ceptibility measurements. We thank Dr. C. Hermes (EMBL Outstation, Hamburg, FRG) for measuring the EXAFS spectra.

Registry No. $[L_4Fe_4(\mu\text{-}O)_2(\mu\text{-}OH)_4]I_4\cdot 3H_2O$, 123567-48-6; $[L_4Fe_4(\mu\text{-}O)_2(\mu\text{-}OH)_4](ClO_4)_4\cdot 3H_2O$, 123567-50-0; $[L_2Fe_2(acac)_2(\mu\text{-}O)](ClO_4)_2$, 118486-84-3; $LFeCl_3$, 86823-88-3.

Supplementary Material Available: Tables of complete crystallographic data, bond distances and angles, and thermal parameters (5 pages); a listing of structure factor amplitudes (43 pages). Ordering information is given on any current masthead page.

Contribution from the Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300, and Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Molybdenum(VI)–Dioxo Complexes with Linear and Tripodal Tetradentate Ligands: Models for the Molybdenum(VI/V) Centers of the Molybdenum Hydroxylases and Related Enzymes. 1. Syntheses and Structures

Carol J. Hinshaw,[1a] Gang Peng,[1a] Raghuvir Singh,[1a] Jack T. Spence,*[,1a] John H. Enemark,[1b] Michael Bruck,[1b] John Kristofzski,[1b] Shannath L. Merbs,[1b] Richard B. Ortega,[1b] and Pamela A. Wexler[1b]

Received January 12, 1989

As models for the molybdenum(VI/V) centers of the molybdenum hydroxylases and related enzymes, 15 new Mo(VI)–dioxo complexes (MoO_2L) with tetradentate ligands have been synthesized and characterized. The effects of coordinating groups (N_2S_2, N_2OS, and N_2O_2), chelate ring size (five and six members), ligand geometry (linear and tripodal), and steric bulk have been investigated. X-ray crystal structures have been obtained for seven of the complexes. While minor differences, attributed to these features, are evident, the structures have remarkably similar Mo–ligand bond lengths and bond angles and all have distorted-octahedral geometry. The oxo groups are cis to one another and to the thiolate or phenolate groups of the ligands. The N atoms are approximately trans to the oxo groups, and the Mo—N bonds are relatively long (>2.34 Å), with the bond length correlated with the size of the trans O=Mo—N bond angle. The Mo=O and Mo—S(thiolate) bond lengths are comparable to those determined by EXAFS spectroscopy for the Mo centers of the enzymes. The relevance of the results to the structures of the Mo centers of the enzymes is discussed.

Introduction

The molybdenum hydroxylases and related enzymes catalyze two-electron-redox processes in which an oxygen atom or a hydroxyl group is added to or removed from the substrate. The most extensively studied of these enzymes are xanthine oxidase (XO),[2a] xanthine dehydrogenase (XDH),[3a] sulfite oxidase (SO),[2a] and nitrate reductase (NR).[2b] The minimal structure of the molybdenum center, as deduced by EXAFS and EPR investigations, for oxidized XO and XDH is $Mo^{VI}O(S)(SR)_2$.[3] A $Mo^{VI}O_2(SR)_{2-3}$ center appears to be present in oxidized SO and NR from *Chlorella vulgaris*.[3a] Some of the thiolate (SR) ligands are probably furnished by the cofactor, Mo-co,[4] and additional oxygen, nitrogen, or thioether ligands may also be present.[3]

The enzyme molybdenum centers cycle between the VI, V, and IV oxidation states during catalysis.[2] The Mo centers undergo reversible reduction, and in most cases, the potentials are pH and anion dependent.[5] Two-electron reduction has been interpreted as generating (omitting SR ligands) $Mo^{IV}O(SH)$ (XO, XDH)[2,3] or $Mo^{IV}O(OH)$ (SO, NR)[2,3] centers that, upon one-electron re-oxidation, give EPR signals which have been interpreted as arising from $Mo^{V}OS$,[3d] $Mo^{V}O(SH)$,[3a,b] and $Mo^{V}O(OH)$[2b,3,6–8] centers.

In contrast to the behavior of the enzyme Mo centers, most Mo(VI)–dioxo complexes undergo irreversible electrochemical reduction, with formation of oxo-bridged dimers[9a] or with deprotonation of amino ligands and loss of an oxo ligand as H_2O.[9b] Recent reports[10–12] indicate these biomimetically undesirable results may be avoided by proper ligand design. Specifically, tetradentate N_2S_2 and N_2O_2 ligands (L) with alkylated (tertiary) nitrogen atoms have been shown to give Mo(VI)–dioxo complexes (MoO_2L) that are reported to undergo reversible one-electron reduction and to stabilize $[Mo^{V}O_2L]^-$, $[Mo^{V}O(S)L]^-$, *cis*-$Mo^{V}O(OH)L$, and *cis*-$Mo^{V}O(SH)L$ species in solution.[11] For one such ligand, both $[Ph_4P][Mo^{V}OSL]$ and *trans*-$Mo^{V}O(SH)L$ have been isolated.[13] These results are clearly relevant for understanding the molybdenum centers of the enzymes.

We report here the syntheses of 15 new Mo(VI)–dioxo complexes with tetradentate N-alkylated ligands in which the effects of coordinating groups (N_2O_2, N_2OS, N_2S_2), chelate ring size (five and six members), ligand geometry (linear and tripodal), and steric bulk have been systematically varied. The complexes have been

(1) (a) Utah State University. (b) University of Arizona.
(2) (a) Hille, R.; Massey, V. In *Molybdenum Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1985; p 443. (b) Adams, M. W. W.; Mortenson, L. E. In ref 2a, p 519.
(3) (a) Cramer, S. P. *Adv. Inorg. Bioinorg. Mech.* **1983**, *2*, 259. (b) Cramer, S. P.; Wahl, R. C.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1981**, *103*, 7721. (c) George, G. N.; Bray, R. C.; Cramer, S. P. *Biochem. Soc. Trans.* **1986**, *14*, 651. (d) Bray, R. C.; George, G. N. *Biochem. Soc. Trans.* **1985**, *13*, 560. (e) Bray, R. C.; Gutteridge, S.; Storter, D. A.; Tanner, S. J. *Biochem. J.* **1979**, *177*, 357.
(4) Johnson, J. L.; Hainline, B. E.; Rajagopalan, K. V.; Arison, B. H. *J. Biol. Chem.* **1984**, *259*, 5414. Hawkes, T. R.; Bray, R. C. *Biochem. J.* **1984**, *219*, 481. Cramer, S. P.; Stiefel, E. I. In *Molybdenum Enzymes*; Spiro, T. E., Ed.; Wiley: New York, 1985; p 411.
(5) Spence, J. T.; Kipke, C. A.; Enemark, J. H.; Sunde, R. A. Submitted for publication.
(6) (a) Bray, R. C. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1980**, *51*, 107. (b) George, G. N.; Bray, R. C. *Biochemistry* **1988**, *27*, 3603.
(7) (a) Bray, R. C. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1980; Vol. 2, p 45. (b) Bray, R. C.; Gutteridge, S.; Lamy, M. T.; Wilkinson, T. *Biochem. J.* **1983**, *211*, 227. (c) Gutteridge, S.; Bray, R. C.; Nottom, B. A.; Fido, R. J.; Hewitt, E. J. *Biochem. J.* **1983**, *213*, 137. (d) Vincent, S. P.; Bray, R. C. *Biochem. J.* **1978**, *171*, 639.
(8) Solomonson, L. P.; Barker, M. J.; Howard, W. D.; Johnson, J. L.; Rajagopalan, K. V. *J. Biol. Chem.* **1984**, *259*, 849.
(9) (a) Stiefel, E. I. *Prog. Inorg. Chem.* **1977**, *22*, 1. (b) Subramanian, P.; Spence, J. T.; Ortega, R. B.; Enemark, J. H. *Inorg. Chem.* **1984**, *23*, 2564. Rajan, O. R.; Spence, J. T.; Leman, C.; Minelli, M.; Sato, M.; Enemark, J. H.; Kroneck, P. M. H.; Sulger, K. *Inorg. Chem.* **1983**, *22*, 3065.
(10) Hinshaw, C. J.; Spence, J. T. *Inorg. Chim. Acta* **1986**, *125*, L17.
(11) Dowerah, D.; Spence, J. T.; Singh, R.; Wedd, A. G.; Wilson, G. L.; Farchione, F.; Enemark, J. H.; Kristofzski, J.; Bruck, M. *J. Am. Chem. Soc.* **1987**, *109*, 5655.
(12) Farchione, F.; Hanson, G. R.; Rodrigues, C. G.; Bailey, T. D.; Bajchi, R. N.; Bond, A. M.; Pilbrow, J. R.; Wedd, A. G. *J. Am. Chem. Soc.* **1986**, *108*, 831.
(13) Singh, R.; Spence, J. T.; Cramer, S. P.; George, G. N. *Inorg. Chem.* **1989**, *28*, 8.

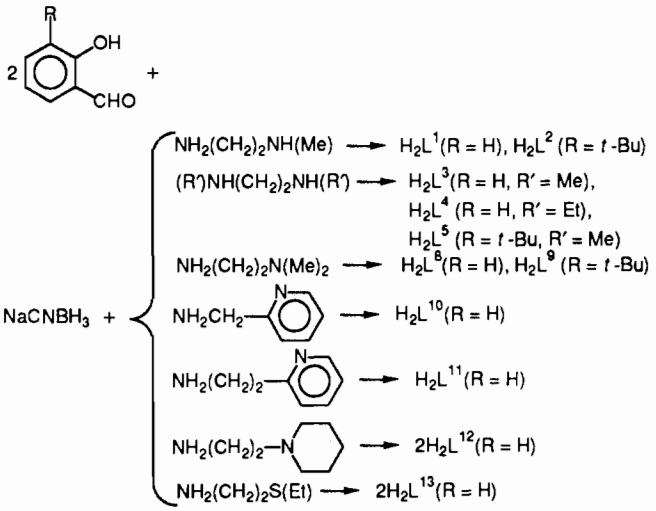
0020-1669/89/1328-4483$01.50/0

characterized by analysis and IR and electronic spectra, and X-ray crystal structures have been obtained for seven of the complexes. The structures have been analyzed, and their relevance to the structures of the enzyme molybdenum centers is discussed. Subsequent papers will report the electrochemical properties of the complexes and the electron paramagnetic resonance (EPR) parameters of their one-electron-reduced (Mo(V)) products.

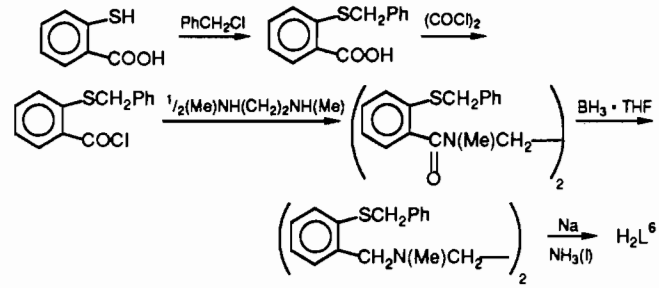
Results

The Mo(VI)–dioxo complexes MoO_2L^n (n = 1–15) with the ligands of Table I have been synthesized and characterized. X-ray crystal structures for complexes **3**, **6**, **8**, **9**, **10**, **14**, and **15** have been obtained.[14]

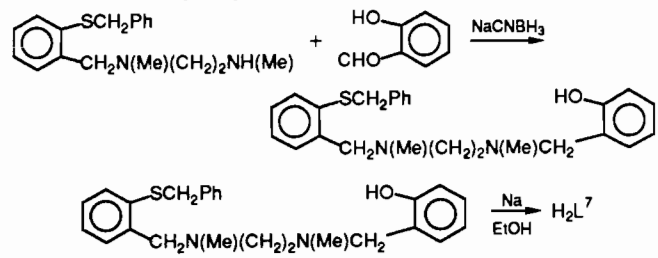
Syntheses. None of the ligands have been previously reported. Ligands H_2L^1–H_2L^5, H_2L^8 and H_2L^9, and H_2L^{10}–H_2L^{13} were obtained by reductive amination of the salicylaldehyde with the appropriate amine with use of the $NaCNBH_3$ method of Borch et al.,[15] as modified for the particular compound:



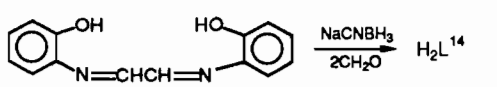
Ligand H_2L^6 was prepared from thiosalicylic acid and *N*,*N*′-dimethylethylenediamine:



Ligand H_2L^7 was prepared from thiosalicylic acid, salicylaldehyde, and *N*,*N*′-dimethylethylenediamine:



Ligand H_2L^{14} was prepared by reductive amination of formaldehyde with glyoxalbis(2-hydroxyanil):



(14) MoO_2L^n complexes are designated by boldface numerals, which correspond to n for the ligands (see Table I).
(15) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.

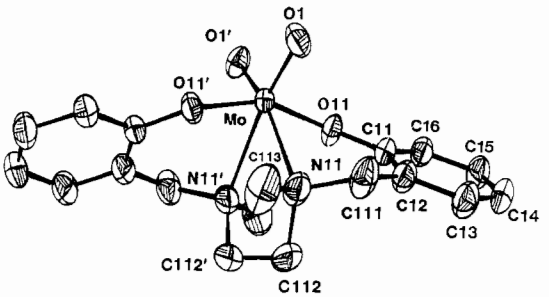


Figure 1. X-ray structure of MoO_2L^3 (**3**) (H atoms omitted for clarity).

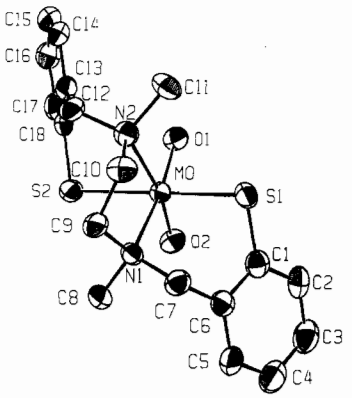


Figure 2. X-ray structure of MoO_2L^6 (**6**) (H atoms omitted for clarity).

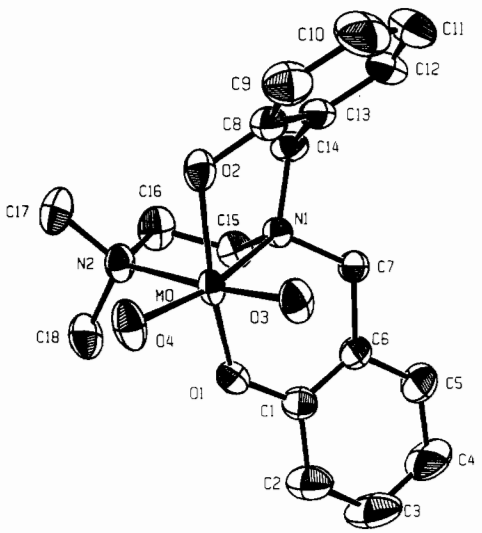


Figure 3. X-ray structure of MoO_2L^8 (**8**) (H atoms omitted for clarity).

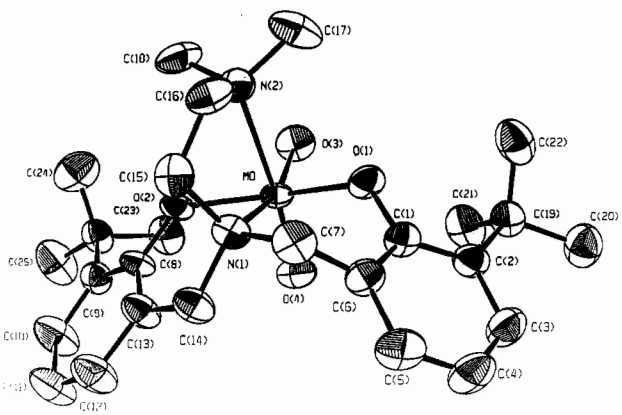


Figure 4. X-ray structure of MoO_2L^9 (**9**) (H atoms omitted for clarity).

Ligand H_2L^{15} was prepared from propylene sulfide and *N*,*N*′-dimethylethylenediamine:

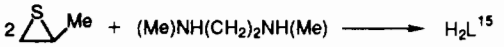
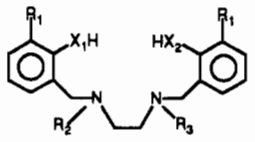
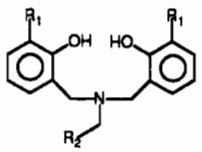


Table I. Ligands H_2L^n



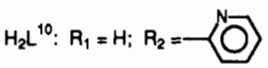
n = 1–7

H_2L^1: R_1, R_2 = H; R_3 = Me; $X_1 = X_2 = O$
H_2L^2: R_1 = *t*-Bu; R_2 = H; R_3 = Me; $X_1 = X_2 = O$
H_2L^3: R_1 = H; R_2, R_3 = Me; $X_1 = X_2 = O$
H_2L^4: R_1 = H; R_2, R_3 = Et; $X_1 = X_2 = O$
H_2L^5: R_1 = *t*-Bu; R_2, R_3 = Me; $X_1 = X_2 = O$
H_2L^6: R_1 = H; R_2, R_3 = Me, $X_1 = X_2 = S$
H_2L^7: R_1 = H; R_2, R_3 = Me; X_1 = S; X_2 = O

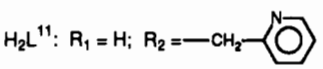


n = 8–13

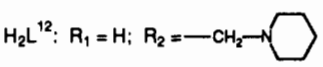
H_2L^8: R_1 = H; R_2 = $-CH_2N(Me)_2$
H_2L^9: R_1 = *t*-Bu; R_2 = $-CH_2N(Me)_2$



H_2L^{10}: R_1 = H; R_2 =

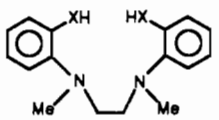


H_2L^{11}: R_1 = H; R_2 =

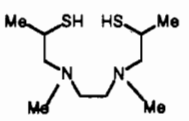


H_2L^{12}: R_1 = H; R_2 =

H_2L^{13}: R_1 = H; R_2 = CH_2SEt



H_2L^{14}: X = O
ref 11 compd: X = S



H_2L^{15}

Table II. IR[a] and Electronic Spectroscopic Data[b,c]

complex	IR (Mo=O), cm^{-1}	electronic λ_{max}, nm (log ϵ)
1	926, 905	330 (3.67), 291 (sh)
2	923, 887	336 (3.74), 301 (3.69)
3	925, 910	330 (3.74), 280 (3.84)
4	923, 905	328 (3.88), 292 (sh)
5	917, 902	335 (3.73), 285 (3.86)
6	932, 900	423 (3.62), 330 (4.07)
7	930, 902	490 (sh), 328 (4.17)
8	924, 903	338 (3.68), 286 (3.69)
9	910, 890	351 (3.47), 295 (3.57)
10	948, 935	359 (sh), 346 (3.69), 297 (sh)
11	948, 928	610 (1.26), 357 (sh), 347 (3.80)
12	945, 930	358 (sh), 351 (3.44), 334 (3.45)
13	925, 905	357 (sh), 348 (3.73), 291 (sh)
14	932, 912	316 (4.03), 286 (sh)
15	920, 891	376 (4.12), 356 (sh)

[a] KBr disk. [b] In MeCN (**1–5**, **8–13**); in DMF (**6**, **14**, **15**). [c] sh = shoulder.

X-ray Structures. The complexes (Figures 1–7) have distorted-octahedral geometry with approximate C_2 symmetry (**3**, **6**, **14**) or C_1 symmetry (**8**, **9**, **10**). The two oxo groups are cis to one another and to the two thiolate (**6**, **15**) or phenolate groups (**3**, **8**, **9**, **10**, **14**) of the tetradentate ligands. The two tertiary amine nitrogen atoms are cis to one another and approximately trans to the terminal oxo groups. Tables III and IV give selected bond angles and distances for the complexes.

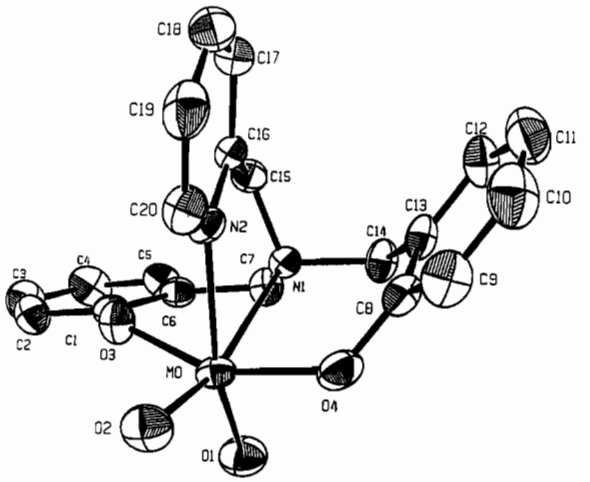


Figure 5. X-ray structure of MoO_2L^{10} (**10**) (H atoms omitted for clarity).

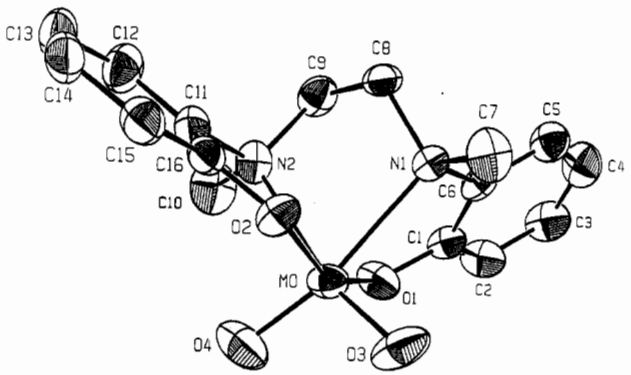


Figure 6. X-ray structure of MoO_2L^{14} (**14**) (H atoms omitted for clarity).

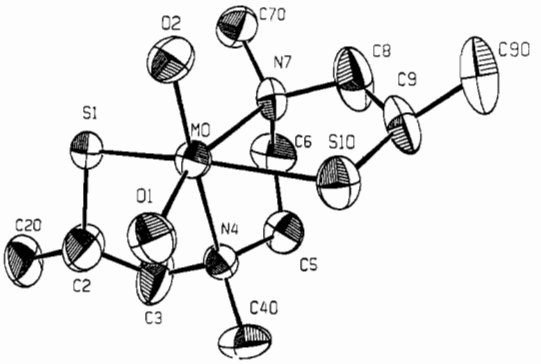


Figure 7. X-ray structure of MoO_2L^{15} (**15**) (H atoms omitted for clarity). Only one of the two disordered configurations at C9 is shown.

The structure of **15** is somewhat unusual because the ligand contains two chiral carbon centers. The crystallized complex is a disordered mixture of diastereomers that differ only in their absolute stereochemistry at C9. Half of the molecules have the stereochemistry shown in Figure 7 with the methyl group denoted by C90 on the same side of the chelate ring as O2 on the Mo atom. The other half of the molecules have the opposite chirality at C9. This inversion at C9 is revealed by two distinct sets of coordinates for C9, which are separated by about 1 Å. The inversion at C9 has little effect on the coordinates of S10, C8, and C90, which are crystallographically well-behaved.

A comparison of structure **3** with structure **6**, and of structure **14** with that of the previously reported corresponding N_2S_2 com-

Table III. Selected Bond Distances (Å)[a]

	3	6	8	9	10	14	15	$MoO_2(S_2N_2)$[11]
Mo–O_t[b]	1.702 (1) [O1]	1.690 (4) [O1] 1.698 (4) [O2]	1.707 (2) [O3] 1.700 (2) [O4]	1.702 (4) [O3] 1.701 (5) [O4]	1.699 (2) [O1] 1.704 (2) [O2]	1.699 (3) [O3] 1.702 (3) [O4]	1.707 (5) [O1] 1.692 (5) [O2]	1.697 (2) [O1] 1.697 (2) [O2]
Mo–O_l[b]	1.934 (1) [O11]		1.953 (3) [O1] 1.967 (2) [O2]	1.977 (5) [O1] 1.954 (5) [O2]	1.941 (2) [O3] 1.970 (2) [O4]	1.956 (2) [O1] 1.970 (2) [O2]		
Mo–S		2.458 (2) [S1] 2.448 (2) [S2]					2.419 (2) [S1] 2.422 (2) [S10]	2.413 (1) [S1] 2.414 (1) [S2]
Mo–N	2.390 (1) [N11]	2.434 (5) [N1] 2.481 (5) [N2]	2.385 (2) [N1] 2.429 (2) [N2]	2.394 (5) [N1] 2.429 (6) [N2]	2.387 (2) [N1] 2.349 (2) [N2]	2.419 (3) [N1] 2.413 (3) [N2]	2.447 (6) [N4] 2.424 (6) [N7]	2.460 (2) [N1] 2.441 (2) [N2]

[a] The numbers in parentheses are the estimated standard deviations in the last digit. The atom labels for individual ligand atoms (see Figures 1–7) appear in brackets. [b] O_t = terminal oxo group; O_l = ligand oxygen atom.

plex,[11] allows the effects of oxygen vs sulfur coordination in otherwise identical ligands to be evaluated. The only significant differences among these complexes are in the average Mo–N bond lengths, with the N_2O_2 complexes having shorter Mo–N bonds than the N_2S_2 complexes (2.406 Å (**3**) vs 2.467 Å (**6**) and 2.416 Å (**14**) vs 2.451 Å[11]), presumably a result of the larger size of sulfur. In all the complexes, the long Mo–N distance (>2.34 Å) is attributed to the trans effect of the oxo groups.

The effect of linear vs tripod coordination may be evaluated by comparison of structure **3** with structures **8** and **9**. No significant differences in Mo–ligand bond lengths or bond angles are observed. In both **8** and **9**, the terminal ring Mo–N bond is longer than the tripodal Mo–N bond (2.429 vs 2.390 Å); however, this difference is similar to the range of Mo–N distances in **3** (2.427–2.384 Å).

Structures **14** and **15** have three five-membered chelate rings, while all other structures have one five-membered and two six-membered rings. A comparison of structure **3** with structure **14** and of structure **6** with that of the previously reported corresponding N_2S_2 complex (five-membered rings)[11] indicates the O–Mo–O (phenolate oxygen) and trans S–Mo–S bond angles for the complexes with six-membered rings are significantly larger (156.5° (**3**) vs 151.0° (**14**): 165.7° (**6**) vs 159.9°,[11] respectively). The longer average Mo–S bond length in **6** (2.453 Å) compared to that of the N_2S_2 complex with five-membered rings (2.413 Å)[11] is probably a result of the larger size and greater flexibility of the six-membered rings. Structure **15** also has the smaller S–Mo–S bond angle (159.3°) and shorter average Mo–S bond length (2.420 Å) characteristic of the complexes with five-membered rings. This structural feature occurs for other tetradentate N_2S_2 complexes,[11,16,17,20f] NS_3 (two thiolate S, one thioether S)[16,17] complexes, and a S_4 (two thiolate S, two thioether S) complex[18] having five-membered rings.

The introduction of the sterically demanding *tert*-butyl group in the 3-position of the aromatic rings (**8**) has no significant effect on the bond angles or distances at Mo. Comparison of structure **15** with the previously reported structure for the corresponding complex without methyl groups on the α-carbon[16] indicates the only significant effect of the methyl groups is an increase in the average Mo–N bond distance (2.436 vs 2.402 Å).

Structure **10** is unique in having an aromatic N (pyridine) as a terminal ring coordinating group. The terminal ring Mo–N bond of **10** (2.349 Å) is considerably shorter than the terminal ring Mo–N bonds of **8** or **9** (2.429 Å), and the N–Mo–N bond angle of **10** is smaller (70.6 vs 73.2°). There are no significant differences between the Mo–N tripodal bond lengths in these complexes. It is not certain whether the short Mo–N (pyridine) bond is a result of stronger bonding of Mo to the sp^2 N of **10** (as compared to the sp^3 N of **8** and **9**) or whether the longer Mo–N bonds of **8** and **9** are a steric consequence of the two methyl groups on N. An average Mo–N sp^2 bond length of 2.32 Å is found in MoO_2(8-hydroxyquinoline)$_2$,[19] while a Mo–N sp^3 bond length of 2.362 Å is observed in the complex corresponding to **3** having secondary amine nitrogen atoms (NH vs NMe).[9b] An examination of the structures of **8** and **9** (Figures 2 and 3) suggests some steric repulsion between the methyl groups on the terminal ring N and the aromatic rings may exist, which could be the reason for the aromatic rings adopting the staggered configuration, instead of the pseudobutterfly ring configuration of **10** (Figure 4). A molecular dynamics calculation for **8** and **10** resulted in minimum-energy structures close to the X-ray structures. Furthermore, when the staggered configuration of **8** was constrained to the pseudobutterfly configuration of **10**, the van der Waals energy term was significantly larger than for **10**, and accounted for approximately 90% of the total dynamic energy. Convergence to the minimum energy structure for **8** reduced this term to approximately 20% of the total dynamics energy, indicating steric hindrance is responsible for the difference in configurations of the ligands.

A feature of interest is the relationship between the Mo—N bond length and the trans O=Mo—N bond angle. As expected, the larger this angle, the longer the Mo—N bond length (with the exception of the pyridine Mo—N bond of **10**), a result of the strong trans effect of the oxo group.[22]

The maximum point symmetry of the inner coordination environment about the Mo atom in these $MoO_2(N_2X_2)$ complexes is C_{2v}. Inspection of the bond angles about the atoms (Table IV) shows that complexes **6** and **10** have approximate C_{2v} point symmetry at **3**, **8**, and **9** have approximate C_s symmetry and **14**, **15**, and the previously reported N_2S_2 complex[11] have approximate C_2 symmetry.

For Mo(VI)–dioxo complexes with tetradentate ligands, within the limits explored, the effects on the Mo–ligand bond lengths and bond angles of the coordinating group, ligand geometry, chelate ring size, and steric bulk are relatively minor, and the structures reported here are also quite similar in these respects to those of other tetradentate Mo(VI)–dioxo complexes found in the literature (vide infra).

Discussion

X-ray structures have been obtained for seven new Mo(VI)–dioxo complexes with tetradentate ligands. In addition, the structures for 16 Mo(VI)–dioxo complexes with tetradentate ligands have been reported previously.[9b,16–18,20] Together, these include ligands with N_2S_2, N_2O_2, N_2OS, NS_3 (two thiolate S, one

(16) Bruce, A.; Corbin, J. L.; Dahlstrom, P. L.; Hyde, J. R.; Minelli, M.; Stiefel, E. I.; Spence, J. T.; Zubieta, J. *Inorg. Chem.* **1982**, *21*, 917.
(17) Berg, J. M.; Hodgson, K. O.; Cramer, S. P.; Corbin, J. L.; Elsberry, A.; Pariyadath, N.; Stiefel, E. I. *J. Am. Chem. Soc.* **1979**, *101*, 2774. Berg, J. M.; Hodgson, K. O.; Bruce, A. E.; Corbin, J. L.; Pariyadath, N.; Stiefel, E. I. *Inorg. Chim. Acta* **1984**, *90*, 25.
(18) Kaul, B. B.; Enemark, J. H.; Merbs, S. L.; Spence, J. T. *J. Am. Chem. Soc.* **1985**, *107*, 2885.
(19) Atovmyan, L. O.; Sokolova, Y. A. *Zh. Strukt. Khim.* **1971**, *12*, 851.
(20) (a) Atovmyan, L. O.; Krasochka, O. N. *J. Chem. Soc. A* **1970**, 1670. (b) Gullotti, M.; Pasini, A.; Zanderighi, G. M.; Ciani, G.; Sironi, A. *J. Chem. Soc., Dalton Trans.* **1981**, 902. (c) Villa, A. C.; Coghi, L.; Manfredotti, A. G.; Guastini, C. *Cryst. Struct. Commun.* **1974**, *3*, 551. (d) Butcher, R. J.; Penfold, B. R. *J. Cryst. Mol. Struct.* **1976**, *6*, 1. (e) Menzer, B. K.; Bannett, M. C.; Ledon, H. J. *Inorg. Chem.* **1980**, *19*, 2061. (f) Berg, J. M.; Spira, D.; Wo, K.; McCord, B.; Lye, R.; Co, M. S.; Belmont, J.; Barnes, C.; Kosydar, K.; Raybuck, S.; Hodgson, K. O.; Bruce, A. E.; Corbin, J. L.; Stiefel, E. I. *Inorg. Chim. Acta* **1984**, *90*, 35.
(21) Hofer, E.; Holzbach, W.; Wieghardt, K. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 282. Wieghardt, K.; Hahn, M.; Weiss, J.; Swiridoff, W. *Z. Anorg. Allg. Chem.* **1982**, *492*, 164. Bristow, S.; Collison, D.; Garner, C. D.; Clegg, W. *J. Chem. Soc., Dalton Trans.* **1983**, 2495.
(22) Berg, J. M.; Holm, R. H. *J. Am. Chem. Soc.* **1985**, *107*, 920.

Table IV. Selected Bond Angles (deg)[a]

	3	6	8	9	10	14	15	$MoO_2(S_2N_2)$[11]
O_t–Mo–O_t[b]	108.25 (7)	109.2 (2)	107.3 (1)	107.6 (2)	108.2 (1)	108.4 (2)	108.6 (3)	107.4 (1)
O_t–Mo–O_l	156.59 (6)		156.5 (1)	156.2 (2)	156.14 (8)	151.0 (1)		
S–Mo–S		165.69 (7)					159.27 (7)	159.91 (2)
N–Mo–N	74.74 (7)	74.0 (1)	73.20 (9)	73.3 (2)	70.59 (7)	74.12 (9)	74.4 (2)	74.52 (5)
O_t–Mo–O_l	95.66 (5) [O1, O11] 97.99 (5) [O1, O11]		94.4 (1) [O3, O1] 94.1 (1) [O3, O2] 99.8 (1) [O4, O1] 98.5 (1) [O4, O2]	94.1 (2) [O4, O1] 94.2 (2) [O4, O2] 99.2 (2) [O3, O1] 99.5 (2) [O3, O2]	95.96 (9) [O4, O1] 97.74 (9) [O4, O2] 98.02 (9) [O3, O1] 96.13 (9) [O3, O2]	101.8 (1) [O3, O1] 94.4 (1) [O3, O2] 96.2 (1) [O4, O1] 101.2 (1) [O4, O2]		
O_t–Mo–S		93.2 (1) [O1, S1] 95.1 (1) [O1, S2] 94.8 (1) [O2, S1] 93.5 (1) [O2, S2]					99.9 (2) [O1, S1] 91.3 (2) [O1, S10] 91.6 (2) [O2, S1] 101.3 (2) [O2, S10]	88.92 (7) [O1, S1] 102.39 (7) [O1, S2] 103.04 (7) [O2, S1] 89.50 (7) [O2, S2]
O_t–Mo–N	163.09 [O1, N11'] 88.55 [O1, N11]	87.9 (2) [O1, N2] 89.1 (2) [O2, N1] 161.5 (2) [O1, N1] 162.7 (2) [O2, N2]	95.1 (1) [O3, N1] 84.4 (1) [O4, N2] 168.1 (1) [O3, N2] 157.6 (1) [O4, N1]	94.1 (2) [O4, N1] 84.9 (2) [O3, N2] 167.4 (2) [O4, N2] 158.2 (2) [O3, N1]	91.72 (8) [O1, N1] 89.64 (8) [O2, N2] 162.04 (8) [O1, N2] 159.89 (8) [O2, N1]	90.1 (1) [O3, N1] 88.4 (1) [O4, N2] 161.7 (1) [O3, N2] 161.0 (1) [O4, N1]	90.3 (2) [O2, N7] 88.1 (3) [O1, N4] 161.2 (2) [O2, N4] 159.5 (2) [O1, N7]	92.45 (8) [O1, N2] 87.89 (8) [O2, N1] 161.38 (8) [O1, N1] 158.27 (8) [O2, N2]

[a] The atom labels for the individual ligand atoms coordinated to the Mo atom appear in brackets (see Figures 1–7). [b] O_t = terminal oxo group; O_l = ligand oxygen atom.

thioether S), S_4 (two thiolate S, two thioether S), NO_3 (three phenolate O), and N_4 (porphyrin) coordinating atoms, aliphatic and aromatic carbon bridges, linear and tripodal ligand geometries, five- and six-membered chelate rings, secondary (protonated), tertiary (alkylated), and aromatic nitrogen atoms, and ligands with steric bulk. While minor differences, which may be attributed to these features, are evident, the structures, with the exception of the Mo(VI)–dioxo porphyrin complex,[20e] have remarkably similar Mo–ligand bond lengths, bond angles, and geometries (distorted octahedral). A comparison with structures of Mo(VI)–dioxo complexes having other geometries (distorted-trigonal-prismatic, tetradentate N_4 (porphyrin),[20e] approximately tetrahedral, bidentate N_2O_2[21] trigonal-bipyramidal, tridentate NS_2[22] and skewed-trapezoidal, bidentate N_2S_2[23]) indicates no significant differences in Mo=O and Mo—S bond lengths exist. However, the structures of these other geometries have average Mo—N bond lengths that are considerably shorter (2.20, 2.145, 2.244, 2.269 Å, respectively) than those in the tetradentate, distorted-octahedral structures (2.407 Å). The only known Mo(VI)–dioxo complex with tetradentate ligands and a Mo—N bond cis to an oxo ligand has a bond length of 2.033 Å.[20b] Such a bond is also present in the complex MoO_2Br_2(bpy), which has a distorted geometry, with a Mo–N length of 2.259 (18) Å.[24] Thus, a Mo—N bond length >2.30 Å appears to be diagnostic of the presence of the trans O=Mo—N structure (as well as a distorted-octahedral geometry), with the bond length correlated to the size of the trans O=Mo—N bond angle.

EXAFS from the enzymes[2,3,6,8] yield Mo=O and Mo—S(thiolate) bond lengths that are comparable to those in Mo(VI)–dioxo complexes in several geometries, including distorted-octahedral complexes with tetradentate ligands (Mo=O = 1.66–1.75 Å for the enzymes and 1.684–1.750 Å for the complexes; Mo—S = 2.33–2.54 Å for the enzymes and 2.393–2.458 Å for the complexes). The somewhat greater range for Mo—S(thiolate) bond lengths in the enzymes may reflect the constraints of the protein, or it may be a result of the error in EXAFS measurements (2.33 ± 0.07 Å for SO, while no estimated error is reported for the 2.54-Å length in XO[3]). The X-ray structural studies of model Mo(VI) complexes discussed above imply that the Mo—N distance should be able to distinguish among possible coordination geometries of the molybdenum atom in the enzymes. Unfortunately, Mo—N bonds are not easily detectable by EXAFS spectroscopy in the presence of Mo—S bonds, and Mo—N bonds cannot be distinguished from Mo—O bonds.[3,25] EXAFS Mo—N(O) bond lengths of 2.12 Å (*Escherichia coli* NR), 2.07–2.10 Å (*Chlorella* NR) and 1.90 Å (*Desulfovibrio gigas* protein) have been reported.[3] However, the uncertainty in these bond lengths (if the bonds are indeed Mo—N bonds) is large. Mo—N(O) bonds have also been postulated for other enzymes (XDH and SO, for example), but the evidence for their presence is minimal.[3] Thus, neither the complete ligand set nor precise metrical data for bonds other than Mo=O, Mo=S, and Mo—S(thiolate) are known for any molybdenum enzyme, and no conclusions concerning the geometries of the molybdenum centers may yet be drawn from a comparison with known structures. In the absence of enzyme X-ray structures, identification of Mo—N bonds and precise determination of their lengths by EXAFS spectroscopy would provide important data with respect to the geometries of these centers.

Experimental Section

Starting Materials and Reagents. Reagent grade solvents, distilled and dried by standard methods, were used in all preparations.

Salicylaldehyde, *N,N'*-dimethylethylenediamine, *N,N*-dimethylethylenediamine, 2-(aminomethyl)pyridine, 2-(2-aminoethyl)pyridine, 1-(2-aminoethyl)piperidine, 2-(ethylthio)ethylamine, paraformaldehyde,

(23) Stiefel, E. I.; Miller, K. F.; Bruce, A. E.; Corbin, J. L.; Berg, J. M.; Hodgson, K. O. *J. Am Chem. Soc.* **1980**, *102*, 3624.
(24) Fenn, R. U. *J. Chem. Soc.* **1964**, 69.
(25) EXAFS analysis of the tetradentate N_2S_2 Mo(VI)–dioxo complex of ref 11, for which the X-ray structure is known, did not detect the presence of Mo–N bonds: Cramer, S. P.; George, G. N. Private communication.

2-*tert*-butylphenol, ethylmagnesium bromide, sodium cyanoborohydride, thiosalicylic acid, benzyl chloride, oxalyl chloride, borane–tetrahydrofuran, glyoxalbis(2-hydroxyanil), hexamethylphosphoramide, acetylacetone, and propylene sulfide were purchased from Aldrich Chemical Co. *N*-Methylethylenediamine and *N,N'*-diethylethylenediamine were purchased from Alfa Products. Sodium molybdate dihydrate was purchased from J. T. Baker Co. 2-Hydroxy-3-*tert*-butylbenzaldehyde was prepared by the procedure of Gasiraghi et al.[26]

Syntheses. Ligands. Ligand syntheses were monitored by IR and NMR spectroscopy in all cases. In particularly difficult syntheses, analyses were obtained.

***N,N'*-Bis(2-hydroxybenzyl)-*N*-methyl-1,2-diaminoethane (H_2L^1).** *N*-Methylethylenediamine (2.20 mL, 25.0 mmol) was dissolved in 50 mL of MeOH. Salicylaldehyde (3.10 g, 25.3 mmol) was added to the stirred solution. The yellow Schiff base began to form immediately, and stirring was continued for 2 h at room temperature. The solution was acidified to pH 5 with methanolic HCl. The second 1 equiv of salicylaldehyde (3.10 g, 25.3 mmol) was added to the solution with stirring, followed by $NaBH_3CN$ (3.11 g, 49.5 mmol). The reaction mixture was stirred 108 h at room temperature. The solvent was removed, and the residue was taken up in H_2O, extracted with $CHCl_3$ and dried over $MgSO_4$ and the $CHCl_3$ removed to give an orange oil, yield 80%.

***N,N'*-Bis(2-hydroxy-3-*tert*-butylbenzyl)-*N*-methyl-1,2-diaminoethane (H_2L^2).** *N*-Methylethylenediamine (0.66 mL, 7.5 mmol) was dissolved in 30 mL of MeOH. 3-*tert*-Butylsalicylaldehyde (1.60 g, 9.0 mmol) was added to the solution with stirring, and the yellow Schiff base began to form immediately. The pH was adjusted to 5.5 with methanolic HCl, then the second 1 equiv of aldehyde (1.60 g, 9.0 mmol) was added, followed by $NaBH_3CN$ (1.40 g, 22.3 mmol), and the reaction mixture was stirred at room temperature for 72 h. The solvent was removed, and the residue was taken up in H_2O, the solution was extracted with ethyl ether, the ether extracts were dried over $MgSO_4$, and the ether was removed to give a tan oil, yield 50%.

***N,N'*-Bis(2-hydroxybenzyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^3), *N,N'*-Bis(2-hydroxybenzyl)-*N,N'*-diethyl-1,2-diaminoethane (H_2L^4), and *N,N'*-Bis(2-hydroxy-3-*tert*-butylbenzyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^5).** These ligands were prepared by the method of Borch et al.,[15] with use of the appropriate stoichiometry, from salicylaldehyde and *N,N'*-dimethylethylenediamine, salicylaldehyde and *N,N'*-diethylethylenediamine, and 2-hydroxy-3-*tert*-butylbenzaldehyde and *N,N'*-dimethylethylenediamine, respectively. The resulting yellow viscous oils were purified by preparation of the hydrochloride salts: the oils were taken up in MeOH, and HCl gas was bubbled through. The solvent was removed and the resulting material triturated with Et_2O and dried under vacuum to give white solids, yields of purified products 60%, 70%, and 50%, respectively.

***N,N'*-Bis(2-mercaptobenzyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^6).** Thiosalicylic acid (9.7 g, 50 mmol) was dissolved in 50 mL of 2 M NaOH in 60 mL of EtOH. Benzyl chloride (7.0 g, 55 mmol) was added dropwise with stirring. Stirring was continued for 1 h, giving a clear light yellow solution. After the solution was cooled to −20 °C, the pH was adjusted to 2.5 with HCl and the white solid collected by filtration and washed with small portions of H_2O, EtOH, and Et_2O and dried under vacuum to give a 90% yield of *S*-benzylthiosalicylic acid; mp 185–188 °C.

To a stirred solution of the *S*-benzylthiosalicylic acid (3.8 g, 15.5 mmol) in 50 mL of dry benzene was added 6.2 mL (71 mmol) of oxalyl chloride. A few drops of DMF were added, and the reaction proceeded rapidly to give a clear yellow-green solution. After the solution was stirred for 1 h, the solvent was removed with a rotavaporator and the yellow solid was washed with small portions of Et_2O. The product (*S*-benzylthiosalicylic acid chloride) was dried under vacuum: Yield 98%; mp 113–117 °C.

To a solution of 1 M NaOH (24.5 mL) and 50 mL of benzene containing *N,N'*-dimethylethylenediamine (0.63 mL, 5.8 mmol) were added dropwise *S*-benzylthiosalicylic acid chloride (3.0 g, 1.14 mmol) in 50 mL of benzene and 30 mL of CH_2Cl_2 over a 1-h period. The solution was cooled to 0 °C, and the aqueous layer was collected and extracted with 3 × 30 mL of CH_2Cl_2. The solvent was removed under vacuum, giving a viscous yellow-white oil. Trituration with Et_2O gave a white solid (*N,N'*-bis(2-(benzylthio)benzoyl)-*N,N'*-dimethyl-1,2-diaminoethane): yield 85%; mp 122–124 °C.

A solution of *N,N'*-bis(2-(benzylthio)benzoyl)-*N,N'*-dimethyl-1,2-diaminoethane (2.5 g, 4.6 mmol) in 10 mL of THF was added to 27.6 mL of 1.0 M borane in THF dropwise over 15 min under N_2. The temperature was maintained at 0 °C during the addition. The mixture was refluxed for 1.5 h under N_2 and cooled and 10 mL of 1:1 HCl–H_2O added to destroy excess borane. THF was removed by distillation, and the aqueous solution remaining was neutralized with 1 M NaOH to pH 7.5 and the solution extracted with 3 × 40 mL of CH_2Cl_2. The extracts were combined, the solvent was removed under vacuum, and the white solid was washed with small portions of Et_2O and dried under vacuum, giving a 78% yield of the product (*N,N'*-bis(2-(benzylthio)benzyl)-*N,N'*-dimethyl-1,2-diaminoethane), mp 58–60 °C.

To a solution of *N,N'*-bis(2-(benzylthio)benzyl)-*N,N'*-dimethyl-1,2-diaminoethane (1.09 g, 2.0 mmol) in anhydrous liquid NH_3 was added fresh Na over 30 min until a permanent blue color was obtained. The blue solution was stirred for 30 min, the color was destroyed by addition of the minimum amount of NH_4Cl, and the NH_3 was allowed to evaporate under N_2. The solid was dissolved in 20 mL of H_2O, and the solution was extracted with 20 mL of CH_2Cl_2 and neutralized with 1 N NaOH to pH 7.0. The solution was extracted with 3 × 30 mL of CH_2Cl_2, the extracts were combined, and the solvent was removed under vacuum, giving a viscous oil. Trituration with Et_2O gave H_2L^6 as a solid: yield 70%; mp 124–125 °C. It was recrystallized from CH_2Cl_2–Et_2O. Anal. Calcd for $C_{18}H_{24}N_2S_2$: C, 65.02; H, 7.36; N, 8.42; S, 19.28. Found: C, 65.09; H, 7.36; N, 8.38; S, 19.41.

***N*-(2-Hydroxybenzyl)-*N'*-(2-mercaptobenzyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^7).** Salicylaldehyde (1.14 g, 9.3 mmol) was added with stirring to a solution of *N*-(2-(benzylthio)benzyl)-*N,N'*-dimethyl-1,2-diaminoethane in 50 mL of MeOH. The bright yellow Schiff base formed immediately, and stirring was continued for 1 h. The pH was lowered to 5.0 with 1:1 HCl–MeOH, and 0.88 g (14 mmol) of $NaCNBH_3$ was added slowly. After the mixture was stirred at room temperature for 72 h, the solvent was removed by rotavaporation and the residue dissolved in 50 mL of H_2O. The solution was extracted with 3 × 80 mL of CH_2Cl_2, and the extracts were combined and dried over $MgSO_4$. The solvent was removed by rotavaporation giving a light yellow oil (*N*-(2-benzylthio)benzyl-*N'*-(2-hydroxybenzyl)-*N,N'*-dimethyl-1,2-diaminoethane) in 75% yield. To a solution of this product (1.0 g, 2 mmol) in 20 mL of EtOH was slowly added fresh Na metal (2 g, 87 mmol), and the solution was refluxed for 4 h. The solvent was removed and the residue dissolved in 20 mL of H_2O. The aqueous solution was extracted with 20 mL of CH_2Cl_2 and the extract discarded. The aqueous solution was adjusted to pH 7.0 with 1 M NaOH and extracted with 3 × 30 mL of CH_2Cl_2. The extracts were combined and dried over $MgSO_4$, and the solvent was removed, giving a viscous oil. Tritrating with Et_2O and drying under vacuum gave a yellow solid (H_2L^7) in 25% yield.

***N,N*-Bis(2-hydroxybenzyl)-*N',N'*-dimethyl-1,2-diaminoethane (H_2L^8).** *N,N*-Dimethylethylenediamine (5.50 mL, 50.0 mmol) was dissolved in 75 mL of MeOH. Salicylaldehyde (6.11 g, 50.0 mmol) was added to the solution with stirring. The bright yellow Schiff base began to form immediately, and stirring was continued for 1.5 h at room temperature. The pH was lowered to 5 with methanolic HCl, and $NaBH_3CN$ (5.00 g, 80.0 mmol) was slowly added. The reaction mixture was stirred at room temperature for 72 h, during which time the color faded to light yellow. The second 1 equiv of salicylaldehyde (6.11 g, 50.0 mmol) was added, followed by $NaBH_3CN$ (5.00 g, 80.0 mmol), and stirring was continued for 72 h at room temperature. The solvent was removed, and the residue was taken up in 150 mL of H_2O. This solution was extracted with $CHCl_3$, the $CHCl_3$ extracts were dried over $MgSO_4$, and the $CHCl_3$ was removed to yield an off-white solid, yield 37%.

***N,N*-Bis(2-hydroxy-3-*tert*-butylphenyl)-*N',N'*-dimethyl-1,2-diaminoethane (H_2L^9).** *N,N*-Dimethylethylenediamine (0.80 mL, 7.3 mmol) was dissolved in 50 mL of MeOH, and 3-*tert*-butylsalicylaldehyde (1.30 g, 7.3 mmol) was added, resulting in a bright yellow solution, which was stirred at room temperature for 2 h. The pH was lowered to 5–6 with methanolic HCl, and $NaBH_3CN$ (0.70 g, 11.1 mmol) was added. After the mixture was stirred at room temperature for 2 h, the pH was checked and adjusted to 5, and additional $NaBH_3CN$ (0.50 g, 8.0 mmol) was added. The reaction mixture was stirred for 72 h at room temperature. The solvent was removed, and the residue was taken up in 50 mL of H_2O. The solution was extracted with $CHCl_3$, the $CHCl_3$ extracts were dried over $MgSO_4$, and the $CHCl_3$ was removed to yield a yellow oil (the monocondensed intermediate). This oil was redissolved in MeOH, the pH adjusted to 5 with methanolic HCl, and the second 1 equiv of 3-*tert*-butylsalicyaldehyde added, followed by addition of $NaBH_3CN$ (1.00 g, 15.9 mmol). The reaction mixture was stirred at room temperature for 68 h. Workup as above yielded a yellow oil. The yellow oil was dissolved in 25 mL of $CHCl_3$ and the solution washed three times with 10-mL portions of pH 2–3 aqueous HCl. The $CHCl_3$ layer was allowed to stand at room temperature, and slow evaporation of the solvent produced large off-white needles, which were washed with pentane and dried over Drierite in a vacuum desiccator; yield 20%.

***N,N*-Bis(2-hydroxybenzyl)-2-(aminomethyl)pyridine (H_2L^{10}).** 2-(Aminomethyl)pyridine (5.00 g, 46.2 mmol) was dissolved in 100 mL of MeOH. Salicylaldehyde (5.64 g, 46.2 mmol) was added with stirring,

(26) Gasiraghi, G.; Casnati, G.; Cornia, M.; Pochini, A.; Puglia, G.; Sartori, G.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 1* **1978**, 318.

and the bright yellow solution was stirred at room temperature for 1 h. $NaBH_3CN$ (3.00 g, 47.7 mmol) was added and the solution acidified to pH 5 with methanolic HCl. After the mixture was stirred overnight at room temperature, the orange solution had a pH of 8. The pH was lowered to 5 with methanolic HCl, and the color became yellow. $NaBH_3CN$ (4.00 g, 63.7 mmol) was added, and the mixture was refluxed for 1 h and stirred overnight at room temperature. The solvent was removed, the residue was taken up in 100 mL of H_2O, and the solution was extracted with $CHCl_3$. The $CHCl_3$ extracts were dried over $MgSO_4$, and the $CHCl_3$ was removed to give an orange semisolid. This material was washed with acetone to give a white solid, the monocondensed product, in 6% yield. The white solid (0.64 g, 3.0 mmol) was slurried in 150 mL of MeOH, and 1 equiv of salicylaldehyde (0.37 g, 3.0 mmol) was added. The reaction mixture was warmed until all solids went into solution. $NaBH_3CN$ (1.00 g, 15.9 mmol) was added, the pH was adjusted to 6 with methanolic HCl, and the reaction mixture was stirred at room temperature for 72 h. The solvent was removed, the residue was taken up in 50 mL of H_2O, the solution was extracted with $CHCl_3$, and the $CHCl_3$ extracts were dried over $MgSO_4$. The solvent was removed to give an off-white solid: yield of second step 64%; total yield 3.8%.

***N,N*-Bis(2-hydroxybenzyl)-2-(2-aminoethyl)pyridine (H_2L^{11}).** 2-(2-Aminoethyl)pyridine (3.00 g, 24.6 mmol) was added to 100 mL of MeOH. Salicylaldehyde (3.00 g, 24.6 mmol) was added to the stirred solution. The bright yellow solution was stirred at room temperature for 1 h, followed by addition of $NaBH_3CN$ (1.60 g, 25.5 mmol) and reduction of the pH to 6 with methanolic HCl. After 3 h of stirring at room temperature, the pH was lowered to 5 and stirring continued overnight at room temperature. $NaBH_3CN$ (1.60 g, 25.5 mmol) was added to this reaction mixture, and the pH was increased to 6–7 with methanolic NaOH. Salicylaldehyde (3.00 g, 24.6 mmol) was added, and the solution was stirred at room temperature for 72 h. The solvent was removed, the residue was taken up in 40 mL of H_2O, and the solution was extracted with ethyl acetate. The ethyl acetate extracts were dried over $MgSO_4$, and the ethyl acetate was removed to give a yellow oil. The oil was taken up in a small amount of acetone and the solution allowed to stand for several days at room temperature, after which time a white solid was deposited; this was washed with acetone and dried in vacuo; yield 33%.

***N,N*-Bis(2-hydroxybenzyl)-1-(2-aminoethyl)piperidine (H_2L^{12}).** 1-(2-Aminoethyl)piperidine (2.00 g, 15.6 mmol) was dissolved in 50 mL of MeOH. Salicylaldehyde (1.90 g, 15.6 mmol) was added, and the bright orange Schiff base solution was stirred at room temperature for 1.5 h. The pH was lowered to 6–7 with methanolic HCl, and $NaBH_3CN$ (1.00 g, 15.9 mmol) was added. The reaction mixture was stirred overnight at room temperature. The yellow solution was acidified further to a pH of 6, and the yellow color faded. Stirring was continued for 2.5 h at room temperature. $NaBH_3CN$ (1.00 g, 15.9 mmol) and salicylaldehyde (1.90 g, 15.6 mmol) was added, and the reaction mixture was stirred at room temperature for 72 h. The solvent was removed, the residue was taken up in 50 mL of H_2O, the solution was extracted with $CHCl_3$, the $CHCl_3$ extracts were dried over $MgSO_4$, and the $CHCl_3$ was removed to give a pale yellow solid. Washing with acetone yielded a white solid. NMR spectroscopy (DMSO-d_6) indicated that the second condensation had not been achieved; mainly the monocondensed product was present. This product (0.56 g, 2.4 mmol) was slurried in 50 mL of MeOH. One equivalent of salicylaldehyde (0.29 g, 2.4 mmol) was added. After the mixture was stirred overnight at room temperature, the amine had still not dissolved. The reaction mixture was warmed until the solution cleared, then excess $NaBH_3CN$ was added, and the reaction mixture was stirred overnight at room temperature; all of the yellow color had disappeared. Workup as above gave a white solid, yield from initial starting materials 9%.

***N,N*-Bis(2-hydroxybenzyl)-2-(ethylthio)ethylamine (H_2L^{13}).** 2-(Ethylthio)ethylamine hydrochloride (2.50 g, 17.7 mmol) was dissolved in 50 mL of MeOH. One equivalent of NaOH (0.70 g, 17.5 mmol) was added to the solution, and a white solid formed. Salicylaldehyde (2.16 g, 17.7 mmol) was added, and the bright yellow Schiff base began to form immediately. The reaction mixture was stirred at room temperature for 4 h, the pH was adjusted to 6 with methanolic HCl, and $NaBH_3CN$ (2.50 g, 39.8 mmol) was added. The reaction mixture was stirred overnight at room temperature. The solvent was removed, the residue was taken up in 50 mL of H_2O, the solution was extracted with ethyl ether, and the ethyl ether extracts were dried over $MgSO_4$. The ether was removed to give a yellow oil, the monocondensed product. This oil was redissolved in MeOH (50 mL), and salicylaldehyde (2.16 g, 17.7 mmol) was added to the solution with stirring. The pH was adjusted to 6–7 with methanolic HCl, and $NaBH_3CN$ (3.00 g, 47.7 mmol) was added. The reaction mixture was stirred overnight at room temperature, the pH lowered to 6, and the reaction mixture again stirred overnight at room temperature. Workup as above gave a light yellow oil, which became semicrystalline on standing. Washing with 10 mL of acetone gave a white solid, yield from initial reactants 25%.

***N,N'*-Bis(2-hydroxyphenyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^{14}).** To a solution of glyoxalbis(2-hydroxyanil) (5.6 g, 23 mmol), CH_2O (38 mL, 460 mmol), and $NaCNBH_3$ (8.79 g, 140 mmol) in 180 mL of MeCN under N_2 was added 4.5 mL of acetic acid over 10 min, and stirring was continued for 6 h. An additional 4.5 mL of acetic acid was added, and the solution was allowed to stand for 2 h. After removal of solvent, the residue was treated with 50 mL of H_2O and neutralized (pH 7) with 1:1 HCl–H_2O. It was extracted with 3 × 100 mL portions of CH_2Cl_2, and the extracts were collected and dried over $MgSO_4$. The CH_2Cl_2 was removed with a rotavaporator, giving H_2L^{14} as a white solid: yield 45%; mp 112 °C. Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.28. Found: C, 70.43; H, 7.56; N, 10.06.

***N,N'*-Bis(2-methyl-2-mercaptoethyl)-*N,N'*-dimethyl-1,2-diaminoethane (H_2L^{15}).** Propylene sulfide (44 mL, 560 mmol) in 30 mL of benzene was added dropwise to a warm solution of *N,N'*-dimethylethylenediamine (30 mL, 280 mmol) in 80 mL of benzene. The solution was stirred for 10 h and then refluxed for 6 h. The solution was cooled and extracted with 30 mL of H_2O and the benzene phase dried over anhydrous $MgSO_4$. The solution was filtered and the solvent removed under vacuum. The product (H_2L^{15}) was obtained in 80% yield as a colorless liquid.

Complexes. MoO_2L^1 (1). $MoO_2(acac)_2$ (0.51 g, 1.6 mmol) was dissolved in 15 mL of MeOH. The unpurified ligand (H_2L^1; 0.70 g, 2.5 mmol) was dissolved in 15 mL of MeOH and the solution added with stirring to the $MoO_2(acac)_2$ solution. The yellow color of the solution intensified, and after 30 min of stirring at room temperature, a yellow precipitate began to form. Stirring was continued overnight at room temperature. The yellow precipitate was filtered, washed with 3 × 5 mL of MeOH, and dried under vacuum. The crude product was recrystallized from hot acetone–MeOH; yield 25%. Anal. Calcd for $MoC_{17}H_{20}N_2O_4$: C, 49.55; H, 4.85; N, 6.97. Found: C, 49.72; H, 5.01; N, 6.47.

MoO_2L^2 (2). $MoO_2(acac)_2$ (1.64 g, 5.0 mmol) was dissolved in 20 mL MeOH. The unpurified ligand (H_2L^2; 2.00 g, 5.0 mmol) was dissolved in 10 mL of MeOH and the solution added with stirring to the $MoO_2(acac)_2$ solution. The reaction mixture was refluxed for 4 h, cooled, and held overnight at −20 °C. The yellow precipitate was filtered, washed with 3 × 3 mL portions of MeOH, and dried under vacuum; yield 3%. Anal. Calcd for $MoC_{25}H_{36}N_2O_4$: C, 57.25; H, 6.92; N, 5.34. Found: C, 56.07; H, 7.19; N, 5.08.

MoO_2L^3 (3). $MoO_2(acac)_2$ (0.65 g, 2.0 mmol) was dissolved in 15 mL of MeOH. The ligand (H_2L^3; 0.60 g, 2.0 mmol) was dissolved in 30 mL of MeOH, and the solution was added with stirring to the $MoO_2(acac)_2$ solution. The yellow color intensified, and a yellow precipitate began to form in about 1 min. The reaction mixture was stirred overnight at room temperature. The yellow precipitate was filtered, washed three times with small portions of MeOH, and dried in vacuo; yield 88%. Anal. Calcd for $MoC_{18}H_{22}N_2O_4$: C, 50.76; H, 5.21; N, 6.57. Found: C, 50.61; H, 5.40; N, 6.54.

MoO_2L^4 (4). $MoO_2(acac)_2$ (0.65 g, 2.0 mmol) was dissolved in 15 mL of MeOH. The ligand dihydrochloride (H_2L^4·HCl; 0.66 g, 1.6 mmol) and NaOMe (0.22 g, 4.0 mmol) were dissolved in 15 mL of MeOH, and the solution was added with stirring to the $MoO_2(acac)_2$ solution. The yellow color itensified, and the reaction mixture was stirred at room temperature for 3 h. The volume was reduced under vacuum by 10 mL, and a yellow precipitate began to form. The reaction mixture was stirred an additional 1 h at room temperature, then the volume was reduced by half, and the reaction mixture was kept at −20 °C overnight. The yellow solid was filtered, washed with 3 × 3 mL of MeOH, and dried under vacuum. The yellow solid was recrystallized from CH_2Cl_2–MeOH to give shiny yellow flakes, yield 12%. Anal. Calcd for $MoC_{20}H_{28}N_2O_4$: C, 52.89; H, 5.73; N, 6.17. Found: C, 52.68; H, 5.98; N, 5.92.

MoO_2L^5 (5). $MoO_2(acac)_2$ (0.51 g, 1.6 mmol) was dissolved in 20 mL of MeOH. The unpurified ligand (H_2L^5; 0.80 g, 1.9 mmol) was dissolved in 8 mL of MeOH, and the solution was added with stirring to the $MoO_2(acac)_2$ solution. The solution changed from pale yellow to a deep yellow-orange. The reaction mixture was refluxed for 2 h; a yellow precipitate began to appear between 1 and 2 h. The solution was cooled and held at −20 °C overnight. The yellow product was filtered, washed with 3 × 5 mL of MeOH, and dried under vacuum; yield 50%. Anal. Calcd for $MoC_{26}H_{38}N_2O_4$: C, 58.02; H, 7.06; N, 5.20. Found: C, 58.82; H, 7.23; N, 5.20.

MoO_2L^6 (6). $[(n\text{-}Bu_4)N]_4Mo_8O_{26}$ (1.09 g, 0.46 mmol) was dissolved in 25 mL of hot MeOH. After the solution was cooled to room temperature, the ligand (H_2L^6; 0.46 mmol) in 5 mL of CH_2Cl_2 was added dropwise with stirring. After the reaction mixture was stirred for 1 h, the solid was collected by filtration, washed with 3 × 20 mL portions of MeOH, and dried under vacuum; yield 60%. It was recrystallized from CH_2Cl_2–Et_2O by vapor diffusion (Et_2O into a solution of **6** in CH_2Cl_2).

Table V. Summary of Crystal and Refinement Data for Complexes

	3	6	8	9	10	14	15
space group	$C2/c$	$P2_1/c$	$P2_12_12_1$	$P2_12_12$	$P2_1/n$	$P2_1/n$	$P2_1/c$
a, Å	19.003 (5)	8.018 (2)	7.896 (1)	26.116 (5)	14.532 (2)	11.218 (2)	7.230 (15)
b, Å	7.437 (2)	13.323 (4)	12.932 (2)	13.030 (4)	7.1766 (6)	8.020 (1)	23.793 (71)
c, Å	17.468 (5)	17.998 (4)	17.736 (3)	8.458 (2)	17.843 (3)	18.535 (3)	12.001 (22)
β, deg	132.14 (1)	96.59 (2)			104.44 (1)	103.97 (1)	106.28 (15)
V, Å^3	1830.5	1909.9	1811.0	2878.3	1802.1	1618.1	1981.7
Z	4	4	4	4	4	4	4
fw	426.33	454.42	426.33	580.01	446.32	398.27	479.73
D(calcd), g cm^{-3}	1.55	1.58	1.56	1.34	1.65	1.63	1.61
radiation, Å	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)	Mo Kα (λ = 0.71073)
temp, °C	23 ± 1	23 ± 1	23 ± 1	23 ± 1	23 ± 1	23 ± 1	23 ± 1
μ, cm^{-1}	7.2	8.9	7.3	4.7	7.4	8.1	12.6
scan type	2θ–θ	2θ–θ	2θ–θ	2θ–θ	2θ–θ	2θ–θ	2θ–θ
scan speed, deg min^{-1}	1.5–15.0	2–8	2.5–12.0	2–8	2–8	2–8	2–8
scan width, deg	2.3 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.0 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.0 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.0 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.2 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.0 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)	2.2 + ($2\theta_{K\alpha_2}$ – $2\theta_{K\alpha_1}$)
max 2θ, deg	50.0	50.0	55.0	55.0	55.0	50.0	50.0
no. of unique rflns	2700	3388	4175	3752	4157	2874	3526
no. of unique data used with $F_o^2 > 3.0\sigma(F_o^2)$	2475	1979	3789	2764	3210	2157	1872
R	0.021	0.038	0.033	0.058	0.028	0.029	0.044
R_w[a]	0.030	0.042	0.047	0.068	0.036	0.036	0.049
GOF[b]	1.45	1.14	1.49	1.84	1.21	1.23	1.27

[a] $R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$. [b] GOF = $[\sum w(|F_o| - |F_c|)^2/(NO - NV)]^{1/2}$; NO = number of deviations; NV = number of variables.

Anal. Calcd for $MoC_{18}H_{22}N_2O_2S_2$: C, 47.16; H, 4.84; N, 6.11; S, 13.99. Found: C, 47.36; H, 5.00; N, 6.07; S, 13.81.

MoO_2L^7 (7). This complex (deep orange) was prepared in a manner identical with that for **6**. Anal. Calcd for $MoC_{18}H_{22}N_2O_3S$: C, 48.87; H, 5.01; N, 6.33; S, 7.25. Found: C, 48.66; H, 4.95; N, 6.09; S, 7.31.

MoO_2L^8 (8). $MoO_2(acac)_2$ (0.65 g, 2.0 mmol) was dissolved in 20 mL of MeOH. The ligand (H_2L^7; 0.60 g, 2.0 mmol) was dissolved in 65 mL of MeOH and the solution added with stirring to the $MoO_2(acac)_2$ solution. A bright yellow precipitate began to form almost immediately. The reaction mixture was stirred overnight at room temperature, and the yellow precipitate was filtered, washed with 4 × 5 mL of MeOH, and dried under vacuum; yield 30%. Anal. Calcd for $MoC_{18}H_{22}N_2O_4$: C, 50.74; H, 5.16; N, 6.57. Found: C, 50.80; H, 5.43; N, 6.59.

MoO_2L^9 (9). $MoO_2(acac)_2$ (0.14 g, 0.4 mmol) and the ligand (H_2L^8; 0.17 g, 0.4 mmol) were placed as solids together in a flask. To the flask was added 30 mL of MeOH. The ligand is not very soluble; the slurry was refluxed for 2.5 h, after which time all solids had dissolved to form a bright yellow solution, which deposited a yellow precipitate upon cooling. The flask was kept at −20 °C for several hours. The yellow solid was filtered, washed three times with MeOH and dried under vacuum; yield 76%. Anal. Calcd for $MoC_{26}H_{38}N_2O_4$: C, 58.02; H, 7.06; N, 5.20. Found: C, 57.93; H, 6.98; N, 5.30.

MoO_2L^{10} (10). $MoO_2(acac)_2$ (0.62 g, 1.9 mmol) and the ligand (H_2L^{10}; 0.61 g, 1.9 mmol) were placed together in the reaction flask. MeOH (20 mL) was added, and the solution began to develop a bright yellow color immediately. The reaction mixture was stirred overnight at room temperature, giving a bright yellow solid, which was isolated, washed with 3 × 10 mL of MeOH, and dried under vacuum; yield 47%. Anal. Calcd for $MoC_{20}H_{18}N_2O_4$: C, 53.82; H, 4.06; N, 6.28. Found: C, 53.89; H, 4.25; N, 6.16.

MoO_2L^{11} (11). $MoO_2(acac)_2$ (0.65 g, 2.0 mmol) and the ligand (H_2L^{11}; 0.67 g, 2.0 mmol) were placed together in the reaction flask, and 30 mL of MeOH was added. The slurry was stirred at room temperature for 48 h, after which time all solids had dissolved, giving a yellow-orange solution. The volume was reduced to 15 mL, and stirring at room temperature was continued. After a total of 72 h a yellow precipitate had been deposited. Stirring was continued an additional 24 h, and a yellow solid was isolated, washed three times with small portions of MeOH, and dried under vacuum; yield 51%. Anal. Calcd for $MoC_{21}H_{20}N_2O_4$: C, 54.80; H, 4.38; N, 6.08. Found: C, 54.67; H, 4.54; N, 5.86.

MoO_2L^{12} (12). $MoO_2(acac)_2$ (0.45 g, 1.4 mmol) and the ligand (H_2L^{12}; 0.47 g, 1.4 mmol) were placed in the reaction flask. To the flask was added 20 mL of MeOH, and the slurry began to develop a bright yellow color. After the mixture was stirred 12 h at room temperature, a yellow precipitate had formed. Stirring was continued for a total of 60 h, and the volume was reduced by half. The yellow product was isolated, washed with 3 × 3 mL of MeOH, and dried under vacuum; yield 44%. Anal. Calcd for $MoC_{21}H_{26}N_2O_4$: C, 54.07; H, 5.63; N, 6.01. Found: C, 53.89; H, 5.44; N, 5.92.

MoO_2L^{13} (13). $MoO_2(acac)_2$ (0.65 g, 2.0 mmol) was dissolved in 10 mL of MeOH. The ligand (H_2L^{13}; 0.70 g, 2.2 mmol) was dissolved in 10 mL of MeOH and added with stirring to the $MoO_2(acac)_2$ solution. The yellow color intensified. After the mixture was stirred at room temperature for 65 h, no solid had been deposited. The solvent was removed and the residue triturated three times with ethyl ether (50 mL total), giving a yellow-orange solid, which was dried under vacuum and recrystallized from CH_2Cl_2–MeOH at −20 °C to give a slightly greenish yellow solid, yield 40%. Anal. Calcd for $MoC_{18}H_{21}NO_4S$: C, 48.76; H, 4.78; N, 3.16; S, 7.14. Found: C, 48.65; H, 4.65; N, 3.17; S, 7.14.

MoO_2L^{14} (14). This complex was prepared in a manner identical with that for **6**; yield 75%. Anal. Calcd for $MoC_{16}H_{18}N_2O_4$: C, 48.25; H, 4.56; N, 7.03. Found: C, 47.43; H, 4.78; N, 6.90.

MoO_2L^{15} (15). To a solution of $MoO_2(acac)_2$ (3.63 g, 11.1 mmol) in 40 mL of MeOH (solution by heating) was added dropwise the ligand (H_2L^{15}; 3.66 g, 11.2 mmol) in 10 mL of MeOH, which gave a color change from light yellow to bright orange. Stirring was continued for 3 h, during which time a bright yellow solid precipitated. The solid was collected by filtration, washed with small portions of MeOH and Et_2O, and dried under vacuum; yield 36%. Anal. Calcd for $MoC_{10}H_{72}N_2O_2S_2$: C, 33.14; H, 6.11; N, 7.73; S, 17.88. Found: C, 33.39; H, 5.91; N, 7.78; S, 17.80.

X-ray Structure Determinations. Crystal and refinement data are summarized in Table V. Preliminary examination and data collection were performed on a Syntex $P2_1$ diffractometer equipped with a graphite crystal incident beam monochromator. The scan range (in degrees) was determined as a function of 2θ to correct for the separation of the Kα doublet.[27] The diameter of the incident beam collimator was 0.75 mm, and the ratio of peak-counting time to background-counting time was 2:1.

Data Reduction. Lorentz and polarization corrections were applied to the data. The linear absorption coefficients are 12.6 cm^{-1} or less for Mo Kα radiation, and no absorption corrections were made. Intensities of equivalent reflections were averaged.

Structure Solution and Refinement. The structures were solved by using the Patterson heavy-atom method, which revealed the position of the Mo atom. The hydrogen atoms were included in the refinement and restrained to ride on the atom to which they are bonded, with fixed thermal parameters. The geometry of the hydrogens was idealized, and the bond lengths were fixed at 0.95 Å. The structures were refined by full-matrix least squares, where the function minimized is $\sum w(|F_o| - |F_c|)^2$. The standard deviation on F^2, $\sigma(F^2)$, was defined as $\sigma(F^2) = (\sigma^2(I) + (pF^2)^2)^{1/2}$. The weights for each reflection were calculated by using the counterweighting scheme given by $w = 4(F_o^2)/\sigma^2(F_o^2)$, where the uncertainty factor, p, was set to the value 0.040. Scattering factors were taken from Cromer and Waber.[28] Anomalous dispersion effects were included in F_c;[29] the values for $\Delta f'$ and $\Delta f''$ were those of Cromer.[30]

(27) *Nicolet P3: Data Collection Operation Manual*; Nicolet XRD Corp.: Madison, WI, 1982.
(28) Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.2B.
(29) Ibers, J. A.; Hamilton, W. C. *Acta Crystallogr.* **1964**, *17*, 781.

Reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. All calculations were performed on a VAX computer using SDP/VAX or a PDP-11 computer using SDP-PLUS.[31]

Molecular Dynamics. Molecular dynamics calculations were performed with the software Biograf (version 2.0) from Biodesign, Inc., on an Ardent Titan Computer.

Acknowledgment. Financial support for this work by National Science Foundation Grant CHE-8402136 and National Institutes of Health Grant GM-08347 (J.T.S.) and National Institutes of Health Grant GM-37773 (J.H.E.) is gratefully acknowledged. Dr. Daniel L. Comins, Department of Chemistry, North Carolina State University, is thanked for his help and advice concerning ligand syntheses. The complete structures were determined in the Molecular Structure Laboratory of the Department of Chemistry of the University of Arizona.

Registry No. **1**, 105810-22-8; **2**, 123641-32-7; **3**, 105810-21-7; **4**, 105810-24-0; **5**, 105821-52-1; **6**, 123641-33-8; **7**, 123641-34-9; **8**, 123641-35-0; **9**, 123641-36-1; **10**, 123674-06-6; **11**, 123641-37-2; **12**, 123674-07-7; **13**, 123641-38-3; **14**, 123641-39-4; **15**, 84191-23-1; H_2L^1, 123641-18-9; H_2L^2, 123641-19-0; H_2L^3, 121788-87-2; H_2L^4, 121788-88-3; H_2L^5, 123641-20-3; H_2L^6, 123674-04-4; H_2L^7, 123641-21-4; H_2L^8, 123641-22-5; H_2L^9, 123641-23-6; H_2L^{10}, 123674-05-5; H_2L^{11}, 123641-24-7; H_2L^{12}, 123641-40-7; H_2L^{13}, 123641-25-8; H_2L^{14}, 123641-26-9; H_2L^{15}, 123641-27-0; $MoO_2(acac)_2$, 17524-05-9; $[(n\text{-}Bu)_4N]_4Mo_8O_{26}$, 59054-50-1; CH_2O, 50-00-0; *N*-methylethylenediamine, 109-81-9; salicylaldehyde, 90-02-8; 3-*tert*-butylsalicylaldehyde, 24623-65-2; *N*,*N*′-dimethylethylenediamine, 110-70-3; *N*,*N*′-diethylethylenediamine, 111-74-0; thiosalicylic acid, 147-93-3; benzyl chloride, 100-44-7; *S*-benzylthiosalicylic acid, 1531-80-2; *S*-benzylthiosalicylic acid chloride, 1531-81-3; *N*,*N*′-bis(2-(benzylthio)benzoyl)-*N*,*N*′-dimethyl-1,2-diaminoethane, 123641-28-1; *N*,*N*′-bis(2-(benzylthio)benzyl)-*N*,*N*′-dimethyl-1,2-diaminoethane, 123641-29-2; *N*-(2-(benzylthio)benzyl)-*N*,*N*′-dimethyl-1,2-diaminoethane, 123641-30-5; *N*-(2-(benzylthio)-*N*′-(2-hydroxybenzyl)-*N*,*N*′-dimethyl-1,2-diaminoethane, 123641-31-6; 2-(aminomethyl)pyridine, 3731-51-9; 2-(2-aminoethyl)pyridine, 2706-56-1; 1-(2-aminoethyl)piperidine, 27578-60-5; 2-(ethylthio)ethylamine hydrochloride, 54303-30-9; glyoxal bis(2-hydroxyanil), 1149-16-2; propylene sulfide, 1072-43-1; hydroxylase, 9046-59-7.

Supplementary Material Available: Listings of positional parameters (Tables 3.1, 6.1, 8.1, 9.1, 10.1, 14.1, and 15.1), general temperature factors, *U*'s (Tables 3.2, 6.2, 8.2, 9.2, 10.2, 14.2, and 15.2), and additional bond distances and angles (Tables 3.3, 3.4, 6.3, 6.4, 8.3, 8.4, 9.3, 9.4, 10.3, 10.4, 14.3, 14.4, 15.3, and 15.4) (31 pages); listings of observed structure factors (Tables 3.5, 6.5, 8.5, 9.5, 10.5, 14.5, and 15.5) and unobserved structure factors (Tables 3.6, 6.6, 8.6, 9.6, 10.6, 14.6, and 15.6) (68 pages). Ordering information is given on any current masthead page.

(30) Cromer, D. T. *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.3.1.
(31) Frenz, B. A. In *Computing in Crystallography*; Schenk, H., Olthof-Hazelkamp, R., van Konigsveld, H., Bassi, G. C., Eds.; Delft University Press: Delft, Holland, 1978; pp 64–71.

Contribution from the Department of Chemistry, Princeton University, Princeton, New Jersey 08544-1009, and Department of Pharmacology, University of Connecticut Health Center, Farmington, Connecticut 06032

Influence of Thiolate Ligation on the Heme Electronic Structure in Microsomal Cytochrome P-450 and Model Compounds: Resonance Raman Spectroscopic Evidence

Pavel Anzenbacher,*[,†,§] Ruby Evangelista-Kirkup,[†,∥] John Schenkman,[‡] and Thomas G. Spiro*[,†]

Received January 27, 1989

Resonance Raman spectra are reported for rat liver microsomal (RLM3) cytochrome P-450 in oxidized, reduced, and CO-bound forms, and are compared with spectra of model compounds. Contrary to a previous report, thiolate coordination per se is sufficient to shift the ν_4 porphyrin mode frequency of Fe^{II} heme to 1341 cm^{-1}, from the normal 1357-cm^{-1} frequency when bound to imidazole in a five-coordinate complex. A corresponding downshift of ν_3 from 1471 to 1463 cm^{-1} is noted. Other bands can also be assigned on the basis of expected shifts upon increased back-bonding to the porphyrin ring. No additional protein influences are required to explain the RR spectra. The CO-bound and oxidized forms are likewise adequately modeled by protein-free complexes with thiolate ligation. In these cases the shifts relative to complexes with imidazole ligands are small. These differential frequency shifts are discussed in terms of the donor properties of the thiolate ligand, which are transmitted to the porphyrin ring for Fe^{II} heme, but are accommodated by the ferric ion of Fe^{III} heme and by the CO ligand in the Fe^{II}–CO adduct (as seen in the C–O and Fe–CO stretching frequencies). There is a striking difference between the thiolate influence on the porphyrin ground state of the CO adduct (minimal effect, small vibrational frequency shifts) and on its excited state (major perturbation resulting in a split Soret band with a low-energy 450-nm component).

Introduction

The cytochromes P-450 are ubiquitous heme enzymes that catalyze the insertion of one O atom from O_2 into organic substrates, the other O atom being reduced to water.[1] They have attracted widespread attention because of the importance of this reaction for numerous metabolic processes and because of its intrinsic chemical interest. The mechanism of the reaction has been worked out in considerable detail. It involves one-electron reduction of the Fe^{III} substrate-bound enzyme to the Fe^{II} state, which binds O_2. Addition of a second electron induces O—O heterolysis and generates a putative intermediate, analogous to the Fe^{IV}=O cation radical intermediate compound I of horseradish peroxidase,[2] which transfers the O atom to the substrate, regenerating the Fe^{III} enzyme. An important feature of the enzyme is that the proximal ligand of the heme Fe atom is the thiolate side chain of a cysteine residue. A variety of spectroscopic information about this ligand has been assembled,[3] and the coordination of Cys-357 to the heme Fe is evident in the X-ray crystal structure of the camphor-metabolizing enzyme $P\text{-}450_{cam}$ in sub-

* To whom correspondence should be addressed.
† Princeton University.
‡ University of Connecticut Health Center.
§ Current address: Department of Biochemistry, Charles University, Albertov 2030 128 40 Prague 2, Czechoslovakia.
∥ Current address: Colgate-Palmolive Co., 909 River Road, Piscataway, NJ 08854.

(1) (a) Sligar, S. G.; Murray, R. I. *Cytochrome P-450 Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 443–479. (b) Guengerich, F. P.; MacDonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9–16. (c) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315–336. (d) Groves, J. T. *Adv. Inorg. Biochem.* **1979**, 119–145.
(2) (a) Dunford, H. B.; Stillman, J. S. *Coord. Chem. Rev.* **1976**, *19*, 187–251. (b) Hewson, W. D.; Hager, L. P. In *The Porphyrins*: Dolphin, D., Ed.; Academic: New York, 1979; Vol. 7, pp 295–332. (c) Frew, J. E.; Jones, P. *Adv. Inorg. Bioinorg. Mech.* **1984**, *3*, 175–212. (d) Yonetani, T.; Yamamoto, H.; Erman, J. E.; Leigh, J. S.; Reed, G. H. *J. Biol. Chem.* **1971**, *247*, 2447–2455. (e) Mauk, M. R.; Giroti, A. W. *Biochemistry* **1974**, *13*, 1757–1763.
(3) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255–1276.

0020-1669/89/1328-4491$01.50/0