1 h provided enol ether **7** in 99% yield. Heating of a solution of **7** in toluene (0.05 M) containing 30 equiv of propylene oxide (to prevent acid-catalyzed decomposition) at 160 °C for 22 h effected Cope rearrangement, leading to the *cis*-hydrindene **8**, which without purification was heated at reflux with wet Me_2SO (7.5 M water) containing 3 equiv of sodium chloride[8] for 4 h to give ketone **9** in 71% overall yield from **7**.

Treatment of **9** with 9-borabicyclo[3.3.1]nonane (9-BBN, 2.15 equiv) in THF at 23 °C for 19 h followed by oxidation with alkaline hydrogen peroxide (2 equiv, 23 °C, 30 min) gave the two epimeric bicyclic diols **10** contaminated with 1,5-cyclooctanediol. Since chromatographic separation of **10** from the latter was not easy, the mixture of alcohols was oxidized by using 2.5 equiv of pyridinium dichromate (PDC) and powdered Linde 3-Å molecular sieves[9] (1 g/mmol of PDC) in methylene chloride at 23 °C for 2.5 h to afford after chromatography diketone **11**, mp 39-40 °C (76% overall from **9**); none of the position-isomeric diketone could be detected in the reaction mixture. Highly selective hydroboration of the cyclopentene olefinic linkage in the diene **9** is to be expected in view of the known deactivating effect of vinylic bromine in hydroboration.[10]

Construction of the bridged ring utilized methodology previously developed in these laboratories specifically for the preparation of the D ring of gibberellic acid.[11] Treatment of **11** with lithium di-*n*-butylcuprate (11 equiv) in 33% hexane-diethyl ether at −78 °C for 2.5 h followed by inverse quenching with pH 8 ammonia-ammonium chloride buffer afforded tricyclic ketone **12**,[12] mp 112-113 °C, in 67% yield; only 4% of **13**, the result of cyclization involving the five-ring keto function, was isolated by chromatography.[13] This high degree of selectivity was predicted based upon the greater degree of strain in **13** with respect to **12**.[14] Protection of the hydroxy function of **12** using excess methoxyethoxymethyl (MEM) chloride and diisopropylethylamine in methylene chloride at 23 °C for 24 h provided in 96% yield the tricyclic ketone **4**, which was identical with a sample of **4** prepared by an alternate sequence[3] on the basis of ^1H NMR, infrared, and mass spectral and thin-layer chromatographic comparison. The establishment of this new route to **4** provides another synthetic pathway to gibberellic acid that is both direct and stereocontrolled.

Prior to the synthesis of **5**, the simpler norbornene **14** was prepared[15] and converted to the corresponding trimethylsilyl enol ether **15**.[16] Attempted Cope rearrangement of **15** in various solvents (benzene, toluene, xylene) at temperatures ranging from 90 to 150 °C yielded only silylated aldol dimers of **14**. In addition, thermolysis of **15** in the gas phase by passage at ca 0.1 torr through a tube heated to 425 °C afforded 2-(trimethylsiloxy)-1,3-butadiene and a mixture of 2- and 1-(2-bromoallyl)cyclopentadienes, fragments derived from a retro-Diels-Alder reaction of **15**. Evidently, a delicate balance exists between Cope rearrangement and retro-Diels-Alder pathways in this system. The utilization of substrate **7**, which contains an additional carbomethoxy group relative to **15**, was expected to favor rearrangement over fragmentation because electron delocalization from the trimethylsiloxy donor group to the withdrawing carbomethoxy group of **8** should provide driving force for the Cope but not the retro-Diels-Alder pathway.

The synthesis of **4** reported herein is conceptually quite different than previous routes. Additionally, modification of this sequence should allow for entry into a general class of *cis*-hydrindenes otherwise not readily available.[17]

Supplementary Material Available: ^1H NMR, infrared, and mass spectral data and detailed experimental procedures (35 pages). Ordering information is given on any current masthead page.

(8) Krapcho, A. P.; Weimaster, J. F.; Eldridge, J. M.; Jahngen, E. G. E., Jr.; Lovey, A. J.; Stephens, W. P. *J. Org. Chem.* **1978**, *43*, 138.
(9) Herscovici, J.; Antonakis, K. *J. Chem. Soc., Chem. Commun.* **1980**, 561.
(10) Yamamoto, Y.; Toi, H.; Moritani, I. *Chem. Lett.* **1974**, 485.
(11) (a) Corey, E. J.; Kuwajima, I. *J. Am. Chem. Soc.* **1970**, *92*, 395. (b) Corey, E. J.; Narisada, M.; Hiraoka, T.; Ellison, R. A. *Ibid.* **1970**, *92*, 396.
(12) During the course of these studies, other syntheses of **12** have been reported; see: Stork, G.; Boeckmann, R. K., Jr.; Taber, D. F.; Still, W. C.; Singh, J. *J. Am. Chem. Soc.* **1979**, *101*, 7107.
(13) Tricyclic ketone **12** was readily separated from **13** and the other byproducts of the reaction (which stem from replacement of bromine in **11** by *n*-butyl (15%) and hydrogen (6%)) by column chromatography on silica gel. The R_f values obtained by using 50% ether-toluene were 0.21, 0.25, 0.51, and 0.40, respectively, for **12**, **13**, and the butyl and hydrogen halogen replacement products.
(14) MM2 conformational calculations (performed by Jay W. Ponder in these laboratories) indicated that **13** is strained by 2.5 kcal/mol relative to **12**.
(15) Treatment of the mixture of 2- and 1-(2-bromoallyl)cyclopentadienes and methyl vinyl ketone with boron trifluoride etherate (in methylene chloride at −78 °C) afforded **14** in 51% yield.
(16) Addition of a THF solution of **14** to lithium diisopropylamide in THF at −78 °C followed by quenching with a 1:1 (v/v) solution of trimethylsilyl chloride and triethylamine, previously centrifuged to settle triethylamine hydrochloride, provided **15** in 96% yield.

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Oxygen-18 Labeling Evidence against a Hexacoordinate Phosphorus Intermediate in the Alkaline Hydrolysis of Ethyl Ethylene Phosphate

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The role of the hexacoordinate phosphorus intermediate in the reactions of phosphate esters has been the subject of much speculation but little experiment. Westheimer and co-workers[1] observed that the fraction of exocyclic cleavage for methyl ethylene phosphate increases linearly with hydroxide ion concentration from ~0% at pH 11-13 to about 15% cleavage in 10 M alkali. They suggest this result is consistent with the required pseudorotation of a dianionic pentaoxyphosphorane intermediate. They further indicate that an explanation involving a hexacoordinate phosphorus intermediate, although unsupported, cannot be eliminated.

Ramirez[2] and Gillespie et al.[3] indicate that the hydrolysis of methyl ethylene phosphate is second order in hydroxide (as suggested by Kluger et al.'s observation of an increase in exocyclic cleavage with strong base[1]) and argue for formation of a hexacoordinate intermediate in strong alkali as shown in Scheme I. This hypothesis has gained widespread recognition and has even been presented in a text[4] as a quite reasonable mechanistic possibility. The more recent preparation of stable hexacoordinated phosphorus anions, $(PhO)_6P^-$,[5] and $(CH_3O)_6P^-$,[6] and the kinetic data supporting a hexacoordinate intermediate in the hydrolysis of $(ArO)_5P$[7] suggest that the earlier hypothesis for the involvement of a hexacovalent intermediate in the strong alkali (hydrolysis of methyl ethylene phosphate is certainly quite reasonable. However, we now present ^{18}O labeling results that argue against the formation of such a species in the hydrolysis of a related five-membered ring phosphate ester, ethyl ethylene phosphate.

A 50-μL sample of 0.155 M ethyl ethylene phosphate in dry dioxane was added to 0.49 mL of 5.0 M NaOH (72% $H_2{}^{18}O$/22%

(1) Kluger, R.; Covitz, F.; Dennis, E.; Williams, L. D.; Westheimer, F. T. *J. Am. Chem. Soc.* **1969**, *91*, 6066.
(2) Ramirez, F.; Tasaka, K.; Hershberg, R. *Phosphorus* **1972**, *2*, 41.
(3) Gillespie, P.; Ramirez, F.; Ugi, I.; Marquarding, K. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 91.
(4) Emsley, J.; Hall, D. "The Chemistry of Phosphorus"; Wiley: New York, 1976; p 338.
(5) Lerman, C. L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1976**, *98*, 179.
(6) Denney, D. B.; Denney, D. Z.; Ling, C.-F. *J. Am. Chem. Soc.* **1976**, *98*, 6755. See also Ramirez et al. and Chang et al. (Ramirez, F.; Nowakowski, M.; Maracek, J. F. *Ibid.* **1974**, *96*, 7269). Chang, B. C.; Denney, D. B.; Powell, R. L.; White D. W. *J. Chem. Soc., Chem. Commun.* **1971**, 1070) for other stable hexacoordinate oxyphosphorus species.
(7) Archie, W. C., Jr.; Westheimer, R. H. *J. Am. Chem. Soc.* **1973**, *95*, 5955. See also: Ramirez, F.; Ramanathan; Desai, N. B. *J. Am. Chem. Soc.* **1963**, *85*, 3465.

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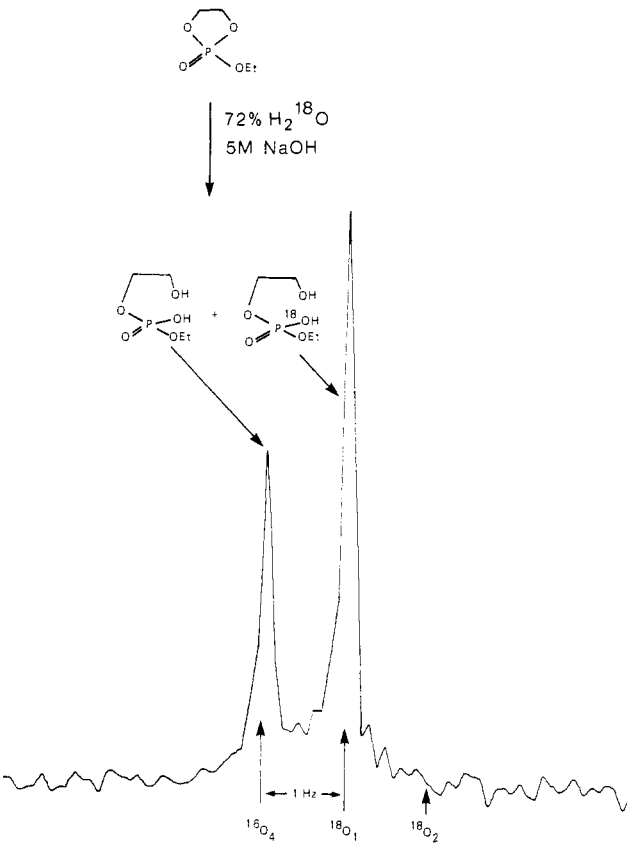
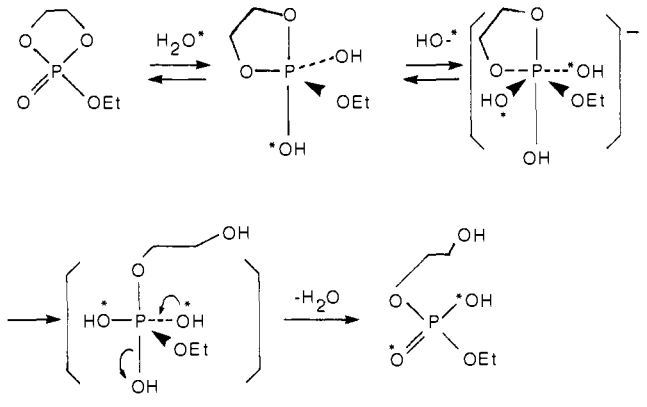


Figure 1. 32.37-MHz ^{31}P NMR spectrum of ethyl 2-hydroxyethyl phosphate, **1**, showing no ^{18}O (1.359 ppm) and mono-^{18}O labeled (1.331 ppm) signals, on a Bruker WP-80 spectrometer; chemical shifts relative to 15% H_3PO_4 in D_2O.

Scheme I



D_2O) at room temperature, and the solution was quickly frozen in an acetone/dry ice bath. To the almost frozen solution, was carefully added 66.6 μL of 36 N H_2SO_4. The pH ~2 solution was added to a tris-base buffer solution. Up to this point everything was carried out within a minute. Some inorganic precipitate was filtered through a nitric acid and EDTA solution treated disposable pipet plugged with EDTA-treated cotton and Chelex-100 (Bio-Rad) ion exchange resin. The final pH of the solution was about 9, and the ^{31}P NMR spectrum of the sample is shown in Figure 1. Only the endocyclic product ethyl 2-hydroxyethyl phosphate, **1**, was observed. The two signals represent the mono-^{18}O-labeled ester **1** and the unlabeled (^{16}O) ester **1**. The magnitude of the upfield shift for the ^{18}O-labeled ester is consistent with the expected ^{18}O isotope shift on the ^{31}P chemical shift for a singly ^{18}O-labeled compound.[8-10] If two ^{18}O atoms had been

(8) Cohn, M., Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200. Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913.

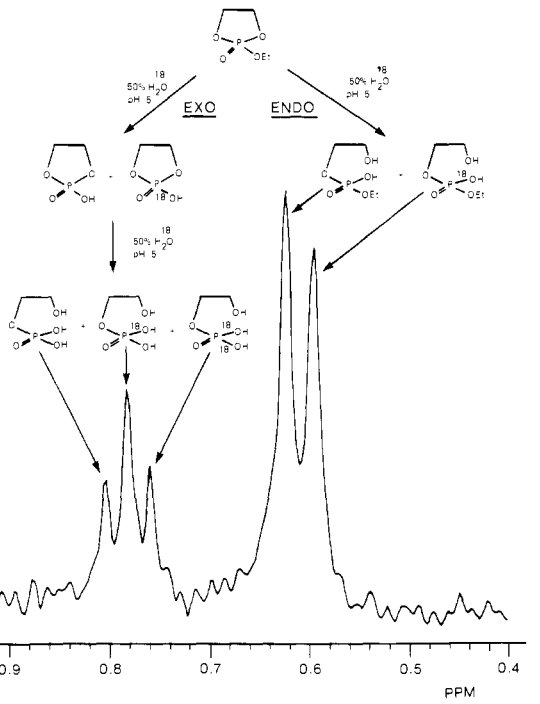


Figure 2. 190.3-MHz ^{31}P NMR spectrum of 2-hydroxyethyl phosphate (triplet, with ^{18}O labeling shown) and ethyl 2-hydroxyethyl phosphate (doublet), on a Nicolet 470-MHz spectrometer. Scheme II, shown for generation of phosphate ester products from ethyl ethylene phosphate, pH 5, 50% $H_2{}^{18}O$. Products were identified by titration of the triplet ^{31}P signals for the monoester and lack of titration of the doublet signal for the diester.

incorporated into the product as would be required[11] for a mechanism involving a hexacoordinate intermediate, then another ^{18}O-labeled signal would have been observed upfield of the singly ^{18}O-labeled signal. Within the S/N (±5%) of the spectrum we can rule out any hexacoordinate intermediate.[11] In contrast to the results for methyl ethylene phosphate in strong alkali we observed no exocyclic cleavage product. (Also none is observed in dilute alkali between pH 8 and 13.) Whether this is due to the poorer leaving-group ability of ethoxide relative to methoxide or is due to some C–O cleavage in strong alkali for methyl ethylene phosphate is not known. Note, however, that endocyclic alkoxide leaving is predicted to be much more stereoelectronically favorable than exocyclic alkoxide leaving,[12,13] consistent with our results.

Similar labeling results were obtained in dilute acid (pH 2–7) where both exocyclic cleavage product, 2-hydroxyethyl phosphate (25% at pH ~5) and endocyclic cleavage product were observed by ^{31}P NMR (Figure 2). Again, for the endocyclic product, only unlabeled and singly ^{18}O-labeled ^{31}P signals were observed. For the exocyclic product, 2-hydroxyethyl phosphate, three ^{31}P signals

(9) Lowe, F.; Sproat, B. S. *J. Chem. Soc., Chem. Commun.* **1978**, 565.
(10) Gorenstein, D. G.; Rowell, R. *J. Am. Chem. Soc.* **1980**, *102*, 6165.
(11) It is possible to envision a mechanism involving a hexacoordinate intermediate that does not isotopically equilibrate the original phosphoryl oxygen and the two water oxygens so that loss of two oxygens from this intermediate does not yield a doubly ^{18}O-labeled **1**. However, this would require a violation of the principle of microscopic reversibility, assuming an octahedral intermediate (i.e., the three HO(P) groups should be equivalent in the hexacoordinate intermediate of Scheme I, and loss of labeled or unlabeled oxygen should be energetically equivalent, ignoring a small ^{18}O isotope effect). If the phosphoryl oxygen could always be chemically distinguished from hydroxyl oxygen in the pentacovalent and hexacoordinate intermediates, then it is conceivable that our observed labeling results could not rule out a hexacoordinate intermediate. This would be possible, for example, if the phosphoryl oxygen was always anionic while the labeled solvent oxygen molecules were always neutral hydroxyl groups in the intermediates. However, it is highly unlikely that tautomerization ($^-$O–P–OH ⇌ HO–P–O$^-$) is going to be slower than hydroxide attack on a pentacoordinate intermediate.
(12) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B.; Momii, R. *J. Am. Chem. Soc.* **1977**, *99*, 4170.
(13) Gorenstein, D. G. Luxon, B. A.; Findlay, J. B. *J. Am. Chem. Soc.* **1979**, *101*, 5869.

are observed in the expected ratio for incorporation from solvent of no ^{18}O, one ^{18}O, and two ^{18}O atoms. (Scheme II, Figure 2) The first-formed exocyclic product is ethylene phosphate (mono-^{18}O labeled from one water molecule), which under our conditions rapidly further hydrolyzes to 2-hydroxyethyl phosphate (incorporating a second water molecule giving some di-^{18}O-labeled product.)

These results confirm that *no* oxygen exchange from solvent occurs during the course of the reaction or with starting material or products and that there is 100% P–O cleavage for all products at pH 2–15 (other results not reported). In addition, most significantly we have found no evidence under any conditions for formation of a hexacoordinate intermediate.

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Registry No. Ethyl ethylene phosphate, 823-31-4; ethyl 2-hydroxyethyl phosphate, 5178-07-4.

EXAFS and Raman Evidence for Histidine Binding at the Active Site of Protocatechuate 3,4-Dioxygenase

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The coordination environment at the active site iron of the the catechol-cleaving dioxygenases has been examined by a number of spectroscopic probes. One of these enzymes, protocatechuate 3,4-dioxygenase (PCD), which catalyzes the intradiol cleavage of protocatechuic acid to β-carboxy-*cis,cis*-muconic acid, has been isolated from a number of microbial genera,[2-6] with the crystalline enzyme from *Pseudomonas aeruginosa* being most extensively studied.[7-10] Previous resonance Raman studies[9,11] provided evidence that the ferric ion is coordinated by two tyrosines; however, the identity of other iron ligands is not firmly established. Based upon EPR[12] and sulfhydryl titration,[13] suggestions were advanced that sulfur binding was possible, although the original proposal of tetrahedral sulfur ligation is no longer viable. Instead, histidyl coordination was proposed due to the similarity between reduced PCD and deoxyhemerythrin Mössbauer spectra.[14]

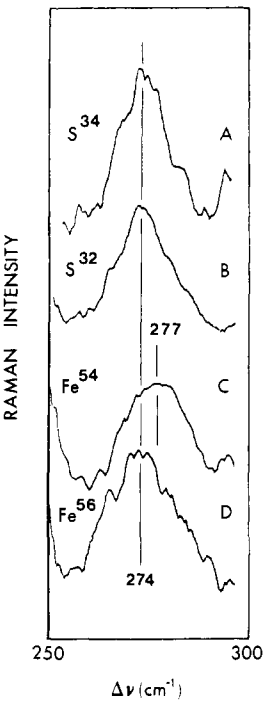

Figure 1. Resonance Raman spectra of native and labeled PCD: (A) $^{56}Fe^{34}S$; (B) $^{56}Fe^{32}S$; (C) $^{54}Fe^{32}S$; (D) $^{56}Fe^{32}S$. Spectra A, B and C, D, are measured from samples in a partitioned, rotating cell. Laser wavelength is 514.5 nm; power is 500 mW.

A Raman peak at 274 cm^{-1} in the native enzyme disappears when the enzyme is reversibly inhibited by 3-chloro-4-hydroxybenzoate (3-ClHB); yet, Raman peaks assigned to tyrosines suffer no concomitant intensity reduction.[9] This observation suggests that displacement or modification of nontyrosine groups is being monitored. Although the 274-cm^{-1} frequency is below that of ca. 350 cm^{-1} found for ν(Fe–S) in iron–sulfur proteins[15-17] and cytochrome P-450_{cam},[18,19] a frequency shift to lower energies is conceivable in a highly ionic environment provided by tyrosinate coordination. Characterization of this vibration, at least in terms of sulfur participation, becomes feasible by a study of the ^{34}S isotope shift. On the other hand, a mode at 267 cm^{-1} is identified as ν(Cu–ImH) (ImH = imidazole) in the UV resonance Raman study of oxyhemocyanin,[20] and imidazole ligation in PCD becomes an attractive alternative assignment of the 274-cm^{-1} peak, if a $^{54}Fe/^{56}Fe$ isotope shift is observed with no accompanying sulfur isotope shift.

A complementary probe of local geometry about a metal ion is provided by extended X-ray absorption fine structure (EXAFS). It is now appreciated that coordinated histidine is characterized by a prominent peak in the Fourier transform of EXAFS data.[21,22] Consequently, combined Raman and EXAFS studies further delineate the active-site structure of PCD.

(1) Present address: Union Carbide Corp., Bound Brook, NJ 08805.
(2) Stanier, R. Y.; Ingraham, J. L. *J. Biol. Chem.* **1954**, 210, 799–808.
(3) Wells, M. C. C. Ph.D. Thesis, University of Texas at Austin, University Microfilms, Ann Arbor, MI, 1972.
(4) Hou, C. T.; Lillard, M. O.; Schwartz, R. D. *Biochemistry* **1976**, *15*, 582–8.
(5) Durham, D. R.; Stirling, L. A.; Ornston, L. N.; Perry, J. J. *Biochemistry* **1980**, 19, 149–55.
(6) Bull, C.; Ballou, D. P. *J. Biol. Chem.* **1981**, *256*, 12673–80.
(7) Fujisawa, H.; Hayaishi, O. *J. Biol. Chem.* **1968**, *243*, 2673–81.
(8) May, S. W.; Phillips, R. S.; Oldham, C. D. *Biochemistry* **1978**, *17*, 1853–60.
(9) Felton, R. H.; Cheung, L. D.; Phillips, R. S.; May, S. W. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 844–50.
(10) May, S. W.; Phillips, R. S. *Biochemistry* **1979**, *18*, 5933–9.
(11) Que, L., Jr.; Epstein, R. M. *Biochemistry* **1981**, *20*, 2545–9.
(12) Blumberg, W. E.; Peisach, J. *Ann. N.Y. Acad. Sci.* **1973**, *222*, 539–60.
(13) Fujiwara, M.; Nozaki, M. *Biochim. Biophys. Acta.* **1973**, *327*, 306–12.
(14) Que, L., Jr.; Lipscomb, J. D.; Zimmermann, R.; Munck, E.; Orme-Johnson, N. R.; Orme-Johnson, *Biochim. Biophys. Acta.* **1976**, *452*, 320–34.
(15) Long, T. V.; Loehr, T. M.; Allkins, J. R.; Lovenberg W. *J. Am. Chem. Soc.* **1971**, *93*, 1809–11.
(16) Tang, S. P. W.; Spiro, T. G.; Mukai, K.; Kimura, T. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 869–74.
(17) Tang, S. P. W.; Spiro, T. G.; Antanaitis, C.; Moss, T. H.; Holm, R. H.; Herskovitz, T.; Mortensen, L. E. *Biochem. Biophys. Res. Commun.* **1975**, *62*, 1–6.
(18) Felton, R. H.; Yu, N. T. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. III, pp 347–93.
(19) Champion, P. M.; Stallard, B. R.; Wagner, G. C.; Gunsalus, I. C. *J. Am. Chem. Soc.* **1982**, *104*, 5469–72.
(20) Larrabee, J. S.; Spiro, T. G. *J. Am. Chem. Soc.* **1980**, *102*, 4217–23.
(21) Bunker, G.; Stern, E. A.; Blankenship, R. E.; Parson, W. W. *J. Biophys. Soc.* **1982**, *37*, 539–51.
(22) Co, M. S.; Scott, R. A.; Hodgson, K. O. *J. Am. Chem. Soc.* **1981**, *103*, 986–8.

0002-7863/82/1504-6132$01.25/0