reasonably high velocity and to competitively reduce the enzyme inactivation by the inhibitor during the assay. The assay was run for 30 s and treated as described in the enzyme assay section. Inactivation of the enzyme was measured by comparing the velocity at time zero to that at the indicated incubation times. Under the conditions of the assay the uninhibited enzyme retained 96% of the initial activity after 4 min of incubation.

Determination of Adduct Formation. The ultraviolet spectra were recorded for solutions containing 5-nitro-2′-deoxyuridine 5′-phosphate [**2**] at a concentration of 5.4×10^{-5} M, 0.024 M tris–acetate buffer (pH 6.8), 0.012 M magnesium chloride, and 0.5 mM ethylenediaminetetraacetic acid, and concentrations of 2-mercaptoethanol (**5**) varying from 0 to 3.6×10^{-2} M concentration. Changes in the absorbance at 335 nm were used to determine the mole fraction of adduct **8** formation. Similarly, changes in absorbance at 335 nm were observed for 6.8×10^{-5} M solutions of 5-nitrouridine (**6**) treated in the same manner.

Acid dissociation constants (K_a apparent) for **2** and **6** were determined by ultraviolet absorption changes of solutions of varying pH and by titration (**6**).

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Communications to the Editor

Reaction of CS_2 with Metal Cluster Carbonyls of the Iron Triad: Synthesis and X-ray Structure of $[Fe_5(CO)_{13}S_2(CS)]$ Containing a Six-Electron-Donor Thiocarbonyl Group

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The thiocarbonyl ligand (CS) has been observed to bond to metal centers in the linear μ_1,[1] the bridging μ_2[1,2] and the triply bridging, μ_3, modes[3] (Figure 1a–c). It may also bridge two metal centers linearly by donation to one metal atom from the carbon and to the second from the sulfur[4] (Figure 1d). There are also examples of coordination to four metal atoms.[3,5] In $[\{(\eta^5\text{-}C_5H_5)Co\}_3(\mu_3\text{-}S)(\mu_3\text{-}CSCr(CO)_5)]$,[3] the Co_3 triangle is capped by the carbon while the sulfur donates to an independent chromium center (Figure 1e), and in $[Fe_4(CO)_{12}(CS)S]$[5] the carbon atom caps an Fe_3 triangle with the sulfur bonded to the fourth iron atom which is terminally bonded to the triangular Fe_3 unit (Figure 1f). In these bonding modes the thiocarbonyl ligand may be considered to donate two or four electrons to the metal unit.

We now report the preparation and characterization of the complex $[Fe_5(CO)_{13}S_2(CS)]$ in which a further bonding mode for the thiocarbonyl ligand has been established. In this complex the thiocarbonyl group is bonded to four iron atoms arranged in a square and may be formally regarded as a six-electron donor. A further point of interest is the Fe_5 unit which is unusual in that it contains a square plane of four iron atoms with the fifth iron atom terminally bonded to this (Figure 2).

The reaction between $[Fe_3(CO)_{12}]$ and excess CS_2 in hexane solution (80 °C, under a 10-atm CO/Ar (1:1) pressure, 18 h) gave a mixture of products. After removal of the solvent, the mixture was dissolved in CH_2Cl_2 and separated by TLC using silica plates and hexane as eluant. Four products **1–4** were obtained. Component **1** was found to be $[Fe_3(CO)_9S_2]$,[6] component **2** was identified as $[Fe_4(CO)_{12}(C_2S_4)]$, component **4** was $[Fe_4(CO)_{12}(CS)S]$,[5] and component **3** has now been identified as $[Fe_5(CO)_{13}S_2(CS)]$.

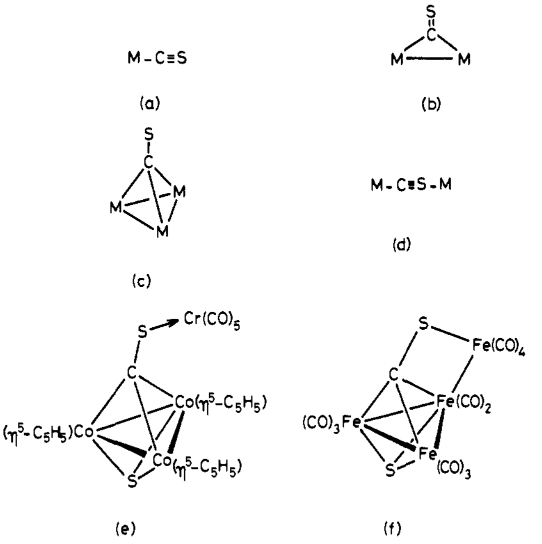

Figure 1. Modes of bonding of the thiocarbonyl ligand.

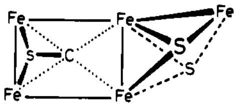

Figure 2. The cluster core in $[Fe_5(CO)_{13}S_2(CS)]$.

The reaction conditions employed and the product distribution are similar to those reported[5] previously for the preparation of $[Fe_4(CO)_{12}(CS)S]$, except that the reaction time is longer. Under these conditions lower yields of **2** and **4** and higher yields of **1** and **3** (although **3** is obtained in only 2% yield) are obtained. Crystallization was achieved by the slow cooling of a benzene/hexane solution giving black platelets. Spectroscopic and analytical data have been obtained for **3**,[7] and full details of the molecular structure have been established through single-crystal X-ray structure analysis[8,9] (Figure 3).

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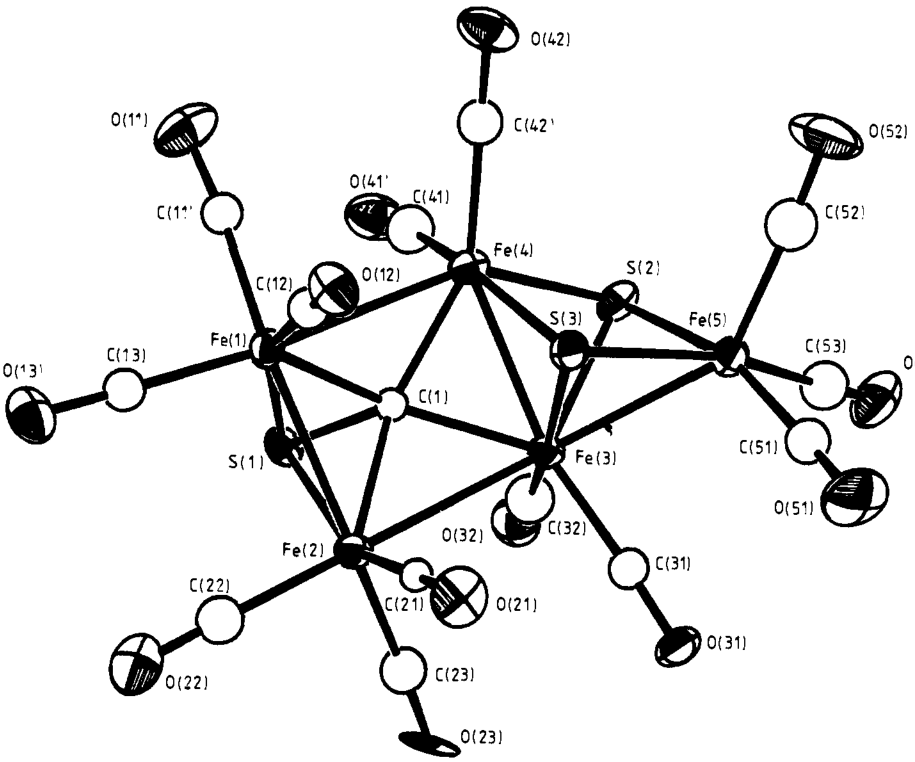

Figure 3. A perspective ORTEP plot of $Fe_5(CO)_{13}(CS)S_2$ showing 50% probability ellipsoids.

The four iron atoms Fe(1)–Fe(4) describe a square. The fifth, Fe(5), is terminal to the square, bonded to Fe(3), and is situated above the Fe_4 plane. Fe(3), Fe(4), and Fe(5) form an Fe_3 unit (μ_3-S) capped on each side, with no appreciable S···S interaction (S···S, 2.87 Å). The carbonyl ligands are all terminal with three bonded to each of Fe(1), Fe(2), and Fe(5) and two to each of Fe(3) and Fe(4).

The thiocarbonyl ligand is bonded so that the carbon is situated almost exactly central to and slightly below the Fe_4 plane. The average Fe–C distance is 1.96 Å with small variations to either side of this. The sum of the single bond covalent radii is 1.95 Å. Thus, an interaction occurs with all four atoms of the Fe_4 square. The sulfur is symmetrically situated across the Fe(1)–Fe(2) bond, and it is further below the Fe_4 plane than the carbon. The C–S bond length, at 1.71 Å, is shorter than the typical single bond, at 1.81 Å, but significantly longer than a typical double bond, for example, at 1.55 Å in CS_2.[10]

In $[Fe_5(CO)_{15}C]$,[11] the carbide is equally central to the Fe_4 square comprising the base of the Fe_5 square pyramid, but the average Fe_4–C distance is 1.89 Å, somewhat less than in $[Fe_5(CO)_{13}S_2(CS)]$ (**3**). However, the apical iron atom is also bonded to the carbide and tends to bring this more into the Fe_4 basal plane. Also, in **3** the below-plane sulfur exerts an opposite influence. These factors are reflected in the greater out-of-plane position of the carbon in **3** at 0.53 Å, cf. in $[Fe_5(CO)_{15}C]$ at 0.1 Å.

The Fe–Fe bond lengths of the Fe_4 square in **3** vary. For single-bond interactions, Fe(2)–Fe(3) and Fe(1)–Fe(4) are both rather long (2.78 Å), Fe(1)–Fe(2) is middling (2.64 Å), and Fe(3)–Fe(4) is short (2.46 Å). However, $[Fe_4(CO)_8(\mu_2\text{-}CO)_3(\mu_4\text{-}NEt)(\mu_2\text{-}O\cdot\mu_2\text{-}NEt)]$ which contains an Fe_4 square and has appreciable numbers of bridging and capping ligands also shows short Fe–Fe single bonds, varying between 2.484 and 2.552 Å,[12] while $[Fe_2(CO)_9]$ has the Fe–Fe bond at 2.523 Å.[13] The short Fe(3)–Fe(4) bond is bridged by both the (μ_3-S) groups and the (μ_4-CS), thus matching the short bonds in the highly bridged species mentioned above. Longer bonds have also been observed (e.g. $[\{(\eta_3\text{-}C_3H_5)Fe(CO)_3\}_2]$ at 3.14 Å[14]) and the thiocarbonyl carbon "setting into" the plane would cause this, related to the delocalized nature of its bonding. Thus, the Fe–Fe bond lengths are consistent with single bonds around the Fe_4 square.

For the cluster to obey the effective atomic number rule, the thiocarbonyl group must donate six electrons (formally) to the metal core. The previous maximum observed for the thiocarbonyl group is four electrons. However, it is noteworthy that one of the carbonyl ligands in $[\{(\eta^5\text{-}C_5H_5)Nb\}_3(CO)_7]$[15] formally donates six electrons if this compound is to be electron precise.

The bonding arrangement of the thiocarbonyl to the Fe_4 framework is similar to that frequently postulated for the bonding of carbon monoxide to a metal surface.[16] The comparison between the thiocarbonyl and carbonyl groups is of interest. As anticipated, the thiocarbonyl group tends to be a better σ and π donor than the carbonyl group to metal atoms. This is in keeping with the lower stability of p_π–p_π bonding between carbon and sulfur relative

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(8) Crystal data: $C_{14}O_{13}Fe_5S_3$; M 751.57; orthorhombic; $a = 34.570$ (9), $b = 11.244$ (4), $c = 11.891$ (5) Å; $U = 4622.1$ A^3; $Z = 8$, $D_c = 2.16$ g cm^{-3}; D_m is not measured; graphite monochromated Mo Kα radiation, $\lambda = 0.71069$ Å, μ(Mo Kα) = 33.54 cm^{-1}; space group *Pbcn*. The Fe atoms were located by multisolution $\sum_2$ sign expansion and the remaining atoms from a subsequent electron-density-difference synthesis. The structure was refined by blocked-cascade least-squares (Fe, S, and O atoms anisotropic), using 1147 unique observed intensities $[F > 3\sigma(F)]$ recorded on a Stoe four-circle diffractometer (1833 intensities measured to a $2\theta_{max} = 47°$) with an ω/θ scan technique and a crystal with dimensions 0.115 × 0.154 × 0.192 mm. The current residuals are $R = 0.059$ and $R^1 = [\sum w^{1/2}\Delta/\sum w^{1/2}|F_o|] = 0.051$. The weighting scheme employed was $w = [\sigma^2(F) + 0.002\,F^2]^{-1}$. Calculations were performed on an IBM 370/165 by using a modified version of the SHELX 76 program package.

(9) Selected bond lengths (Å) and bond angles (deg) for $Fe_5(CO)_{13}(CS)S_2$: Fe(1)–Fe(2) = 2.641 (5), Fe(1)–Fe(4) = 2.782 (5), Fe(2)–Fe(3) 2.778 (5), Fe(3)–Fe(4) 2.462 (4), Fe(3)–Fe(5) 2.626 (5), Fe(1)–S(1) 2.259 (6), Fe(2)–S(1) 2.259 (5), Fe(3)–S(2) 2.251 (6), Fe(3)–S(3) 2.248 (6), Fe(4)–S(2) 2.186 (5), Fe(4)–S(3) 2.296 (6), Fe(5)–S(2) 2.203 (6), Fe(5)–S(3) 2.244 (5), Fe(1)–C(1) 1.93 (2), Fe(2)–C(1) 1.97 (2), Fe(3)–C(1) 2.01 (2), Fe(4)–C(1) 1.94 (2), S(1)–C(1) 1.71 (2); Fe(1)–S(1)–Fe(2) 71.5 (2), Fe(1)–C(1)–S(1) 76.5 (7), Fe(2)–C(1)–S(1) 75.2 (6), Fe(3)–C(1)–S(1) 131.8 (9), Fe(4)–C(1)–S(1) 134.4 (9).

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to carbon and oxygen in the parent molecules.

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Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, bond lengths, and bond angles for $Fe_5(CO)_{13}(CS)S_2$ (5 pages). Ordering information is given on any current masthead page.

Copper Site of Deoxyhemocyanin. Structural Evidence from X-Ray Absorption Spectroscopy

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Structural models for the copper site of oxyhemocyanin from *Megathura crenulata*,[1] *Helix pomatia*,[1] and *Busycon canaliculatum*[2] have recently been proposed using X-ray absorption spectroscopic (XAS) information. In this communication we report analogous XAS studies on the copper site of deoxyhemocyanin from *Megathura crenulata*. Extended X-ray absorption fine structure (EXAFS) has been analyzed as described previously in detail for the oxyprotein by using an imidazole group fitting technique.[1,3] This study on the deoxyprotein shows that each copper atom is coordinated to two imidazoles with an average Cu-N distance of 1.95 Å. No evidence of a copper-copper interaction within 4 Å is observed. A structural model for the binuclear binding site is presented from these EXAFS results. The model suggests two copper atoms each coordinated only to two histidines. Upon oxygenation, the two copper atoms are pulled together to 3.55 Å,[1] bridged by a low-Z atom, and the coordinated dioxygen with the dioxygen bound end-to-end to the binuclear copper site as a peroxide ion.[4]

Hemocyanin is a copper-containing protein found in molluscs and arthropods which functions as a dioxygen carrier. The smallest functional subunit contains two copper atoms and binds one molecule of dioxygen reversibly. The oxygenated form of the protein has been extensively studied over the past two decades.[5] However, studies on the other native form (deoxy) are relatively rare, since this derivative contains Cu(I) which is spectroscopically undetectable by both EPR and visible absorption techniques. Recently, an EXAFS study on hemocyanin from *Busycon canaliculatum* was reported.[2] The authors suggested a Cu-Cu interaction at 3.4 Å in deoxyhemocyanin. However, they also noted that the peak which was fitted with copper could also be fitted with low-Z scatterers.

Megathura crenulata hemocyanin was collected from live giant keyhole limpets (obtained from Pacific Biomarine, Venice, CA) as previously described.[6] The deoxygenated protein was prepared[7] by anaerobically dialyzing a purified sample at 4 °C for 3-4 days, in a 0.1 M phosphate buffer at pH 8.5 with 0.1 M $MgCl_2$. The buffer was kept deoxygenated by continuously bubbling N_2 through the solution. The colorless protein solution was finally centrifuged at 100000g for 3 h. The gel was transferred to an EXAFS cell which was kept in an anaerobic chamber continuously circulated with N_2. The protein remained colorless at the end of data collection period and turned blue on exposure to air. XAS data were collected both at room temperature and -70 °C as described previously[8] by using synchrotron radiation at the Stanford Synchrotron Radiation Laboratory. Radiation was monochromatized by using a Si[220] crystal monochromator on the wiggler beam line IV. Energy was calibrated relative to a copper foil by assigning 8980.3 eV to the first inflection point on the copper K-absorption edge.

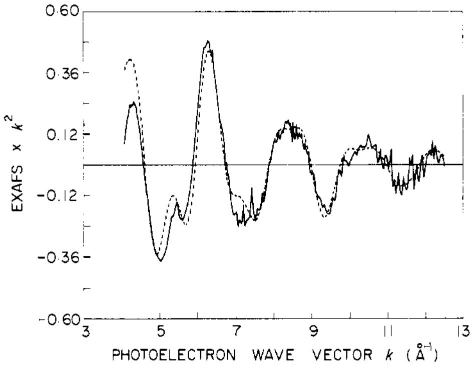

Figure 1. The normalized, background-subtracted EXAFS data (solid line) for deoxyhemocyanin from *M. crenulata*. The fit over a k range of 4-12 Å^{-1} (dashed line) is obtained by fitting with two imidazoles, giving a Cu-N distance of 1.95 Å.

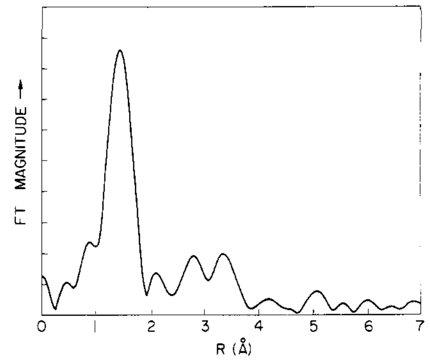

Figure 2. Fourier transform of the data in Figure 1, from a k range of 4-12 Å^{-1}, k^3 weighted. The first peak can be Fourier filtered and fit with two nitrogen atoms at a Cu-N distance of 1.95 Å.

The X-ray absorption edge spectrum of deoxyhemocyanin was examined for evidence concerning the oxidation state of copper. This method has been used previously in the investigation of the oxidation state of the coppers in cytochrome c oxidase[9] and hemocyanin.[1,2,6] Absorption edge spectra for deoxyhemocyanin indicate the absence of the small preedge feature at ca. 8979 eV, which is normally assigned to the 1s → 3d bound state transition for the Cu(II) state.[6] The transition occurring at ca. 8983 eV (assigned in the literature as "1s → 4s") is consistent with Cu(I) models, which average 8983 eV, 4 eV lower than the same transition generally assigned to Cu(II).[6] Hence, edge studies are consistent with the copper in deoxyhemocyanin being present in the reduced [Cu(I)] form.

EXAFS data were processed as previously described.[8] The normalized, background-subtracted data, presented in Figure 1, are averages of 15 room temperature scans, each scan comprising data collected by 20 NaI scintillation detectors. The signal-to-noise level of the data was among the best of any data that we have

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