

5, 6, and 7 (see Scheme I) form stable complexes with $\text{Cu}(\text{ClO}_4)_2$ which have not yet been characterized.²⁸ The complexes formed and the successful implementation of Diels-Alder chemistry of the acetylene diimidazole demonstrate the viability of this approach. Further elaboration of these systems to structurally fixed multiple imidazole groups, e.g., through Diels-Alder reaction of 4 with 5, and to imidazole combinations with other ligands through Diels-Alder and carbene chemistry is underway.

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Supplementary Material Available: Listings of atomic coordinates and isotropic thermal parameters (Table I), bond lengths (Table II), bond angles (Table III), anisotropic thermal parameters (Table IV), H-atom coordinates and isotropic thermal parameters (Table V), and magnetic properties (Table VII) and an additional view of the crystal structure (4 pages); listing of observed and calculated structure factors (Table VI) (7 pages). Ordering information is given on any current masthead page.

(24) ^1H NMR (300 MHz, CDCl_3) δ 3.78 (s, 6 H), 6.95 (s, 2 H), 7.08 (s, 2 H); ^{13}C NMR (75 MHz, CDCl_3) δ 33.66, 81.82, 122.10, 129.94, 131.29; MS (70 eV), m/z (rel intensity) 186.0905 (M^+ , 74) (calcd for $\text{C}_{10}\text{H}_{10}\text{N}_4$ 186.0905).

(25) ^1H NMR (300 MHz, CDCl_3) δ 3.19 (s, 6 H), 6.87 (s, 2 H), 7.03 (s, 2 H), 7.78 (s, 2 H); ^{13}C NMR (75 MHz, CD_3OD) δ 33.97, 116.25, 124.14, 127.78, 140.47, 154.64; MS (70 eV), m/z (rel intensity) 228.101 (calcd for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}$ 228.0978).

(26) ^1H NMR (300 MHz, CDCl_3) δ 2.11 (d, $J = 6.5$ Hz, 1 H), 2.44 (d, $J = 6.5$ Hz, 1 H), 2.84 (s, 6 H), 4.06 (br s, 2 H), 6.77 (s, 2 H), 7.00 (m, 2 H), 7.07 (s, 2 H).

(27) ^1H NMR (300 MHz, CDCl_3) δ 3.34 (s, 6 H), 6.61 (s, 2 H), 6.80 (s, 2 H), 7.03 (s, 2 H).

(28) Preliminary crystal structure data indicates that 5 forms a complex with $\text{Cu}(\text{II})$ in which the two furan rings lie on the same side of Cu and parallel to each other, in contrast to the complex of 2.

The $[\text{MoFe}_6\text{S}_6(\text{CO})_{16}]^{2-}$ Ion: A New Model for the FeMo-Cofactor of Nitrogenase

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The iron-molybdenum cofactor (FeMo-co) of nitrogenase² consists of a small dissociable cluster of approximate composition $\text{MoFe}_6\text{-}_7\text{S}_8\text{-}_{10}$ ^{2,3} that appears to constitute the site at which dinitrogen is reduced.⁴ Despite its importance and the application of a host of spectroscopic techniques,^{3d,5-9} the structure of FeMo-co

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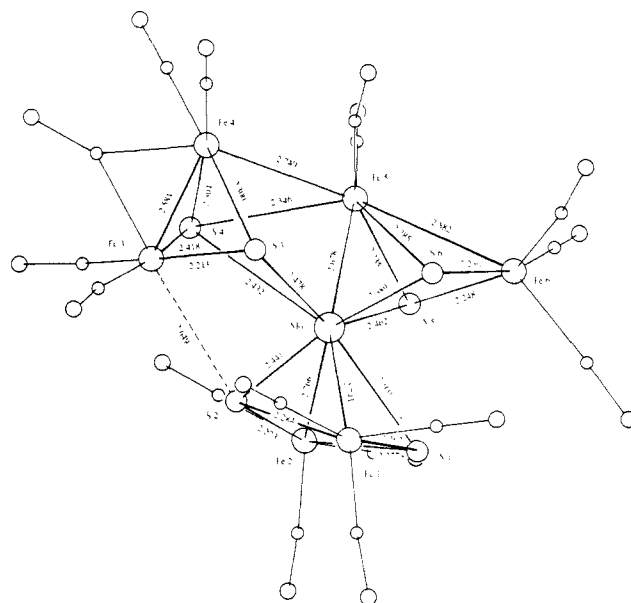


Figure 1. A ball and stick drawing of the structure of the $[\text{MoFe}_6\text{S}_6(\text{CO})_{16}]^{2-}$ ion, illustrating the heavy atom numbering scheme and showing selected distances.

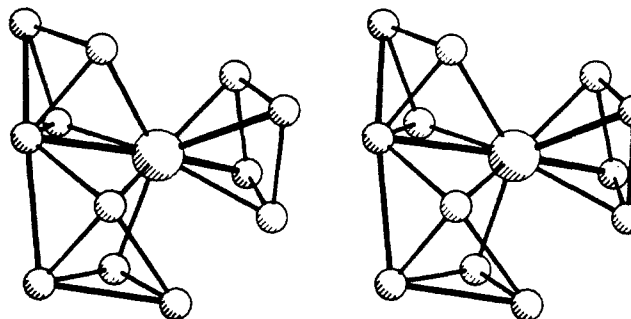


Figure 2. Stereoview of the MoFe_6S_6 core of the $[\text{MoFe}_6\text{S}_6(\text{CO})_{16}]^{2-}$ ion

remains one of the major unresolved problems in contemporary bioinorganic chemistry. None of the many attempts to produce synthetic analogues¹⁰ of FeMo-co has resulted in the preparatio

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of clusters possessing the correct stoichiometry, although a variety of novel Mo-Fe-S clusters has been synthesized. These include the well-studied MoFe_3S_4 "cubane"^{10a,b,d} and "linear"^{10a-c,e} MoS_2Fe units, the more recently reported triply-bridged MoS_2Fe_2 unit,¹¹ the $\text{S}_2\text{Fe}_3\text{S}_2\text{Mo}$ unit containing a discrete 3Fe core,^{11b} and the "doubly-capped" hexamers with $\text{Mo}_2\text{Fe}_6\text{S}_6$ cores.¹² Efforts in our laboratory have focussed on the development of an alternative strategy for the preparation of Mo-Fe-S clusters of higher nuclearity.^{11b,13} Herein we describe the synthesis, structure, and some properties of the $[\text{MoFe}_6\text{S}_6(\text{CO})_{16}]^{2-}$ ion, the closest synthetic approach yet to the core stoichiometry and structure of FeMo-co.

Slow addition of an amber solution of $(\text{Et}_4\text{N})[\text{Mo}(\text{CO})_4\text{I}_3]^{14}$ (1 equiv) in THF to a green solution of $[\text{Fe}_2\text{S}_2(\text{CO})_6]^{2-}$ (2 equiv) (prepared from 2 equiv of $\text{Fe}_2\text{S}_2(\text{CO})_6$ and 4 equiv of $\text{LiBEt}_3\text{H}^{15}$) in THF at -78°C under anaerobic conditions results in the formation of a dark green solution that evolves CO and turns brown upon slowly warming to room temperature. Addition of quaternary cations (e.g., Ph_4As^+) followed by straightforward workup and recrystallization affords the corresponding salts of $[\text{MoFe}_6\text{S}_6(\text{CO})_{16}]^{2-}$ (I) in ca. 30% yield.¹⁶

Although its stoichiometry suggests that I is derived simply from coordination of discrete $[\text{Fe}_2\text{S}_2(\text{CO})_6]^{2-}$ units to Mo, the crystal structure¹⁷ of the Ph_4As^+ salt of I reveals an unexpectedly complex structure, as shown in Figure 1; a stereoview of the MoFe_6S_6 core is shown in Figure 2. Anion I has only C_1 symmetry and is best viewed as consisting of a distorted trigonal prismatic MoS_6 core (containing S(1), S(3), S(4) and S(2), S(5) and S(6) triangles with a twist angle of 13.7°) in which all sulfur atoms also bridge two or three iron atoms; S-Mo-S angles range from 75 to 148° . The six iron atoms are arranged about a pseudo-2-fold axis in groups of two and four. In one direction along this axis is an isolated $\text{Fe}_2\text{S}_2(\text{CO})_6$ unit, containing Fe(1), Fe(2), S(1), and S(2). The severe distortion of the Fe_2S_2 unit toward planarity (all atoms within 0.2 \AA being coplanar), the long Fe(1)-Fe(2) distance of $3.419(2)\text{ \AA}$, and the short Mo-Fe(1) and Mo-Fe(2) distances of $2.741(5)$ and $2.766(5)\text{ \AA}$, respectively, suggest that the Fe-Fe bond of the $[\text{Fe}_2\text{S}_2(\text{CO})_6]^{2-}$ unit has been replaced by two Mo-Fe bonds. In the other direction along the pseudo-2-fold axis is a zigzag chain of four bonded iron atoms (Fe(3) to Fe(6)); maximum deviation from the MoFe_4 plane $\pm 0.4\text{ \AA}$ bridged to each other and to molybdenum by sulfur atoms S(3) to S(6). The Fe-Fe bonds of the parent $\text{Fe}_2\text{S}_2(\text{CO})_6$ units have lengthened by ca. 0.1 \AA to $2.581(5)\text{ \AA}$ and $2.582(6)\text{ \AA}$ for Fe(3)-Fe(4) and Fe(5)-

Fe(6), respectively, with formation of a new Fe(4)-Fe(5) interaction at $2.749(4)\text{ \AA}$. In addition, Fe(5) is directly bonded to the molybdenum at a distance of $2.678(3)\text{ \AA}$. A carbonyl bridging Fe(3) and Fe(4), two terminal CO's on Fe(3), (4), (5) and three on Fe(6), and a long ($2.649(6)\text{ \AA}$) S(2)-Fe(3) interaction complete the coordination of the iron atoms.

Notable features of the structure include the following. (i) The Mo-Fe distances divide into two sets, three at ca. 2.7 \AA and three at 3.0 - 3.6 \AA . This is similar to the case for FeMo-co, for which EXAFS measurements^{7a} have indicated the presence of 3 ± 1 iron atoms at ca. 2.68 - 2.70 \AA from molybdenum, with the rest too distant to be detected. (ii) The cluster contains both triply and quadruply bridging sulfur atoms that link the iron atoms to molybdenum in a highly irregular structure. The importance of such a low-symmetry structure is enhanced by a recent ENDOR study^{9a} demonstrating inequivalent iron sites in both protein-bound FeMo-co and NMF solutions of FeMo-co. (iii) The Mo-S distances (av 2.43 \AA) are longer than those for both the "linear" MoS_2Fe clusters^{10a-c,e} (ca. 2.2 \AA) and the MoFe_3S_4 "cubane" clusters^{10a,d} (ca. 2.35 \AA), with formal Mo oxidation states of $+6$ and $+3$ or $+4$, respectively, and shorter than those for the doubly capped hexamers¹² (2.58 - 2.65 \AA), with a formal Mo oxidation state of 0. These data are consistent with an oxidation state of ca. $+2$ for the Mo atom in I (cf. $\text{Mo}(i\text{-Pr}_2\text{dte})_2(\text{CO})_2$, with average Mo-S distance of 2.45 \AA ¹⁸) and suggest that the oxidation required by the stoichiometry (Mo(II) plus three $[\text{Fe}_2\text{S}_2(\text{CO})_6]^{2-}$ "ligands" should yield a tetraanion) is centered on the iron atoms. The average Mo-S distance is also significantly longer than that observed for FeMo-co (2.37 \AA),^{7a} consistent with a higher formal oxidation state of Mo in the latter.

Preliminary physical and spectroscopic properties of I are consistent with oxidation of the Fe sites rather than Mo, although it is impossible to assign formal oxidation states at this time. The IR spectrum (MeCN; $\nu(\text{CO})$ 2048 (w), 2019 (m), 1996 (s), 1978 (m), 1948 (mw, br), 1937 (mw, br), 1760 (w, br)) is complex, consistent with retention of the low symmetry structure of I in solution. The electronic spectrum shows only a gradually rising absorption into the UV, with shoulders (ϵ , $\text{M}^{-1}\text{ cm}^{-1}$) at 495 (7000), 415 (13800), and 296 (30000) nm. Variable temperature magnetic susceptibility measurements (4 - 300 K) show that $(\text{Ph}_4\text{As})_2\text{I}$ is essentially diamagnetic, except for the presence of small amounts ($<5\%$) of paramagnetic impurities. The ⁵⁷Fe Mössbauer spectrum of $(\text{Ph}_4\text{As})_2\text{I}$ at 298 and 77 K is comprised of at least three sharp overlapping quadrupole doublets in the -0.8 to $+1.1\text{ mm/s}$ region (versus Fe metal at room temperature). A substantial fraction of the Fe sites appears to have isomer shifts $> 0.1\text{ mm/s}$, consistent with at least partial oxidation of some of the Fe atoms, but a detailed analysis will be required to fit the spectrum. Electrochemical studies (dc and differential pulse polarography at DME; 50 mM $(\text{Bu}_4\text{N})(\text{PF}_6)$ in MeCN) show only a quasi-reversible e^- reduction at -1.00 V versus SCE, in addition to the reduction of Ph_4As^+ at -1.32 V .

Cluster I is thus the first synthetic Mo-Fe-S cluster to approximate not only the core stoichiometry but also the Mo-Fe distance distribution of the FeMo-cofactor. The low symmetry of I, even in the absence of any polypeptide-imposed constraints, suggests that the structures proposed to date for FeMo-co^{10a,11b,19} may possess unnecessarily high symmetry. Although the presence of the remaining abiological CO ligands to iron and the resulting low oxidation state of iron preclude consideration of I as a detailed model for FeMo-co, it is clear that partial decarbonylation and Fe oxidation has occurred during the synthesis of I. Preliminary results indicate that I readily undergoes further oxidative decarbonylation with a variety of reagents; the structures and properties of the products will be the subject of future communications.

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(16) Anal. Calcd. for $(\text{Ph}_4\text{As})_2\text{I}$, $\text{C}_{64}\text{H}_{40}\text{As}_2\text{Fe}_6\text{MoO}_{16}\text{S}_6$: C, 41.82; H, 2.19; As, 8.15; Fe, 18.23; Mo, 5.22; S, 10.47. Found: C, 41.74; H, 2.27; As, 8.10; Fe, 17.56; Mo, 5.23; S, 10.72.

(17) (a) Crystals of $(\text{Ph}_4\text{As})_2\text{I}$ were obtained by slow cooling of a MeOH/*i*-PrOH solution. X-ray diffraction measurements were performed on a Nicolet P3m diffractometer by using graphite-filtered Mo $K\alpha$ radiation; data were collected by using the θ - 2θ technique over the range $3.5^\circ < 2\theta < 60^\circ$. A total of 9150 reflections having $I > 2.5\sigma(I)$ out of 14577 independent reflections measured were used in the refinement. $(\text{Ph}_4\text{As})_2\text{I}$ crystallizes in the triclinic space group $P\bar{1}$, with $a = 12.473(12)\text{ \AA}$, $b = 12.836(13)\text{ \AA}$, $c = 22.360(22)\text{ \AA}$, $\alpha = 90.96(2)^\circ$, $\beta = 97.58(2)^\circ$, $\gamma = 99.52(2)^\circ$, $V = 3497\text{ \AA}^3$, $Z = 2$. The structure was determined by using SHELXS-86,^{17b} and the core structure was confirmed independently by a Patterson map. Least-squares refinement gave $R = 8.0\%$ and $R_w = 8.1\%$ with anisotropic thermal parameters for all non-hydrogen atoms but without accounting for hydrogen atom contributions; an empirical absorption correction^{17c} was applied to the data. (b) Sheldrick, G. M. SHELXS-86, a fortran program for the solution of crystal structures from diffraction data, University of Göttingen, Göttingen, BRD, 1986. (c) Walker, N.; Stuart, D. *Acta Crystallogr., Sect. A: Found Crystallogr.* **1983**, *A39*, 158.

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Supplementary Material Available: Tables of positional and thermal parameters and bond length and angles for $(\text{Ph}_4\text{As})_2\text{I}$ (10 pages); table of observed and calculated structure factors (36 pages). Ordering information is given on any current masthead page.

Catalysis and Thermodynamics of the Phosphoenolpyruvate/Phosphonopyruvate Rearrangement. Entry into the Phosphonate Class of Naturally Occurring Organophosphorus Compounds

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Organophosphates are most commonly found in nature as oxygen esters, diesters, and anhydrides of phosphoric acid. Phosphonates, which contain a P-C linkage, by comparison are more sparse in the natural environment. Nonetheless, they have been discovered in a variety of organisms (ranging from bacteria to man) and have been shown to occur in a number of structural forms.¹

Studies of the biosynthetic pathways leading to the phosphonolipid precursor, 2-aminoethylphosphonate (AEP)^{2,6} (1) in *Tetrahymena pyriformis*, and to the antibiotics, fosfomycin⁷ (2), and bialaphos⁸ (3) in certain strains of *Streptomyces* have suggested that phosphoenolpyruvate (PEP)⁹ is the key precursor (see Scheme I) of naturally occurring phosphonates. Twenty years ago Warren postulated that the P-C bond in 1 is formed from PEP by its isomerization to phosphonopyruvate (P-pyr).⁴ In this communication we wish to report the discovery of the reversible rearrangement reaction of PEP and P-pyr which takes place in the active site of a Mg^{2+} activated phosphomutase found in *T. pyriformis*.

The phosphomutase was isolated from *T. pyriformis* cells that were cultivated and harvested according to published procedures.⁶ Cell lysis was carried out in buffered solutions at pH 8.2 (4 °C) with a French Pressure Cell Press (8000 psi).¹⁰ The cell debris was removed at 17 000 g (10 min), and *Streptomycin* sulfate (2% wt/v) was added to the supernatant to precipitate the nucleic acids.

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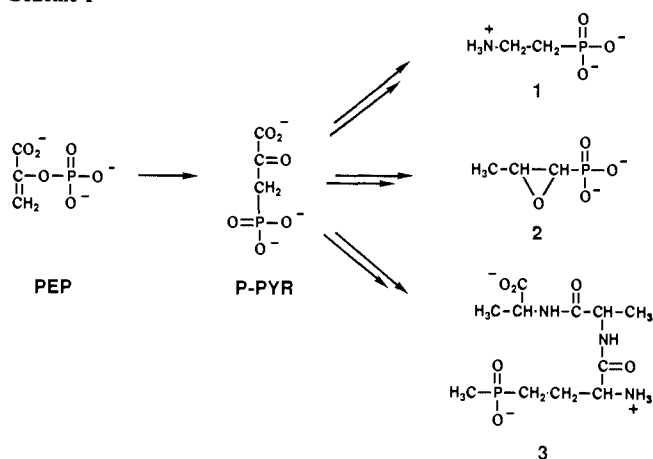
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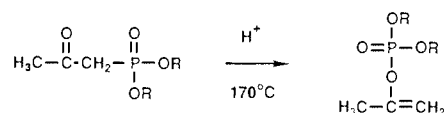
(9) Abbreviations used: phosphoenolpyruvate (PEP), phosphonopyruvate (P-pyr), (N-2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES), ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), adenosine 5'-diphosphate (ADP), orthophosphate (P_i), 2-aminoethylphosphonate (AEP), dihydrionicotinamide adenine dinucleotide (NADH), high pressure liquid chromatography (HPLC), nuclear magnetic resonance (NMR).

(10) Cell lysis was carried out with 25 mL of packed cells in 100 mL of suspension buffer (pH 8.2) containing 50 mM K^+ HEPES, 1 mM EDTA, 10 mM β -mercaptoethanol, 0.8% BSA, 1 mM 1,10-phenanthroline, 1 mM benzamidine-HCl, 50 μM phenylmethylsulfonyl fluoride, and 50 $\mu\text{g}/\text{mL}$ trypsin inhibitor.

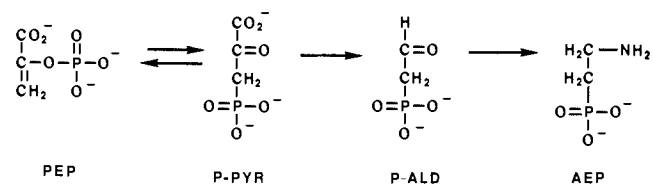
Scheme I



Scheme II



Scheme III



The P-pyr \rightarrow PEP phosphomutase activity (measured with synthetic P-pyr¹¹ and with the ADP/pyruvate kinase-NADH/lactate dehydrogenase coupled spectrophotometric assay at pH 7.8, 25 °C) was found in the 60-70% $(\text{NH}_4)_2\text{SO}_4$ protein fraction. The phosphomutase was purified as a $\sim 40\,000$ D protein having a specific activity of 30 unit/mg (5 mM MgCl_2 , 50 mM K^+ Hepes, pH 7.5, 25 °C) by using the following sequence of column chromatographies at 4 °C: DEAE-Sephadex (0.1 M NaCl to 0.4 M NaCl in 10 mM MgCl_2 and 20 mM triethanolamine pH 7.5), Sephadex G-100 (10 mM MgCl_2 , 20 mM triethanolamine pH 7.5), and Superose-12 (Pharmacia) FPLC (10 mM MgCl_2 , 20 mM triethanolamine pH 7.5). In the absence of Mg^{2+} ion the phosphomutase was found to be catalytically inactive.

The thermodynamically favored direction of the phosphomutase-catalyzed PEP/P-pyr isomerization reaction (20 mM PEP or P-pyr, 10 mM MgCl_2 , 50 mM K^+ Hepes, pH 8.0, 25 °C) was determined by using ³¹P NMR (Figure 1) and HPLC (Altex C-18 reversed phase analytical column, 3% triethylamine, 25 mM P_i , 1.5% methanol, pH 6.5 as isocratic eluent, A^{231nm}) techniques. The PEP/P-pyr ratio at equilibrium was found to be ≥ 500 . Because PEP is the highest energy naturally occurring organophosphate, this finding was not anticipated. Specifically, compared to a simple phosphate ester (for which the standard free energy of hydrolysis is 2-3 kcal/mol) PEP releases ca. 10 kcal/mol more standard free energy upon hydrolysis.¹² This energy release is brought about largely as a result of the conversion of the phosphoenol moiety in PEP to the ketone moiety in pyruvate and the relief of the repulsive interaction which takes place between the charged carboxylate and phosphate moieties present in the PEP.¹³ Both of these factors are expected to also contribute to the en-

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