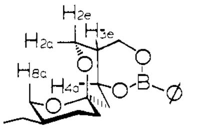
low-field resonance, δ 97.2, suggestive of equivalent ketal centers in each isomer.[11] Acid-catalyzed equilibration (0.1 N p-TSA in THF, room temperature) of talaromycin A (0.5 mg) quantitatively converted the less-stable axial hydroxymethylene isomer into talaromycin B and thus proved the presence of the ketal functionality.[12] The ketal's relative configuration[13] was established by observing the nuclear Overhauser enhancement of H_{2a} (3.7%)[14] on irradiation of H_{8a} in talaromycin A. Similar irradiation of H_{2a} in **1** gave enhancements of H_{2e} (15%), H_{3e} (6.3%), H_{4a} (2.3%), and also H_{8a} (4.0%).[15] These enhancements verified the ring conformations shown in **1** and also established the three-dimensional positions of H_{8a} and H_{2a}, two protons that would be proximal only if both oxygens occupied axial positions in the spiro-fused ketal.



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The structural characterization of the talaromycins has shown COSY to be a powerful method for resolving isomeric mixtures (Figure 1) and for identifying spin systems isolated by regions devoid of protons (Figures 2 and 3). Even though this experiment gives comparable data to a series of double resonance experiments, the opportunity to obtain all the information in a single experiment, even in highly congested regions where decoupling is difficult, provided an important advantage in the talaromycin assignment. This ability to verify all the proton-coupling connectivities of a molecular structure allows for a formal assignment of all the protons and leads to a nonempirical establishment of structure.

The spiro ketal present in the talaromycins is a structural feature common to many bacterial polyether antibiotics[16] as well as to several insect pheromones.[17] The talaromycins constitute the first example of such functionality being elaborated in fungi through what appears to be a polyketide pathway. The toxicity of the talaromycins is still under investigation, but initial data suggest that they block outward potassium fluxes and thus lead to muscle dysfunction.[18]

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(11) The ^{13}C chemical shift of the spiro ketal carbon does not appear to have any predictive value as to configuration, but the thermodynamic stability of such systems has been explored: Pothier, N.; Rowan, D. D.; Deslongchamps, P.; Saunders, J. K. *Can. J. Chem.* **1981**, *59*, 1132–1139. Francke, W.; Reith, W.; Sinnwell, V. *Chem. Ber.* **1980**, *113*, 2686–2693.

(12) Opening of the ketal results in two equivalent primary alcohols that will close to the thermodynamically most stable isomer. At this point we have no direct data as to whether only one isomer is naturally occurring and the second is a function of our isolation procedure, but we suspect that both isomers occur naturally.

(13) CD analysis of the dibenzoates (benzoyl chloride, pyridine) of the talaromycins has indicated that the absolute configuration indicated in **1** is correct.

(14) Preirradiation (selective 180°) of the desired signal in talaromycin A (1.5 mg in 0.5 mL $CDCl_3$) was followed after a delay (0.7 s) by a 90° observed pulse. This spectrum (500–1000 transients) was acquired simultaneously with a spectrum where the selective pulse was applied 3 ppm upfield of Me_4Si, and the two spectra were computer subtracted to observe the enhancements: Solomon, I. S. *Phys. Rev.* **1955**, *99*, 559–565. Hall, L. D.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1980**, 368–370.

(15) The phenyl boronic esters, **1**, were more easily dried and provided better observation of small NOE. The small chemical shift difference between H_{2a} and H_{8a} in talaromycin B did not allow for quantitative measurements of the NOE's.

(16) Westley, J. W. *Adv. Appl. Microbiol.* **1977**, *22*, 177–222. Westley, J. W. "The Polyether Antibiotics: Carboxylic Acid Ionophores"; Westley, J. W., Ed.; Marcel Dekker: New York, 1982: Chapter 1.

(17) Baker, R.; Herbert, R.; Howse, P. E.; Jones, O. T.; Francke, W.; Reith, W. *J. Chem. Soc., Chem. Commun.* **1980**, 52–53. Francke, W.; Reith, W.; Bergström, G.; Tengö, J. *Naturwissenschaften* **1980**, *67*, 149–150. Baker, R.; Herbert, R. H.; Parton, A. H. *J. Chem. Soc., Chem. Commun.* **1982**, 601–603.

(18) The molecular action of the talaromycins is still under investigation by Dr. Stuart Taylor, Department of Pharmacology, The Mayo Foundation, Rochester, MN.

(19) ^{1}H NMR data obtained with an NTC-360 spectrometer with a 1280/293B data system and standard NMCFT software.

Acknowledgment. The funds for the purchase of the NTC 360 spectrometer were partially provided by NSF. We thank Research Corp. for partial support, Professor Steven Weinreb, Pennsylvania State University, for obtaining the ^{13}C NMR of the mixed sample, and Dr. Jerry Golik and Professor Koji Nakanishi, Columbia University, for helping to obtain and interpret the CD spectra of the benzoates of the talaromycins.

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(20) Baumann, R.; Wider, G.; Ernst, R. R.; Wüthrich, K. *J. Magn. Reson.* **1981**, *44*, 402–406.

Transient Raman Scattering Observation of Surface Reactions in Aqueous TiO_2 Colloids

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Absorption of light by semiconductor particles in an aqueous colloid creates mobile electrons (e^-) and holes (h^+) that may migrate to the particle surface and undergo redox processes with adsorbed chemical species. Both e^- and h^+ come to the surface apparently because the particle dimensions are small with respect to the characteristic band-bending length. The optimization and mechanism of such reactions have been intensively studied in recent years, principally by using transient absorption spectroscopy of the reaction products.[1–7] We, as well as others, have recently discovered that the kinetics of the reaction precursor species e^- and h^+ can be observed via radiative recombination emission.[8–9]

Transient Raman scattering analysis of chemical reactions provides direct structural information about reactive intermediates. In addition, variations in the local molecular environment of a given species are sometimes observed as small shifts in the spectra, for example, in heme proteins[10] and on the hydrocarbon:water boundary in aqueous micelles.[11] We now report application of this technique to semiconductor surface redox processes in aqueous colloids.

In our experiment e^- and h^+ are created by absorption of an ultraviolet laser pulse at a frequency above the semiconductor band gap ($\lambda \leq 3800$ Å for TiO_2). After a time delay Δt a Raman spectrum is generated by a second pulse at a frequency below the band gap. The semiconductor is transparent at the Raman probe wavelength, which is chosen to give electronic resonance Raman enhancement for a specific chemical species. In this respect, our experiment differs from recent observations of enhanced Raman scattering by stable, transparent species in absorbing metal colloids.[12–14] Our apparatus has been described recently.[15]

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(1) Inone, T.; Fujishima, A.; Konishi, S.; Honda, K. *Nature* (*London*) **1979**, *277*, 637.

(2) Kraentler, B.; Bard, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 5985.

(3) Borgarello, E.; Kiwi, J.; Pelizzetti, E.; Visca, M.; Grätzel, M. *Nature* (*London*) **1981**, *289*, 159.

(4) Kalyanasundaram, K.; Borgarello, E.; Duonghong, D.; Grätzel, M. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 987.

(5) Fujihira, M.; Satoh, Y.; Osa, T. *Nature* (*London*) **1981**, *293*, 206.

(6) Humphry-Baker, R.; Lilie, J.; Grätzel, M. *J. Am. Chem. Soc.* **1982**, *104*, 422.

(7) Duonghong, D.; Ramsden, J.; Grätzel, M. *J. Am. Chem. Soc.* **1982**, *104*, 2977. We thank Professor Grätzel for a preprint of this paper.

(8) Rossetti, R.; Brus, L. E. *J. Phys. Chem.* **1982**, *86*, 4470.

(9) Henglein, A. *Ber. Bunsenges. Phys. Chem.* **1982**, *86*, 241.

(10) Lyons, K. B.; Friedman, J. M.; Fleury, P. A. *Nature* (*London*) **1978**, *275*, 565.

(11) Beck, S. M.; Brus, L. E. *J. Am. Chem. Soc*, in press.

(12) von Raben, K. U.; Chang, R. K.; Laube, B. L. *Chem. Phys. Lett.* **1981**, *79*, 465.

(13) Creighton, J. A.; Blatchford, C. G.; Albrecht, M. G. *J. Chem. Soc., Faraday Trans. 2* **1979**, *75*, 790.

(14) Kerker, M.; Siiman, O.; Bumm, L. A.; Wang, D. S. *App. Optics* **1980**, *19*, 3253.

(15) Beck, S. M.; Brus, L. E. *J. Chem. Phys.* **1981**, *75*, 4934.

0002-7863/82/1504-7322$01.25/0

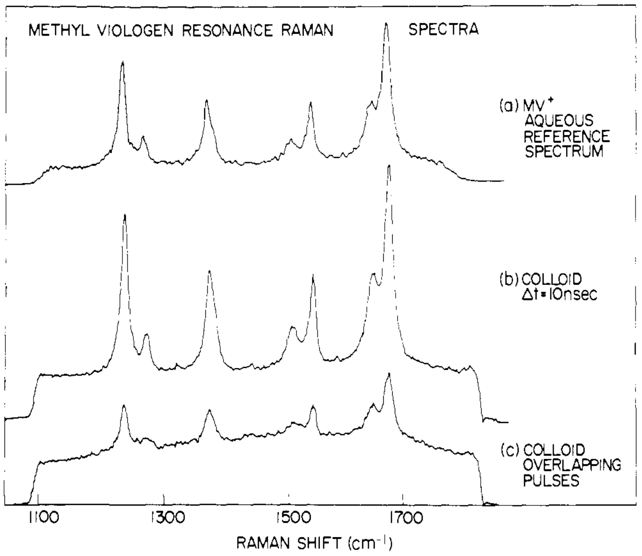

Figure 1. Partial 395-nm resonance Raman spectra of reduced methyl viologen MV^+: (a) aqueous (steady state) MV^+ made by reduction with $Na_2S_2O_4$ at pH 10; (b) transient spectrum observed at time delay $\Delta t \simeq$ 10 ns following 320-nm excitation of TiO_2 colloidal particles at pH 10.9 (the initial methyl viologen dichloride concentration is 4×10^{-3} M); (c) same as b, except that the 320- and 395-nm pulses ($\simeq$8 ns fwhm) are overlapping to give an effective Δt on the order of 5 ns. Continuous scattering underlying the MV^+ lines is due to TiO_2 luminescence. Other species present (e.g., MV^{2+}) are not observed as they do not exhibit a resonance Raman effect at 395 nm.

We study two TiO_2 surface processes whose kinetics have been observed by Grätzel and co-workers[7] using transient absorption:

$$SCN^- + h^+ \rightarrow SCN\cdot \xrightarrow{SCN^-} (SCN)_2^- \quad (1)$$

$$MV^{2+} + e^- \rightarrow MV^+ \quad (2)$$

$(SCN)_2^-$ is probed by 532-nm Raman scattering, while MV^+ (reduced methyl viologen) is observed by 395-nm Raman scattering. The second step in reaction 1 occurs at a near-diffusion-controlled rate.

Transparent, colorless TiO_2 colloids (2.56 g of TiO_2/L) were prepared by hydrolysis of titanium isopropoxide.[7,16] In reaction 1 at acidic pH, this colloid was used directly after removal of isopropanol by evaporation. In the reduction of MV^{2+}, at neutral and basic pH, the colloid was further stabilized with 0.3% polyvinyl alcohol. TiO_2 colloids loaded with surface Pt were prepared by dissolving TiO_2 evaporation residues in Pt colloids.[7,16] The TiO_2 particles are reported to have hydrodynamic radii of $\simeq$200 Å.

Figure 1 shows MV^+ spectra generated following absorption by TiO_2 at 320 nm. The Raman probe wavelength, 395 nm, is near the peak of an intense absorption band of MV^+, and a strong resonance Raman enhancement occurs.[17] The dependence of the MV^+ signal on pH and Δt qualitatively agrees with previous observations via absorption spectroscopy.[7] There is a microsecond time scale rise time that shortens at basic pH.[18] The best MV^+ spectra at short time delays are observed near pH 11 in Figure 1b and c. Figure 1c, for time superimposed 320- and 395-nm pulses (pulse widths $\simeq$8 ns), shows weak MV^+ spectra on top of structureless luminescence from the TiO_2 particles. Figure 1b shows a stronger MV^+ signal at $\Delta t \simeq$ 10 ns.[19] Figure 1a shows a reference MV^+ aqueous spectrum made by chemical reduction of MV^{2+} with dithionite. There is no spectral difference between MV^+ made in the surface reaction and the MV^+ aqueous species, at our present resolution, for these transitions. Platinum surface-loaded TiO_2 colloids 1% and 2% (by weight) were also studied. In the former case, the small Pt particles are reported to have $\simeq$30-Å diameters, while in the latter case, the Pt colloidal preparation yields 15-Å diameter spheres. We did not observe changes in either the rise times or the MV^+ Raman spectra.

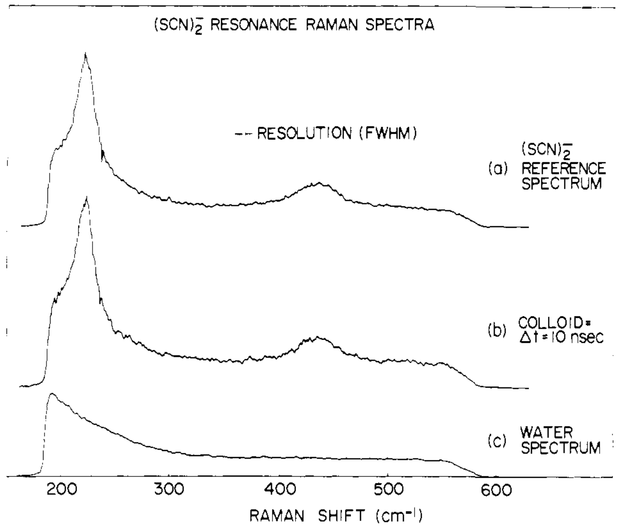

Figure 2. Partial 532-nm resonance Raman spectra of aqueous $(SCN)_2^-$ radical anion: (a) transient spectrum (Δt = 100 ns) following 355-nm excitation of a neutral pH solution 2×10^{-2} M in both benzoquinone and KSCN. The S═S fundamental at 221 cm^{-1} and its overtone at 435 cm^{-1} are on top of continuous H_2O Raman scattering; (b) transient spectrum (Δt = 10 ns) following 355-nm excitation of a concentrated (3×) TiO_2 colloid at pH 1. KSCN concentration is 4×10^{-2} M; (c) H_2O Raman spectrum.

In our study of reaction 1, a concentrated (3 times normal) TiO_2 colloid at pH 1 was excited at 355 nm in the presence of 4×10^{-2} M KSCN. The TiO_2 particles have a net positive charge at this pH, and the SCN^- ions are attracted to the surface.[7,20] The $(SCN)_2^-$ Raman signal in Figure 2b grows approximately as the convolution of the two laser pulses, showing no detectable kinetic rise time, in contrast to the MV^+ results. This result is in agreement with the transient absorption studies. The observed spectrum[21] consists of the S═S stretch (221 cm^{-1}) and a weaker, broad overtone (435 cm^{-1}) on top of the continuous H_2O Raman signal, which appears by itself in Figure 2c. An aqueous $(SCN)_2^-$ reference signal in Figure 2a was generated by oxidation of SCN^- with triplet benzoquinone.[22] As in the case of MV^+, there is no detectable difference between this aqueous reference spectrum and the spectrum observed in the surface reaction.

These experiments demonstrate that it is possible to obtain transient Raman spectra of semiconductor surface reactions in colloidal solutions. The high surface area and homogeneous optical nature of such solutions are favorable for spectroscopic analysis. The Raman spectra confirm the assignment of the 10^{-8}-s time scale products, MV^+ and $(SCN)_2^-$, previously observed by transient absorption spectroscopy.[7] The nearby dielectric particle and the ionic strength and static electric field of the surface ionic double layer have no apparent effect on the molecular Raman spectra under our present conditions.

If the products we observe were adsorbed when created and then immediately desorbed, they could diffuse some tens of angstroms away from the surface at Δt = 5 ns. The unshifted

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(16) Duonghong, D.; Borgarello, E.; Grätzel, M. *J. Am. Chem. Soc.* **1981**, *103*, 4685.

(17) Resonance Raman spectra of MV^+ have been described by the following: (a) Lee, P. C.; Schmidt, K.; Gordon, S.; Meisel, D. *Chem. Phys. Lett.* **1981**, *80*, 242. (b) Forster, M.; Hester, R. E. *Ibid.* **1982**, *85*, 287. Our spectra at 395 nm differ somewhat from these spectra obtained at other laser wavelengths.

(18) We have not attempted to quantitatively verify the rise time kinetics, which are reported in ref 7 to vary with excitation flux as well as pH.

(19) The increase of MV^+ signal between traces 1c and 1b reflects both the intrinsic MV^+ rise time and the finite laser pulse widths.

(20) We observe the TiO_2 absorption spectrum to shift red as a function of KSCN concentration. This suggests that adsorbed SCN^- has a specific chemical effect on the valence and/or conduction band energies.

(21) $(SCN)_2^-$ resonance Raman spectra and assignments were previously reported: Jensen, N. H.; Wilbrandt, R.; Pagsberg, P.; Hester, R. E.; Ernstbrunner, E. *J. Chem. Phys.* **1979**, *71*, 3326.

(22) Beck, S. M.; Brus, L. E. *J. Am. Chem. Soc.* **1982**, *104*, 1103.

spectra observed suggest that $(SCN)_2^-$ and MV^+ are fully solvated by H_2O at this time. Alternately, it could be that SCN· and MV^+ are initially created by electron tunnelling in a fully solvated condition, as has been suggested for reactions on similar semiconductor electrodes.[23] Further experiments at shorter times would be interesting.

Acknowledgment. We thank G. Schwartz and B. Miller for useful discussions.

Registry No. TiO_2, 13463-67-7; SCN^-, 302-04-5; MV^{2+}, 4685-14-7; $(SCN)_2^-$, 34504-17-1.

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[23] Morrison, S. R. "Electrochemistry at Semiconductor and Oxidized Metal Electrodes"; Plenum: New York, 1980; Section 6.2.1.

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^1H and ^2H NMR Studies of the Catechol Dioxygenases

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Catechol 1,2-dioxygenase (CTD) and protocatechuate 3,4-dioxygenase (PCD) catalyze the oxidative cleavage of catechols to *cis,cis*-muconic acids.[1,2] The active sites of these enzymes consist of mononuclear high-spin ferric centers[3,4] coordinated to at least two tyrosinates.[5-8] Resonance Raman studies show that substrate coordinates to the iron in these enzymes, but the mode of catechol binding, that is, whether it is coordinated in a monodentate or chelated configuration to the iron, is not established.[6,9-12] We report therein the first NMR studies of CTD and PCD enzyme–substrate complexes that elucidate the coordination mode of the substrate in these enzymes.

CTD from *Pseudomonas arvilla*[13] has an $\alpha\beta Fe^{3+}$ composition and a molecular weight of 63 000.[14] Figure 1 shows NMR spectra[15] of CTD complexes with catechol and 4-methylcatechol, which are both good substrates for the enzyme. Resonances outside the diamagnetic 0–14 ppm window are observed due to protons on ligands coordinated to the high-spin ferric ($S = {}^5/_2$) center. These resonances arise from contact interactions and provide information regarding the coordination environment of the iron.[16,17]

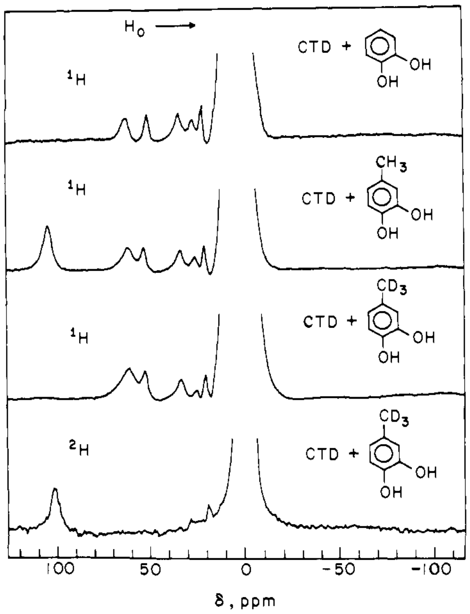

Figure 1. ^1H and ^2H NMR spectra of CTD–substrate complexes in 50 mM potassium phosphate pH 7.5 at 30 °C under argon: [CTD], 1.6 mM; [S], 3.1 mM. Downfield shifts are positive.

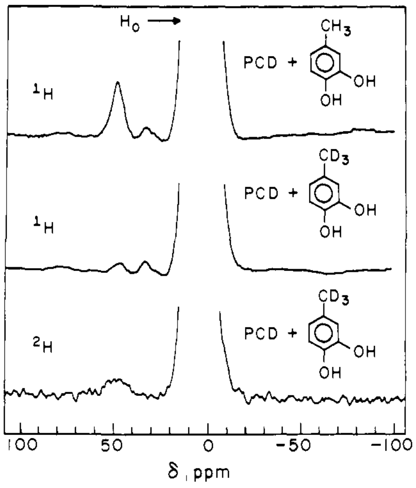

Figure 2. ^1H and ^2H NMR spectra of PCD–substrate complexes in 50 mM potassium phosphate pH 7.5 at 40 °C under argon: [PCD], 0.2 mM; [S], 13 mM.

The focus of this study is on the iron–catecholate interaction. In our study of model ferric catecholate complexes,[18] catecholates coordinated in a monodentate fashion exhibit shifts very similar to those of coordinated phenolates. That is, methyl groups ortho and para to the coordinated oxygen are shifted downfield ca. 100 ppm, while meta-methyl groups are shifted upfield ca. 30 ppm. Chelated catecholate complexes, on the other hand, exhibit different shifts because of the presence of dual pathways for spin delocalization (i.e., through the two oxygens) with methyl groups

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[1] Nozaki, M. *Top. Curr. Chem.* **1979**, *78*, 145–186.

[2] Que, L., Jr. *Struct. Bonding (Berlin)* **1980**, *40*, 38–72.

[3] Que, L., Jr.; Lipscomb, J. D.; Zimmermann, R.; Münck, E.; Orme-Johnson, W. H.; Orme-Johnson, N. R. *Biochim. Biophys. Acta* **1976**, *452*, 320–334.

[4] Kent, T.; Münck, E.; Widom, J.; Que, L., Jr., unpublished results.

[5] Que, L., Jr.; Heistand, R. H., II; Mayer, R.; Roe, A. L. *Biochemistry* **1980**, *19*, 2588–2593. Que, L., Jr.; Epstein, R. M. *Ibid.* **1981**, *20*, 2545–2549.

[6] Tatsuno, Y.; Saeki, Y.; Iwaki, M.; Yagi, T.; Nozaki, M.; Kitagawa, T.; Otsuka, S. *J. Am. Chem. Soc.* **1978**, *100*, 4614–4615.

[7] Keyes, W. E.; Loehr, T. M.; Taylor, M. L. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 941–945.

[8] Bull, C.; Ballou, D. P.; Salmeen, I. *Biochem. Biophys. Res. Commun.* **1979**, *87*, 836–841.

[9] Felton, R. H.; Cheung, L. D.; Phillips, R. S.; May, S. W. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 844–850.

[10] Que, L., Jr.; Heistand, R. H., II *J. Am. Chem. Soc.* **1979**, *101*, 2219–2221.

[11] Que, L., Jr.; Lipscomb, J. D.; Münck, E.; Wood, J. M. *Biochim. Biophys. Acta* **1977**, *485*, 60–74.

[12] May, S. W.; Phillips, R. S.; Oldham, C. D. *Biochemistry* **1978**, *17*, 1853–1860.

[13] CTD was prepared from *Pseudomonas arvilla* (ATCC 23974) with use of a procedure modified from that of Fujiwara et al. Enzyme with specific activity greater than 30 was used for the experiments. Fujiwara, M.; Golovleva, L. A.; Saeki, Y. Nozaki, M.; Hayaishi, O. *J. Biol. Chem.* **1975**, *250*, 4848–4855.

[14] Nakai, C.; Kagamiyama, H.; Saeki, Y.; Nozaki, M. *Arch. Biochem. Biophys.* **1979**, *195*, 12–22.

[15] Enzyme–substrate complexes (1–2 mM) were prepared under argon in potassium phosphate buffer pH 7.5 in H_2O or D_2O (pH reading uncorrected). NMR spectra were recorded on a Bruker WM-300 spectrometer. 300-MHz ^1H NMR spectra were obtained by using 10-μs 90° pulses, an 83-kHz band width with 8K data points, and 40 000 transients. The solvent resonance was suppressed with a 30-ms presaturation pulse. 46-MHz ^2H NMR spectra were obtained by using 30-μs 90° pulses, a 15-kHz band width with 4K data points, and 100 000 transients. Chemical shifts are reported in parts per million, referenced to 4,4-dimethyl-4-silapentanesulfonate (DSS), with downfield shifts taken as positive.

[16] La Mar, G. N.; Eaton, G. R.; Holm, R. H.; Walker, F. A. *J. Am. Chem. Soc.* **1973**, *95*, 63–75.

[17] La Mar, G. N., Horrocks, W. D., Jr.; Holm, R. H., Eds. "NMR of Paramagnetic Molecules. Principles and Applications"; Academic Press: New York, 1973.

[18] Heistand, R. H., II; Lauffer, R. B.; Fikrig, E.; Que, L., Jr. *J. Am. Chem. Soc.* **1982**, *104*, 2789–2796.

0002-7863/82/1504-7324$01.25/0