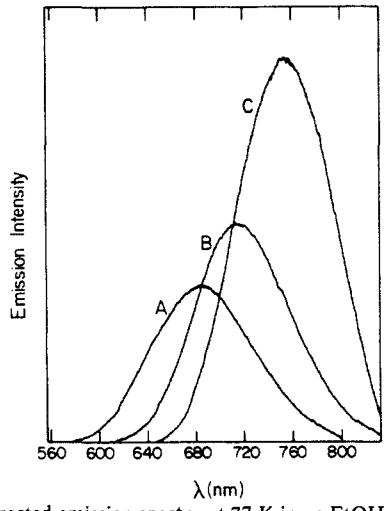

Figure 1. Corrected emission spectra at 77 K in an EtOH/MeOH glass: (A) $Pt_2(pop)_4Cl_2^{4-}$; (B) $Pt_2(pop)_4Br_2^{4-}$; (C) $Pt_2(pop)_4(SCN)_2^{4-}$.

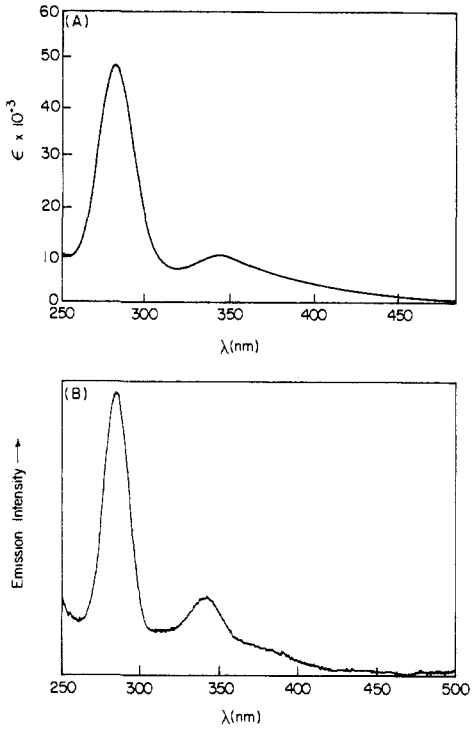

Figure 2. (A) Absorption spectrum of $Pt_2(pop)_4Cl_2^{4-}$ in aqueous solution. (B) Corrected excitation spectrum of $Pt_2(pop)_4Cl_2^{4-}$ at 77 K in an EtOH/MeOH glass.

Table I. Emission Spectral Data for $Pt_2(pop)_4X_2^{n-}$ at 77 K

	Ph_4As^+ salt: EtOH/MeOH glass		K^+ salt: solid	
X/n	λ_{max}, nm	τ, μs	λ_{max}, nm	τ, μs
Cl/4[a]	685	22.1	*b*	*b*
Br/4[a]	715	15.3	765	13.1
SCN/4	754	17.6	751	18.6
py/2	691	23.0	*b*	*b*

[a] Also characterized in 50% saturated LiX(aq) glass at 77 K. λ_{max} = 650 nm, τ = 13.9 μs for X = Cl; λ_{max} = 697 nm, τ = 13.3 μs for X = Br. [b] No detectable emission.

Why have there been no previous examples of such emission? We think that the key in the present case is that the metal–metal bond is bridged by the four pop ligands, and metal–metal dissociation, a characteristic[2] deactivation process of d^7–d^7 excited states, is therefore prevented. The excited states of these complexes could still decay by dissociation of the axial ligands,[7] but this apparently is not an efficient process at 77 K. However, deactivation by axial-ligand dissociation may explain why our efforts to detect emission in fluid solution have thus far been unsuccessful.

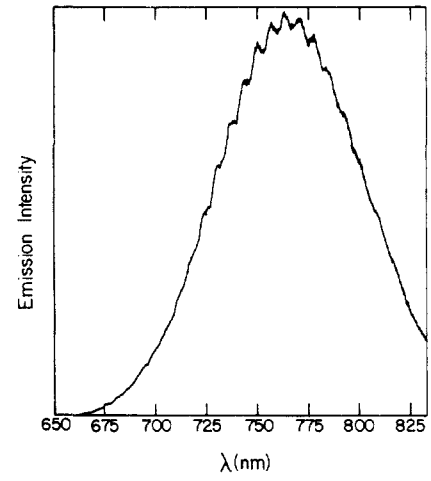

Figure 3. Corrected emission spectrum of solid $K_4[Pt_2(pop)_4Br_2]\cdot 2H_2O$ at 5 K. The vibronic spacing is 125 cm^{-1}.

Acknowledgment. We thank Mike Hopkins for assistance with the helium temperature measurements. This research was supported by National Science Foundation Grant CHE84-19828 (H.B.G.) and the Caltech President's Fund (V.M.M.).

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Mechanisms of Hemin-Catalyzed Alkene Epoxidation. The Effect of Catalyst on the Regiochemistry of Epoxidation

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Several mechanisms have been proposed for the epoxidation of alkenes by cytochrome P-450 or relevant model hemin compounds.[1-5] Oxidants in these systems have been dioxygen or other oxygen atom donors such as peracids, iodosylbenzenes, or hypochlorite. Although all proposed mechanisms implicate the high-valent iron(IV) porphyrin cation radical $(Fe^{IV}{=}O)^+$ as the oxidizing species, they differ with regard to the nature of its reaction with alkenes. Among these are direct oxygen atom transfer (eq 1), free radical addition followed by fast ring closure (eq 2), electrophilic addition followed by fast ring closure (eq 3), reversible electrocyclic metallooxetane formation followed by

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(2) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9.
(3) (a) Ortiz de Montellano, P. R.; Kunze, K. L. *Biochemistry* **1981**, *20*, 7266. (b) Ortiz de Montellano, P. R.; Mangold, B. L. K.; Wheeler, C.; Kunze, K. L.; Reich, N. O. *J. Biol. Chem.* **1983**, *258*, 4203.
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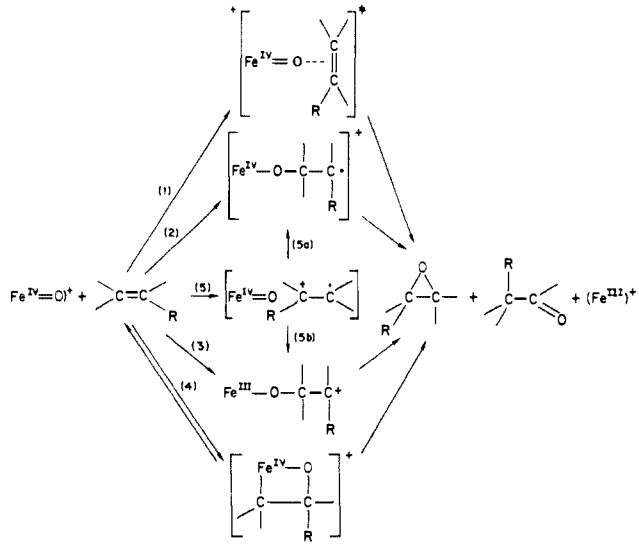
Table I. Ratios of *exo*- to *endo*-Epoxynorbornane Obtained from Catalyzed Epoxidation Reactions with (Tetraarylporphinato)iron(III) Chlorides as Catalysts[a]

aryl group in catalyst	ratio of *exo*- to *endo*-epoxynorbornane[b] using C_6F_5IO	using iodosylxylene[c]
Ph-	35	58
	49	43
mesityl-	23	23
C_6F_5-	16	
2,4-$(CF_3)_2C_6H_3$-	5	3
2,6-$Cl_2C_6H_3$-	9[d]	10
C_6Cl_5-	6	5

[a] Approximately 1–3% norcamphor and 2–5% cyclohexene-4-carboxaldehyde were also obtained. [b] Product ratios determined by gas–liquid chromatography. *endo*-Epoxynorbornane was isolated by preparatory GLC and identified by NMR spectroscopy. For ratios higher than 30, the data are rather inaccurate due to small peaks for the endo isomer. [c] 2,4-Dimethyliodosylbenzene. [d] Similar results were obtained in methylene chloride/methanol/water, 80:18:2, where all reagents are soluble.

Scheme I

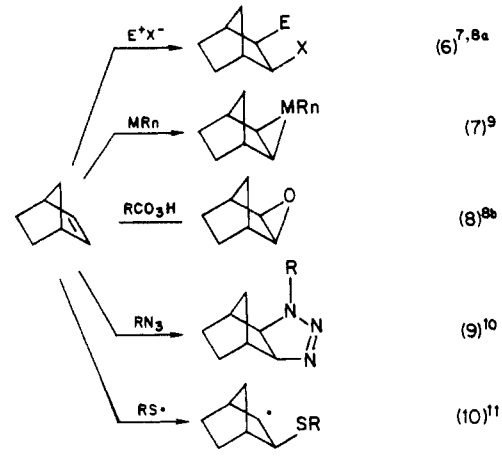


dissociation to epoxide or other products (eq 4), and electron transfer followed by collapse to radical (eq 5a), or to carbocation (eq 5b) (Scheme I).

We have recently prepared a series of metalloporphyrins containing substituents of varying electronegativity.[6a,b] The effect of these structural changes in the catalysts upon the distribution of products in norbornene epoxidation provides evidence against some of these proposed mechanisms.

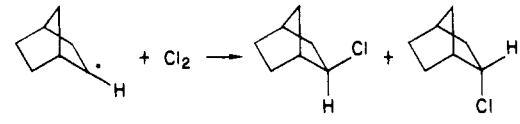
Table I shows the ratios of *exo*- to *endo*-epoxynorbornane produced (in high total yield in most cases) when 0.32 M equivalent (insoluble) of perfluoroiodosylbenzene or 2,4-dimethyliodosylbenzene and 1.0 M norbornene in 0.1 mL of methylene chloride were made 0.001 M in the appropriate catalyst.[6a] It is clear that the exo/endo ratio is virtually independent of the structure of iodosylbenzenes, indicating that they are not involved in the product-determining step. This accords with the proposal that the direct oxidizing species is the two-electron oxidized "oxohemin", symbolized by $(Fe^{IV}{=}O)^+$.

The mechanistic significance of the production of endo epoxide derives from the well-known behavior of norbornene, 2-norbornyl radical, and 2-norbornyl carbocation toward various reagents. Both addition of electrophiles or radicals to norbornene and the formation of norbornene–metal complexes occur exclusively on the exo side as do peracid epoxidation and electrocyclic additions.



It therefore appears that our results are incompatible with the exclusive occurrence of direct electrophilic, radical, or molecular attack on norbornene.

In contrast, the reaction of 2-norbornyl radical with chlorine gives a 3:1 ratio of exo to endo product. However, alkyl free



radicals generally do not rearrange.[13] The rearrangements commonly observed in hemin-catalyzed epoxidation reactions,[1b,2] including the rearrangements to norcamphor and cyclohexene-4-carboxaldehyde observed here, are inconsistent with the free-radical pathways 2 and 5a.

The remaining mechanism, eq 5b, accommodates the observations that regiochemistry (endo product)[14] shows free radical behavior[15] whereas hydride migration and ring opening are typical of carbocation reactions,[16,17] as detailed below. The decrease in exo/endo product ratio as the catalyst is made more electron deficient and thus more reactive could result from lower selectivity in the more reactive caged pair. A similar trend was observed with norbornyl radical which reacts with Cl_2 to give a 3:1 ratio of exo/endo product and carbon tetrachloride to give a 42:1 ratio.[12] A mechanism involving a carbocation intermediate has previously been suggested by Groves[1b] to explain the hydride migration observed during styrene epoxidation.

There remains the possibility that the mechanism of epoxidation could change from electron transfer to one of the other mechanisms when the ionization potential of the alkene is high. For example, ethylene, with a very high ionization potential, is nevertheless epoxidized by using our catalyst systems. The possibility of multiple oxidation mechansims is under study.

In summary, the production of substantial amounts of *endo*-

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(7) (a) Traylor, T. G. *Acc. Chem. Res.* **1969**, *2*, 152. (b) Tidwell, T. T.; Traylor, T. G. *J. Org. Chem.* **1968**, *33*, 2614.

(8) (a) Brown, H. C. *The Nonclassical Ion*, Plenum Press: New York, 1977. (b) Brown, H. C.; Kawakami, J. H.; Ikegami, S. *J. Am. Chem. Soc.* **1970**, *92*, 6914.

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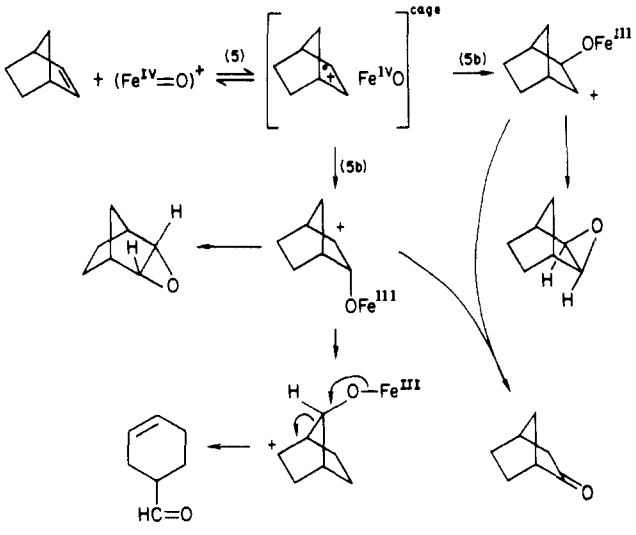
(14) Steric effects cannot be used to explain the exo/endo ratios observed here since the bulky tetramesitylhemin yields less endo product than does the fluoro- or chloro-substituted hemin.

(15) This proposal assumes that the norbornene radical cation will react as a free radical with the same stereochemistry as does the norbornyl radical. We hope to verify this assumption.

(16) Stearns et al. (Stearns, R. A.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1985**, *107*, 4081) have presented convincing evidence for electron transfer in the oxidation of quadricyclane catalyzed by cytochrome P-450.

(17) These conclusions also apply to the epoxidation of norbornene catalyzed by bleomycin, in which endo epoxide is obtained.[18]

(18) Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 493.



2,3-epoxynorbornane during hemin-catalyzed epoxidation is inconsistent with direct attack of the "oxohemin" on norbornene. The results are best explained as an electron transfer from the alkene followed by radical collapse to give a carbocation.

Acknowledgment. This work was supported by National Science Foundation Grant CHE-81-20969, National Institutes of Health Grant AM-07233, and Fogarty Fellowship (T.G.T.) and National Institutes of Health Grant GM-29198 (D.D.).

Blue Copper Proteins. The Copper Site in Azurin from *Alcaligenes denitrificans*

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The blue copper proteins have been the focus of many spectroscopic and structural studies,[1] with much of the interest centering on the nature of the copper site. X-ray crystallographic results have previously been reported for two single-copper proteins, plastocyanin and azurin. The structure of the oxidized, Cu(II), form of poplar plastocyanin has been refined at high resolution,[2] but structural analysis of azurins (from two bacterial species, *Pseudomonas aeruginosa*[3,4] and *Alcaligenes denitrificans*[5]) have been reported only at medium resolution.

We have now refined the structure of *Alcaligenes denitrificans* azurin at high resolution (1.8 Å)[6] and we wish to report the details of the refined copper site. The refinement has clearly shown that its geometry is closer to a distorted trigonal-planar or trigonal-bipyramidal arrangement rather than the distorted tetrahedron usually quoted. This distinction may be crucial for a proper understanding of the spectroscopic and functional properties of azurin, in particular, and blue copper proteins in general.

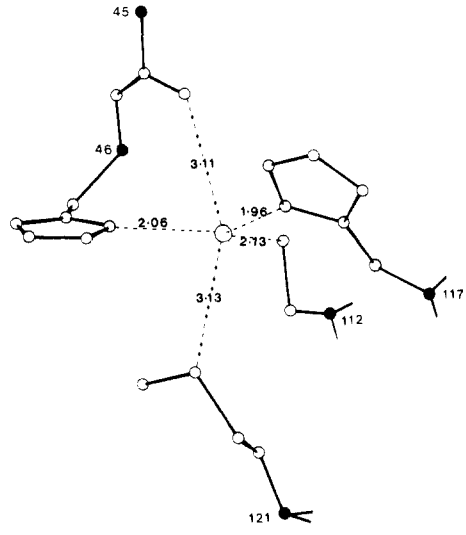

Figure 1. Copper site in azurin. Distances given are the mean of those found for the two independent molecules in the asymmetric unit.

Table I. Bond Lengths and Angles for the Copper Site in Azurin from *Alcaligenes denitrificans*

bond lengths, Å	1	2	bond angles, deg	1	2
Cu-O(45)	3.14	3.08	O(45)-Cu-N$_{\delta 1}$(46)	72	75
Cu-N$_{\delta 1}$(46)[a]	2.08	2.04	O(45)-Cu-S$_\gamma$(112)	103	104
Cu-S$_\gamma$(112)[a]	2.10	2.16	O(45)-Cu-N$_{\delta 1}$(117)	78	82
Cu-N$_{\delta 1}$(117)[a]	1.98	1.94	O(45)-Cu-S$_\delta$(121)	146	148
Cu-S$_\delta$(121)	3.13	3.13	N$_{\delta 1}$(46)-Cu-S$_\gamma$(112)[a]	137	136
			N$_{\delta 1}$(46)-Cu-N$_{\delta 1}$(117)[a]	100	106
other distances			N$_{\delta 1}$(46)-Cu-S$_\delta$(121)	77	74
S$_\gamma$(112)···N(47)	3.54	3.44	S$_\gamma$(112)-Cu-N$_{\delta 1}$(117)[a]	121	117
N$_{\epsilon 2}$(46)···O(10)	2.68	2.63	S$_\gamma$(112)-Cu-S$_\delta$(121)	110	105
			N$_{\delta 1}$(117)-Cu-S$_\delta$(121)	93	97

[a] Bond lengths and angles within the trigonal plane.

Our azurin was purified from *Alcaligenes denitrificans* NCTC 8582[7] and crystallized in its oxidized, Cu(II), form at pH 6.0. The unit cell data, and the results of the medium-resolution (2.5 Å) analysis have been published previously.[8,5] One notable feature is that the crystallographic asymmetric unit contains two molecules of azurin, thus giving two copies of the same structure and a valuable internal check on the reliability of structural observations.

Refinement of the structure was based on diffractometer data to 1.8-Å resolution (2θ = 50.7°). Because of the relatively low ratio of observations to parameters for an asymmetric unit containing over 2000 atoms, all X-ray data between 10 and 1.8 Å were used, with no σ cutoff, and only negative intensities and misset reflections excluded.[9] For the same reason, restrained least-squares procedures[10] were used for the refinement, with protein bond lengths and angles being restrained close to standard values.[11] No restraints were, however, imposed on any of the distances or angles involving the copper atom, and the two molecules in the asymmetric unit were allowed to refine quite independently.

(1) For recent reviews, see: (a) Adman, E. T. In *Topics in Molecular and Structural Biology. Metalloproteins*; Harrison, P. M., Ed.; Macmillan: New York; Vol. 1, in press; (b) Gray, H. B.; Solomon, E. I. In *Copper Proteins*; Spiro, T. G., Ed.; Wiley: New York, 1981; Vol. 3, pp 1-39. (c) Fee, J. A. *Struct. Bonding (Berlin)* **1975**, *23*, 1-60.

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(6) Full details of the refinement and the refined protein structure and its comparison with plastocyanin will be published elsewhere.

(7) The strain number, NCTC 8582, is important as this organism has recently been reclassified as belonging to the genus *Alcaligenes faecalis* and should not be confused with the type strain *Alcaligenes faecalis* NCIB 8156, on whose azurin numerous other studies have been performed.

(8) Norris, G. E.; Anderson, B. F.; Baker, E. N.; Rumball, S. V. *J. Mol. Biol.* **1979**, *135*, 309-312.

(9) From an estimated total of 25 200 unique reflections to 1.8-Å resolution, 15 330 (i.e., 61%) were measured with $I > 2\sigma_I$ and 18 680 (74%) with $I > \sigma_I$. The inclusion of all nonnegative intensities increased the number of observed data to 21 980 (87% of total).

(10) Programs used were PROLSQ, the restrained least-squares program of Hendrickson and Konnert (See: Hendrickson, W. A.; Konnert, J. H. *Biomolecular Structure, Function, Conformation and Evolution*; Srinivasan, R., Ed.; Pergamon: Oxford, 1980; Vol 1, pp 43-57), and TNT, a restrained least-squares procedure using fast Fourier methods: D. Tronrud and L. Ten Eyck, Institute of Molecular Biology, University of Oregon.

(11) In the final protein model, root mean square (rms) deviations from standard values were 0.017 Å for bond lengths and 3.1° for bond angles.

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