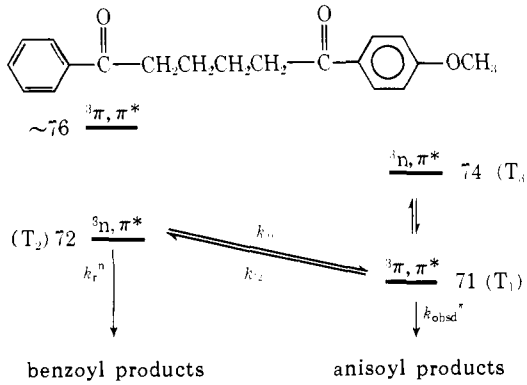
Scheme I



measured for γ-hydrogen abstraction by triplet 1,4-dibenzoylbutane.[8] The slightly endothermic energy transfer from anisoyl to benzoyl should then proceed at a rate $>10^8$ sec^{-1}, much faster than the rate of decay of *p*-methoxyphenyl ketones.[5] Therefore, we expected that the lowest triplets of the two chromophores in **1** might approach thermal equilibrium before undergoing significant reaction or decay. The results verify this supposition. Most of the photoproducts of **1** clearly arise from the molecule's second triplet. Since the anisoyl group has double the extinction coefficient of the benzoyl group at 313 nm, T_2 must be populated by thermal activation of the lowest triplet. Furthermore, the comparable quenching efficiencies toward the two chromophores indicate that the two lowest triplets are nearly equilibrated, since they display almost the same lifetime.[14]

If, for the moment, we ignore the problem of whether T_1 and T_3 are equilibrated, we can first determine whether the results are quantitatively consistent with excitation being equilibrated between the two chromophores. Under conditions such that T_1 and T_2 are totally equilibrated, the relative yields of products derived from γ-hydrogen abstraction by the two aroyl groups are described by eq 1. The various rate constants are defined in Scheme I. The value of the rate ratio k_r^n/k_{obsd}^π has been shown to average 200/1 for several pairs

$$\frac{\Phi \text{ benzoyl}}{\Phi \text{ anisoyl}} = \frac{\chi_2 k_r^n}{\chi_2 k_{obsd}^\pi} \quad (1)$$

$$\chi_2 = 1 - \chi_1 = k_{12}/(k_{12} + k_{21}) \quad (2)$$

of ketones at 25°.[5] Since the quenching data indicate that T_1 and T_2 are very nearly equilibrated, the observed product ratios indicate a χ_2 value of 0.09 at room temperature and thus a 1.4 kcal energetic difference between T_1 and T_2, well within the range of uncertainties in our Scheme I estimate.

If we now return to the problem of equilibration of excited states within the *p*-methoxybenzoyl chromophore, the validity of our previous conclusion[5] that such ketones react from equilibrium concentrations of their n,π* triplets depends on reversible internal conversion of the two triplets being much faster than any irreversible decay modes of either individual triplet. Our present observation that *inter*chromophore energy transfer is sufficiently rapid to allow almost complete equilibration before any chemical reaction certainly lends support to the supposition of rapid *intra*chromophore energy transfer. The temperature effects observed for **1** also are consistent with the interpretation that the anisoyl derived products arise from T_3. An Arrhenius plot of the data in Table I yields a $\Delta\Delta H^{\ddagger}$ of 1.70 ± 0.05 kcal/mol and a $\Delta\Delta S^{\ddagger}$ of 0. If all the chemistry of triplet **1** arises from two n,π* triplets 1.7 kcal apart, each of which abstracts a similar secondary hydrogen with the same activation energy (~4 kcal/mol),[15] then T_3 and T_1 are indeed 3 kcal apart, as previously deduced.[5]

Although these temperature effects are nicely consistent with the equilibrium description of reactivity, they alone cannot rule out the possibility that T_1 actually reacts with a coincidental 3 kcal higher activation energy than T_2. The only evidence *against* reaction from the π,π* triplet, perhaps induced by vibronic coupling, is that presented in our previous papers.[5]

(14) P. J. Wagner, *Mol. Photochem.*, 3, 23 (1971).

(15) J. C. Scaiano, J. Grotewold, and C. M. Previtali, *J. Chem. Soc., Chem. Commun.*, 390 (1972); (b) F. D. Lewis, *Mol. Photochem.*, **4**, 501 (1972); (c) L. Giering, M. Berger, and C. Steel, paper submitted to *J. Amer. Chem. Soc.* We thank Professors Lewis and Steel for informing us of their results prior to their publication.

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Neighboring Group Effect in Heme–Carbon Monoxide Bonding[1,2]

Sir:

One of the factors contributing to the stability of oxymyoglobin relative to oxyheme is thought to be that the protein holds the imidazole "snugly in place"[3] on the iron. This implies some special stability to the geometrically positioned proximal imidazole group relative to the binding of free imidazole to iron in a simple heme–base interaction.[4] In order to quantitatively probe this "chelating" or "neighboring group" effect, we have prepared a series of simple heme compounds having the base covalently attached to a side chain on the porphyrin in such a way as to lead to approximately the same base-to-heme geometry as that in myoglobin.[6] Typical structures are shown in Figure 1.

Compounds **1** and **2** were synthesized by coupling mesoporphyrin IX to one equivalent mole of 3-(3-pyridyl)-1-propanol *via* acid chloride, followed by tlc purification and iron insertion. Compound **3** was then prepared from peptide coupling of **2** to the requisite amine by DCC method.[7,8] All compounds had nmr,

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

(2) Previous papers in this series are (a) H. Diekmann, C. Chang, and T. G. Traylor, *J. Amer. Chem. Soc.*, **93**, 4068 (1971); (b) C. K. Chang and T. G. Traylor, *ibid.*, **95**, 5810 (1973).

(3) J. H. Wang in "Heamatin Enzymes," Part 1, J. E. Falk, R. Lemberg, and R. K. Morton, Ed., Pergamon Press, New York, N. Y., 1961, p 98.

(4) Other factors such as the nature of the proximal base and the solvent[5] or hydrophobic pocket effect will be discussed in subsequent papers. See also ref 2b.

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

(6) C. K. Chang and T. G. Traylor, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2647 (1973).

(7) 3-(3-Pyridyl)-1-propanol was obtained from Aldrich and redistilled over calcium hydride before use. 4-(Aminomethyl)imidazole·2HCl was synthesized according to Turner's procedure, mp 243°.[8]

(8) R. A. Turner, C. F. Huebner, and C. R. Scholz, *J. Amer. Chem. Soc.*, **71**, 2801 (1949).

Table I. Equilibria of Base-Hemochromes and Carbon Monoxide

Compound	Conditions	k^{CO} (M^{-1})[a]	$P_{1/2}^{CO}$ (mm)	Ref
Mesoheme Me ester + pyridine	[Heme] = 3.5×10^{-5} M; [Py] = 1.3 M; Temp = 20°	7,950	12.5	This work, 9
Deuteroheme Me ester + pyridine		3,500	28	
Protoheme Me ester + pyridine		2,950	33	
Protoheme	[Heme] = 10^{-4} M in 0.002 N KOH; Temp = ~24°		2.4	10a
Protoheme + pyridine	[Heme] = 5×10^{-5} M; pH 11, temp = 21–24°			
	[Py] = 5×10^{-5} M		0.8	10b
	[Py] = 1×10^{-4} M		0.4	
	[Py] = 5×10^{-4} M		0.29	
Py-mesoheme-py (**1**)	[Heme] = 5×10^{-5} M in $CHCl_3$; Temp = 23°	82,000	1.1	This work
Py-mesoheme (**2**)		440,000	0.20	
Py-mesoheme-Im (**3**)		750,000	0.12	
Protoheme + piperidine	In piperidine, 23°		5.8	5
Horse myoglobin	pH 7.0, 20°	22×10^6	0.034	11a
Sperm whale myoglobin	pH 8.6, 25°	39×10^6	0.021	11a

[a] Conversion of the values of $P_{1/2}$ into the equilibrium constant K (M^{-1}) involves the solubility coefficients of the gases, for example, at 25° 1 Torr of CO gives concentrations of 1.24×10^{-6} M in H_2O, 9.93×10^{-6} M in benzene, or 11.1×10^{-6} M in $CHCl_3$.

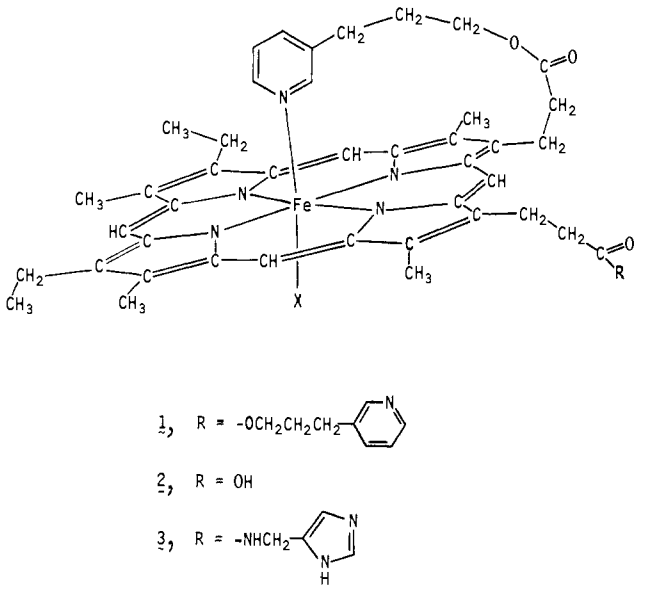

Figure 1. In the **1-3** series, X = no substituent, H_2O, or second base, Fe^{II}. In the **1**-CO-**3**-CO series, X = CO and Fe^{II}.

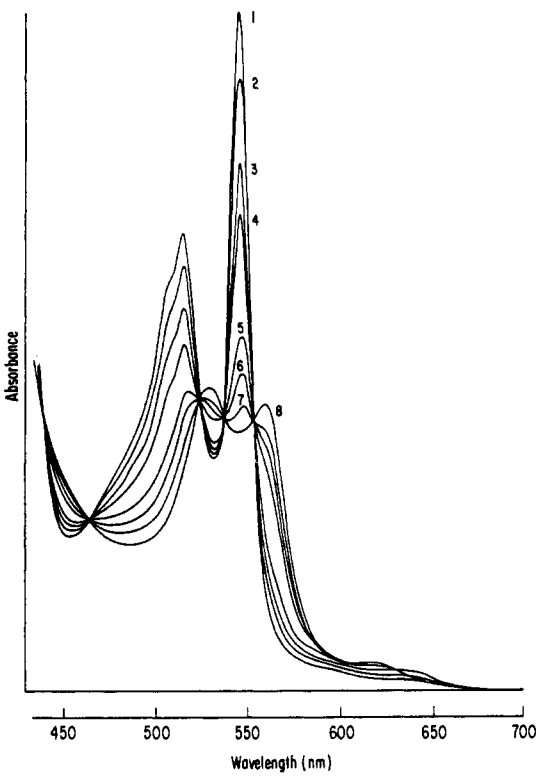

Figure 2. CO titration plot for Py_2-mesoheme (**1**) [CO] = 0, 0.11, 0.34, 0.57, 3.0, 5.6, 10.5, and 760 mm for curves 1–8, respectively.

ir, and C, H analyses consistent with the indicated structures.

The carbon monoxide affinities as $P_{1/2}$, the pressure for half-saturation of the hemes, or as K for the equilibrium

$$\text{base-heme} + CO \overset{K}{\rightleftharpoons} \text{base-heme-CO} \qquad (1)$$

are listed in Table I along with those of external base complexes formed from a variety of hemes and the indicated base. The half-saturation values were determined by the usual visible spectra procedures.[11b] Figure 2 shows typical CO titration spectra for these measurements.

The effect of having the pyridine covalently attached to mesohemin appears first upon reduction of a 5×10^{-5} M solution of the dipyridine compound **1**. A clean dipyridine hemochrome spectrum, independent of concentration, is observed.[12] The external pyridine equilibrium constant is such as to require about 5×10^{-2} M to achieve a complete dipyridine hemochrome spectrum.[14] This observation indicates that these hemochromes are not aggregated or polymerized, be-

(9) W. S. Caughey, J. O. Alben, and C. A. Beaudreau, "Oxidases and Related Redox Systems," T. E. King, H. S. Mason, and M. Morrison, Ed., Wiley, New York, N. Y., 1965, p 97.

(10) (a) J. H. Wang, A. Nakahara, and E. B. Fleischer, *J. Amer. Chem. Soc.*, **80**, 1109 (1958); (b) A. Nakahara and J. H. Wang, *ibid.*, **80**, 6526 (1958).

(11) (a) F. Antonini and M. Brunori, "Hemoglobin and Myoglobin and Their Reactions with Ligands," North Holland Publishing Co., Amsterdam, 1971, p 221; (b) p 170; (c) p 23.

(12) Absorption spectrum of **1**: α band (547 nm, $\epsilon m M$ = 28) and β band (517 nm, $\epsilon m M$ = 18.6). The ratio α/β = 1.5 is comparable to that of pyridine mesohemochrome.[13]

(13) P. K. Warme and L. P. Hager, *Biochemistry*, **9**, 1599 (1970).

(14) The bis-pyridine complex **1** is much more stable toward oxidation in nonpolar solvents than are the simple dipyridine hemochromes.[9]

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.

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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.