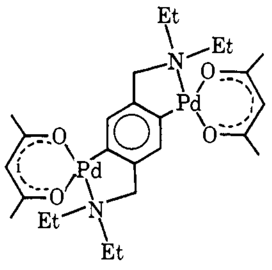
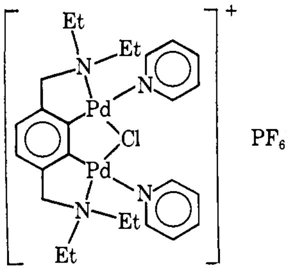
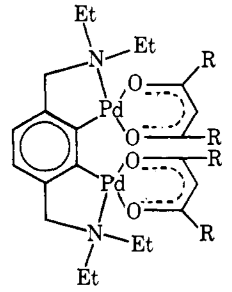


III

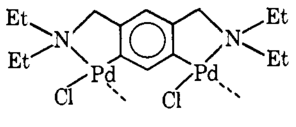


IV

molecule from C_{2v} symmetry. For instance, the nmr spectrum of the bisacetylacetonato derivative, Va, mp 195–197°, while confirming the presence of all ligands shown, indicated nonequivalence of the ethyl groups and of benzylic methylenes: τ 3.20 (s, aromatic CH), 4.62 (s, acetylacetonato CH), 5.00 (d, J = 13.5) and 6.38 (d, J = 13.5, nonequivalent benzylic H's), 6.3–7.6 (complex, unresolved multiplet, ethyl CH_2), 7.75 and 7.93 (singlets, both acetylacetonate CH_3), and 8.26 (m, overlapping triplets, ethyl CH_3) in a 1:1:1:1:4:3:3:6 ratio. Molecular models suggest that coplanarity of the acetylacetonate rings with the benzene ring would entail prohibitive steric crowding. This can be relieved by tilting of both rings so that the coordination planes for both palladium atoms are fixed parallel to each other, but at an angle with regard to the benzene ring. This would make the ethyl groups and the benzylic hydrogens nonequivalent. The same effect was observed with the 2,2,6,6-tetramethyl-3,5-heptanedionato derivative, Vb, mp 204–205° dec.

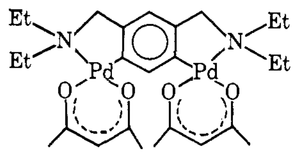


Va, R = Me
b, R = *t*-Bu

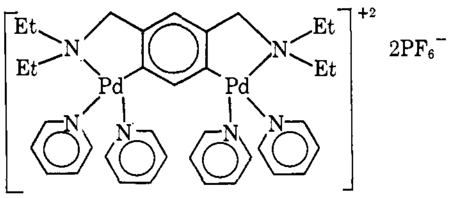


VI

A representative of 1,3-dipalladiobenzenes, VI, was obtained as an insoluble, polymeric solid by the reaction of tetrachloropalladate ion with *N,N,N′,N′*-tetraethyl-*m*-xylylenediamine. It was characterized by conversion to the bisacetylacetonato derivative VII, the nmr spectrum of which was confirmatory: τ 2.75 (s, aromatic 2-H), 3.43 (s, aromatic 5-H), 4.71 (s, acetylacetonato CH), 6.12 (s, benzylic CH_2), 7.1 (m, two overlapping quadruplets, J = 7.0, ethyl CH_2), 8.00 and 8.09 (singlets, both acetylacetonato CH_3), and 8.50 (t, J = 7.0, ethyl CH_3) in a 1:1:2:4:8:6:6:12 ratio. The assignment of the τ-2.75 singlet to the 2-H is based on its large upfield shift (2.8 ppm, relative to the benzyl CH_2, with the τ-3.43 singlet remaining unshifted) in the nmr spectrum of the tetrapyridine cation VIII, isolated as the hexafluorophosphate salt, mp 205–208° dec: τ 1.8 (m, pyridine 2-H), 2.6 (t, J = 8, pyridine 4-H), 3.1 (m, pyridine 3-H), 3.68 (s, aromatic 5-H), 5.80 (s, aromatic 2-H), 6.35 (s, benzyl CH_2), 7.58 (m, two overlapping quadruplets, J = 7.0, ethyl CH_2), and 8.90 (t, J = 7.0) in the correct 8:4:8:1:1:4:8:12 ratio. The shift is presumably a consequence of the pyridine ring current and it is almost twice as large as that in analogous monopalladio systems.[8]



VII



VIII

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[8] An upfield shift of 1.3–1.4 ppm for the aromatic H adjacent to Pd has been observed in 2-(dialkylaminomethyl)phenylpalladium(II) derivatives and in analogous systems upon formation of the bispyridine cation: S. Trofimenko, unpublished results.

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Determination of Iron Coordination in Nonheme Iron Proteins Using Laser-Raman Spectroscopy. II. *Clostridium pasteurianum* Rubredoxin in Aqueous Solution[1]

Sir:

Three-dimensional X-ray techniques can provide structural data of the highest information content for crystalline proteins and enzymes. The extent to which the structures displayed by these complex molecules in the crystalline phases resemble those adopted in the aqueous solutions that are presumably their physiological environments is generally an open question. Here we present evidence that bears on this important point for the nonheme iron protein rubredoxin, isolated from *Clostridium pasteurianum*.[2]

The biological functions of the rubredoxins are obscure, although it is known that they can substitute for ferredoxins in some electron-transfer reactions[2] and that rubredoxin from *Pseudomonas oleovorans* is a component of the ω-hydroxylation system of that aerobe.[3] This participation in oxidation–reduction processes focuses attention on the iron atom and the residues to which it binds to the protein. In an X-ray study of crystalline rubredoxin, Herriott, Sieker, Jensen, and Lovenberg[4] conclude that an electron density map based on 2.5-Å resolution data shows that the single iron is tetrahedrally coordinated to four cysteinyl sulfur atoms. Recently, we suggested the possibility of characterizing the mode of binding of iron in nonheme

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[1] Paper I: T. V. Long, II, and T. M. Loehr, *J. Amer. Chem. Soc.*, **92**, 6384 (1970).

[2] W. Lovenberg and B. E. Sobel, *Proc. Nat. Acad. Sci. U. S.*, **54**, 193 (1965).

[3] J. A. Peterson and M. J. Coon, *J. Biol. Chem.*, **243**, 329 (1968).

[4] J. R. Herriott, L. C. Sieker, L. H. Jensen, and W. Lovenberg, *J. Mol. Biol.*, **50**, 391 (1970).

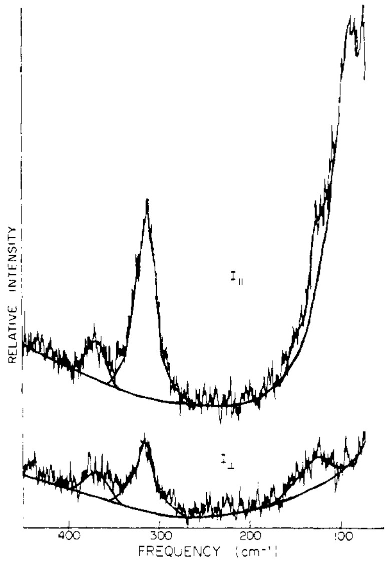

Figure 1. The laser-Raman spectrum of *Clostridial* rubredoxin in aqueous solution in two polarization modes:[8] argon-ion laser excitation (power at sample, 5 mW); spectral resolution, 10 cm^{-1}; amplification scale, 1 × 10^{-9}; rise time, 3 sec; scan, 50 cm^{-1} min^{-1}.

iron proteins using laser-Raman spectroscopy.[1] This earlier observation may now be confirmed and extended.

Oxidized rubredoxins from *Pseudomonas oleovorans*[3] and *Peptostreptococcus elsdenii*[5] show negative Cotton effects at 632 and 502 nm, and a similar circular dichroism spectrum is anticipated for *Clostridial* rubredoxin.[1,6] The 632.8-nm line of a He-Ne laser coincides with the first of these absorptions, and it excites an apparent resonance or preresonance Raman effect from crystalline rubredoxin.[1] Raman shifts are observed at 365 and 311 cm^{-1}, and these are assigned as the ν_3 and ν_1 stretching vibrations of the $Fe-S_4$ tetrahedron that is the optically active chromophore. The tetrahedral bending modes, which would probably lie below 150 cm^{-1}, were not discriminated in the spectrum of the solid material because of strong elastic scattering.

The laser-Raman spectrum of an aqueous solution of oxidized *Clostridial* rubredoxin is shown in Figure 1.[7] In contrast to our original report, this spectrum was excited by the 488.0-nm line of an Ar^+ laser, which lies well within the strong absorption band of the molecule centered at 490 nm. Raman lines are measured at 368 and 314 cm^{-1}, at energies identical with those observed for the crystalline material within experimental error. Although the noise level of the spectrum prevented accurate determination of depolarization ratios, the band at 368 cm^{-1} is definitely depolarized ($\rho = 0.9$) while that at 314 cm^{-1} is polarized ($\rho = 0.3$).[8] This is consistent with their identification as the ν_3 and ν_1 fundamentals of a tetrahedron, which have T_2 and A_1 symmetries, respectively. Furthermore, a broad band is clearly evident in the depolarized spectrum ($I_{\perp}$ mode, bottom of Figure 1) with a maximum at 126 cm^{-1}. This shift appears to have a weak shoulder at *ca.* 150 cm^{-1}, and these features are assigned as the ν_2 (E) and ν_4 (T_2) bending modes, respectively, of the $Fe-S_4$ tetrahedron. Although these frequencies are somewhat higher than the corresponding values for $FeCl_4^-$(aqueous) (ν_2 = 106 cm^{-1} and ν_4 = 133 cm^{-1}),[9] the ligating sulfurs are bound to the protein backbone, and the increase in energy is within reason. The total band contour in this region is depolarized ($\rho = 0.8$). The origin of the polarized band at *ca.* 86 cm^{-1} is uncertain. The existence of an artifact may not be discounted, although no grating ghosts or laser lines are present in this region. It is also possible that this is an intramolecular mode of the protein, but such might be expected to be weak.

Several important conclusions may be drawn from the measurements reported above. First, *the tetrahedral coordination about the iron atom by four cysteinyl sulfurs that is known to exist in crystalline oxidized rubredoxin*[4] *appears to be maintained upon dissolution.* The energies of the ν_1 and ν_3 fundamentals recorded for the protein in aqueous solution are identical with those observed for the solid material.[1] This description of the coordination geometry is accompanied by the caveat that the Raman data do not preclude the possibility of slight deviations from strict tetrahedral symmetry about the iron in either the solid or solution phases. The nonzero value of the depolarization ratio for the A_1 line may reflect such a distortion or it may arise from a coupling of this vibration to protein modes. However, any modification of the polyhedron about the iron, such as a conversion to an octahedral structure with water molecules filling two coordination sites or a modification in the binding sites to the protein, should sensitively affect the vibrational spectrum. Eaton and Lovenberg[10] have recently concluded that the coordination about the iron in reduced rubredoxin (aqueous solution) is also tetrahedral. The strict maintenance of this structural feature regardless of phase suggests that the metal ion and its ligands may be important in stabilizing the principal elements of the tertiary structure of the molecule.

Second, this study reinforces our interpretation of the results from the crystalline material.[1] The observation of the 365- and 314-cm^{-1} lines with both Ar^+ and He-Ne laser excitation confirms their identification as Raman shifts. Moreover, the depolarization measurements and the resolution of the bending modes

(5) N. M. Atherton, K. Garbett, R. D. Gillard, R. Mason, S. J. Mayhew, J. L. Peel, and J. E. Stangroom, *Nature* (*London*), 212, 590 (1966).

(6) W. Lovenberg, *Protides Biol. Fluids, Proc. Colloq.*, **14**, 165 (1966).

(7) The spectra were measured on a Spex Ramalog laser-Raman spectrophotometer. Attempts to obtain a spectrum of crystalline rubredoxin using Ar^+ laser excitation were unsuccessful because the intense absorption of the laser radiation by the material resulted in local heating and pyrolysis even at quite low levels of laser power at the sample. The spectrum of the aqueous solution was obtained from several microliters of the protein solution contained in a silica capillary tube held at a right angle to the incident laser beam. Cavitation of the solution from absorptive heating resulted for laser power levels (at sample) of greater than 5 mW.

(8) The depolarization ratio of a Raman band is defined as $\rho = I_{\perp}(\text{obsd}_{\perp})/I_{\parallel}(\text{obsd}_{\perp})$, where $I_{\perp}(\text{obsd}_{\perp})$ is the integrated intensity of the Raman scattering whose polarization is perpendicular to the polarization of the incident radiation and which is observed at a 90° angle to the plane of polarization of the incident radiation, and $I_{\parallel}(\text{obsd}_{\perp})$ is the integrated intensity of the scattered light with a polarization parallel to that of the incident radiation, which is observed at a 90° angle to the plane of polarization of the incident radiation. In the present experiment, polarized incident radiation was employed, and the scattered light passed through a Polaroid analyzer that was rotated so as to pass radiation of the desired polarization, with subsequent polarization scrambling before entrance into the spectrometer. Symmetric (A_1) vibrations may exhibit any depolarization values less than 0.75, while asymmetric modes should have $\rho = 0.75$.

(9) T. V. Long, presented in part at the 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968.

(10) W. A. Eaton and W. Lovenberg, *J. Amer. Chem. Soc.*, **92**, 7195 (1970).

substantiate our assignments of the 365- and 314-cm^{-1} bands as the antisymmetric and symmetric stretching modes, respectively, of the Fe–S$_4$ tetrahedron. In this regard, one notes that Heath and Martin[11] have recently assigned the infrared-active Fe–S stretch to a band at 348 cm^{-1} in the spectrum of tris(dithioacetylacetonato)iron(III), a compound in which the ferric ion is surrounded by an octahedron of sulfurs. It is interesting that the intensity enhancement of the vibrational lines of the iron–sulfur polyhedron of rubredoxin occurs upon irradiation in either the 632- or the 502-nm band of the chromophore. A resonance or preresonance Raman effect has similarly been invoked to account for the appearance of only the Fe–Fe stretching mode in the laser-excited Raman spectrum of $[(C_5H_5)Fe(CO)]_4$.[12]

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(11) G. A. Heath and R. L. Martin, *Aust. J. Chem.*, **23**, 1721 (1970).

(12) A. Terzis and T. G. Spiro, *Chem. Commun.*, 1160 (1970).

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Ion Radicals. XX. Aromatic Nitration. A New Preparation of 3-Nitroperylene and 1-Nitropyrene[1,2]

Sir:

3-Nitroperylene is ordinarily prepared by the nitration of perylene. The procedure is tedious, requires caution against polynitration, and entails the use of large volumes of solvent. For example, the best procedure in the literature for the mononitration of perylene uses approximately 1 g of perylene in over 1.5 l. of a dilute solution of nitric acid in acetic anhydride. The nitration takes 24 hr at 15°, and involves extensive work-up after hydrolyzing the acetic anhydride.[3]

In contrast with this, we have found that perylene can be mononitrated within a matter of minutes by treating the perylene cation radical with nitrite ion.

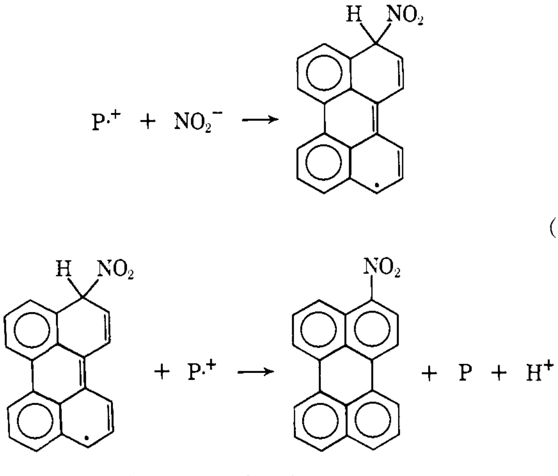
Perylene cation radical is readily obtained as a solid by two methods: anodic oxidation[4] and oxidation with iodine in the presence of silver perchlorate.[5] According to Williams,[4] the former gives a crystalline 1:1 complex of perylene and perylene cation radical perchlorate. In our hands, the method gave solid mixtures of perylene and the cation radical perchlorate of variable composition but containing approximately 30% of perylene. The solid is, nevertheless, suitable for studying reactions of the cation radical.[6] The iodine–silver perchlorate method precipitates a mixture of silver iodide and perylene cation radical perchlorate. This mixture is also suitable for studying certain of the cation radical reactions[6] and, in particular, the reaction with nitrite ion.

A sample of perylene cation radical–silver iodide ($P\cdot^+$/AgI) mixture was prepared and found by iodimetry[6] to contain 99% of the stoichiometrically required cation radical. Of this, 3.7 g (6.3 mmol) was suspended in 450 ml of dry acetonitrile, and an excess of dry sodium nitrite was added. The color of the stirred mixture changed rapidly from purple to orange-red. The mixture was stirred for 10 min and filtered. The filtrate was evaporated to dryness on a rotary evaporator and chromatographed on alumina (Woelm, neutral; 5.5 cm × 50 cm column). The blue fluorescent perylene band was eluted with 50:50 benzene–cyclohexane. The orange fluorescent 3-nitroperylene band was eluted with benzene, giving 642 mg (69% of theory) of brick-red 3-nitroperylene, mp 208–209°. Chromatographing a second time and crystallizing from benzene gave mp 209–209.5° (lit.[3] mp 210–211°).

Since only two products are obtained, perylene and 3-nitroperylene, the stoichiometry of the reaction is undoubtedly as in eq 1, where P refers to perylene

$$2P\cdot^+ + NO_2^- \longrightarrow P + P\text{-}NO_2 + H^+ \qquad (1)$$

and P-NO$_2$ to 3-nitroperylene. The simplest interpretation of the reaction (since, in the absence of kinetic data, distinction between mono- and dication reaction cannot be made[7]) is given in eq 2. This stoichiometry, and the easy oxidation of perylene by iodine in the presence of silver ion, suggests that oxidation of perylene by an excess of iodine–silver nitrite should convert all of the perylene into 3-nitroperylene. This has been achieved with a fair degree of success, as follows.



(2)

Perylene (57 mg, 0.23 mmol) and iodine (114 mg, 0.45 mmol) were placed in 50 ml of dry acetonitrile and 138 mg (0.9 mmol) of dry silver nitrite was added. The reaction mixture turned orange-red almost immediately. After 15 min of stirring, perylene was found to be absent by tlc. Working up as described above gave 45 mg (66% of theory) of 3-nitroperylene. The stoichiometry of reaction in this case is presumed

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(1) Supported by the National Science Foundation, Grant No. GP-25989X.

(2) Part XIX: H. J. Shine, L. Hughes, and P. Gesting, *J. Organometal. Chem.*, **24**, 53 (1970).

(3) M. J. S. Dewar and T. Mole, *J. Chem. Soc.*, 1441 (1956).

(4) D. F. Williams, Abstracts, Fourth Molecular Crystal Symposium, Enschede, Holland, July 1968, and private communication.

(5) Y. Sato, M. Kinoshita, M. Sano, and H. Akamatu, *Bull. Chem. Soc. Jap.*, **42**, 3051 (1969).

(6) Manuscript in preparation.

(7) Y. Murata and H. J. Shine, *J. Org. Chem.*, **34**, 3368 (1969).