Functional and Structural Model for the Molybdenum–Pterin Binding Site in Dimethyl Sulfoxide Reductase. Synthesis, Crystal Structure, and Spectroscopic Investigations of Trichloro(*quinonoid*-N(8)*H*-6,7-dihydropterin)oxomolybdenum(IV)

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Dimethyl sulfoxide is the substrate to the molybdenum-dependent enzyme dimethyl sulfoxide reductase, which is a member of the large group of molybdenum-containing non-nitrogenase redox enzymes. The active site of these enzymes is thought to possess a so-called molybdopterin, a hydrogenated pterin with an unusual side chain containing a dithiolene group. Up to now the enzyme reactivity was mostly attributed to molybdenum and to the coordination of these sulfur ligands in the side chain. The pterin moiety was not taken into account as playing an active part essential for the enzyme reaction. We demonstrated for the first time a possible coordination of a hydrogenated pterin to molybdenum with a complex of *quinonoid*-dihydro-L-*bio*pterin bound to molybdenum in the oxidation state +IV. Now we report the synthesis, crystal structure, and spectroscopic data for trichloro(*quinonoid*-N(8)*H*-6,7-dihydropterin)oxomolybdenum(IV), $[MoOCl_3(H^+\text{-}q\text{-}H_2Ptr)]$ (**1**) (dihydropterin = H_2Ptr).[1] Crystal data: $a = 9.966(3)$ Å, $b = 14.408(4)$ Å, $c = 17.362(5)$ Å, $V = 2493(2)$ Å^3, $Z = 8$, orthorhombic, space group *Pbca*, $R_1 = 0.059$ and $wR_2 = 0.150$. **1** is synthesized in a redox reaction between $Mo(VI)O_2Cl_2$ and tetrahydropterin [$H_4Ptr\cdot 2HCl$] and contains a cationic quinonoid dihydropterin coordinated via the N(5) and O(4) atoms to the molybdenum atom. The crystal structure of **1** containing the hydrogenated pterin exhibits an unusually short Mo–N(5) bond length of 2.013(3) Å, as compared to 2.324(6) Å for the corresponding bond in oxidized pterin. **1** is able to quantitatively reduce the substrate dimethyl sulfoxide to dimethyl sulfide under strict exclusion of oxygen. This reaction can be monitored by ^{13}C-NMR spectroscopy. A simplified in vivo reaction cycle for the enzyme center of DMSO reductase is proposed as a working hypothesis.

Introduction

A large number of life processes are catalyzed by metalloenzymes, and molybdenum is one of the metal centers responsible for important reactions in the metabolism of bacteria, algae, fungi, plants, and mammals. In addition to nitrogenase, there is a large group of molybdenum-containing enzymes catalyzing oxidation and reduction of various substrates.[2–6]

Several bacterial species, e.g. *Rhodobacter sphaeroides*[7] and *Rhodobacter capsulatus*,[8] possess the ability to reduce dimethyl sulfoxide to dimethyl sulfide with the help of the molybdenum-dependent enzyme dimethyl sulfoxide reductase. Although the different molybdenum enzymes have molecular weights of at least 100 000, most of them possess a common cofactor with a molecular weight of about 1500. This cofactor contains the active metal center coordinated by different ligands. One possible ligand found in the vicinity of molybdenum is a pterin unit. Rajagopalan and co-workers proposed a model for the cofactor consisting of a C(6)-substituted tetrahydropterin, where two sulfur atoms of a dithiolene group in the sidechain, but not the pterin nucleus, are coordinated to the molybdenum(VI) atom[9–12] (Figure 1).

The oxidation state of the molybdenum atom is thought to switch between +VI and +IV in the enzyme reactions, with a short living EPR-detectable state +V. In the model of a *common molybdenum cofactor* there is no differentiation between the oxidation states and possibly changing ligands.

The oxidase and reductase activity in the molybdenum enzymes is generally coupled with the uptake or release of oxygen atoms, the so-called oxygen atom transfer reaction.[13,14]

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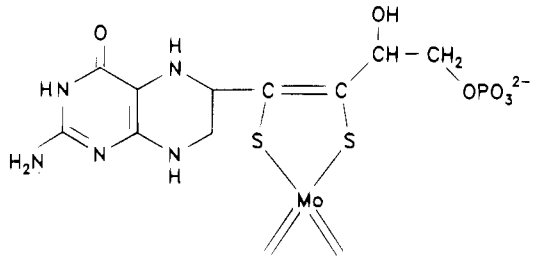
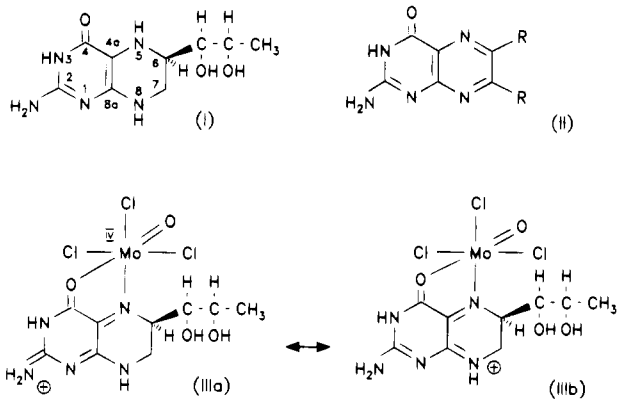


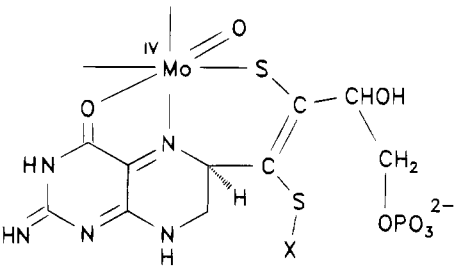
Figure 1. Proposal of a common molybdenum cofactor.

Scheme 1



Terminal oxygen and/or sulfur atoms and the sulfur atoms of the dithiolene group in the coordination sphere of the molybdenum atom should solely be responsible for maintaining the proper potentials for a reversible reaction.

The redox chemistry of the dithiolene group with regards to metal ions is known only in part, and there exist only a few isolated Mo–dithiolene complexes with additional ligands and even less information on their redox properties.[15–17] On the other hand, the redox properties of hydrogenated pterins have been well-known for quite a long time.[18–22] Their direct participation in enzyme reactions, e.g. 6*R*-tetrahydro-L-biopterin [H_4Bip] (**I**)[1] in hydroxylases, is well established,[19,20] and there are existing sophisticated enzyme systems in metabolism for synthesizing and reducing hydrogenated pterins.[23–27] Still not clear are the reasons for the additional necessity of iron e.g. in human phenylalaninehydroxylase, and of copper in bacterial hydroxylases.[19–21] Interaction of H_4Bip (**I**) with metal ions can be assumed.[28–31] In the established enzyme systems, the oxidation state of the pterins switches in two-electron steps



X = PROTEIN

Figure 2. Proposal of an extended molybdenum cofactor in its reduced form.[32]

between the tetrahydro- and the quinonoid–dihydropterin forms. The fully oxidized pterins (**II**) are generally unreactive and are not used in nature for redox reactions (see Scheme 1).

Neither an isolated compound nor any crystal structure of a complex between a metal and a hydrogenated pterin was known before we demonstrated with the first molybdenum–dihydropterin complex, trichloro(*quinonoid*-N(8)*H*-6,7-dihydro-L-biopterin)oxomolybdenum(IV) [$MoOCl_3(H^+$-*q*-H_2Bip)] (III) that chelating coordination of molybdenum by the hydrogenated pterin nucleus through O(4) and N(5) is possible.[32] In contrast to several structurally characterized metal complexes of oxidized pterins with mostly the same coordination type,[33–39] this complex displays an unusually short metal–N(5) distance. $MoOCl_3(H^+$-*q*-H_2Bip) (**III**) exhibits an interesting behavior in solution, which can be monitored by ^{13}C-NMR spectroscopy. When the compound is dissolved in solvents such as methanol or dimethyl sulfoxide, a slow equilibrium reaction takes place, resulting in the starting compound tetrahydro-L-biopterin [H_4Bip] and a Mo(VI) complex of yet unknown composition in addition to the still present $MoOCl_3(H^+$-*q*-H_2Bip) complex. For the first time it could be shown that a quinonoid–dihydrobiopterin might be converted to a tetrahydrobiopterin in the presence of a transition metal. This structure prompted us, supported by spectroscopical data, to propose a participation of the pterin nucleus in the enzyme reaction of molybdenum dependent oxidoreductases and to formulate a model of an extended *molybdenum cofactor* in its reduced form (Figure 2).[32,40]

In this paper a new molybdenum–quinonoid–dihydropterin complex and its structural and spectroscopical characterization are described. The complex is able to quantitatively reduce the substrate dimethyl sulfoxide to dimethyl sulfide in the absence of oxygen. This reaction can be monitored by ^{13}C-NMR spectroscopy.

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On the basis of the reactivity investigations and the proposal of the extended molybdenum cofactor model, we present a simplified model of an in vivo reaction cycle for the active center of dimethyl sulfoxide reductase as a working hypothesis.

Experimental Section

Starting Materials. MoO_2Cl_2 was prepared according to Wasmuht in a slightly modified way,[41] $MoCl_4$ by a method described in the literature.[42] Pterin [Ptr] and tetrahydropterin [H_4Ptr·2HCl] were synthesized according to well known literature methods.[43,44] Toluene, cyclohexane, diethylether and methanol (p.a.) were purchased from Merck, dried over molecular sieves (3 or 4 Å) and degassed prior to use. Dimethyl sulfide (purum) and chloramin T (*N*-chlorobenzenesulfonamide, sodium salt, purum) were from Fluka and used as received. DMSO-d_6 (Uvasol) from Merck has been distilled under nitrogen and stored over a molecular sieve (3 Å) under nitrogen. Preparative manipulations were carried out in a dry nitrogen or argon atmosphere.

Preparation of $Mo^{IV}OCl_3(H^+$-*q*-H_2Ptr) (1). Bright yellow MoO_2Cl_2 (0.398 g, 2 mmol) is dissolved in ca. 100 mL of dry methanol at room temperature. H_4Ptr·2 HCl (0.48 g, 2 mmol) is added as a solid. Almost immediately the bright yellow solution changes its color to deep red and no solid tetrahydropterin remains in solution after 15 min. The reaction is almost quantitative. Isolation of the product can be achieved either by removing the solvent and gaseous HCl in vacuo or by slow precipitation with a toluene/cyclohexane mixture added carefully to the reaction solution at 4 °C. The dark red precipitate was filtered off, washed with ether and dried in high vacuum at 50 °C. Yield: 0.691 g (90%) of a fine, dark red powder.

Although the crystallographic data clearly support the composition without any solvent molecule, the elemental analysis is not in good agreement due to a small solvent content of the powder, to the incomplete combustion of the pteridines in elemental analysis procedures, and to the moisture sensitivity of the powder. The best agreement is achieved by assuming the presence of half a molecule of methanol and of water per molecule of **1**. Powders, prepared by precipitation with toluene/cyclohexane, always contain a residual quantity of toluene, even after drying at high vacuum and elevated temperatures. Presence of solvents in the powders can be demonstrated by NMR spectroscopy. Anal. Calcd for $C_6H_8N_5Cl_3MoO_2$: C, 18.74; H, 2.08; N, 18.22; Cl, 27.67. Anal. Calcd for $C_6H_8N_5Cl_3MoO_2 \cdot {}^1/_2CH_6O_2$ ($^1/_2$ methanol and $^1/_2$ water per molecule of **1**): C, 19.05; H, 2.68; N, 17.09; Cl, 26.01. Found: C, 19.00; H, 2.56; N, 16.40; Cl, 26.83.

Fast atom bombardment mass spectrometry (FAB-MS): highest mass peak in the isotope distribution is $m/z = 349.9$ for $[C_6H_8N_5Cl_2MoO_2]^+$; a second molecule ion is found at $m/z = 329.0$ for $[C_6H_6N_5ClMoO_3]^+$. Both isotope distributions of the molecule ions are in good agreement to the calculated intensity patterns.[45]

Infrared spectral data (KBr, pellet, range 4000−600 cm^{-1}), cm^{-1}: ν_{C-H}, ν_{O-H}, ν_{N-H} = 3346 m, 3302 m, 3158 m, 2943 w, 2860 w, 2705 w, $\nu_{C=O}$ = 1696, $\nu_{C=N}$, $\nu_{C=C}$, ν_{C-O} = 1651 vs, 1619 vs, 1603 vs, 1502 s, 1440 w, 1395 w, 1336 m, δ_{C-H}, δ_{C-H}, others = 1248 w, 1199 w, 1164 w, 775 m, 701 w, $\nu_{Mo=O}$ = 985m (ν = stretching mode, δ = bending mode).

Electronic spectrum UV−vis (quartz, methanol, range 200−600 nm), λ_{max}, nm (ϵ_M, M^{-1} cm^{-1}): 486 (4453), 306 (2712), 267 (5699), 220 (20 062).

Physical Measurements. ^{13}C-NMR spectra were measured on a Varian Gemini 200 spectrometer at 50.3 MHz. Chemical shifts are reported relative to the residual proton impurity of the solvent DMSO-d_6 (39.5 ppm relative to TMS). Infrared spectra were recorded on a Biorad FTS 45 FT-IR spectrophotometer as KBr-pellets. Fast atom bombardment mass spectrometry (FAB-MS) studies were performed on a ZAB-VSEQ spectrometer (Vacuum Generators); the samples were dissolved in DMSO and then mixed with 3-NBA, applied to a standard stainless steel VG FAB target, and introduced through the vacuum lock.

Table 1. Crystal Data and Structure Determination Parameters of $Mo^{IV}OCl_3(H^+$-*q*-H_2Ptr) (**1**)

formula	$C_6H_8N_5Cl_3MoO_2$
fw	384.46
cryst syst	orthorhombic
space group	*Pbca* (No. 61)
a, Å	9.965(2)
b, Å	14.408(6)
c, Å	17.362(3)
V, Å^3	2493(2)
formula units (*Z*)	8
calcd density (ρ_x), g·cm^{-3}	2.049
absorption coeff (μ(Mo Kα), cm^{-1}	16.734
cryst dimens, mm	0.28 × 0.16 × 0.15
temp, K	295(2)
abs cor	numerical
max abs cor	0.7896
min abs cor	0.7224
av abs cor	0.7735
R values	
R_1[a]	0.0586
wR_2[b]	0.1501
goodness of fit on F^2	1.180

[a] For all 2752 unique data with an intensity $F_o^2 \geq 2\sigma(F_o^2)$. [b] For all 5879 unique data. $w = 1/\sigma^2(F_o^2) + (0.0287p)^2 + 8.40p$ with $p = (\max F_o^2 + 2F_o^2)/3$.

EI mass spectra were recorded on a QMS511 spectrometer (Balzers, FL). The GC instrument was a HRGC 5160 Mega Series of Carlo Erba Strumentatione using a fused silica capillary column: inner bore 0.25 mm, film thickness 0.25 μm, and phase DB1. Electronic absorption spectra were measured on a Cary 1E UV/visible spectrophotometer.

X-ray Crystallographic Procedures. Suitable crystals of the product were obtained by slow diffusion of a toluene/cyclohexane mixture into a concentrated solution in methanol. A dark red, almost opaque crystal of irregular shape was separated from dendritic aggregates, cut to the approximate size 0.28 × 0.16 × 0.15 mm and mounted on the tip of glass fiber with epoxy cement. Crystal and intensity data were collected on an Enraf-Nonius CAD4 diffractometer equipped with graphite monochromated Mo Kα radiation (λ = 0.710 73 Å). A total of 25 reflections in the range 15.2° ≤ 2θ ≤ 24.4° were used to determine the crystal system (orthorhombic) and an accurate cell for the data collection was calculated. A total of 6577 intensity data (including 72 standard reflections, monitored at an interval of every 3 h to check the crystal stability) of one-eighth of the limiting sphere were collected in the range 2° ≤ 2θ ≤ 72°. An $\omega-2\theta$ scan (zigzag mode) was applied; *hkl* range: 0−16, 0−23, 0−28. To control orientation three standard reflections were remeasured every 400 reflections. Lorentz and polarization corrections were applied, and a negligible −0.7% loss of intensity data was observed. Numerical absorption correction with five indexed crystal faces was performed, using the Enraf-Nonius CAD4 software and the MoLEN program system.[46] A total of 626 reflections, which were systematically absent, were omitted from the data set. The phase problem was solved with the Patterson interpretation routine of SHELXS86[47] using 5879 unique data in the space group *Pbca*. All hydrogen atom positions could be identified in the difference Fourier synthesis. Finally, the refinement was performed with all F_o^2 unique data and 187 parameters using the crystal structure refinement program SHELXL-93.[48] H-atom positions were refined isotropically. Largest shift to esd was 0.046 (mean value 0.004). The maximum and minimum heights of the final difference Fourier map were +1.12 and −1.32 e·Å^{-3} respectively. All calculations were performed on a Micro-Vax 3100 computer. Crystal and refinement data are summarized in Table 1. Positional parameters are compiled in Table 2, and interatomic distances and angles are presented in Table 3.

Oxo Transfer from DMSO to the $Mo^{IV}O$ Moiety. A 100 mg sample of $Mo^{IV}OCl_3(H^+$-*q*-H_2Ptr) **1** (0.26 mmol) was dissolved in 0.5

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Table 2. Positional Parameters ($\times 10^4$) and Equivalent Isotropic and Isotropic Displacement Parameters U_{eq}/U_{iso} [Å^2 × 10^3] of $Mo^{IV}OCl_3(H^+$-*q*-$H_2Ptr)$ (**1**), Where $U_{eq} = {}^1/_3 \sum_i\sum_j \mathbf{U}_{ij}\mathbf{a}^*_i\cdot\mathbf{a}^*_j\cdot\mathbf{a}_i\cdot\mathbf{a}_j$

atom	*x*	*y*	*z*	U_{eq}/U_{iso}
Mo	252(1)	1568(1)	1518(1)	30(1)
Cl(1)	759(1)	1151(1)	198(1)	46(1)
Cl(2)	1280(1)	3013(1)	1236(1)	45(1)
Cl(3)	−618(1)	2316(1)	2631(1)	46(1)
O(1)	1398(3)	897(2)	1933(2)	46(1)
N(1)	286(4)	4647(3)	−1024(2)	33(1)
C(2)	186(4)	3820(3)	683(2)	30(1)
N(2)	−967(4)	3573(3)	−373(3)	43(1)
N(3)	1222(4)	3199(2)	−633(2)	31(1)
C(4)	2437(4)	3388(3)	−940(2)	25(1)
O(4)	3413(3)	2830(2)	−916(2)	33(1)
C(4a)	2597(4)	4264(3)	−1300(2)	26(1)
C(8a)	1489(4)	4883(3)	−1312(2)	28(1)
N(5)	3825(3)	4418(2)	−1595(2)	28(1)
C(6)	3919(6)	5273(3)	−2061(4)	49(1)
C(7)	3003(5)	6022(3)	−1815(3)	47(1)
N(8)	1668(4)	5713(2)	−1621(2)	34(1)
H(3)	1055(46)	2711(16)	−378(20)	47(15)
H(21)	−1613(61)	3921(42)	−444(36)	67(19)
H(22)	−1017(48)	3135(32)	−226(27)	26(13)
H(61)	4659(48)	5459(34)	−2028(28)	36(14)
H(62)	3330(85)	5156(58)	−2683(53)	147(34)
H(71)	2933(52)	6416(36)	−2217(31)	49(16)
H(72)	3421(72)	6317(47)	−1288(40)	88(22)
H(8)	1090(46)	6048(33)	−1612(27)	30(13)

Table 3. Interatomic Distances (Å) and Bond Angles (deg) in $Mo^{IV}OCl_3(H^+$-*q*-$H_2Ptr)$ (**1**)

Mo−Cl(1)	2.421(2)	O(4)−C(4)	1.262(5)	N(5)−C(6)	1.477(6)
Mo−Cl(2)	2.372(1)	N(1)−C(2)	1.333(5)	N(5)−C(4a)	1.346(5)
Mo−Cl(3)	2.377(1)	N(1)−C(8a)	1.343(6)	N(8)−C(7)	1.444(6)
Mo−O(1)	1.661(3)	N(2)−C(2)	1.319(6)	N(8)−C(8a)	1.323(5)
Mo−O(4)	2.281(3)	N(3)−C(2)	1.369(6)	C(4)−C(4a)	1.417(5)
Mo−N(5)	2.013(3)	N(3)−C(4)	1.351(5)	C(6)−C(7)	1.476(7)
				C(4a)−C(8a)	1.419(6)
N(2)−H(21)	0.82(6)	C(6)−H(61)	0.79(5)		
N(2)−H(22)	0.68(5)	C(6)−H(62)	1.24(9)		
N(3)−H(3)	0.85(1)	C(7)−H(71)	0.90(5)		
N(8)−H(8)	0.75(5)	C(7)−H(72)	1.09(7)		

Cl(1)−Mo−Cl(2)	86.13(5)	Cl(2)−Mo−N(5)	159.7(1)
Cl(1)−Mo−Cl(3)	163.33(5)	Cl(3)−Mo−O(1)	99.3(1)
Cl(1)−Mo−O(1)	97.1(1)	Cl(3)−Mo−O(4)	84.66(9)
Cl(1)−Mo−O(4)	80.16(9)	Cl(3)−Mo−N(5)	90.5(1)
Cl(1)−Mo−N(5)	92.1(1)	O(1)−Mo−O(4)	166.6(1)
Cl(2)−Mo−Cl(3)	85.80(5)	O(1)−Mo−N(5)	92.7(2)
Cl(2)−Mo−O(1)	107.6(1)	O(4)−Mo−N(5)	74.4(1)
Cl(2)−Mo−O(4)	85.36(8)		
C(2)−N(1)−C(8a)	117.2(4)	N(3)−C(4)−C(4a)	117.1(4)
C(2)−N(2)−H(21)	117(4)	N(5)−C(6)−C(7)	114.4(4)
C(2)−N(2)−H(22)	118(4)	N(5)−C(6)−H(61)	108(4)
H(21)−N(2)−H(22)	124(6)	N(5)−C(6)−H(62)	110(4)
C(2)−N(3)−C(4)	121.2(4)	C(7)−C(6)−H(61)	108(4)
C(2)−N(3)−H(3)	115(3)	C(7)−C(6)−H(62)	93(4)
C(4)−N(3)−H(3)	123(3)	H(61)−C(6)−H(62)	124(5)
C(6)−N(5)−C(4a)	113.8(4)	N(8)−C(7)−H(72)	106(4)
Mo−N(5)−C(4a)	120.1(3)	C(6)−C(7)−H(71)	107(3)
Mo−N(5)−C(6)	125.5(3)	C(6)−C(7)−H(72)	107(4)
C(7)−N(8)−C(8a)	119.9(4)	H(71)−C(7)−H(72)	116(5)
C(7)−N(8)−H(8)	121(4)	N(8)−C(7)−C(6)	114.3(4)
C(8a)−N(8)−H(8)	118(4)	N(8)−C(7)−H(71)	108(3)
Mo−O(4)−C(4)	111.2(2)	N(5)−C(4a)−C(4)	114.6(4)
N(1)−C(2)−N(2)	119.1(4)	N(5)−C(4a)−C(8a)	126.8(4)
N(1)−C(2)−N(3)	123.8(4)	C(4)−C(4a)−C(8a)	118.6(4)
N(2)−C(2)−N(3)	117.0(4)	N(1)−C(8a)−N(8)	120.0(4)
O(4)−C(4)−N(3)	123.3(4)	N(1)−C(8a)−C(4a)	122.0(4)
O(4)−C(4)−C(4a)	119.7(4)	N(8)−C(8a)−C(4a)	117.9(4)

mL of deaerated DMSO-d_6 under argon. ^{13}C-NMR spectra were recorded every 3 h. The identity of the dimethyl sulfide [DMS] (MW: 68) formed was confirmed after 25 h by mass spectrometry. The EI−MS spectrum showed a signal of DMS-d_6 at $M^+ = m/z$ 69. After several weeks the complete oxidation of **1** to a $Mo^{VI}O_2$ complex and fully oxidized pterin was checked by NMR-spectroscopy. Then an equal amount of $H_4Ptr\cdot 2HCl$ was added to this solution under exclusion of oxygen. The exclusive formation of the $Mo^{IV}O$−*quinonoid*-dihydropterin complex was again checked by NMR spectroscopy, and no remaining H_4Ptr was detected.

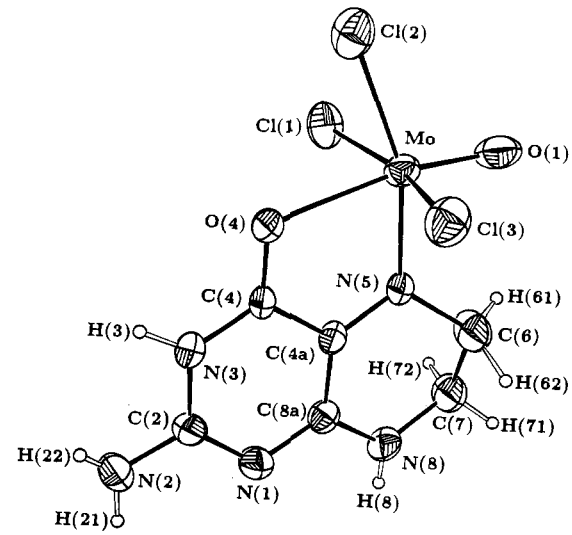


Figure 3. ORTEP representation of $MoOCl_3(H^+$-*q*-$H_2Ptr)$ (**1**), showing 50% probability ellipsoids.

In a parallel experiment DMS was isolated after several weeks by distillation from DMSO using its low boiling point (36−37 °C). The quantitative determination of DMS was achieved by gas chromatography and a gravimetric method using chloramin T, resulting in the formation of *S,S*-dimethyl-*N*-(*p*-tolylsulfonyl)sulfilimine.[49] Commerical DMS was used as a standard. The ratio of starting complex $Mo^{IV}OCl_3(H^+$-*q*-$H_2Ptr)$, **1**, to DMS produced was determined to be almost 1:2.

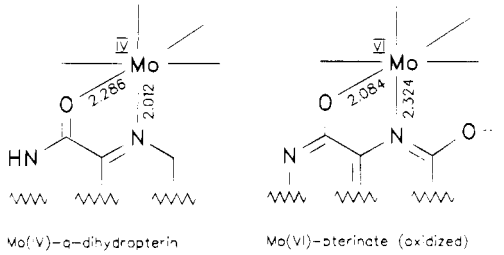
Results and Discussion

X-ray Structure of $MoOCl_3(H^+$-*q*-$H_2Ptr)$ (1). The crystal structure of trichloro(*quinonoid*-N(8)*H*-6,7-dihydropterin)oxomolybdenum(IV) (**1**), presented in Figure 3, consists of separated mononuclear molecules. The molybdenum atom shows a distorted octahedral coordination geometry. It is ligated in a chelating mode by N(5) and O(4) of the pterin ligand. In contrast to several structurally characterized metal complexes of oxidized pterins with the same coordination type,[33−38] this complex displays an unusually short metal−N(5) distance. The structure of **1** confirms the difference between hydrogenated pterins and oxidized pterins coordinated to molybdenum, with an Mo−N(5) distance of 2.013(3) Å compared to 2.324(6) Å in the same bond for an oxidized pterin.[33] Such a short Mo−N(5) distance has also been previously found in the structure of **III**.[32]

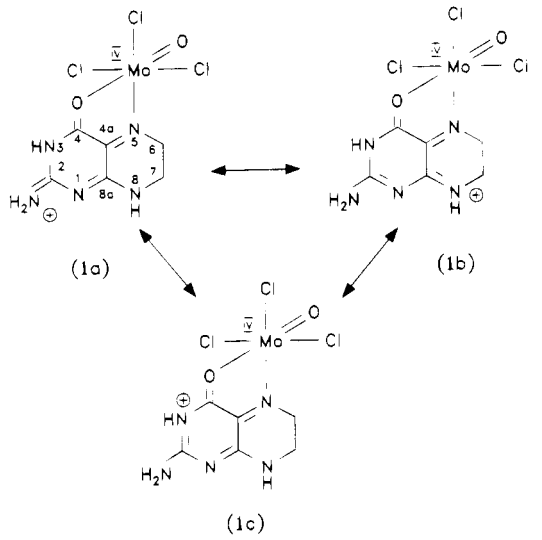
The terminal oxo ligand to molybdenum O(1), with a Mo−O(1) distance of 1.661(3) Å, is trans located to the atom O(4) of the pterin. The long Mo−O(4) distance of 2.281(3) Å reflects the charge state of the pterin ligand. This fact, together with the proton at N(3), clearly found in the difference Fourier synthesis, provides evidence, that the pterin nucleus in **1** is not a N(3)-deprotonated pterinate. In a complex where Mo is coordinated by xanthopterinate, a N(3)-deprotonated oxidized pterin, Burgmayer and Stiefel found a Mo−O(4) distance of 2.084(5) Å.[33] The discussed differences in the Mo−pterin coordination are summarized in the Chart 1. The octahedral coordination in **1** is completed by three chlorine atoms in a *meridional* arrangement.

(49) Večeřa, M.; Petránek, J. *Collect. Czech. Chem. Commun.* **1956**, *21*, 912.

Chart 1



Scheme 2



The bond lengths between the atoms N(2)−C(2)−N(1)−C(8a)−N(8) all range between 1.319(6) and 1.343(6) Å, showing clearly double bond character and indicating the presence of a mesomeric system. Comparison of the C(4a)−C(8a) bond distances in **1** (1.419(6) Å), with the corresponding values in a fully oxidized pterin[50] (1.359(2) Å), and with several tetrahydrogenated pterins[51] (range 1.376−1.402(3) Å) indicates a more pronounced single bond character for this bond in **1**. The same comparison of the bond distance N(5)−C(4a) in **1** (1.346(5) Å) with the corresponding bond in the fully oxidized pterin[50] (1.373(2) Å) and the single bonds in tetrahydrogenated pterins[51] (1.386(6)-1.464 Å) clearly speaks for a higher degree of double bond character for this bond in **1**. This analysis of the bond lengths in the pterin nucleus therefore allows in analogy to **III**[40] the formulation of metal complex **1** as the mesomeric structures **1a**, **1b**, and **1c** containing a monoprotonated quinonoid dihydropterin coordinated to Mo(IV) (Scheme 2).

The reactivity of **1**, described in the section on ^{13}C-NMR spectroscopy, confirms this interpretation. **1c** is a less probable mesomeric form, since the bond distance of 1.369(6) Å for N(3)−C(2) is longer than other distances in the mesomeric system. Even the neighboring N(3)−C(4) bond is shorter (1.351(5) Å), implying the N(3)−C(2) to possess more single bond character. In a protonated tetrahydropterin, the N(3)−C(2) bond distance is found to be 1.344 Å,[51] indicating that, in this case, the positive charge is delocalized over the centers N(1), N(2), N(3), and C(2). However the mesomeric form of the hydrogenated pterin in **1c**, denoted in the pterin literature as an *endo para* quinonoid 7,8-dihydro-(6*H*)-pterin tautomeric form, has been proposed by several authors to be reduced to the tetrahydro form by a redox reaction catalyzed by the enzyme dihydropteridine reductase [DHPR (human)].[26,27,52] It is interesting to note that we found in the first crystal structures containing quinonoid dihydropterins, which delocalize a positive charge, a preference for the so called *exo para* tautomer **1a,b**. Complex **1** therefore can be formulated as cationic N(2)−N(8)-mesomeric trichloro(*quinonoid*-N(8)-*H*,6,7-dihydropterin)oxomolybdenum(IV).

There are two other formal electronic structures possible in our complex, which can be considered. If we denote our complex as $[Cl_3Mo^{IV}O]^-[H_3Ptr]^+$, to emphasize that the Mo(IV) center carries a formal negative charge, the two other possibilities are $[Cl_3Mo^{V}O][H_3Ptr]$ and $[Cl_3Mo^{VI}O]^+[H_3Ptr]^-$.

The $[Cl_3Mo^{V}O][H_3Ptr]$ formulation as a spin-paired complex between Mo(V) and a trihydropterin radical is very uncommon. We have described iron complexes with pterin radicals[29,30,31] and found stable pterin radicals only with hydrogenated pterins, which were substituted in position N(5) with a methyl group. In all cases we were able to record the spectra of pterin radicals using EPR. This was not possible with our molybdenum complex, and also we experienced no problems in recording the NMR spectra. Therefore, we must have a completely spin-paired system in this formulation, which may be consistent with the found mesomeric system between N(2) and N(8).

The $[Cl_3Mo^{VI}O]^+[H_3Ptr]^-$ formulation as an Mo(VI) complex of a deprotonated tetrahydropterin is formally a reasonable formulation and can explain the short Mo−N(5) bond. Similar bond lengths were found in Mo−amido complexes $Mo^{VI}(NR_2^-)$.[53] But this formal electronic structure cannot explain the NMR results as will be discussed in the section on ^{13}C-NMR spectroscopy. Furthermore the tetrahydro formulation does not need or possess a mesomeric system between N(2) and N(8).

As noted, the structures of the molecules in **1** (Figure 3) and **III**[32] are basically identical in respect to the first coordination sphere of the molybdenum atom. Only in the second coordination sphere of molybdenum in the structure of **III** can a weak interaction in solution between a Mo-coordinated chlorine atom and an OH group from the *quinonoid*-dihydrobiopterin side chain be considered. The properties of both complexes might be expected to be very similar with regard to spectroscopy and in particular reactivity. This is, however, not the case, as shown in the following section on ^{13}C-NMR spectroscopy.

^{13}C-NMR Spectroscopy. $Mo^{IV}OCl_3(H^+$-*q*-$H_2Ptr)$ (**1**) was synthesized in a redox reaction from $Mo^{VI}O_2Cl_2$ and tetrahydropterin (H_4Ptr·2HCl) in analogy to $MoOCl_3(H^+$-*q*-$H_2Bip)$ (**III**), and both complexes are nearly structurally identical with respect to the first coordination sphere of the molybdenum atom. But, in contrast to **III**, complex **1** is able to reduce the substrate dimethyl sulfoxide [DMSO] to dimethyl sulfide [DMS] under mild conditions, as can be shown by ^{13}C-NMR spectroscopy. This reaction consists of two concomitant steps. The DMS evolved was also identified by mass spectrometry. Quantitative determination of the DMS is described in the experimental section.

In Figure 4 the upper spectrum a displays the ^{13}C-NMR signals of the carbon atoms in $MoOCl_3(H^+$-*q*-$H_2Ptr)$ (**1**), which appear in the same range as in **III**.[32] The signal of C(7) is hidden by the signals of the deuterated solvent. A comparison of the signals with those of the starting compound H_4Ptr and fully oxidized pterin [Ptr] (Table 4) clearly reveals the formation of a new product with the pterin nucleus in a new oxidation state.

(50) Bieri, J. H.; Hummel, W.-P.; Viscontini, M. *Helv. Chim. Acta* **1976**, *59*, 2374.

(51) Bieri, J. H.; Viscontini, M. *Helv. Chim. Acta* **1977**, *60*, 447. Bieri, J. H.; Viscontini, M. *Helv. Chim. Acta* **1977**, *60*, 1926; Bieri, J. H. *Helv. Chim. Acta* **1977**, *60*, 2303. Prewo, R.; Bieri, J. H.; Ganguly, S. N.; Viscontini, M. *Helv. Chim. Acta* **1982**, *65*, 1094. Antoulas, S.; Prewo, R.; Bieri, J. H.; Viscontini, M. *Helv. Chim. Acta* **1986**, *69*, 210.

(52) Ayling, J. E.; Dillard, S. B.; Turner, A. A.; Bailey, S. W. In ref 27, p 43.

(53) Lappert, M. F.; Power, P. P.; Sanger, A. R.; Srivastava, R. C. *Metal and Metalloid Amides*; Ellis Horwood: New York, 1980; Chapter 8.

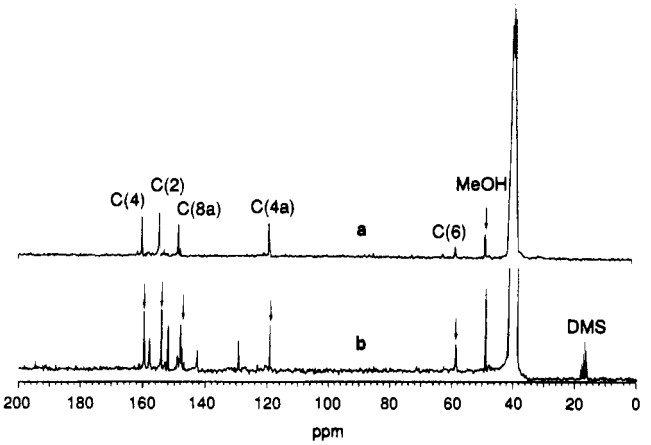


Figure 4. ^{13}C-NMR spectrum of $MoOCl_3(H^+$-q-$H_2Ptr)$ (**1**) in DMSO-d_6, room temperature, argon: (a) after 3 h; (b) after 20 h. Arrows in spectrum b indicate signals of **1**. The six new signals correspond to Ptr. DMS = dimethyl sulfide.

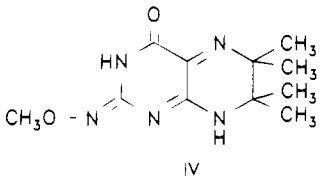
Table 4. ^{13}C-NMR Data for **1**, H_4Ptr, and Ptr in DMSO-d_6 and **IV**[54] in D_2O at pH 4.5[a]

compd	C(4)	C(2)	C(8a)	C(4a)	C(6)	C(7)
1	160.1	154.4	148.3	119.0	58.1	ca. 40[b]
H_4Ptr·2HCl	156.8	153.4	151.8	85.2	ca. 40[b]	37.0
Ptr	158.6	152.9	149.3	129.9	143.0	147.9
IV[54]	159.7	150.2	149.9	146.2	65.6	57.1

[a] Chemical shifts are reported in ppm. Reference is the signal of the solvent DMSO-d_6 = 39.5 ppm. [b] Hidden by DMSO-d_6.

The chemical shift of the atom C(4a) is a very characteristic indicator for the oxidation state of the pterin nucleus and, as we now learn, also for metal complexation. The resonance of C(4a) in **1** is found at 119.0 ppm and is shifted, with respect to the starting compound H_4Ptr, more than 30 ppm downfield.

In the spectrum of the only isolated neutral quinonoid dihydropterin (**IV**), characterized by single crystal X-ray dif-



IV

fraction, which contains a fixed *exo para* quinonoid tautomeric form by blocking the N(2)-atom as a methoxyimino group[54] (Table 4), a resonance at 146.2 ppm has been assigned to the C(4a) atom. Compared to this value, a coordination of a quinonoid dihydropterin to Mo(IV) shifts the C(4a) signal about 27 ppm to higher field. The strong influence of metal complexation upon the dihydropterin resonances is confirmed by the shift of the C(6) signal, which should not be substantially changed on going from tetrahydro to the quinonoid dihydro state. However, the resonance of C(6) shifts downfield from about 40 ppm in protonated tetrahydropterin to 58 ppm in **1**. These results indicate a strong electronic interaction between the metal and the mesomeric quinonoid bonding system in the dihydropterin, implying an electronic charge transfer presumably from the Mo(IV) center to the mesomeric quinonoid system N(5)−C(4a)−C(8a)−N(8)−N(1)−C(2)−N(2), resulting in an additional shielding of C(4a) and to a smaller extent of C(8a), both in comparison to the uncomplexed and fixed quinonoid dihydropterin system.

The alternative electronic structure formulation $[Cl_3Mo^{VI}O]^+[H_3Ptr]^-$ as a Mo(VI) complex of a deprotonated tetrahydropterin has difficulties in explaining the NMR results. As indicated in Table 4, a tetrahydro form (and even a deprotonated tetrahydro form is still a tetrahydro oxidation state) should possess a C(4a) signal in the ^{13}C-NMR spectrum, which is shifted downfield to a maximal value of 103 ppm. This is the shift, that the C(6) atom shows upon formal deprotonation and complexation (40−58 ppm). But the observed C(4a) signal is shifted to 119 ppm.

The picture is completed by the analysis of the resonances of C(4), the neighboring atom to the Mo ligating oxygen atom O(4), which resemble one other in **1** (160.1 ppm) and in uncomplexed quinonoid dihydropterin (159.7 ppm). This is in accordance with the long, and therefore weak, Mo−O(4) bond, indicating only minor electronic interaction between these atoms.

In the solutions of **III** in DMSO, disproportionation of the complex into a Mo(VI) compound and H_4Bip is observed to a large extent. In contrast, there is almost no change in the spectrum of **1** in DMSO after 7 h, other than a very small amount of H_4Ptr being formed. Satellite signals of very low intensity appear for each main resonance in the spectrum of **1**, which may be an indication of an intermediate product with DMSO coordinated to the molybdenum atom. After about 8 h, signals of deuterated DMS and Ptr begin to rise and after 20 h the spectrum in Figure 4b is obtained. DMS was not formed in control experiments with the pure substances H_4Ptr, q-H_2Ptr (generated in solution by H_2O_2 oxidation of H_4Ptr[55]), or an Mo(IV) compound ($MoCl_4$) in DMSO solutions. After 24 h, the reaction reaches about 70% completion and then slows down. Reasons may be the high concentration of the complex in the NMR tube (0.52 M) and competition of the DMS evolved in the coordination to the molybdenum atom. The reaction runs faster at elevated temperatures and steady removal of DMS, but also with more pterin byproducts, as monitored by NMR spectroscopy. After several weeks (faster at elevated temperatures), the solution adopts a bright reddish color and complex **1** can no longer be detected by NMR spectroscopy. The resulting DMSO solutions contains Ptr, DMS, and presumably a monomeric $Mo^{VI}O_2$ complex of yet unknown composition. A quantitative determination of the deuterated DMS, additionally identified by mass spectrometry, revealed a 1:2 ratio of complex **1** to DMS produced. Hence two molecules of DMSO have been reduced to DMS: one by the oxidation of Mo(IV) to Mo(VI), an oxo transfer reaction which is important in relation to enzyme action, the second, in a concomitant step, by the oxidation of quinonoid dihydropterin to Ptr.

In natural enzyme systems, employing hydrogenated pterins, e.g. phenylalaninehydroxylase, the quinonoid dihydropterins will normally not be oxidized to pterins, but reduced again to tetrahydropterins by another enzyme, e.g. dihydropteridine reductase,[19,20] or other reducing agents (NADPH, cytochrome *c*, etc.). The oxidation of quinonoid dihydropterin to oxidized pterin in our in vitro experiment seems to be catalyzed by the metal center, since quinonoid dihydropterin alone does not reduce DMSO under the conditions applied. In vivo, this reaction is of minor importance, but also happens in biological enzyme systems to a small extent, otherwise no oxidized pterins would be detectable in the urine of mammals.

In molybdenum enzymes, the effective electron transfer to the substrates, e.g. DMSO→DMS, will be carried out by the Mo(IV)/Mo(VI) shuttle. The presumed Mo(VI) species in the reacted solution of complex **1** can not be detected by NMR spectroscopy. This indicates the presence of an MoO_2Cl_2 complex additionally coordinated by two DMSO molecules.

(54) Noar, J. B.; Venkataram, U. V.; Bruice, T. C.; Bollag, G.; Whittle, R.; Sammons, D.; Henry, R.; Benkovic, S. J. *Bioorg. Chem.* **1986**, *14*, 17.

(55) Matsuura, S.; Murata, S.; Sugimoto, T. In ref 22, p 81.

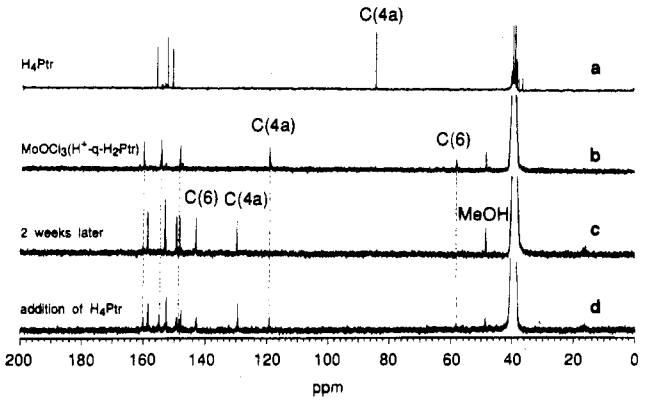


Figure 5. ^{13}C-NMR spectra of H_4Ptr (a) $MoOCl_3(H^+$-*q*-H_2Ptr) (**1**) (b), same solution 2 weeks later (c), and solution after addition of H_4Ptr (d). DMSO-d_6, room temperature, argon. Experiments were typically 0.52 M with respect to the concentration of **1**.

This formulation is confirmed by the additional experiment described below.

An Mo(V) dimeric species, a type well-known from the literature,[13] can also possibly exist in the reaction solution, but only as an intermediate, not as main product. The possible presence of this intermediate may provide an additional explanation for the long reaction time. The evidence that the main product is a Mo(VI) complex is given by the stoichiometry of the overall process and the two electron steps involved. No evidence was found in the NMR spectra, that would confirm the presence of one electron oxidized or reduced hydrogenated pterin species in a large amount. This species should be paramagnetic and would, therefore, strongly disturb the NMR experiment.

In a further experiment, we tried to "regenerate" the enzyme model complex, a crucial demand for a functional model. Figure 5 displays, in addition to the ^{13}C-NMR spectra of H_4Ptr (a) and $MoOCl_3(H^+$-*q*-H_2Ptr) (**1**) (b) the spectrum of the fully reacted oxidized complex **1** after 14 days (c). Spectrum **c** exhibits only the six signals of oxidized Ptr and presumably contains an additional molybdenum complex with an $Mo(VI)O_2$ moiety. After adding H_4Ptr under argon to the solution in spectrum c, no H_4Ptr but only signals of the "regenerated" complex **1** could be detected, in addition to the still present signals of Ptr (d). The reaction "back" to complex **1** is very fast and unequivocal.

It is now evident, that a continuous reaction of an enzyme substrate (DMSO) can be achieved under constant supply of new or "regenerated" tetrahydropterin, with a simple model system containing a $Mo^{IV}O$ moiety *and* quinonoid dihydropterin without additional sulfur ligands.

Electronic and Infrared Spectroscopy. The UV/visible spectrum of $MoOCl_3(H^+$-*q*-H_2Ptr) (**1**) closely resembles that of $MoOCl_3(H^+$-*q*-H_2Bip) (**III**). The red color of the complex results mainly from a strong charge transfer absorption at λ_{max} = 487 nm with an ϵ_M of 4453 M^{-1} cm^{-1} (Figure 6). The large ϵ_M indicates an MLCT character for this broad absorption.[56] No isolated complexes of metals and hydrogenated pterins exist, and therefore no secure assignment or comparison with other data can be made. But there are some hints: Viscontini and Okada described an intermediate, fast declining absorption at about λ_{max}~500 nm in a reaction between Fe^{3+} ions and *N*(5)-methyltetrahydropterin[28] and Vonderschmitt and Scrimgeour noticed a transient, purple complex in a reaction of Cu^{2+} ions

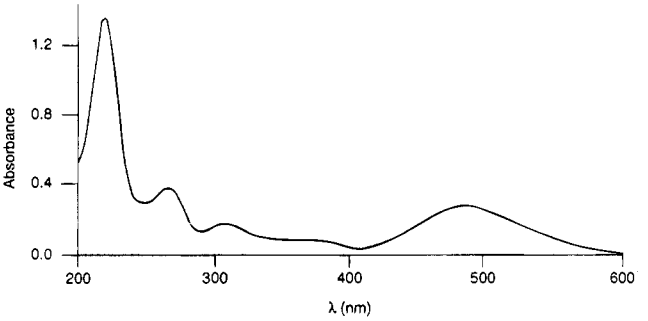


Figure 6. UV−vis spectrum of $MoOCl_3(H^+$-*q*-H_2Ptr) (**1**) in methanol (concentration 6.8 × 10^{-5} M).

with H_4Ptr.[57] Similar observations have been made by Burgmayer et al.[58]

It is interesting to note, that a red, purple color is mostly attributed to sulfur containing ligands coordinating to Mo(V) or Mo(IV)[13] and to Mo_2O_5-dimers,[14] but not to mononuclear Mo(V) or Mo(IV) complexes with nitrogen or oxygen donor ligands. Rajagopalan and co-workers reported the UV/vis spectra of DMSO reductase in concentrated solutions.[7] They found absorbance peaks at 720, 550, 470, and 350 nm in the so-called "oxidized" enzyme, and peaks at 640 and 374 nm with a shoulder at 430 nm in the anaerobically dithionite "reduced" enzyme. The 470 nm peak is also found in rat liver sulfite oxidase, as reported in the same paper. The authors drew attention to the similarities in the spectra with the above-mentioned molybdenum model complexes with sulfur ligands. As our results with molybdenum−dihydropterin complexes show, this is not necessarily the only explanation.

In the spectrum of $MoOCl_3(H^+$-*q*-H_2Ptr) (**1**), there are three additional distinguishable absorptions that must be assigned to the quinonoid form of the dihydropterin [λ_{max}, nm (ϵ_M, M^{-1} cm^{-1}): 306 (2712), 267 (5699), 220 (20 062)]. An additional shoulder at 360 nm may be assigned to small amounts of fully oxidized Ptr due to trace amounts of oxygen in solution. An absorption at about 300 nm seems to be typical for a quinonoid dihydropterin in its neutral form.[59,60] This would, in our case, provide support for the outwards "neutral" betaine formulation of complex **1**.

There are three main features in the infrared spectra of quinonoid dihydropterins coordinated to a $Mo^{IV}O$ moiety. In **1** we observe them to almost the same extent as in **III**.[32] The first is the moderate shift of the C=O stretch from 1730 to 1696 cm^{-1} upon complexation to the metal. This fact and the strong decrease in intensity of the absorption band are in good accordance with the double bond found between C(4) and O(4) in the crystal structure of **1**. It also allows a clear differentiation to the metal-pterinate complexes, where this band disappears.[33,35] The second diagnostic feature is the appearance of several strong new bands in the region between 1500−1660 cm^{-1} due to mixed modes from C=C and C=N vibrations.[61] But again, in contrast to metal complexes of oxidized pterins, the strongest bands are found at 1651, 1619, and 1603 cm^{-1}, a region where the former complexes show no absorption bands. Only one strong new band at 1502 cm^{-1} in the infrared spectrum of **1** can also be

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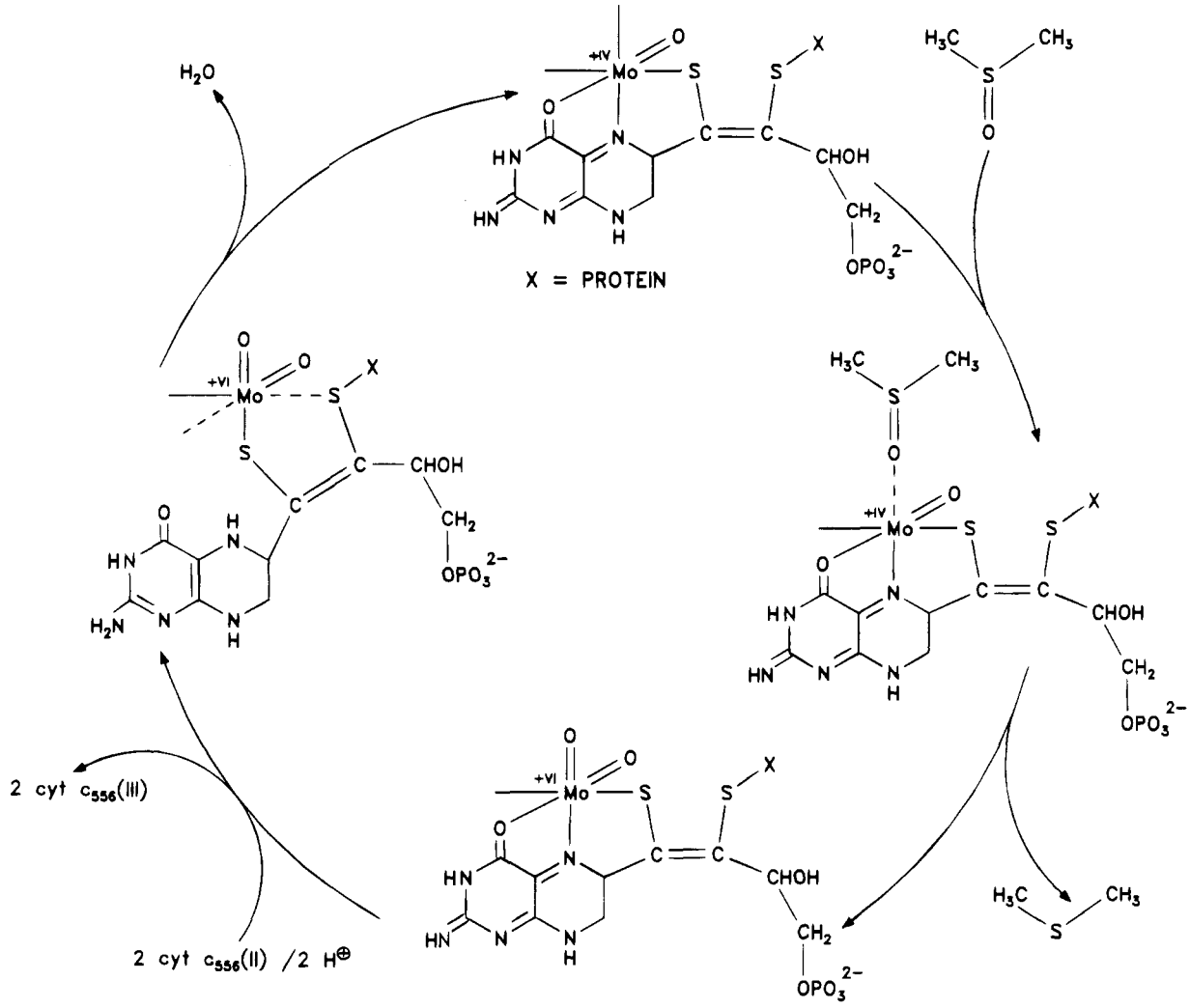

Figure 7. Model of a simplified in vivo reaction cycle for the active center in the enzyme dimethyl sulfoxide reductase.

found in metal complexes of oxidized pterins.[33,35] The third feature is the typical single band of the terminal Mo=O stretch at 985 cm^{-1}.

Mass Spectrometry. The FAB-MS spectrum of **1** confirms the X-ray structure. The highest mass peak in the isotope distribution (m/z = 349.9 for $[C_6H_8N_5Cl_2MoO_2]^+$) is due to the loss of a chlorine anion under FAB-MS conditions, resulting in a detectable molecule ion, that carries a positive charge on the quinonoid dihydropterin ligand. A second molecule ion is found at m/z = 329.0 for $[C_6H_6N_5ClMoO_3]^+$. This peak can be explained by a redoxreaction under FAB-MS conditions. A loss of two chlorine anions and two protons from **1**, and an uptake of one oxygen atom results in a $Mo^{VI}O_2Cl$ moiety coordinated by a N(3)-deprotonated pterin ligand.

Conclusions

A redox reaction between $Mo^{VI}O_2Cl_2$ and tetrahydropterin ($H_4Ptr \cdot 2HCl$) almost quantitatively yields the complex $Mo^{IV}OCl_3(H^+\text{-}q\text{-}H_2Ptr)$ (**1**). The structure of complex **1** confirms the uniqueness of quinonoid dihydropterins coordinated to molybdenum in the oxidation state +IV. The capability of **1** to reduce the enzyme substrate dimethyl sulfoxide to dimethyl sulfide under mild conditions strongly indicates a participation of the pterin nucleus in the enzyme reaction of molybdenum dependent oxidoreductases. This has been proposed earlier (Figure 2)[32] and is now supported by the characterization of complex **1** and its reducing properties. In the proposed model of an extended molybdenum cofactor in its reduced (Mo(IV)) form, the dithiolene moiety is formulated to adopt a *trans* stereochemistry. This coordination mode would allow a facile ligand degradation to urothione, the known catabolic product of molybdopterin containing enzymes.[11,12] It should be noted that the *cis*-dithiolene formulation is also possible, shown in the top molecule of Figure 7.

All spectroscopic and structural investigations indicate the molybdenum–N(5)–pterin bond to be the site of interaction between the metal and the hydrogenated pterin. The Mo–O(4)–pterin bond can be neglected considering electronic interaction. It may have only a stabilizing effect as a chelate, but it may be not strong enough to prevent the release of the hydrogenated pterin during enzyme action. These structural considerations, the reduction of the enzyme substrate DMSO to DMS by complex **1** mediated through the oxidation of Mo(IV) to Mo(VI) and the "regeneration" of the enzyme model complex with the continuous supply of tetrahydropterin prompt us to propose a simplified in vivo reaction cycle for the active center of dimethyl sulfoxide reductase as shown in Figure 7. We want to emphasize that this proposal should only be used as a working hypothesis and does not imply to be complete and established.

The starting compound (top) in Figure 7 would be a Mo(IV) monooxo species coordinated by a chelating quinonoid dihydromolybdopterin. One sulfur atom of the dithiolene group in the pterin sidechain is coordinated to the Mo(IV) atom. In the first step a DMSO molecule coordinates, e.g. under displacement of a labile amino acid ligand, to the molybdenum atom and is instantaneously reduced to DMS and released from the now Mo(VI)–dioxo moiety. The $Mo^{VI}O_2$–*quinonoid*-dihydro-

molybdopterin complex may no longer be stable, leading to the partial displacement of the dihydropterin ligand, or alternatively the reduction of the *quinonoid*-dihydropterin nucleus supports this decoordination. Anyway, the result of the following step is a reduction of the *quinonoid*-dihydropterin nucleus by the identified electron donor to DMSO reductase, cytochrome c_{556},[62] to a tetrahydropterin derivative, where the pterin nucleus is not coordinated to the molybdenum atom. In this stage, coordination of both sulfur atoms of a dithiolene moiety, adopting a *cis* stereochemistry, occurs. The last step is performed in an analogous manner to the formation of **1**, from a $Mo^{VI}O_2$ compound ($Mo^{VI}O_2Cl_2$) and H_4Ptr. Effectively, the reduction of the $Mo^{VI}O_2$ moiety to a $Mo^{IV}O$ moiety by oxidation of the tetrahydropterin nucleus to a N,O-coordinated quinonoid dihydropterin nucleus under release of a water molecule will close the reaction cycle. The reduction of Mo(VI) to Mo(IV) may also be mediated trough the reactive dithiolene group. The formation of this $Mo^{IV}O$–*quinonoid*-dihydromolybdopterin complex is the starting point for the next cycle.

It should again be noted, that this cycle can be described in essentially the same manner with a *trans* configuration of the dithiolene moiety. A possible intermediate Mo(V) stage in the enzyme cycle has been omitted. This does not imply that it does not exist.

Is it possible to generalize the function of the molybdenum–hydrogenated pterin combination? The tetrahydropterin form can act as a reversible electron donor to reduce the stable Mo(VI) state or an oxidized sulfur component (e.g. a disulfide) to a dithiolene group.

The evolving quinonoid dihydropterin stage may be useful as a stabilizing chelate ligand to the reactive Mo(IV) state. On the other hand this ligand does not coordinate as strongly as other chelating ligands found in biosystems, e.g. tetrapyrrole macrocycles and *o*-quinones.

Thus the reactivity toward substrates is not hindered and can be tuned very finely as shown by the differences between our simple models $MoOCl_3(H^+$-*q*-$H_2Bip)$ (**III**) and $MoOCl_3(H^+$-*q*-$H_2Ptr)$ (**1**). Free quinonoid dihydropterins are very unstable compounds in solution with half-lives in the range of seconds to minutes. Coordination to Mo(IV) stabilizes this oxidation state, and we can therefore speak of a mutual electronic stabilization between the Mo(IV) and the quinonoid dihydropterin state. This hypothesis can also explain the short Mo–N(5) bond.

A significance of the quinonoid dihydropterin derivatives for the enzyme reaction in molybdenum containing *oxidase* enzymes can also be assumed. Further investigations in this direction are underway in our laboratory.

Parts of this work have been presented at the 10th International Symposium on Chemistry and Biology of Pteridines and Folates, held March 21–26 1993, Orange Beach, AL.[63]

Note Added in Proof. Since submission of the manuscript, several significant papers of relevance to our contribution have appeared. Burgmayer and co-workers have claimed that their complexes of molybdenum coordinated by hydrogenated pterins have to be formulated as Mo^{VI} complexes coordinated by deprotonated tetrahydropterins.[64] The compounds show structural and spectroscopic features similar to the one presented in this paper. The lack of oxygen transfer activity with DMSO of their complexes was given as one reason for their formulation as Mo^{VI} complexes coordinated by deprotonated tetrahydropterins. The structure of a hyperthermophilic tungstopterin enzyme, aldehyde ferredoxin oxidoreductase, from *Pyrococcus furiosus* has been solved by Chan and co-workers.[65] Surprisingly the enzyme center consists of tungsten coordinated by four sulfur atoms from two *cis*-dithiolene groups of two molybdopterins. The noncoordinating hydrogenated pterin forms an unusual tricyclic system with an additional pyrano ring and is formally in a tetrahydro oxidation state. Very recently the structure of aldehyde oxidoreductase from *Desulvovibrio gigas*, a member of the xanthine oxidase protein family, has been determined.[66] In this case a molybdenum center is coordinated by the sulfur atoms of a *cis*-dithiolene groups of one molybdopterin cytosine dinucleotide and additionally by three or four oxygen atoms. As in the tungsten dimolybdopterin cofactor, the pterin system adopts a tricyclic system including a pyrano ring that originates formally from an intramolecular addition of a side chain hydroxyl group to the double bond of a dihydropterin system. Preliminary investigations in our laboratory using Mo(VI)-dioxo compounds and pyranopterins without a dithiolene group showed reaction patterns similar to those described in this paper. Pyranopterins seem to react in a manner similar to tetrahydrogenated pterins.

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Supporting Information Available: Listings of anisotropic thermal parameters (1 page). Ordering information is given on any current masthead page.

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