determining, leading to an intermediate having a stretched C_1–C_2 bond, *e.g.*, **15**, which may retain enough interaction between the orbitals at C_1 and C_2 to allow the stereospecificity observed with **12–14**, yet able to discriminate between C_4 and C_5 migration. This suggestion of a potential well on the energy surface is in accord with that made by Benson[12] and not with that by Salem[3c] for the possibly structurally related cyclopropane energy surface.

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Hoffmann and Salem), or an ethoxyoxaspiroheptene *via* a carbonyl-cyclopropane rearrangement were considered and discarded as major contributors in view of all the stereochemical data.

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(13) Fellow of the Alfred P. Sloan Foundation, 1971–1973.

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Synthesis of the Central Iron Core of Rubredoxin

Sir:

Rubredoxin from *Micrococcus aerogenes*, an anaerobic bacterium, is a linear polypeptide of 53 residues and contains an iron that is coordinated to the cysteine residues at positions 6, 9, 38, and 41 in the molecule (Figure 1).[1] The chelate structure of this specific protein has been studied by various physical techniques,[2,3] but considerably more work exists on the rubredoxin from *Clostridium pasteurianum*,[4–8] which includes the preparation of several simple inorganic models.[9–11] A detailed X-ray study of this last rubredoxin showed recently that the metal was in a strained tetrahedral configuration and was located at one side of the molecule.[12] Furthermore, the iron connected two separate, secondary hairpin turns of the main chain that involves the regions between residues 5–10 and 37–42. Most importantly, the large, middle peptide section consisting of residues 11–36 was not in bonding contact with the metalloorganic region. Such information suggested that the synthesis of a small, model peptide area might produce an "active site" or, at least, give evidence as to the stability of the existing central core of rubredoxin.

We now wish to report the partial realization of this idea, in terms of the rubredoxin from *M. aerogenes*, by two related chemical approaches. In the first, the protected pentapeptide R_{6-10}, methyl N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucinyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (I),[13] on reaction with hydrazine gave the corresponding hydrazide II.[14] This compound was joined by the organic azide procedure[15] to the pentapeptide R_{38-42}, methyl *S*-*p*-methoxybenzyl-L-cysteinyl-L-prolyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (III), to yield the decapeptide, methyl N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-prolyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (IV). Alternatively, the same compound was prepared by the mild base hydrolysis of I to the pentapeptide acid (V), followed by a mixed carbonic anhydride[16] coupling with the amine III. The addition of sodium to a liquid ammonia solution of IV cleaved the *S*-*p*-methoxybenzyl protecting groups[17] and generated the partially deblocked peptide, methyl N^{α}-*tert*-butyloxycarbonyl-L-cysteinyl-L-threonyl-L-leucyl-L-cysteinylglycyl-L-cysteinyl-L-prolyl-L-leucyl-L-cysteinylglycinate (VI). If the decapeptide IV was hydrolyzed to the acid VII, then the remaining blocking groups could be removed by warming with trifluoroacetic acid–anisole to afford the salt, L-cysteinyl-L-threonyl-L-leucyl-L-cysteinylglycyl-L-cysteinyl-L-prolyl-L-leucyl-L-cysteinylglycinate trifluoroacetate (VIII). Iodometric titration of both VI and VIII showed the presence of 3.50 and 3.60 free cysteinyl groups, respectively.[18]

Both VI and VIII (0.1 mmol) were suspended separately in water (20 ml), mercaptoethanol (20 mmol) was added, and the pH was adjusted to 10 by addition of triethylamine. The clear liquid was deaerated with nitrogen, and an aqueous solution of ferrous ammonium sufate (0.5 mmol) was introduced, after which the reaction was cooled to 0°. The admission of air into the flask produced an immediate dark red-brown coloration. The failure to see any precipitate under these conditions means that the ferrous ion has been incorporated into a chelate structure and subsequently is oxidized to the ferric state. Each solution was passed through a Sephadex LH-20 column, previously equilibrated to a pH value of 9.5 (μ = 0.06), to yield one brown and two pale yellow fractions. The former possessed a continuous band spectrum, which was different in shape from those produced by mixing the various reactants in any two combinations.[19] It should be noted that a match to the exact spectrum of rubredoxin would not be expected (Figure 2), as no aromatic residues are present in compounds VI and VIII. Lyophilization of either product produced a pale-green

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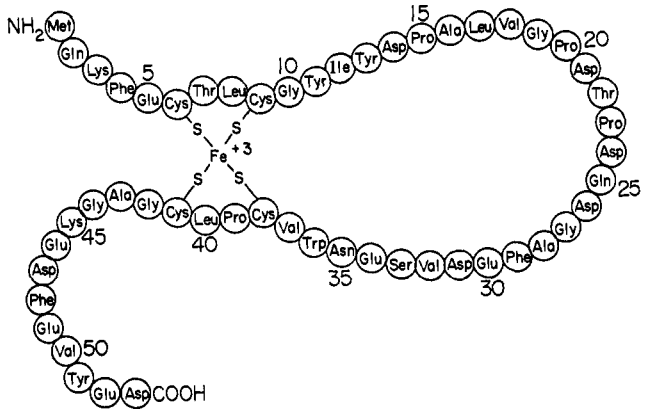

Figure 1. The amino acid sequence of the rubredoxin from *Micrococcus aerogenes*.

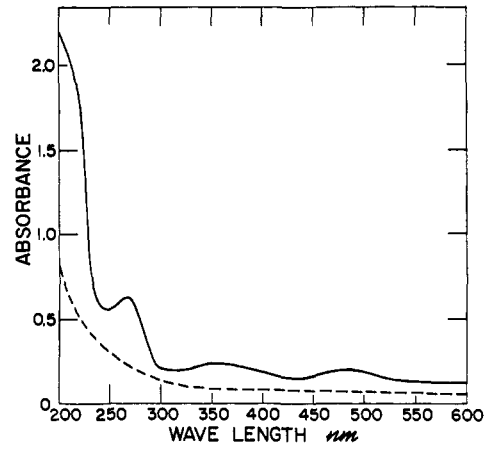

Figure 2. Absorption spectrum of native rubredoxin (—) and the iron complex (– – –) in water.

powder, which was partially insoluble in water and contained starting peptide and iron salts. The failure to obtain a stable, solid adduct implies the existence of an incorrect geometry for these two synthetic substrates. A biological assay is unavailable here, but the more complex rubredoxin from an aerobic bacterium, *Pseudomonas oleovarans*, functions in a ω-hydroxylation scheme.[20]

The conclusions reached are as follows: it is possible to form a complex between the ferric ion and the cysteines of the two peptides, whose visible colors are similar to that of rubredoxin, and, secondly, electron-transfer models are possible using existing, simple structural features found in native proteins.[21,22] We plan to extend this work to the synthesis of such peptides as Cys-x-x-Cys-Gly-Gly-Gly-Gly-Cys-x-x-Cys, since this unit represents an optimum distance between the cysteinyl residues, as measured on an actual molecular model.

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Additions and Corrections

Bonding and Valence Electron Distributions in Molecules. An X-Ray and Neutron Diffraction Study of the Crystal and Molecular Structure of Tetracyanoethylene Oxide [*J. Amer. Chem. Soc.*, **93**, 5945 (1971)]. By D. A. MATTHEWS,* J. SWANSON, M. H. MUELLER, and G. D. STUCKY, University of Illinois, School of Chemical Sciences, Urbana, Illinois 61801, and Argonne National Laboratory, Argonne, Illinois 60439.

On page 5946, column 2, the sentence beginning on the 13th line of the second paragraph under the heading **(a) X-Ray** should read: The polarization correction used for the monochromatic radiation was $(\cos^2 2\theta + \cos^2 2\theta_m)/(1 + \cos^2 2\theta_m)$, where θ_m is the Bragg angle for the monochromator crystal.

A Four-Parameter Equation for Predicting Enthalpies of Adduct Formation [*J. Amer. Chem. Soc.*, **93**, 6014 (1971)]. By RUSSELL S. DRAGO,* GLENN C. VOGEL, and TERENCE E. NEEDHAM, William A. Noyes Laboratory, University of Illinois, Urbana, Illinois 61801.

On page 6015, eq 2 should read: $\Delta = (A'PA)^{-1}A'PF$. On page 6026, the first display equation in the left-hand column should read: $-\Delta H = H_A H_B + k[(1/H_A)(1/H_B)]$.

^{31}P–^{11}B Constant as a Qualitative Measure of Dative Bond Strength [*J. Amer. Chem. Soc.*, **93**, 6821 (1971)]. By R. W. RUDOLPH* and C. W. SCHULTZ, Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48104.

The first sentence on page 6822 should read: Therefore, sign inversion does not occur.

Singlet–Triplet Resonance Interaction in the A_2 States of Formaldehyde [*J. Amer. Chem. Soc.*, **93**, 7098 (1971)]. By C. G. STEVENS, A. M. GARCIA, and J. C. D. BRAND,* Photochemistry Unit, University of Western Ontario, London, Ontario, Canada.