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Synthesis of Structural Analogues of the Oxidized Sites in the Xanthine Oxidoreductase Enzyme Family

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Our recent research in the biomimetic chemistry of molybdenum and tungsten[1] has produced structural and/or functional analogues of a variety of active sites in the DMSO reductase (DMSOR) and sulfite oxidase (SO) enzyme families as classified by Hille.[2] Analogue sites include those of DMSO reductase,[1,3a] nitrate reductase,[3b] selenate reductase,[3c] formate dehydrogenase,[3d] and sulfite oxidase.[3e] In these molecules, pyranopterindithiolene cofactor ligand binding is simulated by one (SO family) or two (DMSOR family) ene-1,2-dithiolate or benzene-1,2-dithiolate (bdt) ligands. Site analogues provide unconstrained structures with accurate metric details and kinetic/mechanistic information on oxygen-atom transfer reactions. The third major enzyme group is the xanthine oxidoreductase (XO) family,[2] whose members, primarily XO itself and aldehyde oxidoreductase, are hydroxylases.[4] The X-ray structure of *P. putida* quinoline 2-oxidoreductase discloses the square pyramidal (SP) oxidized active site structure **1a**[5] (Figure 1) characterized by one cofactor ligand, apical and basal oxo ligands, and a *basal* sulfido ligand bound to Mo^{VI}. The structure of a Mo^{IV} catalytic intermediate of bovine milk XO[6] is consistent with this structure. EXAFS analysis of oxidized XO at two pH values affords the coordination units and dimensions of **1a** and protonated **1b**.[7]

While synthetic non-tetrahedral species containing the *cis*-M^{VI}-OS group (M = Mo, W) are known,[8] the $M^{VI}O_2S$ group in such molecules has remained elusive. Here we report synthesis of the initial structural analogues of **1a** and **1b** with use of tungsten to stabilize the M^{VI} state. Reactions are summarized in Figure 1.[9] A light yellow solution of $(Et_4N)_2[WO_3(bdt)]$[10] (**2a**, 0.20 mmol) was frozen at −78 °C in an evacuated 20-mL container. Gaseous H_2S was admitted to the headspace for 5 s, the temperature was raised to −30 °C, and the melted mixture was stirred and became bright orange. The solvent was removed, the residue was washed with ether and recrystallized (acetonitrile/ether) to afford $(Et_4N)_2[WO_2S(bdt)]$[9] (∼60%) as deep-orange plate-like crystals. The two ordered anions **3** have similar metric features and the distorted square pyramidal stereochemistry of **1a**, most notably with apical oxo and basal sulfido ligands (Figure 2). The structure is summarized by two large basal angles (O2−W1−S2, 146.3(4)°; S1−W1−S3, 146.8(1)°), apical-basal angles of 104.4(3)−106.3(3)°, and the indicated bond distances. The conformance of bond lengths (≤0.05 Å) and stereochemistry delimits **3** as the initial structural analogue of unprotonated site **1a**.

Given the usual instability of protonated sulfido complexes, an analogue of site **1b** was sought by another route. Treatment of **2a** with 1 equiv of Pr^i_3SiCl in acetonitrile followed by standard workup affords green-brown $(Et_4N)[WO_2(OSiPr^i_3)(bdt)]$ (68%) with basal silyloxide ligation.[11] Reaction of this complex with Pr^i_3SiSH (2 equiv, 30 min, acetonitrile/THF), product isolation, and recrystallization (acetonitrile/ether) yielded $(Et_4N)[WOS(OSiPr^i_3)(bdt)]$[9]

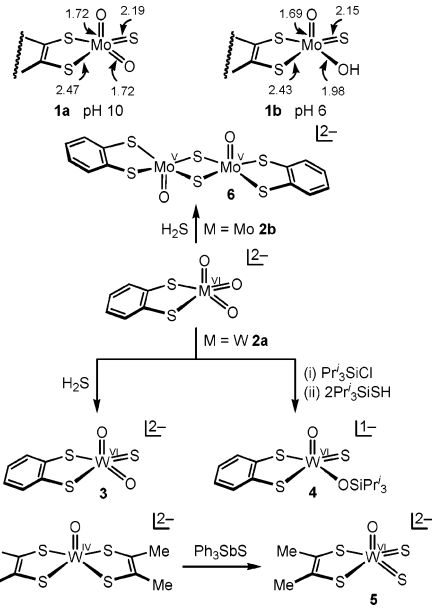

Figure 1. Schematic structures of the active site of oxidized XO at two pH values (**1a**, **1b**) with bond lengths (Å) determined by EXAFS and reactions affording mononuclear W^{VI} complexes **3**−**5** and Mo^{V} dimer **6**.

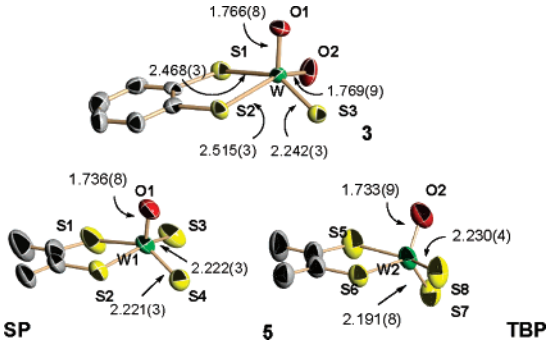

Figure 2. Structures and bond lengths (Å) of **3** and the approximate square pyramidal and trigonal bipyramidal configurations of **5**.

(78%). This new method for oxygen/sulfur substitution proceeds with the apparent stoichiometry $[WO_2(OSiPr^i_3)(bdt)]^{1-} + 2Pr^i_3SiSH \rightarrow [WOS(OSiPr^i_3)(bdt)]^{1-} + (Pr^i_3Si)_2O + H_2S$. The structure of **4** is distorted square pyramidal (S−W−O_{Si}, 149.5(2)°; S−W−S, 143.6(1)°) with apical oxo (W−O, 1.753(6) Å) and basal sulfido (W−S, 2.153(3) Å) ligation. These and the basal bond distances (mean W−S_{bdt}, 2.44 Å; W−O_{Si}, 1.902(6) Å) render **4** a structural analogue of **1b**, in which the silyloxide group simulates protonation. In effect, **4** is a silylated derivative of **3** with retention of stereochemistry.

To investigate the incorporation of the native metal into XO site analogues, a procedure closely analogous to that for **3** was followed

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Table 1. Computed Relative Energies for $[WO_nS_{3-n}L]^{2-}$ Complexes

	$\Delta E^{a,b}$ (kcal/mol)	
$[WO_nS_{3-n}L]^{2-}$	$O_{ap} - S_{ap}$[c]	O_{ap} – TS[d]
n=2, L = bdt (**3**)	−0.8	−1.8
n=2, L = dt[e]	−0.7	−1.5
n=1, L = bdt	−0.9	−1.6
n=1, L = dt (**5**)	−0.5	−1.4

[a] Negative differences favor the first (O_{ap}) structure. [b] Corrected for zero-point energies. [c] O_{ap} = apical oxo, S_{ap} = apical sulfido, SP geometry. [d] TS = transition state, TBP geometry. [e] dt = $H_2C_2S_2^{2-}$.

utilizing Mo^{VI} complex **2b**. The crystalline product was identified as $(Et_4N)_2[Mo_2O_2S_2(bdt)_2]$.[9] The centrosymmetric dimer **6** (Figure 1) contains the $[Mo^V_2O_2(\mu_2\text{-}S)_2]^{2+}$ core, a precedented structural element in oxothiomolybdate systems.[12] This outcome is another example of the autoreduction of non-tetrahedral Mo^{VI} by an anionic sulfur ligand environment, here obviating isolation of $[MoO_2S(bdt)]^{2-}$. Isoelectronic molybdenum and tungsten complexes with identical ligands are isostructural and nearly isometric, justifying the description of **3** and **4** as site structural analogues.

The existence of **2a** and **3** implies the series $[WO_nS_{n-3}(S_2C_2R_2)]^{2-}$ (n = 0−3) with bdt or another dithiolene ligand, a matter pursued by the reaction of $(Et_4N)_2[WO(S_2C_2Me_2)_2]$[13] with Ph_3SbS (1.4 equiv, acetonitrile/THF), which afforded red $(Et_4N)[WOS_2(S_2C_2Me_2)]$[9] (56%). The two independent anions **5** have related but distinct structures (Figure 2). One approaches SP stereochemistry with basal dithiolene and two sulfido ligands, an axial oxo ligand, and two large basal angles (S1−W1-S4, 141.1(1)°; S2−W1-S3, 150.3(1)°). The other approximates a trigonal bipyramidal (TBP) structure with axial sulfido, axial−equatorial dithiolene, equatorial oxide and sulfide, and identifiable axial (S5−W2-S8, 154.79(1)°) and equatorial (107.9(3)−126.2(3)°) angles. The existence of **5**-SP and **5**-TBP in the same crystal indicates that the energy difference between them is slight.

Structure preferences for the mixed terminal chalcogenido species $[WO_nS_{3-n}(S_2C_2R_2)]^{2-}$ (n = 1, 2) were investigated by density functional calculations (Table 1).[14,15] The calculations indicate that for all given ligand combinations diastereoisomeric SP complexes with apical oxo and sulfido ligation are near-equienergetic with the former slightly, but consistently, more stable. The O_{ap} and S_{ap} isomers can interconvert through an easily accessible TBP transition state that lies less than 2 kcal/mol above the most stable SP structure; the transition state normal mode corresponds to a torsion of pyramidal WO_nS_{3-n} with respect to the planar dithiolene chelate ring, a process equivalent to a half-Berry pseudorotation.

Comparing computation to experiment, the optimized geometries of the relevant DFT models reproduce well the crystallographic structures of **3** and **5**-SP (as approximated by the simpler dt ligand), with the largest bond deviations appearing in selected W−S contacts that are ca. 0.05 Å too long. Remarkably, the calculated TBP solution transition-state structure is also found, at similar metrical accuracy, as **5**-TBP. The DFT energy predictions are consistent with the observation of **3** and **5** as predominantly O_{ap} SP isomers in the solid state and with the existence of **5**-TBP, which presumably represents the stabilization of an energetically low-lying structure by weak lattice forces.

In summary, we have prepared the initial structural analogues of oxidized active sites **1a** and **1b** of the XO enzyme family. Ongoing studies focus on reduced site representations and the reactivity of oxidized and reduced analogues.

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Supporting Information Available: X-ray crystallographic files in CIF format for the structures of compounds containing anions **3**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) All operations were carried out under pure dinitrogen atmosphere. X-ray structures were solved and refined by standard methods. $(Et_4N)_2$[**3**]: absorption spectrum (acetonitrile), λ_{max} (ϵ_M) 316 (5650), 363 (sh, 2890), 490 (sh, 1100) nm. IR(KBr): 901, 845, 421 cm^{-1}. ^1H NMR (CD_3CN, anion): δ 6.56 (q, 2), 7.03 (q, 2). Anal. Calcd (Found) for $C_{22}H_{44}N_2O_2S_3W$: C, 40.74 (40.38), 6.84 (6.75), 4.32 (4.26); S, 14.83 (14.76). Triclinic ($P\bar{1}$, 298 K): a = 11.430(2) Å, b = 15.430(3) Å, c = 24.460(5) Å, α = 105.81(3)°, β = 102.96(3)°, γ = 90.01(3)°, Z = 6, $R_1(wR_2)$ = 0.0665(0.1518). (Et_4N)[**4**]: absorption spectrum (acetonitrile), λ_{max} (ϵ_M) 301 (11400), 360 (5880), 500 (sh, 750) nm. IR(KBr): 949, 911, 477 cm^{-1}. Orthorhombic (*Pbca*, 193 K): a = 15.742(5) Å, b = 14.364-(5) Å, c = 26.503(9) Å, Z = 8, $R_1(wR_2)$ = 0.0540(0.1117). $(Et_4N)_2$[**5**]: absorption spectrum (acetonitrile), λ_{max} (ϵ_M) 263 (sh, 11000), 338 (sh, 3450), 477 (2700), 675 (630) nm. IR (KBr): 893, 440 cm^{-1}. Monoclinic (*C*2/c, 193 K): a = 19.982(1) Å, b = 17.956(1) Å, c = 41.086(2) Å, β = 103.22(1)°, Z = 8, $R_1(wR_2)$ = 0.0634(0.1658). $(Et_4N)_2$[**6**], monoclinic ($P2_1/n$, 293 K): a = 9.310(2) Å, b = 12.180(2) Å, c = 15.660(2) Å, β = 90.48(3)°, Z = 2, $R_1(wR_2)$ = 0.0755(0.2043). The structures containing **3** and **5** have 3 and 2.5 independent anions in the asymmetric unit, one or one-half of which is disordered.
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