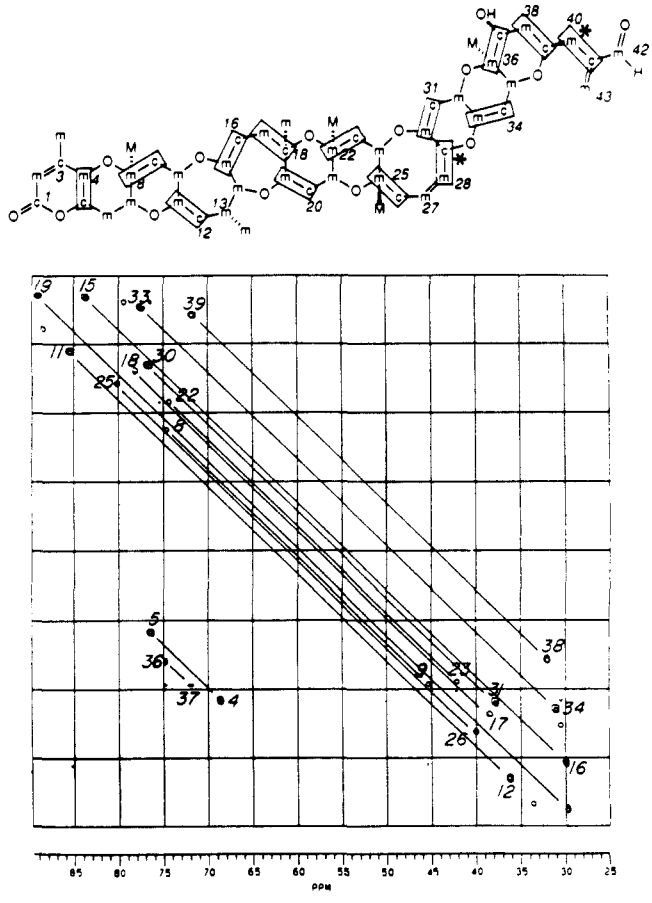

Figure 1. Partial ^{13}C NMR spectrum (90–25 ppm) of 2D INADEQUATE of $^{13}CH_3{}^{13}CO_2Na$ labeled BTX-B (3.2 mM) in C_6D_6, 125.13 MHz Bruker AM-500. The C_2 units that originate from the same acetates are enclosed in squares. The asterisked carbon pairs 28/29 and 40/41 are not in the figure because they fall out of the range shown, but the connectivities were evident. m = $^{13}CH_3CO_2Na$, c = $CH_3{}^{13}CO_2Na$, and M = $^{13}CH_3SCH_2CH_2CH(NH_2)CO_2H$.

labeling experiments the yield of BTX-B is frequently less then 1 mg. The ^{13}C NMR peak heights showed the incorporation level of acetates in BTX-B to be 2.0–2.5%. The erratic growth of these dinoflagellates has been a major obstacle in obtaining the toxins. This was especially true with respect to labeling experiments. In some instances the addition of sodium acetate to the *G. breve* culture led to the destruction of the entire culture, whereas in some instances it actually enhanced the growth of the culture.

Of the 50 carbons of BTX-B, 16 carbons showed enrichment from [1-^{13}C]acetate, 30 carbons from [2-^{13}C]acetate, and 4 carbons from methyl-^{13}C-methionine (Figure 1), thus accounting for the origin of all carbons. The doubly labeled BTX-B molecule is an ideal model for demonstrating the INADEQUATE ^{13}C NMR technique[11] in biosynthetic studies. Namely, its single carbon chain contains many contiguous carbon pairs, e.g., C-8/C-9 in **1**, in which one carbon (C-9) is linked only to hydrogens and carbon (C-10) thus appearing around 35 ppm, whereas the other (C-8) is linked to an oxygen and appears around 80 ppm. This large difference in chemical shifts gives a spectrum in which the carbons correlated by connectivity are clearly separated (Figure 1).[12] These 2D INADEQUATE measurements[13] on BTX-B

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(12) Cross peaks for the acetate connectivity patterns of C-1, C-2, C-3, and 3-Me were absent from the 2D INADEQUATE spectrum, presumably due to longer t_1's of the carbons within this ene lactone moity.

(13) For the INADEQUATE experiment, 125.13 MHz Bruker AM-500, internal reference C_6D_6 at 25 °C (128.0 ppm) was used;[4] the residual artifacts in 2D matrix were removed by symmetrization.[14]

Table I. Carbon Assignments of BTX-B[a]

carbon	ppm	carbon	ppm	carbon	ppm
1	162.51	18	78.04	35	63.65
2	116.54	19	89.02	36	74.92
3	159.43	20	29.76	37	71.93
4	68.63	21	74.85	38	32.01
5	76.47	22	74.25	39	71.70
6	30.53	23	42.16	40	32.34
7	79.37	24	75.39	41	148.79
8	74.69	25	80.03	42	193.53
9	45.39	26	40.01	43	134.40
10	83.89	27	126.94	3-Me	16.70
11	85.43	28	136.41	8-Me	15.84
12	36.24	29	80.35	13-Me	18.33
13	33.59	30	76.73	18-Me	22.12
14	88.41	31	37.85	22-Me	20.53
15	83.59	32	69.78	25-Me	18.33
16	29.95	33	77.48	36-Me	14.00
17	38.48	34	31.09		

[a] Measurements were performed with Bruker AM-500, 125.13 MHz, and Bruker WM-250, 62.89 MHz, in C_6D_6 at 25 °C.

incorporating [1,2-$^{13}C_2$]acetate enabled one to assign remaining carbons and also to confirm assignments of many other carbons. This led to the assignments of all carbons (Table I) as well as elucidation of their biosynthetic origins. It should be noted that the single carbon chain which constitutes the backbone of the ladder-like oxacyclic skeleton is not a simple polyketide. Indeed there are six m–m moieties and even two contiguous m–m–m moieties, one of which is extended by an additional Me group (13-Me). Further studies of this unprecedented biosynthetic pattern are being carried out.

Acknowledgment. This work was supported by NIH grant AI 10187; financial support from Merck Sharpe and Dohme is also gratefully acknowledged. We thank J. C. Cesarelli for technical assistance.

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Thiol as an Electron Donor in Molybdenum Oxo-Transferase Analogue Reaction Systems: Observations by ^{19}F NMR Spectroscopy and Biological Implications

John P. Caradonna,[1] Edgar W. Harlan, and R. H. Holm*

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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The molybdenum oxo-transferases (hydroxylases)[2] catalyze the two-electron oxidation or reduction of substrates X/XO in processes which may be formally represented as $X + H_2O \rightleftharpoons XO + 2H^+ + 2e^-$. Our recent investigations have demonstrated that (i) the complexes MoO_2(L-NS_2) (**1**) and MoO(L-NS_2)(DMF) (**2**) are reasonable structural representations of certain enzyme sites;[3] (ii) stoichiometric reaction 1 occurs with a variety of substrates, including sulfoxides and *N*-oxides that are enzyme substrates;[4,5] (iii) the catalytic cycle 2 (XO = R_2SO) operates

(1) NIH Postdoctoral Fellow, 1985–1987.

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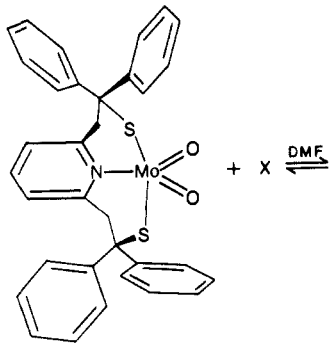
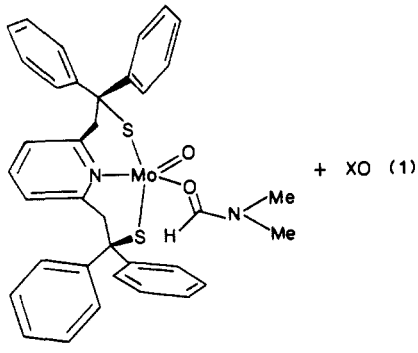


1



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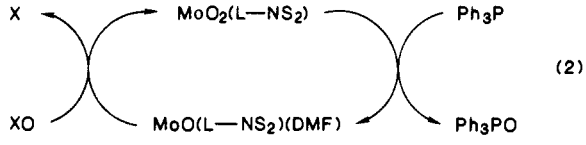


(2)

and is capable of at least 500 turnovers in DMF solution.[4] Throughout, our working hypothesis has been that at least some enzymatic processes proceed via forward or reverse direct oxo atom transfer, viz., $MoOL_n + XO \rightleftharpoons MoO_2L_n + X$. The catalytic transfer of ^{18}O from nicotinamide *N*-oxide to xanthine by milk and liver xanthine oxidase[6] constitutes proof of one such direct reaction. Here the enzyme acts as a reductase toward the alternative substrate, the *N*-oxide, and an oxidase toward its natural substrate, xanthine.

With cycle 2 and related information as the basis, we proposed a reaction cycle for the enzymatic reduction of sulfoxides[4,7] as catalyzed by, e.g., *d*-biotin sulfoxide reductase.[8] All proposed steps follow from reactions demonstrated in the analogue system except for the reduction of the $E_{ox}(Mo^{VI}O_2)$ to the $E_{red}(Mo^{IV}O)$ form of the enzyme, which is presumed to contain the indicated groups. In cycle 2 this step is effected by the nonphysiological reductant Ph_3P. Demonstration of sulfoxide reduction by liver cytosolic enzymes[9] and yeast methionine reductase[10] in reconstituted systems including the cysteinyl-containing redox protein thioredoxin[11] implicates thiol as a physiological reductant ($2Cys{\cdot}SH \rightleftharpoons Cys{\cdot}SS{\cdot}Cys + 2H^+ + 2e^-$), as shown in cycle 3. We

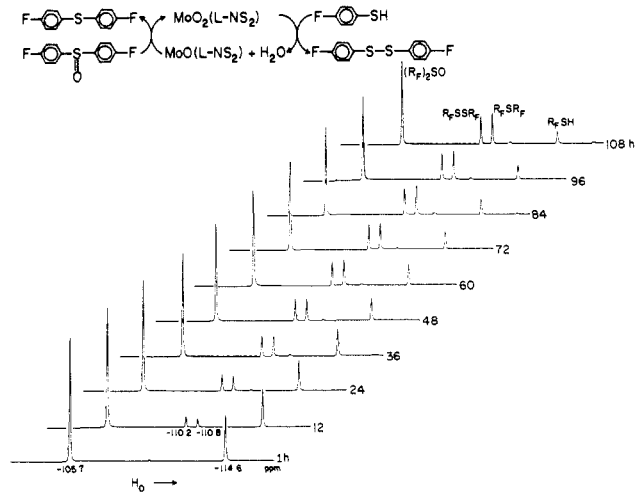
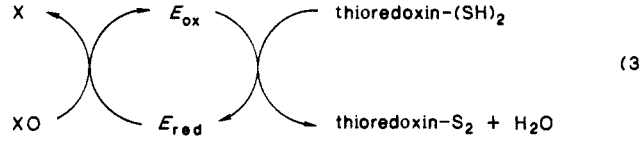


Figure 1. ^{19}F NMR (282.4 MHz) of a reaction system in DMF solution at 25 °C initially containing the mole ratio $R_FSH:(R_F)_2SO:MoO_2(L\text{-}NS_2)$ = 12.5:12.5:1 (R_F = *p*-FC_6H_4) and $[MoO_2(L\text{-}NS_2)]$ = 40 mM. Spectra over the 1–108-h interval are shown. Signal assignments are indicated; chemical shifts are relative to $CFCl_3$ external standard. Acquisition parameters were adjusted to afford meaningful integrated signal intensities for $T_1 \leq 20$ s, as determined for a set of *p*-fluorophenyl compounds.

present evidence that thiol is a viable electron donor in the Mo-mediated reduction of sulfoxides.



(3)

An anaerobic reaction system with the initial mole ratio $R_FSH:(R_F)_2SO$:**1** = 12.5:12.5:1 (R_F = *p*-FC_6H_4) in DMF solution[12] was examined. The fluorine-substituted components[13] and their products allowed ready monitoring of the reactions by ^{19}F NMR spectroscopy. Spectra over a 108-h period are provided in Figure 1; all signals are fully resolved. Over this period, R_FSH (−114.6 ppm) and $(R_F)_2SO$ (−105.7 ppm) are consumed, and known R_FSSR_F[14] (−110.2 ppm) and R_FSR_F[13] (−110.8 ppm) are progressively generated in exactly equimolar amounts. No other signals were observed in the −90 to −130 ppm range, the sum of the integrated intensities of all signals was constant (±10%), and the system remained homogeneous. At 108 h, product signal intensities indicated 3.8 ± 0.2 turnovers at [**1**] = 40 mM. In a control experiment, an anaerobic solution with $[R_FSH] = [(R_F)_2SO]$ = 500 mM in DMF[12] at 25 °C for 120 h showed no reaction.

We have previously demonstrated that **1** is reduced to **2** in DMF by PhSH.[5] Here, 15 mM **1** in DMF (λ_{max} 385, 449 nm)[3] was treated with 2.5 equiv of R_FSH, which caused the gradual appearance of the characteristic spectrum of **2** (λ_{max} 365, 528, 734 nm).[3] After 62 h, reaction 4 was 63% complete. At longer times

$$MoO_2(L\text{-}NS_2) + 2R_FSH \rightarrow MoO(L\text{-}NS_2)(DMF) + R_FSSR_F + H_2O \quad (4)$$

the spectrum of **2** decayed, presumably because of degradation induced by excess thiol. The reduction of **1** is much slower than the reaction of **2** with substrate. Thus, the reaction of 0.44 mM **2** with 2.7 equiv of $(R_F)_2SO$ in DMF gave the absorbance ratio A_{385}/A_{449} = 1.18 (vs. 1.13 for authentic **1**) after 1 h, demonstrating complete reaction. Further, at the mol ratio Ph_2SO:**2** > 10:1, $k = 1.4 \times 10^{-3}$ s^{-1} for the reaction **2** + $Ph_2SO \rightarrow$ **1** + Ph_2S (DMF,

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23 °C),[4] corresponding to $t_{1/2}(2) \approx 8$ min.

The foregoing results demonstrate the reaction cycle set out in the figure, whereby thiol acts as the electron donor in the reduction of sulfoxide. The system is catalytic, with ca. 4 turnovers in 108 h, but overall reaction 5 is slow by reason of the sluggish

$$(R_F)_2SO + 2R_FSH \rightarrow R_FSR_F + R_FSSR_F + H_2O \quad (5)$$

rate of reduction of Mo(VI) by thiol. Other synthetic systems capable of the same reactions doubtless can be devised. Thus, $MoO(S_2CNEt_2)_2$ (**3**) has been isolated in good yield from the reaction of $MoO_2(S_2CNEt_2)_2$ (**4**) with PhSH,[15] and **3** has been shown to reduce sulfoxides[16-18] with formation of **4** and sulfides. The instability of **2** in the presence of excess thiol is obviated to an extent in a catalytic reaction system, where it is oxidized to **1** at the same rate at which it is formed. In the present system **1** is cleanly reduced at an appreciable rate only by arenethiols, whose relatively acidic character[19] presumably promotes protonation of an oxo ligand.

The principal result forthcoming from the present work is that thiols are thermodynamically capable of reducing a $Mo^{VI}O_2$ species to a $Mo^{IV}O$ state which executes reductase reactions on enzymic substrates. Electron transfer may occur via intervening redox centers (heme, Fe/S, flavin), including possibly the pterin component of the Mo cofactor.[22,23] Inasmuch as all $Mo^{IV}O$ complexes with a labile binding site yet tested are capable of reducing Me_2SO,[5] it is apparent that catalysis depends critically on the potentials of the external electron donor and the Mo(VI) center. Elsewhere we have shown that coordinated thiolate sulfur (present in oxo-transferases[26]) raises Mo(VI) reduction potentials,[5] one apparent advantage of this effect being to render this state reducible in catalysis by physiological reagents. With the provisos that certain sulfoxide-reducing enzyme systems utilize other donors[27,28] and that the only enzyme with a partially characterized catalytic site (liver aldehyde oxidase[27]) known to reduce sulfoxides[29] may contain the $Mo^{VI}OS$ group when oxidized, the present results improve the viability of thiols and thioredoxin as endogenous electron donors in reductase reactions of Mo oxotransferases.

Lastly, the ^{19}F NMR method for following oxo transfer catalysis is particularly effective, and applications will be presented subsequently with high-turnover systems and other substrates. The present system is not intended to be catalytically useful. Rather, it is employed to demonstrate that electrons can be transferred from thiol to substrate via a currently credible representation of an oxo-transferase active site.

Acknowledgment. This research was supported by National Science Foundation Grants CHE 81-06017 and 85-21365.

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Chemical Regulation of Distance: Characterization of the First Natural Host Germination Stimulant for *Striga asiatica*

Mayland Chang,[†] David H. Netzly,[‡] Larry G. Butler,[‡] and David G. Lynn*[†]

Department of Chemistry, The University of Chicago Chicago, Illinois 60637
Department of Biochemistry, Purdue University West Lafayette, Indiana 47907
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Striga asiatica, "witchweed", an obligate parasitic plant which attaches to the roots of corn, *Sorghum*, and other grasses, causes severe damage to crop yields around the world. The seeds of this parasite require a germination stimulus,[1] and once germinated *Striga* survives for less than 2 weeks in the absence of a host. In vitro, *Striga* seeds germinate only within a 0.75-cm zone of the roots of both corn and *Sorghum*[2] and produce roots that are no more than ~3 mm in length. Several years ago, the sesquiterpene strigol was isolated from cotton, a nonhost plant, and found to be a potent germination stimulus for *Striga*.[3] However, a stable sesquiterpene which could accumulate in the soil may stimulate germination at too great a distance for host attachment.[4] We describe here the identification of the first germination stimulant for *Striga* from the root exudate of a natural host and provide an explanation of how this compound would define the distance away from the host root at which *Striga* germination occurs.

Sorghum bicolor (L.) Moench cv. IS 8768 seeds (10 g) were grown aseptically on moist filter paper in the dark at 27 °C for 7 days. The roots were dipped in 0.5% $HOAc/CH_2Cl_2$ (100 mL) for 2 s,[5] and the extract was evaporated in vacuo to give 15 mg of a biologically active crude exudate.[6] The ^1H NMR spectrum

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