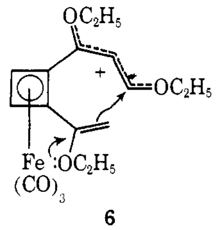

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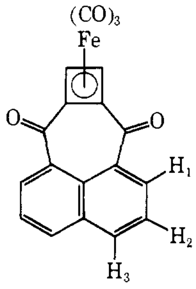

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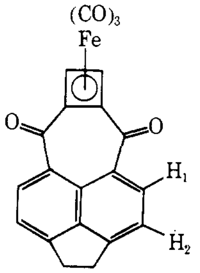

8

(golden plates, dec pt 230–240°) in 75 and 42%[12] yields, respectively. The pmr spectra[4] ($CDCl_3$) were particularly valuable in establishing that cyclization had taken place across peri positions. Thus the cyclobutadiene protons in each appeared as a singlet (**7**: δ 5.18; **8**: δ 5.16] while the naphthalenic protons appeared as incipient AMX [δ_{H_1} 8.89, δ_{H_2} 7.64; δ_{H_3} 8.17; J_{12} = 7.7 Hz, J_{13} = 1.9 Hz, J_{23} = 7.6 Hz] and AX [δ_{H_1} 8.90, δ_{H_2} 7.48 (somewhat broadened, W_h = 2.0 Hz); J_{12} = 7.5 Hz] patterns in **7** and **8**, respectively.[13] Chemical shifts were assigned on the basis that the lowest field absorption in each compound corresponds to H_1, which is situated for effective deshielding by a carbonyl group.[14] The ethano protons in **8** absorbed as a broadened singlet at δ 3.47 (W_h = 2.0 Hz). In addition the infrared spectrum ($CHCl_3$) of each diketone [**7**: 1508, 1563, 1601, 1637, 2006, and 2066 cm^{-1}; **8**: 1505, 1566, 1587, 1600, 1626, 2002, and 2064 cm^{-1}] was consonant with the indicated functionality.[15]

The foregoing results are especially useful in defining some of the limitations of approach to fused-ring cyclobutadienes from 1,2-disubstituted complexes. In the case of diketone **1a**, the stereochemical disposition of substituents is undoubtedly a crucial factor in the failure to cyclize. X-Ray crystallographic studies of tetramethylcyclobutadienenickel dichloride dimer[17] and tetraphenylcyclobutadieneiron tricarbonyl[18] have shown that external ring-substituent angles are close to 135°. If this structural feature be maintained in **1a**, ring closure would be inhibited because of the large distance to be overcome between interacting centers of substitutents and because of angle strain generated during product formation. Although alternate experimental conditions for effecting the closure have not been exhausted, this particular approach would appear to hold little promise for the synthesis of cyclobutadienocyclopentadienyl derivatives without serious modification.

On the other hand facile fusion of a cycloheptatrienyl ring to a complexed cyclobutadiene suggests the efficacy of the general approach and presents the possibility that substituent geometry may facilitate fusion of medium rings. We are currently testing the latter suggestion as a route to larger cyclobutadienopolyenes and derivatives.

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[12] This is a minimum yield after one recrystallization. The total yield is significantly higher, but an unidentified side product has prevented complete separation.

[13] A singlet at δ 8.57 in the spectrum of **7** suggested the presence of a very minor and as yet unidentified side product.

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[15] Diketones **7** and **8** are of special interest in being potential precursors of cyclobutadiene analogs of pleiadene,[16a] acepleiadene,[16b] and acepleiadylene.[16c] Hopefully, the chemistry of the cyclobutadienoid counterparts can be developed for comparison with that of the benzo forms.

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The Possible Determination of Iron Coordination in Nonheme Iron Proteins Using Laser-Raman Spectroscopy. Rubredoxin

Sir:

The isolation[1] from *Clostridium pasteurianum* of ferredoxin, the initial member of the class of nonheme iron proteins that participate in electron-transfer reactions, has generated substantial interest in the structure and biochemistry of proteins of this type.[2–5] Of particular importance is the nature of iron coordination in these proteins, since this may be a primary determinant of their apparently unique metabolic and photosynthetic roles.[6,7] Briefly summarized, it is generally thought that sulfhydryl groups from cysteinyl residues and inorganic sulfides[8] (with the exception of rubredoxin)[9] constitute at least part of the iron environment.[6,7] Electron paramagnetic resonance[10–13] techniques have been applied to the problem, but so far with little success. Circular dichroism studies did not

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at first appear fruitful,[14a] but a more recent careful investigation of reduced rubredoxin by Lovenberg and Eaton[14b] has been interpreted as indicating tetrahedral coordination about the iron. X-Ray crystallographic examinations of several nonheme iron proteins have been reported,[15-17] but only in the case of rubredoxin from *Clostridium pasteurianum*[8, 18, 19] has the resolution been sufficient to define the environment of the iron. Herriott, Sieker, and Jensen[19] report that the electron density map at 2.5-Å resolution clearly shows that the iron atom is coordinated to four sulfur atoms that occupy tetrahedral vertices. We now present evidence to indicate that laser-Raman spectroscopy may constitute a powerful method for studying this problem.

While investigating the Laser-Raman spectra of some Fe(II) and Fe(III) complexes, such as bis(*o*-phenanthroline)iron(II) sulfate,[20] $(C_2H_5)_4NFeCl_xBr_{4-x}$ (x = 0–4),[21] and aqueous solutions of $FeCl_3$,[22] we became convinced that the spectra associated with the iron-containing moiety were particularly intense, presumably as a consequence of a resonance or preresonance effect. Illustrative of this phenomenon, the Raman spectrum of solid $(C_2H_5)_4NFeBr_4$ excited by the 632.8-nm emission of a helium–neon laser contains only vibrational lines of the chromophoric $FeBr_4^-$ anion, while those of the tetraethylammonium ion are presumably of insufficient intensity to be observed above self-absorption effects. This raised the intriguing possibility that Raman measurements of iron-containing metalloproteins and metalloenzymes might yield only the spectra of the iron chromophores and their associated polyhedra under He–Ne excitation.

To test these suppositions, rubredoxin seemed to be an especially convenient choice. First, the laser-Raman results can be compared with those anticipated on the basis of the X-ray structural determination.[19] Second, the observation of a resonance or preresonance Raman effect is dependent on excitation within an electronic absorption band of the material.[23] The circular dichroism spectrum of rubredoxin from *Peptostreptococcus elsdenii*[14] exhibits a distinct negative Cotton effect centered at 632 nm, which is virtually coincident with the laser wavelength. Although the laser-Raman spectrum was obtained from a sample of rubredoxin from *Clostridium pasteurianum*, there is no detectable difference in the absorption spectra of the material from these two bacterial species.[14, 18]

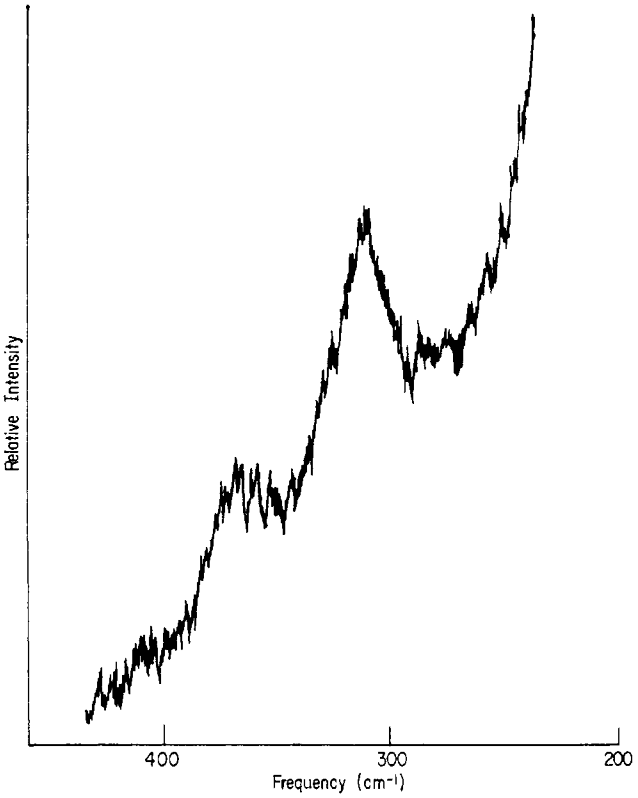

Figure 1. The laser-Raman spectrum of rubredoxin (400–200 cm^{-1}): spectral resolution, 10 cm^{-1}; scan, 5 cm^{-1}/min; time constant, 10 sec; range, 200 cps; laser power, 68 mW.

A portion of the laser-Raman spectrum of solid rubredoxin is contained in Figure 1.[24] The spectrum was searched under high-sensitivity conditions from 100 to 4000 cm^{-1}, but no lines other than the two observed at 365 and 311 cm^{-1} could be discriminated above background. Small sample sizes (*ca.* 2 mg) necessitated sample arrangements which were less than ideal, and this is at least in part responsible for the large Rayleigh scattering which obscures the low-frequency region. The spectrum presented is that of the amorphous, noncrystalline protein which was lightly pressed to an adhesive surface attached to a goniometer head and mounted at an angle of 20° to the incident laser beam. The spectrum of rubredoxin which was crystallized from a pH 6 solution of $(NH_4)_2SO_4$ was also measured, and the two lines in the 300–400-cm^{-1} region were again the only observable Raman shifts. The intensities of the effect for the crystallized material are enormously enhanced, but the intensities decay over a short period, concomitant with a photolytic bleaching of the material in the laser beam.

Since only two lines are measured for a material which could exhibit a complex spectrum, there are a number of possible assignments for these lines. Yet, the only coherent argument that occurs to us is one that invokes the resonance effect discussed above. For the unit containing tetrahedrally disposed sulfurs about the iron, which is believed to exist in this molecule,[19] one would expect four Raman-active modes: the symmetry and antisymmetric stretching modes, ν_1 and ν_3,

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respectively, and the two bending modes, ν_2 and ν_4. We suggest that the lines at 365 and 311 cm^{-1} correspond to ν_3 and ν_1, respectively, of the Fe–S_4 tetrahedron. The intensity pattern is identical with that found for $FeCl_4^-$,[21,22,25] where the analogous modes appear at 385 and 330 cm^{-1}. Additionally, the infrared-active metal–sulfur stretching vibrations have been assigned to lines at 340 cm^{-1} for the tris(ethylxanthato)cobalt(III) and tris(ethylxanthato)chromium(III) complexes.[26] In cysteine, aminoethanethiol, and mercaptopropionic acid complexes of Hg(II), strong Raman-active bands which appear at *ca.* 330 cm^{-1} are assigned to Hg(II)–S stretching modes,[27] and the assignment of bands in this frequency region to iron–sulfur stretches is not unreasonable.

A search has not revealed vibrational measurements on model compounds in which Fe(III) is tetrahedrally surrounded by sulfurs. Unfortunately, ferric ion catalyzes the oxidation of cysteine to cystine,[28] so this system may not be readily studied. A preliminary laser-Raman spectrum of potassium thioferrite, $KFeS_2$, which contains polymeric Fe–S_4 tetrahedra[29] and has been suggested as a possible ferredoxin model,[30] contains bands at 360 and 303 cm^{-1} which possibly bear analogy to the rubredoxin modes, but other lines at both higher and lower frequencies also appear in this spectrum. The bending modes could not be resolved because of the strong elastic scattering at relatively low frequencies. For $FeCl_4^-$, ν_2 and ν_4 are found at 106 and 133 cm^{-1}, respectively,[21,23] and slightly lower values for these lines might be expected for the Fe–S_4 polyhedron.

Similar studies of a variety of these materials are in progress with the goal of identifying systematic structural analogies and differences, particularly including those systems for which single-crystal X-ray data are available. To the extent that metal–ligand frequencies may be transferred between simple model compounds and these more complex systems, frequency comparisons may permit structural predictions in lieu of diffraction results.

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Steric Hindrance and Solvation Effects with Hydrogen-Bonded Adducts

Sir:

In an earlier report,[1] the enthalpy of hydrogen-bond formation between 1,1,1,3,3,3-hexafluoroisopropyl alcohol (HFIP) and γ-collidine (2,4,6-trimethylpyridine) was reported to be the same in both CCl_4 and hexane. Since CCl_4 is known to interact with pyridine and pyridine derivatives,[2] it was suggested that this observation stems from steric interaction between CCl_4 and the 2,6-methyl groups of γ-collidine.[3] In addition, the same enthalpies of reaction were reported for HFIP–pyridine and HFIP–collidine, which suggested a steric interaction between HFIP and collidine.[3]

More recently, Arnett, *et al.*,[4] have suggested that the pyridine–CCl_4 interaction is small since the same enthalpies were obtained for the phenol–pyridine reaction using CCl_4 as solvent (I) and pure base as solvent (II). In order to understand these solvation effects as well as to determine the strength of the pyridine–CCl_4 interaction, we have redetermined the HFIP solution reaction enthalpies and have included in our study the acid 2,2,2-trifluoroethanol (TFE) with the bases pyridine and γ-collidine.

Concentration studies of the heats of solution, ΔH_{sol}, of pyridine and γ-collidine in CCl_4 and hexane as a function of concentration demonstrate that pyridine has a greater tendency to self-associate in either solvent.[5,6] The concentration studies also indicate that both bases self-associate less in CCl_4 than in hexane. The greater self-association of neat pyridine than neat collidine is also shown by the $\Delta H_{\text{sol},\infty}$ values found in Table I. This association could be between the nitrogen lone pair of one molecule and a hydrogen or the ring π^* system of another molecule.[7]

Table I. The Heats of Solution at Infinite Dilution of Pyridine and γ-Collidine in CCl_4 and Hexane

Solvent	Base	$\Delta H_{\text{sol},\infty}$, kcal mol^{-1}
CCl_4	Pyridine	+0.25 (±0.002)
Hexane	Pyridine	+1.96 (±0.012)
CCl_4	γ-Collidine	−0.34 (±0.003)
Hexane	γ-Collidine	+0.93 (±0.012)

The data of Table I indicate the occurrence of steric repulsion in the CCl_4–collidine solvation. The observed $\Delta H_{\text{sol},\infty}$ of pyridine and γ-collidine in CCl_4 is the resultant of an endothermic term due to dissociation of base molecules and an exothermic term due to CCl_4–base interactions. In hexane, the observed $\Delta H_{\text{sol},\infty}$'s should be due mainly to the endo-

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