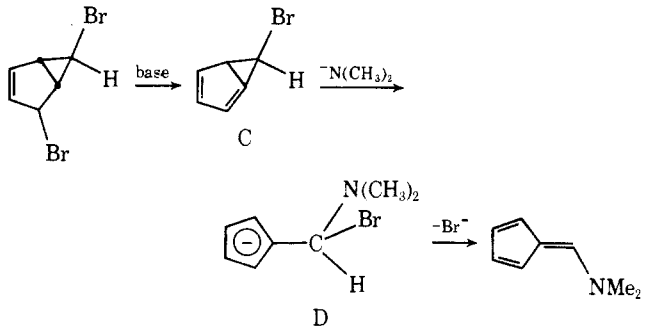
Scheme II



[3.1.0]hexadiene, C, opens the highly strained cyclopropane ring to give the intermediate D. Rapid elimination of Br^- yields the 6-substituted fulvene.

Acknowledgments. We thank Professor F. Mallory for suggesting the nucleophilic attack at the cyclopropane ring. We are grateful to the Research Foundation of the City University of New York for financial support of this work (FRAP Award No. 10596).

Supplementary Material Available. Tables II (NMR spectra) and III (mass spectra) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-5946.

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Ferrous Porphyrin–Mercaptide Complexes. Models for Reduced Cytochrome P-450

Sir:

Cytochrome P-450, a protoheme-containing monooxygenase system gives, upon reduction in the presence of CO, an anomalous Soret band at about 450 nm. During the enzymatic cycle of cytochrome P-450 ferric cytochrome P-450 first combines with a substrate, followed by a one-electron reduction to form a ferrous cytochrome P-450–substrate complex which can bind either oxygen or CO reversibly.[1-3] It is suggested that the "activated" oxygen, formed after the addition of the second electron to the O_2–P-450 complex, interacts with the substrate to give rise to hydroxylated product, water, and ferric cytochrome P-450. Thus, cytochrome P-450 not only functions as an electron transporter but resembles the oxygen carriers hemoglobin and myoglobin, in terms of its capability toward O_2 binding.

The axial ligands of the heme iron in cytochrome P-450 are of great interest, since they hold the key to our understanding of the enzymic function and the underlying principles that enable the single complex protoheme to perform various functions ranging from oxygen transport, oxidation catalysis, to electron transport. The possibility of axial sulfur ligation in cytochrome P-450 has been repeatedly expressed in the literature based on EPR evidence.[3-5] Although well-characterized five-coordinate thiolates of ferric protoporphyrin dimethyl ester (Fe^{III}PPDME) and *meso*-tetraphenylporphyrin (Fe^{III}TPP) have been prepared and shown to exhibit similar EPR parameters to those of high-spin ferric cytochrome P-450,[6,7] model studies on sulfur ligated ferrous porphyrins have been scarce, and the CO-P-450 type spectrum is difficult to duplicate.[8,9] Recently, Stern and Peisach[10] reported that the combination of reduced heme, thiol, CO, and a strong base under stringent mixing procedure could result in the partial appearance of a 450-nm Soret peak, thus implying a mercaptide anion at the fifth coordination site. However, the failure to produce a complete 450-nm peak as well as the inability to duplicate similar results by sequential addition of reagents was puzzling and has been attributed to the "transient nature" of the intermediate species. We chose to study the interaction between ferrous porphyrins and thiols in order to assess the role of sulfur ligands in cytochrome P-450.

Under basic conditions, thiols readily reduce Fe(III) porphyrins to Fe(II) porphyrins. When a protohemin–DMSO solution (~10 μM) was mixed with an equal volume of a solution of *n*-BuSH (1 M)–NMe_4OH (2 M) in EtOH, under CO, the visible spectrum exhibited two Soret peaks at 450 (50%) and 412 nm (50%). The ratio of the two was independent of the mode or temperature of mixing but somewhat dependent on the concentration of thiol or base at low concentrations. With the same procedure, mesoheme gave predominantly the normal CO–mesoheme Soret peak at 408 nm (95%) and a small shoulder at about 440 nm, while 2,4-diacetyldeuteroheme resulted in an intense peak at 470 nm with less than 10% of the normal peak at 430 nm.[11] DMSO as solvent appears to be important to bring about the long wavelength Soret peak.[12] We have used several other bases and find that their ability to produce this spectrum follows the order: KH > NaH, $NMe_4OH \gg$ *n*-BuLi, 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU), 1,8-bis(dimethylamino)naphthalene (Proton Sponge). These findings suggest that the interaction between RS^- and Fe(II) porphyrin is weak and occurs only under the most thermodynamically favorable conditions, when interaction between the mercaptide and counterion is minimal. This situation can be likened to the CN^-–Fe(II) porphyrin interaction in which the repulsion between the negatively charged ion and the high charge density at the iron engenders extremely low binding affinity. It is expected that the peripheral electronic effect of porphyrin and the solvation and/or ion dissociation of mercaptide salt in solution become very crucial here.

To enhance the activity of the mercaptide anion, crown ether complexes[13,14] of potassium *n*-butyl mercaptide were used. Under these conditions we observed 100% conversion to the long wavelength Soret band with protoheme and 2,4-diacetyldeuteroheme and about 95% with mesoheme in DMSO as solvent. The reagent was prepared by stirring the mercaptide salt (~0.5 M) in DMSO under argon or CO and then adding dibenzo-18-crown-6[15] (~0.2 M). The CO binding to the heme is reversible and can be achieved in

Table I. Absorption Maxima of Fe(II) Hemeproteins and Model Compounds

	P-450[a] substrate free		P-450 + S[a]		FePPDME–BuSK–crown ether in DMSO[b]		Sperm whale Mb[c]		FePPDME–Bu-imidazole in DMF[d]	FePPDME in DMSO
	λ, nm	ε, m*M*	λ, nm	ε, m*M*	λ, nm	ε, m*M*	λ, nm	ε, m*M*	λ, nm	λ, nm
Reduced	411	(71)	408	(73)	408	(104)	434	(115)	419	421
(deoxy)	540	(14)	540	(14)	540	(17)	556	(11.8)	528	522
					560				558	553
+ CO			447[e]	(106)	458[f]	(88)	423	(187)	420	412
			550	(12)	552	(15)	542	(14)	535	534
							579	(12.2)	565	564
$+O_2$			418	(62)	428	(148)	418	(128)	417	
			552	(14)	551	(18)	543	(13.6)	536	
			580		581	(15)	581	(14.6)	572	

[a] Bacterial cytochrome P-450, with D-camphor (50 μ*M*) as substrate, ref 3. [b] The spectrum with O_2 was recorded 1 min after exposure to O_2. [c] E. Antonini and M. Brunori, "Hemoglobin and Myoglobin in their Reactions with Ligands", North Holland Publishing Co., Amsterdam, 1971, p 19. [d] Reference 16, oxygenated spectrum was recorded at −45°. [e] Wavelength varies from 446 to 451 nm, depending on the source and preparation. [f] Wavelength varies from 449 to 458 nm, depending on solvent and thiol; see text.

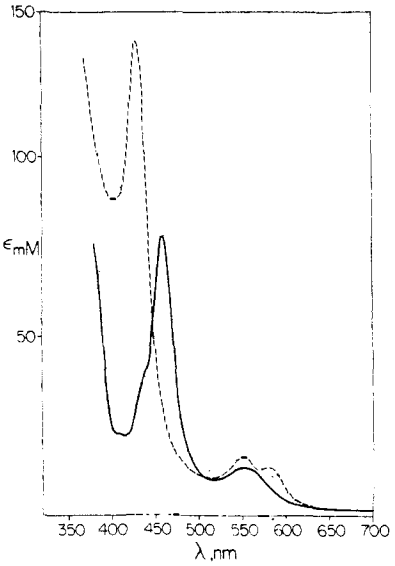

Figure 1. Visible absorption spectra of Fe(II) protoporphyrin IX dimethyl ester with a mixture of *n*-BuSK and dibenzo-18-crown-6 in DMSO: (a,—) in the presence of 1 atm of CO; (b,· · · under argon or in vacuo; (c,- - -) 1 min after b was exposed to O_2. All spectra were recorded at 23°.

three different ways. (1) A degassed DMSO solution of protohemin (or Fe^{III}PPDME) is reduced with either dithionite or Pd–CaH_2[16] under CO to give a typical 412-nm carboxy protoheme spectrum and then mixed with the mercaptide solution to afford curve a in Figure 1. (2) Protohemin in DMSO is mixed with the mercaptide solution under argon with or without prior reduction of the hemin to give a new spectral species with the Soret peak at 408 nm (curve b). Aliquots of CO can then be added until curve a is observed (clean isosbestic points were observed during this conversion). (3) Protohemin in DMSO is simply mixed with the mercaptide solution under CO to give curve a. CO can be removed by brief evacuation which results in the spectral change a → b. Both species with spectra a and b are diamagnetic at 300 K (μ_{eff} = 0)[17] and EPR quiet at 77 K, suggesting hexacoordinate low-spin Fe(II). We tentatively assign the species a to be [RS-Fe^{II}-CO]$^-$ and species b [RS-Fe^{II}-SR]$^{2-}$ or [RS-Fe^{II}-SRK]$^-$. The presence of a second mercaptide ion at the sixth site is suggested by CO-titration experiments which showed that with [RS] = 0.1 *M*, $P_{1/2}$ is 17 Torr, while with [RS] = 0.15 *M*, $P_{1/2}$ is 21 Torr. Similar ligand competition phenomena have been studied with N-base hemochrome and CO interactions.[18-20] Addition of pyridine to b results in total conversion to a typical dipyridine hemochrome spectrum.

Exposure of b to O_2 at 1 atm and 23° gave curve c. This spectral change is reversible (c → a) if the oxygen atmosphere is replaced by CO within 3 min after exposure to O_2.[21] The nature of this new spectral species c and its relationship to the oxygenated P-450 enzyme is subject to future clarification. The spectral resemblance of our model system to various forms of reduced cytochrome P-450 is given in Table I.

The 458-nm peak of a shifts to higher energy when butanol is added to DMSO. In 50/50 DMSO-BuOH the peak is at 449 nm with decreased ε (*M*).[22] This could relate to the dielectric constant of the solvent system as a similar observation has been reported.[10] The nature of thiol is also important. Alkyl thiols invariably give a peak at 458 nm in DMSO. Benzyl mercaptan brought about 90% conversion to a peak at 454 nm, while benzenethiol gave no evidence of the long wavelength Soret band. Since thiols can be oxidized to disulfides and thiol ethers by atmospheric oxygen and DMSO[23] we have deliberately substituted for butanethiol its oxidation products in the above system yet no P-450-like spectrum could be achieved. By similar substitution, the participation of phenoxide or carboxylate anions as the axial ligands for P-450, a situation which might arise in the protein complex, was also ruled out. Finally the possibility of DMSO or DMSO anion ligation is unlikely since a curve a type spectrum was obtained by using the crown ether complex of BuSK in DMF with protoheme (457 nm).[24]

These results are consistent with the assumption that the fifth axial ligand in cytochrome P-450 is a mercaptide anion of unusual coordinating power, probably a deprotonated cysteine residue.

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(21) When the species c was allowed to stand at room temperature under 1 atm of O_2 for longer period of time, it lost reversibility toward CO binding and was presumably oxidized to an Fe(III) species. The spectrum (curve c) also changed with time.
(22) The 412-nm Soret peak appeared when the *n*-BuOH content was higher than 20%. Nonpolar solvents were tried but results were inconclusive since mercaptide salt was precipitated.
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Aromatic Hydrocarbon–Diene Exciplexes. The Importance of Reversible Exciplex Formation

Sir:

The interaction of aromatic hydrocarbon excited singlet states with ground state dienes and olefins has attracted considerable interest in the past decade.[1-8] The initial observation of fluorescence quenching by Hammond and coworkers[1a] and subsequent observations of long-wavelength emission[3,4] and cycloaddition[5-8] have been attributed to the formation of an exciplex intermediate (eq 1).[1-9]

$$A^* + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (AD)^* \begin{cases} \xrightarrow{k_r} A + D + h\nu' \\ \xrightarrow{k_n} A + D \\ \xrightarrow{k_a} \text{adducts} \end{cases} \quad (1)$$

Rate constants for fluorescence quenching generally decrease with increasing diene ionization potential, as shown in Figure 1 for quenching of naphthalene[1a] and *trans*-stilbene. Evans[2] attributed the variation in rate constant with ionization potential to a charge-transfer mechanism for exciplex formation. The free energy change for electron transfer as given in eq 2[2,10] correctly predicts a linear relation-

$$\Delta G_{ET} \propto \text{ionization potential (D)} - \text{electron affinity (A*)} \quad (2)$$

ship between log k_q and diene ionization potential for endothermic electron transfer. An electron-transfer mechanism does not satisfactorily explain (a) the large variation in excited aromatic sensitivity to diene ionization potential (slopes in Figure 1), (b) the absence of significant rate enhancement by polar solvents,[1c] and (c) the anomalously rapid quenching rates of cyclic dienes for some aromatic hydrocarbons (e.g., naphthalene, anthracene)[1b,c] but not for others (e.g., *trans*-stilbene, phenanthrene).

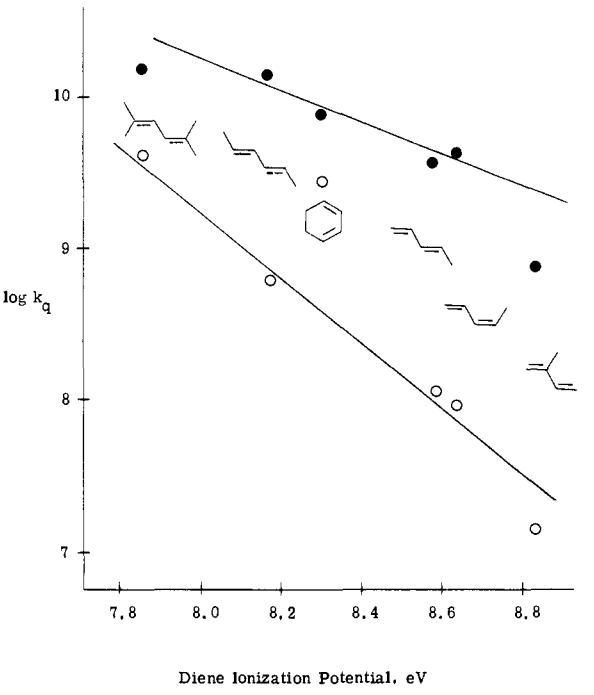

Figure 1. Correlation of quenching rate constants for *trans*-stilbene (●) and naphthalene (○)[1a] with diene vertical ionization potential.[1b]

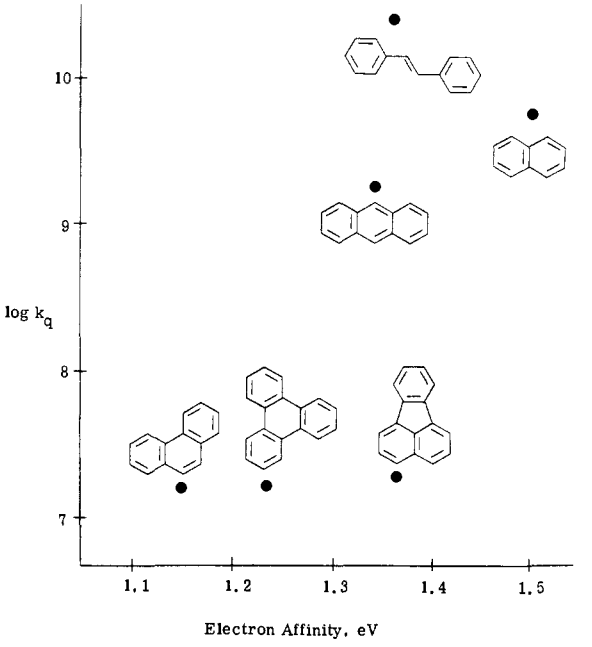

Figure 2. Correlation of 2,5-dimethyl-2,4-hexadiene quenching rate constants with excited state electron affinities.

For the interaction of a series of excited aromatic hydrocarbons with a single diene, it should be possible to correlate quenching rate constants with excited state electron affinity (eq 2).[10] Our attempts at such a correlation are shown in Figure 2. Even allowing for the large errors involved in estimating electron affinities[10] from singlet energies[11] and ground state reduction potentials,[12] the results proved less than gratifying. However, the recent kinetic analysis of α-cyanonaphthalene olefin exciplexes by Ware[13] indicates that reversible exciplex formation can lead to ob-