chem.* **29,** 1662 (1990).
302. Eagle, A. A., Tiekink, E. R. T., and Young, C. G., *J. Chem. Soc., Chem. Commun.*, p. 1746 (1991).
303. Eagle, A. A., and Young, C. G., unpublished results.
304. Enemark, J. H., Roberts, S. A., and Sullivan, E. P., Jr., unpublished results.
305. Wedd, A. G., unpublished results.
306. Johnson, J. L., and Rajagopalan, K. V., *J. Clin. Invest.* **58,** 543 (1976).
307. Johnson, J. L., and Rajagopalan, K. V., *J. Biol. Chem.* **252,** 2017 (1977).
308. Toghrol, F., and Southerland, W. M., *J. Biol. Chem.* **258,** 6762 (1983).
309. Satoh, T., and Kurihara, F. N., *J. Biochem.* (*Tokyo*) **102,** 191 (1987).
310. del Campillo-Campbell, A., and Campbell, A., *J. Bacteriol.* **149,** 469 (1982).
311. Adams, M. W. W., and Mortenson, L. E., *in* "Molybdenum Enzymes" (T. G. Spiro, ed.), p. 519. Wiley, New York, 1985.
312. Yamamoto, I., Okubo, N., and Ishimoto, M., *J. Biochem.* (*Tokyo*) **99,** 1773 (1986).

313. Johnson, J. L., *in* "Molybdenum and Molybdenum-Containing Enzymes" (M. P. Coughlan, ed.), p. 345. Pergamon, Oxford, 1980.
314. Hille, R., and Massey, V., *in* "Molybdenum Enzymes" (T. G. Spiro, ed.), p. 443. Wiley, New York, 1985.
315. Barber, M. J., Coughlan, M. P., Rajagopalan, K. V., and Siegel, L. M., *Biochemistry* **21,** 3561 (1982).
316. Ljungdahl, L. G., *in* "Molybdenum and Molybdenum-Containing Enzymes" (M. P. Coughlan, ed.), p. 463. Pergamon, Oxford, 1980.
317. Meyer, O., and Schlegel, H. G., *Annu. Rev. Microbiol.* **37,** 277 (1983).
318. Deli, J., and Speier, G., *Transition Met. Chem.* **6,** 227 (1981).
319. Wuebbens, M. M., and Rajagopalan, K. V., *J. Biol. Chem.* **268,** 13493 (1993).
320. Pitterle, D. M., and Rajagopalan, K. V., *J. Biol. Chem.* **268,** 13499 (1993).
321. Pitterle, D. M., Johnson, J. L., and Rajagopalan, K. V., *J. Biol. Chem.* **268,** 13506 (1993).
322. Holm, R. H., and Donahue, J. P. *Polyhedron* **12,** 571 (1993).
323. Hille, R., Kim, J. H., and Hemann, C. *Biochemistry* **32,** 3973 (1993).
324. Yang, L., Coury, L. A., Jr., and Murray, R. W. *J. Phys. Chem.* **97,** 1694 (1993).
325. Schmitz, R. A., Albracht, S. P. J., and Thauer, R. K. *Eur. J. Biochem.* **209,** 1013 (1992).
326. Schmitz, R. A., Albracht, S. P. J., and Thauer, R. K. *FEBS Lett.* **309,** 78 (1992).
327. Mukund, S., and Adams, M. W. W. *J. Biol. Chem.* **268,** 13592 (1993).
328. Johnson, J. L., Rajagopalan, K. V., Mukund, S., and Adams, M. W. W. *J. Biol. Chem.* **268,** 4848 (1993).

NOTE ADDED IN PROOF. Several significant papers related to this chapter have appeared since submission of the manuscript. Wuebbens and Rajagopalan have shown that the intermediate biosynthetic precursor (Z) to molybdopterin in *E. coli* is an oxygen sensitive, 6-alkyl pterin with a 4-carbon phosphorylated side chain but with neither of the sulfur atoms that are present in molybdopterin (*319*). Active converting factor, which orchestrates the incorporation of the sulfur atoms into precursor Z to form molybdopterin, has been characterized by Pitterle and Rajagopalan (*320*), and the *in vitro* synthesis of molybdopterin from precursor Z using purified converting factor has been accomplished (*321*). Holm and Donahue have tabulated a general thermodynamic scale for oxygen atom transfer reactions (*322*). Hille and co-workers have provided experimental evidence that the species that gives rise to the Rapid Type 1 EPR signal in xanthine oxidase is a dead-end complex (*323*). Their results support the proposal of Scheme 8 that the Rapid Type 1 EPR signal is due to the catalytically incompetent species 27a. Yang *et al.* have carried out electrocatalytic studies of electron transfer processes in sulfite oxidase under sulfite-saturated conditions by using mediator oxidants. The rate of intramolecular electron transfer between the molybdenum and heme centers was found to decrease as the concentration of chloride in the buffer increased (*324*). The same effect was previously observed in steady-state and flash photolysis studies (Section VII.B). The molybdenum and tungsten formylmethanofuran dehydrogenase enzymes from *M. wolfei* (Section VIII.D) have both been purified and their metal centers definitively identified by the hyperfine splittings from ^{97}Mo (*325*) and ^{183}W (*326*) observed in the EPR spectra of isotopically enriched samples. A formaldehyde ferredoxin oxidoreductase (FOR) containing tungsten has been isolated from the anaerobic Archeon *Thermococcus literalis* (*327*). The enzyme is postulated to have an as yet unknown role in peptide fermentation. The presence of both FOR and AOR (Section VIII.C.) in *T. litoralis* and *P. furiosus* permits the organisms to grow under conditions of varying peptide/saccharide availability (*327*). Molybdopterin has been identified as the organic component of the tungsten cofactors in four enzymes from hyperthermophilic Archea (*328*).