9. Models for the Active Center of Pterin-Containing Molybdenum Enzymes: Crystal Structure of a Molybdenum Complex with Sulfur and Pterin Ligands

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The first crystal structure of a molybdenum complex **9** with a hydrogenated pterin *and* a sulfur ligand contributes to the discussion about the active center of molybdenum and tungsten enzymes containing a molybdopterin cofactor. Complex **9** was synthesized through a redox reaction of $[Mo^{VI}O_2(LN\text{-}S_2)]$ (**8**; LN-S_2 = pyridine-2,6-bis(methanethiolato)) with 5,6,7,8-tetrahydropterin (**7**)·2 HCl ($H_4Ptr \cdot 2$ HCl). The complex crystallizes, with a non-coordinating Cl-atom acting as a counterion, in the monoclinic space group $C2/c$ (No. 15) with cell dimensions $a = 22.900(5)$, $b = 10.716(2)$, $c = 17.551(4)$ Å, $\beta = 120.36(3)°$, and $Z = 8$. We interpret **9** as $[Mo^{IV}O(LN\text{-}S_2)(H^+\text{-}q\text{-}H_2Ptr)]Cl$ (q = quinonoid; H_2Ptr = dihydropterin), *i.e.*, a Mo^{IV} monooxo center coordinated by a pyridine-2,6-bis(methanethiolato) ligand and a protonated dihydropterin. The spectroscopic properties of this new complex are comparable to those of other crystalline molybdenum complexes of hydrogenated pterins without additional S-coordination. The slightly H_2O-soluble complex **9** reacts with the natural enzyme substrate DMSO very slowly, possibly due to the lack of easily dissociable ligands at the metal center.

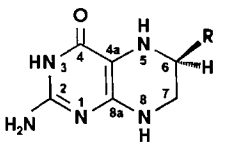
Introduction. – The chemistry and structure of metalloenzymes containing hydrogenated pterins[1]) are currently intensive fields of research. The 6β-5,6,7,8-tetrahydro-L-biopterin[2]) (**1**; H_4Bip[3])) plays an important role as a cofactor in the iron- or copper-dependent phenylalanine hydroxylases and other amino-acid hydroxylases [2] and in the iron-containing NO synthase [3]. A large group of molybdoenzymes utilizes molybdopterin, a hydrogenated pterin component, in the molybdenum cofactor **2** (Mo-co **2**). These molybdenum enzymes catalyze O-transfer reactions such as those of dimethyl-sulfoxide reductase, sulfite oxidase, and xanthine oxidase [4]. Mo-co is also present in the formylmethanofuran dehydrogenase of methanogenic bacteria [5], and recently molybdopterin has been identified as a component of the active center of several tungsten-containing enzymes [6]. The proposed structures of Mo-co **2** had two main features: a hydrogenated pterin and coordination to the molybdenum ion by an enedithiol group attached as a side chain to the pterin. The oxidation state of the pterin in Mo-co has been a point of

[1]) Trivial name; name according to IUPAC rules: pterin = 2-aminopteridin-4(3*H*)-one and 2-aminopteridin-4-ol (enol form), respectively.

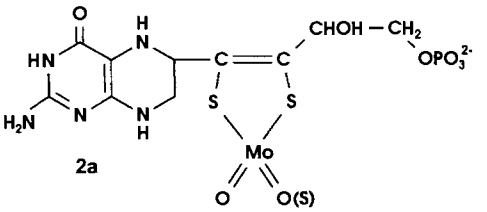
[2]) Name assigned according to the designation rules proposed in [1a]. Name according to the IUPAC rules: (1′*R*,2′*S*,6*R*)-2-amino-6-(1′,2′-dihydroxypropyl)-5,6,7,8-tetrahydropteridin-4(3*H*)-one (**1**), shortened to tetrahydrobiopterin [H_4Bip].

[3]) The proposals towards a normalization of pteridine nomenclature and abbreviations have been applied as suggested in [1b].

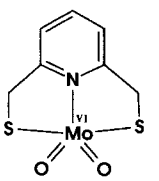
controversy. *Rajagopalan et al.* have proposed a tetrahydrogenated pterin **2a**. However, their current view is a quinonoid dihydropterin **2b** for xanthine oxidase and a different tautomeric dihydropterin form for other (Mo-co)-containing systems [7].



1 R = MeCH(OH)CH(OH)
7 R = H



2a



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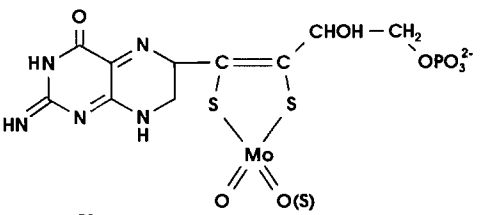
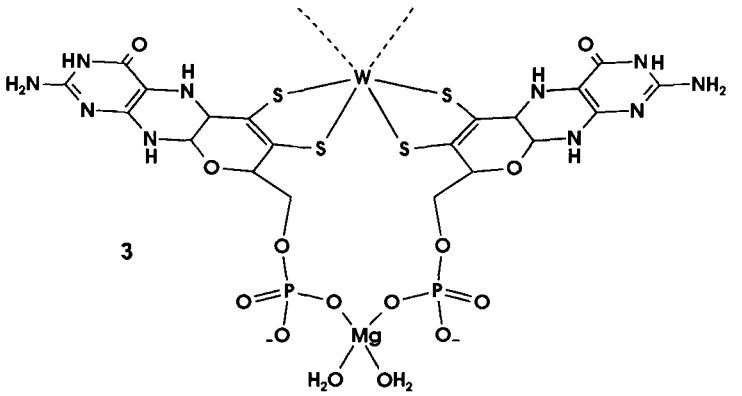


2b

The very recent X-ray crystallographic structures of metal-molybdopterin enzymes revealed at least three different structural types [8–10], schematically indicated in **3**–**5**. Common to all of the crystal structures is the coordination of the metal center by the S-donors from the enedithiol groups of one or, surprisingly, two pterins. Another unexpected finding is the pterin portion, which is present in a previously unreported tricyclic form including a pyrano ring.

In the enzyme center **3** of the tungsten- and molybdopterin-containing aldehyde ferredoxin oxidoreductase from *Pyrococcus furiosus*, a hyperthermophilic archaeon, the two vacant coordination sites may be filled by glycerol or other oxo ligands to complete a trigonal prismatic coordination polyhedron [8]. The molybdenum center **4** in the aldehyde oxido-reductase from *Desulvovibrio gigas*, a member of the xanthine oxidase protein family, is coordinated by three O-atoms beside the S-atoms of one molybdopterin cytosine dinucleotide [9]. In the monooxomolybdenum(VI) center **5** of DMSO reductase from *Rhodobacter sphaeroides*, an unusual O-atom coordination of a side-chain serine (S 147) is found in addition to two molybdopterin guanine dinucleotides [10]. A Mo–S distance of 2.9 Å for one of the Mo–S bonds indicates a particularly weak bound. Furthermore, the X-ray structure of the reduced Mo^{IV} form of the enzyme revealed, beside the lack of terminal O-atom coordination, a different coordination mode **6** of the pterins; a Mo–S distance of 3.7 Å suggests the partial or complete loss of a bonding interaction in the Mo^{IV} form. In the crystal structures of **3** and **4**, H-bonds are found from the S-atoms of nearby Fe_xS_x (x = 2,4) clusters to pterin NH H-atoms. This arrangement, say the authors, can provide a possible electron pathway, implicating an active participation of the pterin in the redox chemistry of the enzymes. Despite three crystal structures of molybdopterin-containing enzymes, up to now this is the only indication for the possible function of the pterin part of the cofactor.



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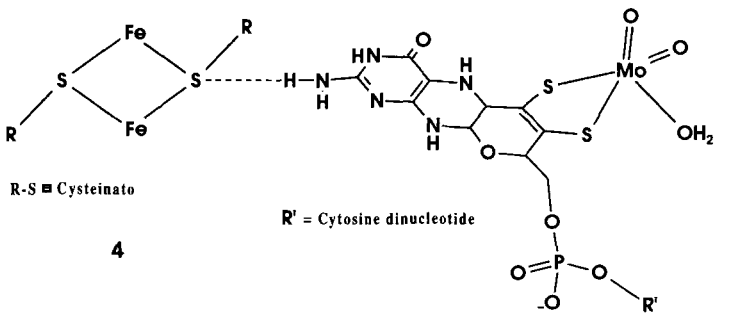
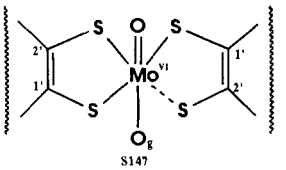


R-S ≡ Cysteinato

R' = Cytosine dinucleotide

4



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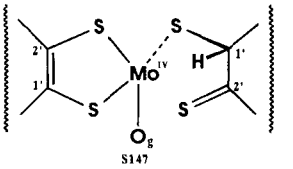

6

We have demonstrated reactions of $Mo^{VI}O_2Cl_2$ with tetrahydro-L-biopterin (**1**) and tetrahydropterin[4]) (**7**; H_4Ptr), which we interpreted as redox reactions that formed Mo^{IV} centers with coordinated, stabilized quinonoid dihydropterins [11] [12]. One product, $[MoOCl_3(H^+\text{-q-}H_2Ptr)]$ (q = quinonoid; H_2Ptr = dihydropterin) [12], reduces dimethyl sulfoxide to dimethyl sulfide, which is the reaction catalyzed by the molybdopterin-dependent molybdenum enzyme, dimethyl sulfoxide reductase [13]. Up to now, our model compounds do not contain S-ligands, which are thought indispensable to the enzyme function whether as redox-active group itself or for maintaining the optimal potentials at the reactive molybdenum center [4]. Herein, we investigate if coordination by S-donors to the Mo-atom will permit additional coordination by a hydrogenated pterin.

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[4]) Name according to IUPAC rules; tetrahydropterin [H_4Ptr] = 2-amino-5,6,7,8-tetrahydropteridin-4(3*H*)-one.

Results and Discussion. – Proof for the coordination of hydrogenated pterin to a already S-coordinated Mo-atom was achieved by the reaction of the known molybdenum complex $[MoO_2(LN\text{-}S_2)]$ (**8**; $LN\text{-}S_2$ = pyridine-2,6-bis(methanethiolato)) [14] with tetrahydropterin·2 HCl (**7**·2 HCl), resulting in $[Mo^{IV}O(LN\text{-}S_2)(H^+\text{-}q\text{-}H_2Ptr)]Cl$ (**9**). The poor solubility of **8** in most organic solvents led to prolonged reaction times, resulting in undesired side reactions of the highly reactive hydrogenated pterin and the formation of insoluble polymers. However, the ionic complex **9** remained in solution so that, after completion of the reaction and removal of the side products by filtration, evaporation of the burgundy-red filtrate afforded the crude complex **9**. A few deeply red, almost opaque crystals, suitable for X-ray crystallography, were obtained by slow diffusion of a toluene/cyclohexane mixture into the filtered reaction solution.

X-Ray Structure of $[Mo^{IV}O(LN\text{-}S_2)(H^+\text{-}q\text{-}H_2Ptr)]Cl$ (**9**). The molecular structure was determined by single-crystal X-ray diffraction analysis. The molecular structure of **9** (*Fig. 1*) shows a mononuclear Mo-center that is coordinated, in a distorted octahedral arrangement, by the tripodal pyridine-2,6-bis(methanethiolato) ligand and by the N(5),O(4)-chelating dihydropterin ligand. The octahedral arrangement is completed by the coordination of a terminal oxo anion. The Cl^- counterion, which is non-coordinating, displays several pterin N–H···Cl H-bonds within the crystal structure. The planes of the pyridine ring and the pterin heterocyclic ring which is planar, except for the atoms C(6) and C(7), are almost perpendicular to each other. Bond distances and angles are summarized in the *Table*.

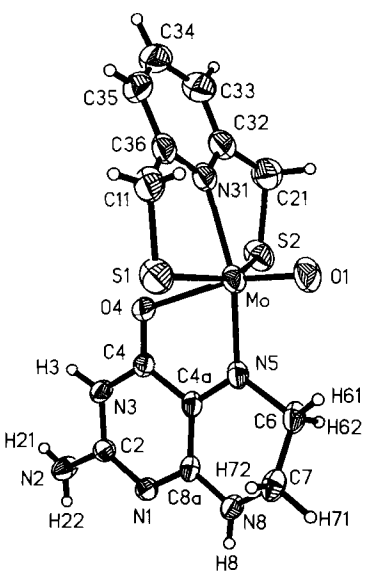


Fig. 1. *Structure of the cation of* **9** *in the crystal.* Counterion is Cl^- (not shown). ORTEP representation showing 50% probability ellipsoids. Arbitrary numbering.

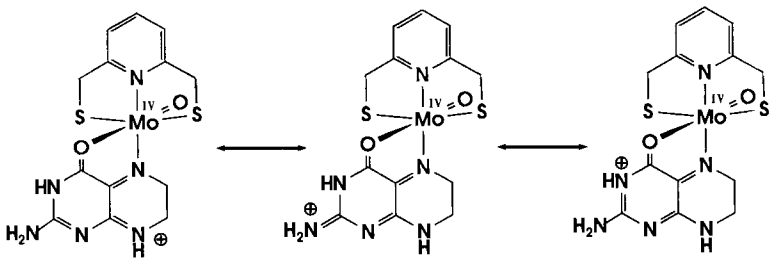
Table. *Selected Interatomic Distances* [Å] *and Bond Angles* [°] *in* **9**

Bond distances [Å]		Bond angles [°]	
Mo–O(4)	2.302(3)	O(4)–Mo–N(5)	72.7(1)
Mo–N(5)	2.015(3)	O(1)–Mo–N(5)	93.8(1)
Mo–O(1)	1.676(3)	N(31)–Mo–O(1)	102.4(2)
Mo–N(31)	2.218(4)	N(31)–Mo–N(5)	163.6(1)
Mo–O(4)	2.302(3)	O(4)–Mo–O(1)	166.5(1)
Mo–S(2)	2.390(2)	S(2)–Mo–N(5)	95.3(1)
Mo–S(1)	2.393(1)	S(1)–Mo–N(5)	98.2(1)
N(1)–C(2)	1.336(5)	N(5)–C(4a)–C(4)	113.8(3)
N(1)–C(8a)	1.355(4)	C(2)–N(1)–C(8a)	117.3(3)
C(2)–N(2)	1.308(4)	N(2)–C(2)–N(1)	119.1(3)
C(2)–N(3)	1.365(4)	N(2)–C(2)–N(3)	117.4(3)
N(3)–C(4)	1.359(4)	C(4)–N(3)–C(2)	122.0(3)
C(4)–O(4)	1.233(4)	O(4)–C(4)–C(4a)	119.7(3)
C(4)–C(4a)	1.408(5)	N(3)–C(4)–C(4a)	116.0(3)
N(5)–C(4a)	1.348(4)	C(4a)–N(5)–C(6)	112.7(3)
N(5)–C(6)	1.488(5)	C(4a)–N(5)–Mo	121.4(3)
C(6)–C(7)	1.491(7)	C(7)–C(6)–N(5)	111.3(3)
C(7)–N(8)	1.473(5)	N(8)–C(7)–C(6)	110.4(4)
N(8)–C(8a)	1.312(5)	C(8a)–N(8)–C(7)	117.9(3)
C(4a)–C(8a)	1.421(5)	N(5)–C(4a)–C(8a)	126.4(3)

The Mo-atom is displaced from the center of the ideal octahedron in the direction of the terminal oxo ligand, caused by the *trans* effect of this terminal oxo function, leading to a relative short Mo–O(1) bond distance. This also results in a relative elongation of the opposite Mo–O(4) bond. Additionally, the small bite angle of the chelating pterin ligand (72.7(1)°) contributes to the distortion of the coordination octahedron. The bond distances of the coordinating atoms of the pterin ligand to the metal center are almost identical to other known [Mo^{IV}-q-H_2Ptr] complexes [11] [12]. The Mo–O(4) bond distance is only slightly longer, probably because of the higher sterical demand of the LN-S_2 ligand. The short Mo–N(5) bond distance of 2.015(3) Å, known only in Mo-amido complexes [15], is the same, indicating strong interactions of the Mo-center with the pterin ligand. The observed bond distances in the hydrogenated pterin nucleus support the formulation of the oxidation state of the ligand as a monocationic N(2)–N(3)–N(8)-mesomeric form of a monoprotonated quinonoid 6,7-dihydropterin, that we have proposed earlier [11] [12]. The explanation for this dihydropterin oxidation state is a two-electron oxidation of the starting compound tetrahydropterin (**7**) with a concomitant reduction of Mo^{VI} to Mo^{IV}.

The possible forms of the cation **9** are displayed in *Fig. 2*. The mesomeric forms **9a** ↔ **9b** may be preferred, indicated by the slightly longer bond distance C(2)–N(3) compared with C(2)–N(1) and C(2)–N(2). The resulting overall positive charge of the complex, due to the protonation of the N(2), N(8), or N(3) atom in the quinonoid dihydropterin nucleus, is neutralized in **9** by the Cl^- counterion.

Controversy has occurred over the formulation of the oxidation states of the Mo-atom and the hydrogenated pterins in the complex [$MoOCl_3$(H^+-q-H_2Bip)] [11]. *Burgmayer* and coworkers have synthesized similar complexes including an interesting dimeric species with the same structural and spectroscopic features as our complex [16]. They



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

Fig. 2. *Schematic formulation of the possible mesomeric structures of the pterin heterocycle in the cationic complex* **9**. Counterion is Cl^- (not shown).

formulate the complexes as Mo^{VI} centers coordinated by N(5)-deprotonated tetrahydropterin anions implying no Mo^{VI}/tetrahydropterin redox reaction. One of their arguments in favor of Mo^{VI} coordinated by the N(5)-deprotonated tetrahydropterin anion was the lack of oxo transfer to their complexes from DMSO, a reaction typical of Mo^{IV} complexes. We have demonstrated the reduction of DMSO to dimethyl sulfide by [$MoOCl_3(H^+$-q-$H_2Ptr)$] [12].

Spectral Properties of [$Mo^{IV}O$(LN-S_2)(H^+-q-H_2Ptr)]Cl (**9**). The UV/VIS spectrum of the deeply red solution of **9** in MeOH displays absorptions of the quinonoid dihydropterin at λ_{max} 219 (ε = 20900) and 267 (11000) nm and a shoulder at *ca.* 310 (*ca.* 5000) nm. The absorption at 380 (4500) nm is attributed to the coordinated LN-S_2 ligand. A strong charge-transfer absorption at 495 (8600) nm is interpreted as a metal-to-ligand charge-transfer transition from Mo^{IV} to the N(5) atom of the pterin ligand [17].

The ^{13}C-NMR data (see *Exper. Part*) of **9** in DMSO confirms earlier assignments. The chemical shifts appear in the same range as found for [$MoOCl_3(H^+$-q-$H_2Bip)$] [11] and [$MoOCl_3(H^+$-q-$H_2Ptr)$] [12]. The change of the chemical shifts of the C-atoms C(4a) and C(6), which are neighboring atoms to N(5), which coordinates the Mo-atom, is large and provides evidence for the oxidation state of the heterocyclic system and the influence of metal complexation. The strongly shifted values – up to +35 ppm compared with a non-coordinated tetrahydropterin [11] and up to −20 ppm compared with a non-coordinated quinonoid dihydropterin system [18] – indicate intensive interactions between the Mo-atom and the pterin *via* the Mo–N(5) bond.

Complex **9** reacts with the enzyme substrate DMSO to a very small extent and only over a large period of time, in contrast to [$MoOCl_3(H^+$-q-$H_2Ptr)$] [12] as proven by NMR spectroscopy.

Conclusion. – Complex **9** is a new structural model compound for the possible interactions of molybdenum and hydrogenated pterins. The structure demonstrates the concurrent coordination of thiolato ligands and hydrogenated pterin to a Mo-atom in the enzyme relevant oxidation state +IV. The very slow reaction of **9** with the enzyme substrate DMSO, in contrast with that of [$MoOCl_3(H^+$-q-$H_2Ptr)$] [12] containing the easily dissociable Cl^- ligand, indicates the need for such a labile binding site in a reactive model complex. Labile binding sites are also seen in the metal centers of the protein structures of the recently solved X-ray structures of molybdopterin-containing enzymes [8–10]. However, the crystal structures of these proteins do not show a N(5),O(4) coordi-

nation of the hydrogenated pterin to the metal atoms. This leads to a question of the relevance of this observed coordination mode in model complexes for the active site in molybdopterin-containing enzymes. By analogy with a former proposal [11], such a coordination mode may represent the structure of a very short-lived intermediate compound as indicated in *Fig. 3*. If such an intermediate is not required for the catalytic function of the enzymes, then nature may have tuned its ligand design to prevent this coordination mode by incorporating an enedithiol unit that preferentially binds to the molybdenum center.

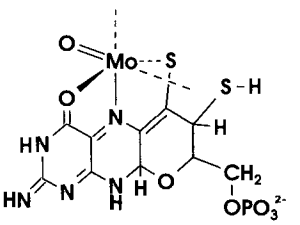


Fig. 3. *Schematic proposal of a possible short-lived intermediate in the reaction course of molybdopterin-dependent molybdenum and tungsten enzymes*

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Experimental Part

General. $[MoO_2(LN\text{-}S_2)]$ (**8**) was prepared according to a published procedure [14], and $H_4Ptr \cdot 2$ HCl (**7**·2 HCl) was synthesized by a known method [19]. All solvents were of *puriss.* grade and made O_2-free by bubbling through Ar. MeOH was dried over Mg and distilled under Ar. NMR Spectra: *Bruker AS400*; δ in ppm. UV/VIS Spectra: *Perkin-Elmer Lambda 15*; λ_{max} [nm] (ε [M^{-1} cm^{-1}]).

(2-Amino-3,4,6,7-tetrahydro-4-oxopteridin-8(or N^2 or 3)-ium-κ^2-N^5,O^4)oxo[pyridine-2,6-bis(methanethiolato)-κ^2-S,S']molybdenum(IV) Chloride ($[Mo^{IV}O(LN\text{-}S_2)(H^+\text{-}q\text{-}H_2Ptr)]Cl$; **9**). White $H_4Ptr \cdot 2$ HCl (**7**·2 HCl; 64 mg, 0.26 mmol) is added in one portion under exclusion of O_2 to a vigorously stirred suspension of orange $[MoO_2(LN\text{-}S_2)]$ (**8**; 79 mg, 0.26 mmol) in MeOH (100 ml). After *ca.* 4 h at r.t., the suspension adopts a burgundy-red color which intensifies during the course of the reaction. Stirring is continued for another 5 days after which the dark red residue is filtered off under inert conditions. The residue consists of a mixture of starting compounds and several polymeric by-products. The burgundy-red filtrate is evaporated and co-evaporated several times with O_2-free MeOH to remove excess HCl. The dark red residue obtained is suspended under Ar in a minimal amount (*ca.* 3 ml) of O_2-free MeOH, to remove excess $H_4Ptr \cdot 2$ HCl, and filtered off immediately. The resulting dark red and very powdery product is dried at 50°/high vacuum. Yield *ca.* 50%. Diffusion of a toluene/cyclohexane mixture into the reaction solution yielded a few crystals of **9** suitable for X-ray crystallography. UV/VIS (MeOH): 219 (20900), 267 (11000), *ca.* 310 (sh, *ca.* 5000), 380 (*ca.* 4500), 495 (8600). ^{13}C-NMR (100 MHz, $(D_6)DMSO$, 30°; ref.: solvent, 39.5 ppm; for numbering, see *Fig. 1*): 168.8 (C(32),C(36)); 157.7 (C(4)); 154.2 (C(2)); 150.1 (C(8a)); 144.3 (C(34)); 120.9 (C(33),C(35)); 118.1 (C(4a)); 59.1 (C(6)); 42.8 (C(11),C(21)); 41.4 (C(7)). Anal. calc. for $C_{13}H_{15}ClMoN_6O_2S_2$ (482.82): C 31.9, H 3.2, Cl 8.6, N 16.6, S 12.4; found: C 32.3, H 3.1, Cl 7.3, N 17.4, S 13.2.

Crystal-Structure Determination of **9**. Data for the single-crystal X-ray structure determination of **9**: $a = 22.900(5)$, $b = 10.716(2)$, $c = 17.551(4)$ Å, $\beta = 120.36(3)°$, $V = 3716.3(2.9)$ Å^3, $Z = 8$, crystal system monoclinic, space group $C2/c$, No. 15, $\rho_{calc.} = 1.726$ gcm^{-3}. A single crystal of the size 0.57 × 0.37 × 0.32 mm was fixed on the tip of a glass fiber with epoxy cement. Crystal and intensity data were collected on an *Enraf-Nonius CAD4* diffractometer equipped with graphite monochromated MoK_α radiation ($\lambda = 0.71073$ Å). To determine the crystal system (monoclinic) and an accurate cell for the data collection, 25 reflections were used. At r.t. (295 K), 13224 intensity data (including 309 systematic absences) were collected in the range $4° \leq 2\Theta \leq 64°$. An ω–2θ scan (zigzag mode) was applied, *hkl* range: $-33 \rightarrow 34$, $-15 \rightarrow 15$, $-18 \rightarrow 26$. Numerical absorption correction was performed, using the *Enraf-Nonius-CAD4* software and the MolEN program system [20]. The phase problem was solved with the *Patterson* interpretation routine of SHELXS86 [21] using 6421 unique data in the space group $C2/c$. All

H-atom positions could be identified in the difference *Fourier* synthesis. Finally, the refinement was performed with all F^2 unique data and 283 parameters using SHELXL93 [22]. H-Atom positions were refined isotropically, except for H(3), which had to be refined as a riding model. The maximum and minimum heights of the final difference *Fourier* map were 1.393 and -0.639 e·Å^{-3}, resp. Goodness-of-fit for $F^2 = 1.090$, $R = 0.0488$, $wR_2 = 0.1137$ [$I > 2\sigma(I)$]; $R = 0.1294$, $wR_2 = 0.1629$ (for all data). All calculations were performed on a *Micro-Vax-3100* computer. The atomic coordinates and all bond lengths and angles have been deposited with the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, England, as supplementary publication No. CCDC-10/30.

REFERENCES

[1] a) M. Viscontini, in 'Pteridines and Biogenic Amines in Neuropsychiatry, Pediatrics, and Immunology', Eds. R.A. Levine, S. Milstien, D.M. Kuhn, and H.-Ch. Curtius, Lakeshore Publishing Comp. Grosse Pointe, Michigan, USA, 1989, p. 33; b) J. Ferré, K.B. Jacobson, W. Pfleiderer, *Pteridines* **1990,** *2,* 129.

[2] T.A. Dix, S.J. Bencovic, *Acc. Chem. Res.* **1988,** *21,* 101; S.O. Pember, K.A. Johnson, J.J. Villafranca, S.J. Bencovic, *Biochemistry* **1989,** *28,* 2124; S. Kaufmann, in 'Chem. Biol. Pteridines, Proc. 8th Int. Symp. Pteridines and Folic Acid Derivatives', Eds. B.A. Cooper and V.M. Whitehead, Walter de Gruyter, Berlin, 1986, p. 185; J.M. Zaleski, K.E. Loeb, A.M. Orville, T.J. Kappock, J.P. Caradonna, J.D. Lipscomb, E.I. Solomon, *J. Inorg. Biochem.* **1995,** *59,* 364; W. Meyer, J. Haavik, H. Winkler, A.X. Trautwein, H.-F. Nolting, *ibid.* **1995,** *59,* 382.

[3] M.A. Tayeh, M.A. Marletta, *J. Biol. Chem.* **1989,** *264,* 19654; N.S. Kwon, C.F. Nathan, D.J. Stuehr, *ibid.* **1989,** *264,* 20496; C. Nathan, *FASEB J.* **1992,** *6,* 3051; S. Moncada, R.M.J. Palmer, F.A. Higgs, *Pharmacol. Rev.* **1992,** *43,* 109; J.S. Stammler, D.J. Singel, J. Loscalzo, *Science* **1992,** *258,* 1898.

[4] J.L. Johnson, K.V. Rajagopalan, *Proc. Natl. Acad. Sci. U.S.A.* **1982,** *79,* 6856; J.L. Johnson, B.E. Hainline, K.V. Rajagopalan, B.H. Arison, *J. Biol. Chem.* **1984,** *259,* 5414; C.D. Garner, S. Bristow, in 'Molybdenum Enzymes, Met. Ions Biol.', Ed. T.G. Spiro, J. Wiley, New York, 1985, Vol. 7; S.J.N. Burgmayer, E.I. Stiefel, *J. Chem. Educ.* **1985,** *62,* 943; R.S. Pilato, E.I. Stiefel, in 'Bioinorganic Catalysis', Ed. J. Reedjik, Marcel Dekker, New York, 1993, p. 131; J.H. Enemark, C.G. Young, *Adv. Inorg. Chem.* **1994,** *40,* 1; R.H. Holm, *Coord. Chem. Rev.* **1990,** *100,* 183.

[5] P.A. Bertram, R.A. Schmitz, D. Linder, R.K. Thauer, *Arch. Microbiol.* **1994,** *161,* 220.

[6] C.R. Woese, O. Kandler, M.L. Wheelis, *Proc. Natl. Acad. Sci. U.S.A.* **1990,** *87,* 4576; R. Hekinian, M. Fevrier, J.L. Bischoff, P. Picot, W.C. Shanks, *Science* **1980,** *207,* 1433; R.A. Schmitz, M. Richter, D. Linder, R.K. Thauer, *Eur. J. Biochem.* **1992,** *207,* 559.

[7] K.V. Rajagopalan, S. Kramer, S. Gardlik, *Polyhedron* **1986,** *5,* 573; S. Gardlik, K.V. Rajagopalan, *J. Biol. Chem.* **1990,** *265,* 13047; K.V. Rajagopalan, J.L. Johnson, *ibid.* **1992,** *267,* 10199.

[8] M.K. Chan, S. Mukund, A. Kletzin, M.W.W. Adams, D.C. Rees, *Science* **1995,** *267,* 1463.

[9] M.J. Romão, M. Archer, I. Moura, J.J.G. Moura, J. LeGall, R. Engh, M. Schneider, P. Hof, R. Huber, *Science* **1995,** *270,* 1170.

[10] H. Schindelin, C. Kisker, J. Hilton, K.V. Rajagopalan, D.C. Rees, *Science* **1996,** *272,* 1615.

[11] B. Fischer, J. Strähle, M. Viscontini, *Helv. Chim. Acta* **1991,** *74,* 1544.

[12] B. Fischer, H. Schmalle, E. Dubler, A. Schäfer, M. Viscontini, *Inorg. Chem.* **1995,** *34,* 5726.

[13] N.R. Bastian, C.J. Kay, M.J. Barber, K.V. Rajagopalan, *J. Biol. Chem.* **1991,** *266,* 45; A.G. McEwan, S.J. Ferguson, J.B. Jackson, *Biochem. J.* **1991,** *274,* 305.

[14] J.M. Berg, R.H. Holm, *Inorg. Chem.* **1983,** *22,* 1768.

[15] M.F. Lappert, P.P. Power, A.R. Sanger, R.C. Srivastava, in 'Metal and Metalloid Amides', Ellis Horwood, New York, 1980, Chapt. 8.

[16] S.J.N. Burgmayer, M.R. Arkin, L. Bostick, S. Dempster, K.M. Everett, H.L. Layton, K.E. Paul, C. Rogge, A.L. Rheingold, *J. Am. Chem. Soc.* **1995,** *117,* 5812.

[17] A.B.P. Lever, in 'Inorganic Electronic Spectroscopy', 2nd edn., Elsevier, New York, 1984.

[18] J.B. Noar, U.V. Venkataram, T.C. Bruice, G. Bollag, R. Whittle, D. Sammons, R. Henry, S.J. Benkovic, *Bioorg. Chem.* **1986,** *14,* 17.

[19] A. Bobst, M. Viscontini, *Helv. Chim. Acta* **1966,** *49,* 875.

[20] 'MolEN, an Interactive Structure Solution Procedure', *Enraf-Nonius*, Delft, The Netherlands, 1990.

[21] G.M. Sheldrick, *Acta Crystallogr., Sect. A* **1990,** *46,* 467.

[22] G.M. Sheldrick, 'SHELXL-93, A Program for Structure Refinement', University of Göttingen, Germany, 1993.