Brookhart,[13] in general these complexes readily rearrange to the more stable U conformation. However, for the (1,1,5-trimethylpentadienyl)iron tricarbonyl cation, steric interactions between substituents on the 1- and 5-positions lead to an equilibrium between $\eta^5$-bound U and S conformations.[13c] However, the 2,4-dimethylpentadienyl group is known to favor the U conformation when bound to lithium, potassium, or magnesium[14,15] (contrary to other pentadienyl groups used in the above-mentioned studies). Thus, while the 1,1,5-trimethylpentadienyl ligand bound to $Fe(CO)_3^+$ *partially* adopts the S conformation *due to* its particular substituents, one of the 2,4-dimethylpentadienyl ligands in $Mo(2,4\text{-}C_7H_{11})_2(PEt_3)$ is *totally* present in the S form *despite* an inherent bias for the U conformation. Clearly a powerful driving force is operative here in favor of the unusual $\eta^5$-"S" form. Exactly why this preference occurs here is not clear, however. Molybdenum is smaller than zirconium or niobium (the average M-P bond distances are 2.520 (3), 2.721 (2), and 2.628 (2) Å, while the average M-C bond distances involving the "U" 2,4-$C_7H_{11}$ ligands are 2.309 (6), 2.460 (3), and 2.399 (2) Å, respectively), and thus the greater interligand repulsions in a syn-eclipsed "U" structure could be reduced if one ligand adopted the "S" conformation. However, the marked contrast between the zirconium or niobium complexes and the molybdenum complex, particularly the apparent absence of any S ⇄ U equilibria,[16] may be indicative of some electronic influences. In this regard, the present situation is reminiscent of the occurrence of $\eta^4$-butadiene complexes involving both cis and trans conformations.[17] Much more remains to be learned about these systems, and further studies are under way.

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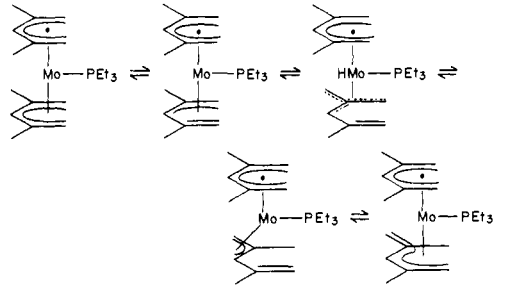
(16) (a) It might be expected that the implementation of a more normal pentadienyl ligand, i.e., one for which the S conformation is favored relative to the U conformation, might lead to an $\eta^5$-bound S pentadienyl ligand for the less crowded zirconium and/or niobium compounds. Preliminary $^1H$ and $^{13}C$ NMR data for $Zr(C_5H_7)_2(P(C_2H_5)_3)$ indicate that two $\eta^5$-"U"-pentadienyl ligands are present; however, the situation regarding niobium has not been resolved yet. (b) While the most obvious way to form the $\eta^5$-"S"-pentadienyl ligand would involve simple rotation around an "internal" carbon-carbon bond of the pentadienyl fragment, alternatives do exist, e.g.:




Similar reactions are known for (pentadienyl)cobalt compounds.[16c] (c) Wilson, D. R.; Ernst, R. D.; Kralik, M. S. *Organometallics* **1984**, *3*, 1442.

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**Registry No.** $[ZrCl_3(P(C_2H_5)_3)_2]_2$, 77061-31-5; $ZrCl_4(P(C_2H_5)_3)_2$, 77061-35-9; $NbCl_4(P(C_2H_5)_3)_2$, 73135-97-4; $MoCl_4(P(C_2H_5)_3)_2$, 61567-15-5; $Zr(2,4\text{-}C_7H_{11})_2(P(C_2H_5)_3)$, 97551-48-9; $Nb(2,4\text{-}C_7H_{11})_2(P(C_2H_5)_3)$, 97551-49-0; $Mo(2,4\text{-}C_7H_{11})_2(P(C_2H_5)_3)$, 97551-50-3; $Zr(C_5H_7)_2(P(C_2H_5)_3)$, 97551-51-4; 2,4-$C_7H_{11}$, 78395-91-2.

**Supplementary Material Available:** A listing of the positional and bonding parameters for the non-hydrogen atoms of the zirconium, niobium, and molybdenum compounds and a description of pertinent physical and spectroscopic data for these compounds (8 pages). Ordering information is given on any current masthead page.

## Control of Heme Protein Redox Potential and Reduction Rate: Linear Free Energy Relation between Potential and Ferric Spin State Equilibrium

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The cytochrome $P\text{-}450_{cam}$ monooxygenase system isolated from *Pseudomonas putida* grown on camphor is an ideal system to study the regulation of electron transfer between two redox centers on separate protein molecules. Cytochrome P-450 catalyzed monooxygenation reactions require an external source of two electrons ($SH + O_2 + 2e^- + 2H^+ \rightarrow SOH + H_2O$), which are transferred from NADH to the heme iron of cytochrome $P\text{-}450_{cam}$ through the combined action of the FAD-flavoprotein putidaredoxin reductase and the iron-sulfur protein putidaredoxin.[1] The mechanisms of regulation of the first electron-transfer step (ferric to ferrous reduction) in the various P-450 systems has been the subject of intense interest.[2] In the case of the camphor monooxygenase system, camphor binding produces dramatic changes in the visible and near-UV regions of the heme optical spectrum.[1] Mössbauer[3] and electron spin resonance studies[4] have linked these optical changes to a shift in the spin state of the heme iron from a low spin ($S = {}^1/_2$) configuration in the absence of substrate to a high spin ($S = {}^5/_2$) configuration for the substrate-bound hemoprotein. This dynamic spin state change has also been observed for cytochrome P-450 model metalloporphyrin compounds.[5] In addition, experimentation has shown that camphor binding in-

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**Table I**

| substrate | $K_D$, μM | $K_{spin}$ | $E_{obsd}$, mV | $k_F$, $s^{-1}$ | $\Delta E_A$, meV |
|---|---|---|---|---|---|
| TMCH | 34.0 | 0.28 | −242 ± 5 | 0.7 | −121 |
| norcamphor | 150.0 | 0.85 | −206 ± 9 | 5.1 | −72 |
| *d*-fenchone | 17.5 | 1.30 | −208 ± 10 | 6.0 | −68 |
| *d*-3-bromocamphor | 48.0 | 3.0 | −197 ± 10 | 8.0 | −60 |
| *l*-camphoroquinone | 12.5 | 6.7 | −183 ± 15 | 30.0 | −26 |
| adamantanone | 1.3 | 24.0 | −175 ± 15 | 43.0 | −17 |
| *d*-camphor | 0.84 | 32.0 | −170 ± 11 | 84.0 | |
| no substrate | | 0.08 | −303 ± 10 | 0.15 | −163 |

creases the reduction potential for the ferric/ferrous couple by about 125 mV.[2f] We report herein investigations of a variety of camphor analogues which vary the free energies of substrate binding, ferric spin state, and redox equilibria. All of the compounds listed in Table I were found to bind to cytochrome P-450$_{cam}$ as judged by the characteristic changes in the optical spectrum yielding the dissociation constants ($K_D$) and spin-state equilibrium constants ($K_{spin}$) of substrate-saturated hemoprotein that are listed.[6] In all cases, single-site saturation of the hemoprotein was observed. These results show that $K_{spin}$ and $K_D$ are independent quantities. For example, 3-bromocamphor binding is characterized by a dissociation constant of 48 μM and saturated hemoprotein is 75% high spin. On the other hand, tetramethylcyclohexanone binds with essentially the same dissociation constant (34 μM); however, the protein is only 22% high spin when substrate is saturating. Thermodynamically this result indicates that tetramethylcyclohexanone binds less selectively than 3-bromocamphor to the two spin-state conformers of cytochrome P-450$_{cam}$.[7]

A long-standing question in hemoprotein research has been the factors that control the reduction potential for the Fe(III)/Fe(II) couple in protoporphyrin IX chelates. Due to the difference in net charge of Fe(III) and Fe(II), the local dielectric constant can play an important role.[9] However, metal ligand effects are perhaps more important.[10] Since the ligand field is expected to also contribute to the ferric spin-state equilibrium position, one might expect a correlation between the ferric spin state and redox potential in P-450 hemoproteins. A complete quantitative treatment of this question has been hampered by the heretofore lack of cases where the spin state is intermediate between a predominantly high spin and a predominantly low spin value.[2a] Measurement of the Fe(III)/Fe(II) reduction rate and equilibrium potential, $E_{obsd}$, of cytochrome P-450$_{cam}$ saturated with various substrate analogues, Table I, was carried out under anaerobic conditions by stopped flow spectrophotometry with putidaredoxin as reductant.[2i,j] Under the conditions employed, the reaction monitored is electron transfer between putidaredoxin and cytochrome P-450$_{cam}$ in the dienzyme complex: $Pd^r{\cdot}P\text{-}450_{cam}{}^o \rightleftharpoons Pd^o{\cdot}P\text{-}450^r$. Activation energies for the ferric–ferrous reduction of the P-450$_{cam}$ heme are reported relative to that for the camphor-saturated protein at 25 °C. All reduction potential determinations and the observed strictly monophasic reduction kinetics followed theoretical predictions closely with no evidence for irreversible protein destruction. Figure 1 shows a plot of $E_{obsd}$ vs. the free energy change of the ferric spin state equilibrium $\Delta G = -(RT)\ln(K_{spin})$. These results demonstrate an excellent linear free energy relationship between the reduction potential and the free energy change of the ferric spin equilibrium. Thus, despite the lack of any correlation between substrate affinity and the ferric spin state equilibrium, the observed redox potential of cytochrome P-450 displays a striking linear free energy relationship with the ferric spin state equilibrium, suggesting that the highly distorted heme environment of cytochrome P-450 allows utilization of the ligand field stabilization energy to regulate the oxidation–reduction potential of the center in response to substrate binding. This linear free energy relationship also extends to the activation energy for the ferric–ferrous reduction rate for P-450$_{cam}$, demonstrating kinetic control of electron input to the heme center.

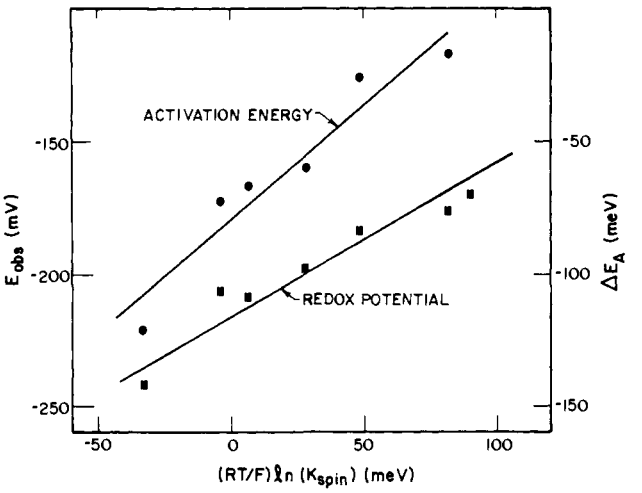


**Figure 1.** Free energy correlation between the P-450$_{cam}$ ferric spin state equilibrium and the redox potential and activation energy of the ferric–ferrous reduction rate. Oxidation–reduction potential of the P-450$_{cam}$ heme center was determined from the forward- and back-electron-transfer rates in a enzyme complex of putidaredoxin and cytochrome P-450$_{cam}$. The reduction potential of putidaredoxin when complexed to P-450$_{cam}$ is −196 mV.[2f] Differential activation energies for the ferric–ferrous reduction of P-450$_{cam}$, $Pd^r{\cdot}P\text{-}450_{cam}{}^o \rightarrow Pd^o{\cdot}P\text{-}450_{cam}{}^r$, were obtained from the kinetic data in Table I by using $k = AE^{-E_A/RT}$ and are presented relative to the activation energy obtained with camphor bound cytochrome and assume identical pre-exponential factors.

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(6) Difference spectra are characterized by a peak at 390 nm, trough at 417 nm, and isosbestic point at 406 nm. Dissociation constants were determined by iterative curve fitting using the free substrate concentration and maximal absorbance change, $\Delta A_{max}$.[2a] Spin-state equilibrium constants were determined from $\Delta A_{max}$ values relative to that obtained with camphor as substrate, $\Delta A'_{max}$, via

$$\frac{\Delta A'_{max}}{\Delta A_{max}} = \frac{K_0 - K'_{spin}}{K_0 - K_{spin}} \frac{1 + K_{spin}}{1 + K'_{spin}}$$

where $K_0$ is the spin-state equilibrium constant for cytochrome P-450$_{cam}$ in the absence of substrate and has a value of 0.084 at 20 °C.[2a] $K'_{spin}$ and $K_{spin}$ are the spin-state equilibrium constants for camphor-saturated and camphor-analogue-saturated cytochrome, respectively.

(7) A variety of spectroscopic studies[3,8] of camphor-bound cytochrome P-450$_{cam}$ and high spin Fe(III) model compounds strongly suggest that these species are primarily a five-coordinate heme iron, with thiolate as the only axial ligand. Low spin P-450 complexes are thought to have water as a sixth ligand. thus, the spin-state change observed on substrate binding is not due to the direct ligation of camphor to the metal. A likely explanation is that the high and low spin forms of ferric enzyme represent two conformational states of the protein and with substrate binding the relative populations of the conformers change.

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