with excess nickel carbonyl and triethylamine (2 mol equiv) in benzene at 65 °C for 10 min.[4]

Attempts to obtain complete conversion of **21** to **1** directly during reaction of **20*E*** were not successful. Higher reaction temperatures, longer times, and the use of polar solvents led to decomposition of **1** and lower overall yields. The most efficient procedure is a two-step process. The methanesulfonate ester **20*E*** (0.30 mmol) was cyclized with excess nickel carbonyl in benzene at 65 °C for 25 h as before, and most of the solvent and excess nickel carbonyl were removed into a cold trap at aspirator vacuum to leave about 30 mL. This residual suspension was filtered through Celite and concentrated at oil pump vacuum to leave a yellow oil. The residue was dissolved in a mixture of triethylamine (0.35 mmol), nickel carbonyl (7.8 mmol), and benzene (50 mL) under argon and heated at 65–68 °C for 10 min. Then the solvent and excess nickel carbonyl were removed under vacuum, and the residue was examined by **1H** NMR spectroscopy. Frullanolide (**1**) was present (45% yield) along with a high molecular weight compound tentatively identified as a coupling product of **20*E*** (about 10%), but the intermediate **21** was absent. Isolation of **1** by MPLC gave 28 mg of colorless crystals, 40% yield.

With isomer **20*Z***, reaction with nickel carbonyl in benzene at 58–63 °C for 20 h produced a single lactone (**1**, 10% yield) and a single isomer of the intermediate (**21**, 40%). The crude mixture of **1** and **21** was carried on with nickel carbonyl and triethylamine as above, producing **1** in 31% yield after chromatography. The formation of the same ring fusion isomer (**1**, **21**) from both **20*E*** and **20*Z*** may be accounted for simply by rapid isomerization of the allylic double bond during formation of the (presumed) common allyl–nickel intermediate. The origin of the very high selectivity for the cis ring fusion and the syn orientation of the lactone ring with respect to the angular methyl group is not clear and will be the subject of future study.

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(21) $^1$,h NMR ($CDCl_3$) $\delta$ 5.75 (t, $J$ = 1.5 Hz, 1 H, HC=CBr anti), 5.67 (m, 1 H, HC=CBr syn), 5.10 (br s, 1H, CHO), 2.5–1.9 (7, 8 H, $CH_2$), 1.22 (s, 3H, $CH_3$). $^{13}$C NMR ($CDCl_3$) $\delta$ 136.4, 134.5, 132.6, 117.5, 66.5, 54.2, 41.5, 40.9 33.5, 27.1, 21.2, 19.1, 18.6 (one carbon not observed); IR ($CHCl_3$) 3560(w, OH), 625 (m C=C), 1600 (w, C=C), 1460(m), 1373 (m), 955 (m), 900(m) cm$^{-1}$; mass spectrum, $M$, 284.0781; clacd for $C_{14}H_{21}O^{79}Br$: 284.0776.

## Dioxygenase Model Studies: Reaction of Oxygen with Iron Catecholates

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Protocatechuate 3,4-dioxygenase and catechol 1,2-dioxygenase are high-spin ferric enzymes[1,2] which catalyze the oxidative cleavage of catechols to *cis,cis*-muconic acids. One proposed role for the iron in these enzymes is the binding and activation of molecular oxygen after substrate reduction of the ferric center to the ferrous oxidation state.[1] However, Mössbauer studies[3,4] of the enzyme–substrate complexes and an oxygenated intermediate show the active-site iron to remain high-spin ferric in these states. On the basis of these observations, we have proposed an alternative mechanism[5] which does not involve the ferrous oxidation state; instead, we suggest that coordination of the catechol to the ferric center activates the catechol to attack by oxygen, resulting in the formation of semiquinone and superoxide (or the hydroperoxy radical). We have explored the reaction of oxygen with model catecholate complexes and report our results in this communication.

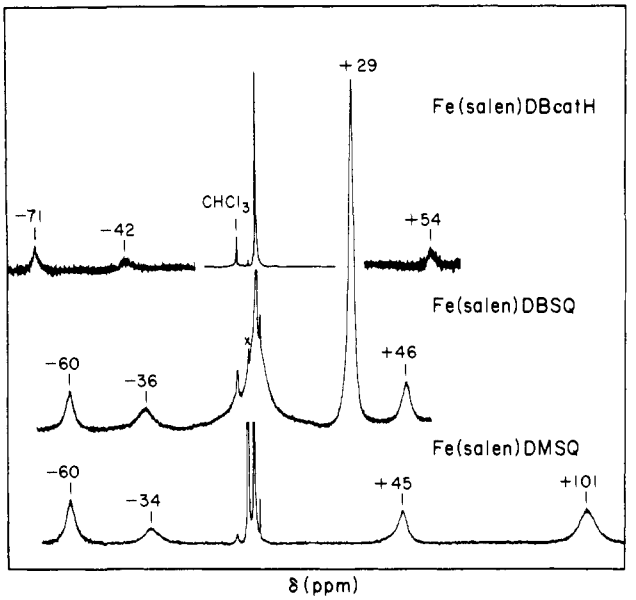


**Figure 1.** 80-MHz $^1$H NMR spectra of (A) Fe(salen)DBcatH, (B) Fe(salen)DBSQ, and (C) Fe(salen)DMSQ in $CDCl_3$ solution. These spectra exhibit resonances in the diamagnetic region due to (A) bound and free $DBcatH_2$. (A one-fold excess of $DBcatH_2$ was added to ensure complete formation of Fe(salen)$DB_{cat}$H.[14]) (B) 3,5-Di-*tert*-butyl-*o*-benzoquinone from slight decomposition of Fe(salen)DBSQ, and (C) THF of crystallization. Spectra were obtained at ambient temperature on a Varian CFT-20 spectrometer operating at 80 MHz in the FT mode.

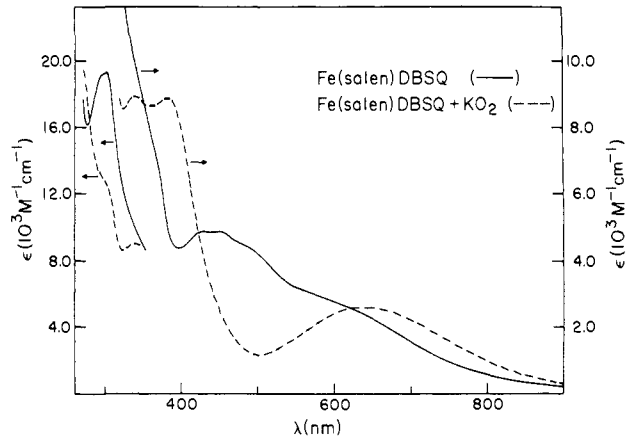


**Figure 2.** Electronic spectra of Fe(salen)DBSQ in THF solution before and after addition of 1 equiv of K(18-crown-6)$O_2$. The latter spectrum is identical with that of [Fe(salen)DBcat]$^-$. Spectra were obtained in 0.1-mm cells on a Cary 219 spectrophotometer.

Fe(salen)DBcatH,[6] a high-spin ferric complex synthesized from the ligand exchange reaction of Fe(salen)OAc with excess 3,5-di-*tert*-butylcatechol,[7] yields an NMR spectrum with paramagnetically shifted resonances (Figure 1). Upon introduction of oxygen, these resonances are replaced by features identified as

† National Institutes of Health predoctoral trainee, 1978–1981.

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(6) Abbreviations used: salen, ethylenebis(salicylaldimine); $DBcatH_2$, 3,5-di-*tert*-butylcatechol: OAc, acetate; DBSQ, 3,5-di-*tert*-butyl-*o*-semiquinone anion; THF, tetrahydrofuran; PhOH, phenol; $catH_2$, catechol; saloph, *o*-phenylenebis(salicylaldimine); DMSQ, 4,5-dimethoxy-*o*-semiquinone anion.

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arising from Fe(salen)DBSQ,[8] the product of a one-electron oxidation; similar conclusions are derived from visible spectra. In a separate experiment, [Fe(salen)DBcat]$^-$ is generated in solution from Fe(salen)DBcatH ($\lambda_{max}$ 416 nm in $CH_2Cl_2$) by the addition of 1 equiv of KO-*t*-Bu; this complex ($\lambda_{max}$ 640 nm in THF) does not exhibit NMR resonances. No immediate change in the NMR ($CD_3CN$ solution) or visible (THF solution) spectrum is observed upon exposure to oxygen. Furthermore, Fe(salen)DBSQ, synthesized by the oxidative addition of 3,5-di-*tert*-butyl-*o*-benzoquinone to Fe(salen),[8,9] reacts with $KO_2$ in THF (solubilized by 18-crown-6[10]) to yield the green [Fe(salen)DBcat]$^-$ (Figure 2). These observations, i.e.,

$$\text{Fe(salen)DBcatH} + O_2 \rightarrow \text{Fe(salen)DBSQ} + HO_2\cdot$$

$$[\text{Fe(salen)DBcat}]^- + O_2 \rightarrow \text{no reaction}^{11}$$

$$\text{Fe(salen)DBSQ} + O_2^-\cdot \rightarrow [\text{Fe(salen)DBcat}]^- + O_2$$

indicate that catecholates coordinated to ferric centers can react with molecular oxygen only under certain conditions.

The NMR spectrum of Fe(salen)DBcatH is typical of Fe(salen)X complexes[12] which exhibit isotropically shifted resonances because of the paramagnetic center. Resonances at −71, +54, and −42 ppm are assigned to H-4, H-5, and H-6 of the salicylaldimine protons, respectively, this alternating shift pattern being indicative of dominant contact interactions. Catecholate and phenolate complexes exhibit similar shifts;[13] this shows that the catH$^-$ ligand behaves like PhO$^-$ ligands and is consistent with the similarity of their optical spectra.[14] A monodentate catecholate structure has been established for crystalline Fe(saloph)catH,[2] and our solution studies suggest that the solid-state structure persists in solution.[14]

The semiquinone complexes also exhibit a shift pattern consistent with dominant contact interactions, though the shifts are smaller because of the antiferromagnetic coupling of the semiquinone free radical with the high-spin ferric center to yield a net $S = 2$ state[8] (Figure 1). In addition to salen resonances, features arising from the semiquinone ligand are also observed, indicating that unpaired electron density persists on the ligand. For Fe(salen)DBSQ, the *tert*-butyl resonances are found at −1 and +29 ppm and assigned to the groups at the 3 and 5 positions,[15] respectively, in accordance with their $a_H$ values determined from the ESR spectrum of the free semiquinone.[17] Further confirmation of our assignments comes from Fe(salen)DMSQ[18] which exhibits neither of these resonances; instead, a broad signal is found at +101 ppm and assigned to the methyl groups. The other protons are presumably too broad to be observed.

[Fe(salen)DBcat]$^-$, unlike the other Fe(salen)X complexes, does not exhibit an NMR spectrum, though Evans' susceptibility measurements[20] show that the complex remains high-spin ferric. The absence of NMR resonances indicates a significant increase in the electron spin–lattice relaxation time ($T_{1e}$) compared to the other Fe(salen)X complexes; this increase in $T_{1e}$ probably arises from a decrease in the zero-field splitting of the complex.[21,22] Such line-broadening effects due to changes in zero-field splitting have been observed in studies of ferric porphyrin complexes with axial substituents of increasing ligand field strength.[22] These observations together with the established affinity of catecholates for iron[23] suggest that on deprotonation the catecholate ligand chelates to the iron, giving rise to a six-coordinate complex. The chelated structure is consistent with the resonance Raman spectrum of the green complex in $CD_3CN$, which exhibits peaks at 1262, 1323, and 1472 cm$^{-1}$, typical of other chelated catecholate complexes.[7,24,25] Indeed, preliminary X-ray crystallographic studies on K$^+$[Fe(salen)cat]$^-$ confirm the suggested structure.[14]

The reactivity of Fe(salen)DBcatH with oxygen contrasts the stability of [Fe(salen)DBcat]$^-$. Fe(salen)DBcatH reacts readily with molecular oxygen; the conversion to the semiquinone complex in $CDCl_3$ solution is estimated to be greater than 80% from NMR spectra. This reactivity may be ascribed either to the monodentate coordination of the catecholate or to the dismutation of the hydroperoxy radical formed, which can act as the driving force for the reaction. [Fe(salen)DBcat]$^-$, on the other hand, does not react readily with oxygen, indicating that the normally air-sensitive catecholate dianion has become stabilized to oxidation by chelation to the metal. Indeed, the observed reduction of Fe(salen)DBSQ by superoxide confirms the oxidative stability of [Fe(salen)DBcat]$^-$. Further evidence for this comes from cyclic voltammetric studies on [Fe(salen)DBcat]$^-$ and Fe(salen)DBSQ; the two complexes show quasi-reversible voltammograms for the reaction

$$\text{Fe(salen)DBSQ} + e^- \rightleftarrows [\text{Fe(salen)DBcat}]^-$$

$$E_{1/2}(CH_3CN) = -180 \text{ mV vs. SCE}$$

This is compared to an $E_{pc}$ of −1134 mV vs. SCE for the reduction of DBSQ.[26] Similar shifts in the potential of the SQ$^-$/cat$^{2-}$ couple upon chelation have been observed for Fe(III),[27] Co(III),[28] Pd(II),[29] and Pt(II)[29] complexes, among others, indicating that chelation stabilizes the cat$^{2-}$ state.

Our model studies have thus provided some insights into the role of the iron center in the intradiol cleaving catechol dioxygenases. These enzymes have been shown to have two tyrosinates coordinated to the active site iron.[30,31] Using salen as a mimic for the iron coordination environment,[14] we have synthesized two catecholate complexes to model the enzyme–substrate interaction. Both complexes are high-spin ferric, indicating that the bound catecholates have not reduced the iron in this coordination environment. Thus, substrate reduction of the ferric center in the dioxygenases during the catalytic process as proposed by one mechanism[1] would appear unlikely. On the other hand, the observed reaction of a ferric catecholate complex with oxygen provides a precedent for the alternative mechanism[5] we have proposed. Furthermore, the difference in the reactivities of the

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monodentate and the chelated catecholate complexes is striking and has implications as to the nature of the enzyme–substrate interaction in these enzymes. Resonance Raman studies on the dioxygenases indicate that the substrate catechol is chelated to the active-site iron;[7,25] our results suggest that this form is not reactive. However, studies on substrate analogues indicate a preference of the iron center for the coordination of one catecholate hydroxyl over the other.[30] Perhaps oxygen binding induces protonation of the catecholate, rendering the complex reactive with oxygen. Further studies on both model and enzyme systems are in progress.

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## Observation of a Novel Effect of pH on the Anionic Inhibition of Carbonic Anhydrase: Implications for Enzymatic Catalysis

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The inhibition by anions of the catalysis of the reversible hydration of $CO_2$ and dehydration of $HCO_3^-$ by carbonic anhydrase (EC 4.2.1.1) was noted by the earliest investigators of this extremely powerful catalyst.[1] The catalytic properties of this enzyme have recently been reviewed.[2] Carbonic anhydrase activity is quite pH dependent; at low pH, catalysis of $CO_2$ hydration is negligible, while at high pH, $CO_2$ hydration is catalyzed with maximal efficiency. The reverse holds for $HCO_3^-$ dehydration activity. An ionization with p$K_a$ near neutrality appears to control this change in activity.[2]

The mechanism and pH dependence of the anionic inhibition of the $HCO_3^-$ dehydration activity of bovine erythrocyte carbonic anhydrase (BCA) has recently been investigated in these laboratories.[3] We are engaged in a corresponding study of the anionic inhibition of the BCA catalysis of $CO_2$ hydration, covering for the first time a significant range of pH values. The results of this study are sufficiently surprising to warrant a preliminary communication. Using stopped-flow initial rate techniques previously described,[3,4] inhibition by the acetate anion has been investigated over the pH range 6.15–9.00. At each of 9 pH values, a full Lineweaver–Burk plot ($1/V$ vs. $1/[S]$) has been obtained by using inhibitor concentrations spanning the observed $K_i$ values. Primary plots and secondary slope and intercept replots[5] exhibit excellent linearity (correlation coefficients >0.99).

At pH values below 7, the mechanism of inhibition observed is best described in the nomenclature of Cleland[6] as intersecting (slope and intercept linear) noncompetitive inhibition; both the slope and ordinate intercepts of the Lineweaver–Burk plots increase with increasing inhibitor concentration. The slope and intercepts are not necessarily equivalently influenced. Two $K_i$ values can be isolated; one describes the effect of inhibitor on the slope of the Lineweaver–Burk plot and will be referred to as $K_i^{slope}$. The other term, $K_i^{int}$, describes the effect of inhibitor on the ordinate intercept of Lineweaver–Burk plots. In simple one-substrate enzymes under conditions of rapid equilibrium between enzyme species, $K_i^{slope}$ measures the dissociation of enzyme–inhibitor

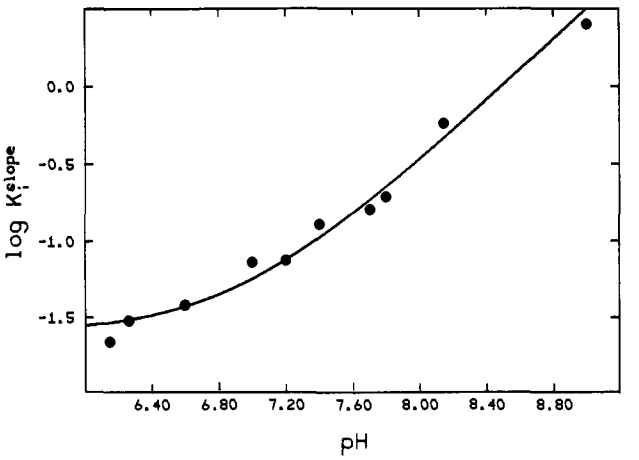


**Figure 1.** Plot of log $K_i^{slope}$ vs. pH for the inhibition of BCA-catalyzed $CO_2$ hydration by $CH_3CO_2^-$. The line is a theoretical curve representing a change from a limit low pH value for $S_i^{slope}$ of 0.025 M to negligible binding at high pH, governed by a single ionization of p$K_a$ 6.9.

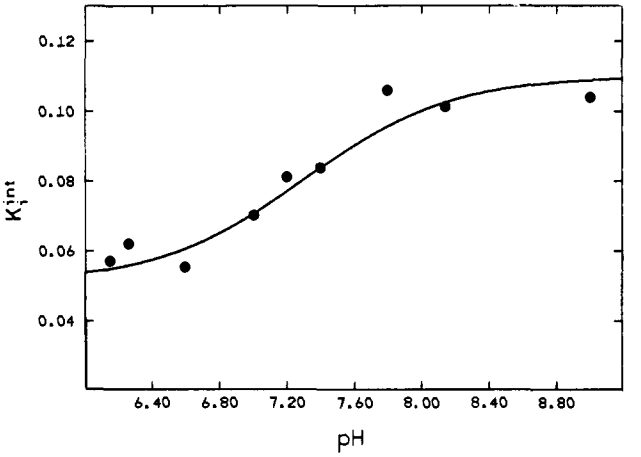


**Figure 2.** Plot of $K_i^{int}$ vs. pH for the inhibition of BCA-catalyzed $CO_2$ hydration by $CH_3CO_2^-$. The line is a theoretical curve representing a change from a limit low pH value for $K_i^{int}$ of 0.05 M to a limit high pH value of 0.11 M, governed by a single ionization of apparent p$K_a$ 7.3 (see ref 8).

complex in the absence of substrate, while $K_i^{int}$ reflects the dissociation of the ternary enzyme–substrate–inhibitor complex.[5]

As the pH increases above 7.0, the mechanism of inhibition changes to a limiting pattern best described[5,6] as linear uncompetitive inhibition; increasing inhibitor concentration increases the ordinate intercept, but not the slope, of the Lineweaver–Burk plot. Such a mechanism of inhibition of carbonic anhydrase has not been previously reported under any experimental conditions. This important and unprecedented result is quite general. With $K_i$ values ranging over four orders of magnitude, the anions $Cl^-$, $N_3^-$, $ClO_4^-$, formate, and hexanoate all exhibit the same uncompetitive pattern at high pH. Anionic inhibition is established extremely rapidly. No change in degree or mechanism of inhibition is observed whether enzyme and inhibitor are preincubated for several minutes or allowed to interact for only 10 ms before initial rate measurements.

For illustration of the smooth transition between the mechanisms observed at low and high pH, log $K_i^{slope}$ values for acetate inhibition of BCA-catalyzed $CO_2$ hydration are plotted vs. pH in Figure 1. The line drawn is a theoretical curve describing the binding represented by $K_i^{slope}$ as controlled by a single ionizable group of p$K_a$ 6.9, with inhibitor binding only to the acidic form of enzyme. This is exactly the pattern observed in studies of the anionic inhibition of BCA-catalyzed $HCO_3^-$ dehydration[3] and *p*-nitrophenyl acetate hydrolysis,[7] both of which display significant

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