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A biomimetic model reaction for the extradiol catechol dioxygenases

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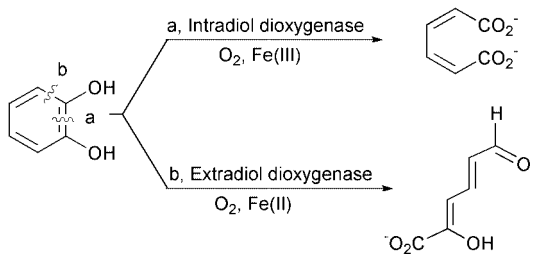
A model reaction is described for extradiol catechol cleavage involving $FeCl_2$ or $FeCl_3$, 1,4,9-triazacyclononane (TACN), pyridine and dioxygen which shows similar cofactor and regio-selectivity to the extradiol catechol dioxygenases.

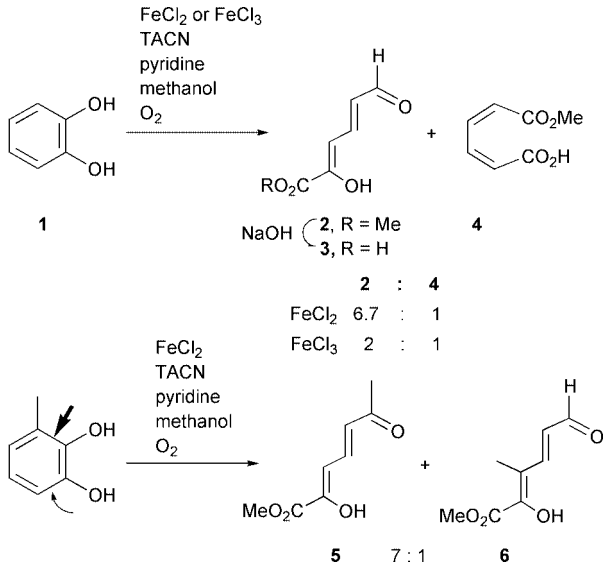
The oxidative cleavage of catechol and other dihydroxy aromatics is a key step in the biodegradation by soil bacteria of naturally occurring aromatic molecules and many aromatic environmental pollutants.[1]

The catechol dioxygenases are a class of non-haem iron enzymes that catalyse the oxidative cleavage of catechols. These enzymes can be divided into two subclasses (Scheme 1): the intradiol dioxygenases, which utilise a non-haem iron(III) cofactor, catalyse the cleavage of the carbon–carbon bond between the two catechol oxygens; and the extradiol dioxygenases, which utilise a non-haem iron(II) cofactor, catalyse the cleavage of the carbon–carbon bond adjacent to the catechol oxygens.[2]

The intradiol cleaving enzymes require non-haem Fe^{3+} to effect oxygen activation, which is ligated by two His and two Tyr moieties.[3] The extradiol cleaving enzymes require non-haem Fe^{2+} as an active site cofactor,[2,4] ligated by two His and one Glu ligands. Many attempts to model the catechol dioxygenases have been reported, but most catalysts give intradiol cleavage products.[5] However, there are three reported examples of extradiol cleavage by synthetic iron complexes. Funabiki *et al.*[6] found that $FeCl_2$ or $FeCl_3$ in THF–H_2O cleaves 3,5-di-*tert*-butylcatechol to substituted 2-pyrones, which were believed to derive from decarboxylation of the extradiol-cleavage intermediate α-keto lactone. Dei *et al.* found that the complex [Fe^{III}(TACN)Cl(dbc)] afforded 2-pyrone upon exposure to O_2 in 35% yield.[7] Ito and Que used the same complex to give an almost quantitative yield of 2-pyrone using a modified procedure.[8] However, none gave the authentic extradiol reaction product. Here, we report the first observation of extradiol ring fission product 2-hydroxymuconic aldehyde by an iron-dependent model reaction shown in Scheme 2. An investigation of the catalytic properties of $FeCl_2$ and $FeCl_3$ in the presence of a series of macrocyclic ligands, namely 1,4,7-triazacyclononane ([9]aneN_3, TACN), 1-oxo-4,7,10-triazacyclododecane ([12]aneN_3O), 1-oxo-4,7-diazacyclononane ([9]aneN_2O), 1,5,9-triazacyclododecane ([12]aneN_3), for extradiol catechol cleavage was carried out in methanol by UV–VIS spectroscopy (0.1 mM of catechol in methanol). Reaction of $FeCl_2$ or $FeCl_3$ in the presence of 1.0 equiv. TACN gave a new product absorbing at 315 nm. Upon addition of NaOH, the absorption maximum shifted immediately to 405 nm (Fig. 1), then over a



Scheme 1



Scheme 2

period of 2 h to 378 nm, the characteristic λ_{max} for the extradiol ring fission product. The free acid form **3** of ring fission product **2** is reported to show an absorption maximum shift from 322 nm (pH 3) to 378 nm (pH 8) on passing from the enol to the enolate form.[9] The UV–VIS data suggested that the initial product was the methyl ester enol **2**, which was converted into the corresponding enolate, then hydrolysed to give the disodium salt of acid **3**. Reaction of $FeCl_2$ or $FeCl_3$ in the presence of each of the other macrocyclic ligands gave no absorbance above 300 nm, indicating no extradiol cleavage.

To confirm the identity of the reaction product, a large scale model reaction of catechol with O_2 in the presence of $FeCl_2$ or $FeCl_3$, pyridine and TACN with a ratio of 1 : 1 : 3 : 1 (cat : Fe : py- : TACN) was carried out in methanol by bubbling dioxygen gas into the solution for 3 h (for $FeCl_2$) or 5 h (for $FeCl_3$).† The ^{1}H NMR spectrum of the reaction product displayed signals

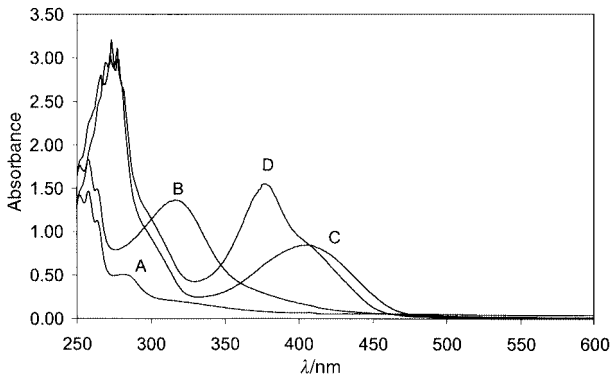


Fig. 1 UV–VIS spectra of (A) mixture of 0.1 mM catechol, 0.1 mM TACN, 0.3 mM pyridine, 0.1 mM $FeCl_2$ in methanol(recorded immediately after mixing), (B) the product **2** formed after 1 h, (C) methyl ester enolate upon adding NaOH (0.3 mM, final conc.) to B(recorded immediately after adding NaOH), (D) hydrolysis product **3** (recorded 2 h after addition of NaOH).

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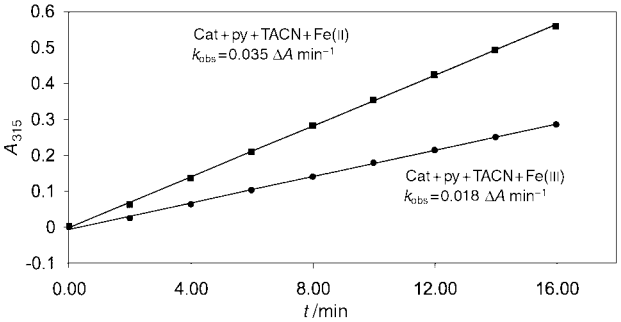

Fig. 2 Kinetic study of the reaction at 23 °C (0.1 mM catechol, 0.1 mM $FeCl_2$ or $FeCl_3$, 0.1 mM TACN, 0.3 mM pyridine in methanol). Absorbance at 315 nm was measured every 2 min for a total of 16 min.

corresponding to 2-hydroxymuconic semialdehyde methyl ester **2** as the major product, by comparison with NMR data for an enzymatic extradiol cleavage product,[11] and muconic acid monomethyl ester **4** as the minor product confirmed by comparison with authentic NMR spectral data (Scheme 2).[12]

This is the first reported reaction to give 2-hydroxymuconic semialdehyde as the major product from a biomimetic model reaction of extradiol dioxygenase. Its derivative 3,5-dibutyl-2-hydroxymuconic semialdehyde has been detected as a minor product previously from a reaction of 3,5-di-*tert*-butylcatechol, identified by Funabiki.[6] The extradiol cleavage product has also been generated using KO_2/DMSO.[10]

No ring fission was observed when either TACN, pyridine or Fe(II)/Fe(III) was omitted, indicating that all these reagents are essential for this reaction. The reaction was found to proceed using DBU in place of pyridine at a lower rate, but not using 2,6-lutidine or 4-dimethylaminopyridine. The absorption maximum at 315 nm was still observed using ethanol instead of methanol, but was not observed when the reaction was attempted in aqueous buffer. No activity was observed using $CuCl_2$, $CoCl_2$ or $MnCl_2$. Similarly, no activity was observed using $FeSO_4$ or $Fe(NH_4)_2(SO_4)_2$.

Interestingly, the reaction in the presence of $FeCl_2$ gave extradiol product **2** (50% yield by NMR spectroscopy using DMF as internal standard) and intradiol product **4** (7.5%) in a ratio of 6.7 : 1, while the reaction in the presence of $FeCl_3$ gave the products in a ratio of 2 : 1. Monitoring of A_{315} (corresponding to extradiol cleavage product only) revealed the reaction is twice as fast in the presence of 0.1 mM $FeCl_2$ (k_{obs} = 0.035 ΔA min^{-1}) than 0.1 mM $FeCl_3$ (k_{obs} = 0.018 ΔA min^{-1}) (Fig. 2). These observations imply that there is some intrinsically higher activity and specificity of Fe^{2+} for extradiol cleavage, which gives some insight into the fact that Fe^{2+} is the cofactor for the extradiol dioxygenase family.[2]

Furthermore, the treatment of 3-methylcatechol under the same conditions gave ketone **5** and aldehyde **6** in a ratio of 7 : 1 (only a trace amount of intradiol cleavage product) by NMR, which demonstrates that the model reaction also shows regioselectivity for the site of C–C cleavage. It is of interest that the model reaction shows the same selectivity as is found for extradiol dioxygenases which cleave 3-substituted catechols,[2] for example, 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) from *Escherichia coli*, which cleaves in the same way to give a ketone ring fission product whose ^{1}H NMR spectrum is almost identical to **5**.[11]

These observations raise the question of what the active iron-containing species during the reaction are. TACN has previously been shown to form a mononuclear, octahedral iron(III) complex with 3,5-di-*tert*-butylcatechol.[7] Monitoring of the $FeCl_3$-containing reaction at 0.4 mM TACN concentration by UV–VIS spectroscopy reveals the immediate formation of a complex (λ_{max} 456, 712 nm), which decays as product is formed, and whose UV–VIS spectrum matches that of the complex formed anaerobically between $FeCl_3$, TACN and catechol, indicating that an Fe(III)–TACN-catechol complex is formed. Monitoring of the $FeCl_2$-containing reaction at 0.4 mM TACN concentration by UV–VIS spectroscopy reveals immediate product formation at 315 nm, but only gradual formation of the Fe(III)–TACN-catechol complex, indicating that some oxidation of iron(II) is taking place during the reaction, but that a separate iron(II) complex is responsible for the majority of extradiol product formation. The formation of distinct iron(II) and iron(III) complexes, whose structures remain to be determined, would account for the different product distributions formed by $FeCl_2$ *cf.* $FeCl_3$.

In conclusion, this model reaction shows several features which closely mimic the extradiol dioxygenases. The extradiol ring fission product **2** is obtained as the major product; the reaction shows an inherent preference in extradiol selectivity for Fe^{2+} over Fe^{3+}, shows a similar regioselectivity to the extradiol dioxygenases, and demonstrates a selectivity for the TACN ligand which parallels the facial tridentate coordination found in the extradiol dioxygenase active site. It will be of interest to study the scope and the mechanism of this model reaction, and compare its properties with the enzyme-catalysed reaction.

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Notes and references

† 11 mg (0.1 mmol) of catechol, 20 mg (0.1 mmol) of $FeCl_2 \cdot 4H_2O$, 24 mg (0.3 mmol) of pyridine and 12.9 mg (0.1 mmol) of TACN were dissolved in 500 mL of methanol in a single-necked round bottom flask. Oxygen gas was bubbled through the reaction mixture with stirring for 3 h. After removing the solvent, 2 mL of 10% HCl was added, and the products was extracted with diethyl ether and dried (Na_2SO_4). 2-hydroxymuconic semialdehyde methyl ester **2**: yield 50% (by NMR based on DMF as the internal standard): δ_H(300 MHz, $CDCl_3$) 9.55 (d, 1H, *J* = 7.8 Hz, 6-CHO), 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-CO_2CH_3); δ_C(75 MHz, $CDCl_3$) 205.1, 194.1, 164.2, 144.6, 139.6, 133.3, 51.2. Muconic acid monomethyl ester **4**: yield 7.5% (by NMR 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, 2- and 5-H), 3.7 (s, 0.45 H, 1-CO_2CH_3).

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