Ed. Engl. **1969**, *8*, 613.

(6) Stirling, C. J. M. *Chem. Rev.* **1978**, *78*, 519.

(7) This enthalpy is made up by the following contributions: bond contribution,[7a] -(C═C) + (C—C) + (C—O) = +22 kcal mol^{-1}; resonance contribution, enolate resonance energy (estimd) − carboxylate resonance energy (estimd) (20 − 34) = −14 kcal mol^{-1}; contribution of ring strain,[7a] −26 kcal mol^{-1}, sum, −18 kcal mol^{-1}.[7b] (a) Adapted from Benson, S. W. "Thermochemical Kinetics", Wiley: New York, 1968. (b) The actual enthalpy should be even more negative, because the lithium cation gains in solvation energy by getting attached to a carboxylate instead of an enolate function.

(8) It might be argued that the difficulty to eliminate HX from systems like 1-halobicyclo[2.2.1]heptane, etc. is also due to an orthogonal orbital arrangement. However, in these cases the elimination leads to an unstable olefin (Bredt's rule; cf. Köbrich, G. *Angew. Chem., Int. Ed. Engl.* **1973,** *12*, 464), whereas in our case the "forbidden" β elimination furnishes the stable acrylic acid anion. An argumentation similar to ours, but concerning a different type of elimination, has been put forward by P. Deslongchamps in a series of excellent papers: Deslongchamps, P.; Chenevert, R.; Taillefer, R. J.; Moreau, C.; Saunders, J. K. *Can. J. Chem.* **1975,** 53, 1601. Deslongchamps, P.; Dube, S.; Lebreux, C.; Patterson, D. R.; Taillefer, R. J. *Ibid.* **1975,** *53*, 2791. Deslongchamps, P.; Taillefer, R. J. *Ibid.* **1975,** *53*, 3029. Stable 2-thietanone anión: Carter, S. D., Stoodley, R. J. *J. Chem. Soc., Chem. Commun.* **1977,** 92.

(9) We have synthesized more than 20 pairs of 3,4-disubstituted cis–trans β-lactone isomers. In all cases, even for extreme steric congestion (e.g., *cis*-3,4-di-*tert*-butyl-, or *cis*-3-*tert*-butyl-4-mesityl (substitution), we always found that $J_{3,4}$(cis) = 6.5 Hz and $J_{3,4}$(trans) = 4.5 ± 0.5 Hz.

(10) Mulzer, J.; Brüntrup, G. *Angew. Chem., Int. Ed. Engl.* **1979,** *18*, 793.

(11) This is of particular interest, because 1-lithio-1-phenylalkenes are configurationally unstable in THF even at −60 °C; cf. Panek, E., Neff, B., Houng Chu, Panek, M. *J. Am. Chem. Soc.* **1975,** *97*, 3996. Similarly, direct alkylation of 3-(2-oxolanonyl) anions often proceeds unstereoselectively; cf. Grieco, P. A. *Synthesis* **1975**, 67.

(12) E.g. hydration of the triple bond in **3h,** oxidation of the double bond in **3f,** derivatization of the ester groups in **3d** and **3g**; subsequently olefins or γ-lactones may be formed according to ref 2 or 10.

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Osmochromes (Osmium Analogues of Hemochromes): Proof of Superoxide and Hydroperoxide Generation from Dioxygen and a Metalloporphyrin Lacking a Free Coordination Site

Sir:

For several years it was believed that iron(II) porphyrins, Fe(P),[1] and heme proteins are autoxidized via an inner-sphere mechanism,[2] the first step being coordination of the dioxygen molecule to the heme iron (eq 1), the second step being the attack of a second heme to the dioxygen ligand (eq 2), and the third step being nucleophilic substitution of the superoxide ion, e.g., by chloride[3] (eq 3a) or proton-assisted dissociation (eq 3b):[2c]

$$\mathrm{Fe(P)L_2} \underset{}{\overset{-\mathrm{L}}{\rightleftharpoons}} \mathrm{Fe(P)L} \overset{\mathrm{O_2}}{\rightleftharpoons} \mathrm{Fe(P)O_2(L)} \qquad (1)$$

$$\mathrm{Fe(P)O_2(L)} \xrightarrow[-2\mathrm{L}]{\mathrm{Fe(P)L}} \mathrm{(P)FeOOFe(P)} \xrightarrow[-2\mathrm{L}]{2\mathrm{Fe(P)L}} \mathrm{2(P)FeOFe(P)} \qquad (2)$$

$$\mathrm{Fe(P)Cl} \xleftarrow[-\mathrm{O_2}^-,\ -\mathrm{L}]{\mathrm{a.}\ +\mathrm{Cl}^-} \mathrm{Fe(P)O_2(L)} \xrightarrow[-\mathrm{HO_2}\cdot]{\mathrm{b.}\ \mathrm{H}^+} \mathrm{Fe(P)L^+} \qquad (3)$$

Steps 3a or 3b should then be followed by the superoxide disproportionation reactions 4a,b or 4b in protic media:

$$\mathrm{O_2}^- + \mathrm{H}^+ \xrightarrow{\mathrm{a}} \mathrm{HO_2}\cdot \xrightarrow{\mathrm{b.}\ 2\times} \mathrm{H_2O_2} + \mathrm{O_2} \qquad (4)$$

If reactions 1-3 were the only possible autoxidation pathways of hemochromes Fe(P)L_2, one could not understand how those cytochromes are autoxidized that do not bind carbon monoxide,[4] e.g., cytochrome b_5, and thus do not offer a free coordination site. Therefore, on the basis of our experiments with osmium porphyrins, we have suggested that cytochromes can be autoxidized by an outer-sphere mechanism producing superoxide;[5] in this paper, we extend this suggestion to hemochromes as well. Equilibrium

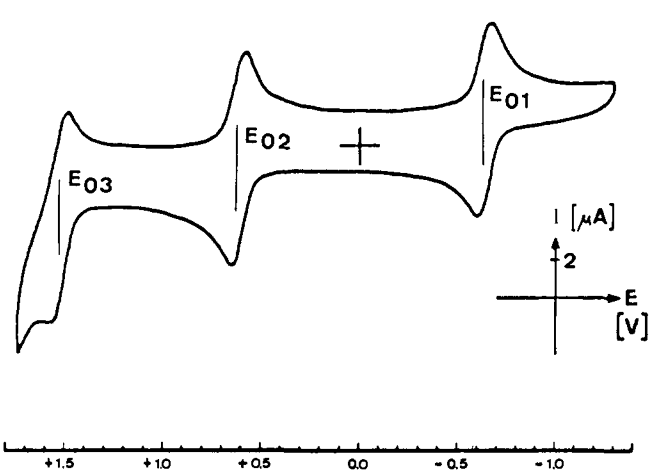

Figure 1. Cyclic voltammogram of Os(OEP)(1-Meim)$_2$ taken in CH_2Cl_2 (Beckman Pt button, scan rate, 0.1 V/s; NBu_4PF_6, 0.1 M, reference, saturated NaCl/calomel electrode): E_{O1}, Os^{II}/Os^{III} step, −0.63 V; E_{O2}, Os^{III}/Os^{IV} step, +0.61 V; ring oxidation, ca. +1.5 V.

$$\mathrm{Fe(P)L_2} + \mathrm{O_2} \underset{\mathrm{b}}{\overset{\mathrm{a}}{\rightleftharpoons}} [\mathrm{Fe(P)L_2}]^+ + \mathrm{O_2}^- \qquad (5)$$

which describes this outer-sphere process may itself lie far on the left, but in protic media the successive superoxide dismutation along steps 4a,b could drive it to the right.

With iron porphyrins,[6a,b] manganese porphyrins,[6c] or ruthenium porphyrins,[6d] the products of direct inner-sphere or outer-sphere electron transfer from the metalloporphyrin to dioxygen, i.e., O_2^- and its dismutation product, H_2O_2, cannot be identified because in those complexes a free coordination site can be offered which causes superoxide dismutase[6a] or catalase activity and thus destroys any O_2^- or H_2O_2 formed; moreover, H_2O_2 attacks the porphyrin carbon skeleton of hemes.[2d] Therefore, porphyrin complexes of those metals cannot serve to discriminate inner-sphere and outer-sphere electron transfer to dioxygen. Nevertheless, Castro has found the rate of the autoxidation of Fe(OEP)py$_2$ to be accelerated by a pH decrease and kinetic data to be interpretable by formation of an outer-sphere charge-transfer complex, [Fe(OEP)py$_2$·O_2], which is protonated.[7] The data also could fit the successive steps 5a and 4a,b. Some recent ruthenium work points also toward this direction.[6d] Fleischer has determined the oxygen uptake of a water-soluble hemochrome to be in accord with hydrogen peroxide formation, but he explains his kinetic results in favor of an inner-sphere process and could not detect the hydrogen peroxide.[6a]

In the course of our research on osmium porphyrins[2a,8,9] we have found that the "osmochromes" (osmium analogues of hemochromes[5]) are very suitable models for the investigation of hemochrome (and hence also cytochrome) autoxidation, for the following reasons. (1) Osmochromes, e.g., Os(OEP)py$_2$ (**1**)[8] or Os(OEP)(1-Meim)$_2$ (**2**)[9,10] are kinetically inert below 100 °C, i.e., stable to axial ligand exchange, e.g., carbon monoxide binding. (2) As a consequence of this, autoxidation does not occur in aprotic media. (3) Hydrogen peroxide does not attack the carbon skeleton of the osmium porphyrin at the low concentrations involved. (4) The osmochrome/osmichrome redox pair is fully reversible as evidenced by cyclic voltammetry (Figure 1),[9c,e] contrary to the substitutionally labile hemochrome/hemichrome pairs.[11] (5) The osmichrome cations are EPR silent, thus allowing a clear distinction of other radicals that might occur during the autoxidation.[12] (6) In the presence of acids, autoxidation does occur at a rate increasing with the strength of the acid present; in this autoxidation quantitative

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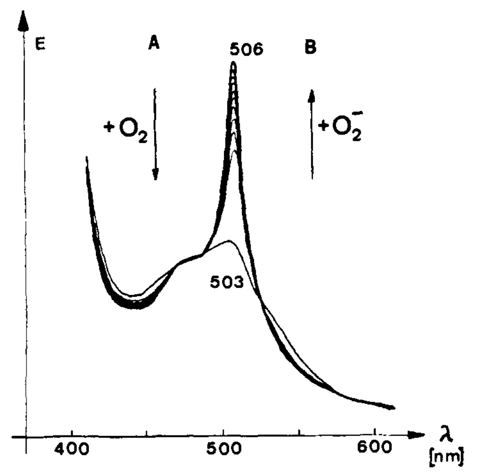

Figure 2. Visible region of the electronic spectrum of Os(OEP)(1-Meim)$_2$ (α band at 506 nm): A, collapse to the osmichrome spectrum (α band at 503 nm) on autoxidation in wet pyridine B, restoration of the osmochrome spectrum on addition of KO$_2$-crown ether in dry pyridine.

formation of the osmichrome salt even in the absence of excess axial base is observed (Figure 2); no oxygen atom is attached to the osmium porphyrin.[9b]

The kinetic inertness of the osmochrome then necessitates the operation of an outer-sphere electron transfer from the osmochrome to the dioxygen molecule in this "acid-induced autoxidation" which consists of the steps 6 and 7 adding up to 8:

$$\mathrm{Os(P)L_2 + O_2 \rightleftharpoons [Os(P)L_2]^+ + O_2^-} \qquad (6)$$

$$\mathrm{2O_2^- + 2HX \rightarrow H_2O_2 + O_2 + 2X^-} \qquad (7)$$

$$\mathrm{2Os(P)L_2 + O_2 + 2HX \rightarrow 2[Os(P)L_2]X + H_2O_2} \qquad (8)$$

The primary autoxidation product is the osmichrome superoxide (eq 6). The superoxide is then dismutated according 7. The evidence for the occurrence of equilibrium 6 and reactions 7 comes from the following observations. (1) Dioxygen-containing solutions of Os(OEP)(1-Meim)$_2$ ($\sim 10^{-3}$ mol L^{-1}) in water-free pyridine do not show an EPR signal. However, on addition of 1% water to such solutions and freezing, an intense signal appears (Figure 3)[12] which is due to 3-4% superoxide in accord with its known *g* values.[13] The water serves to hydrate the superoxide according to[14]

$$\mathrm{O_2^- + \mathit{n}H_2O \rightarrow [O_2(H_2O)_\mathit{n}]^-} \qquad (9)$$

thus shifting the equilibrium 6 to the right; it also induces the dismutation reaction 7, whereby the initial O$_2^-$ concentration slowly diminishes [half-life time of **2** in pyridine-H$_2$O (99:1) was ~45 h; in pyridine-H$_2$O (9:1), however, it was only ~1 min]. (2) Treatment of osmichrome salts in pyridine with KO$_2$-dibenzo-18-crown-6 in pyridine leads to the osmochrome (Figure 2) and evolution of dioxygen (detected by purging the system with argon passing an electrochemical ZrO$_2$ cell sensitive to O$_2$).[9b] Thus, equilibrium 6 lies indeed far to the left. This is also indicated by the redox potentials, E_0[Os(OEP)(1-Meim)$_2$/Os(OEP)(1-Meim)$_2^+$] = −0.49[9b] and E_0[O$_2^-$/O$_2$] = −0.9 V[15] in anhydrous pyridine (SCE). (3) The oxygen consumption of Os(OEP)py$_2$ (**1**) amounts to 0.5 ± 0.05 mol/mol of **1** in dimethylacetamide-benzenesulfonic acid (determined with a Gilson respirometer modified for nonaqueous solutions).[12,16] (4) When a solution of Os(OEP)(1-Meim)$_2$ (**2**) in oxygen-containing, dry pyridine and a solution of TiOSO$_4$ in 25% H$_2$SO$_4$-H$_2$O are mixed, the osmichrome sulfate immediately precipitates. Extraction with CH$_2$Cl$_2$ yields a porphyrin-free aqueous phase showing absorption at 407 nm due to the peroxotitanyl cation. The extinction indicates the presence of H$_2$O$_2$ in yields of 16-71% based on initial osmochrome.[9b] The yields increase with decreasing initial osmochrome concentration owing to interference of

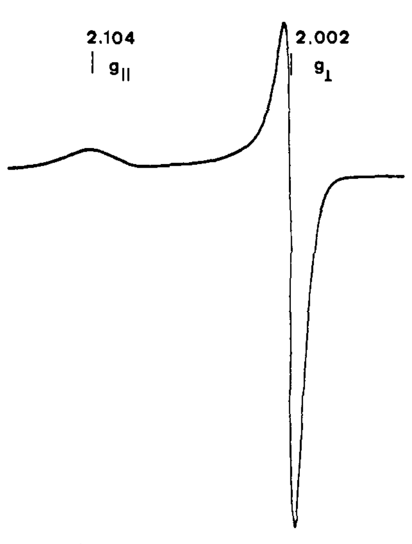

Figure 3. Electron spin resonance spectrum of autoxidizing Os(OEP)(1-Meim)$_2$ in pyridine-H$_2$O (99:1) frozen at 77 K. A Bruker X band spectrometer BER 420 (microwave energy control, 200 μA; field modulation amplitude, 1 G) was used. Concentration determination of O$_2^-$ was done by double integration of signals like the one shown in comparison with aqueous CuSO$_4$-HCl-NaClO$_4$ (1 mM:0.01 M:2 M) as external standard. The first integral (absorption curve) was obtained by the commercial Bruker Physics computing equipment ("EPR-Measure") and the second integral by cutting the plotted absorption curve and weighing. Just after mixing and freezing, 3.5 ± 1% O$_2^-$ was indicated.

$$\mathrm{2Os(OEP)(1\text{-}Meim)_2 + H_2O_2 + 2HX \rightarrow 2[Os(OEP)(1\text{-}Meim)_2]X + 2H_2O} \qquad (10)$$

which under certain circumstances, e.g., on autoxidations in acetic acid (X = OAc),[12] leads to lower dioxygen consumptions than normally occurring according eq 8.

These findings demonstrate that outer-sphere electron transfer to dioxygen can occur from metalloporphyrins with blocked axial positions when the superoxide is removed from the equilibrium. The acid-induced autoxidation operates with a variety of inert ruthenochromes or osmochromes having redox potentials between 0 and −0.63 V in CH$_2$Cl$_2$ vs. SCE, although apart from **2** only in a few cases can transient superoxide be detected by EPR spectroscopy.[9b,17] The given range of potentials also applies to hemochromes.[11] Therefore, we suggest that the latter also take the autoxidation pathway of steps 5a and 4 under quasi-inert conditions (large excess of L)[7] or in aqueous media (where the lifetime of O$_2^-$ is short).[6a]

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References and Notes

(1) Abbreviations used: (P)$^{2-}$, general porphinate dianion; L, neutral donor ligand; (OEP)$^{2-}$: octaethylporphyrinate dianion; py, pyridine; 1-Meim, 1-methylimidazole; HX, monoprotic acid; SCE, saturated NaCl/calomel electrode.

(2) For recent reviews, see (a) J. W. Buchler, *Angew. Chem., Int. Ed. Engl.*, **17**, 407 (1978); (b) C. E. Castro, in "The Porphyrins", Vol. V, D. Dolphin, Ed., Academic Press, New York, 1978, pp 1-28; (c) B. R. James, ref 2b, pp 207-303; (d) A. F. McDonagh in "The Porphyrins", Vol. VI, Academic Press, New York, 1979, p 313.

(3) W. J. Wallace, J. C. Maxwell, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **57**, 1104 (1974).

(4) R. Lemberg and J. Barrett, "Cytochromes". Academic Press, London, 1973.

(5) J. W. Buchler, *Hoppe-Seyler's Z. Physiol. Chem.*, **359,** 1065 (1978).

(6) (a) E. B. Fleischer and R. V. Ferra, *Inorg. Biochem.*, **10,** 91 (1979); (b) R. F. Pasternack and B. Halliwell, *J. Am. Chem. Soc.*, **101,** 1026 (1979); (c) J. S. Valentine and A. E. Quinn, *Inorg. Chem.*, **15,** 1997 (1976); (d) D. R. Paulson, A. W. Addison, D. Dolphin, and B. R. James, *J. Biol. Chem.*, **254,** 7002 (1979).

(7) M. M. L. Chu, C. E. Castro, and G. M. Hathaway, *Biochemistry*, **17,** 481 (1978).

(8) (a) J. W. Buchler and P. D. Smith, *Angew. Chem., Int. Ed. Engl.*, **13,** 745 (1974); (b) A. Antipas, J. W. Buchler, M. Gouterman, and P. D. Smith, *J. Am. Chem. Soc.*, **100,** 3015 (1978).

(9) (a) J. W. Buchler, W. Kokisch, and P. D. Smith, manuscript in preparation; (b) W. Kokisch, Doctoral Thesis, Technische Hochschule Aachen, 1979; (c) W. Kokisch, Diplomarbeit, Technische Hochschule Aachen, 1976; (d) P. D. Smith, unpublished experiments, 1976/1977; (e) P. D. Smith, Doctoral Thesis, Technische Hochschule Aachen, 1976; (f) J. W. Buchler, W. Kokisch, and P. D. Smith, *Struct. Bonding* (*Berlin*), **34,** 79 (1978).

(10) Os(OEP)(1-Meim)$_2$,[9a–d] [Os(OEP)(1-Meim)$_2$]PF$_6$,[9d] and [Os(OEP)py$_2$]BPh$_4$[9e] were characterized by elemental analyses, ^{1}H NMR, optical, and infrared spectra. For selected spectral data, see ref 9f. Additionally, the effective magnetic moments have been determined for [Os(OEP)py$_2$]ClO$_4$ by the NMR method (1.52 μ_B) and of [Os(OEP)(NH$_3$)$_2$]BF$_4$ with a Faraday balance (Dr. H. Lueken, Aachen; 1.85 μ_B, Curie law, 70–300 K).[9e]

(11) G. M. Brown, F. R. Hopf, T. J. Meyer, and D. G. Whitten, *J. Am. Chem. Soc.*, **97,** 5385 (1975).

(12) J. Billecke and J. W. Buchler, unpublished experiments.

(13) R. C. Bray, G. N. Mautner, E. M. Fielden, and C. I. Carle in "Superoxide and Superoxide Dismutase", A. M. Michelson, J. M. McCord, and I. Fridovich, Eds., Academic Press, London, 1977, p 61.

(14) M. R. Green, H. A. O. Hill, and D. R. Turner, *FEBS Lett.*, **103,** 176 (1979).

(15) M. E. Peover and B. S. White, *Electrochim. Acta*, **11,** 1061 (1966). Neither the osmochrome nor the dioxygen potential is altered by addition of 1% water to pyridine solutions. Obviously the electrodes do not indicate eq 9 although the latter exists in the bulk of the solution.

(16) J. Billecke, Diplomarbeit, Technische Hochschule Aachen, 1977.

(17) A more detailed report of this work will appear in "Superoxide and Superoxide Dismutase", Elsevier, New York, in press.

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A Nonoctahedral Dioxo Molybdenum Complex with a Coordinated Partial Disulfide Bond

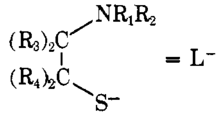
Sir:

EXAFS studies on the oxidized forms of sulfite oxidase[1] and xanthine oxidase[2] have implicated the presence of terminal oxygen atoms at an Mo-O distance of ~1.7 Å and approximately three sulfur atoms in the Mo(VI) coordination sphere. The striking similarity of the EXAFS patterns for oxidized sulfite oxidase[1] and $MoO_2[CH_3SCH_2CH_2N(CH_2CH_2S)_2]$[3,4] led to the suggestion that the Mo coordination sphere of the enzyme may contain two cis oxo groups, two mutually trans thiolates each cis to both oxo donors, and one thioether linkage trans to an oxo group as found in the crystallographic study of the complex.[4] The above and other structural discussions[5] on the Mo(VI) state in Mo oxidases have uniformly been based on the assumptions of a conventional octahedral structure and two independently acting thiolate donor atoms. In this paper we present the results of studies on the seemingly simple Mo(VI) complex $MoO_2[CH_3NHCH_2C(CH_3)_2S]_2$ (**1**), which reveal a distinctly nonoctahedral structure and a partial S-S bond within the "Mo(VI)" coordination sphere. Moreover, the ^{1}H NMR spectra of this and related Mo(VI) complexes demonstrate stereochemical nonrigidity which is sensitive to the nature of the ligand. Since these structural features must be considered in discussions of the Mo coordination sphere in enzymes, we also discuss in a preliminary way some of the potential biochemical implications of these findings.

Table I. Bond Distances and Angles in $MoO_2[SC(CH_3)_2CH_2NHCH_3]_2$ and Related Complexes

	1	**4**	**5**
	Bond Distances, Å		
Mo-S(1)	2.420 (3)	2.420 (1)	2.411 (3)
Mo-S(2)	2.409 (3)	2.409 (1)	2.411 (3)
Mo-O(1)	1.723 (5)	1.699 (2)	1.694 (6)
Mo-O(2)	1.711 (5)	1.705 (2)	1.712 (7)
Mo-N(1)	2.262 (9)	2.372 (2)	2.382 (9)
Mo-N(2)	2.277 (8)	2.510 (2)	2.374 (9)
S(1)-S(2)	2.764 (5)	[4.71]	[4.75]
	Bond Angles, deg		
S(1)-Mo-S(2)	69.8 (19)	154.3(3)	160.9 (1)
O(1)-Mo-O(2)	122.2 (3)	107.9 (1)	106.3 (3)
N(1)-Mo-N(2)	144.0 (3)		
S(1)-Mo-O(1)	107.7 (3)		
S(2)-Mo-O(2)	107.5 (2)		
S(1)-Mo-O(2)	118.3 (2)		
S(2)-Mo-O(1)	120.4 (2)		
S(1)-Mo-N(1)	72.9 (3)		
S(2)-Mo-N(2)	74.6 (2)		
S(1)-Mo-N(2)	142.6 (2)		
S(2)-Mo-N(1)	141.0 (2)		
O(1)-Mo-N(1)	81.8 (3)		
O(2)-Mo-N(2)	83.0 (3)		
O(1)-Mo-N(2)	80.5 (3)		
O(2)-Mo-N(1)	80.3 (3)		

Complex **1**, containing the ligand $(CH_3)NHCH_2C(CH_3)_2S^-$, was prepared as part of our program to design and synthesize new bi-, tri-, tetra- and pentadentate ligands containing N, S, and O donor atoms. The variety of ligand types is such that some arrangement of the donor atom sets is likely to be analogous to that present in Mo enzymes. In this paper we restrict our attention to complexes **1**-**3** of the bidentate ligands derived from cysteamine.



complex (MoO_2L_2)	ligand (L^-)
1	$R_1 = R_3 = H$; $R_2 = R_4 = CH_3$
2	$R_1 = R_2 = R_4 = CH_3$; $R_3 = H$
3	$R_1 = R_2 = R_4 = H$; $R_3 = CH_3$

The Mo(VI) complexes were prepared by the reaction

$$MoO_2(acac)_2 + 2LH \rightarrow MoO_2L_2 + 2\ acacH$$

where acac = acetylacetonate.

Crystals of **1** were grown at 0 °C in CH_3OH directly from the reaction mixture. The compound crystallizes in the monoclinic space group $P2_1/c$ with $a = 10.688$ (3), $b = 11.923$ (2), $c = 14.032$ (3) Å; $\beta = 106.65$ (2)°. The structure was solved by standard heavy-atom methods and was refined using full-matrix least squares to $R = 4.9\%$ and $R_w = 5.4\%$ as described previously[6] using 1516 reflections with $F^2_{obsd} > 3\sigma(F^2_{obsd})$. All nonhydrogen atoms were refined anisotropically and hydrogen atoms were included as fixed contributions in the final least-squares cycles.

The structure of **1** displayed in the figure has noncrystallographic twofold symmetry with the C_2 axis bisecting the S-Mo-S, O-Mo-O, and N-Mo-N bond angles. Distances and angles are displayed in Table I along with corresponding values for $MoO_2[(CH_3)_2NCH_2CH_2N(CH_2CH_2S)_2]$[4] (**4**) and MoO_2(8-mercaptoquinolinato)$_2$[7] (**5**), other complexes containing $MoO_2N_2S_2$ coordination spheres.

Inspection of Table I and Figure 1 reveals the nonoctahedral nature of the structure. There are no atoms present at sites