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# Transient Inverted Metastable Iron Hydroperoxides in Fenton Chemistry. A Nonenzymatic Model for Cytochrome P450 Hydroxylation

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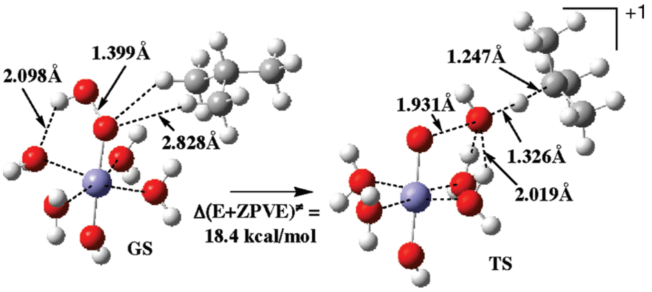
*Received March 12, 2010*



Quantum mechanical calculations (DFT) have provided a mechanism for the oxidative C$-$H bond cleavage step in Fenton-like hydrocarbon hydroxylation. A transition structure for hydrocarbon oxidation by aqueous solvated cationic iron(III) hydroperoxides ($(H_2O)_nFe^{III}OOH$) is presented that involves a novel rearrangement of the hydroperoxide group (FeO$-$OH → FeO$\cdots$HO) in concert with hydrogen abstraction by the incipient HO• radical with activation barriers ranging from 17 to 18 kcal/mol. In every hydroperoxide examined, the activation barrier for FeO$-$OH isomerization, in the absence of the hydrocarbon, is significantly greater than the overall concerted activation barrier for C$-$H bond cleavage in support of the concept of O$-$O bond isomerization in concert with hydrogen abstraction. The transition structure for the oxidation step in simple anionic iron(III) hydroperoxides has been shown to bear a remarkable resemblance to model porphyrin calculations on cytochrome P450 hydroxylation.

## Introduction

Hydroperoxides continue to play a central role in both synthetic chemistry and biological oxidations.[1] Our longstanding interest in oxygen chemistry continues with a theoretical study of the novel rearrangement of a series of iron(III) hydroperoxides to metastable inverted hydroperoxides that provide hydrogen-bonded hydroxyl radicals as the primary oxidant. We have recently identified a novel isomerization of a series of hydroperoxides, including peroxynitrous acid,[2] vinyl and phenyl hydroperoxides.[3] An exceptional opportunity exists for resonance stabilization of an oxyradical where the O$-$O bond cleavage produces an incipient oxygen radical that is conjugated to a carbon$-$carbon double bond. Simple vinyl hydroperoxides ($H_2C{=}CH{-}OOH$) have atypically low O$-$O BDE (20.2 kcal/mol, G3) due to the delocalization of the initially produced O-centered radical to a more stable C-centered radical. The electronic spin density prefers to reside on the carbon atom, resulting in a stabilization energy of 23.1 kcal/mol ($\Delta E_{\delta\pi}$, G3). Phenyl hydroperoxide exhibits the same behavior with an O$-$O BDE of only 20.2 kcal/mol when calculated relative to its C-centered radical.[3]

We have suggested a new mechanism for the C$-$H oxidation step in the hydroxylation of hydrocarbons catalyzed by a metastable isomer of peroxynitrous acid, [ONO$\cdots$HO*].[2] We were particularly intrigued by the observation that certain metal hydroperoxides undergo what we termed a "somersault" rearrangement (MO$-$OH → MO$\cdots$HO), where the hydroxyl radical can remain strongly bound to the MO• radical fragment that arises upon homolytic O$-$O bond cleavage.[3] When the MO• fragment, resulting from O$-$O bond cleavage, can realize some form of unique stabilization by localizing its spin density in a lower lying orbital, the activation barrier for such a somersault rearrangement of the $-$OOH fragment can be significantly lower

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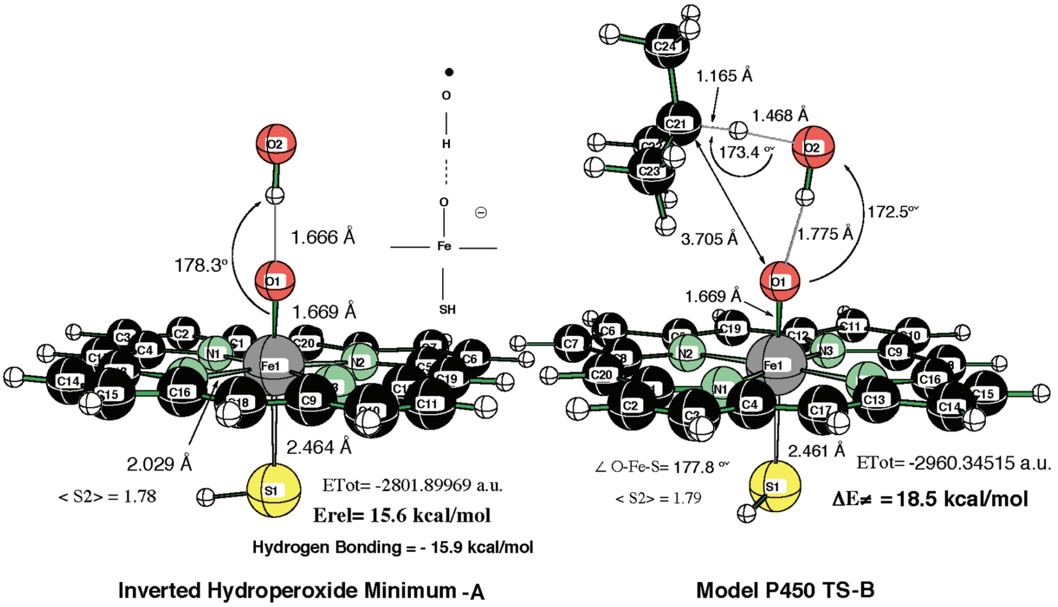
DOI: 10.1021/jo1004668



**Inverted Hydroperoxide Minimum -A** **Model P450 TS-B**

**FIGURE 1.** Inverted hydroperoxide product minimum A produced by the somersault rearrangement of model Cpd O (FeO−OH) and the model P450 transition state B for H-abstraction from isobutane by the resulting bound HO• radical in concert with hydroperoxide rearrangement.

than the O−O bond dissociation limit.[3] Upon the basis of these observations, we proposed[3] a new potential pathway for what transpires in the so-called "black box" in the cytochrome P450 oxidation sequence.[4] This new mechanism involves the intermediacy of a metastable "inverted" iron hydroperoxide produced by homolytic O−O bond cleavage (FeO−OH → FeO···HO$^\bullet$) of the hydroperoxide moiety in P450FeOOH$^{2+}$,Cpd 0[3]. This type of somersault or vaulting motion in our model Cpd O produces a stabilized inverted hydroperoxide minimum A (Figure 1), with the HO• hydrogen bonded to its open shell oxyradical fragment [Por(SH)Fe(III)−OOH$^-$] → [Por(SH)Fe(III)−O···HO$^-$]. The activation barrier for this somersault rearrangement, in the absence of a hydrocarbon substrate, is 2.9 kcal/mol greater[3] than that for the concerted oxidation in Figure 1.

This *bound* HO• radical A is 15.6 kcal/mol higher in energy than its ground state hydroperoxide (FeO−OH) form, and the HO• radical is strongly hydrogen bonded to the ferryl oxygen (−15.9 kcal/mol).[3] Upon the basis of this model, we have suggested that the hydrogen abstraction step in P450 hydroxylation is a concerted hydrogen abstraction followed along the reaction pathway by a subsequent hydroxyl transfer to the resulting carbon-centered radical to produce the overall product, an alcohol. The novel O−O bond rearrangement produces a developing HO• fragment that concomitantly abstracts a hydrogen atom from a C−H bond, as shown in the model P450 TS-B (Figure 1). The geometry in TS-B for H-abstraction closely resembles that of the rearranged hydroperoxide minimum A, with a nearly linear FeO··· H−O$^\bullet$ array for the bound hydroxyl radical (172.5°). This avoids the formation of a hydroxyl radical as a discrete free radical intermediate. We extend these fundamental concepts to the oxidation of hydrocarbons by aqueous iron(III) hydroperoxides in Fenton-type oxidations that can potentially serve as a model for enzymatic hydroxylation.

One of the oldest reactions for the oxidation of saturated hydrocarbons in all of organic chemistry utilized the so-called Fenton reagent.[5] More than a century ago, the oxidation of organic compounds by the action of iron(II) and hydrogen peroxide was reported and the original Fenton reagent consisted of ferrous salts and $H_2O_2$ in aqueous acidic solution. It was discovered that $Fe^{2+}$ and $H_2O_2$ could readily oxidize a hydrocarbon to its corresponding alcohol. Many of today's concepts concerning high-valent iron and the continuing role that it plays in both chemical and biological oxidation systems derive from the continuing mechanistic studies of the Fenton reagent. Perhaps more importantly, the extensive breadth of the overall chemistry of Fe−$H_2O_2$ systems has provided both precedence and valuable insight for numerous enzymatic mechanisms.

Nearly 40 years after the initial discovery[5] of this nonenzymatic hydrocarbon oxidation, the involvement of a ferryl ion, $FeO^{2+}$, was introduced by Bray and Gorin.[6] This proposed oxidant was followed almost immediately by the suggestion of Haber and Weiss[7] that the hydroxyl radical was the actual oxidizing agent. The mechanism of this oxidative process remains the subject of serious controversy even today. There is not even agreement as to the actual oxidizing reagent, and many aspects of the C−H bond cleavage step are in question.

Proponents of the involvement of hydroxyl radicals[8] have vigorously refuted the claims by others that neither HO• nor carbon free radicals were involved.[9] Solvent effects are also

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important and can influence the reaction mechanism. For example, in acetonitrile, in contrast to water, iron(III) is reduced by hydrogen peroxide and dioxygen can be produced, leading to the conclusion that a mechanism involving only free radicals seemed to be unjustified.[9b]

More recent studies have tried to put this controversy in perspective, but the view has persisted that hydroxyl radicals are not necessarily involved.[10] The initial kinetic study by Rush and Koppenol[11a] on the reaction of ferrous ion with hydrogen peroxide prompted a recent comprehensive reinvestigation by Kremer[11b] that identified an error in the earlier investigation, casting serious doubt upon the involvement of free radicals. A related kinetic study provided indirect evidence of the involvement of hydroxyl radicals, but evidence for its involvement remains elusive.[11d]

The second candidate for the primary oxidant, the ferryl ion, $Fe^{IV}{=}O^{2+}$, has been the subject of recent DFT and Car–Parrinello MD simulations.[12] DFT calculations on water-ligated $Fe^{2+}$ hydrogen peroxide complexes suggest that the ferryl ion is much more readily derived from $H_2O_2$ coordinated to penta aqua iron(II) than is free HO• radical. A free energy barrier of 22 kcal/mol[12a] was suggested for hydrogen abstraction from methane by the ferryl ion. However, the aqua oxoiron(IV) intermediate, $[(H_2O)_5Fe^{IV}{=}O]^{2+}$, was recently generated in solution and its candidacy as the primary oxidant was called into question, but support for this primary oxidant was forthcoming in the presence of a penta dentate ligand.[12b] Spectroscopic, as well as chemical, evidence supported by DFT calculations have provided results that tend to exclude this ferryl $Fe^{IV}{=}O^{2+}$ species as the principle oxidant in the Fenton reaction.[13] However, DFT calculations support the direct formation of $Fe^{IV}{=}O$ from $[(H_2O)_6Fe^{IV}{=}O]^{2+}$ and $H_2O_2$ under Fenton conditions, although support continues for the involvement of hydroxyl radicals. Thus, considerable evidence has been presented for and against the two primary consensus oxidizing reagent candidates for this reaction.[14] We sense a definite relationship between our P450 hydroxylation mechanism[3] and how the Fenton reaction may proceed and now present an alternate mechanism for this century old oxidation reaction involving a metastable FeO–OH moiety that can also serve as a model for enzymatic hydroxylation.

## Results and Discussion

Many of the basic tenets for precedence in enzymatic reactions involving high-valent iron systems have been gleaned from mechanistic studies on this relatively primitive oxidative system. It was postulated earlier by Walling[8a] that Fenton-type reactions must start with some type of association or complex formed between the peroxide used and a metal ion. Our approach to predicting the primary oxidant in the Fenton reaction is predicated upon the assumption that a relatively simple FeO–OH could, in the absence of a porphyrin ring, also undergo the somersault rearrangement to produce an inverted metastable oxidant [FeO···HO].[3] Both $Fe^{2+}$ and $Fe^{3+}$ salts have been invoked as possible oxidation states in this reaction with the higher-valent iron being derived from the facile reduction of $H_2O_2$ by $Fe^{2+}$ at low pH (eq 1). Dependence of pH on the rate of the Fenton reaction has been recently reported.[11c]

$$2Fe^{2+} + H_2O_2 \rightarrow 2Fe^{3+} + 2HO^{-} \qquad (1)$$

It has been noted that the ligand environment of the iron ions plays an important role in the reaction mechanism. For example, it has been suggested that addition of chloride anions to a "naked" iron(III) cation switches the reaction mechanism from one involving hydroxyl radicals to a different mechanism.[15] Consequently, we examined initially the simplest neutral iron hydroperoxide ($Cl_2FeO{-}OH$) that might possibly be involved in the Fenton oxidation upon addition of $FeCl_2$ to an oxidative mixture of aqueous $H_2O_2$. $Cl_2FeO{-}OH$ can also be formed by Lewis acid enhanced $H_2O_2$ protolysis after coordination of $H_2O_2$ to Fe(III), producing a sharp reduction in p$K_a$.

**Activation Barriers for Neutral Dichloroiron(III) Hydroperoxide.** The sextet state for this dichloroiron(III) hydroperoxide, **GS-1**, was 2.2 kcal/mol lower in energy than the quartet (Figure 2). The O–O BDE in sextet dichloroiron(III) hydroperoxide is relatively high, 43.0 kcal/mol, while that of the quartet is somewhat lower (29.2 kcal/mol). The sextet TS for the somersault isomerization, in the absence of a substrate, also had a relatively high activation barrier of 41.0 kcal/mol. The O–O bond was elongated to 2.556 Å in **TS-1**, and as noted previously,[3] the OH group hydrogen atom is H-bonded to the ferryl oxygen (2.221 Å). In the TS, the O–O–H bond angle is typically less than 90° to ensure some element of intramolecular hydrogen bonding to the ferryl oxygen that facilitates O–O bond cleavage attending the FeO–OH isomerization.[3]

The resulting minimum, on the product side of O–O bond isomerization, **Min-1**, containing the hydroxyl radical H-bonded to the $Cl_2FeO$• fragment, was found to be 40.3 kcal/mol higher in energy than its **GS-1** minimum. Although the somersault rearrangement is complete and the HO• radical is in a nearly linear array, it is very weakly H-bonded (2.0 kcal/mol) to the neutral ferryl oxo fragment. Such a weakly bound HO• is not likely to produce a useful oxidizing agent because this would essentially be a free hydroxyl radical that would exhibit the anticipated indiscriminate reactivity of such a high-energy open shell species.

**Activation Barriers for Negatively Charged Dichloroiron(III) Hydroperoxide.** The active site of cytochrome P450, in its resting state, consists of an iron porphyrin with the iron(III) coordinated to a water molecule and a cysteinyl axial ligand that renders it negatively charged,[4] as modeled in Figure 1. To make a more direct comparison with the negatively charged ferryl intermediate in P450 hydroxylation,[3]

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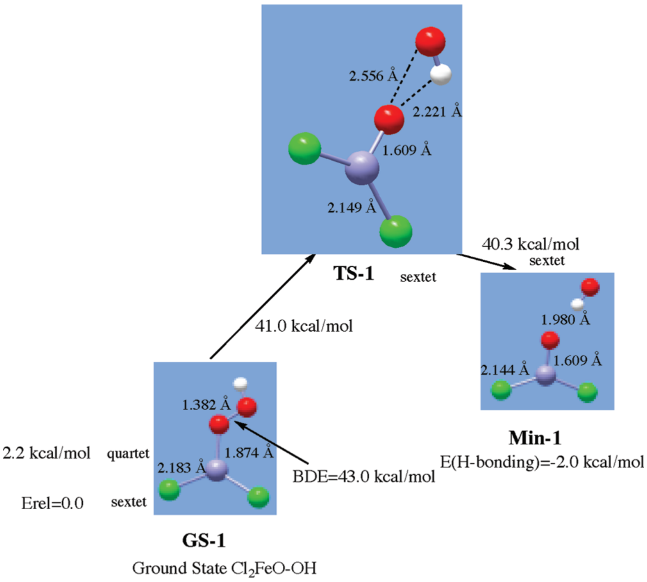

**FIGURE 2.** Ground state $Cl_2FeO-OH$ and its transition state ($\nu_I$ = 118.6*i* $cm^{-1}$) for the somersault isomerization to a bound hydroxyl radical intermediate **Min-1** (ECP basis set on Fe and the 6-311+G(d,p) basis set on all other atoms).

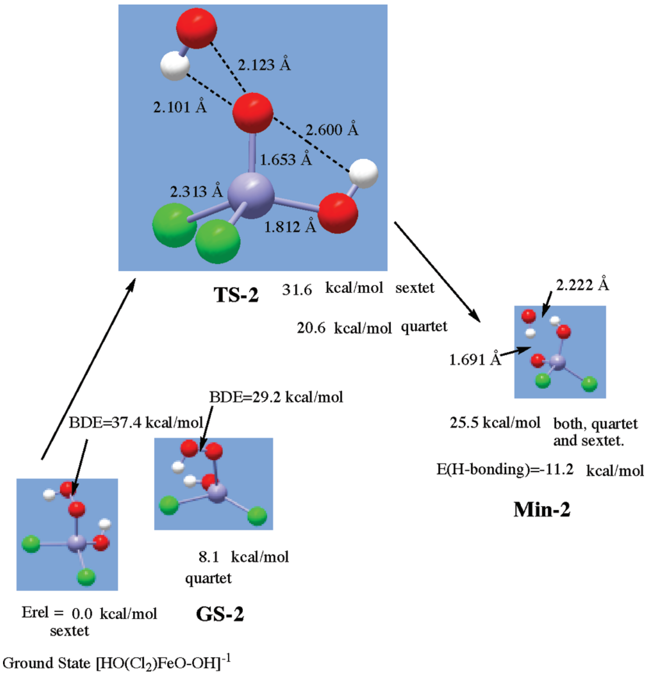

**FIGURE 3.** Anionic ground state **GS-2** $[HO-Cl_2FeOOH]^-$ and its transition state, **TS-2** ($\nu_I$ = 805.1*i* $cm^{-1}$), for the somersault isomerization to a bound hydroxyl radical intermediate, **Min-2** (ECP basis set on Fe and the 6-311+G(d,p) basis set on all other atoms).

Cpd II, we examined the effect of a negative charge on the somersault rearrangement of the FeO−OH moiety in $Cl_2FeO-OH$. When we added a hydroxyl anion to the above iron(III) hydroperoxide to produce a negative charge, the sextet of $[HO-(Cl_2)FeOOH]^-$ was 8.1 kcal/mol more stable than the quartet. The O−O BDE for this anionic complex, **GS-2**, was slightly reduced to 37.4 kcal/mol for the sextet and 29.2 kcal/mol for the quartet (Figure 3). Consequently, the activation barrier for the FeO−OH rearrangement ($\nu_I$ = 278.5*i* $cm^{-1}$) of the more stable hydroperoxide (**TS-2**) is greater for the sextet (31.6 kcal/mol, with ZPVE) than for the quartet (20.6 kcal/mol) because of the relative energetic requirements for O−O bond elongation in the TS. In this case, the H-bonded isomeric hydroxyl radical product intermediate (**Min-2**) is 25.5 kcal/mol higher in energy (for both the sextet and quartet) than the GS anionic hydroperoxide. In addition, the hydroxyl radical is much more strongly H-bonded (−11.2 kcal/mol) to the anionic oxy radical $[HOCl_2FeO\bullet]^-$ due to the overall negative charge on the system. Thus, both neutral and anionic gas phase transition structures and rearrangement product minima can be readily located for the simplest iron(III) hydroperoxide, $Cl_2FeO-OH$, potentially involved in the classical Fenton oxidation. This is an important observation that has a direct bearing on our recently disclosed new mechanism for the P450 oxidation step[3] because this demonstrates that this type of novel isomerization can take place with a relatively simple FeO−OH in the absence of the electronic influence of a porphyrin ring.

The next question that we addressed is the nature of the transition structure for H-abstraction for this negatively charged inverted hydroperoxide $[HOCl_2FeO\cdots HO]^-$ and how this TS compares to that for the comparable TS involving our P450 model TS-B (Figure 1). Prereaction complex **GS-3** had the isobutane H-bonded to both the distal oxygen and the axial $HO^-$ ligand (Figure 4). The O−O bond in **GS-3** ($r_{O-O}$ = 1.449 Å) homolytically cleaves in **TS-3** (O−O−H angle = 101.81°), and a complete somersault isomerization of the FeO−OH group provides an inverted hydroperoxide with an FeO···H−O angle of 164.4°. The HO• radical is H-bonded to the proximal oxygen, very reminiscent of the TS we find in P450 oxidation (Figure 1).[3] The activation energy for concerted H-abstraction (**TS-3**) was calculated to be 25.8 kcal/mol (with ZPVE) with an ECP basis set on iron and 22.5 kcal/mol (with ZPVE) with an all-electron calculation with the 6-311+G(d,p) basis set on all atoms including iron. This was a very early TS with C−H bond elongation of only 1.135 Å possibly due to further stabilization of the HO• radical by a H-bonding interaction (2.15 Å) with the axial $HO^-$ ligand.

Again we see a contraction of the Fe−O distance in the TS of 0.25 Å. The relatively small magnitude of the single imaginary frequency is consistent with heavy atom motion in the TS.[16] The eigenvectors indicate a pendulum motion of the OH group as it abstracts the H-atom with little involvement of the axial $HO^-$ ligand. The activation barrier for somersault rearrangement (**TS-2**) in the absence of isobutane is 7.5 kcal/mol lower than that for the concerted rearrangement and oxidative C−H bond cleavage by the incipient HO• radical (**TS-3**).

This rather simplified TS involving an Fe(III) center, in the absence of the porphyrin ring, bears a striking resemblance to the TS that we observed with our P450 model heme. The corresponding barrier for C−H-abstraction from isobutane with the P450 model (Figure 1) was 17.8 kcal/mol ($\Delta\Delta E^{\ddagger}$ = +6.3 kcal/mol). More importantly, it also involved the

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(16) For a detailed discussion of how the magnitude of the imaginary frequency in the TS for oxygen atom transfer is related to the nature of the TS, see: Bach, R. D.; Dmitrenko, O. *J. Phys. Chem. B* **2003**, *107*, 12861.

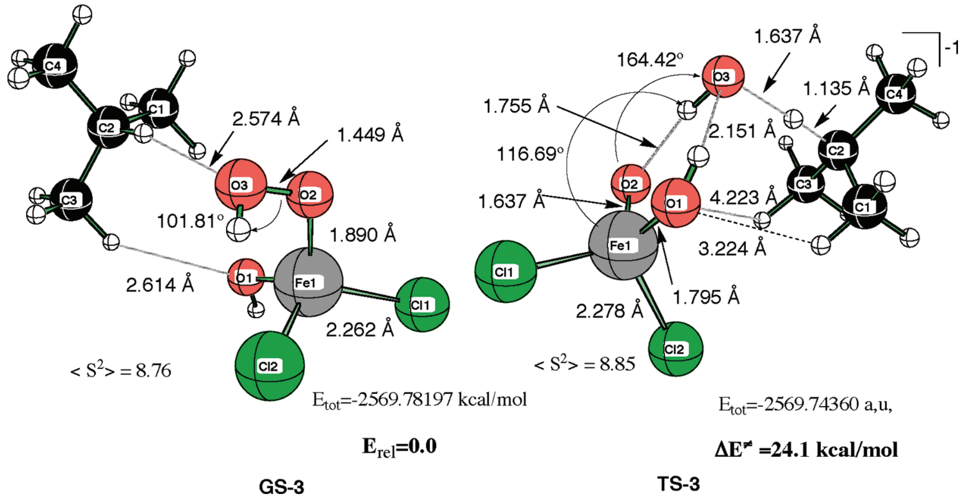

**FIGURE 4.** Anionic ground state **GS-3** and transition state **TS-3** ($\nu_i$ = 91.4*i* $cm^{-1}$) for concerted somersault rearrangement and C−H-abstraction from isobutane. This is an all-electron calculation with the 6-311+G(d,p) basis set on all atoms including the Fe atom.

complete somersault rearrangement to the inverted FeO··· HO radical with a nearly linear O−H−O arrangement in concert with H-abstraction. For comparison with experimental data, the $FeCl_3$-catalyzed oxidation of cyclohexane with $H_2O_2$ has been estimated to be 14 kcal/mol.[15]

**Activation Barriers for Neutral Aqueous Solvated Iron(III) Hydroperoxides.** We next turned our attention to what might take place under actual Fenton reaction conditions. In acidic aqueous $H_2O_2$ solution, a likely candidate derived upon oxidation of $FeCl_2$ would be an aqua iron(III) hydroperoxide such as $[(H_2O)_5Fe^{III}OOH]^{2+}$] that might perhaps be in equilibrium with its monocation derived from the loss of a proton from an axial water to produce $[HO-Fe^{III}OOH\cdot 4H_2O]^+$. Since conventional wisdom suggests[14] that the actual oxidant is most likely a cation, we examined the effect of positive charge upon the O−O bond dissociation energy (BDE) and the barrier for the somersault isomerization.[3] We retained a model system based upon iron(III) hydroperoxide, $Cl_2FeO-OH$, but maintained an approximately octahedral geometry around the iron atom with specific waters of solvation. The charge on the system was varied from 0 to $2^+$ by successfully replacing $Cl^-$ with water molecules. We utilized isobutane as the hydrocarbon substrate and located the transition structures (TS) for abstraction of the tertiary hydrogen atom. In each case, a prereaction complex of ground state FeO−OH weakly hydrogen bonded to isobutane was located with full geometry optimization.

For the neutral tri aqua iron(III) reactant **GS-4**, $Cl_2FeOOH\cdot 3H_2O$, an O−O bond dissociation energy (BDE) of 39.3 kcal/mol was found. As anticipated,[3] the somersault activation barrier (with ZPVE) of 30.9 kcal/mol (**TS-4**) was lower than the O−O bond dissociation limit, reflecting the internal H-bonding ($r_{O-H}$ = 2.196 Å) in the OOH group that maintains an O−O−H bond angle in the TS of 88.3° (Figure 5). The O−O bond elongation of 2.000 Å is typical for this type of FeO−OH isomerization.[3] The waters of solvation are very loosely bound to the iron center (≥2 Å), and in **TS-4**, the weakly bound HO• is H-bonded to the equatorial water molecules with H···O distances of 1.85 and 1.96 Å (not shown).

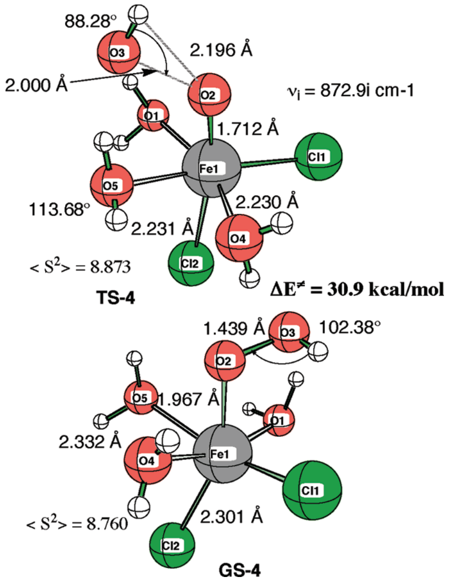

**FIGURE 5.** Neutral ground state reactant, **GS-4**, and transition structure, **TS-4**, for isomerization of FeO−OH to produce FeO···HO [ECP basis set on Fe and 6-311+G(d,p) on all other atoms].

The barrier for the hydrogen abstraction step by **GS-4**, weakly bound to isobutane (**GS-4a**), was calculated to be 28.6 kcal/mol (**TS-4a**, Figure 6). The O−O bond distance (1.896 Å) is considerably less than that in the **TS-4** (2.000 Å), and the C−H distance in **TS-4a** (1.255 Å) is consistent with a fairly late TS reflective of the relatively high C−H activation barrier. For *neutral* reactant **GS-4a**, the C−H-abstraction barrier is only slightly lower than **TS-4** for FeO−OH isomerization ($\Delta\Delta E^{\ddagger}$ = 2.3 kcal/mol).

**Activation Barriers for Positively Charged Aqueous Solvated Iron(III) Hydroperoxides.** Removal of a chloride ion from dichloroiron(III) hydroperoxide **GS-4** provides a system that has a unit of positive charge, **GS-5** $[Cl-FeOOH\cdot 4H_2O]^+$, with an O−O BDE of 39.7 kcal/mol. We found that the sextet for this cation was 3.97 kcal/mol more stable than the corresponding quartet minimum. The FeO−OH rearrangement barrier (Figure 7) for this tetra aqua sextet system was 36.4 kcal/mol (with ZPVE). The O−O bond distance in **TS-5**

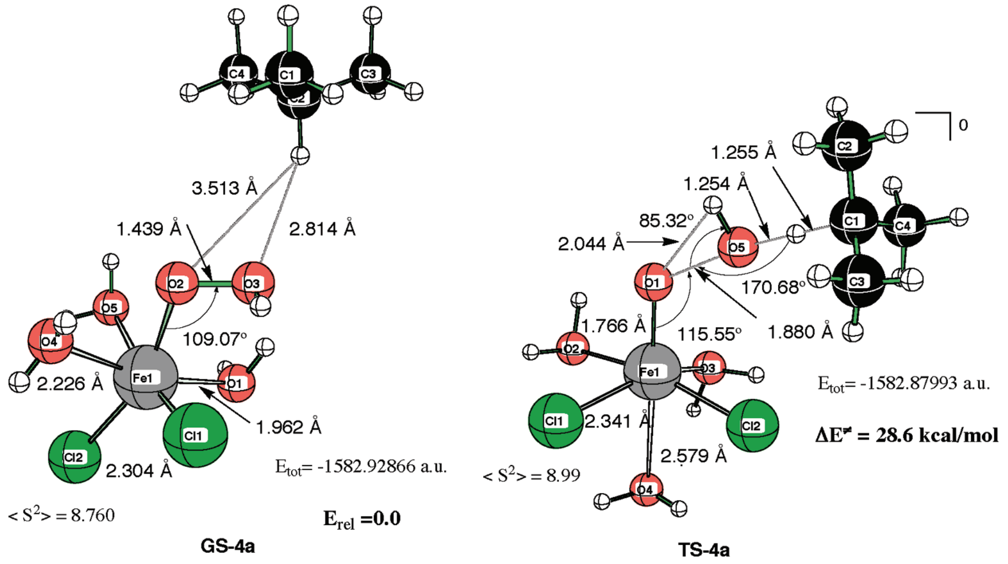

**FIGURE 6.** Neutral ground state reactant complex, **GS-4a**, and transition structure, **TS-4a**, for isomerization of FeO−OH in concert with hydrogen abstraction from isobutane [ECP basis set on Fe and 6-311+G(d,p) on all other atoms].

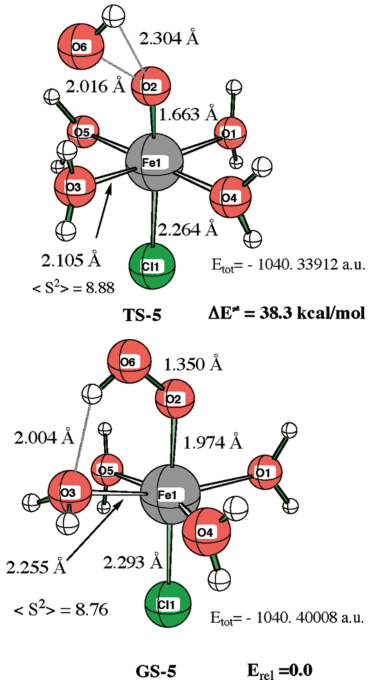

**FIGURE 7.** Ground state reactant, [Cl−FeO−OH·4H$_2$O]$^+$, **GS-5**, and transition structure, **TS-5**, for isomerization of FeO−OH to produce FeO···HO (ECP basis set on Fe and 6-311+G(d,p) on all other atoms).

of 2.016 Å is as anticipated,[3] but the contracted O−O−H bond angle (94.3°) and intramolecular H-bonding (2.304 Å) provide only a modest reduction in the rearrangement barrier relative to the O−O BDE. A general trend that is developing is that the Fe−O distance to the FeO−OH group in the GS contracts in the TS for isomerization, for example, 1.97 to 1.66 Å (Figure 6). This isomerization barrier appeared at the outset to be much greater than what one could anticipate for an effective oxidizing agent unless, as noted below, a concerted process for C−H bond breaking with a much lower oxidation barrier is found.

As anticipated, the barrier for the hydrogen abstraction step with [Cl−FeO−OH·4H$_2$O]$^+$, **TS-6**, was reduced ($\Delta\Delta E^{\ddagger}$ = 15.7 kcal/mol) to 21.8 kcal/mol (with ZPVE), supporting the suggestion of FeO−OH isomerization in concert with hydrogen abstraction (Figure 8). The TS comes a little earlier than **TS-4a** with the transferring hydrogen atom being nearly equally disposed between the C−H and developing O−H bonds. However, the nature of the TS for the neutral (**GS-4a**) and the positively charged (**GS-6**) oxidants has radically changed relative to negatively charged **TS-3**. When the developing HO• radical attending the somersault rearrangement is strongly H-bonded to a negatively charged ferryl oxygen as in **TS-3** and in our P450 model TS-B (Figure 1), the O···H−O angle approaches 180° in both the ground state product of FeO−OH rearrangement and in the TS for oxidation.

With the neutral and positively charged TSs, some semblance of the O−O bond remains, and as we have emphasized, the O−O−H internal angle remains about 90° to effect H-bonding in both the GS for isomerization and in the TS for concerted C−H bond oxidation as noted in **TS-4a** and in **TS-7**. Most importantly, the activation barrier for concerted C−H bond cleavage (**TS-6**) is 15.7 kcal/mol lower than the somersault barrier (**TS-5**) in the absence of the substrate.

Replacing the Cl$^-$ with HO$^-$ of **GS-6**, either by hydrolysis or by loss of a proton from a water molecule in the penta aqua system, produces **GS-7** [HO−FeOOH·4H$_2$O]$^+$ that had a O−O BDE (38.6 kcal/mol) comparable to negatively charged **GS-5**. The sextet (all-electron calculation) was found to be 5.5 kcal/mol more stable than the corresponding quartet. When all atoms, including the Fe, had the 6-311+G(d,p) basis set, the somersault barrier was 34.7 kcal/mol (with ZPVE). The Fe−O bond shortens from 1.920 Å in the ground state to 1.668 Å in the resulting isomerization product, **Min-7**. The single imaginary frequency ($\nu_I$ = 1609.8$i$ cm$^{-1}$) for **TS-7** is particularly intriguing because a value of this magnitude suggests largely light atom (hydrogen) motion in the TS.[16] The largest bond-breaking motion involves O−O bond elongation, with the proximal oxygen

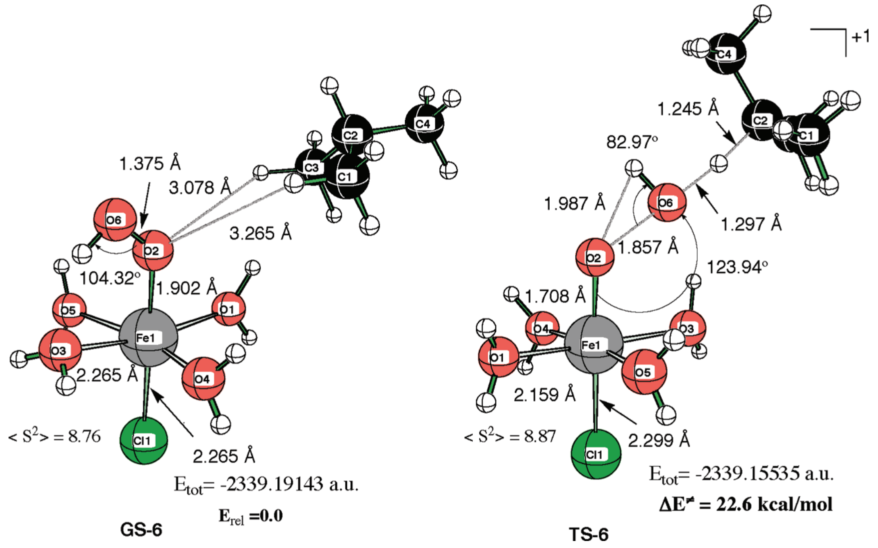

**FIGURE 8.** Ground state complex (**GS-6**) and transition structure (**TS-6**) for hydrogen abstraction from isobutane in concert with FeO−OH isomerization in $(CH_3)_3C-H\cdots[Cl-FeO-OH\cdot 4H_2O]^+$). This is an all-electron calculation with the 6-311+G(d,p) basis set on all atoms including the Fe.

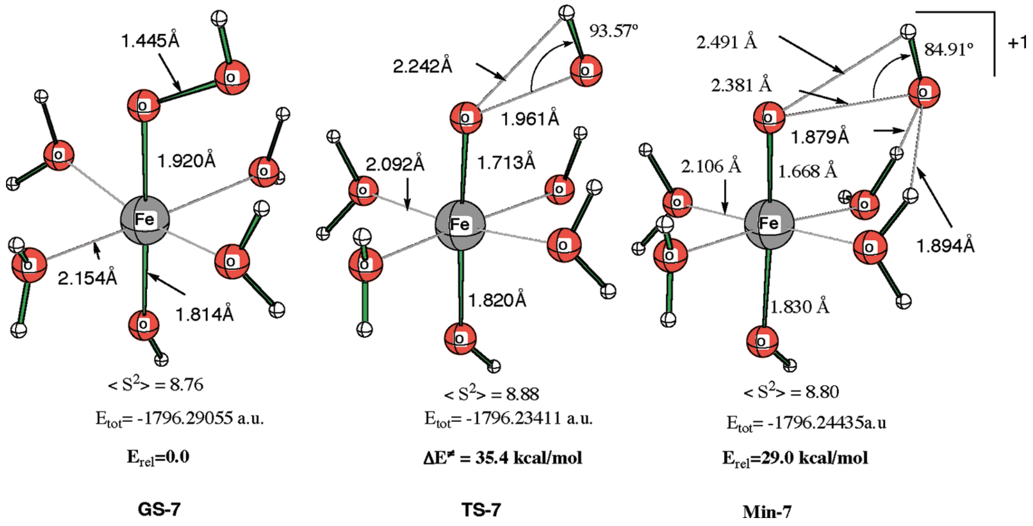

**FIGURE 9.** Reactant, **GS-7**, transition structure (**TS-7**), and product minimum for FeO−OH rearrangement in $[HO-FeO-OH\cdot 4H_2O]^+$ to produce the HO• radical hydrogen bonded to the ferryl oxygen in **MIN-7** (all-electron geometries).

interacting with both the oxygen and the hydrogen of the HO•, suggesting oxygen motion that should give rise to a much smaller negative imaginary frequency (50−100 is more typical). However, the major contribution to the eigenvectors was from a contraction of the Fe−O−H angle of the axial $HO^-$ ligand and a simultaneous elongation of the Fe proximal oxygen bond in consonance with homolytic O−O bond cleavage. Thus, the light atom motion indicated by the large imaginary frequency (1609.8*i* $cm^{-1}$)[16] that dominated HO• expulsion in the TS was that of the wagging hydrogen of the axial OH ligand that affected O−O bond cleavage.

**MIN-7** was only 6.4 kcal/mol lower in energy than **TS-7** (Figure 9), and hence its lifetime may be too short for a bimolecular collision with isobutane with such a small barrier to revert back to its ground state, **GS-7**. This supports formation of a stabilized prereaction complex. The stabilization energy for the HO• radical hydrogen bonded to the ferryl oxygen radical $[(4H_2O)HO\cdot FeO\bullet]^+$ in **MIN-7** is 12.2 kcal/mol (with ZPVE). In this positively charged rearrangement product, the linear array of FeO···HO was conspicuously absent.

The HO• radical maintains a weak bond to the proximal oxygen ($r_{O-O}$ = 1.879 Å), and instead of the linear array of anionic H-bonding (**Min-2**), it is further stabilized by H-bonds to at least two waters and an H-bond to the proximal oxygen (2.491 Å).

These combined data suggest that isobutane forms a weak complex with **GS-7** prior to the H-abstraction or initial oxidation step that supports our overall concerted pathway for hydrocarbon hydroxylation.

The reactivity of solvated $[(H_2O)_4Fe^{(III)}(OH)OOH]^+$ with an axial hydroxide ligand toward isobutane oxidation proved to be quite comparable to that with the axial chloride

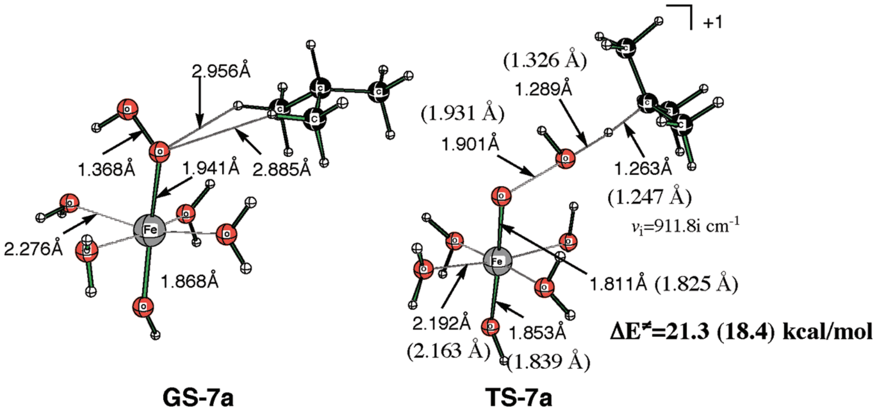

**FIGURE 10.** Transition structure (**TS-7a**) for hydrogen abstraction from isobutane in concert with FeO−OH isomerization in $(CH_3)_3C{-}H\cdots[HO{-}FeO{-}OH\cdot4H_2O]^+$ reactant complex, **GS-7a**. Values in parentheses are with the all-electron basis set.

ligand (**GS-5**) since both axial ligands bear a negative charge. Their activation barriers for FeO−OH rearrangement were comparable, so one might expect the activation energy for the concerted isobutane oxidation in reactant complex **GS-7a** (Figure 10) to also be quite close. The barrier for C−H bond cleavage relative to prereaction complex $(CH_3)_3C{-}H\cdots[HO{-}FeO{-}OH\cdot4H_2O]^+$, **GS-7a**, was calculated to be 26.0 kcal/mol (21.3 with ZPVE) when the ECP basis set was used for Fe. The activation energy was reduced to 22.6 kcal/mol (18.4 kcal/mol with ZPVE) as indicated in **TS-7a** with an all-electron calculation employing the larger basis set on Fe, as well. We do observe a change in geometry when the basis set on iron was also 6-311+G(d,p), as demonstrated in Figure 10.

The (HO−H) bond-making step to produce a water molecule was again comparable to the bond-breaking step (C−H) in **TS-7a**. It is noteworthy that the activation barrier for H-abstraction by this positively charged simple iron(III) aqueous hydroperoxide is essentially identical to that for H-abstraction by the much more elaborate anionic porphyrin complex model TS-B for P450 oxidation shown in Figure 1. Both transition states were calculated at the same level of theory and involve the somersault rearrangement of the initial FeO−OH hydroperoxide in concert with H-abstraction to produce a water molecule and a carbon-centered radical. The O−O bond length (1.901 Å) was also less elongated ($\Delta r$ = 0.06 Å) than the comparable O−O distance for the somersault rearrangement in the absence of the isobutane (**TS-7**). The O−O−H angle of 79.9° and the intramolecular O−H hydrogen bonding distance (1.98 Å) are similar to that in rearrangement **TS-7**. The single imaginary frequency of **TS-7a** exhibited the largest (essentially equal) negative eigenvectors corresponding to O−O bond breaking and O−H bond making (water formation) with a relatively large contribution for C−H bond cleavage. Significantly, the proximal (ferryl) oxygen is interacting with both the oxygen and hydrogen of the *bound* HO• radical, supporting the above intramolecular hydrogen bonding interaction of the −OOH moiety in the TS. There is also a minor contribution from the Fe−O bond contraction in the TS. The oxidation barrier (**TS-7a**) is 17.0 kcal/mol lower than the FeO−OH rearrangement barrier (**TS-7**). These combined data lend additional support to the concept of a concerted but nonsynchronous process where the outward and upward motion of the developing HO• radical places it in a nearly linear alignment (175.0°) with the C−H bond to effect hydrogen abstraction concomitantly with O−O bond elongation.

Recent kinetic data[17] has provided evidence for the involvement of an aqueous Fe(III) hydroperoxide in Fenton chemistry when an excess of $H_2O_2$ in acidic media is present (2). In such an acidic aqueous medium, we would anticipate that monocation **GS-7** may also be in equilibrium with penta aqua dication **GS-8** ($[FeOOH\cdot5H_2O]^{2+}$) simply by reversible addition of a proton to the axial OH group.

$$\mathrm{Fe^{III}(aq) + H_2O_2 \rightleftharpoons Fe^{III}(^-O2H)_{aq} + H^+} \qquad (2)$$

An all-electron calculation suggests that the sextet is 15.6 kcal/mol lower in energy than the corresponding quartet. The increase in positive charge to $2^+$ causes a surprisingly large increase in the ECP O−O BDE (62.0 kcal/mol), but this O−O bond energy in **GS-8** was reduced to 52.0 kcal/mol with the all-electron basis set. Since the O−O BDE values are reflected in the homolytic O−O bond cleavage, the somersault isomerization barriers are also relatively high for FeO−OH isomerization in dication **GS-8**. The FeO−OH rearrangement barrier with the ECP basis set on Fe ($\Delta E^{\ddagger}$ = 54.1 kcal/mol) was reduced somewhat to 45.0 kcal/mol (with ZPVE) with the all-electron basis set on iron. In **TS-8** (Figure 11), the O−O bond was elongated to 2.063 Å and all five water molecules have Fe−O distances greater than 2 Å.

The vectors of the single imaginary frequency ($\nu_I$ = 603.4$i$ cm$^{-1}$) suggest only O−O bond elongation with a minor contribution from Fe−O elongation. An interaction of the ferryl oxygen with both the oxygen and hydrogen of the developing HO• radical is in evidence. Although we were able to locate a rearrangement product minimum, the HO• radical is only weakly bound to one of the equatorial waters upon complete homolytic O−O bond cleavage and is 37.4 kcal/mol higher in energy than **GS-8**. The Fe−O bond also shortens considerably (0.33 Å) in the rearrangement TS as noted above.

Isobutane is only weakly bound to dication **GS-8** with one of its methyl groups H-bonding to an equatorial water molecule

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[17] Rachmilovich-Calis, S.; Masarawa, A.; Meyerstein, N.; Meyerstein, D.; van Eldik, R. *Chem.—Eur. J.* **2009**, *15*, 8303.

as shown (**GS-8a**) in Figure 12. The gas phase binding energy for isobutane to FeO−OH is only 5.9 kcal/mol and possibly reflects largely van der Waals interactions.

Considering the magnitude of the above FeO−OH isomerization barriers (Figure 11) for the penta aqua GS hydroperoxide **GS-8**, we were pleasantly surprised to calculate a hydrogen abstraction barrier of only 21.6 kcal/mol with the ECP basis set on Fe. The activation barrier (Figure 12) was reduced to 14.0 kcal/mol (with ZPVE) with an all-electron calculation (**TS-8a**). This striking result places the C−H bond cleavage by the developing HO• radical 29.7 kcal/mol lower than the corresponding somersault rearrangement barrier, leaving no doubt about the overall concerted nature of this hydrocarbon oxidation. The barrier is systematically lowered as the positive charge on the system increases, and the TS comes earlier along the reaction coordinate as noted by the shortening of the O−O distance from 1.901 Å in **TS-7a** to 1.683 Å in **TS-8a**. The C−H distance in the developing *tert*-butyl radical is also shorter in the latter, more highly charged TS. These data also corroborate our earlier findings in the barriers for hydrogen abstraction in P450, where we noted that the all-electron calculation using a 6-311+G(d,p) basis set on Fe (58 basis functions) instead of the ECP basis set (22 basis functions) apparently always results in lower barriers for the FeO−OH isomerization and consequently the hydrogen abstraction step, as well.

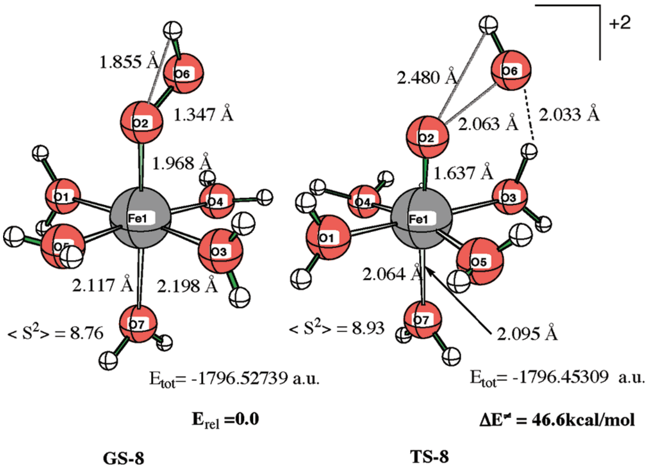


**FIGURE 11.** Reactant, transition structure, and product minimum for FeO−OH rearrangement in **GS-8** $[FeO-OH\cdot 5H_2O]^{2+}$ to produce the HO• hydrogen bonded to the ferryl oxygen in **TS-8** (all-electron geometries).

To date, no one has been able to get a Fenton-like reagent nor a heme ferryl species to react with methane at atmospheric pressure,[15] yet hydroxyl radical readily attacks it. This is one of the major inconsistencies that must be addressed by the proponents of a free hydroxyl radical as the primary oxidant in Fenton chemistry because HO• readily oxidizes methane. We have reported an activation barrier for the gas phase hydrogen abstraction by hydroxyl radical itself to be only 2.3 kcal/mol ($CH_4$ + HO• → $H_3C$• + $H_2O$).[3] Consequently, the magnitude of the activation barrier for hydrogen abstraction in **TS-8a** (16.9 kcal/mol) is not representative of a free hydroxyl radical but rather one that is tempered in its reactivity by being bound to its ferryl precursor in this concerted TS for hydrogen abstraction. Out data are therefore consistent with experiment where the barrier for the oxidation step in the Fenton oxidation of cyclohexane was estimated to be 14 kcal/mol.[15] The corresponding activation barrier for the all-electron oxidation of methane with monocation $[HO-Fe^{III}O-OH\cdot 4H_2O]^+$ is 39.9 kcal/mol (36.1 with ZPVE). Thus, it is not surprising that the oxidation of methane has never been accomplished at atmospheric pressure under classical Fenton reaction conditions.[14]

## Summary

Several important reactivity trends for these iron(III) hydroperoxides are in evidence. As the positive charge on the solvated oxidant $(H_2O)_nFe^{III}OOH)$ increases, the O−O bond dissociation energy and the somersault barrier increase, but the activation energy for the oxidation step, FeO−OH rearrangement in concert with C−H bond cleavage, decreases. The activation energy for the somersault rearrangement in monocation $[HO-Fe^{III}O-OH\cdot 4H_2O]^+$ is 17.0 kcal/mol greater than that for concerted rearrangement

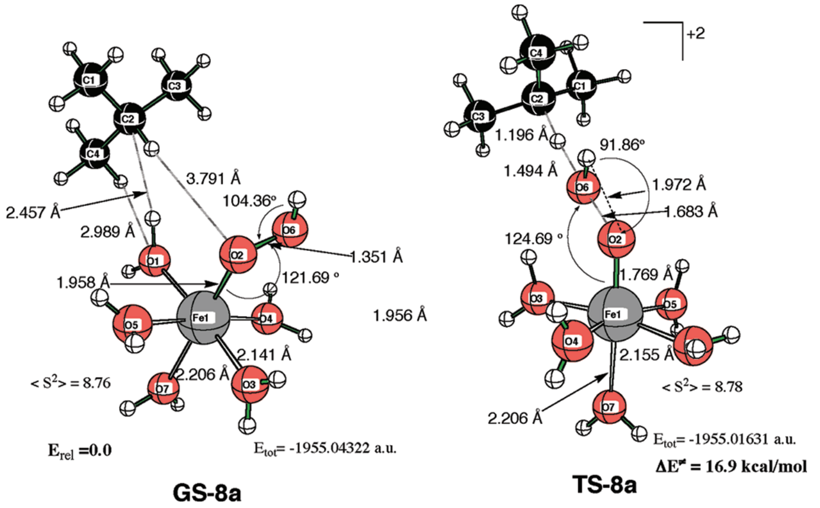
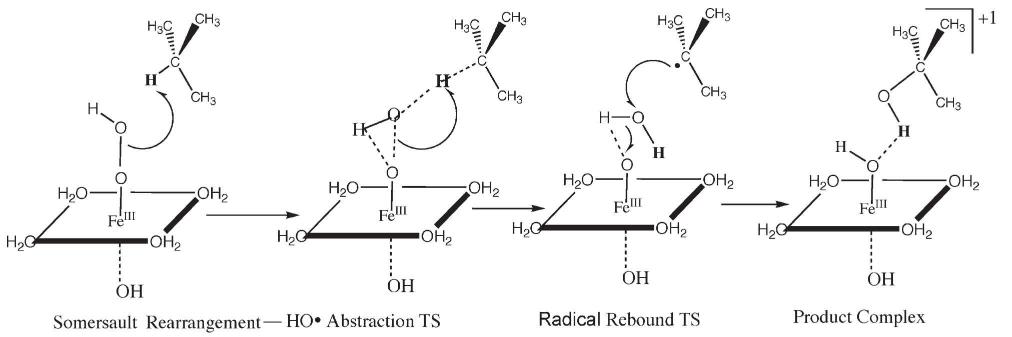


**FIGURE 12.** Transition structure (**TS-8a**) for hydrogen abstraction from isobutane in concert with FeO−OH isomerization in $(CH_3)_3C-H\cdots[FeO-OH\cdot 5H_2O]^{2+}$ reactant complex, **GS-8a** (all-electron geometries).

**SCHEME 1**



attending H-abstraction from isobutane by the developing HO• radical. This $\Delta\Delta E^{\ddagger}$ increases to 29.7 kcal/mol for concerted oxidation of isobutane by dication $[FeO{-}OH{\bullet}5H_2O]^{2+}$. Both **TS-7a** and **TS-8a** appear to be viable candidates for the rate-limiting steps in classical Fenton-type oxidation reactions. As the positive charge on the system increases, the transition state comes much earlier along the reaction pathway. Because the C−H-abstraction barriers are so much lower than their corresponding O−O isomerization barriers, these are obviously nonsynchronous concerted reactions where the GS FeO−OH forms a prereaction complex with the hydrocarbon and hydrogen abstraction occurs concomitantly with O−O bond elongation in concert with FeO−OH isomerization. We propose that the product-forming hydroxylation step (Scheme 1) is comparable to that in the P450 mechanism,[3] where we have suggested that the incipient carbon radical is hydroxylated by the weakly bound developing water molecule albeit with the *obligatory transfer of a hydrogen atom from the $H_2O$ to the ferryl oxygen accompanying C−O bond formation*. This is a highly exothermic reaction overall where the *tert*-butyl alcohol product, complexed to $[HO{-}FeOH{\cdot}4H_2O]^+$, is −100.6 kcal/mol lower in energy than **TS-7a**.

To date, we have been unable to locate a TS for the hydroxylation or C−O bond-making step with this tetra aqua system. The PES is very flat, and we cannot locate the obligatory shallow well on either side of this TS, but we were able to do so for our less exothermic P450 model.[3] The dynamics of this process may well carry the second portion of the reaction trajectory, involving C−O bond formation in product *tert*-butyl alcohol formation, through any shallow minimum that exists along the reaction pathway.

Upon the basis of these theoretical data and the close analogy to the mechanism of hydrocarbon oxidation by peroxynitrous acid[2] and P450 hydroxylation,[3] we propose that the actual oxidant in Fenton-type reactions is the developing *bound* hydroxyl radical attending the "somersault"[3,18] isomerization of the FeO−OH moiety to the inverted metastable iron(III) hydroperoxide [FeO···HO]. The somersault rearrangement of the FeO−OH group is quite general irrespective of the charge on the system. The positive charge on the oxidant may vary from one $[HO{-}Fe^{III}OOH{\cdot}4H_2O]^+$ to two $[(5H_2O)Fe^{III}OOH]^{2+}]$ depending upon the pH and the reaction media. This concerted mechanism (Scheme 1) provides a rational for why some[8] thought that a hydroxyl radical must be involved and others[9] could find no evidence that supported a HO• radical or a free carbon radical. We close with the caveat that the overall area of Fenton chemistry is so diverse that under some conditions hydroxyl radical may well be produced and this had led to this controversy.

## Computational Details

Quantum chemistry calculations were carried out using the Gaussian03 suite of programs[19] utilizing gradient geometry optimization.[20] Calculations were performed using the UB3LYP hybrid density functional[21] in combination with the Los Alamos effective core potential coupled with a double-ζ LANL2DZ basis set[22] for iron (22 basis functions) and a 6-311+G(d,p) basis set for all other atoms. The GEN (allows different user-specified basis sets for different atoms to be used) and PSEUDO=READ (requests that a model potential, LANL2DZ, be substituted for the core electrons) keywords have been used to perform these calculations in utilizing the Gaussian program. Where specified, the 6-311+G(d,p) basis set was applied additionally to the Fe atom (58 basis functions) and is described in the text as the "all-electron" basis set. All structures were fully optimized, followed by complete frequency analyses. The sextet spin state was found to be lower in energy than the quartet in every case examined.

**Acknowledgment.** This work was supported by the National Science Foundation (CHE-0138632) and by (see Supporting Information) the National Computational Science Alliance under CHE050085 and CHE050039N and utilized the NCSA IBM P690 and NCSA Xeon Linux Supercluster. GridChem is also acknowledged for computational resources (www.gridchem.org) (see ref 22).

**Supporting Information Available:** Total energies and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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