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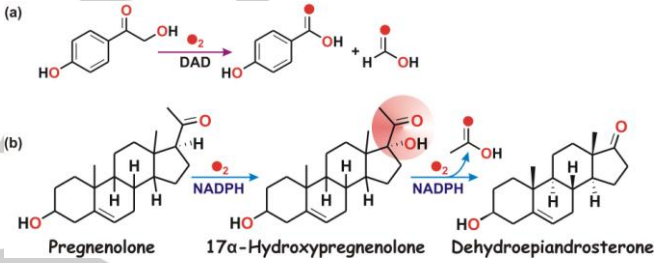
Aliphatic C-C Bond Cleavage of α-Hydroxy Ketones by a Dioxygen-Derived Nucleophilic Iron-Oxygen Oxidant

Shrabanti Bhattacharya,[a] Rubina Rahaman,[a] Sayanti Chatterjee[a] and Tapan Kanti Paine[a],*

Abstract: A nucleophilic iron-oxygen oxidant, formed in situ in the reaction between an iron(II)-benzilate complex and O_2, oxidatively cleaves the aliphatic C-C bonds of α-hydroxy ketones. In the cleavage reaction, α-hydroxy ketones without any α-C-H bond afford a 1:1 mixture of carboxylic acid and ketone. Isotope labeling studies establish that one of the oxygen atoms from dioxygen is incorporated into the carboxylic acid product. Furthermore, the iron(II) complex cleaves an aliphatic C-C bond of, 17-α-hydroxyprogesterone, affording androstenedione and acetic acid. The O_2-dependent aliphatic C-C bond cleavage of α-hydroxy ketones containing no α-C-H bond bears similarity to lyase activity of the heme enzyme, cytochrome P450 17A1 (CYP17A1).

The selective oxidative cleavage of C-C bonds are important reactions in the biodegradation of toxic compounds and biosynthesis of natural products.[1-4] These reactions are catalyzed by dioxygen-activating metalloenzymes with the incorporation of oxygen atoms from dioxygen. Based on the nature of substrates, these reactions are classified into two categories: aromatic ring cleavage and aliphatic C-C bond cleavage. The aromatic C-C bond cleavage reactions by ring-cleaving dioxygenases have been extensively studied over the last several decades.[1, 5-7] These studies provided useful mechanistic insights into the role of metal ion and ligand environment in controlling the selectivity of ring fission reactions. On the contrary, the aliphatic C-C bond cleaving oxygenases have received less attention.[2, 8] The aliphatic C-C bond cleaving oxygenases are structurally diverse and act on a wide range of substrates.[9] Moreover, structurally and functionally distinct enzymes cleave the aliphatic C-C bonds of similar type of substrates. One such example is the oxidative aliphatic C-C bond cleavage of α-hydroxy ketones. 2,4'-Dihydroxyacetophenone dioxygenase (DAD),[10, 11] a bacterial nonheme iron enzyme involved in the catabolism of 4-hydroxyacetophenone, catalyzes the oxygenative cleavage of 2,4'-dihydroxyacetophenone into 4-hydroxy benzoate and formate (Scheme 1a). On the other hand, the heme enzyme cytochrome P450 17A1 (CYP17A1),[12-14] cleaves the C17–C20 bond (lyase) of 17α-hydroxypregnenolone in the biosynthesis of steroid hormone, dehydroepiandrosterone from pregnenolone (Scheme 1b).[15] Initial binding of the α-hydroxy ketone prior to dioxygen activation has been proposed in the oxidative cleavage reaction by DAD.[16] For CYP17A1,[3, 17-22] an iron-peroxide species is implicated to initiate the lyase reaction. Only very recently, the proposed intermediate in the lyase step has been trapped and spectroscopically characterized as 'iron(III)-peroxo-hemiacetal' species.[23] Although both the enzymes cleave the C-C bond of α-hydroxy ketones, the substrates are different. DAD acts on a α-hydroxy ketone with α-C-H bond, whereas the α-hydroxy ketone unit in 17α-hydroxypregnenolone does not contain any α-C-H bond. Thus the cleavage pathways are expected to be different in the two cases.



Scheme 1. Aliphatic C-C bond cleavage reactions catalyzed by (a) 2,4'-dihydroxyacetophenone dioxygenase (DAD) and (b) cytochrome P450 17A1 (CYP17A1).

Synthetic model complexes provide useful insights into the mechanistic aspects of aliphatic C-C bond cleavage reactions.[2, 24] We recently reported the C-C bond cleavage of α-hydroxy ketones containing α-H atom by iron(II) complexes of nitrogen donor polydentate ligands.[25, 26]. But the iron(II) complexes were unable to cleave the C-C bond of the α-hydroxy ketones without α-C-H bond. Since a nucleophilic iron(III)-hydroperoxide species has been invoked as a reactive intermediate in the C-C bond cleavage reaction by CYP17A1, metal based nucleophilic iron-oxygen species is expected to cleave the C-C bond of α-hydroxy ketones. In this direction, we have investigated the reactivity of an iron(II)-benzilate complex, [(Tp^{Ph2})Fe^{II}(benzilate)] (**1**)[27] supported by a facial N_3 ligand (Scheme 2), toward various α-hydroxy ketones as model substrates. As an outcome of our investigation, we present herein the C-C bond cleavage of α-hydroxy ketones with dioxygen by complex **1** and a mechanism of the oxidative transformation reaction.

The iron(II)-benzilate complex [(Tp^{Ph2})Fe^{II}(benzilate)] (**1**) reductively activates dioxygen and performs a number of bioinspired oxidations.[27-30] A putative iron(II)-hydroperoxide species, intercepted by external substrates, has been proposed as the active oxidant. Since the iron-oxygen oxidant from **1** has been reported to exhibit nucleophilic reactivity, the oxidant is likely to react with electrophilic carbonyl compounds. Although ketones are not oxidized by complex **1**, aldehydes are converted to mixtures of carboxylic acids and alcohols (Experimental Section and Table S1 in the Supporting Information, SI). In the reaction with substituted benzaldehydes, the yields of the

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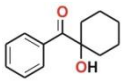
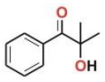
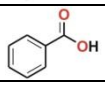
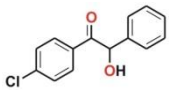
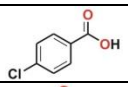
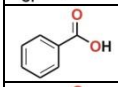
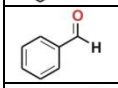
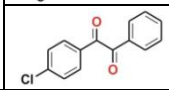
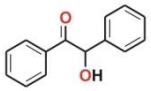
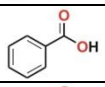
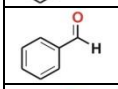
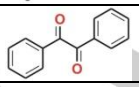
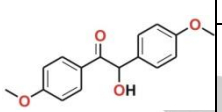
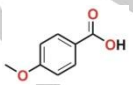
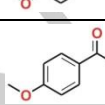
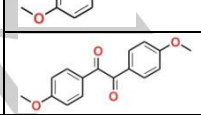
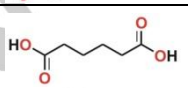
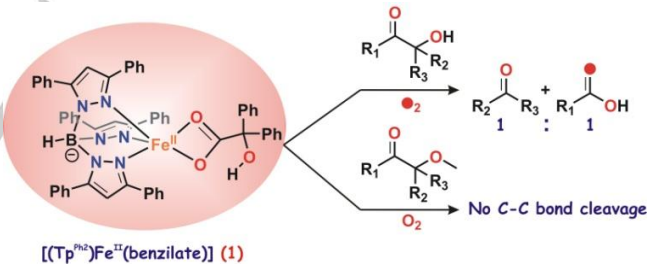
corresponding carboxylic acids are higher than the alcohols suggest that two different pathways are operational. The nucleophilic oxidant directly oxidizes benzaldehydes to benzoic acid, and in another pathway, benzaldehyde is converted to equimolar amounts of benzyl alcohol and benzoic acid via Cannizzaro mechanism.[28] The yield of the major product, benzoic acid was used to obtain the relative rates for the competitive oxidations of pairs of aldehydes. Hammett analysis on 1:1 mixtures of benzaldehyde and *para*-substituted benzaldehydes (*p*-X-C_6H_4CHO, where X = -NO_2, -Br, -Me, -OMe) gives a ρ value of +0.82 (Figure S1). Thus the iron-oxygen species from **1** is a nucleophilic oxidant, which oxidizes aldehyde substrates.

Table 1. Aliphatic C-C bond cleavage products of different α-hydroxy ketones in the reaction with complex **1** and dioxygen.

α-Hydroxy ketone(s)	Product(s)	% Yield/% Intra-ligand hydroxylation
		50(±3)/35
		50(±3)
		58(±2)/27
		60(±2)
		91(±3)/43
		80(±3)
		8(±3)
		6(±3)
		75(±2)/46
		10(±2)
		12(±3)
		64(±3)/53
		8(±2)
		14(±3)
		68(±3)/30

The reactivity of **1** was then tested toward different α-hydroxy ketones. Two different types of α-hydroxy ketones were investigated: one without α-C-H bond, and the other with α-C-H bond (Table 1). The complex oxidatively cleaves the C-C bond of α-hydroxy ketones and the metal-coordinated benzilate undergoes two-electron oxidation to form benzophenone quantitatively. The reaction of **1** with 1-hydroxycyclohexyl phenyl ketone (1 equiv) affords benzoic acid and cyclohexanone with 50% yield (Figures S2 and S3). It is known that complex **1** reacts with O_2 to undergo intramolecular ligand hydroxylation (90%) in the absence of any substrate.[28] In the reaction with 1-hydroxycyclohexyl phenyl ketone, 35% ligand hydroxylation is estimated (Figure S4). Therefore, 50% active oxidant is involved in the C-C bond cleavage reaction, and the rest of the oxidant hydroxylates the *ortho* position of one of the phenyl rings of Tp^{Ph2} ligand. Thus intra-ligand hydroxylation causes low yields of the C-C cleavage products. When 2-hydroxy-2-methylpropiophenone is used as a substrate, benzoic acid and acetone are obtained in 58(±2)% and 60(±2)% yield, respectively along with 27% ligand hydroxylation (Figures S5-S7). No appreciable change in product yield is observed upon increasing the concentration of substrate.

The reaction between **1** and 1-hydroxycyclohexyl phenyl ketone when carried out with $^{18}O_2$, (methyl) benzoate derived from the substrate displays an ion peak at *m/z* = 138, which is two mass unit higher than that obtained in the reaction with O_2. This supports the incorporation of one oxygen atom of O_2 into the product (Figure S8). The other product cyclohexanone, however, does not contain any labeled oxygen from $^{18}O_2$. In a mixed labeling experiment with $^{16}O_2$ and $H_2{}^{18}O$, no incorporation of labeled oxygen is observed into the product.



Scheme 2. Oxidative C-C bond cleavage of α-hydroxy ketones by complex **1**.

To check the importance of the O-H group in the C-C bond cleavage pathway, reaction of **1** with 2-methoxy-2-methylpropiophenone was carried out under oxygen atmosphere (Scheme 2, Experimental Section in SI). In the reaction, the methoxy derivative of 2-hydroxy-2-methylpropiophenone remains unreacted without any detectable C-C bond cleavage product. When a natural substrate of CYP17A1, 17-α-hydroxyprogesterone is used, the C-C bond cleavage of the α-hydroxy ketone moiety takes place to an extent of 10% forming androstenedione and acetic acid (Figure 1 and Scheme 3). A labeling experiment with $^{18}O_2$ reveals that acetic acid contains one labeled oxygen atom while androstenedione does not contain any labeled oxygen product (Figure 1).

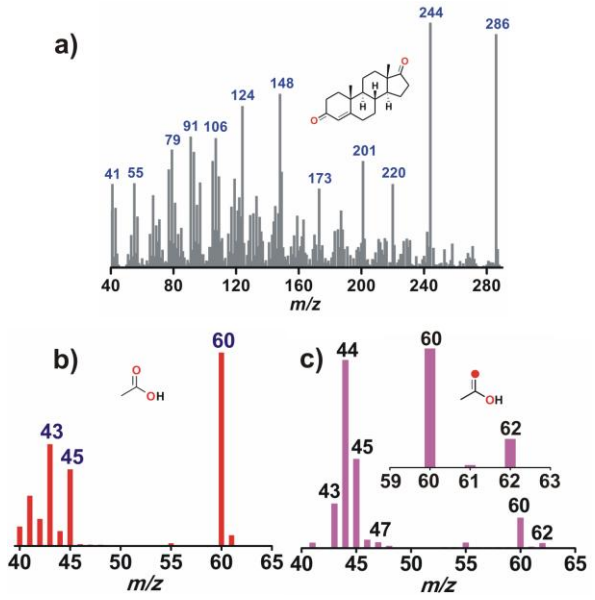
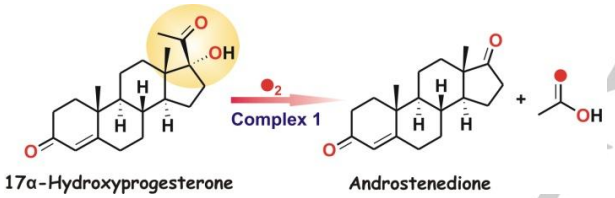
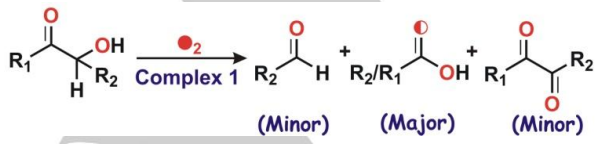


Figure 1. GC-mass spectra of (a) androstenedione, and of acetic acid formed in the reaction between **1** and 17α-hydroxyprogesterone with (b) $^{16}O_2$ and (c) $^{18}O_2$. Inset c: molecular ion peak for acetic acid at *m/z* = 60 (^{16}O) and 62 (^{18}O).



Scheme 3. Layase of the C17-C20 bond of 17-α-hydroxyprogesterone by complex **1**.

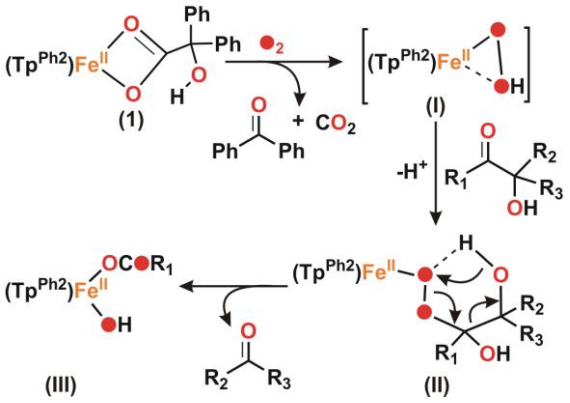
On the contrary, the α-hydroxy ketones with α-C-H bond afford the corresponding acids as the major cleavage products, whereas aldehydes are obtained in trace amounts (Scheme 4). The reaction of **1** with benzoin (1 equiv) yields 75(±2)% benzoic acid, 10(±2)% benzaldehyde and 12(±3)% benzil (Table 1, Figure S9). 1-(4-Chlorophenyl)-2-hydroxy-2-phenylethanone forms a mixture of 4-chlorobenzoic acid 91(±3)%, benzaldehyde 8(±3)%, benzoic acid 80(±3)%, and 4-chlorobenzil 6(±3)% (Figure S10). 4,4'-Dimethoxy benzoin is converted to 4-methoxybenzoic acid 64(±3)%, 4-methoxybenzaldehyde 8(±2)% and 4,4'-dimethoxybenzil 14(±3)% (Figure S11). In all these reactions, intramolecular ligand hydroxylation is also observed (Figure S12). When 2-hydroxycyclohexanone is made to react with complex **1** and dioxygen, adipic acid is obtained as the sole product (Figure S13).



Scheme 4. C-C bond cleavage of α-hydroxy ketones containing α-C-H bond.

To understand the difference in C-C bond cleavage reactivity of two different types of α-hydroxy ketones, time-dependent ^{1}H NMR experiments were carried out for the reaction between **1** and benzoin (Figure S14). In the reaction, the yield of benzoic acid increases with time, whereas that of benzaldehyde remains constant after 3 min. Thereafter benzoin is linearly converted to benzoic acid. Thus benzoic acid is not derived from benzaldehyde via oxidation by a metal-oxygen species. For unsymmetrical substrate 4-chlorobenzoin, the yield of 4-chlorobenzoic acid is slightly (8-11%) higher than that of benzoic acid. While benzaldehyde is detected as a minor product, 4-chlorobenzaldehyde is not observed in the reaction. Therefore benzaldehyde and 4-chlorobenzoic acid are formed (with about 8% yield for each product) via a pathway similar to that for the α-hydroxy ketones without α-C-H bond. The labeling experiment with $^{18}O_2$ for the reaction of **1** with 4-chlorobenzoin reveals partial incorporation of labeled oxygen into 4-chlorobenzoic acid and benzoic acid, whereas benzaldehyde does not contain any labeled oxygen (Figure S15). The partial incorporation of labeled oxygen atom into carboxylic acid products suggests that a metal−oxygen intermediate species exchanges its oxygen atom with water in the reaction pathway.[26] A mixed labeling experiment with $^{16}O_2$ and $H_2{}^{18}O$ for the reaction between **1** and 4-chlorobenzoin further supports that labeled oxygen atom from water is incorporated both into benzoic acid and 4-chlorobenzoic acid (Figure S16).

All these observations indicate that the C-C bond cleavage reaction of benzoin (or substituted benzoin) by complex **1** takes place via two different pathways. In one pathway, it forms a 1:1 mixture of benzoic acid and benzaldehyde and in other pathway benzoin forms two equivalents of carboxylic acids. With benzoin-type substrates, only 8-10% reaction takes place in the former pathway. For cyclic substrate, 2-hydroxycyclohexanone, only the second pathway is followed to afford the dicarboxylic acid.



Scheme 5. Proposed mechanism for the oxidative C-C bond cleavage of α-hydroxy ketones with dioxygen by a biomimetic iron(II)-benzilate complex.

With the results discussed above, a mechanistic proposal is put forward in Scheme 5. The reaction of complex **1** with dioxygen proceeds via the formation of an iron(II)-hydroperoxide (**I**) species.[28] In the enzymatic system, it has been shown that the hydroxy group of substrate and the oxygen atoms of peroxide could be responsible in initiating the aliphatic C-C

bond cleavage.[23] The experimental result from the reaction of **1** with O_2 and the methoxy derivative of α-hydroxy ketone strongly supports the role of hydroxy group. In analogy to the "peroxo-hemiacetal" intermediate observed in CYP17A1,[23] an iron(II)-alkylperoxo species (**II**) is thus proposed to form upon nucleophilic iron(II)-hydroperoxide to the electrophilic carbonyl carbon of α-hydroxy ketone. Intermediate **II** further undergoes C-C bond cleavage to yield an iron(II)-benzoate-hydroxide species (**III**) and ketone. For benzoin-type substrates, the minor pathway involves the above mechanism. The major pathway, which forms two equivalents of carboxylic acid with incorporation of oxygen atom from water, involves the formation of iron(II)-α-hydroxy ketone complex after replacing the hydroperoxide group of intermediate **I** (Scheme S1). Benzoin is likely to undergo enolization and subsequent formation of a planar chelate ring at the iron(II) center is a driving force to replace hydroperoxide. Detection of hydrogen peroxide in the reaction of **1** and O_2 with benzoin supports this hypothesis (Experimental section, SI). The substrate coordinated species (**II'**) then reacts with dioxygen to afford the cleavage products following a mechanism (Scheme S1) similar to that reported recently by us.[26] The resulting iron(II)-benzoate product rapidly gets oxidized to form iron(III)-phenolate-benzoate complex. The above mechanism explains the formation of two equivalents of carboxylic acid as well as the formation of diketone.

In conclusion, the reactivity of a nucleophilic iron-oxygen oxidant from an iron(II)-benzilate complex toward different α-hydroxy ketones has been investigated. Hammett analysis and interception studies with external substrates suggest formation of a putative iron(II)-hydroperoxide species. The oxidant cleaves the aliphatic C-C bonds of α-hydroxy ketones to afford carboxylic acids and ketones. Isotope labeling studies establish that that one of the oxygen atoms from dioxygen is incorporated into carboxylic acid. Furthermore, the iron(II) complex cleaves the C17-C20 bond of 17-α-hydroxyprogesterone affording androstenedione and acetic acid. Although the coordination environment and spin state of iron-oxygen oxidant are different than those in heme systems, the iron complex discussed here shows reactive similarities to the lyase activity of cytochrome P450 17A1 (CYP17A1). Additionally, this work demonstrates that the O_2-dependent aliphatic C-C bond cleavage pathway depends on the nature of substrate.

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Keywords: iron • dioxygen • nucleophilic oxidant • C-C bond cleavage • α-hydroxy ketone

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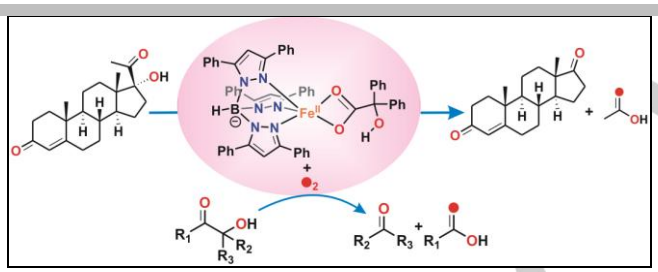
Cleavage of Aliphatic C-C Bonds. A nucleophilic iron-oxygen oxidant from an iron(II)-benzilate complex cleaves the aliphatic C-C bonds of α-hydroxy ketones including the C17-C20 lyase of 17-α-hydroxyprogesterone.



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Aliphatic C-C Bor Cleavage of α-Hydrox Ketones by a Dioxyge Derived Nucleophil Iron-Oxygen Oxidant