cause, at 5×10^{-5} M external pyridine, it is largely dissociated from mesoheme.[15]

The effect upon carbon monoxide binding of having a pyridine neighboring group in the fifth or in the sixth position can be examined separately. Studies by Wang on pure protoheme[10a] and protoheme–pyridine mixtures[10b] show that the binding constant for carbon monoxide to mesoheme increases with increasing pyridine concentration up to approximately 10^{-3} M before it decreases (see Table I). The comparably high CO binding strength of the monopyridine mesoheme (**2**) therefore demonstrates that our internal pyridine of stoichiometric concentration chelates at the fifth position at least as effectively as a tenfold excess of external pyridine.

In the sixth position, pyridine competes with carbon monoxide, as shown by the drastic decrease in CO binding strength as the pyridine concentration increases from 5×10^{-4} to 1.3 M. The decreased binding constant observed when our monopyridine compound is converted to a dipyridine compound ($P_{1/2}^{CO}$ = 1.1 mm) further illustrates the enhanced chelation of the internally bound pyridine.

These results demonstrate, in carbon monoxide binding, a substantial neighboring group effect in the fifth and sixth positions. Such effects are probably important in determining the behavior of five-liganded hemes in proteins such as myoglobin as well as those of six-liganded hemes in, *e.g.*, cytochrome *c*.[16]

We have also studied the effect of the distal imidazole upon carbon monoxide binding by preparing **3**, in which the imidazole can occupy a position similar to its position in myoglobin but cannot approach the iron itself. The approximately twofold increase in binding constant in going from **2** to **3** is consistent with the proposed hydrogen bonding[11c] between this imidazole and the CO ligand.

The fact that the best of our pyridine compounds has a CO binding constant of only about $^1/_{30}$ that of myoglobin means that there are other important attributes of the protein in addition to the neighboring group effect which contribute to carbon monoxide bonding. The nature of another dominant effect is discussed in the following communication.

(15) Further evidence against the aggregation phenomenon is the fact that both the parent compounds and the Fe^{II}–CO complexes move as single spots on tlc plates.

(16) Other obvious advantages of covalently bound ligands are the simplicity of getting pure O_2 or CO complexes and the simplication of ligand (*e.g.*, CO, O_2, etc.) equilibria and kinetic schemes and their determination.

C. K. Chang, T. G. Traylor*
Department of Chemistry, Revelle College
University of California, San Diego
La Jolla, California 92037

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Proximal Base Influence on the Binding of Oxygen and Carbon Monoxide to Heme[1,2]

Sir:

We wish to report a strikingly large effect of the structure of the proximal nitrogen base upon the differential affinity of heme complexes for oxygen on the one hand and carbon monoxide on the other.

The importance of histidine in myoglobin function is well established. The function of such bases as imidazole and pyridine in increasing the ability of heme to bind CO and O_2 has been discussed in terms of π-electorn donation.[3] Although there is some indication that imidazole is superior to pyridine in binding oxygen[4,5] no explanation of this superior behavior has been offered nor is there an explanation for the observation that myoglobin, unlike other transition metal complexes, binds oxygen almost as well as it does carbon monoxide. This peculiar behavior allows life even in CO polluted air.

In this report we compare the oxygen and carbon monoxide affinities of heme compounds having either pyridine or imidazole as a fifth ligand.

The Fe^{III} counterparts of compounds **1a** and **2a** in Figures 1 and 2 were synthesized from pyrrohemin[8] and mesohemin, with the appropriate bases by methods previously described.[2] These were reduced in methylene chloride or chloroform using a small amount of aqueous sodium dithionite in a pH 7.0 phosphate buffer, all under argon. Cooling to −45° in liquid propane precipitated the frozen aqueous phase leaving a solution of either **1a** or **2a**, having a typical visible spectrum for five-liganded Fe^{II} porphyrins.[9] To each of these solutions was added aliquots of gases while keeping the measuring cuvette at controlled temperature. The spectra of a series of experiments with **2a** are shown in Figure 3. Although the pyridine compound **2a** is seen to oxidize rather quickly, the identical spectra with and without 1 atm of oxygen indicates that the $P_{1/2}$ for oxygen binding is much larger than 760 mm. The $P_{1/2}$, pressure for half-saturation, values are shown in Table I.

Table I. Pressure for Half-Saturation of Hemes with O_2 and CO

	$P_{1/2}$ of O_2, mm	$P_{1/2}$ of CO, mm	Ref
Im–Pyrroheme, **1a**	0.2 (CH_2Cl_2, −45°)	0.088 (CH_2Cl_2, 25°)	This work
Py–Mesoheme, **2a**	>760[a] (CH_2Cl_2, −45°)	0.20 ($CHCl_3$, 23°)	2
Sperm whale myoglobin	0.48 (pH 8.6, 25°)	0.021 (pH 8.6, 25°)	10
Reconstituted sperm whale deutero-myoglobin	0.21 (pH 7, 20°)		11

[a] No O_2 complex formation.

(1) This work was supported by the National Institutes of Health, Grant USPHS HE 13581.

(2) Previous paper in this series: C. K. Chang and T. G. Traylor, *J. Amer. Chem. Soc.*, **95**, 8475 (1973).

(3) (a) J. H. Wang, "Hematin Enzymes," J. E. Falk, R. Lemberg, and R. K. Morton, Ed., Pergamon Press, 1961, p 76; (b) J. H. Wang, *Accounts Chem. Res.*, **3**, 90 (1970).

(4) A. H. Corwin and Z. Reyes, *J. Amer. Chem. Soc.*, **78**, 2437 (1956).

(5) Similar, but much smaller dependence upon the nature of the base has been observed in cobalt-porphyrin complexation with oxygen.[6,7] However, the pyridine–cobalt porphyrins bind oxygen.

(6) D. V. Stynes, H. C. Stynes, J. A. Ibers, and B. R. James, *J. Amer. Chem. Soc.*, **95**, 1142 (1973), and references therein.

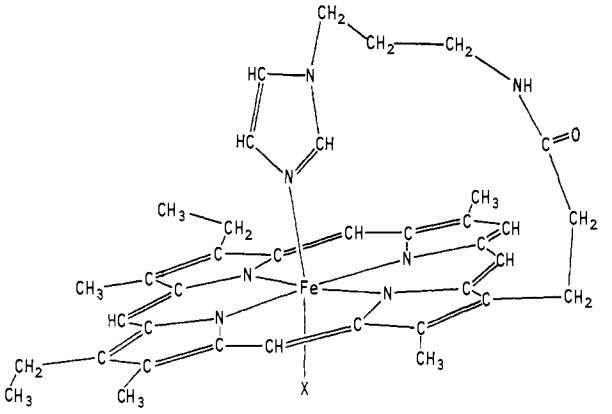
(7) F. A. Walker, *J. Amer. Chem. Soc.*, **95**, 1150, 1154 (1973).

(8) (a) C. K. Chang and T. G. Traylor, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2647 (1973); (b) C. K. Chang and T. G. Traylor, *J. Amer. Chem. Soc.*, **95**, 5810 (1973).

(9) See ref 8 for spectra of **1a,b,c** at 25° and −45° and Figures 3 and 4 for spectra of **2a,b,c,d**. The α/β bond extinction ratio of 1.2–1.3 is consistent with five-liganded iron.

(10) M. Keyes, H. Mizukmi, and R. Lumry, *Anal. Biochem.*, **18**, 126 (1967).

(11) F. Antonini and M. Brunori, "Hemoglobin and Myoglobin and Their Reactions with Ligands," North Holland Publishing Co., Amsterdam, 1971, p 229.

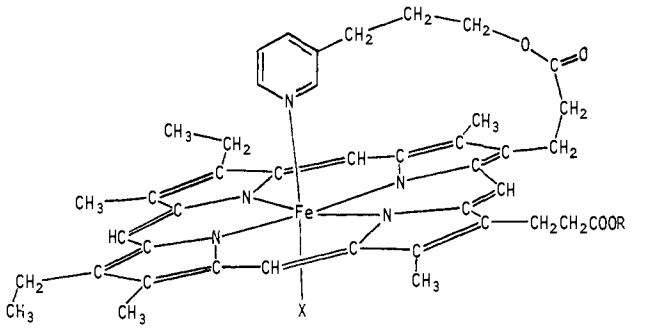


1a, X = no substituent or H_2O, Fe^{II}

1b, X = CO, Fe^{II}

1c, X = O_2, Fe^{II}

Figure 1. Structure of pyrroheme-*N*-[3-(1-imidazolyl)propyl]-amide and its derivatives.



2a, X = no ligand or H_2O, Fe^{II}, R = H

2b, X = CO, Fe^{II}, R = H

2c, X = the R group, Fe^{II}, R = $-CH_2CH_2CH_2$-(N)

2d, X = no ligand, Fe^{II}, R = $-CH_2CH_2CH_2$-(N)

Figure 2. Monopyridinepropanolmesoheme and its CO complex.

The most striking thing about this table is that a pyridine ligand, which brings about strong CO binding, is completely ineffective in oxygen binding.[12] Taking a minimum of 760 mm as the $P_{1/2}$ for **2a**, we see that changing pyridine to imidazole increases K^{O_2} by at least 760/0.2 = 3800.[14]

(12) Corwin and Bruck[13] observed that "treatment of either proto- or meso-hemochrome with an imidazole-saturated pyridine solution in air changed the intense hemochrome spectrum almost instantaneously into that of the corresponding hemochrome which had been oxygenated in the crystalline state. No such change was observed when pyridine without dissolved imidazole was used." This interesting "imidazole" effect can now be better understood in terms of the π basicity effect. However, their oxygen complexes were obtained from heme-(base)$_2$ complexes whereas ours are formed from heme–base, *five-liganded* compounds analogous to myoglobin.

(13) A. H. Corwin and S. D. Bruck, *J. Amer. Chem. Soc.*, **80**, 4736 (1958).

(14) The small difference of the porphyrin nucleus between mesoheme and pyrroheme should not have significant contribution to the binding constant. In fact, mesoheme is known to have higher affinity toward gaseous ligands than does deuteroheme, and this would imply an even greater O_2 binding ratio between **1a** and **2a**.

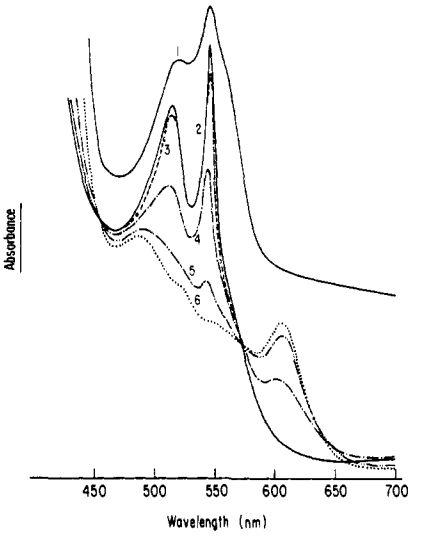


Figure 3. Changes in absorption spectra during oxidation of Py-mesoheme **2a** in CH_2Cl_2: 1, at 25°, *in vacuo*; 2, at −45°, *in vacuo*; 3, 30 sec after reaction with 1 atm of O_2 at −45°; 4, after 1.5 min; 5, after 3 min; 6, after 5 min.

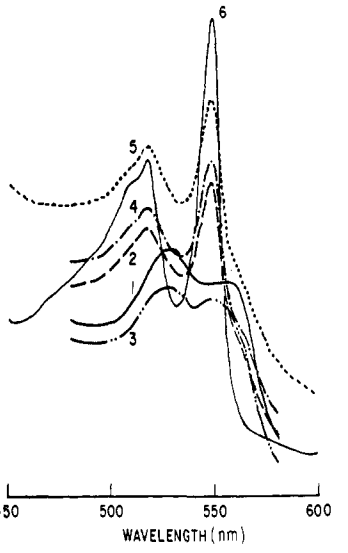


Figure 4. Spectra of hemochromes imbedded in polystyrene films: 1, original CO–heme–Py film; 2, flushed with argon for 1 hr; 3, regenerated CO complex; 4, flushed with argon again for 0.5 hr; 5, exposed in pure O_2 for 0.5 hr; 6, closed film. These curves and curve 1 of Figure 3 are vertically displaced for clarity.

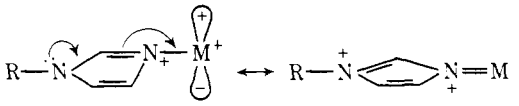
To further illustrate this ability of imidazole to favor O_2 over CO, we incorporated compounds **1a** and **2c**[2] into polystyrene films. The films of **2c** were prepared in two ways. (1) "Closed film," a benzene solution containing 10^{-4} *M* oxidized **2c** and about 10% polystyrene, was reduced with dithionite solution, dried, and evaporated under vacuum. The spectrum of this film is shown in Figure 4, curve 6. This spectrum was unchanged by exposure to carbon monoxide or oxygen, indicating a very immobilized hexacoordinate Fe^{II} compound. (2) "Open film," a solution like that for the closed film, was treated with carbon monoxide to give the typical pyridine–Fe–CO spectrum similar to that shown in Figure 4, curve 1. After evaporation of the benzene with a stream of carbon monoxide the Figure 4, curve 1 spectrum was obtained. Purging this dry film with argon removed carbon monoxide

leaving a pentacoordinate Fe^{II} compound, **2d**, curve 2. The rest of the spectra in Figure 4 clearly show that this pentacoordinate iron, which reversibly binds carbon monoxide, is neither oxygenated nor oxidized by oxygen. The film of **1a** was prepared by the same procedure as for the "open film" of **2c** and its ability to bind oxygen has been reported previously.[8a]

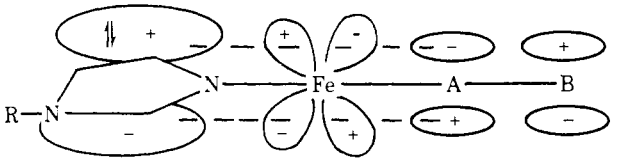
Thus, both the pyridine film **2d** and the imidazole film **1a** bound CO reversibly and both films were stable indefinitely toward oxidation by 1 atm of oxygen.[15] However, the pyridine compound **2d** in such a film showed no tendency to bind oxygen whereas the film of **1a** bound oxygen just as it did at −45° in methylene chloride[8b] or as a pure solid compound.[8a] These results imply that the factors influencing oxidation and binding are not necessarily the same. Having prepared a simple, stable heme–oxygen complex, we can now study these factors in detail.

The fact that our compound **1a** binds oxygen and carbon monoxide almost as strongly as does myoglobin tells us that the neighboring group effect plus the basic nature of imidazole are the most important attributes of the myoglobin protein.

Although **1a** and **2a** have similar affinities for CO, they differ by at least 3800 in affinity for O_2. A possible explanation of the extraordinary differentiation between O_2 and CO by the imidazole compound might be in the π basicity (sideways basicity) of imidazole as discussed by Wang for hemes[3a] and described more generally elsewhere.[16]



We suggest, in addition, that it is the unusually large π basicity of imidazole compared to other π bases serving to make the iron back-bond more strongly with the empty electronegative π^* orbitals of oxygen, which is important.



This π back-bonding to AB becomes more important as the σ basicity of the ligand AB decreases and the π electronegativity of AB increases. Since the σ basicity (protic basicity) decreases in the order CO > O_2, we might expect O_2 to depend more strongly upon the π basicity of the proximal base than does CO. This, rather than a solvent effect, is probably responsible for the difference in selectivity between NO and CO displayed by myoglobin and by the piperidine–heme complexes reported recently.[17-19]

(15) These results are reminiscent of the results reported for another model by J. H. Wang, *J. Amer. Chem. Soc.*, **80**, 3168 (1958).

(16) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," Wiley, New York, N. Y., 1958, pp 183–185.

(17) D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *J. Amer. Chem. Soc.*, **95**, 4087 (1973), and references given therein.

(18) Our results could also explain the instability of the pyridine–Fe^{II}–O_2 complex recently reported by Baldwin and Huff[19] although it is difficult to compare their nonheme iron complex with iron porphyrins.

(19) J. E. Baldwin and J. Huff, *J. Amer. Chem. Soc.*, **95**, 5757 (1973).

However, there is an important solvent effect on oxidation rates which we will discuss separately.

C. K. Chang, T. G. Traylor*

Department of Chemistry, Revelle College
University of California, San Diego
La Jolla, California 92037

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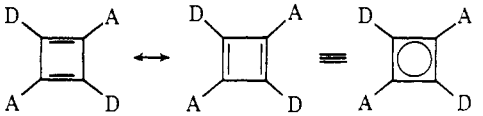
Rapid Double Bond Shift in a Donor–Acceptor Substituted Cyclobutadiene. Evidence from 584-Å and X-Ray Photoelectron Spectroscopy

Sir:

In contrast to cyclobutadiene, whose fleeting existence has thus far precluded a determination of its ground-state geometry and spin multiplicity, donor–acceptor substituted cyclobutadienes[1] are perfectly stable molecules whose chemistry is now well established.[2] The available data regarding their structure are considerably more varied.

Both the observed equivalence of the N–CH_2 proton nmr signals down to −46° (for A = $COOC_2H_5$, D = $N(C_2H_5)_2$) and HMO calculations, which yield identical π bond orders within the four-membered ring, point to the resonance hybrid shown below.



1

A recently published X-ray structure analysis for **1** appears to substantiate this formulation in that all ring CC bond lengths were found to be equal.[3]

However, the poor agreement factor reported in this study (R = 0.12) leaves doubts as to the correctness of the proposed structure. These doubts are reinforced by the anomalous uv spectrum[4] of **1**; the long wavelength transition, observable over a span of 8000 cm^{-1} (λ_{max} 25,500 cm^{-1}, ϵ_{max} 2.37), is strongly nonvertical, indicating that the ground- and first excited-state potential surfaces are markedly different. Since **1** is formally a nonbenzenoid hydrocarbon, it is tempting to attribute this disparity to the effect of double bond fixation operating in the ground state. MINDO/2 calculations by Weiss and Murrell[5] support this view and indicate a low barrier for the interconversion of the energetically equivalent valence tautomers.

In order to throw light on these apparently inconsistent results, the 584-Å and X-ray photoelectron spectra of **1** have been measured and compared with those of the

(1) R. Gompper and G. Seybold, *Angew. Chem., Int. Ed. Engl.*, 7, 824 (1968); M. Neuenschwander and A. Niederhauser, *Helv. Chim. Acta*, **53**, 519 (1970).

(2) R. Gompper and G. Seybold in "Aromaticity, Pseudo-Aromaticity, Anti-Aromaticity," E. D. Bergmann and B. Pullman, Ed., The Israel Academy of Sciences and Humanities, Jerusalem, 1971, p 215; R. Gompper and G. Seybold in "Topics in Nonbenzenoid Aromatic Chemistry," in press.

(3) H. J. Lindner and B. v. Gross, *Angew. Chem., Int. Ed. Engl.*, **10**, 490 (1971). Professor Lindner has informed us that further refinement is in progress and will soon be published.

(4) G. Seybold, Ph.D. Thesis, Universität München, 1969.

(5) R. Weiss and J. N. Murrell, *Tetrahedron*, **27**, 2877 (1971).