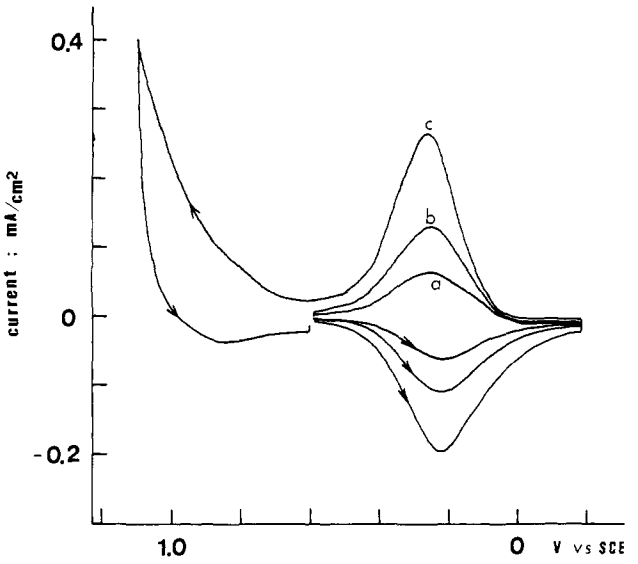

Figure 1. Cyclic voltammograms of a iron–ruthenium cyanide modified Pt electrode with 3 mC/cm^2 as the total charge consumed in the reduction in a solution of 0.5 M K_2SO_4 (pH 4.0). The scan rates of the electrode potential were (a) 5 mV/s, (b) 10 mV/s, and (c) 20 mV/s.

important result that the peak potentials obtained here are nearly equal to those observed in the reduction of PB.[1-4] This indicates that the Coulombic interactions as well as the electron-exchange interactions in the RP crystal are almost the same as those in PB with respect to the high-spin iron ions. The electron-transfer mechanism in RP crystal can be formulated as follows by assuming the formula $Fe_4^{3+}[Ru^{II}(CN)_6]_3$.[5]

$$Fe_4^{3+}[Ru^{II}(CN)_6]_3 + 4e^- + 4K^+ \rightleftharpoons K_4^+Fe_4^{2+}[Ru^{II}(CN)_6]_3 \quad (1)$$

As is observed at the PB electrode,[2,4] not only K^+ ions but also NH_4^+, Rb^+, and Cs^+ ions can be expected to transport through the crystal of RP because of its open lattice with a cell constant of 10.3 Å.[5]

It has been previously reported that the oxidation of PB occurs at about 0.9 V vs. SCE, yielding Berlin green.[2-4] However, no distinct oxidation wave is observed for RP, as shown in Figure 1. A shoulder observed at 0.8 V might be due to the oxidation of the ruthenium ions in the crystal. It is noteworthy that a fairly large background current commencing at about 1.0 V is observed in the anodic scan of electrode potential. We believe that this large background current involves oxygen evolution. It is well known that the mixed oxide of ruthenium electrodes exhibit the lowest overpotential for oxygen evolution.[11] If the anodic background as shown in Figure 1 is due to oxygen evolution, the electrode prepared here should be one of the best catalysts for oxygen evolution. The electrochemistry of the RP electrode has been examined in a 1.0 M KCl solution (pH 4.0). Again, a large anodic background current commenced at 0.8 V. In this case, chlorine evolution might have occurred. Exactly the same results were obtained at RP-modified SnO_2 and glassy carbon electrodes.

Figure 2 shows the absorption spectrum of the film at a SnO_2 electrode obtained in situ in a 1.0 M KCl solution (pH 4.0). The spectrum observed at 0.6 V can be attributable to the intervalence charge-transfer band in $Fe_4^{3+}[Ru^{II}(CN)_6]_3$, i.e., $Fe^{3+}\cdots N-C\cdots Ru^{II} \rightarrow Fe^{2+}\cdots N-C\cdots Ru^{III}$.[5,6] Robin reported a similar spectrum with a colloidal form of RP prepared by adding a stoichiometric amount of Fe^{3+} to a $Ru(CN)_6^{4-}$ solution.[10]

The spectrum observed at −0.2 V shows no band in the visible region. The purple is completely bleached out during the potential scan from 0.6 to −0.2 V. Continuous cyclic scans of the potential between 0.6 and −0.2 V resulted in no degradation of the wave up to 10^5 cycles.

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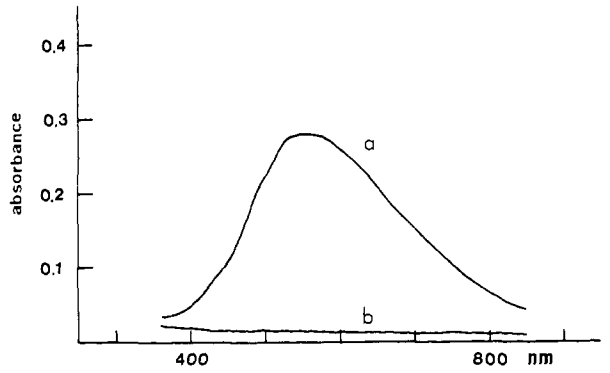

Figure 2. Absorption spectra obtained on a SnO_2 electrode with 3.1 mC/cm^2 of the iron–ruthenium cyanide at two different electrode potentials in 1.0 M KCl (pH 4.0): (a) at 0.6 V vs. SCE; (b) at −0.2 V vs. SCE.

Numerous applications to catalysis and electrochromism can readily be imagined from the results discussed above.

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Registry No. $Fe_4^{3+}[Ru^{II}(CN)_6]_3$, 41898-61-7.

Ferric Nitrilotriacetate: An Active-Center Analogue of Pyrocatechase

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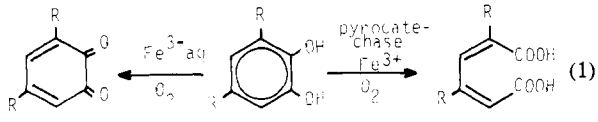
Pyrocatechase (catechol 1,2-dioxygenase, EC 1.13.11.1),[1] a nonheme iron(III) dioxygenase, catalyzes the intradiol ring cleavage of catechol **1** by dioxygen; both atoms of the O_2 are incorporated into the product, *cis,cis*-muconic acid (**2**) (eq 1b, R = H). The substrate catechol is known to be initially bound



3 1 2

to iron[2] and oxidized subsequently via a yet unknown mechanism. Recent comprehensive reviews on ferric dioxygenases are available.[3,4]

However, with 3,5-di-*tert*-butylcatechol—chosen for the stability of its oxidation products—it was demonstrated that oxidation in the presence of Fe(III) ions selectively leads to the quinone **3**[5] (eq 1a, R = *t*-Bu). Hitherto, only a mixture of 3,5-di-*tert*-butylcatechol with Fe^{2+}, bpy, and pyridine in THF gave some muconic acid besides the quinone as the main product.[6] Catechol

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Table I. Oxidation of 3,5-Di-*tert*-butylcatechol Catalyzed by [Fe(NTA)]

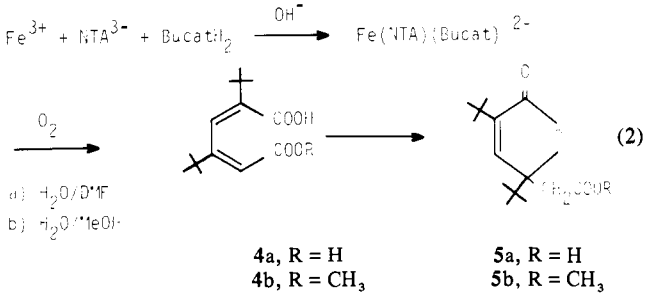
reaction	$BucatH_2$, mmol	NTA^{3-}, mmol	Fe^{3+}, mmol	solvent[a]	time, days	product, %[b] Bucat (1)	Buqu (3)	lactone (5)	turnover[c]
1	5	5	5	DMF	4	0	10	81	0.8
2	5	0.5	0.5	DMF	5	0	3	84	8.4
3	5	0.05	0.05	DMF	7	5	2	80	80
4	5	5	5	MeOH	3	0	8	86	0.9
5	5	0.5	0.5	MeOH	4	0	5	83	8.3
6	5	0.05	0.05	MeOH	5	21	30	41	41
7	5		5	MeOH	5	10	80		
8	5		0.05	MeOH	5	73	18		
9	3			MeOH	5	82	4		
10	3		1	DMF	5	8	75		

[a] 1 part aqueous 0.6 M borate buffer pH 8.5, 2 parts MeOH or DMF. [b] Based on original $BucatH_2$ identification: (5a) IR (CH_2Cl_2) 2958, 1752, 1729, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (1 H), 6.91 (1 H), 2.91 (d, J = 14.4 Hz, 1 H), 2.76 (d, J = 14.4 Hz, 1 H), 1.19 (9 H), 0.95 (9 H); found C, 65.80, H, 8.83; calcd C, 66.11, H, 8.72. (5b) IR 2952, 1747, 1719, 1700, 1630 cm^{-1}; ^1H NMR δ 6.94 (1 H), 3.56 (3 H), 2.89 (d, J = 13.8 Hz, 1 H), 2.80 (d, J = 14.1 Hz, 1 H), 1.21 (9 H), 0.95 (9 H); found C, 66.96, H, 9.03; calcd C, 67.14, H, 9.01. [c] Mol of ring-cleavage product/mol of Fe^{3+}.

cleavage was reported also for several copper(II) systems.[7-9] At least some of these, however, do not require O_2 for the cleavage reaction but for regeneration of the cupric ion, the latter being the oxidizing agent. We now report the first nonenzymatic ferric complex capable of catalyzing the dioxygenase reaction.

Mixtures of ferric perchlorate, sodium nitrilotriacetate (Na_3NTA), and 3,5-di-*tert*-butylcatechol ($BucatH_2$), molar ratios 1:1:1, 1:1:10, and 1:1:100, respectively, in aqueous borate buffer pH 8.5 (1 part) and MeOH or DMF (2 parts)[10] were incubated with O_2 at 23 °C until the solution changed color from blue-green to orange (3-7 days). Removal of MeOH under vacuum was followed by CH_2Cl_2 extraction from basic and then from acidic solution. If necessary, product separation was completed by chromatography on silica gel with $CHCl_3$.

Generally, isolation yielded traces of unreacted catechol, a minor amount of quinone, and the lactones **5** (eq 2) as the main product



4a, R = H 5a, R = H
4b, R = CH_3 5b, R = CH_3

(Table I). The latter result from the muconic acids **4** as primary ring-cleavage products.

Typical results are listed in the table. With aqueous DMF as solvent (reactions 1-3), >80% ring cleavage of 3,5-di-*tert*-butylcatechol was observed within 4-7 days, the reaction yielding mainly the lactone **5a**, i.e., the Markovnikov product of γ-lactonization of the *cis,cis*-muconic acid **4a** (cisoid form). The acid **4a** and lactones arising from the anti-Markovnikov reaction of **4a** and from the transoid isomer of **4a**, respectively, are present as byproducts.

In the presence of MeOH (reactions 4-6), the muconic acid half ester (eq 2, **4b**, two isomers) is formed initially, the lactone ester **5b** representing the main fraction amongst the isolated lactones.

Although the ring cleavage is fairly slow, it by far exceeds stoichiometry with respect to iron(III), with the highest observed turnover (mol of ring cleavage/mol of Fe^{3+}) being 80 in reaction 3; the turnover of pyrocatechase is 1800.[3]

In the absence of NTA, only the quinone was found as oxidation product (reactions 7, 8, 10; see eq 1a). With Fe^{3+} also being absent, no reaction at all took place, showing that borate, besides serving as a buffer, is also effectively protecting catechol from autoxidation by forming the borate complex (reaction 9).

In the pyrocatechase reaction cycle the substrate catechol is coordinated initially to the active-center high-spin ferric ion.[1,3,4] Concomitant spectral changes are a large increase of long-wavelength absorbance (around 650 nm, vs. λ_{max} 440 nm for the native enzyme) and the disappearance of the EPR signal at g = 4.3 of the native enzyme.

With striking similarity, the orange of [Fe(NTA)] changes to blue-green (e.g., λ_{max} 688 nm in reaction 4) upon addition of 3,5-di-*tert*-butylcatechol (eq 2). The EPR signal at g = 4.3 of [Fe(NTA)] greatly diminishes and becomes superimposed on a new broad peak centered around g = 4.2; signals at g = 9.1, 7.55, and 5.5 are seen additionally.[11] All signals are weak at 77 K and probably not detectable with biological material (signals near g = 6 were reported for the enzyme substrate complex of protocatechuate 3,4-dioxygenase at 12 K[4]). The complex species present in these solutions can be viewed as an analogue of the enzyme-substrate complex of pyrocatechase. From stoichiometric mixtures of the components in aqueous DMF under anaerobic conditions with piperidine as base, we were able to obtain crystals of the ternary complex [Fe(NTA)(Bucat)](pip H)$_2$·DMF, characterized as follows: found C, 55.64, H, 8.10, N, 7.90; calcd C, 55.85, H, 8.09, N, 7.89; vis λ_{max} 660 nm in H_2O (ϵ_0 2250 M^{-1} cm^{-1}); IR (KBr) ν_{CH} 2960 (s), ν_{CO} 1625, (vs, br), ν_{CO} 1245 (m) cm^{-1}.

The EPR spectrum is the same as for the reaction mixtures, both in DMF and aqueous solution. The IR and visible spectra are consistent with a catecholate[4,13,14] rather than a semiquinone[12,14,15] chelate ligand. In view of the as yet unknown reaction mechanism of nonheme ferric intradiol dioxygenases, it

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seems of interest to note that Que and co-workers[16] did not observe ring cleavage with [Fe(salen)(Bucat)]$^-$, although in this complex, too, the catecholate appears to be stabilized relative to the semiquinone form. This may be due to the fact that the tetragonal salen ligand does not allow catechol and oxygen to be bound to the iron in adjacent positions, thus preventing reactions between these two components other than electron exchange.[16] (Vice versa, this may be the reason for the nonheme nature of this class of dioxygenases.) Further, neither ferric semiquinone nor monodentate catecholate[16] complexes were found to yield ring-cleavage reactions with O_2. Proposed mechanisms involving such species[4,16] now seem less likely since we here show that chelated catecholate in [Fe(NTA)(Bucat)]$^{2-}$ does undergo the dioxygenase reaction.

Ferric semiquinones, however, appear to serve as intermediates of the alternative reaction pathway, iron(III)-catalyzed catechol autoxidation (eq 1a; M. G. Weller and U. Weser, unpublished results). The formation of these complexes (e.g., tris(3,5-di-*tert*-butylsemiquinone)iron(III)) is favored by resonance stabilization. Such stabilization is lost with "hard" ligands present in mixed complexes. In our studies, the hard phenolate ligands in pyrocatechase (Tyr residues of the protein)[14] are replaced by the hard NTA. Such "hardening" of a nonheme ferric center, both in the enzyme and model system, finally leads to catechol ring cleavage upon reaction with O_2. Mechanistic studies of the oxygenation are under way.

Acknowledgment. The award of a Deutsche Forschungsgemeinschaft Grant No. Gi132/1-1 to M.G.W. is gratefully acknowledged.

Registry No. 1 (R = *t*-Bu), 1020-31-1; **5a**, 22802-86-4; **5b**, 22961-94-0; Fe, 7439-89-6; [Fe(NTA)(Bucat)](pipH)$_2$, 81770-38-9; H_3NTA, 139-13-9.

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Biosynthesis of Riboflavin. Incorporation of D-[1-^{13}C]Ribose

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The terminal step of riboflavin (**3**) biosynthesis consists of the transfer of four carbon atoms from one molecule of 6,7-dimethyl-8-ribityllumazine (**2**) to a second molecule of **2**. This reaction is catalyzed by the enzyme riboflavin synthase and has been studied in considerable detail (for a review see ref 1). The biosynthetic precursor of **2** is 5-amino-6-ribitylamino-2,4-(1*H*,3*H*)-pyrimidinedione 5′-phosphate (**1**),[2-4] which in turn arises from guanosine triphosphate (GTP).[5] The formation of the pyrazine ring of **2** from **1** requires the addition of four carbon atoms; duplication of this four-carbon moiety by riboflavin synthase ultimately produces the xylene moiety of the riboflavin molecule.[1]

Table I. Relative ^{13}C Abundance in Riboflavin Tetraacetate Derived from Riboflavin Biosynthetically Labeled by Feeding D-[1-^{13}C]Ribose to *Ashbya gossypii*

carbon	chemical shift, ppm[a]	rel ^{13}C abundance, %
2	159.3	0.9
4	154.4	0.8
4a	136.1	0.9
5a	134.6	3.3
6	133.0	10.3
7	137.0	0.8
8	148.1	3.7
9	115.5	1.1
9a	131.2	0.6
10a	150.7	0.6
7-Me	19.4	0.9
8-Me	21.4	9.1
1′	45.0	19.4
2′	69.4	2.9
3′	70.5	0.6
4′	69.0	1.3
5′	61.9	1.1
$\underline{C}H_3CO$	20.3, 20.7, 20.8, 21.0	av 1.0[b]
$CH_3\underline{C}O$	169.7, 169.8, 170.3, 170.6	av 0.9

[a] Relative to Me_4Si = 0. [b] Standard.

Various hypotheses for the origin of the four-carbon moiety involved in the formation of **2** have been proposed. Thus, diacetyl or acetoin,[6,7] a tetrose,[8] a pentose,[9] or a hexose[10] have been discussed as the ultimate source of these four carbon atoms. More recently, it has been suggested that one molecule of the ribitylaminopyrimidine **1** (R = H) donates its ribitol moiety, which is subsequently inserted into a second molecule of **1** to form the pyrazine ring.[11,12] We decided to reexamine this issue by feeding ^{13}C-labeled precursors and analyzing the product **3** by ^{13}C NMR spectroscopy.

It had been shown earlier that the isotope from [1-^{14}C]ribose is incorporated into one or both of the methyl groups of **3** and into carbon atoms 6 and/or 9.[9] However, the incorporated isotope could not be traced to individual atoms in these experiments.

We therefore studied the incorporation of [1-^{13}C]ribose into riboflavin by the flavinogenic fungus *Ashbya gossypii*. The organism was grown in 200 mL of complete medium[13] containing glucose (10 g/L) until the onset of flavinogenesis. D-[1-^{13}C]Ribose (250 mg, 90% ^{13}C, Los Alamos Stable Isotope Resource) was added, and the culture was incubated for another 24 h. Riboflavin was isolated and purified by column chromatography. Acetylation ($HClO_4$/acetic anhydride)[14] gave the 2′,3′,4′,5′-tetraacetate (**4**), which was purified by column chromatography and crystallization from water (65 mg). ^{13}C NMR spectra of **4** were recorded in $CDCl_3$ on a Varian XL-200 Fourier transform spectrometer. The signal assignments for **3** and **4** (Table I) rest firmly on chemical shift and multiplicity arguments,[15] the spectral analysis of specifically ^{13}C- and ^{15}N-labeled flavins[16,17] and isoalloxazines,[18] the

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