Extradiol Oxidative Cleavage of Catechols by Ferrous and Ferric Complexes of 1,4,7-Triazacyclononane: Insight into the Mechanism of the Extradiol Catechol Dioxygenases

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Abstract: The major oxygenation product of catechol by dioxygen in the presence of $FeCl_2$ or $FeCl_3$, 1,4,7-triazacyclononane (TACN), and pyridine in methanol is the extradiol cleavage product 2-hydroxymuconic semi-aldehyde methyl ester (Lin, G.; Reid, G.; Bugg, T. D. H. *J. Chem. Soc. Chem. Commun*. **2000**, 1119−1120). Under these conditions, extradiol cleavage of a range of 3- and 4-substituted catechols with electron-donating substituents is observed. The reaction shows a preference in selectivity and rate for iron(II) rather than iron(III) for the extradiol cleavage, which parallels the selectivity of the extradiol dioxygenase family. The reaction also shows a high selectivity for the macrocyclic ligand, TACN, over a range of other nitrogen- and oxygen-containing macrocycles. Reaction of anaerobically prepared iron−TACN complexes with dioxygen gave the same product as monitored by UV/vis spectroscopy. KO_2 is able to oxidize catechols with both electron-donating and electron-withdrawing substituents, implying a different mechanism for extradiol cleavage. Saturation kinetics were observed for catechols, which fit the Michaelis−Menten equation to give $k_{cat}^{app} = 4.8 \times 10^{-3}$ s^{-1} for 3-(2′,3′-dihydroxyphenyl)propionic acid. The reaction was also found to proceed using monosodium catecholate in the absence of pyridine, but with different product ratios, giving insight into the acid/base chemistry of extradiol cleavage. In particular, extradiol cleavage in the presence of iron(II) shows a requirement for a proton donor, implying a role for an acidic group in the extradiol dioxygenase active site.

Introduction

The oxidative cleavage of catechol and other dihydroxy aromatics is a key step in the biodegradation by soil bacteria of naturally occurring aromatic molecules and many aromatic environmental pollutants.[1] The catechol dioxygenases are a class of non-heme iron enzymes that catalyze the oxidative cleavage of catechols. These enzymes can be divided into two subclasses (Figure 1): the intradiol dioxygenases, which utilize a non-heme iron(III) cofactor, that catalyze the cleavage of the carbon−carbon bond between the two catechol hydroxyl oxygens; and the extradiol dioxygenases, which utilize a non-heme iron(II) cofactor, that catalyze the cleavage of the carbon−carbon bond adjacent to the catechol oxygens.[2]

The three-dimensional crystal structures of three extradiol catechol dioxygenases, 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC) from *Pseudomonas* LB400,[3] catechol 2,3-dioxygenase (XylE) from *Pseudomonas putida* mt-2,[4] and protocatechuate 4,5-dioxygenase (LigAB) from *Sphingomonas paucimobilis*[5] have been determined, and in each case the mononuclear iron(II) center is ligated by two histidine ligands and one glutamic acid ligand. In contrast the mononuclear iron(III) center of the intradiol-cleaving protocatechuate 3,4-dioxygenase from *Pseudomonas aeruginosa* and catechol 1,2-dioxygenase from *Acinetobacter calcoaceticus* is ligated by two histidine ligands and two tyrosine ligands.[6]

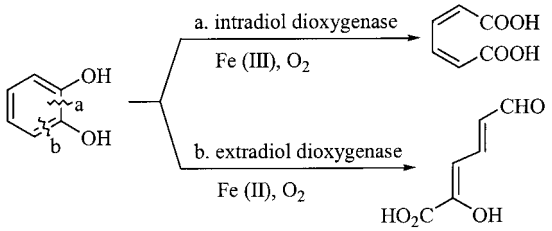

Figure 1. Enzyme-catalyzed reactions of the extradiol and intradiol dioxygenases.

Many attempts to model the catechol dioxygenases have been reported, most of which give products consistent with intradiol cleavage.[7] In all of these cases, the ligands used to prepare the iron(III) complexes are tetradentate, thus resembling the coordination environment of iron(III) center in the active site of

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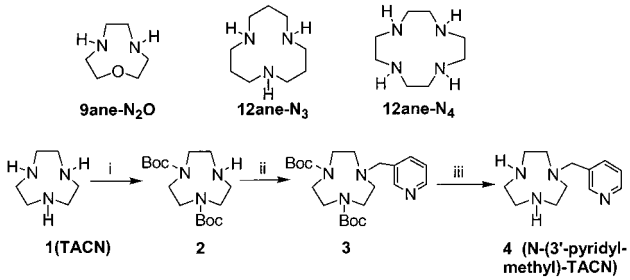
the intradiol dioxygenases. Que and co-workers have found a correlation between the Lewis acidity of the iron center in a series of iron(III) complexes of tripodal ligands, with the product yield and reactivity,[7] and proposed a substrate activation rather than oxygen activation mechanism. In this mechanism an iron(III)-catecholate species undergoes ligand to metal charge transfer to give an iron(II)-semiquinone intermediate. The attack of O_2 on the activated substrate yields a transient alkyl-peroxy radical which combines with the equally short-lived iron(II) center to generate an alkyl-peroxy iron(III) species. The peroxy adduct decomposes by a Criegee-type rearrangement to muconic anhydride.

However, there are a few reported examples of extradiol-type cleavage by synthetic iron complexes to give pyrone products. Funabiki et al. found that $FeCl_2/FeCl_3$ complexes with bipyridine/pyridine prepared in situ cleave 3,5-di-*tert*-butyl catechol to 2-pyrones, which are believed to derive from decarbonylation of the extradiol-cleavage intermediate α-keto lactone.[8] Dei et al. found that the complex [Fe^{III}(TACN)Cl-(dbc)] yielded 2-pyrones upon exposure to O_2 in 35% yield.[9] Que et al. used the same complex to give an almost quantitative yield of 2-pyrone using a modified procedure.[10] We have reported that the oxygenation of catechol by O_2 in the presence of $FeCl_2/FeCl_3$, TACN (1,4,9-triazacyclononane), and pyridine in methanol gave authentic 2-hydroxymuconic semi-aldehyde methyl ester in about 50% yield.[11] In these model reactions, ligands TACN and bipyridine/pyridine are tridentate, resembling the coordination environment of the iron(II) center in the active site of extradiol dioxygenases. However, the mechanism of this model reaction remains unclear. Extradiol catechol cleavage has also been reported using KO_2.[14]

Previous studies in our laboratory have focused on the reaction mechanism of the extradiol enzyme 2,3-dihydroxyphenyl propionate 1,2-dioxygenase (MhpB) from *Escherichia coli*. Mechanistic studies using substrates containing cyclopropyl radical traps have established that, following the ligation of the catechol and dioxygen by the iron(II) center, the catalytic mechanism proceeds via single electron transfers to give a semiquinone−iron(II)−superoxide intermediate.[12] ^{18}O-labeling studies have established the existence of a seven-membered ring lactone intermediate, formed by Criegee rearrangement of the hydroperoxide.[13] Synthesis and evaluation of carba-analogues of putative proximal and distal hydroperoxide intermediates have provided evidence in support of a proximal hydroperoxide intermediate for MhpB. The reaction is completed by attack of iron(II)−hydroxide upon the lactone to give the extradiol ring fission product.[13]

We report here, the syntheses of tridentate ligands and studies of the TACN/$FeCl_2$ model reaction, including evidence of formation of an intradiol cleavage product and an extradiol cleavage product; the preference of iron(II) over iron(III), substrate specificity, kinetics, and regioselectivity. These studies give new insights into the questions of why iron(II) and iron(III) are employed respectively by the extradiol and intradiol dioxygenases, and why they exhibit different active-site coordination chemistry.

Scheme 1. Macrocyclic Ligands, Synthetic Route for *N*-(3′-Pyridyl)-TACN (4)[a]



[a] Reagents and conditions: i) Boc-ON,$CHCl_3$, 92%; ii) K_2CO_3, CH_3CN, 3-$BrCH_2$-pyridine, 45%; iii) CF_3CO_2H, CH_2Cl_2, quant.

Results

Synthesis of Macrocyclic Ligands. TACN was synthesized according to a literature procedure,[16] and commercially available 12ane-N_3 (1,5,9-triazacyclododecane) was tested for activity. Since the extradiol dioxygenase active site contains N, N, O coordination, 9ane-N_2O and 12ane-N_3O were synthesized according to literature methods.[17,18] Since the active site of BphC also contains additional acid/base residues[3], monosubstituted *N*-(3′-pyridylmethyl)-TACN **4**, was designed and synthesized as shown in Scheme 1. N,N′-DiBoc-TACN **2** was obtained by reacting TACN **1** with 2 equiv of Boc-ON reagent in 92% yield.[19] Treatment of **2** with 3-bromomethyl pyridine gave **3** in 45% yield. Removal of Boc groups was achieved by stirring with 2% trifluoroacetic acid in dichloromethane to give **4** in quantitative yield.

Assay of Monosubstituted TACN and N_2O, 12ane-N_3 in Model Reaction. We have developed a simple method to scan for the extradiol cleaving activity of specific ligands on the basis of the observation that extradiol cleavage of catechol in buffer pH 8.0, yields a bright yellow color with UV/vis absorption at 375 nm. When catechol was used in the model reaction catalyzed by TACN/$FeCl_2$, the initial extradiol cleavage product shows an absorbance at 315 nm in methanol and then shifts to 405 nm by adding pH 8.0 Tris buffer; with addition of NaOH solution, the absorbance completely shifts to 378 nm after 2 h (Figure 2).

All assays with other macrocyclic ligands showed no absorbance above 300 nm, which indicates that (1) three nitrogen atoms here are essential for the occurrence of the reaction; (2) substitution of H on one nitrogen atom of TACN disrupts the extradiol-cleaving activity; (3) the increase of one carbon in each bridge between two nitrogen atoms also leads to the loss of activity. The extradiol cleavage reaction is therefore highly selective for the macrocyclic ligand.

Metal Dependence. The replacement of $FeCl_2/FeCl_3$ by $MnCl_2$, $CoCl_2$, CuCl showed no reaction at all. The replacement

Funabiki, T.; Yamazaki, T.; Fukui, A.; Tanaka, T.; Yashida, S. *Angew. Chem., Int. Ed.* **1998**, *37*, 513−515; Koch, W. O.; Krüger, H-J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2671−2674; Mialane, P.; Tchertanov, L.; Banse, F.; Sainton, J.; Girerd, J-J. *Inorg. Chem.* **2000**, *39*, 2440−2444.

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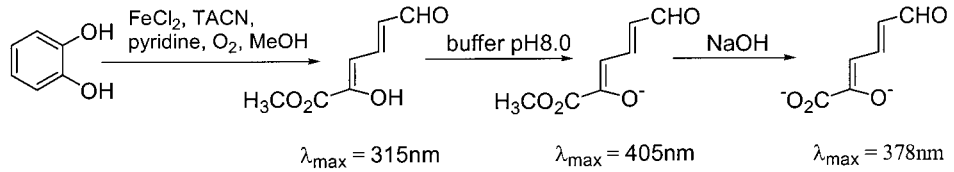


Figure 2. Intermediates and respective λ_{max} values for $FeCl_2$/TACN model reaction.

Table 1. Metal Dependence of Model Reaction[a]

metal salt[a]	base[a]	substrate[a]	solvent	rate (ΔA/min)
$FeCl_2$	pyridine	catechol	CH_3OH	0.033
$FeCl_2$	pyridine	DHP	CH_3OH	0.098
$FeBr_2$	pyridine	catechol	CH_3OH	0.053
$FeBr_2$	pyridine	DHP	CH_3OH	0.133
$FeCl_2$	DBU	catechol	CH_3OH	0.026
$FeCl_2$	2,6-lutidine	catechol	CH_3OH	no reaction
$FeCl_2$	DMAP	catechol	CH_3OH	no reaction
$MnCl_2$	pyridine	catechol	CH_3OH	no reaction
$CoCl_2$	pyridine	catechol	CH_3OH	no reaction
CuCl	pyridine	catechol	CH_3OH	no reaction
$FeSO_4$	pyridine	catechol	CH_3OH	no reaction
$(NH_4)_2Fe(SO_4)_2$	pyridine	catechol	CH_3OH	no reaction

[a] Reactions were carried out with 0.1 mM of metal salt, 1 mM of TACN, 0.3 mM of base, and 0.1 mM of substrate in methanol (DHP = 3-(2′,3′-dihydroxyphenyl)propionic acid) at 23 °C.

Table 2. Effect of Changing Reaction Conditions on Rate[a]

	concentration (mM)				rate[a]	
solvent	$FeCl_3$	TACN	pyridine	catechol	ΔA/min	nmoles/min
MeOH	0.1	0.1	0.3	0.1	0.017	0.94
solvent	$FeCl_2$	TACN	pyridine	catechol	ΔA/min	nmoles/min
MeOH	0.1	0.1	0.3	0.1	0.033	1.83
MeOH	0.1	0.1	0.15	0.1	0.036	2.00
MeOH	0.1	0.1	0.1	0.1	0.024	1.33
MeOH	0.1	0.1	0.1	0.05	0.018	1.00
MeOH	0.1	0.05	0.3	0.1	0.021	1.17
MeOH	0.1	0.05	0.15	0.1	0.021	1.17
MeOH	0.05	0.05	0.3	0.1	0.013	0.72
MeOH	0.05	0.1	0.3	0.1	0.036	2.00
EtOH	0.1	0.1	0.3	0.1	0.026	1.44
2-Propanol	0.1	0.1	0.3	0.1	0.016	0.89
MeOH	0.1	0.1	0.3	0.1[b]	0.047	2.61
MeOH	0.1	0.1	–	0.1[b]	0.046	2.56

[a] Methanol, $FeCl_2$ or $FeCl_3$, TACN, pyridine, catechol were added in this order into a cuvette, and extradiol product formation was measured at 315 nm over a total of 6 min at 23 °C. [b] Catechol was replaced by monosodium catecholate.

of $FeCl_2$ by $FeSO_4$ or $Fe_2(NH_4)_2(SO_4)_2$ also showed no reaction. $FeBr_2$ gave the characteristic UV absorbance of 315 nm under the standard reaction conditions, and kinetic studies showed that reaction with $FeBr_2$ is faster than that with $FeCl_2$ (Table 1). Since $FeBr_2$ is a softer Lewis acid than $FeCl_2$, this may indicate a preference for a soft Lewis acid for extradiol cleavage.

Optimization of the TACN/$FeCl_2$ Model Reactions. Optimization of the reaction was carried out by changing the ratio between all the four reagents, and by changing the base. The results are listed in Table 2. No extradiol ring cleavage was observed when either TACN, pyridine, or $FeCl_2$/$FeCl_3$ was omitted. The ratio of reagents found to give optimum activity was a 1:1:1.5:1 ratio of catechol:TACN:pyridine:$FeCl_2$. The reaction was found to proceed using DBU in place of pyridine but at a lower rate. The absorption maximum at 315 nm was still observed using ethanol or 2-propanol instead of methanol. The lower rate of the reaction in ethanol and 2-propanol indicates that the final step, alcoholysis of the seven-membered ring α-keto lactone, is becoming more rate-determining upon changing solvent from methanol to ethanol then to 2-propanol, due to increased steric hindrance. No reaction was observed with the reaction carried out in *tert*-butyl alcohol. Also, no reaction was observed when solvent was changed to H_2O, THF, or Et_2O.

Substrate Specificity. The TACN/$FeCl_2$ model reaction was found to operate on a range of substituted catechols. Highest rates were observed with electron-donating substituents, whereas catechols with electron-withdrawing substituents were not processed. This indicates that an electronic effect plays an important role in the TACN/$FeCl_2$ model reaction. A similar pattern of results was found for the reaction catalyzed by *E. coli* 2,3-dihydroxyphenyl propionate 1,2-dioxygenase (MhpB): catechols with electron-withdrawing substituents were not turned over. The results are listed in Table 3. The similar substrate dependence is consistent with a similar mechanism for enzymatic and model reactions.

In contrast, the KO_2/DMSO reaction reacts with catechols containing either electron-donating substrates or electron-withdrawing substrates at similar rates (Table 3). Furthermore, all of the catechols with electron-donating alkyl substituents give products that have an absorbance maximum at 377 nm. This means that KO_2 reacts with 3-substituted catechols via 1,6-cleavage to give substituted 2-hydroxymuconic semi-aldehydes rather than ketone derivatives. It suggests a different mechanism for the KO_2/DMSO reaction, probably via a dioxetane intermediate.

When 3,5-di-*tert*-butyl catechol was used as substrate in the TACN/$FeCl_2$ reaction, mixtures of 2-pyrones were obtained (Scheme 2), as observed by Dei et al.[9] No 2-hydroxymuconic semi-aldehyde derivatives were found, suggesting that the mechanism for 2-pyrone formation diverges from the extradiol mechanism prior to the formation of the α-keto lactone intermediate.

Assay of $Fe^{III}(TACN)Cl_3$ in the Model Reaction. $Fe^{III}(TACN)Cl_3$ was prepared using the method of Wieghardt et al.,[15] and was used in place of $FeCl_3$ and TACN in the model reaction. The complex was not soluble in methanol; however, when pyridine was added to the suspension of FeIII(TACN)Cl_3 and catechol in methanol, the complex gradually dissolved with time, and finally gave the characteristic absorbance of the extradiol cleavage product at λ_{max} 315 nm (Scheme 3).

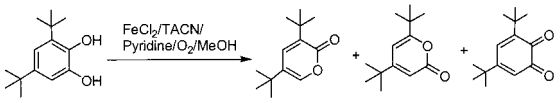
Characterization of Reaction Products and Regioselectivity. The identity of the reaction products was confirmed by carrying out the reaction of 11 mg of catechol with 1 equiv of TACN, 1 equiv of $FeCl_2 \cdot 4H_2O$, and 3 equiv of pyridine in 500 mL of methanol by bubbling O_2 under the surface of the solution for 3 h. The ^1H NMR spectrum of the reaction extraction product displayed signals corresponding to 2-hydroxymuconic semi-aldehyde methyl ester **5** as the major product by comparison to the extradiol cleavage product of enzymatic reaction of 2,3-dihydroxyphenylpropionic acid and 2,3-dihydroxyphenylpropionate 1,2-dioxygenase. Muconic acid monomethyl ester **6** was identified as the minor product confirmed by comparison to the NMR spectrum of an authentic sample (Scheme 4). The ratio of extradiol cleavage product to intradiol cleavage product

Table 3. Substrate Selectivity for $FeCl_2$/TACN and KO_2 Reactions and for MhpB-Catalyzed Enzymatic Reaction[c]

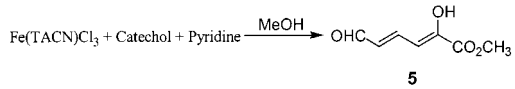
Catechol			λ_{max} (nm)				V_{rel}	
	R_1	R_2	$FeCl_2$/TACN		MhpB	KO_2	$FeCl_2$/TACN	MhpB[a]
			In methanol	+ NaOH			(ΔA/min)	(%)
	H	H	315	375	375	373	0.033	64
	CH_3	H	317	388	385	378	0.13	58
	OCH_3	H	305	374	NT	377	0.12	NT
	H	CH_3	313	403	379	388, 413	0.019	35
	$CH_2CH_2CH_3$	H	320	386	392	377	0.12	85
	CH_2CH_2Ph[a]	H	320	386	392	377	0.016	44
	OCH_2Ph[b]	H	308	368	348	NT	0.13	23
	$CH_2CH_2CO_2H$[b]	H	317	398	394	380	0.11	100
	$CH{=}CHCO_2H$[b]	H	no RFP	-	454	377, 479	-	99
	CH=CHPh[b]	H	no RFP	-	no RFP	377	-	-
	OH	H	no RFP	-	no RFP	347, 468	-	-
	CHO	H	no RFP	-	no RFP	NT	-	-
	CO_2H	H	no RFP	-	no RFP	no RFP	-	-
	H	CN	no RFP	-	no RFP	430	-	-
	H	CO_2H	no RFP	-	no RFP	392	-	-

[a] See Spence E. L.; Kawamukai M.; Sanvoisin J.; Braven H.; Bugg T. D. H.; *J. Bacteriol*. **1996**, *178*, 5249. [b] See refs 12 and 13. [c] NT, not tested; RFP, ring fission product.

Scheme 2. Reaction of 3,5-Di-*tert*-butyl Catechol with TACN/$FeCl_2$/Pyridine



Scheme 3. Reaction of Catechol with Fe(TACN)Cl_3 Complex



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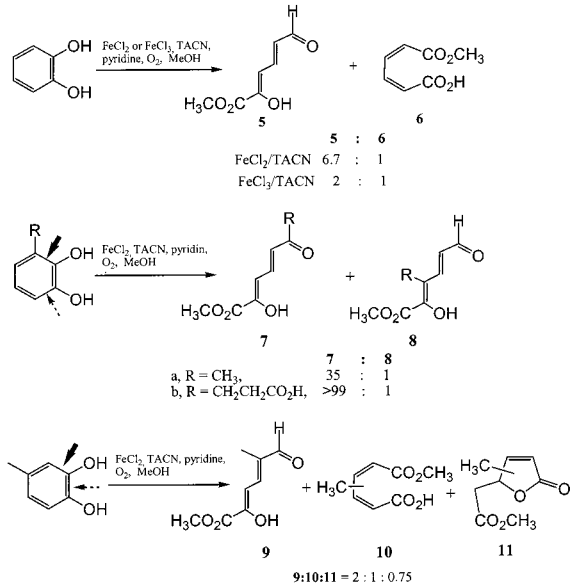
determined by integration of ^{1}H NMR signals was 7:1. When $FeCl_2$ was replaced by $FeCl_3$, the major product was still the extradiol cleavage product, but the ratio between extradiol and intradiol products was reduced to 2:1. When the solvent was changed from methanol to ethanol, the reaction slowed, and the ^{1}H NMR spectrum of the reaction product displayed peaks consistent with the ethyl ester of 2-hydroxymuconic semialdehyde.

The regioselectivity of the TACN/$FeCl_2$ reaction was investigated using 3-methylcatechol and 4-methylcatechol. In the case of 3-methylcatechol, only extradiol cleavage products were observed: the ketone product of 2,3-cleavage and the aldehyde product of 1,6-cleavage were obtained in a 35:1 ratio, indicating a highly regioselective reaction. In the case of 4-methyl catechol, the NMR spectrum of the crude product showed only one extradiol product, arising from 2,3-cleavage, and the intradiol products. Again, the extradiol cleavage is highly regioselective, but in this case intradiol cleavage is a competing reaction. When 3-(2′,3′-dihydroxyphenyl)propionic acid was used as substrate, the aldehyde product was not detectable in the NMR spectrum, only the ketone product was obtained. These data indicate that the reaction shows high regioselectivity for extradiol cleavage.

Reaction Kinetics. The addition of the four reagents and solvent was always carried out in the order: methanol, $FeCl_2$/$FeCl_3$, TACN, catechol, pyridine. The rate of product formation at 315 nm is linear over time up to 6 min when the final

Scheme 4. Product Ratios for Cleavage of Catechol and Substituted Catechols



concentration of $FeCl_2$/$FeCl_3$, TACN, catechol are each 0.1 mM. The effect of changing the relative concentration of the four reagents led to varied behavior. There was no change of initial rate when the concentration of pyridine was reduced from 3 to 1.5 equiv, but in the presence of 1.0 equiv, a reduced rate was observed. Halving the concentration of TACN gave a reduced rate; halving the $FeCl_2$ concentration gave no change in rate at 0.1 mM TACN, but a reduction in rate at 0.05 mM TACN was observed.

The initial rates of $FeCl_2$-containing reaction were slower than that of $FeBr_2$-containing reaction with catechol, DHP, respectively. All of the results are shown in Table 2.

The variation of rate with changing catechol concentration was measured at 23 °C, keeping the final concentrations of $FeCl_2 \cdot 4H_2O$ (0.1 mM), TACN (0.1 mM), pyridine (0.3 mM)

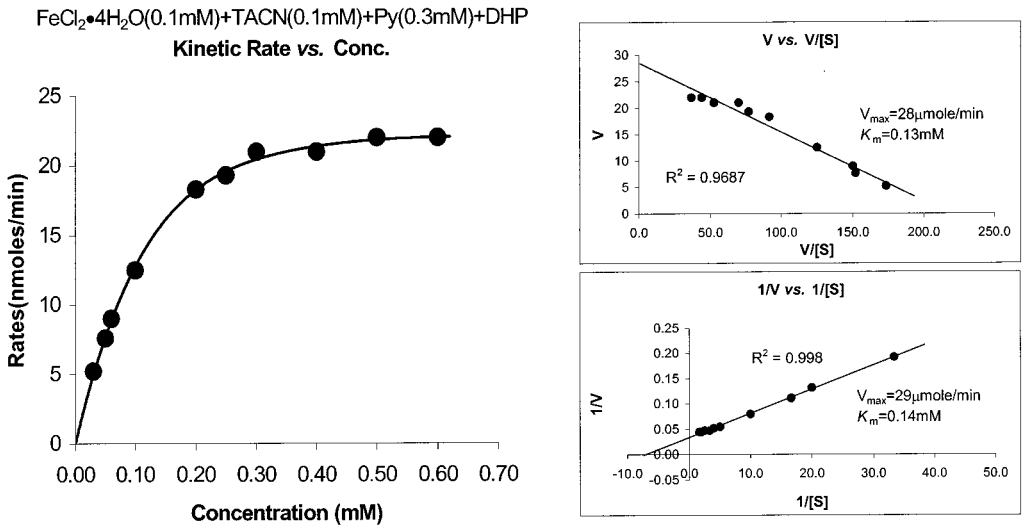


Figure 3. Kinetic behavior of model reaction with substrate 2,3-dihydroxyphenylpropionic acid. Assays carried out at constant final concentrations of $FeCl_2 \cdot 4H_2O$ (0.1 mM), TACN (0.1 mM), pyridine (0.3 mM), varying the concentration of DHP, recording the initial rates at each concentration up to 6 min at 23 °C.

Table 4. Steady-State Kinetic Parameters for (A) $FeCl_2 \cdot 4H_2O$ (0.1 mM) + TACN (0.1 mM) + Pyridine (0.3 mM) + DHP in MeOH (apparent K_m, k_{cat} Values; See Figure 3); (B) MhpB-Catalyzed Reaction (Ref 24)

substrate		ϵ_{RFP} ($M^{-1}\,cm^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}\,s^{-1}$)	$\Delta\Delta G$ (kJ/mol)
3-(2′,3′-dihydroxyphenyl)propionate	A	8100 (317 nm)	140	4.8×10^{-3}	34.3	26.0
	B	15600 (394 nm)	26	29	1.1×10^6	

constant, but changing the concentration of DHP. Saturation kinetics were observed by plotting initial rates versus concentration of catechol (Figure 3). Apparent K_m (0.14 mM) and k_{cat} ($4.8 \times 10^{-3}\ s^{-1}$) values were obtained by applying Lineweaver−Burk or Eadie−Hofstee plots shown in Table 4. This strongly suggests that the complex of iron−TACN, the "active site" of the model reaction, is formed before combining with the substrate.

Investigation of Intermediate Catechol−Iron−TACN Complexes. Dei et al. have previously described the preparation of Fe^{III}(TACN)(3,5-di-*tert*-butylcatechol)Cl from $Fe^{III}(TACN)Cl_3$ under anaerobic conditions; using the same procedure we prepared anaerobically Fe^{III}(TACN)(catechol)Cl and Fe^{II}-(TACN)(catechol)Cl from $FeCl_3$ and $FeCl_2$ respectively, using triethylamine as base.

The Fe^{III}(TACN)(catechol)Cl complex was found to exhibit a UV/vis spectrum containing peaks at 451 and 720 nm (see Figure 4A), similar to the UV/vis data (λ_{max} 480, 520, 782 nm) reported by Funabiki et al. for a complex of 3,5-di-*tert*-butylcatechol with Fe^{III}(bipyridine)(pyridine), due to a ligand-to-metal charge-transfer interaction.[8] When the Fe^{III}(TACN)(catechol)-Cl complex was exposed to O_2, the peaks at 451 and 720 nm decayed, and a new peak at 315 nm appeared, corresponding to the extradiol cleavage product.

When the TACN/$FeCl_3$ reaction was carried out at 0.4 mM TACN, catechol, $FeCl_3 \cdot 6H_2O$ concentration, as shown in Figure 4B, the same absorbance peaks were observed immediately and decreased with time, as the extradiol product was formed. These observations demonstrate the formation of the same Fe^{III}-(TACN)(catechol) complex in the $FeCl_3$ model reaction.

The Fe^{II}(TACN)(catechol)Cl complex, prepared anaerobically using triethylamine as base, was found to exhibit a UV/vis spectrum containing one peak at 550 nm (see Figure 4C); however, upon exposure to O_2 only very slow extradiol product formation was observed. The complex was then prepared using pyridine as base. In this case a different Fe^{II}(TACN)(catechol) complex was obtained, which showed no λ_{max} in the range 300−800 nm, but was converted rapidly to extradiol product on exposure to O_2. Attempts to prepare single crystals of this complex using a range of substituted catechols were unsuccessful; however, the Fe^{II}(TACN)(catechol) complex was characterized by ^1H NMR spectroscopy. The NMR spectrum showed signals in the range 0−10 ppm, characteristic of a low-spin iron(II) complex. Signals were observed for TACN (2.9−3.6 ppm), catechol (6.68, 6.76 ppm), and pyridine (7.6, 7.95, 8.6 ppm) ligands, implying a mononuclear Fe^{II}(TACN)(catechol)(pyridine) complex.

When the $FeCl_2$-containing reaction at 0.4 mM TACN/$FeCl_2$/catechol was monitored by UV/vis spectroscopy (Figure 4D), immediate extradiol product formation was observed at 315 nm, and the only other observed species was the gradual formation of the Fe^{III}(TACN)(catechol) complex at 449/720 nm. These data can be rationalized by the formation of an Fe^{II}(TACN)-(catechol) complex (no λ_{max}), followed by its gradual oxidation to an Fe^{III}(TACN)(catechol) complex (λ_{max} 449, 720 nm) under aerobic conditions. These observations give some insight into the oxidation state of the iron center during the reaction. Since oxidation of iron(II) to iron(III) occurs on a slower time scale than extradiol product formation, these observations imply that extradiol cleavage is mediated by an iron(II) complex. The appearance of the same Fe^{III}(TACN)(catechol) complex in the $FeCl_2$/TACN reaction also suggests that the active iron(II) and iron(III) complexes are structurally related.

Reaction of Monocatecholate with TACN/Iron. To explore the role of pyridine in this model reaction, monosodium catecholate and disodium catecholate were prepared by reacting catechol with 1 equiv of NaOH and 2 equiv of NaOH in methanol, respectively. No reaction was observed with disodium catecholate upon treatment with TACN/$FeCl_2$. When sodium monocatecholate was reacted with TACN/$FeCl_2$ or TACN/$FeCl_3$

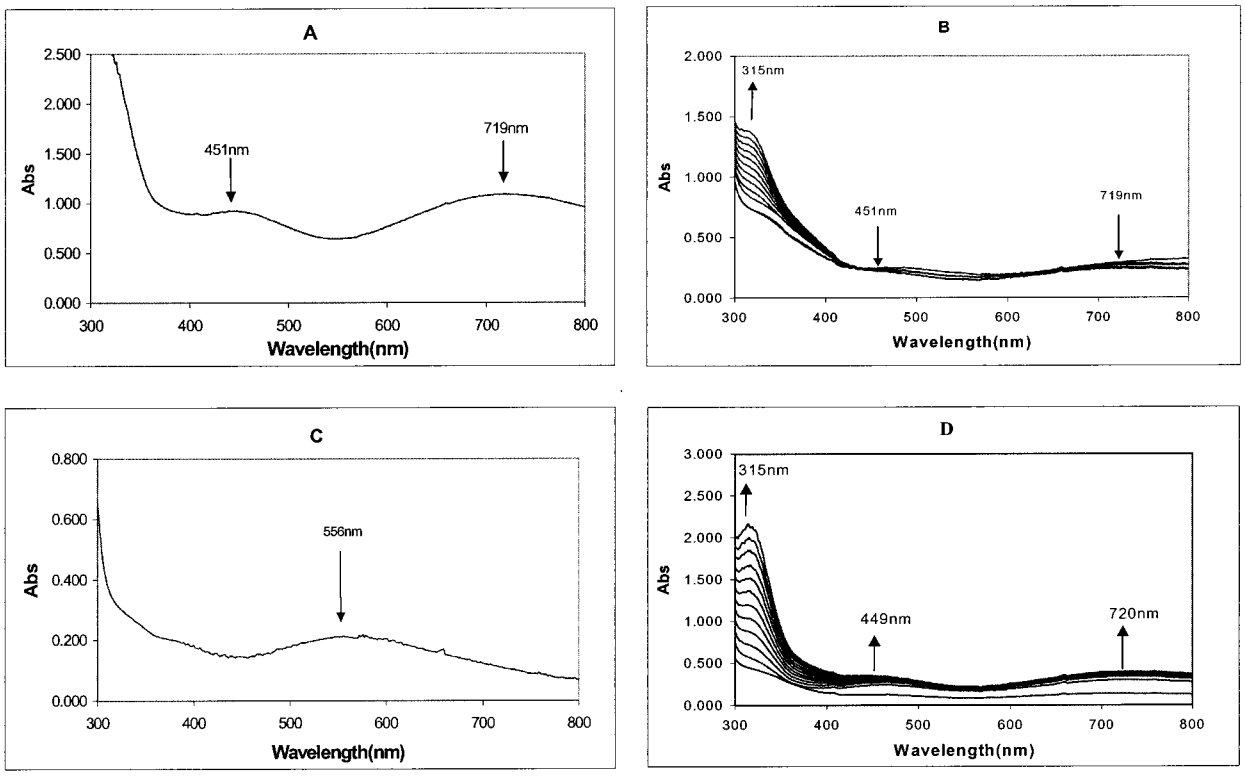
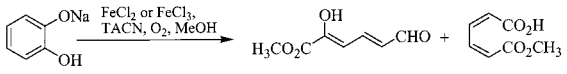


Figure 4. UV/vis spectra of TACN/iron/catechol complexes (A) Fe^{III}(TACN)(catechol) complex generated anaerobically in methanol, using triethylamine as base. (B) UV/vis scanning of TACN/$FeCl_3$ reaction at 0.4 mM concentration in methanol. Scans recorded at 1-min intervals over 0−10 min. Arrows indicate increase in A_{315} (extradiol product formation), and decrease in A_{451} and A_{719} (complex). (C) Fe^{II}(TACN)(catechol) complex generated anaerobically in methanol using triethylamine as base. (D) UV/vis scanning of TACN/$FeCl_2$ reaction at 0.4 mM concentration in methanol. Scans recorded at 1-min intervals over 0−11 min. Arrows indicate increase in A_{315} (extradiol product formation), and increase in A_{451} and A_{719} (complex).

Scheme 5. Product Ratios for the Reaction of Monosodium Catecholate with TACN/$FeCl_2$ and TACN/$FeCl_3$



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	5		6
$FeCl_2$/TACN	1	:	15
$FeCl_2$/TACN/pyridinium.HCl	4	:	1
$FeCl_3$/TACN	3.2	:	1

in methanol, *in the absence of pyridine*, UV/vis spectra showed in both reactions the formation of extradiol ring fission product (RFP) with absorbance at 315 nm. k_{obs} showed the reaction running at the same rate, with or without pyridine (Table 2). No extradiol cleavage was observed in the absence of TACN.

To determine the ratios of products, large-scale reactions were carried out using monosodium catecholate in the absence of pyridine (Scheme 5). With TACN/$FeCl_3$, the NMR spectrum of the crude product indicated that extradiol RFP and intradiol RFP were both formed, in a ratio of 3.2:1. With TACN/$FeCl_2$, the NMR spectrum of the crude product showed that although both extradiol RFP and intradiol RFP were formed, the intradiol RFP was by far the major product. The ratio of intradiol RFP and extradiol RFP was 15:1. When 1 equiv of pyridine hydrochloride was added to the reaction of TACN/$FeCl_2$ and monosodium catecholate, the extradiol RFP was obtained as the major product, and the ratio of extradiol RFP and intradiol RFP was 4:1 (Figure 6). Thus, the presence of 1 equiv of pyridine hydrochloride leads to a 60-fold increase in ratio of extradiol/intradiol products in the TACN/$FeCl_2$ reaction.

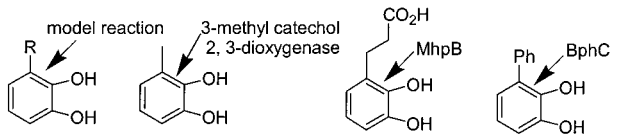

Figure 5. Similar regioselectivity of model reaction to that of extradiol catechol dioxygenases.

Discussion

These results show that the TACN/$FeCl_2$ and TACN/$FeCl_3$ reactions show considerable scope, reactivity, and selectivity for the extradiol oxidative cleavage of catechol substrates. The studies in this paper give new insight into the mechanism of extradiol cleavage by these complexes, and by inference, the catalytic mechanism of the extradiol catechol dioxygenases.

This model reaction is highly selective for the TACN macrocyclic ligand, indicating that the coordination chemistry and precise alignment is important for the extradiol reaction. The tridentate coordination of TACN matches the His, His, Glu tridentate coordination of Fe(II) at the active site of the extradiol dioxygenases.[3] The fact that the *N*,*N*,*N*-TACN ligand works in this reaction suggests that coordination geometry is more important than ligand type. The observation that the mono-N-substituted derivative gives no sign of formation of extradiol cleavage product indicates that all three NH's are required for activity.

Since the extradiol catechol dioxygenases are selective for Fe(II) while the intradiol dioxygenase are selective for Fe(III), it is of considerable interest that both TACN/$FeCl_2$ and TACN/$FeCl_3$ are capable of extradiol cleavage. However, the TACN/$FeCl_2$ reaction shows higher rates and higher selectivity for

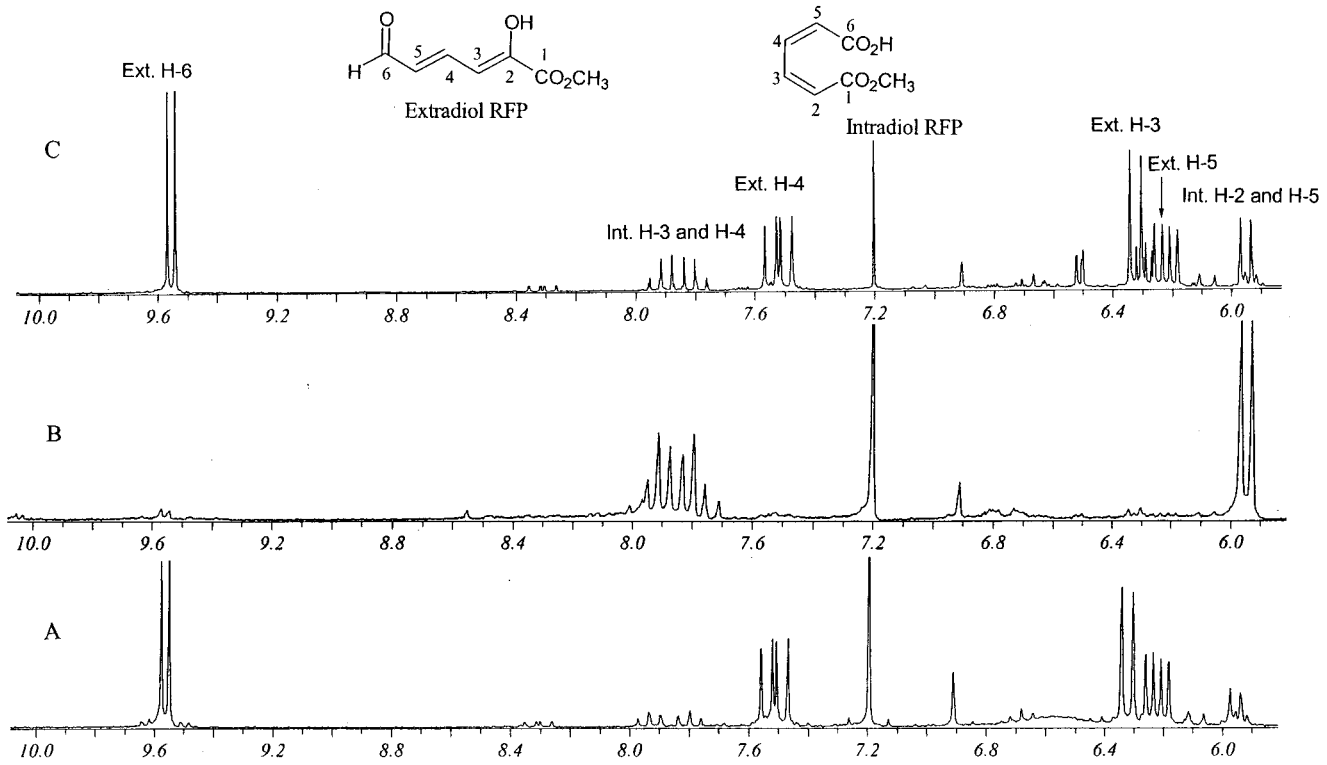

Figure 6. 300 MHz ^{1}H NMR spectra of reaction products: (A) $FeCl_2$ + TACN + Catechol + Pyridine; (B) $FeCl_2$ + TACN + Monosodium catecholate; (C) $FeCl_2$ + TACN + Monosodium catecholate + pyridine hydrochloride (1 equiv).

extradiol cleavage, suggesting that Fe(II) has an inherently higher chemical reactivity for extradiol cleavage than Fe(III). Also, highly regioselective cleavage is observed for 3-methylcatechol, and 3-(2′,3′-dihydroxyphenyl)propionic acid in the TACN/$FeCl_2$ reaction, the same selectivity as observed in extradiol catechol dioxygenases which process 3-substituted catechols (Figure 5). It is interesting to note that there are reported examples of catechol 1,2-dioxygenases which yield some extradiol cleavage product with certain substrates; thus, there are biological examples of Fe (III)-dependent extradiol cleavage.[25,26]

This reaction has wide substrate selectivity for catechols with electron-donating substituents, but not electron-withdrawing substituents, as found for MhpB. The very different pattern of results observed for the KO_2 reaction, suggests that this reaction proceeds via a different mechanism. Since the KO_2 reaction is performed under basic conditions, it probably proceeds via a dioxetane intermediate. Similarities between the TACN/$FeCl_2$ reaction and enzymatic reaction suggests that they probably follow the same reaction mechanism. Both enzymatic and model reactions are sensitive to electron-withdrawing substituents, which could be rationalized by the inhibitory effect of an electron-withdrawing substituent on the Criegee rearrangement step. Saturation kinetics are also consistent with equilibrium binding of catechol to the Fe(II)−TACN complex, similar to the enzyme-catalyzed reaction.

The reaction of monosodium catecholate with TACN/$FeCl_2$ yielded oxidative cleavage products, whereas no extradiol cleavage was observed with the catecholate dianion, which indicates that catechol binds to the iron center as the monoanion. These observations are consistent with EXAFS studies of the 2,3-catechol dioxygenase: catechol complex,[20] which show that the coordination of catechol to the iron(II) center is asymmetric, the catechol binding to the iron center as the monoanion. This observation explains why the model reaction does not proceed using stronger bases such as (dimethylamino)pyridine and 2,6-lutidine. One consequence of catechol monoanion binding is that the semiquinone intermediate will be uncharged, perhaps a neutral semiquinone is required for C−O bond formation with superoxide.

The reactions using monosodium catecholate as substrate showed significant differences between the iron(II) and iron(III) reactions. In the iron(III) reaction, the major product is extradiol RFP in both cases, the ratio of extradiol RFP and intradiol RFP being 2:1 with pyridine and 3.2:1 without pyridine. In the reaction of sodium monocatecholate with TACN/$FeCl_2$, intradiol RFP was found as major product, and the ratio of extradiol RFP and intradiol RFP is 1:15, while in the TACN/$FeCl_2$/pyridine model reaction, extradiol RFP was found as major product, in a 7:1 ratio. The only difference between these two reactions is that there was pyridine in the former reaction and no pyridine in the latter reaction. This indicates that pyridine has two roles in the reaction: *one* equivalent is required as a base to abstract a proton from catechol, and the resulting pyridinium salt is subsequently required as the proton donor for the Criegee rearrangement. This suggestion is confirmed by the addition of pyridine hydrochloride to the reaction of monosodium catecholate with TACN/$FeCl_2$, which resulted in the formation of the extradiol RFP as the major product in a ratio of 4:1 (Figure 6). This result clearly demonstrates the requirement for a proton donor in the extradiol cleavage reaction catalyzed by iron(II), presumably to assist cleavage of the O−O bond in the Criegee rearrangement. It is interesting to note that in each of the extradiol dioxygenase X-ray crystal structures there are additional acid−base residues in the vicinity of the iron(II) center.[3−5] For example, in *Pseudomonas* LB400 BphC, His-195 is located 4.0 Å from the iron(II) center, while on the other side of the active site, Tyr-250 is situated 3.8 Å from the iron(II)

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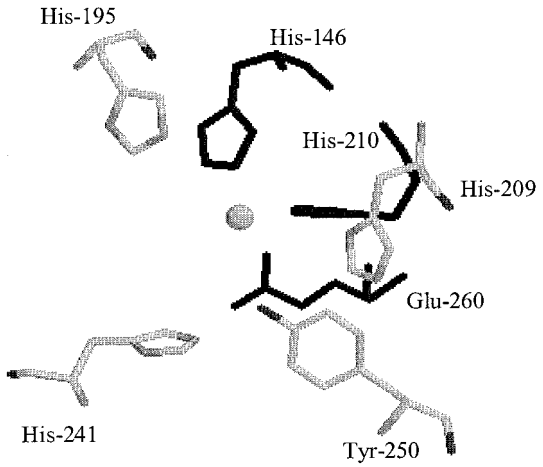

Figure 7. RASMOL representation of BhpC active-site residues, in vicinity of iron(II) cofactor. Darkly shaded residues are the iron(II) ligands His146, His210, and Glu260. Distance in Å: His195−Fe 4.0; Tyr250−Fe 3.8; His241−Fe 4.8; His241−Tyr250 2.7; His209−Fe 6.8; His209−His210 2.8.

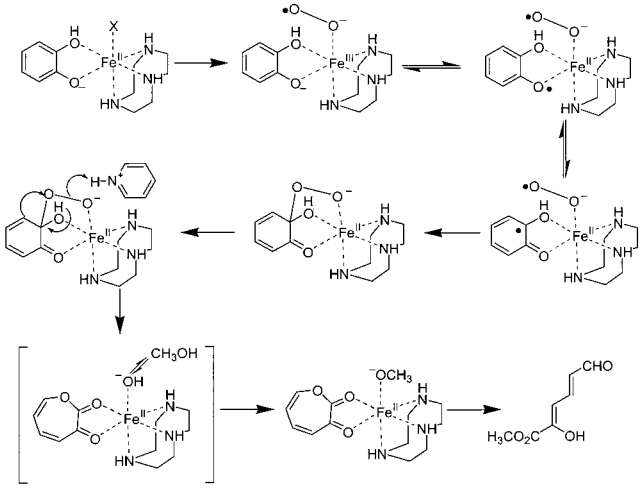

Figure 8. Proposed mechanism for TACN/$FeCl_2$ model reaction (X = pyridine).

center, with a nearby His-241 residue (Figure 7).[3] From this work it seems likely that one of these groups acts as a base to deprotonate the catechol substrate, while the other functions as proton donor for the Criegee rearrangement.

We propose that the mechanism for the iron(II) model reaction is similar to the mechanism of extradiol catechol dioxygenases, since (1) a similar substrate dependence is shown by model and enzymatic reactions and (2) cleavage in the presence of a series of alcohols gives the respective alkyl esters, consistent with a common α-keto lactone intermediate, arising from Criegee rearrangement. We have recently demonstrated the inhibition data of *E. coli* MhpB by carba-analogues of the proximal hydroperoxide, supporting the existence of a proximal hydroperoxide intermediate in the extradiol catechol dioxygenase mechanism.[21] Following the formation of iron(II)−TACN complex, the catechol monoanion combines with the iron center of the complex as shown in Figure 8. A 1-electron transfer from iron(II) to dioxygen forms a transient iron(III) species, and then 1-electron transfer from catechol monoanion to the iron center forms a iron(II)−TACN−semiquinone−superoxide intermediate. Recombination of superoxide forms a proximal peroxy intermediate. By Criegee rearrangement assisted by protonation from a pyridium salt formed earlier, a seven-membered α-keto lactone is obtained. After exchange of solvent methanol with Fe(II)-hydroxide, it is attacked by MeO^- to yield the ring-opened methyl ester.

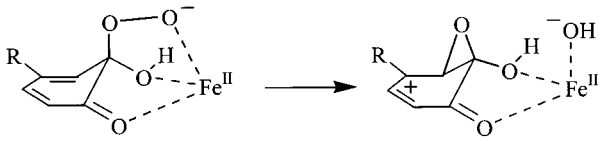

Figure 9. Possible π-participation mechanism for alkenyl migration, which rationalizes the regioselectivity for R = CH_3, and the electronic effect for electron-withdrawing R groups.

The regioselectivity of the TACN/$FeCl_2$ can be rationalized by this mechanism. It is known that 1,2-rearrangements such as the Baeyer−Villiger oxidation are assisted by adjacent alkyl substituents,[22] so the methyl substituent of 3-methylcatechol will favor 2,3-cleavage rather than 1,6-cleavage. In the case of 4-methyl catechol, 2,3-cleavage will result in a transient carbocation intermediate adjacent to the 4-methyl group, which would therefore reduce the activation energy for this pathway (Figure 9).

The mechanism for extradiol cleavage in the iron(III)/TACN reaction must be slightly different from that of iron(II) in view of the different product ratios obtained. The model reaction of TACN/$FeCl_3$ and the reaction of monosodium catecholate with TACN/$FeCl_3$ showed a similar extradiol RFP selectivity and similar ratios between extradiol RFP and intradiol RFP. This indicates that pyridine acts only as a base in the iron(III) reaction and that no proton donor is required in this case. As the iron(III)−TACN complex forms, the catechol monoanion binds with the complex to form iron(III)−TACN-catechol monoanion complex. The attack of O_2 on the activated substrate yields a transient alkyl-peroxy radical (Figure 10). There are two ways the reaction may proceed from this intermediate. One is to form the extradiol RFP by Criegee rearrangement of the hydroperoxide which has ligated to the iron center. The stronger Lewis acidity of Fe(III) will facilitate cleavage of the O−O bond; it then does not require a pyridium salt to act as a proton donor. Proton transfer in this case may either be from the catechol OH ligand or from solvent methanol. The other route is to form intradiol RFP by decomposition of this peroxy adduct via acyl-migration. The increased yield of extradiol RFP in the absence of pyridine may be due to the partial displacement of one ligand in the tridentate hydroperoxide complex by pyridine.

In conclusion, we have shown that both iron(II) and iron(III) are capable of supporting extradiol catechol cleavage but that highest selectivity is observed in the iron(II) reaction in the presence of a tridentate ligand and a proton donor. These observations provide a convincing model for the enzymatic extradiol reaction and suggest roles for other active-site residues in dioxygenase catalysis.

Experimental Section

Chemicals. Deuterated compounds D_2O, $CDCl_3$ were obtained from Isotope Laboratories. Starting materials and reagents $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, *p*-toluenesulfonyl chloride (TsCl), NaH in mineral oil, 3-methylbenzoic acid, 1,5,9-triazacyclododecane (12ane-N_3), 1,4,7,10-tetraazacyclododecane (12ane-N_4), 3-methylcatechol, 4-methylcatechol, 3-methoxy catechol, 2,3-dihydroxybenzaldehyde, 2,3-dihydroxybenzoic

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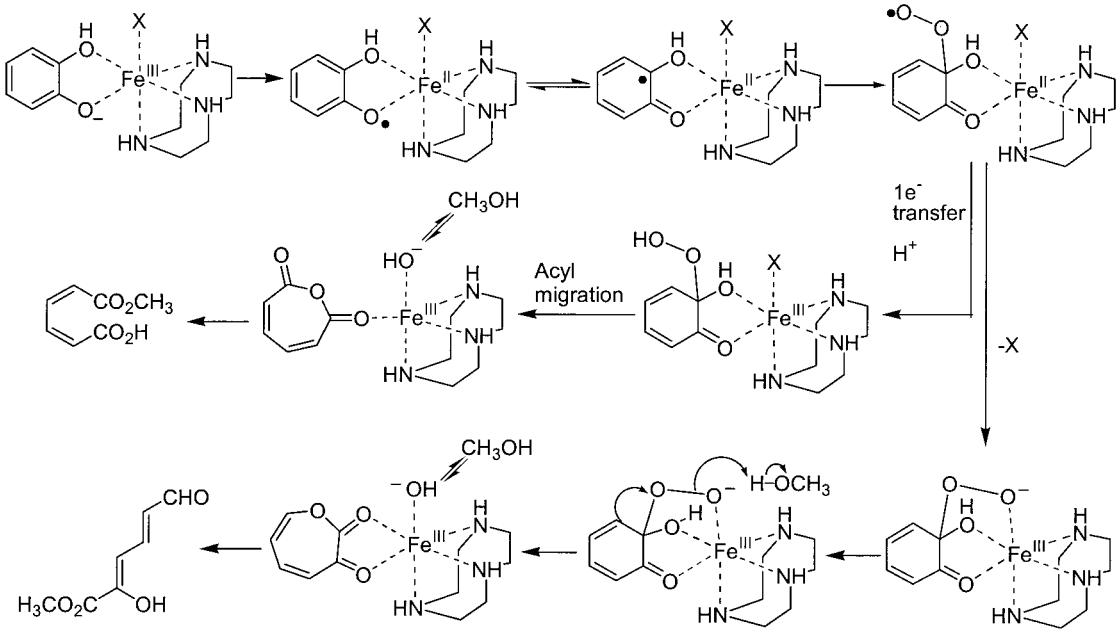

Figure 10. Proposed mechanism for TACN/$FeCl_3$ model reaction (X = Cl or pyridine).

acid, 3,4-dihydroxybenzoic acid, 1,2,3-trihydroxybenzene, 48% HBr (hydrobromic acid) were purchased from Aldrich Chemical Co. and used as obtained without further purification. Catechol was recrystallized from toluene. 1,4,7-Triazacyclononane (TACN) was prepared according to the method of Erhardt et al.[16] 1-Oxo-4,7-diazacyclononane was prepared according to the method of Hancock et al.[17] *N*,*N*′-DiBoc−TACN was prepared according to the method of Kovacs and Sherry.[20] HPLC grade methanol and ethanol were used without further purification, DMF was dried over anhydrous $MgSO_4$. Fe(TACN)Cl_3 was prepared according to the method of Wieghardt.[15] Fe^{III}(TACN)-(catechol)Cl was prepared using the method of Dei et al.[9]

Physical Measurements. ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 spectrometer in deuterated solvents as described below. Mass spectra were recorded on a Micromass AutoSpec mass spectrometer. UV/visible spectra were recorded on a Beckmann DU 7400 UV/visible spectrophotometer equipped with a temperature-control unit.

Preparation of 1,4-DiBoc-7-(3′-pyridylmethyl)-TACN (3). *N*,*N*′-DiBoc-TACN (164.5 mg, 0.5 mmol), anhydrous K_2CO_3 (276 mg, 2 mmol) were suspended in 25 mL of acetonitrile. The suspension was heated to gentle reflux (85 °C). The solution of 3-bromomethyl pyridine-(103.2 mg, 0.6 mmol) in 5 mL of acetonitrile was added dropwise to it. The reaction mixture was heated at reflux for 2 days. The resulting solid was removed by filtration. The acetonitrile was removed in vacuo and the residue dissolved in dichloromethane and purified by flash column to give **4** as a colorless oil (94.5 mg, 45%). ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.38 (2H, m, pyr-H), 7.65 (1H, d, J = 7.7, pyr-H), 7.16 (1H, m, pyr-H), 3.52 (2H, s, -pyr-CH_2−N), 3.25−3.5 (4H, m, TACN), 2.9−3.2 (4H, m, TACN), 2.4−2.6 (4H, m, TACN), 1.37, 1.38 (9H, 2 x s, Boc-H), 1.30 (9H, s, Boc-H) ppm δ_C 194.8, 194.6, 149.9, 148.3, 136.7, 136.1, 123.1, 79.5, 79.3, 57.6, 54.0, 52.6, 52.2, 50.9, 50.3, 49.6, 28.4, 28.3 ppm; MS (FAB, *m*/*z*) 420$[M]^+$, 364$[M - CH_2{=}CH(CH_3)_2]^+$.

Preparation of *N*-(3′-Pyridylmethyl)-TACN (4). 1,4-DiBoc-7-(3′-pyridylmethyl)-TACN (50 mg, 0.24 mmol) was treated with trifluoroacetic acid (0.1 mL) in dichloromethane (5 mL) and stirred at room-temperature overnight. The solvent and excess trifluoroacetic acid were removed in vacuo, to give **4**. ^1H NMR (D_2O, 300 MHz) 7.92 (s, 1H, 2′-**H**), 7.91 (d, 1H, J = 6.0 Hz, 6′-**H**), 7.59 (d, 1H, J = 7.5 Hz, 4′-**H**), 7.45 (dd, 1H, J = 7.5, 6.0 Hz, 5′-**H**), 3.85 (s, 2H,), 3.53 (s, 4H), 3.12 (dd, 4H, J = 6.0, 5.1 Hz), 2.91 (dd, 4H, J = 6.0, 5.1 Hz) ppm; MS (FAB, *m*/*z*) 221$[M + H]^+$.

Preparation of Fe^{II}(TACN)(Catechol) Complex. A modification of the method of Dei et al. was used.[9] A solution of TACN (25.8 mg, 0.2 mmol) in 1 mL of degassed methanol was added to a degassed solution of $FeCl_2{\cdot}4H_2O$ (39.6 mg, 0.2 mmol) in 3 mL of methanol. The solution was gently warmed to 40 °C for 0.5 h under N_2 atmosphere. Then catechol (22 mg, 0.2 mmol) was added to the resulting suspension, followed by pyridine (15.8 mg, 0.2 mmol). The resulting violet solution was stirred at room temperature for 4 h. Methanol was removed under vacuum to give a purple solid. ^1H NMR (CD_3OD, 300 MHz) 8.6 (2H, pyr-αH), 7.95 (1H, pyr-γH), 7.6 (2H, pyr-βH), 6.76 (2H, cat-H), 6.68 (2H, cat-H), 2.92−3.62 (6 × 2H, TACN−H) ppm.

Procedure A: Oxygenation of Catechol Catalyzed by $FeCl_2{\cdot}4H_2O$. Catechol (11 mg, 0.1 mmol), $FeCl_2{\cdot}4H_2O$ (20 mg, 0.1 mmol), pyridine (24 mg, 0.3 mmol), and TACN (12.9 mg, 0.1 mmol) were dissolved in methanol (500 mL) in a single-necked round-bottom flask. Oxygen gas was bubbled through the reaction mixture with stirring for 3 h, the color of the reaction solution changed from deep purple to yellow-green over 3 h. The solvent was removed in vacuo, 10% HCl (2 mL) was added to decompose the iron complex. The products were extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The crude product was analyzed by ^1H NMR, and two products were identified. 2-Hydroxymuconic semi-aldehyde methyl ester (**5**), yield 50% (based on DMF as the internal standard): ^1H NMR (300 MHz, $CDCl_3$) 9.55 (d, 1H, J = 7.8 Hz, 6-C**H**O), 7.51 (dd, 1H, J = 15.3, 11.5 Hz, 4-**H**), 6.31 (d, 1H, J = 11.5 Hz, 3-**H**), 6.21 (dd, 1H, J = 15.3, 7.8 Hz, 5-**H**), 3.86 (s, 3H, 1-COOC**H**$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 205.1, 194.1, 164.2, 144.6, 139.6, 133.3, 51.2 ppm. Muconic acid monomethyl ester (**6**), yield 7.5% (based on DMF as the internal standard): δ_H (300 MHz, $CDCl_3$) 7.93 (dd, 0.15H, J = 10.8, 10.8 Hz, 3-**H**), 7.80 (dd, 0.15H, J = 10.8, 10.8 Hz, 4-**H**), 6.0 (m, 0.3H, 2-**H** and 5-**H**), 3.7 (s, 0.45H, 1-COOC**H**$_3$) ppm. MS(CI, *m*/*z*) 188$[M + NH_4]^+$, 171$[M + H]^+$, 128$[M - 43]^+$.

Oxygenation of Catechol Catalyzed by $FeCl_3{\cdot}6H_2O$. As procedure A, except $FeCl_2{\cdot}4H_2O$ was replaced by $FeCl_3{\cdot}6H_2O$, and the reaction time extended to 5 h. NMR spectrum of crude product showed the formation of extradiol RFP and intradiol RFP, in a ratio of 2:1

Oxygenation of Catechol Catalyzed by TACN/$FeCl_2$ in Ethanol. As procedure A, except methanol was replaced by ethanol. The product 2-hydroxy-6-oxo-hexa-2,4-dienoic acid ethyl ester was identified by NMR analysis as the major product. ^1H NMR ($CDCl_3$, 300 MHz) δ_H 9.56 (d, 1H, J = 8.1 Hz, −C**H**O), 7.52 (dd, 1H, J = 15.6, 11.6 Hz, 4-**H**), 6.31 (d, 1H, J = 11.6 Hz, 3-**H**), 6.2 (dd, 1H, J = 15.6, 8.1 Hz, 5-**H**), 4.31 (q, 2H, J = 7.2 Hz, −OC**H**$_2$−), 1.32 (t, 3H, J = 7.2 Hz, −C**H**$_3$) ppm. MS (CI, *m*/*z*) 188$[M + NH_4]^+$, 171$[M + H]^+$, 153$[M + H-CO]^+$.

Oxygenation of Substituted Catechols Using TACN/$FeCl_2$ in Methanol. As procedure A, except catechol was replaced by a substituted catechol. In each case, the products and product ratios were determined by ^1H NMR spectroscopy.

(a) 3-Methylcatechol. The product was identified by NMR as a 35:1 mixture of 2-hydroxy-6-oxo-hepta-2,4-dienoic acid methyl ester (**7a**) and 2-hydroxy-3-methyl-6-oxo-2,4-dienoic acid methyl ester (**8a**). **7a** ^{1}H NMR ($CDCl_3$, 300 MHz) 7.52 (dd, 1H, J = 16.0, 11.5 Hz, 4-**H**), 6.23 (d, 1H, J = 11.5 Hz, 3-**H**), 6.19 (d, 1H, J = 16.0 Hz, 5-**H**), 3.84 (s, 3H, −OC**H**$_3$), 2.26 (s, 3H, 6-C**H**$_3$) ppm.

(b) 4-Methylcatechol. The product was identified as a mixture of 2-hydroxy-5-methyl-6-oxo-hexa-2,4-dienoic acid methyl ester (**9**) and intradiol cleavage products. ^{1}H NMR ($CDCl_3$, 300 MHz) of **9**: δ_H 9.46 (s, 1H, −C**H**O), 7.28 (d, 1H, J = 11.7 Hz, 4-**H**), 6.52 (d, 1H, J = 11.7 Hz, 3-**H**), 3.87 (s, 3H, −OC**H**$_3$) ppm.

(c) 3-(2′,3′-Dihydroxyphenyl)propionic Acid. The product was identified as 2-hydroxy-6-oxo-nona-2,4-diene 1,9-dioic acid 1-methyl ester **(7a)**,[23] ^{1}H NMR ($CDCl_3$, 300 MHz) 7.65−7.56 (dd, 1H, J = 15.8, 11.4 Hz, 4-**H**), 6.29 (d,1H, J = 11.6 Hz, 3-**H**), 6.27 (d, 1H, J = 15.8 Hz, 5-**H**), 3.90 (s, 3H, −OCH_3), 2.95 (t, 2H, J = 6.7 Hz, 7-**H**), 2.67 (t, 2H, J = 6.7 Hz, 8-**H**) ppm.

(d) 3,5-Di-*tert*-butyl Catechol. The residue was separated by flash column on silica gel. Three products were determined by comparison with literature ^{1}H NMR data.[8] 3, 5-Di-*tert*-butyl-2-pyrone: ^{1}H NMR (300 MHz, $CDCl_3$) 7.14 (d, J = 2.6 Hz, 1H), 7.04 (d, J = 2.6 Hz, 1H), 1.32 (s, 9H), 1.2 (s, 9H) ppm. 4,6-Di-*tert*-butyl-2-pyrone: ^{1}H NMR (300 MHz, $CDCl_3$) 6.01 (s, 2H), 1.26 (s, 9H), 1.19 (s, 9H) ppm.

Preparation of Monosodium Catecholate and Disodium Monocatecholate. Monosodium catecholate and disodium monocatecholate were prepared by stirring catechol with 1 equiv and 2 equiv of NaOH in MeOH under a N_2 atmosphere, respectively. After 0.5 h, methanol was removed in vacuo, and the residue was dried under high vacuum for 2 h.

Procedure B: Oxygenation of Monosodium Catecholate Catalyzed by TACN/$FeCl_2$ in Methanol. Monosodium catecholate (26.4 mg, 0.2 mmol), $FeCl_2 \cdot 4H_2O$ (40 mg, 0.2 mmol), and TACN (25.8 mg, 0.2 mmol) were dissolved in MeOH (400 mL) in a single-necked round-bottom flask. Oxygen gas was bubbled through the reaction mixture with stirring for 3 h. The solvent was removed in vacuo, 10% HCl (2 mL) was added to decompose the iron complex. The product was extracted by diethyl ether. The organic layer was dried (Na_2SO_4) and evaporated to give crude product, whose ^{1}H NMR spectrum showed the formation of intradiol RFP and extradiol RFP, in a ratio of 15:1.

Oxygenation of Monosodium Catecholate Catalyzed by TACN/$FeCl_3$ in Methanol. As procedure B, except $FeCl_2$ was replaced by $FeCl_3$. NMR spectrum of crude product showed the formation of extradiol RFP and intradiol RFP, in a ratio of 3.2:1.

Oxygenation of Sodium Monocatecholate Catalyzed by TACN/$FeCl_2$/Pyridine Hydrochloride in Methanol. Monosodium catecholate (26.4 mg, 0.2 mmol), $FeCl_2 \cdot 4H_2O$ (40 mg, 0.2 mmol), TACN (25.8 mg, 0.2 mmol), pyridine hydrochloride (23.1 mg, 0.2 mmol) were dissolved in MeOH (400 mL) in a single-necked round-bottom flask. Dioxygen was bubbled through the reaction mixture with stirring for 4 h. After removing the solvent, 10% HCl (2 mL) was added to decompose the iron complex, the product was extracted by diethyl ether. The organic layer was dried (Na_2SO_4) and evaporated to give crude products. NMR spectrum displayed the formation of extradiol RFP and intradiol RFP, in a ratio of 4:1.

UV/Vis Screening of the Oxidation Reaction of Catechols by KO_2. The procedure followed was described by Muller and Lingens.[14] To KO_2 (50 mg) was added dimethyl sulfoxide (50 mL) in a mortar. The KO_2 was ground carefully until a fine suspension was obtained. The solution was filtered through a glass wool plug to obtain a clear filtrate. In a 1 mL quartz cuvette was placed 1 mL of this solution, with an addition of 50 μL of substrate solution (DMSO, 2 mg/mL) to begin the reaction. The results were tabulated in Table 3.

Kinetics. The extinction coefficient of 2-hydroxymuconic semialdehyde methyl ester (**5**) (λ_{max}315 nm) was calculated to be 18 000 M^{-1} cm^{-1} by quantitative conversion to 2-hydroxymuconic semialdehyde (λ_{max}375 nm; ϵ_{RFP} 48 400 M^{-1} cm^{-1}) by alkaline hydrolysis, and comparison of UV/vis spectra.[24] The extinction coefficient of DHP extradiol cleavage product 2-hydroxy-6-keto-nona-2,4-diene 1,9-dioic acid 1-methyl ester was calculated to be 8100 M^{-1} cm^{-1} by alkaline hydrolysis to 2-hydroxy-6-keto-nona-2,4-diene 1,9-dioic acid (ϵ_{RFP} 15 600 M^{-1} cm^{-1}).[24] Kinetic data for alternative substrates, reaction conditions, and catechol concentrations were measured at 315 nm, except where noted. All of the components were added to a quartz cuvette in the order: methanol, iron chloride, TACN, catechol, pyridine and mixed and data was collected every minute for 6 min at 23 °C.

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