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# Formation of Bridged $[4Fe\text{-}4S]^{2+}$ Double Cubanes by Site-Specific Reactions: Electron-Transfer Coupling across Sulfur-Containing Bridges of Variable Length

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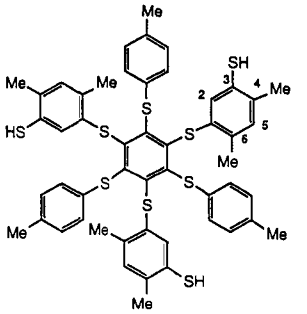
**Abstract:** The first examples of bridged $Fe_4S_4$ double cubanes have been prepared by exploiting the site-specific substitution property of the previously reported single-cubane cluster $[Fe_4S_4(L\cdot S_3)Cl]^{2-}$ (**3**, $L\cdot S_3$ = 1,3,5-tris[(4,6-dimethyl-3-mercaptophenyl)thio]-2,4,6-tris(*p*-tolylthio)benzene(3−)). Reaction of **3** with 0.5 equiv of a bridging dianion afforded the products $\{[Fe_4S_4(L\cdot S_3)]_2[\text{bridge}]\}^{4-}$ with [bridge] = benzene-1,4-dithiolate (**4**), 4,6-dimethyl-1,3-benzenedithiolate (**5**), *p*-xylene-$\alpha,\alpha'$-dithiolate (**6**), *m*-xylene-$\alpha,\alpha'$-dithiolate (**7**), ethane-1,2-dithiolate (**8**), and sulfide (**9**). All clusters except **7**, which was generated in situ, were isolated as microcrystalline $Ph_4P^+$ salts, and the identity and full or substantial purity of all compounds were established by $^1H$ NMR. Clusters **4**–**9** were used to examine, by cyclic and differential-pulse voltammetry, the apparent distance dependence of the coupling of successive electron transfers to subclusters in the series $[4-] \rightleftharpoons [5-] \rightleftharpoons [6-]$ in DMF solutions. Clusters **4**, **6**, and **7** undergo a single two-electron transfer reaction, i.e., essentially statistical behavior for which the (unresolvable) potential separation is 36 mV. The remaining clusters evidence two detectable redox steps with potential separations in the order **5** (40–60 mV) < **8** (70 mV) ≪ **9** (220 mV). Assuming a transoid conformation in solution, which disposes the subclusters at maximum separation for each bridge, the couplings as measured by potential differences are internally consistent with these separations. The strong coupling in **9** is presumably a consequence of close subcluster positions and electronic conjugation through a single-atom bridge. Transoid double cubane **4** places the subcluster centroids at a distance virtually identical with that in *Peptococcus aerogenes* ferredoxin (11.5 Å), which, as **4**, shows no redox coupling between subclusters. It is suggested that deviations from statistical electron transfer are likely to occur in ferredoxins when subcluster environments are sufficiently different and/or centroids of subclusters are separated by less than ca. 11 Å, a range that applies to **5** (10.6 Å), **8** (10.9 Å), and **9** (7.5 Å). The EPR spectra of fully reduced double cubanes are indicative of weak interactions between $S = {}^1/_2$ subclusters, further supporting the double-cubane structure.

In recent years a group of biological clusters has been identified that exhibits significant structural or reactivity properties at a single Fe site in their cubane-type $[Fe_4S_4]^{2+}$ core units. To provide analogue clusters for the elucidation of site-specific properties, we have prepared the tridentate trithiol ligand $L\cdot(SH)_3$ (**1**) and have demonstrated the occurrence of reactions 1 and 2 (X = S, Se).[1-3] The product clusters $[Fe_4X_4(L\cdot S_3)L']^{2-}$ possess the trigonal structure **2** in solution. The ligand has the conformation *ababab*, in which the three coordinating arms are disposed above and the three *p*-tolylthio legs below the central benzene ring. Reaction 2 is one example of a series of site-specific substitution reactions at the unique Fe site of the clusters **2**.[1,2] Of clusters with L′ = $RS^-$ and $Cl^-$, the latter is more readily isolated and purified. The crystal structures of the $Ph_4P^+$ salts of $[Fe_4S_4(L\cdot S_3)Cl]^{2-}$ [1,2] and $[Fe_4Se_4(L\cdot S_3)Cl]^{2-}$ [3] demonstrate the formation of the desired clusters, in which the Fe sites are differentiated in a ratio of 3:1.

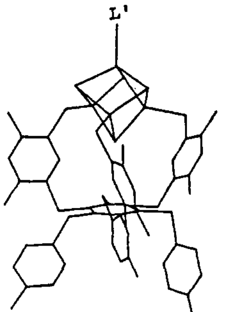
$$[Fe_4X_4(SEt)_4]^{2-} + L\cdot(SH)_3 \longrightarrow [Fe_4X_4(L\cdot S_3)(SEt)]^{2-} + 3EtSH \quad (1)$$

$$\Big\downarrow t\text{-BuCOCl}$$

$$[Fe_4X_4(L\cdot S_3)Cl]^{2-} + t\text{-BuCOSEt} \quad (2)$$



**1**



**2**

Regiospecific reactions at the unique sites of the clusters **2** offer many attractive possibilities for application of synthetic analogue methodology toward the eventual illumination of site-specific features of biological clusters. Apparently viable goals include assembly of an active site analogue of sulfite reductase,[4] binding of substrates and inhibitors as with aconitase,[5] removal of an iron atom as in the inactivation of aconitase[6,7] and isolation of the resultant $Fe_3S_4$ cluster, and reconstitution of the trinuclear site with different metals as with *Desulfovibrio gigas* ferredoxin II in its $Fe_3S_4$ form.[8,9] Several of these possibilities are under investigation in this laboratory. Here, we report a related utilization of **2** but not one in which a site analogue is sought. Instead, the regiospecific reactivity of $[Fe_4S_4(L\cdot S_3)Cl]^{2-}$ is employed to insert bridges between two clusters, thereby permitting the first investigation of the apparent effect of distance on the coupling of electronic features of $[Fe_4S_4]^{2+,1+}$ clusters as manifested in redox potentials and EPR spectra.

The occurrence of weak coupling between or among clusters, as detected by EPR, is not uncommon in both soluble and membrane-bound iron–sulfur proteins and enzymes. The best known and only structurally characterized example is *Peptococcus aerogenes* ferredoxin.[10] This protein has a molecular weight of ca.

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(1) Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1987**, *109*, 2546.

(2) Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 2484.

(3) Stack, T. D. P.; Weigel, J. A.; Holm, R. H., to be submitted for publication.

(4) McRee, D. E.; Richardson, D. C.; Richardson, J. S.; Siegel, L. *J. Biol. Chem.* **1986**, *261*, 10277.

(5) (a) Emptage, M. H.; Kent, T. A.; Kennedy, M. C.; Beinert, H.; Münck, E. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4674. (b) Kent, T. A.; Emptage, M. H.; Merkle, H.; Kennedy, M. C.; Beinert, H.; Münck, E. *J. Biol. Chem.* **1985**, *260*, 6871. (c) Telser, J.; Emptage, M. H.; Merkle, H.; Kennedy, M. C.; Beinert, H.; Hoffman, B. M. *J. Biol. Chem.* **1986**, *261*, 4840.

(6) Kennedy, M. C.; Emptage, M. H.; Dreyer, J.-L.; Beniert, H. *J. Biol. Chem.* **1983**, *258*, 11098.

(7) Emptage, M. H.; Dreyer, J.-L.; Kennedy, M. C.; Beinert, H. *J. Biol. Chem.* **1983**, *258*, 11106.

(8) Moura, I.; Moura, J. J. G.; Münck, E.; Papaefthymiou, V.; LeGall, J. *J. Am. Chem. Soc.* **1986**, *108*, 349.

(9) Surerus, K. K.; Münck, E.; Moura, I.; Moura, J. J. G.; LeGall, J. *J. Am. Chem. Soc.* **1987**, *109*, 3805.

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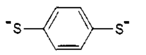
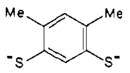
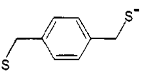
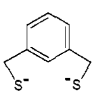
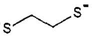
**Table I.** Structural and Redox Properties of Bridged Double Cubanes

| | | distance,[a] Å | | | | | |
|---|---|---|---|---|---|---|---|
| cluster | bridge | S···S | Fe···Fe | Ct···Ct[b] | $E_p$,[c] V | $\Delta E_p$, mV | $K_{com}$ |
| **4** |  | 6.35 | 8.75 | 11.4 | -1.10 | <30 | ~4 |
| **5** |  | 5.50 | 7.87 | 10.6 | -1.10, -1.14 | ~(40-60) | ~7 |
| **6** |  | 8.07 | 12.1 | 15.4 | -1.11 | <30 | ~4 |
| **7** |  | 6.98 | 10.4 | 13.1 | -1.10 | <30 | ~4 |
| **8** |  | 4.38 | 7.79 | 10.9 | -1.13, -1.20 | 70 | 15 |
| **9** | $S^{2-}$ | 0 | 4.20 | 7.46 | -1.28, -1.50 | 220 | $5.4 \times 10^3$ |
| *P. aerogenes* Fd[d] | | 6.43 | 8.87 | 11.5 | | <30[e] | ~4 |

[a] Calculated for the transoid configuration using the indicated angles and distances (S* is a core atom). **4–9**: core dimensions from **3**;[2] S*–Fe–S, 115°. **4–7**: bridge ring C–C, 1.395 Å; angles 120°; $C_{aryl}$–S, 1.78 Å; $C_{alkyl}$–S, 1.81 Å (also for **8**). **4**, **5**: Fe–S, 2.26 Å; Fe–S–C, 106° (mean values from $(Me_4N)_2[Fe_4S_4(SPh)_4]$[23]); S*–Fe–S–$C_{aryl}$, 180°. **6–8**: Fe–S, 2.25 Å; Fe–S–C, 103° (mean values from $(Et_4N)_2[Fe_4S_4(SCH_2Ph)_4]$[48]); S*–Fe–S–$C_{aryl}$, 180°. **9**: Fe–S, 2.20 Å; S–Fe–S, 145°; S*–Fe–S–Fe, 180°. [b] Ct = centroid. [c] Potentials from differential-pulse voltammetry in DMF solutions, vs SCE. [d] Distances calculated from atom coordinates.[10] [e] Reference 35.

6200 Da and contains two $Fe_4S_4$ clusters with core centroids separated by about 12 Å. It and a number of other ferredoxins,[11,12] especially those with similar molecular weights and semiconserved sequence runs and which also contain two clusters, have indistinguishable potentials of individual cluster reduction. When doubly reduced, they present complicated EPR spectra indicative of weak magnetic coupling between clusters in the $[Fe_4S_4]^{1+}$ oxidation level with $S = {}^1/_2$. The behavior of clostridial ferredoxins is prototypical[13] and will be compared with the results for bridged double-cube clusters.

## Experimental Section

**Preparation of Compounds.** All operations and manipulations were performed under a pure dinitrogen atmosphere. Solvents were purified as appropriate and degassed prior to use. $(Ph_4P)_2[Fe_4S_4(L\cdot S_3)Cl]$ (**3**) was prepared as described elsewhere.[2,3]

**$(Ph_4P)_4\{[Fe_4S_4(L\cdot S_3)]_2[bridge]\}$.** A series of bridged double cubanes was prepared by the procedures given below. Bridges are dianionic sulfur ligands. Isolated compounds were obtained as purplish black or red-brown, air-sensitive, microcrystalline solids, which are soluble in DMF and $Me_2SO$ and sparingly soluble in acetonitrile.

(a) **[Bridge] = Benzene-1,4-dithiolate (4).** Compound **3** (0.250 g, 0.124 mmol) was dissolved in about 1 mL of DMF, and 20 mL of acetonitrile was added. Addition of 12 mg (0.062 mmol) of the disodium salt of benzene-1,4-dithiol[14] with stirring resulted in a slight purplish change in color. The reaction mixture was filtered and stored at -20 °C. The microcrystalline solid was collected, washed with ether, and dried in vacuo to give 0.165 g (65%) of product. The compound can be recrystallized by dissolving the solid in a minimum amount of DMF, adding acetonitrile, and cooling to -20 °C. Absorption spectrum (DMF): $\lambda_{max}$ ($\epsilon_M$) 300 (sh, 88 000), 445 (sh, 28 500) nm.

(b) **[Bridge] = 4,6-Dimethyl-1,3-benzenedithiolate (5).** This compound was prepared by the method for **4** but with use of the disodium salt of 4,6-dimethylbenzene-1,3-dithiol.[15] It was recrystallized in the same way as **4**. Absorption spectrum (DMF): $\lambda_{max}$ ($\epsilon_M$) 300 (sh, 94 500), 460 (sh, 36 000) nm.

(c) **[Bridge] = *p*-Xylene-$\alpha,\alpha'$-dithiolate (6).** A solution of compound **3** (0.100 g, 0.050 mmol) in 50 mL of acetonitrile was treated with 0.55 equiv of the disodium salt of *p*-xylene-$\alpha,\alpha'$-dithiol (Aldrich) dissolved in 1 mL of DMF. The reaction mixture was filtered, reduced in volume to 25 mL, and cooled to -20 °C. The microcrystalline solid was collected by filtration, washed with ether, and dried in vacuo, affording 0.045 g (44%) of pure product. Absorption spectrum (DMF): $\lambda_{max}$ ($\epsilon_M$) 300 (sh, 92 000), 440 (31 500) nm.

(d) **[Bridge] = *m*-Xylene-$\alpha,\alpha'$-dithiolate (7).** This cluster was generated in $Me_2SO$ solution by the reaction of 0.525 equiv of the disodium salt of *m*-xylene-$\alpha,\alpha'$-dithiol (prepared from the corresponding dibromide by the thiourea method[16]) with 1.0 equiv of **3** in a 5.1 mM stock solution. The cluster was generated in DMF solution in a similar manner. Isolation and recrystallization of the product cluster resulted in increased contamination with the sulfide-bridged cluster **9**. Consequently, all measurements were made on the cluster generated in situ in $Me_2SO$ or DMF solution.

(e) **[Bridge] = Ethane-1,2-dithiolate (8).** This compound was prepared by the method for **4** but with use of a methanolic solution of the disodium salt of ethane-1,2-dithiol. The product was obtained in >90% purity ($^1H$ NMR); the contaminant is compound **9**. Attempted purification by recrystallization increased the amount of **9**. $^1H$ NMR ($Me_2SO$-$d_6$): $\delta$ 12.8 ($CH_2$), 8.10 (5 H), 7.14 (2′ H), 6.74 (3′ H), 4.96 (2 H), 3.84 (6 Me), 3.59 (4 Me), 2.21 (4′ Me).

(d) **[Bridge] = Sulfide (9).** This compound was prepared by the method for **4** but with use of a methanolic solution of $Li_2S$ and was recrystallized in the same way as **4**. Absorption spectrum (DMF): $\lambda_{max}$ ($\epsilon_M$) 300 (sh, 88 000), 447 (34 000) nm.

**$[Fe_4S_4(L\cdot S_3)(SH)]^{2-}$.** This cluster was generated in situ in $CD_3CN$ solution by the reaction of **3** with 1.1 equiv of $(Et_4N)SH$. $^1H$ NMR ($CD_3CN$): $\delta$ 8.16 (5 H), 7.10 (2′ H), 6.82 (3′ H), 5.13 (2 H), 3.82 (6 Me), 3.75 (4 Me), 2.20 (4′ Me). A small quantity of **9** was detected by NMR.

The preceding compounds that were isolated were not analyzed because of the small scale of the preparations. Additionally, compounds **7** and **8** could not be obtained free of small quantities of **9**. However, the identity and full or substantial purity of all compounds was established by $^1H$ NMR spectroscopy; spectra of all bridged clusters except **8** are presented in the text.

**Physical Measurements.** All measurements were made under strictly anaerobic conditions with degassed solvents. $^1H$ NMR spectra were determined on a Bruker AM-500 spectrometer; chemical shifts are reported relative to $Me_4Si$ internal reference. Electrochemical measurements were recorded in DMF solutions by using standard PAR instrumentation, a platinum working electrode, 0.2 M $(n\text{-}Bu_4N)(ClO_4)$ supporting electrolyte, and SCE reference electrodes. Cyclic voltammograms were recorded at 100 mV/s and differential-pulse voltammograms at 5 mV/s with a 25-mV pulse amplitude. Under these experimental conditions, $E_{1/2}$ (ferrocinium/ferrocene) = +0.48 V.

EPR spectra were determined at 6.5–18.5 K with use of a Bruker ESP 300 spectrometer operating at X-band. Samples of reduced double cubanes were prepared by coulometric reduction in DMF solutions con-

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(10) Adman, E. T.; Sieker, L. C.; Jensen, L. H. *J. Biol. Chem.* **1973**, *248*, 3987; **1976**, *251*, 3801.

(11) Palmer, G. In *The Enzymes*: Boyer, P. D., Ed.; Academic Press: New York, 1975; Vol. XII, Part B, Chapter 1.

(12) Sweeney, W. V.; Rabinowitz, J. C. *Annu. Rev. Biochem.* **1980**, *49*, 139.

(13) Prince, R. C.; Adams, M. W. W. *J. Biol. Chem.* **1987**, *262*, 5125.

(14) Testaferri, L.; Tieco, M.; Tingoli, M.; Chianelli, D.; Montanucci, M. *Synthesis* **1983**, 751.

(15) (a) Wagner, A. W. *Chem. Ber.* **1966**, *99*, 375. (b) Morgenstern, J.; Mayer, R. *Z. Chem.* **1968**, *8*, 106.

(16) Speziale, A. J. *Organic Synthesis*; Wiley: New York, 1963; Collect. Vol. IV, p 401.

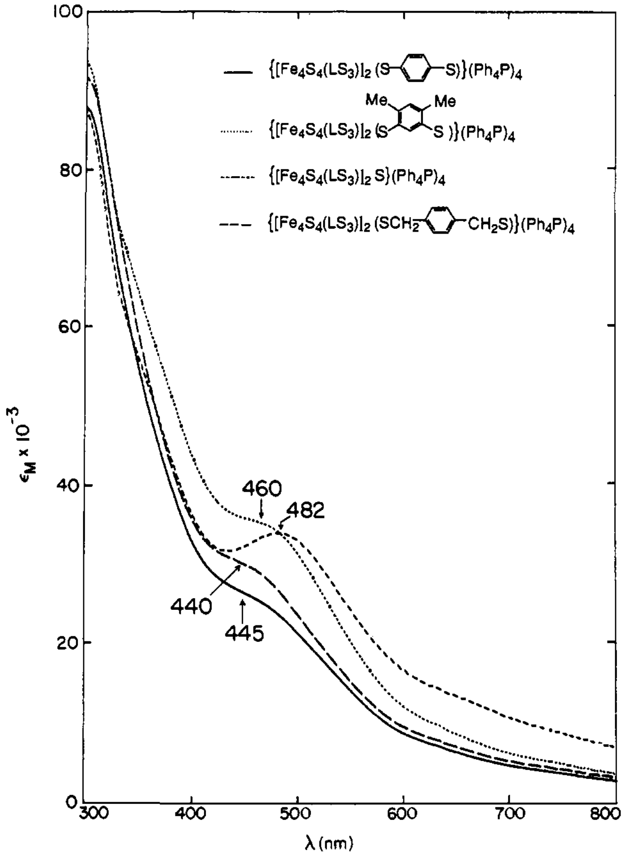

**Figure 1.** UV/visible absorption spectra of bridged double-cubane clusters **4**–**6** and **9** in DMF solutions. Band maxima or shoulders are indicated.

taining the above supporting electrolyte, which had been preelectrolyzed at −1.7 V. Clusters **4**–**6** were used as their $Ph_4P^+$ salts, and clusters **7** and **8** were generated in situ. Typically, a ca. 0.5 mM solution of a double cubane was coulometrically reduced at a Pt gauze electrode at −1.5 V until 2.2 ± 0.1 faraday were passed, assuring complete reduction. After their preparation, solutions were immediately frozen at liquid-nitrogen temperature under a $N_2$/He atmosphere.

## Results and Discussion

### Preparation and Characterization of Double-Cubane Clusters.

These clusters were prepared by reactions 3 and 4, in which the

$$2[Fe_4S_4(L\cdot S_3)Cl]^{2-} \left\{ \begin{array}{ll} + Na_2[SR'S] \rightarrow \underset{\textbf{4-8}}{\{[Fe_4S_4(L\cdot S_3)]_2[SR'S]\}^{4-}} + 2NaCl & (3) \\ + Li_2S \rightarrow \underset{\textbf{9}}{\{[Fe_4S_4(L\cdot S_3)]_2S\}^{4-}} + 2LiCl & (4) \end{array} \right.$$

generalized bridge dianion $[SR'S]^{2-}$ or $S^{2-}$ is inserted between two single cubanes with concomitant displacement of chloride. The six clusters **4**–**9** obtained in this way are listed in Table I. Bridging groups were chosen to vary the distance between subclusters. All clusters were isolated as $Ph_4P^+$ salts except **7**, which was generated in solution. Although isolated compounds were highly microcrystalline, we have thus far been unable to obtain single crystals satisfactory for X-ray diffraction studies. Consequently, establishment of these compounds as bridged double cubanes is based on spectroscopic properties.

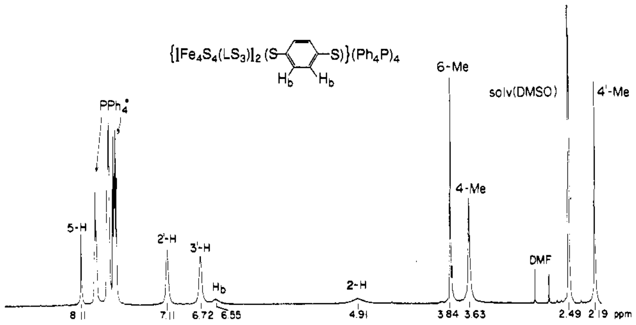

**Figure 2.** ${}^1H$ NMR spectrum of double-cubane cluster **4** in $Me_2SO$-$d_6$ solution at 297 K. Signal assignments are indicated.

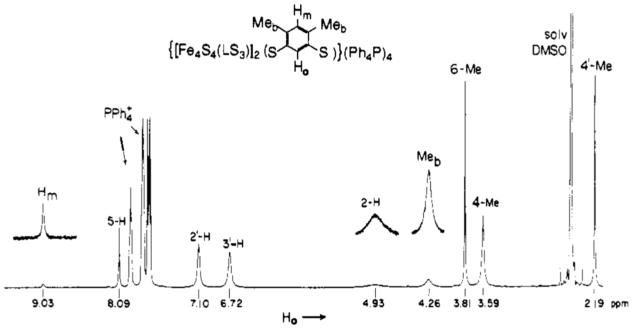

**Figure 3.** ${}^1H$ NMR spectrum of double-cubane cluster **5** in $Me_2SO$-$d_6$ solution at 297 K. Signal assignments are indicated.

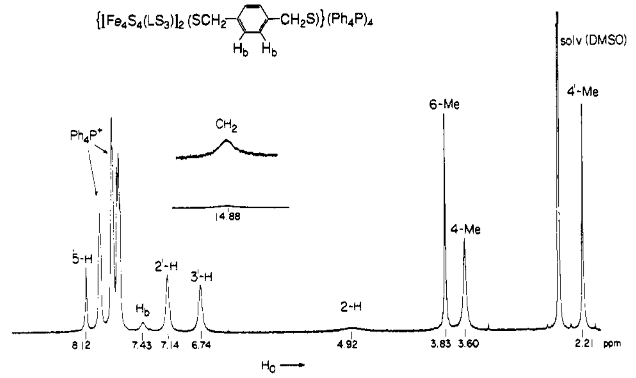

**Figure 4.** ${}^1H$ NMR spectrum of double-cubane cluster **6** in $Me_2SO$-$d_6$ solution at 297 K. Signal assignments are indicated.

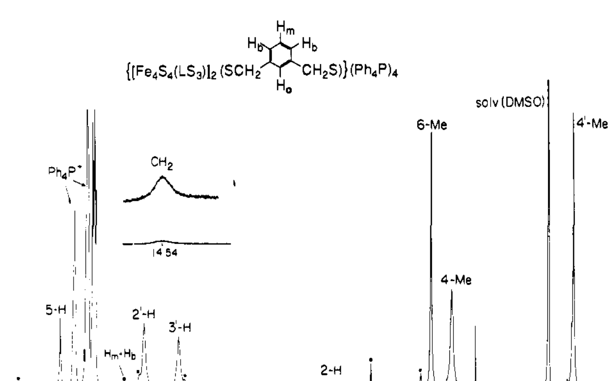

**Figure 5.** ${}^1H$ NMR spectrum of double-cubane cluster **7** in $Me_2SO$-$d_6$ solution at 297 K. Signal assignments are indicated; asterisks denote impurity signals of cluster **9**.

Absorption spectra of four clusters are presented in Figure 1. Features in the 440–460-nm range in DMF solutions are consistent with those of individual clusters $[Fe_4S_4(SR)_4]^{2-}$ ligated mainly or exclusively by arenethiolates[17] and with those of $[Fe_4S_4(L\cdot S_3)L']^{2-}$ ($L' = EtS^-$, $p$-$BrC_6H_4S^-$).[2] In particular, these spectra lack the pronounced shoulder at 490 nm of the precursor chloro cluster **3**.[2] Occurrence of these features as shoulders, rather than as maxima observed for $[Fe_4S_4(SR)_4]^{2-}$, is presumably due to the strong underlying absorption of the ligand, which contains seven thio-bridged benzene rings. Cluster **9** differs from the others in

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(17) DePamphilis, B. V.; Averill, B. A.; Herskovitz, T.; Que, L., Jr.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159.

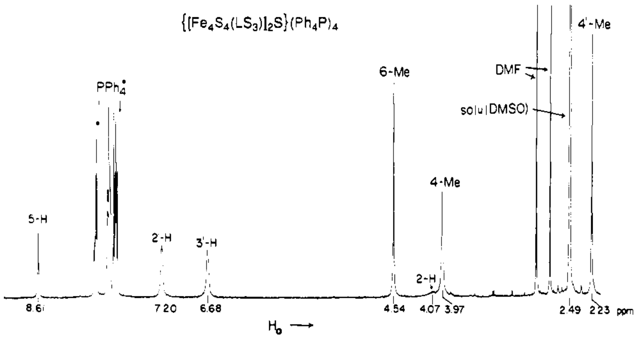
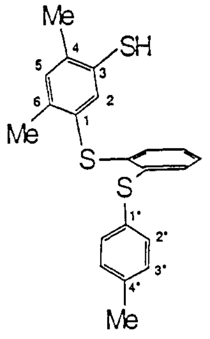


**Figure 6.** $^1$H NMR spectrum of double-cubane cluster **9** in $Me_2SO$-$d_6$ solution at 297 K. Signal assignments are indicated.

having a red-shifted feature, which occurs as a band maximum at 482 nm.

Incisive identification of the clusters as bridged double cubanes follows from the $^1$H NMR spectra shown in Figures 2–6. Signals are isotropically shifted owing to occupation of excited paramagnetic states of the $[Fe_4S_4]^{2+}$ core,[18] which has a singlet ground state. All spectra consist of one set each of trithiolate ligand and bridge resonances, where observable. The former have been assigned following the assignments made previously of the spectra of the single cubanes $[Fe_4S_4(L{\cdot}S_3)L']^{2-}$;[2] the latter are paramagnetically broadened and not all resonances have been detected. The ligand numbering scheme is that in **10**.

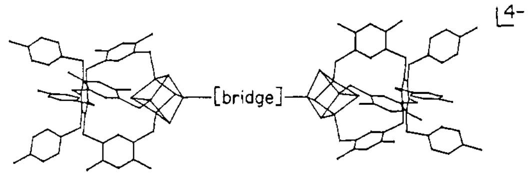


**10**

The following are the most significant NMR features of the set of clusters. (i) Spectra are consistent with magnetically equivalent subclusters in each reaction product and with effective trigonal symmetry of each subcluster. (ii) The chemical shifts of 5 H, which are particularly sensitive to the nature of the ligand at the unique Fe site, occur in the narrow range 8.09–8.12 ppm for **4**–**8**, compared to 8.12 ppm for $[Fe_4S_4(L{\cdot}S_3)(SEt)]^{2-}$ and 8.23 ppm for $[Fe_4S_4(L{\cdot}S_3)Cl]^{2-}$.[2] (iii) One set of signals is observed for each dithiolate in **4**–**6** with isotropic shifts and linewidths larger than those of closely related monothiolates in individual $[Fe_4S_4(SR)_4]^{2-}$ clusters.[18] In the spectrum of **7** (Figure 5), the signals of bridge protons $H_m$ and $H_b$ are broadened and poorly resolved. The signal of $H_o$ was not located, presumably because of paramagnetic broadening. (iv) The isotropic shifts of **9** are substantially larger than those of the other clusters, whose shifts are quite similar, signifying a different type of bridge. From $^1$H NMR spectra, reaction 5 affords a different predominant product

$$[Fe_4S_4(L{\cdot}S_3)Cl]^{2-} + HS^- \rightarrow [Fe_4S_4(L{\cdot}S_3)(SH)]^{2-} + Cl^- \quad (5)$$

than reaction 4, showing that the latter does not produce the hydrosulfide-substituted cluster. Such a cluster could conceivably arise as a result of protic impurities in the reaction mixture, and its existence, in addition to being shown by reaction 5, is given added weight by the isolation and X-ray structural determination of a salt of $[Fe_4S_4(SH)_4]^{2-}$.[19] The collective spectroscopic evidence fully supports the bridged double-cubane structure **11**.



**11**

**Advantages of Site Differentiation.** The earliest indication that bridged double cubanes of the preceding type could be formed was found in the appearance of a broad 5.9 ppm signal from aromatic protons upon the reaction of 2 equiv of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ with 1 equiv of benzene-1,4-dithiol.[20] No resonance near 8 ppm, the expected position for the isotropically shifted $m$-H resonance[18] of a terminal $p$-$HSC_6H_4S^-$ ligand, was found. Numerous attempts to isolate a pure bridged cluster from this reaction were unsuccessful. We have also investigated the system $2[Fe_4S_4(SEt)_4]^{2-}/n\,1,4\text{-}C_6H_4(SH)_2$ in $CD_3CN$ solution. At $n = 1.0$, two signals at 5–6 ppm and at least two methylene signals at 12–13 ppm were observed; at $n = 2.0$, the relative intensities of these signals were reversed and at least one additional signal appeared in the bridge region (5–6 ppm). These observations reveal a mixture of clusters.

The site-differentiated clusters **2** offer substantial advantages in the formation and isolation of pure bridged double cubanes. While thiolate-ligated species such as $[Fe_4S_4(SR)_3Cl]^{2-}$ can be generated in solution,[21] they are always a component of a statistical mixture of products and have never been isolated in pure form. The compound $(Ph_4P)_2[Fe_4S_4(SPh)_2Cl_2]$ has been isolated in pure condition,[22] but the cluster disproportionates in solution. Consequently, a cluster analogous to **3** but with monofunctional ligands only cannot be obtained as a single component in solution. If a bridged double cubane could be obtained by chloride or thiolate substitution of an initial cluster, the possibility of the disproportionation reaction 6 must be recognized. The bridged product

$$2\{[Fe_4S_4(SR)_3]_2(SR'S)\}^{4-} \rightarrow \{[Fe_4S_4(SR)_2]_2(SR'S)_2\}^{4-} + 2[Fe_4S_4(SR)_4]^{2-} \quad (6)$$

may oligomerize by reaction with itself or with the single-cubane product. Such reactions are ones of thiolate substitution between clusters, a reaction type already demonstrated.[23] It is not clear that all components of cluster mixtures could be detected or identified by NMR; proof of only a single component might require determination of molecular weight in solution.[24] Clearly, formation of a bridged double cubane as the only cluster reaction product and its isolation where desired, and repression of disproportionation of this product, are conspicuous attributes of the site-differentiated clusters. We do not insist that bridged double cubanes cannot be prepared or isolated from precursor clusters with identical monodentate ligands. A bridged product could conceivably be trapped by low solubility of an appropriate salt. Further, certain bridges may not readily yield to disproportionation because of their intrinsic stability and/or the inability of the bridge group to stabilize a cluster disproportion product. However, the behavior of the clusters **2** in bridge-forming reactions is both controllable and predictable, and bridge variation beyond the types reported here should be possible using a similar reaction protocol.

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(18) (a) Holm, R. H.; Phillips, W. D.; Averill, B. A.; Mayerle, J. J.; Herskovitz, T. *J. Am. Chem. Soc.* **1974**, *96*, 2109. (b) Reynolds, J. G.; Laskowski, E. J.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5315.

(19) Müller, A.; Schladerbeck, N. H.; Bögge, H. *J. Chem. Soc., Chem. Commun.* **1987**, 35.

(20) Bobrik, M. A.; Que, L., Jr.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 285.

(21) Johnson, R. W.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5338.

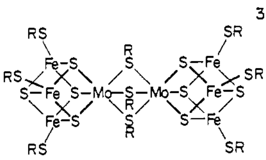
(22) Kanatzidis, M. G.; Baenziger, N. C.; Coucouvanis, D.; Simopoulos, A.; Kostikas, A. *J. Am. Chem. Soc.* **1984**, *106*, 4500.

(23) Que, L., Jr.; Bobrik, M. A.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4168.

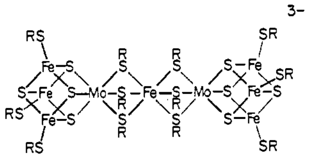
(24) A related problem has recently been encountered with a large, nonrigid dithiol ligand, which could bind to a single $Fe_4S_4$ cluster or form oligomers by binding to different clusters. A molecular weight determination by light scattering supported the former arrangement; cf.: Kuroda, Y.; Sasaki, Y.; Shiroiwa, Y.; Tabushi, I. *J. Am. Chem. Soc.* **1988**, *110*, 4049.

Isolated samples of compounds **4**–**6** are free of cluster impurities by a $^1$H NMR criterion (Figures 2–4). Cluster **7** when generated (Figure 5) and cluster **8** when isolated contain about 10% or somewhat less of sulfide-bridged cluster **9** as an impurity. Use of disodium *o*-xylenedithiolate in reaction 3 leads to about 50% each of cluster **9** and the bridged double cubane. Because of the high level of **9** as an impurity, no attempt to isolate the latter cluster was made. It is apparent that those dithiolates that form stable Fe(II, III) chelate complexes without[25–28] or with sulfide[29,30] are those that afford substantial impurity levels of the sulfide-bridged double cubane. We infer that these ligands partially degrade the $[Fe_4S_4]^{2+}$ core of **3**, freeing sulfide, which is incorporated into double cubane **9** by reaction 4. A single, unsupported sulfide bridge between Fe atoms has been otherwise demonstrated only in complexes of Fe(III) with tetradentate Schiff bases[31] and related ligands.[32]

**Electron Transfer.** In assessing the properties of proteins containing two $Fe_4S_4$ clusters, an implicit point at issue has been the (minimum) separation of clusters required to effect nonstatistical electron transfer at effective parity of cluster environment. With clusters, this matter has been previously illustrated only by the behavior of the double cubanes **12**[33,34] and **13**,[33a] whose subclusters are separated by rigid bridges, which afford the indicated Mo–Mo distances in the fully oxidized forms. Potential differences for successive subcluster reduction in **12** are 210 ± 20 mV whereas in **13** the difference in peak potentials (cyclic voltammetry) could not be resolved, indicating that $\Delta E_p < 50$ mV. In the initial detailed study of the redox behavior of a two-cluster protein, Eisenstein and Wang[35] reported a 31-mV difference in potentials for *Clostridium pasteurianum* ferredoxin and recognized that this was essentially the statistical value. All other investigations of this and similar proteins have detected one two-electron reduction step near −400 mV vs NHE.[36–45] One of these was an NMR method capable of monitoring individual clusters; for two clostridial ferredoxins it was concluded that potential differences could not exceed 10 ± 5 mV.[36] Further, it has been found that the chemical oxidation of doubly reduced ferredoxins takes place in



**12** (3.6 Å)



**13** (6.6 Å)

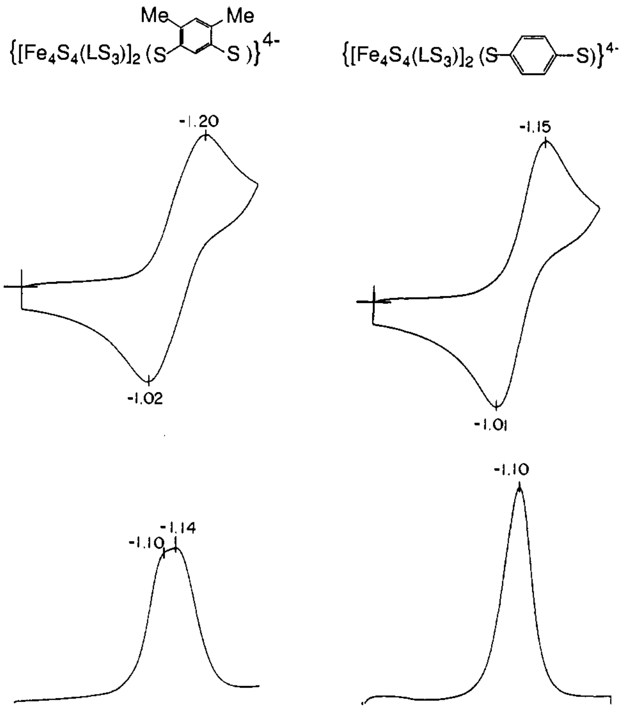


Figure 7. Cyclic and differential-pulse voltammograms of double-cubane clusters **4** and **5** in DMF solutions at a stationary Pt electrode; peak potentials vs SCE are indicated.

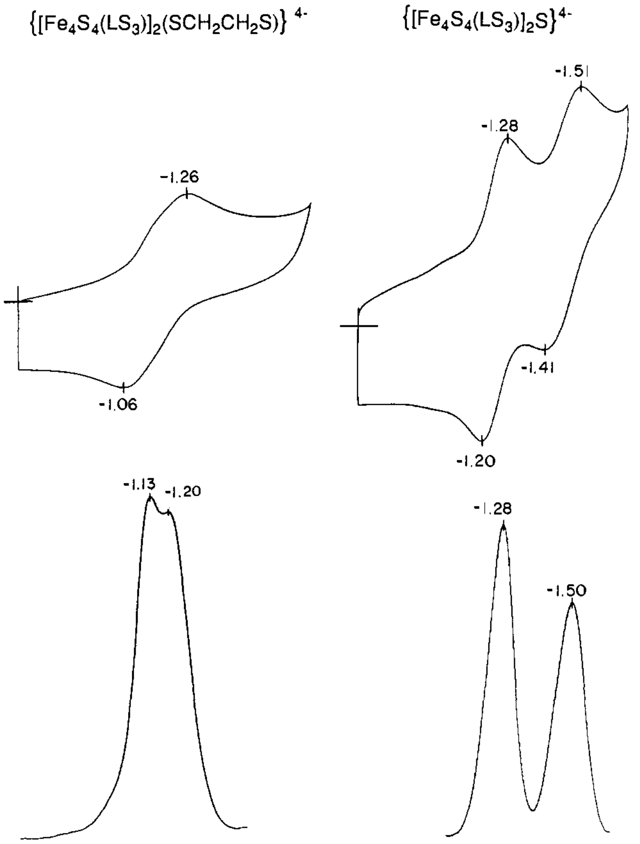


Figure 8. Cyclic and differential-pulse voltammograms of double-cubane clusters **8** and **9** in DMF solutions under the conditions of Figure 7.

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(25) Herskovitz, T.; DePamphilis, B. V.; Gillum, W. O.; Holm, R. H. *Inorg. Chem.* **1975**, *14*, 1426.

(26) Lane, R. W.; Ibers, J. A.; Frankel, R. B.; Papaefthymiou, G. C.; Holm, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 84.

(27) Henkel, G.; Tremel, W.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 319.

(28) Hagen, K. S.; Holm, R. H. *Inorg. Chem.* **1984**, *23*, 418.

(29) Henkel, G.; Tremel, W.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 1033.

(30) Hagen, K. S.; Christou, G.; Holm, R. H. *Inorg. Chem.* **1983**, *22*, 309.

(31) (a) Dorfman, J. R.; Girerd, J.-J.; Simhon, E. D.; Stack, T. D. P.; Holm, R. H. *Inorg. Chem.* **1984**, *23*, 4407. (b) Mukherjee, R. N.; Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 1850.

(32) Corazza, F.; Floriani, C.; Zehnder, M. *J. Chem. Soc., Dalton Trans.* **1987**, 709.

(33) (a) Wolff, T. E.; Power, P. P.; Frankel, R. B.; Holm, R. H. *J. Am. Chem. Soc.* **1980**, *102*, 4694. (b) Christou, G.; Mascharak, P. K.; Armstrong, W. H.; Papaefthymiou, G. C.; Frankel, R. B.; Holm, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2820.

(34) Christou, G.; Garner, C. D.; Miller, R. M.; Johnson, C. E.; Rush, J. D. *J. Chem. Soc., Dalton Trans.* **1980**, 2363.

(35) Eisenstein, K. K.; Wang, J. H. *J. Biol. Chem.* **1969**, *244*, 1720.

(36) Packer, E. L.; Sternlicht, H.; Lode, E. T.; Rabinowitz, J. C. *J. Biol. Chem.* **1975**, *250*, 2062.

(37) Stombaugh, N. A.; Sundquist, J. E.; Burris, R. H.; Orme-Johnson, W. H. *Biochemistry* **1976**, *15*, 2633.

(38) Lode, E. T.; Murray, C. L.; Rabinowitz, J. C. *J. Biol. Chem.* **1976**, *251*, 1683.

(39) Hill, C. L.; Renaud, J.; Holm, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 2549.

(40) Sweeney, W. V.; McIntosh, B. A. *J. Biol. Chem.* **1979**, *254*, 4499.

(41) Carter, K. R.; Rawlings, J.; Orme-Johnson, W. R.; Becker, R. R.; Evans, H. J. *J. Biol. Chem.* **1980**, *255*, 4213.

(42) Elliott, J. I.; Ljungdahl, L. G. *J. Bacteriol.* **1982**, *151*, 328.

(43) Armstrong, F. A.; Hill, H. A. O.; Walton, N. J. *FEBS Lett.* **1982**, *145*, 241.

(44) Harmer, M. A.; Hill, H. A. O. *J. Electroanal. Chem., Interfacial Electrochem.* **1984**, *170*, 369.

(45) Feinberg, B. A.; Tuschel, D. D.; Ryan, M. D. *Bioelectrochem. Bioenerg.* **1984**, *12*, 575.

a single kinetic step.[46] The evidence is conclusive that the potentials of proteins bearing two clusters that have been investigated thus far are not resolvably different or do not exceed the statistical difference of 36 mV at 298 K given by eq 7,[47] which applies to

$$E_1 - E_2 = (2RT/F) \ln 2 \qquad (7)$$

a molecule with two identical and independent redox centers. The issue of coupling in electron transfer has been addressed in this work with use of the double cubanes **11**.

Double-cubane clusters are expected to exhibit the minimal electron-transfer series 8, where the different oxidation levels are

$$[4-] \rightleftharpoons [5-] \rightleftharpoons [6-] \qquad (8)$$

$$[4-] + [6-] \rightleftharpoons 2[5-] \qquad K_{\text{com}} = 4 \qquad (9)$$

represented by net charges of the clusters. The comproportionation constant for statistical behavior is given in eq 9. Representative cyclic and differential-pulse (DP) voltammograms are set out in Figures 7 and 8. Potential data and distances related to cluster separation are collected in Table I. Because of the large number of possible structures of bridged double cubanes, certain assumptions have been made in describing cluster separations. (i) Three viable measures of these separations are bridge S···S, Fe···Fe involving atoms bound to bridge S, and centroid to centroid of the two cubanes. Dimensions used to calculate these separations are given in the table. All Fe–S/S* parameters (S* is a core atom) are taken from single-cube structures.[2,23,48] Of these, the least certain is the S*–Fe–S angle, which is normally quite variable; the assumed value of 115° is the mean S*–Fe–Cl angle in **3**. The bridge angle in **9** (145°) assumes a stronger interaction between subclusters than between neutral half-dimers in complexes with relatively unconstrained Fe(III)–S–Fe(III) bridge angles (121–122°).[31,32] (ii) The transoid arrangement, illustrated for several cases in Figure 9, is considered to be the most likely conformer inasmuch as it minimizes steric and coulombic repulsions between subclusters. Note that the considerable spatial requirements of ligand **1** in the double cubanes **11** will promote the relative stability of the transoid conformer. In this conformation, the dihedral angles S*–Fe–S–C (**4–8**), Fe–S–C–C (**6–8**), and S*–Fe–S–Fe (**9**), involving bonded atoms, are 180°, and the dihedral angles between *all* planes formed by these atom sets are zero except for the Fe–S–C–C planes in **5** and **7**, which are at 120°. The cisoid conformer is recovered from the transoid by rotation by 180° about a C–S (**4**, **5**) or a C–C (**6–8**) bridge bond. Because **9** lacks these bonds, it exists as one conformer under this description, which assumes free rotation about all Fe–S bridge bonds.

As a comparison with the electrochemical behavior of double cubanes, the single cube **3** exhibits in cyclic voltammetry $E_{1/2} = -1.03$ V, $\Delta E_p = 100$ mV, and $i_{pc}/i_{pa} = 1$ (100 mV/s). The half-wave potential is only slightly dependent on scan rate. This redox step is chemically reversible but electrochemically quasi-reversible, properties that apply to the electron-transfer reactions of double cubanes. The reduced clusters contain the $[Fe_4S_4]^{1+}$ core, which has been well characterized in a number of isolated clusters.[49]

All double cubanes show one or two reduction steps in the −1.0- to −1.6-V range in DMF solutions. Potentials of **4–8** are nearly constant at −1.1 to −1.2 V, as would be expected inasmuch as only one of the four ligands of each subcluster is varied. Coulometric reduction at −1.3 to −1.4 V yielded $n$ = 2.0–2.1 e/double cubane. Oxidation of the reduced solutions gave $n$ = 1.7–1.8 e/double cubane, a slightly low value presumably due to some nonelectrochemical reoxidation over the ca. 1 h required for the redox cycle. Cyclic voltammetry of the reduced and reoxidized solutions revealed no features other than those of the double cubanes and diffusion currents within a few percent. In the case of **4**, DP voltammetric peak heights before and after reoxidation were within 1%. These observations are consistent with chemically reversible redox reactions. Although the cyclic voltammetry of **9** was well-behaved, coulometry was unsatisfactory, owing to decomposition of the reduced cluster.

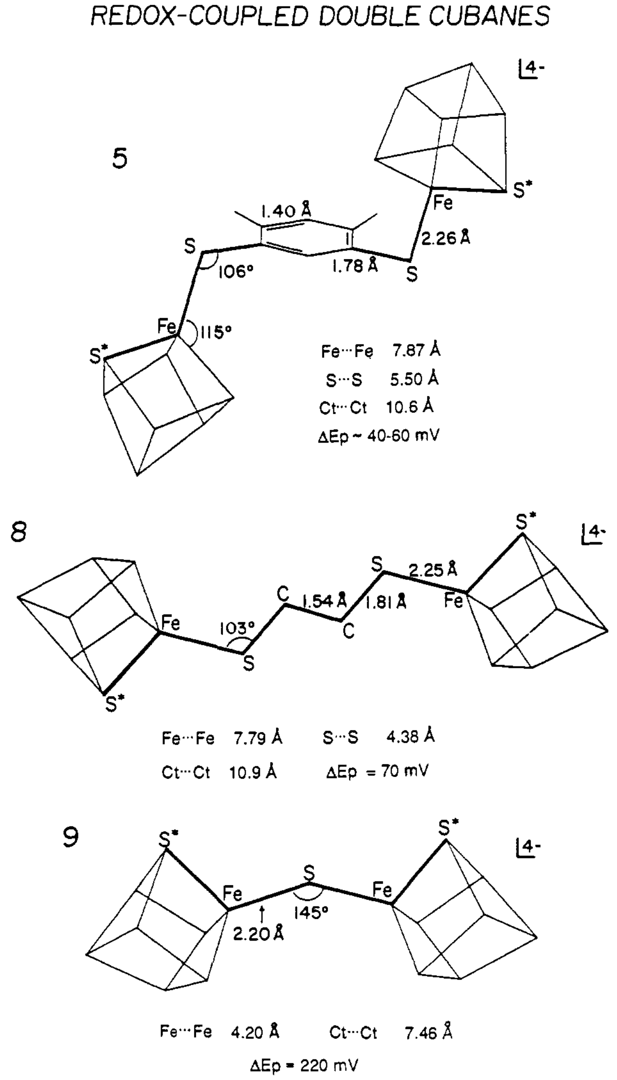

**Figure 9.** Illustration of the transoid conformers of **5** and **8** and the conformer of **9**. Atoms connected by heavy lines lie in the same plane (**8**, **9**), and in **5**, planes of these atoms form a dihedral angle of 120°. Calculated measures of cluster separations and certain parameters used in these calculations are indicated.

Of the six clusters examined, three (**5**, **8**, **9**) exhibited two detectable reduction steps. The cyclic and DP voltammograms of **5** (Figure 7) are those of two barely resolved steps of slightly different potentials.[50] The indicated peak potentials and their separation cannot be accurately determined from the DP voltammogram; the upper limit of 60 mV for $\Delta E_p$ is consistent with the working curves of Richardson and Taube.[50] In the case of **8** (Figure 8), two resolved peaks separated by 70 mV are found in the DP voltammogram. These features are not resolved in the cyclic voltammogram. The sulfido-bridged dimer **9** (Figure 8) displays two fully resolved redox steps separated by 220 mV. Clusters **4** (Figure 7), **6**, and **7** show single two-electron redox steps in cyclic and DP voltammetry.

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(46) (a) Armstrong, F. A.; Henderson, R. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1980**, *102*, 6545. (b) Armstrong, F. A.; Henderson, R. A.; Kim, H. O. W.; Sykes, A. G. *Biochim. Biophys. Acta* **1982**, *681*, 161.

(47) Flanagan, J. B.; Margel, S.; Bard, A. J.; Anson, F. C. *J. Am. Chem. Soc.* **1978**, *100*, 4248.

(48) Averill, B. A.; Herskovitz, T.; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 3523.

(49) Carney, M. J.; Papaefthymiou, G. C.; Whitener, M. A.; Spartalian, K.; Frankel, R. B.; Holm, R. H. *Inorg. Chem.* **1988**, *27*, 346, and references therein.

(50) Richardson, D. E.; Taube, H. *Inorg. Chem.* **1981**, *20*, 1278.

The order of coupling in electron transfer as measured by potential separations is **9** ≫ **8** ≳ **5** > **4**, **6**, **7**. The transoid arrangement is assumed and is depicted in Figure 9 for the three coupled cases. The largest coupling appears in sulfido-bridged cluster **9**, which has the smallest subcluster separation by each of the three measures (Table I). The relatively close positioning of the subclusters is the probable cause of the negative shift of potentials. The interactions in **8** and **5**, which are comparable and at least 150 mV smaller than in **9**, occur over S···S, Fe···Fe, and Ct···Ct distances not exceeding about 5.5, 8, and 11 Å, respectively. At one or more distances longer than those, no potential differences were detectable.

For the purpose of assessing purely distance effects, the set of double cubanes does not have the optimal property of variable separation effected by rigid spacers with repeating units. While electronic coupling through the conjugated bridges of **4** and **5** cannot be discounted, the effect should be smaller in **8**, which has a saturated bridge. In fact, the coupling in this cluster is not less and its subclusters are no closer than those in **5**. With **9**, conjugation of subclusters through the sulfide bridge and their nearness account for the large difference in potentials, which corresponds to an interaction energy of 5.1 kcal/mol. A pertinent observation is afforded by the benzene-1,4-dithiolate-bridged cluster **4**. Here, subcluster separation is very close indeed to that of *P. aerogenes* ferredoxin (Table I). As the protein, this double cubane shows no detectable coupling of its electron-transfer reactions, suggesting that at the distances involved, the conjugated bridge offers no advantage over protein structure and the associated water molecules intervening between the native subclusters. We conclude that dipolar effects are dominant in the electron-transfer coupling of the double-cubane set and suggest that, in two-cluster ferredoxins, statistical or near-statistical differences in potentials implies a subcluster separation comparable to or larger than that in **5**. Near equality of protein potentials could be removed by different subcluster environments and/or closer subcluster juxtaposition. The first factor is absent in *P. aerogenes* ferredoxin,[10,51] which has a pseudo $C_2$ symmetry axis and marked sequence homology in the halves of the molecule. Similar extents of sequence repetition are observed in other ferredoxins containing two $Fe_4S_4$ clusters.[12] Indeed, no protein containing two such clusters is reported to have measurably different potentials. It is likely that any $\{[Fe_4S_4]_2(\mu_2\text{-}S)\}^{2+}$ protein unit would be recognizable in part by a potential separation comparable to that of **9**. Last, the foregoing considerations are offered, as emphasized, under the assumption of the transoid double-cubane conformation. The cisoid arrangement, and thus others that are intermediate between this and the transoid form, still leads to the conclusion that on a distance basis stronger coupling will be found in **4**, **5**, and **8** than in **6** and **7**.[52]

**EPR Spectra.** The spectra of double cubanes **4–8** were examined at 6.5–18.5 K in DMF solutions. Spectra were obtained on samples that had been reduced by 1 and 2 e/double cubane. The former contained mainly half-reduced clusters and gave essentially identical rhombic spectra with apparent *g* values of 2.06, 1.94, and 1.90. These spectra closely resemble those of reduced ferredoxins containing one $S = {}^1/_2$ $Fe_4S_4$ cluster[53] and differ from other analogue clusters[54] in that the latter generally show axial spectra but with average *g* values very similar to half-reduced **4–8**. Spectra of fully reduced clusters are dependent on the identity of the bridge. For example, those of **6** and **7** are poorly resolved in the $g = 2$ region, apparently because of additional resonances that occur at $g = 2.06–1.90$. On the other hand, the spectrum of **4** retains the overall rhombic spectrum of the half-reduced clusters but exhibits an additional weak signal at $g = 2.13$, not unlike a similar feature observed in the spectrum of fully reduced *C. pasteurianum* ferredoxin.[13] Overall, the spectra are roughly similar to, but not as well resolved as, those of fully reduced two-cluster proteins.[11–13,40–42,55] As in those cases they suggest very weak interactions between two paramagnetic centers[11,55] and provide additional evidence for the double-cubane structure **11**. Such spectra as yet are not readily interpretable in terms of subcluster separations.

**Acknowledgment.** This research was supported by NIH Grant GM 28856. We thank J. Sims and S. Gardner of MIT for assistance in obtaining the EPR spectra.

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[51] Carter, C. W., Jr. In *Iron-Sulfur Proteins*; Lovenberg, W., Ed.; Academic Press: New York, 1977; Chapter 6.

[52] In the cisoid conformation double cubanes **4**, **5**, and **8** have the following distances (Å): S···S, 6.35, 5.50, 2.75; Fe···Fe, 7.54, 6.62, 7.23; Ct···Ct, 8.44, 7.46, 10.6. In double cubanes **7** and **8**, there are certain conformations obtained by combinations of rotations about bridge C–C *and* C–S bonds that lead to Fe···Fe and Ct···Ct separations smaller than those listed here or in Table I. These are considered less probable, owing to increased steric and coulombic repulsion between subclusters.

[53] (a) Stombaugh, N. A.; Burris, R. H.; Orme-Johnson, W. H. *J. Biol. Chem.* **1973**, *248*, 7951. (b) Mullinger, R. N.; Cammack, R.; Rao, K. K.; Hall, D. O.; Dickson, D. P. E.; Johnson, C. E.; Rush, J. D.; Simopoulos, A. *Biochem. J.* **1975**, *151*, 75.

[54] Laskowski, E. J.; Frankel, R. B.; Gillum, W. O.; Papaefthymiou, G. C.; Renaud, J.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 1978.

[55] Mathews, R.; Charlton, S.; Sands, R. H.; Palmer, G. *J. Biol. Chem.* **1974**, *248*, 4326.