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.

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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,

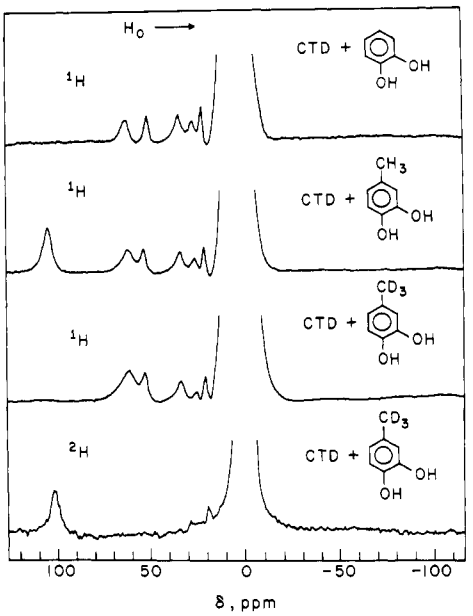

Figure 1. ^1H and ^2H NMR spectra of CTD-substrate complexes in 50 mM potassium phosphate pH 7.5 at 30 °C under argon: [CTD], 1.6 mM; [S], 3.1 mM. Downfield shifts are positive.

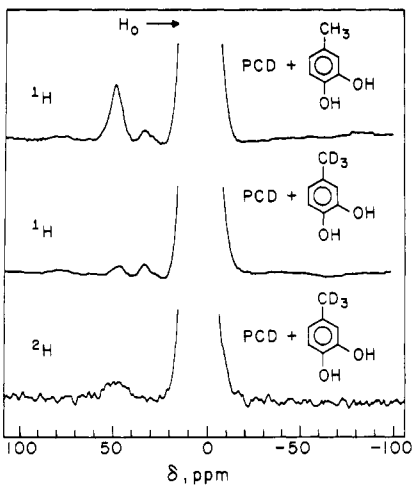

Figure 2. ^1H and ^2H NMR spectra of PCD-substrate complexes in 50 mM potassium phosphate pH 7.5 at 40 °C under argon: [PCD], 0.2 mM; [S], 13 mM.

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]

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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resonating ca. 40–50 ppm downfield.

A comparison of the ^1H NMR spectra of the CTD–catechol and the CTD–4-methylcatechol complexes (Figure 1) shows the appearance of a new feature at 105 ppm for the latter complex. This is assigned to the 4-methyl group on the substrate on the basis of studies with 4-methyl-d_3-catechol.[19,20] The 105 ppm feature is clearly missing in the ^1H NMR spectrum of the CTD-4-methyl-d_3-catechol complex, while the methyl resonance can be observed at 102 ppm in the ^2H NMR spectrum of the same complex.[21] The position of this resonance is consistent with the coordination of 4-methylcatechol to the iron through only one oxygen, the oxygen para to the methyl group. To show the similarity of the ferric center in CTD to our model systems, we have also observed the methyl resonances of the inhibitors in the CTD–*p*-cresol and the CTD–3,5-dimethylphenol complexes; these resonances are found at 87 and −23 ppm, respectively, as expected. At this point, we have not been able to observe the catecholate ring protons in the ^1H and ^2H NMR spectra; perhaps they are too broad. The other features observed in the spectra of the various complexes have yet to be assigned as well. What is clear, however, is that 4-methylcatechol binds in a monodentate configuration to the iron in CTD.

We have also studied the spectra of PCD–substrate complexes. PCD from *Pseudomonas aeruginosa*[22] is an octamer of $\alpha_2\beta_2Fe^{3+}$ units with a molecular weight of 780 000.[24,25] Figure 2 shows NMR spectra of PCD complexed with 4-methylcatechol, which is a pseudosubstrate with a cleavage rate about 1% of that of protocatechuate. Although the higher molecular weight of this enzyme gives rise to broader resonances, deuterium-labeling experiments enable us to assign the feature at 49 ppm unambiguously to the methyl group on the substrate. The isotropic shift observed indicates that, unlike in CTD, 4-methylcatechol chelates to the iron in PCD. This is an unexpected result since the ES complexes of CTD and PCD have such similar spectral properties,[1,2] but the NMR data clearly show that the two complexes are different. Our data at first glance appear to conflict with resonance Raman data on the ES complexes where features consistent with a chelated structure are observed for both complexes.[9,10] However, it has not been demonstrated that the Raman spectra are inconsistent with a monodentate structure.

We have proposed a dioxygenase mechanism[11] postulating a monodentate catecholate iron complex as the species that reacts with dioxygen because of studies demonstrating the large stabilization of the catecholate oxidation state upon chelation to a variety of metal ions, particularly iron.[26–30] According to this mechanism, the CTD ES complex is poised to react with oxygen, while the PCD ES complex would presumably have to undergo a conformational change upon oxygen binding to generate a monodentate substrate configuration. That this conformational change may occur has been suggested by studies with substrate analogues by Lipscomb et al.[31] Whatever the outcome of the mechanistic discussion, this study serves to emphasize the utility of paramagnetic NMR spectroscopy for providing details of the active-site structure in metalloproteins.

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Registry No. AMD, 83719-41-9.

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Synthesis and Structure of Diosmacycloalkanes. Reversible Addition of Ethylene to a Methylene-Bridged Dimer

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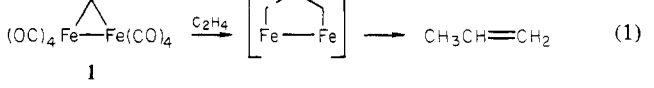
Methylene-bridged dimers and their derivatives have been implicated in olefin metathesis,[1] acetylene polymerization,[2] and other important processes.[3,4] They also serve as models for the surface methylene groups that Pettit and co-workers have shown to be involved in the Fischer–Tropsch reaction[5] and in the hydrogenolysis of linear hydrocarbons.[6] Considerable interest has therefore been aroused by the report (also from Pettit's group[7]) that propene is formed from the reaction of $(\mu\text{-}CH_2)Fe_2(CO)_8$ (**1**) with ethylene, and by their proposal that the reaction (eq 1) involves a di-



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ferracyclopentane intermediate. This hypothesis has stimulated efforts at the synthesis of dimetallacycloalkanes in general and has led to the successful preparation of $((\mu\text{-}CC')CH_2CH_2CH_2)Co_2(CO)_2Cp_2$ (**2**) by Theopold and Bergman.[8] However, although propene is formed upon thermolysis of **2**, generation of the latter by the reaction of ethylene with the corresponding methylene-bridged dimer has not proven possible.[9,10] We now report the

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