

Chemical and Electrochemical Interrelationships of the 1-Fe, 2-Fe, and 4-Fe Analogues of the Active Sites of Iron-Sulfur Proteins

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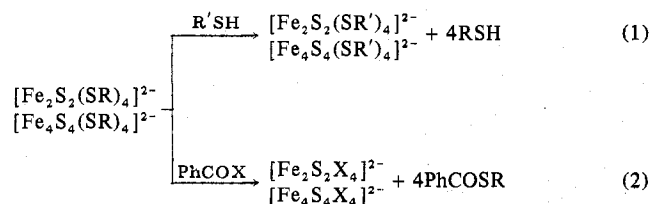
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Previous studies of the reactivity properties of the complexes $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{2-}$ and $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ have demonstrated the occurrence of thiolate ligand substitution reactions with retention of Fe_2S_2 and Fe_4S_4 core structures. Described in this report are reactions which provide chemical and electrochemical interrelationships of 1-Fe, 2-Fe, and 4-Fe synthetic analogues of the active sites of iron-sulfur proteins. These reactions include (i) mononuclear \rightarrow binuclear conversion, $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^- \rightarrow [\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ ($\text{S}_2\text{-}o\text{-xyl} = o\text{-xylyl-}\alpha,\alpha'$ -dithiolate); (ii) binuclear \rightarrow tetranuclear conversion, $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ by spontaneous reaction in protic media and $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$. These transformations are correlated with prior synthetic and ligand-exchange processes to provide an overall reaction scheme starting with a simple iron(II) salt, progressing through intermediate 1-Fe and 2-Fe species and terminating in the 4-Fe complexes with cubane-type stereochemistry. Reaction iii was accomplished using sodium acenaphthylenide in hexamethylphosphoramide as reductant. The product trianion was obtained as oxygen-sensitive, crystalline Me_4N^+ or Et_4N^+ salts and is an analogue of the 4-Fe active sites of reduced ferredoxin proteins. With its isolation synthetic analogues of five of the seven known physiological oxidation levels of 1-Fe, 2-Fe, and 4-Fe sites in iron-sulfur proteins have been obtained in substance.

Introduction

An abundance of physicochemical evidence³⁻⁷ has resulted in definition of the basic structures of three types of active sites in the lower molecular weight, nonheme iron-sulfur redox proteins. These structures, containing as common features approximately tetrahedral coordination around iron atoms and terminal ligation by cysteinate sulfur, are illustrated in Figure 1 and occur in the following types of proteins:⁸ 1, Rd; 2, Fd; 3, Fd, HP. Also shown in this figure are representations of the established structures of synthetic complexes which serve as stereochemical and electronic analogues^{7,9} of protein-active sites in corresponding total oxidation levels: Rd_{red} , $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{2-10}$ (4); Rd_{ox} , $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]^{-10,11}$ (4); 2-Fe Fd_{ox} , $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-12}$ (5), $[\text{Fe}_2\text{S}_2(\text{SC}_6\text{H}_4\text{-}p\text{-Me})_4]^{2-12}$ (6); 4-Fe Fd_{ox} , HP_{red} ; $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-13,14}$ ($\text{R} = \text{alkyl, aryl}$) (7). In addition to these species the complexes $[\text{Fe}(\text{SPh})_4]^{2-15}$ and $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-16a}$ have recently been isolated and are analogues of the sites in Rd_{red} and 4- and 8-Fe Fd_{red} proteins, respectively. Other species not yet isolated, viz., $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{3-4-12}$ and $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{-4-12,17}$ have been detected electrochemically.

In addition to structural and electronic characterization of synthetic analogues 4-7, investigations of their reactivities have been pursued. Of these the best characterized are electron-transfer^{10,12,17} and thiolate substitution processes involving thiols^{12,14} (reaction 1), benzoyl halides¹⁸ (reaction 2), and



water and certain inorganic anions.¹⁹ A common feature of substitution and most redox reactions is the formation of stable products in which the Fe_2S_2 and Fe_4S_4 core structures are retained. However, several prior observations have indicated a further type of reactivity in which core structures are not conserved. The spontaneous binuclear-tetranuclear conversion $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ was noted during the study of removal of cores from holoproteins in the form of their benzenethiolate analogues²⁰ (core extrusion reactions).

Electrochemical examination of the reduction of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ suggested the formation of tetranuclear species.¹² Neither of these observations was pursued. In the present investigation it has been established that the cubane-type 4-Fe structure 7 ($\text{R} = \text{Ph}$) is the terminal stable product in a chemical and electrochemical reaction sequence starting from the simplest analogue 4 and proceeding through the 2-Fe analogues 5 and 6 as intermediates. Also described are the experimental details for preparation and isolation of the reduced tetranuclear complexes $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$, previously accessible only by electrochemical or chemical generation in solution.^{16b}

Experimental Section

Preparation of Compounds. $(\text{Et}_4\text{N})_2[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]$,¹⁰ $(\text{Et}_4\text{N})[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]$,¹¹ $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]$,¹² $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$,¹² $(\text{Et}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]$,¹³ and $(\text{R}_4\text{N})_2[\text{Fe}_4\text{S}_4(\text{SPh})_4]$ ¹³ were prepared by published methods. Tetraalkylammonium tetraphenylborate salts were obtained by combining sodium tetraphenylborate and the tetraalkylammonium halide in hot water and recrystallizing the resulting precipitate from hot acetone or acetone-ether. The syntheses described below were performed under an atmosphere of argon, purified by passage through a tower of BASF catalyst maintained at 110 °C. For use in syntheses and physical measurements reagent grade acetonitrile and tetrahydrofuran (THF) were distilled from calcium hydride and lithium aluminum hydride, respectively. Methanol was distilled from magnesium methoxide. Aldrich reagent grade hexamethylphosphoramide (HMPA) was purified by fractional distillation from sodium. All solvents were carefully deoxygenated before use by repeated evacuation and flushing with argon. Acenaphthylene was recrystallized from methanol.

Mononuclear-Binuclear Conversion: Synthesis of $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ from $[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]$. Sodium hydrosulfide (0.47 g, 8.3 mmol), tetraethylammonium chloride (1.4 g, 8.3 mmol), and sodium methoxide (0.45 g, 8.3 mmol) were dissolved in 50 mL of dry methanol. This solution was added over a 15-min period to a warm (45 °C) solution of 4.3 g (8.3 mmol) of $(\text{Et}_4\text{N})[\text{Fe}(\text{S}_2\text{-}o\text{-xyl})_2]$ in 150 mL of acetonitrile. This solution was left undisturbed at room temperature overnight, after which 3.0 g of a red-black crystalline solid was collected. The UV-visible spectrum of this material is identical in all respects with that of $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]$ prepared by a different method,¹² demonstrating that this product was pure without recrystallization and obtained in 93% yield.

Binuclear-Tetranuclear Conversion: (a) Spontaneous Reaction in Solution. The conversion $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ was followed using a Cary Model 17 spectrophotometer equipped with a thermostated cell holder maintained at 19.0 or 26.0 °C ($\pm 0.1^\circ$). In a typical experiment a 1-3 mM solution of $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$

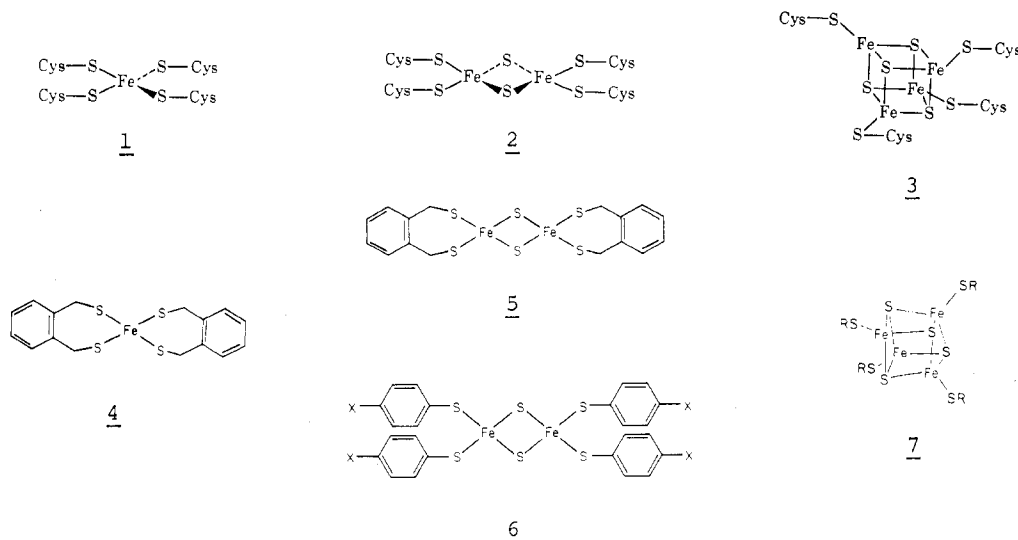


Figure 1. Representations of the structures of the active sites 1–3 of iron–sulfur proteins and their synthetic analogues 4–7 (net charges omitted).

in HMPA was placed in an evacuated quartz cell. The reaction was initiated by anaerobic addition of sufficient degassed aqueous 50 mM Tris–Cl solution adjusted to pH 7.46 or 8.45 such as to give the final solvent composition 80% v/v HMPA–H₂O. Spectra were recorded throughout the course of the reaction. Similar experiments were performed in 80% v/v Me₂SO–H₂O solutions. The effect of benzenethiol on the conversion rate was examined in several cases by inclusion of an ca. 100:1 thiol/dimer mole ratio in the organic solvent prior to addition of the aqueous component. To investigate the stoichiometry of the conversion reaction, 0.5 mL of an 80% HMPA solution whose spectral change was complete was injected into 2.5 mL of concentrated HCl containing several milligrams of NaCl. This mixture was allowed to react for 30 s, resulting in a color change from red–brown to yellow, and was then quickly extracted with ether. The organic phase was analyzed by gas chromatography using a Varian Model 3700 instrument and a 1 ft column of 5% OV-101 on 100–120 mesh Chromasorb-G preconditioned at 325 °C. Temperature programming which included an initial temperature of 70 °C for 1 min followed by a linear increase of 10°/min to a final temperature of 170 °C afforded resolution of benzenethiol and diphenyl disulfide peaks from each other and from some residual HMPA in the ether phase. Response factors of thiol and disulfide were determined relative to naphthalene, and peaks were automatically integrated by a Linear Instruments Model 252A recorder. Control experiments with (Et₄N)₂[Fe₄S₄(SPh)₄] alone demonstrated the necessity of the rapid acid quench and extraction steps to avoid disulfide formation by oxidation of thiol with Fe(III). Three separate experiments yielded PhSH/PhSSPh mole ratio values of 6.0, 6.2, and 6.2.

(b) Electrochemical Reduction. Electrochemical experiments were performed with Princeton Applied Research equipment which included a Model 173 potentiostat–galvanostat with a Model 179 digital coulometer or Model 176 current-to-voltage converter and a Model 175 universal programmer. Voltammograms were recorded with a Model 170 electrochemistry system modified for external input or on a Tektronix 5103N-D15 storage oscilloscope. A Pt disk (Beckman 39273) or a glassy carbon disk (PAR 9333) was used as the working electrode in cyclic voltammetry. Potentials were measured vs. a saturated calomel electrode. The binuclear–tetranuclear conversion was followed by simultaneous coulometry and monitoring of the optical spectra of acetonitrile solutions containing [Fe₂S₂(SPh)₄]²⁻ which were electrolyzed at –1.2 to –1.3 V at a Pt gauze working electrode. The apparatus employed is similar to that described elsewhere.²¹ All solutions contained 0.05 M tetra-*n*-butylammonium tetrafluoroborate or perchlorate as supporting electrolyte.

Tetranuclear Dianion–Trianion Reduction: [Fe₄S₄(SR)₄]³⁻ from [Fe₄S₄(SR)₄]²⁻. In the following preparations it is of utmost importance to maintain *rigorously* anaerobic conditions due to the extreme ease of oxidation of the tetranuclear trianions.

(a) (Me₄N)₃[Fe₄S₄(SPh)₄]. Acenaphthylene (0.550 g, 3.62 mmol) and freshly cut sodium (0.0833 g, 3.62 mmol) were added to 20 mL of HMPA in a Schlenk tube, and the solution was stirred overnight. (Me₄N)₂[Fe₄S₄(SPh)₄] (2.84 g, 3.03 mmol) and tetramethyl-

ammonium tetraphenylborate (1.33 g, 3.38 mmol) were dissolved in a minimum volume of HMPA (~35 mL). To this solution was added the sodium acenaphthylenide solution over a 1-h period, and the reaction mixture was stirred for an additional 2 h. THF (100 mL) was added, causing the separation of the product as black crystals (2.72 g, 95%). Purification was effected by recrystallization from acetonitrile which was initially at 45 °C and then slowly cooled to –20 °C; the product was obtained as well-formed black crystals. Anal. Calcd for C₃₆H₃₆N₃S₈Fe₄: C, 42.78; H, 5.58; N, 4.16; S, 25.38; Fe, 22.10. Found: C, 42.94; H, 5.91; N, 4.16; S, 24.89; Fe, 21.65.

(b) (Et₄N)₃[Fe₄S₄(SPh)₄]. A HMPA solution containing 8.16 mmol of sodium acenaphthylenide in 50 mL was prepared as described in the preceding synthesis. This solution was added dropwise over 100 min to a solution of 1.08 g (6.52 mmol) of dry tetraethylammonium chloride and 6.85 g (6.53 mmol) of (Et₄N)₂[Fe₄S₄(SPh)₄]¹⁷ in 50 mL of HMPA. The reaction mixture was stirred an additional 4 h, 180 mL of THF was added, and the mixture was cooled to –20 °C. The black crystalline product was collected and dissolved in 100 mL of acetonitrile at room temperature and this solution was filtered. Solvent was removed in vacuo at room temperature until crystallization commenced, the solution was warmed to 50 °C and 200 mL of THF at 50 °C was added. Slow cooling to –20 °C afforded separation of product, which after washing with THF was obtained as very thin black crystals (6.50 g, 85%). Anal. Calcd for C₄₈H₃₀N₃S₈Fe₄: C, 48.89; H, 6.84; N, 3.56; S, 21.76; Fe, 18.95. Found: C, 48.79; H, 6.79; N, 3.75; S, 21.64; Fe, 18.75.

(c) (Et₄N)₃[Fe₄S₄(SCH₂Ph)₄]. A HMPA solution containing 4.40 mol (~50% excess) of sodium acenaphthylenide in 20 mL was prepared and added slowly over a 1-h period to a solution of 3.17 g (2.87 mmol) of (Et₄N)₂[Fe₄S₄(SCH₂Ph)₄]¹² and 1.30 g (2.89 mmol) of tetraethylammonium tetraphenylborate in a minimum volume of HMPA (~30 mL). After an additional 2 h of stirring, 150 mL of THF was added and the mixture was cooled to –20 °C. The small black crystals were collected and washed with THF (2 × 10 mL). Purification was achieved by concentrating an acetonitrile solution, initially at 45 °C, to incipient crystallization by solvent removal in vacuo followed by cooling to room temperature. In this manner 2.30 g (65%) of black crystals was obtained after one recrystallization. Anal. Calcd for C₅₂H₈₈N₃S₈Fe₄: C, 50.56; H, 7.20; N, 3.40; S, 20.76; Fe, 18.08. Found: C, 50.64; H, 7.03; N, 3.51; S, 20.71; Fe, 17.97.

Results and Discussion

Throughout the following sections attention is directed to Figure 2. Here are summarized direct syntheses from iron salts of mono-, bi-, and tetranuclear complexes 4–7 (Figure 1) and the chemical and electrochemical relationships among them, which are the subject of this report. This scheme contains all direct or indirect preparative methods for these species thus far developed during the course of our program^{7,9} to synthesize and isolate analogues of active sites 1–3 in all physiologically significant oxidation levels. Designated in the

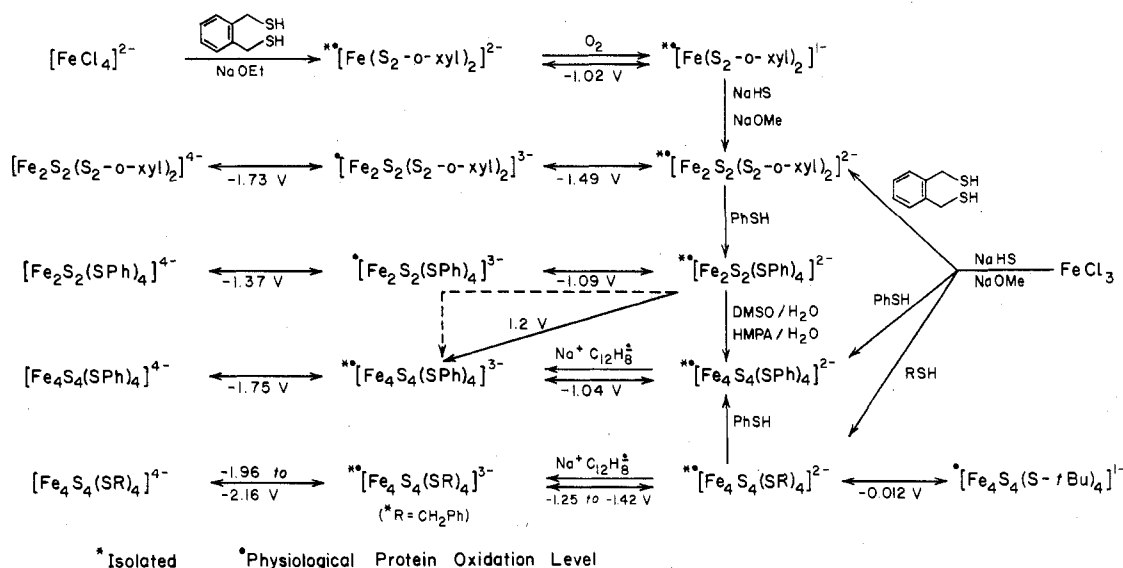
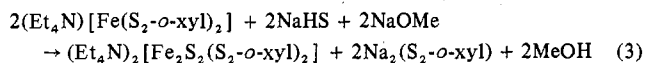


Figure 2. Reaction scheme depicting syntheses of 1-Fe, 2-Fe, and 4-Fe complexes and chemical and electrochemical relationships among them. Double-headed arrows indicate electrochemical reactions but do not necessarily imply reversibility. Potentials are polarographic $E_{1/2}$ values vs. SCE in DMF determined previously.^{10,12,17} Isolated analogues and those isoelectronic with physiological protein oxidation levels are designated.

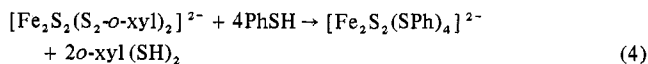
scheme are those analogues which have been isolated (as R_4N^+ or Ph_4As^+ salts) and those which correspond to physiological oxidation levels of protein active sites. The means of assigning site oxidation levels from comparison of protein and analogue physical properties are detailed elsewhere.^{7,10-13,17} With the exception of the oxidation of the mononuclear dianion to the monoanion, all chemical and electrochemical reactions were performed under *anaerobic* conditions. All synthetic analogues are sensitive to dioxygen, especially in solution, but these reactions have not been systematically investigated.

Mononuclear-Binuclear Conversion. The initial iron source for progressive buildup of iron-sulfur structures culminating in the tetranuclear complexes **7** is the tetrachloroferrate(II) ion. As reported recently,¹⁰ reaction of this complex with excess *o*-xyl(SH)₂ and sodium ethoxide in ethanol-acetonitrile solution affords the Rd_{red} analogue $[Fe(S_2-o-xyl)_2]^{2-}$. Controlled aerial oxidation of a solution of the dianion yields $[Fe(S_2-o-xyl)_2]^{-}$,¹¹ the only Rd_{ox} analogue. These two complexes have the mononuclear bis-chelate structure **4** containing distorted tetrahedral Fe(II,III)-S₄ coordination units.^{10,11} Smooth conversion to a binuclear complex is accomplished by treatment of $(Et_4N)[Fe(S_2-o-xyl)_2]$ in acetonitrile with equimolar amounts of sodium hydrosulfide/sodium methoxide in methanol. The isolated red-black crystalline product exhibits an electronic absorption spectrum quantitatively identical with that of $(Et_4N)_2[Fe_2S_2(S_2-o-xyl)_2]$, the 2-Fe Fd_{ox} analogue dianion **5**,^{12,22,23} containing distorted tetrahedral Fe(III) centers bridged by sulfide and previously obtained by the direct synthesis^{12,23} in Figure 2. Its formation occurs, in effect, by removal of one dithiolate ligand of the precursor monoanion with sulfide followed by dimerization in a process with the apparent stoichiometry of reaction 3.



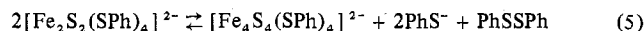
This reaction, which leads to pure product in 93% yield based on $[Fe(S_2-o-xyl)_2]^{-}$, is now the preparative method of choice. Direct synthesis occasionally yields an amorphous product¹² which in some instances has proven difficult to purify by recrystallization.

To facilitate investigation of the reactivity of the $[Fe_2S_2]^{2+}$ core the dianion **5** was converted to the corresponding benzenethiolate complex by the previously reported ligand exchange reaction (4),¹² a specific example of the generalized



reaction (1), and the isolated tetraethylammonium salt was used in experiments described below. The related tetranuclear complex $[Fe_4S_4(SPh)_4]^{2-}$ possesses the cubane-type structure **7**¹⁴ and its solution properties are well characterized.^{12,17,24}

Binuclear-Tetranuclear Conversion. (a) Spontaneous Reaction in Solution. $[Fe_2S_2(SPh)_4]^{2-}$ and related para-substituted complexes **6** are stable in aprotic solvents such as Me₂SO, DMF, and HMPA under anaerobic conditions. However, in mixed aqueous media a slow reaction occurs resulting in formation of tetramer dianions. This reaction was first observed in 80% v/v Me₂SO/H₂O solutions in the course of developing procedures for extruding 2-Fe and 4-Fe cores of protein sites in the form of their benzenethiolate synthetic analogues, and the process involved was proposed to be represented by reaction 5.²⁰



In addition to its interest in the context of the reaction chemistry of the $[Fe_2S_2]^{2+}$ core, binuclear \rightarrow tetranuclear conversion is of significance in core extrusion experiments^{24,25} inasmuch as its occurrence could result in incorrect identification of site structure in a native protein. Because an 80% HMPA/H₂O solvent has been employed in more recent and extensive core extrusion experiments in this^{24,25} and another²⁶ laboratory, the binuclear \rightarrow tetranuclear conversion was examined in this medium. Eight solutions containing 1.2–2.6 mM $[Fe_2S_2(SPh)_4]^{2-}$ with aqueous components buffered at pH 7.46 or 8.50 were studied spectrophotometrically at 19 or 26 °C. A typical experiment is shown in Figure 3. Addition of the aqueous component to the HMPA solution initiates the reaction and causes an initial decrease in the apparent extinction coefficients of the binuclear dianion, curve a, followed by much slower spectral changes, curves b–d. The final

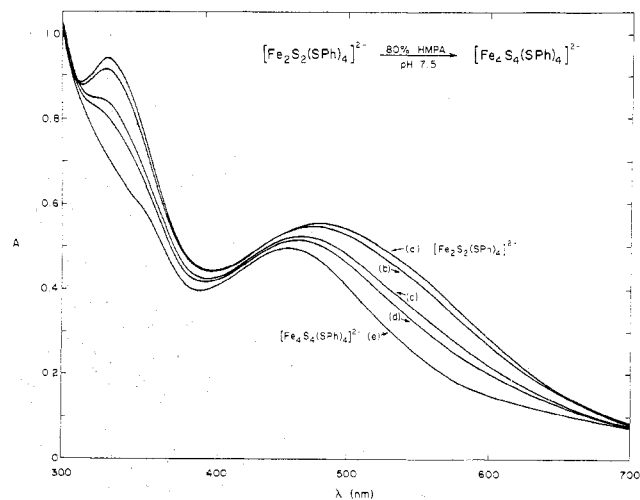
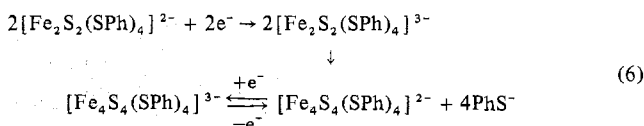


Figure 3. Spectra illustrating the conversion $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ in 80% HMPA- H_2O , aqueous phase pH 7.46, 26.0 $^\circ\text{C}$. Initial 1.21 mM solution of $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$ 5 min (a), 50 min (b), 14.0 h (c), 22.1 h (d), and 118 h (e) after addition of aqueous component.

spectrum, curve e (λ_{max} 458 nm, A_{458}/A_{550} 2.19), is unambiguously that of $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$, which has been measured separately in this medium.²⁴ Using ϵ_{458} 17 200 $\text{M}^{-1} \text{cm}^{-1}$ for the product dianion, the binuclear-tetranuclear conversion is calculated to be 96% complete. The other products of the reaction were identified by acid hydrolysis of solutions whose spectral changes corresponded to $\geq 90\%$ completion of reaction, followed by gas chromatographic analysis of the ether extracts of these solutions. The average PhSH/PhSSR mole ratio from three determinations is 6.1/1, confirming the stoichiometry of reaction 5. Similar results were obtained with other solutions and in a series of related experiments in 80% $\text{Me}_2\text{SO}/\text{H}_2\text{O}$ (pH 7.4–8.3). Thus far we have been unable to extract rate constants and deduce a mechanistic scheme for this reaction, which at some point requires a one-electron reduction of the $[\text{Fe}_2\text{S}_2]^{2+}$ core to achieve the oxidation level of the tetranuclear dianion. However, the following qualitative features of reaction 5 have been observed: (i) conversion rates are faster in 80% Me_2SO than in 80% HMPA at fixed aqueous component pH; (ii) in both solvents rates increase with decreasing pH; (iii) at 1–3 mM binuclear concentration, pH 7.5–8.5, conversion was not complete in < 24 h in either solvent;²⁷ (iv) under the conditions in (iii) but with a PhSH/Fe mole ratio of $\sim 100/1$ the binuclear dianion is stable for 24 h or longer.²⁴ These observations demonstrate that a slow spontaneous binuclear \rightarrow tetranuclear conversion involving an electron transfer step occurs in the presence of a protic solvent and that the reaction rate may be rendered negligible at the indicated pH range by introduction of excess thiol.

(b) Electrochemical Reduction. It has been previously reported that $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ and $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ in aprotic solvents are electrochemically reducible in two one-electron steps^{12,23} to form the three-membered electron-transfer series in Figure 2. No assessment of reduced product stability was made other than the observation that qualitative features of the cyclic voltammograms of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ at a Pt electrode in DMF solution were consistent with reaction scheme 6. Here we provide evidence which indicates that this



scheme obtains upon initial reduction of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$,

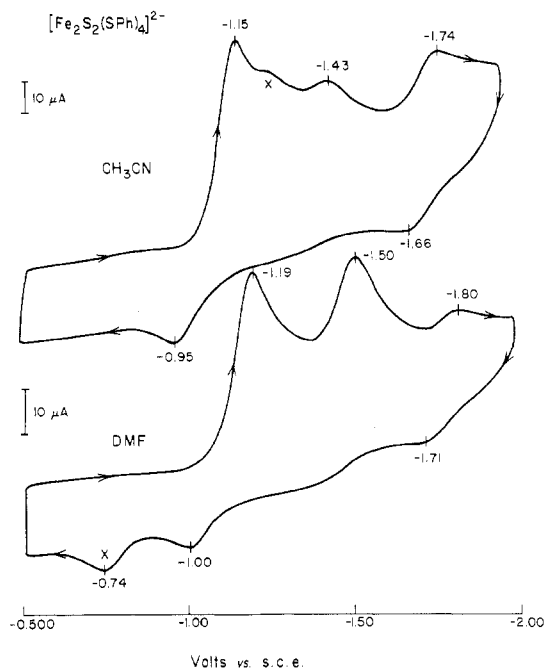


Figure 4. Cyclic voltammograms of 1.0 mM solutions of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ in acetonitrile and DMF solutions at a carbon disk electrode recorded at a scan rate of 100 mV/s. The peaks marked with an \times are unidentified.

whose cyclic voltammograms in acetonitrile and DMF solutions at a carbon disk electrode are shown in Figure 4. The peaks at -1.19 and -1.50 V in DMF correspond to binuclear 2-/3- and 3-/4- reductions,¹² respectively. At -1.80 V the $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ couple appears (vide infra), and the positive scan reveals the tetranuclear 2-/3- couple¹⁷ at -1.00 V. The voltammogram in acetonitrile can be similarly interpreted; as observed previously¹⁷ half-wave or peak potentials occur at slightly less negative potentials in acetonitrile than in DMF. Comparison of the two scans reveals that tetranuclear anion formation is more rapid in acetonitrile as evidenced by the relative heights of the binuclear 3-/4- and tetranuclear 3-/4- features.

Increased conversion of binuclear to tetranuclear species in reaction 6 can be accomplished by repetitive cyclic scans or by holding the potential at a value between the binuclear 2-/3- and 3-/4- potentials. The results of a constant-potential electrolysis experiment in acetonitrile solution with simultaneous coulometry and spectral monitoring are shown in Figure 5. Passage of current corresponding to a coulometric n value of 1.53 e/binuclear dianion affords the spectral change a \rightarrow b. Spectrum b corresponds to $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$, identified by comparison with the spectrum of an isolated salt of this trianion (vide infra) and formed quantitatively. This species appears as the terminal reduction product because the applied potential is more negative than that of the tetranuclear 2-/3- couple. Oxidative electrolysis to zero current above background yielded $n = 0.89$ e and spectrum c, which corresponds to $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$. The absorbance and coulometric data indicate $\geq 90\%$ overall conversion from binuclear dianion to tetranuclear dianion and are consistent with reaction scheme 6. The initial reduced product, $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-}$, was not observed spectrally during electrolysis, indicating spontaneous decay to the tetranuclear dianion.

These results demonstrate that $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ in protic and $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-}$ in aprotic media spontaneously convert to the cubane-type dianion 7, which culminates the progressive buildup in structural complexity from simple mononuclear iron species. In aprotic media the stability of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ toward tetranuclear formation contrasts with the instability

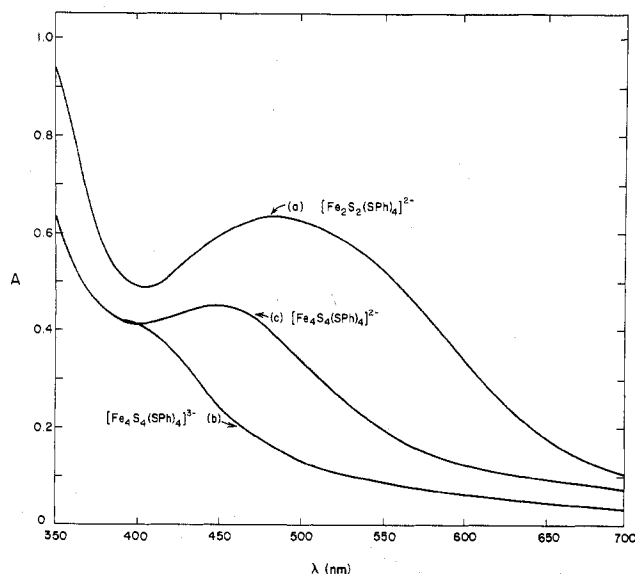


Figure 5. Spectra illustrating the conversion $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ by electrolysis in acetonitrile solution: (a) 1.07 mM (26.1 mg, 30 μmol) $(\text{Et}_4\text{N})_2[\text{Fe}_2\text{S}_2(\text{SPh})_4]$; (b) solution a electrolyzed at -1.25 V, 4.40 C passed; (c) solution b electrolyzed at -0.72 V, 1.29 C passed.

of $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-}$. As noted above, the conversion $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$, reaction 5, can be eliminated in protic solvents by appropriate pH adjustment and introduction of excess thiol. The $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-} \rightarrow [\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ process in reaction scheme 6 is a nonredox conversion involving reactant ($[\text{Fe}_2\text{S}_2]^{+}$) and product ($[\text{Fe}_4\text{S}_4]^{2+}$) cores in the same oxidation level and may be facilitated for this reason. This reaction must be suppressed in order that a species of the general type $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{3-}$, an analogue of 2-Fe Fd_{red} sites, be obtained. Preliminary examination of the $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-,3-}$ couple by cyclic voltammetry in DMF solution at a carbon disk electrode reveals effective reversibility with a unit ratio of anodic and cathodic peak currents.²⁸ Maintenance of potentials sufficient to generate the trianion indicate conversion, but at a slower rate than for $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{3-}$, to a species with the redox characteristics of an alkylthiolate tetramer.¹⁷ Search is continuing for experimental conditions which promote the stability of this and other binuclear trianions.

Tetranuclear Dianion-Trianion Reduction. To complete the investigation of reduced protein site analogues, the chemical reductions of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ ($\text{R} = \text{Ph}, \text{CH}_2\text{Ph}$) were investigated. Earlier voltammetric studies of tetranuclear 2-/3- couples in aprotic solvents have shown that they closely approach electrochemical reversibility.^{12,17} Additionally, $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ and $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$ had been generated electrochemically and chemically (by reduction with sodium acenaphthylenide) in solution.^{16b} Other claims of the formation of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ species in solution by chemical reduction of dianions²⁹ or direct formation from iron salts, sulfide, and thiol³⁰ have appeared. In no case have trianion salts previously been isolated in substance.

Using as a reductant sodium acenaphthylenide, whose half-wave potential (-1.62 V in DMF) is intermediate between those of tetranuclear 2-/3- and 3-/4- couples¹⁷ (Figure 2), and HMPA as the reaction medium, tetranuclear dianions are cleanly reduced to trianions. The latter with $\text{R} = \text{Ph}$ and CH_2Ph were isolated as black, intensely dioxygen-sensitive Me_4N^+ or Et_4N^+ salts, obtained in analytical purity after recrystallization. Also afforded by this method is the highly crystalline compound $(\text{Et}_3\text{MeN})_3[\text{Fe}_4\text{S}_4(\text{SPh})_4]$, for which x-ray results demonstrate retention of the cubane-type

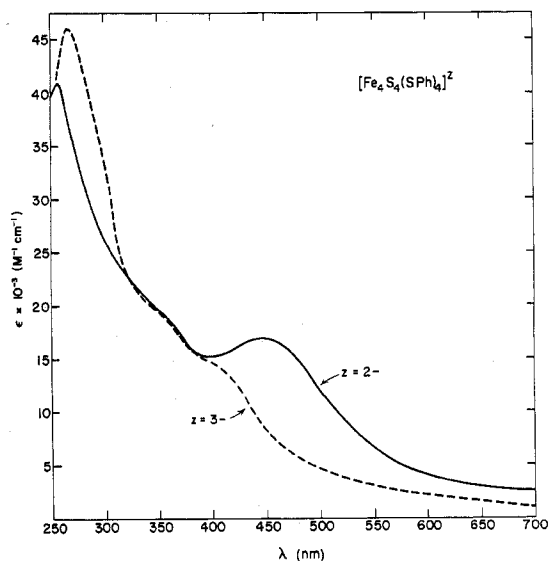


Figure 6. UV-visible absorption spectra of the isolated Me_4N^+ salts of $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ (—) and $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ (- - -).

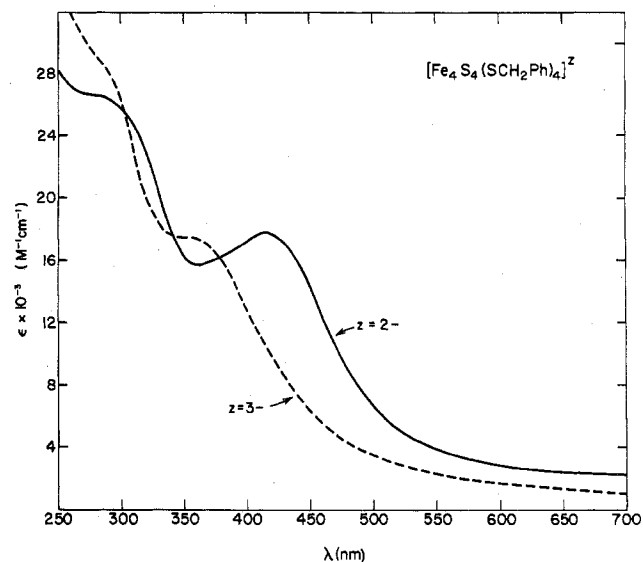


Figure 7. UV-visible absorption spectra of the isolated Et_4N^+ salts of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{2-}$ (—) and $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$ (- - -).

structure.³¹ As is evident from Figures 6 and 7, tetranuclear dianions and trianions are easily distinguished by their absorption spectra. These spectra are nearly identical to those obtained by electrochemical generation^{16b} and to spectrum b of Figure 5 and have the following characteristics (λ_{max} (ϵ), acetonitrile solution): $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$, 267 (46 100), ~ 340 (sh, 20 200), ~ 400 (sh, 14 800); $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]^{3-}$, 358 nm (17 600 $\text{M}^{-1} \text{cm}^{-1}$). Spectral data for dianions are given elsewhere.^{12,17} Elimination of the prominent visible absorption maximum and reduction in absorbance at wavelengths longer than ca. 350 nm are characteristic of the $\text{Fd}_{\text{ox}} \rightarrow \text{Fd}_{\text{red}}$ transition.³² The similar behavior, evident in Figures 6 and 7, together with other protein-trianion comparative physical properties¹⁶ ensures that $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{3-}$ species are correctly designated as analogues of 4-Fe sites of Fd_{red} proteins. Isolation of tetranuclear trianions raises to five the number of analogues corresponding to physiological oxidation levels of sites 1-3 which have been obtained. The remaining two analogues, $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{3-} \equiv 2\text{-Fe } \text{Fd}_{\text{red}}$ and $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{-} \equiv \text{HP}_{\text{ox}}$, persist as objectives; thus far such species have been detected electrochemically only^{12,17,23} (Figures 2 and 4). Structural, electronic, and reactivity properties of tetranuclear

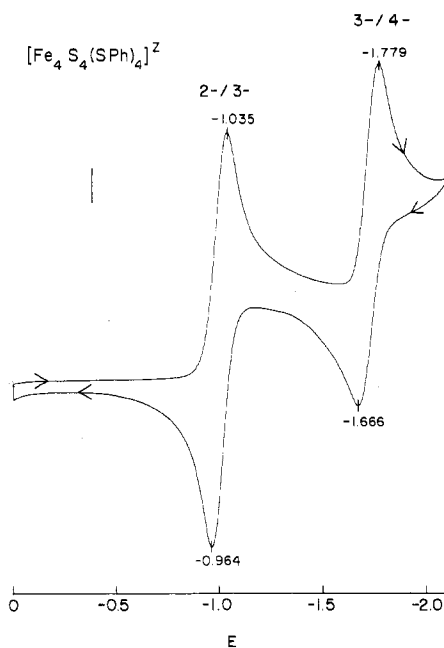


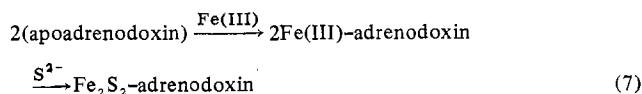
Figure 8. Cyclic voltammogram of a 1.0 mM solution of $(\text{Et}_4\text{N})_2\text{[Fe}_4\text{S}_4(\text{SPh})_4]$ in acetonitrile at a Pt disk electrode recorded at a scan rate of 100 mV/s. This solution contained 0.1 M $(n\text{-Bu}_4\text{N})(\text{ClO}_4)$ supporting electrolyte.

trianions will be described in future reports. In aprotic solvents under anaerobic conditions we have not as yet detected structural instability of the trianion $[\text{Fe}_4\text{S}_4]^+$ core.

Earlier we reported polarographic evidence for the existence of the tetraanions $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{4-}$,¹⁷ whose formation in solution by chemical means has also been claimed.^{29,30,33} Inasmuch as the existence of this species has not been graphically documented, the cyclic voltammogram in Figure 8 is presented. Existence of the three-membered electron-transfer series $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-,3-,4-}$ is evident, with the tetraanion core $[\text{Fe}_4\text{S}_4]^0$ containing formally 4 Fe(II). Because of the highly negative potential of the 3-/4- couple and the lack of evidence for physiological relevance of the 4- oxidation level, isolation and further examination of such species have not been pursued.

Summary

The complete sequence of reactions, starting from a simple Fe(II) salt, progressing through 1-Fe (4) and 2-Fe (5, 6) species, and terminating in the tetranuclear cubane-type structure (7) with multiple oxidation levels, is summarized in Figure 2. Possibly the same series of transformations could be accomplished without the intervening ligand exchange reaction (4) by utilizing the recently prepared dianion $[\text{Fe}(\text{SPh})_4]^{2-}$.¹⁵ However, the corresponding monoanion has not been isolated, rendering the dianion less suitable than $[\text{Fe}(\text{S}_2\text{-o-xyl})_2]^{2-}$ as a component of the complete reaction sequence. The structural conversions in this scheme suggest the possibility of similar reactions of other Fe-S compounds and proteins. Thus the Rd_{ox} -like chromophore formed by Fe(III) and excess 1,4-butanedithiol in aqueous solution reacts with sulfide to form a chromophore of the 2-Fe Fd_{ox} type;³⁴ the absorbing species were not isolated. More interesting is the reaction series (7) of Sugiura et al.,³⁵ who have found that the



unstable 1-Fe complex of the adrenodoxin polypeptide with properties similar to Rd_{ox} spontaneously converts to the native 2-Fe form upon treatment with sulfide. The resemblance of

this conversion to reaction 3 is apparent.

In our initial report of the synthesis and characterization of the 2-Fe Fd_{ox} analogue $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-o-xyl})_2]^{2-}$,²³ it was observed that the Fe_2S_2 core could be considered a fragment of the Fe_4S_4 core and that a 2-Fe structure is formally derivable from a 4-Fe structure by what now might be viewed as the reverse of reactions 5 and 6. On the basis of protein characterization data and possible evolutionary development schemes of ferredoxins,³⁶ it appeared that obligate anaerobic bacterial ancestors of aerobic organisms contained proteins with 4-Fe sites only whereas proteins of the latter (algae, higher plants) were restricted to 2-Fe sites only. Among recent relevant findings³⁶ isolation of a soluble Fe_2S_2 protein from the anaerobe *Clostridium pasteurianum*³⁷ suggests little if any evolutionary division of active site structures. To the extent that relative core stabilities influence the course of biosynthesis of Fe-S proteins³⁸ or precursor protoferredoxins, the equilibrium positions of reactions 5 and 6 suggest that under anaerobic conditions Fe_4S_4 cores may be formed from Fe_2S_2 cores rather than the reverse.

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Registry No. $(\text{Et}_4\text{N})[\text{Fe}(\text{S}_2\text{-o-xyl})_2]$, 57456-65-2; $(\text{Et}_4\text{N})_2\text{[Fe}_2\text{S}_2(\text{S}_2\text{-o-xyl})_2]$, 56083-11-5; $(\text{Et}_4\text{N})_2\text{[Fe}_4\text{S}_4(\text{SPh})_4]$, 55939-70-3; $(\text{Me}_4\text{N})_2\text{[Fe}_4\text{S}_4(\text{SPh})_4]$, 53260-80-3; $(\text{Me}_4\text{N})_3\text{[Fe}_4\text{S}_4(\text{SPh})_4]$, 63115-83-3; $(\text{Et}_4\text{N})_2\text{[Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]$, 52523-51-0; $(\text{Et}_4\text{N})_3\text{[Fe}_4\text{S}_4(\text{SCH}_2\text{Ph})_4]$, 63182-82-1; $(\text{Et}_4\text{N})_3\text{[Fe}_4\text{S}_4(\text{SPh})_4]$, 63115-82-2.

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- that previous half-times of reaction in 80% Me_2SO were underestimated.²⁰
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Formation and Isolation of $\text{FeH}_6\text{Mg}_4\text{X}_4(\text{C}_4\text{H}_8\text{O})_8$ ¹

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Reaction of iron(III) chloride with excess hydrogen and phenylmagnesium bromide resulted in 3.5–4.2 mol of hydrogen absorbed/mol of iron. Vapor-phase extraction of the black products with tetrahydrofuran yielded the yellow crystalline solid (YCS) $\text{FeH}_6\text{Mg}_4\text{X}_4(\text{THF})_8$ where X comprised bromide plus chloride. The extreme sensitivity of the nonvolatile solid to air and water necessitated the development of numerous new manipulative methods. Pyrolysis of the YCS yielded 96% of the THF below 160 °C. Hydrogen evolution was significant above 200 °C. Hydrolysis of the compound initially yielded metallic iron along with hydrogen and magnesium halides.

Attempts to isolate and characterize the iron hydride formed by the reaction of excess phenylmagnesium bromide with iron(III) chloride in diethyl ether under an atmosphere of hydrogen have resulted in diverse experimental observations and controversial conclusions. The reaction was first reported in 1926 by Weichselfelder² who claimed to have prepared FeH_6 . On mixing an ethereal solution of iron(III) chloride with the Grignard under hydrogen, a tan precipitate was rapidly formed and within 10 min it was either consumed or obscured by a black solution from which a viscous black oil later separated. Absorbed hydrogen corresponded to $\text{FeH}_{6.1}$. Hydrolysis of the entire mixture yielded hydrogen corresponding to $\text{FeH}_{5.8}$. When Ray and Sahai³ repeated the reaction, a black precipitate formed. The precipitate was washed with diethyl ether and then hydrolyzed. The H/Fe ratio was 3, and magnesium and organic impurities were slight. Haenny and Levi,⁴ without the support of experimental data, ascribed the hydrogen absorption to hydrogenation of the phenyl group. Wiberg, in a personal communication to Sarry⁵ and also to Tullis,⁶ reported that he had prepared black FeH_6 . Sarry⁵ repeated the synthesis and attempted to wash the product free of excess Grignard with pyridine. While the analysis of the purified black product varied widely, the average composition corresponded to FeH_3 . Considerable magnesium and a minimum of 39% unidentified organic matter were also present. Takegami and Fujmaki,⁷ interested primarily in catalytic hydrogenation, carried out the reaction under a wide variety of conditions. They postulated formation of $\text{Mg}(\text{FeH}_4)_2$ on the basis of hydrogen absorption on reaction and evolution on hydrolysis of an aliquot of the crude mixture. No product was isolated. Kost et al.⁸ obtained black products which they washed with ether. When the $\text{C}_6\text{H}_5\text{MgBr}/\text{FeCl}_3$ reaction ratio was 34, a cream-colored product was isolated which contained H/Mg of 0.2 and no iron. When the reaction ratio was 16, the final black product contained magnesium, iron, and hydrogen in the ratio of 10/1/3, respectively.

A wide variety of experimental approaches resulted in diverse conclusions. Hydrogen consumption on reaction and evolution on hydrolysis varied by more than a factor of 2. A fundamental difficulty was the assumption that valid conclusions could be deduced from incomplete analytical data derived from complex mixtures. Analytical samples were

weighed in only one case.⁵ A total analysis was performed in no instance.

This writer initiated work on the problem when the field of transition metal hydrides was almost exclusively limited to groups 3B, 4B, and 5B elements and palladium. The problem was significant from both an intrinsic and an applied point of view. Since iron was known to form an endothermic hydride, any stoichiometric binary hydridic product from Weichselfelder's reaction was anomalous. As complex hydrides became known, it appeared that back-bonding ligands were the prerequisite for formation of a stable Fe-H bond. The principal established exception was K_2ReH_9 .⁹ The possible uses of hydrides as high-energy fuels and as high-density moderators in nuclear reactors were practical reasons for studying the problem. A timely reason for studying the problem is for the information that may be obtained regarding hydrogenation catalysis.

Experimental Section

The extreme oxidative and hydrolytic sensitivity of the hydridic product and the wide range of results obtained by previous investigators dictate that the experimental procedure be described in detail.

Reactants. Iron(III) chloride was prepared from the elements in an all-glass system. It was purified by vacuum sublimation through a series of three bulbs and then collected in a weighing vessel.¹⁰ All weights reported here, except where indicated, were determined to ± 0.1 mg. At no time was the iron(III) chloride exposed to oxygen or water.

The phenylmagnesium bromide was prepared in the usual manner and then filtered in order to remove metallic magnesium.

The hydrogen used was reported by the manufacturer to contain less than 1 ppm impurity. It was stored in volume-calibrated bulbs equipped with Torricelli-type manometers and was admitted to the reaction vessel via fragile bulb break-seals.

All solvents were dried by sodium benzophenone and then distilled into the vacuum line without exposure to the atmosphere. Diethyl ether and tetrahydrofuran (THF) were dried a second time by condensation in storage vessels sealed to the vacuum line and containing sodium benzophenone. The necessity for this double drying was indicated by the reactivity of the hydridic product.

The Reaction. The hydride formation reactions (Table I) were carried out in a reactor¹⁰ maintained at room temperature except when indicated. The reactor, connected to a standard borosilicate vacuum line via a mercury-protected stopcock, was evacuated to less than 10^{-5} Torr and flamed out to remove adsorbed moisture. The method of