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Ferric Porphyrin Thiolates. Possible Relationship to Cytochrome P-450 Enzymes and the Structure of (*p*-Nitrobenzenethiolato)iron(III) Protoporphyrin IX Dimethyl Ester

Sir:

Four distinct reaction states of the cytochrome P-450 enzyme, each with characteristic electronic properties, have been identified in the *Pseudomonas putida* camphor hydroxylase system. [1-6]The possibility, in one or more reaction states, of axial sulfur ligation[1,7] to the iron protoporphyrin IX prosthetic group present in this[8] and other systems[9] has proved difficult to assess in the absence of known properties of fully characterized sulfur-bound iron porphyrins, which should serve as a guide to those developed by a $[FeN_4S]$ coordination unit present in the enzymes. The *P. putida* cytochrome has in addition to 6 Cys residues, 9 Met, 9 Tyr, and 12 His,[1,10] potentially affording the units $[FeN_4X]$, X = SR, OR, N(Im), one of which should apply to the high-spin component[1,4-6] of Fe^{III}-P-450_{cam}·S (substrate-bound). The low-spin component of this state and low-spin substrate-free Fe^{III}P-450_{cam} are assigned six-coordinate geometry. The simple synthesis of Fe(III) porphyrin thiolates described in the preceding communication,[11] its ready extension to $[Fe^{III}N_4OR]$ analogs, and the availability of other pertinent data permit a more stringent examination of axial coordination[12] in oxidized reaction states than possible heretofore.

The title compound, $Fe(PPIXDME)(SC_6H_4NO_2)$ (**1**), and $Fe(PPIXDME)(OC_6H_4NO_2)$ (**2**)[13] were prepared from $[Fe(PPIXDME)]_2O$[14] and the corresponding thiol and phenol, respectively, in toluene solution.[11] Complex **1** crystallizes in triclinic space group C_i^1-$P\bar{1}$ with a cell of dimensions a = 13.596 (9) Å, b = 14.021 (4) Å, c = 13.307 (4) Å, α = 110.80 (6)°, β = 119.67 (6)°, γ = 62.62 (6)°, Z = 2, and V = 1930 Å^3. Observed and calculated densities are 1.377 and 1.35 (2) g/cm^3, respectively. At the present stage of isotropic refinement, based on 2557 unique data having $F_o^2 > 3\sigma(F_o^2)$ and including no absorption corrections or H atom contributions, the R index is 0.11 for 55 non-H atoms. The structure of the $[FeN_4SC]$ unit, shown in Figure 1, contains these features: the Fe atom appears slightly asymmetrically bonded to the four N atoms and is displaced out of the N_4 (0.43) and 24 atom porphyrin (0.45 Å) mean planes toward the axial ligand (similar displacements are found in other high-spin Fe(III) porphyrins,[15] including hemin chloride,[15a] **3**); Fe-S-C = 101°; the phenyl ring forms dihedral angles of 11, 88, and 100° with the N_4, FeN(1)N(3), and FeN(2)N(4) planes, respectively.

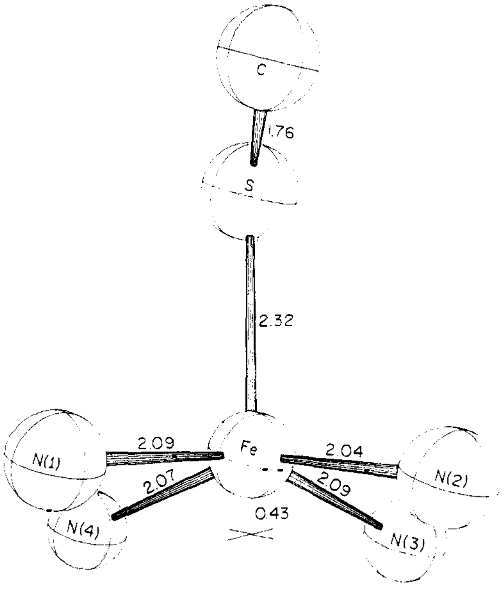

Figure 1. Perspective view of the inner coordination geometry of $Fe(PPIXDME)SC_6H_4NO_2$ (phenyl ring omitted). Estimated standard deviations of bonded distances are 0.01 Å.

Magnetic properties **1** are consistent with its structure and include high-spin susceptibility behavior (χ_M^{corr} = 4.412/(T + 3.2), 20–295°K) with μ = 5.90 BM at 295°K. For **2** μ = 5.90 BM (295°K). The symmetric quadrupole doublet in the Mössbauer spectrum of **1** at 4.2°K becomes increasingly asymmetric due to broadening of the higher velocity component as the temperature is raised, a behavior entirely similar to that observed and interpreted for **3.**[16] As with **3**[17] application of a strong magnetic field at low temperature induces a large magnetic hyperfine interaction (ca. −440 kOe, H_0 = 80 kOe, 4.2°K), expected for high-spin Fe(III).[11] Mössbauer parameters[18] (δ, ΔE_Q) are the following: **1,** 0.29, 0.70; **2,** 0.29, 0.67; **3,** 0.35, 0.75. These values are typical of high-spin Fe(III) porphyrins and those of **1** are close to the values for high-spin Fe^{III}-P-450_{cam}·S[5] (0.35, 0.79 mm/sec, ~200°K). However, as these and other results now reveal, Mössbauer parameters for high-spin complexes measured in zero magnetic field are insufficiently sensitive to distinguish axial sulfur ligands from X = OR,[19] N(Im),[20] and other groups.

The electronic spectra of **1** and **2** (Figure 2) display significant differences, particularly in the Soret and α-band (600–650 nm) regions. In the spectra of **2** and Fe(PPIXDME)OMe[21] the Soret band occurs at somewhat lower energy and the α-band is significantly blue-shifted compared to **1.** Further, in acid-met myoglobin and hemoglobin spectra[22] Soret and α-bands generally occur at ca. 405–410 and 623–635 nm with relative intensities different from that of **1.** Indeed, the spectrum of **1** is in very close agreement with those of high-spin Fe^{III}-P-450_{cam}·S ($\lambda_{max}(\epsilon)$ = 391 (87), 646 nm (4.5 mM))[1-3] and microsomal high-spin P-450.[23] These results are considered to favor axial sulfur (S-Cys) over oxygen or nitrogen coordination in these high-spin cytochromes, with perhaps the most characteristic spectral feature of this ligation mode being the relatively weak α-band at ~646 nm.

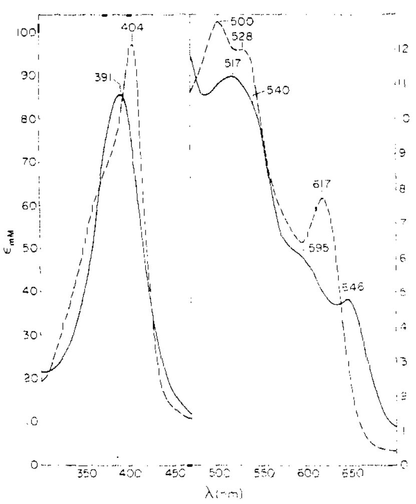

Figure 2. Electronic spectra in dry toluene solution at 25°: Fe(PPIXDME)$OC_6H_4NO_2$, - - - -; Fe(PPIXDME)$SC_6H_4NO_2$, —— (ϵ = 86.8 (391), 11.2 (517), 4.8 m*M* (646 nm)).

The EPR spectra of Fe(III) porphyrin thiolates display a rhombic set of signals[11] similar to those of several oxidized P-450 species,[4,24] whereas **2** and Fe(OEP)OMe afford axial spectra with $g \sim 6$ and 2 (toluene glass, ~95°K). Both S- and O-bonded porphyrins are readily converted to low-spin six-coordinate species with rhombic EPR spectra upon addition of various ligands. These species, particularly those containing thiolate groups, are quite unstable toward reduction to the Fe(II) stage but may be trapped by rapid freezing of freshly prepared solutions. The following *g* values have been obtained at ~95°K for the indicated species: Fe(PPIXDME)($SC_6H_4NO_2$)(N-Me-Im), 2.42. 2.26, 1.91; Fe(PPIXDME)($OC_6H_4NO_2$)(N-Me-Im), 2.61, 2.21, 1.84 (both in toluene glass); [Fe(PPIXDME)(N-Me-Im)$_2$]Cl, 2.90, 2.29, 1.57 (DMF-CH_2Cl_2). For low-spin Fe^{III}-P-450_{cam} g = 2.45, 2.26, and 1.91;[4] values for other low-spin P-450 cytochromes are only slightly different.[24] Very similar results were found for the N-base adducts of Fe(OEP)SPh. Rhombic spectra with *g* values comparable to the preceding first set have been produced upon adding thiols to heme proteins or to porphyrin complexes in the presence of N-bases.[7,25] In this work full characterization of the high-spin complexes adds credence to the identification of the low-spin forms derived from them, which thus far have resisted isolation.

From these results it is postulated that the high-spin form of Fe^{III}-P-450 cytochromes contains one axial sulfur ligand (presumably S-Cys) and a prosthetic group with a structure similar to that of **1** (Figure 1). The low-spin form is concluded to be derived from it by the binding of an imidazole (His) ligand, resulting in axial S-Fe-N coordination.[26] Axial O-Fe-N and N-Fe-N ligation modes do not afford *g* values as close to those of the low-spin cytochromes. The proposed S-Fe high-spin arrangement is at variance with an earlier suggestion[4] that substrate binding by Fe^{III}-P-450_{cam} displaces an axial sulfur ligand. Instead, camphor binding may lead to the displacement of a nitrogenous ligand. Measurements of the electronic and EPR spectra of **1** and Fe(OEP)SPh in the presence of an ~100-fold excess of camphor failed to reveal the formation of low-spin species.

The preceding discriptions of axial coordination in the two reaction states of the oxidized cytochromes are offered with recognition that arylthiolates are not ideal representations of S-Cys ligation, and that certain electronic features of several P-450 states are approached in chloroperoxidase, whose only two Cys residues are reported to form a disulfide.[29] At present only arylthiolate iron(III) porphyrins have proven adequately stable for isolation.[11] Future reports will deal in more detail with comparative structural and electronic properties of five- and six-coordinate iron-porphyrin complexes with variant types of axial ligands.

Acknowledgment. This research was supported at M.I.T. by National Science Foundation Grant GP-40089, at the Francis Bitter National Magnet Laboratory by the National Science Foundation, and at Northwestern University by National Institutes of Health Grant HL-13157.

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(26) Explicit experimental tests of thioether (Met) vs. thiolate (Cys) coordination in the high- and low-spin forms of oxidized P-450 enzymes must await the preparation of well-defined Fe(III) porphyrin complexes containing axial thioether ligand(s). At present evidence against Fe-SR_2(Met) coordination is necessarily indirect but plausible: (i) it is unlikely that five-coordinate thiolate (1) and thioether complexes would afford electronic spectra sufficiently similar that both would compare equally favorably with high-spin P-450; (ii) if, as proposed above, both forms contain a common axial ligand, the divergence of oxidized cytochrome *c* *g* values[27] (3.06, 2.24; 1.24, axial Met-Fe-His coordination[28]) from those of low-spin P-450 argues against thioether binding in either of these forms. In the low-spin form we cannot, strictly speaking, eliminate another conceivable ligation mode, Cys-Fe-Met, if the reported *g* values for Fe(TPP)(SPh)(tetrahydrothiophene) (2.36, 2.27, 1.95)[12] are considered close to the P-450 values. Here we note that there is no precedent for low-spin Fe(III) heme proteins which lack an Im(His) axial ligand.
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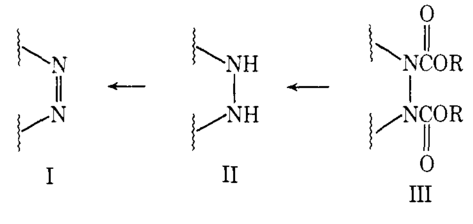
1,2-Bis(β-tosylethoxycarbonyl)diazene. Its Application to the 2,3-Diazabicyclo[2.2.0]hexene System

Sir:

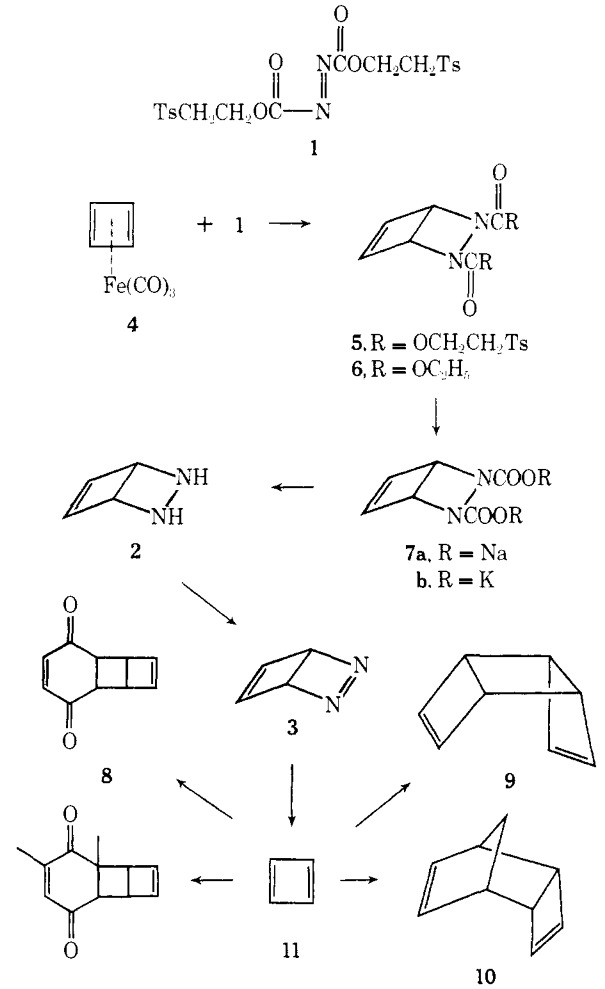
Use of the diazenediyl[1] (azo) group (I) in physical and synthetic organic chemistry is extensive for obvious reasons. Compounds containing this functional group serve as precursors for the study of important reaction intermediates such as trimethylene[2] and are also utilized to form a new carbon–carbon bond, as exemplified in the synthesis of prismane.[3] A general route leading to this functionality involves the oxidation of a 1,2-diazanyl[1] (hydrazo) derivative (II) which is generally prepared from the corresponding alkoxycarbonyl derivative (III). The condition normally required for the hydrolysis of III is rather severe,[4] and, not surprisingly, many functional groups and systems do not survive this operation. 1,2-Bis(β-tosylethoxycarbonyl)diazene (**1**), recently prepared in our laboratory, has overcome this difficulty, as applied to the synthesis of 2,3-diazabicyclo[2.2.0]hex-5-ene (**2**). We believe that this reagent is widely applicable to syntheses of sensitive systems and further report the intriguing behavior of 2,3-diaza-Dewar benzene (**3**) generated from (**2**).



Preparation of 1. Reaction of β-tosylethoxycarbonyl chloride[5] with hydrazine in aqueous dioxane in the presence of magnesium oxide provided a near-quantitative yield of the corresponding 1,2-disubstituted diazane which in turn was oxidized with nitrogen oxide (chiefly N_2O_4) at 0°. The resulting yellow, low melting (ca. 5°) crystals (**1**) (A_2X_2, δ 3.56, 4.73),[6] obtained in 40–50% yield were shown to undergo a Diels–Alder reaction. Typically, a 1:1.5 mixture of **1** and cyclopentadiene provides at room temperature the expected adduct in more than 90% yield (Scheme I).

Preparation of 2. Slow addition of cyclobutadiene iron tricarbonyl (**4**)[7] (10.4 m*M*) in pyridine to a stirred mixture of lead tetraacetate (38.0 m*M*), reagent **1** (6.4 m*M*), and pyridine, produced an adduct (**5**) (AA′XX′, δ 4.97, 6.62 roughly doublets 3.5 Hz apart, A_2X_2, δ 3.47, 4.43) in 45% yield. Its ^{1}H NMR spectrum exhibits absorptions typical of symmetrically substituted cyclobutenes and observed in numerous bicyclo[2.2.0]hexenes, and virtually reproduces that of the ethoxycarbonyl derivative (**6**)[8] (AA′XX′, δ 5.15, 6.70), produced from **4** and diethoxycarbonyldiazene in a similar manner. Thus **5** is formulated as shown. Treatment of **5** with 2 equiv of alkali (sodium or potassium ethoxide) in ethanol at room temperature (or below) immediately and quantitatively generated the disodium or dipotassium salt of *N,N′*-dicarboxylate (**7a** or **7b**) AA′XX′ δ 5.01, 6.82, D_2O). Careful neutralization of these salts with trifluoroacetic acid provided carbon dioxide and an air-sensitive liquid (**2**) and its ^{1}H NMR spectrum (AA′XX′ δ ~ 5.1, 6.91) was consistent with the expected structure.[9,10]

Scheme I



Reactions of 2. Addition of benzoquinone to a methanolic (or tetrahydrofuran) solution of **2** either at −70 or 0° immediately induced gas evolution and provided *endo*-tricyclo[4.4.0.0^{2,5}]deca-3,8-dien-7,10-dione (**8**)[11] in ca. 50% yield but no trace of *syn*-tricyclo[4.2.0.0^{2,5}]octa-3,7-diene (**9**). With 2,6-dimethylbenzoquinone and DDQ, **2** behaved similarly to yield the corresponding adducts, respectively. Oxidation of **2** with aqueous sodium hypochlorite both heterogeneously (methylcyclohexane as solvent) and homogeneously (tetrahydrofuran) led to the formation of **9**. When cyclopentadiene was utilized as a trapping reagent in these experiments, the product was a 1:1 mixture of **9** and tricyclo[4.2.1.0^{2,5}]nona-3,7-diene (**10**)[12] but no anti isomer of **9**.[13]

A straightforward, yet the best interpretation of the