Iron Catalysis

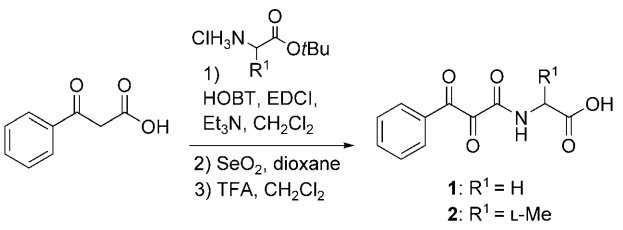
DOI: 10.1002/ange.200806296

Iron-Mediated Cleavage of C–C Bonds in Vicinal Tricarbonyl Compounds in Water**

*Jasmin Mecinović, Refaat B. Hamed, and Christopher J. Schofield**

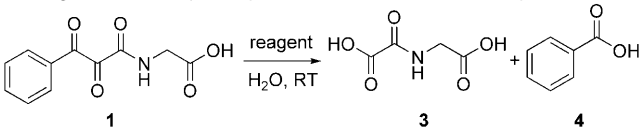
Iron is the second most abundant metal in the Earth's crust and plays an essential role in biology. Iron-containing proteins are involved in biological processes including the modification of nucleic acids, proteins, carbohydrates, and lipids; oxygen transport and sensing; small-molecule metabolism; and electron transfer.[1] Iron and its complexes are also widely used in organic synthesis, for example as catalysts for oxidation, reduction, and coupling reactions.[2–5] Although there are reports of the use of iron salts and complexes for C–C/O/N/S bond formation, these reactions typically require organic solvents and relatively harsh reaction conditions. There are few examples of iron-catalyzed C–C[6–9] and C–O[10] bond-cleavage reactions and, to our knowledge, there are no reported efficient C–C bond-cleavage reactions catalyzed by iron that occur in aqueous solutions in the absence of added ligands/catalysts. Herein, we report the unexpected finding that ferric ions catalyze the cleavage of C–C bonds of vicinal tricarbonyl compounds in water at room temperature in the absence of added ligands.

During our studies on the evaluation of tricarbonyl compounds as inhibitors of a human oxygen-sensing enzyme (prolyl hydroxylase domain 2 (PHD2))[11,12] we found that tricarbonyl compounds **1** and **2** underwent reaction (Scheme 1; Figure S1 in the Supporting Information). ^{1}H NMR spectroscopy (700 MHz) and LC-MS analysis indicated that, in the presence of PHD2 and Fe^{II} (PHD2/Fe^{II}/**1** 1:1:5) **1** reacted to form *N*-oxalylglycine (NOG **3**) and benzoic acid (**4**). Unexpectedly, we found that **3** and **4** (Figure S2 in the Supporting Information) also formed in the absence of PHD2 in an iron-dependent manner (Table 1). When Fe^{III} was substituted for Fe^{II}, it also catalyzed cleavage of **1** (both at unbuffered pH 4 and buffered pH 7; Figure 1) under aerobic conditions. However, when **1** was treated with Fe^{II} at pH 3, little fragmentation was observed (< 5% conversion, 14 h), which is consistent with the slower oxidation of Fe^{II} to Fe^{III} at low pH (Figure S3 in the Supporting Information). Similarly, when Fe^{II} was used in the presence of a 50-fold excess of reducing agent (sodium ascorbate or potassium dithionate), no reaction (< 5% conversion) of **1** took place. Under anaerobic conditions, cleavage of the C–C bond was observed in the presence of Fe^{III}, but not in the presence of Fe^{II}. Taken together, these observations led us to conclude that the active species is Fe^{III} rather than Fe^{II}.



Scheme 1. Synthesis of the tricarbonyls **1** and **2**. EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBT = 1-hydroxy-1*H*-benzotriazole, TFA = trifluoroacetic acid.

Table 1: Screening metal ions for their potential to mediate oxidative cleavage of tricarbonyl compound **1** in water at room temperature.[a]



Entry	Reagent (equiv)	*t* [h]	Conversion [%][b]
1	$FeSO_4$ (2)	2	100
2	$K_4[Fe(CN)_6]$ (2)	5	n.d.
3	$Fe_2(SO_4)_3$ (1)	2	100
4	$Fe_2(SO_4)_3$ (0.1)	9	70
5	$FeCl_3$ (2)	2	100
6	$Fe(NO_3)_3$ (2)	2	100
7	$K_3[Fe(CN)_6]$ (2)	24	traces
8	$CoCl_2$ (2)	5	n.d.
9	$MnSO_4$ (2)	5	traces
10	$NiSO_4$ (2)	5	n.d.
11	$CuSO_4$ (2)	5	n.d.
12	$ZnSO_4$ (2)	5	n.d.
13	$RuCl_3$ (2)	5	n.d.
14	$PdCl_2$ (2)	5	traces
15	$AgNO_3$ (2)	5	n.d.
16	$PtCl_2$ (2)	5	n.d.
17	$Hg(OAc)_2$ (2)	5	n.d.
18	$Ce(SO_4)_2$ (2)	5	n.d.
19	$KMnO_4$ (1)	1.5	100
20	$KRuO_4$ (1)	5 min	100
21	$NaIO_4$ (1)	24	88

[a] Reaction conditions: **1** (500 μM), reagent (metal ion 1 mM) in H_2O, 25 °C. [b] Conversion was determined by ^{1}H NMR spectroscopy and LC-MS analysis. n.d. = not detected.

[*] J. Mecinović, R. B. Hamed, Prof. Dr. C. J. Schofield
Chemistry Research Laboratory
Department of Chemistry, University of Oxford
Mansfield Road, Oxford, OX1 3TA (United Kingdom)
Fax: (+44) 1865-275-674
E-mail: christopher.schofield@chem.ox.ac.uk
Homepage: http://www.chem.ox.ac.uk/researchguide/cjschofield.html

[**] We thank the Newton–Abraham Fund (J.M.), the Ministry of Higher Education, Egypt (R.B.H.), the Biotechnology and Biological Research Council, the European Union, and the Wellcome Trust for funding. We also thank Dr. John M. Brown for helpful discussions on mechanisms.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200806296.

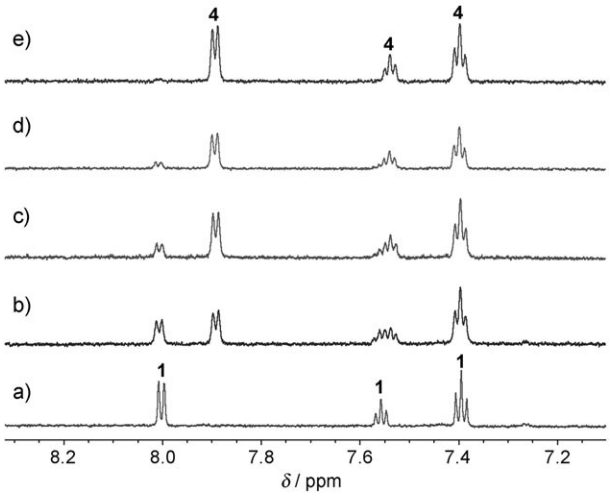


Figure 1. ^{1}H NMR spectra of the iron(III)-mediated cleavage of **1** to give benzoic acid (**4**). Reaction of **1** in the presence of 2 equivalents of Fe^{III} in water at room temperature; a) before the reaction and after reaction times of b) 15 min, c) 45 min, d) 90 min, and e) 2 h.

We investigated the cleavage of **1** to give **3** and **4** in the presence of other first-row transition-metal ions (Table 1). Only iron was found to catalyze the cleavage of **1** in water at room temperature under aerobic conditions. In contrast to $Fe_2(SO_4)_3$, $FeCl_3$, and $Fe(NO_3)_3$ (Table 1, entries 1 and 3–6), $K_3[Fe(CN)_6]$ (Table 1, entry 7) did not catalyze the cleavage of **1**. Under the standard reaction conditions, Ru^{III}, Ag^{I}, Hg^{II}, Pd^{II}, Pt^{II}, and Ce^{IV} reagents did not mediate the cleavage of **1** (< 5% conversion; 5 h, room temperature; Table 1, entries 13–18). Cleavage of **1** was mediated by the strong oxidizing agents $KMnO_4$ (Figure S4 in the Supporting Information), $KRuO_4$, and $NaIO_4$ (Table 1, entries 19–21).

Cleavage of **1** by Fe^{III} also occurred in methanol. ^{1}H NMR spectroscopy and LC-MS analysis (2 equiv of Fe^{III}, CD_3OD) revealed the products to be the *N*-oxalylglycine C1-methyl ester (**5**) and methyl benzoate (≈80% in 3 h; Figure S5 in the Supporting Information).

Other vicinal tricarbonyl compounds[13,14] were then tested to see if they underwent cleavage under the standard conditions. (Unless stated otherwise, the reaction conditions were 2 equivalents of Fe^{III} in H_2O at room temperature; Table 2.) Analogues of **1** (**6** and **7**; Table 2, entries 1 and 2; Figure S6 in the Supporting Information) that lacked a carboxylic acid side chain were cleaved similarly to **1**, thereby excluding the possibility that chelation of iron by a carboxylic acid side chain plays essential role in the cleavage process.

Ethyl 2,3-dioxo-3-phenylpropanoate **8** reacted to form benzoic acid (**4**) and ethanol (Table 2, entries 3; as monitored by ^{1}H NMR spectroscopy, Figure S7 in the Supporting Information). LC-MS analysis revealed oxalate to be the other product (an α-ketoacid intermediate was not observed). In contrast to the cleavage of **1**, which was complete in 2 h, reaction of **8** required 9 h, possibly because of iron chelation by the oxalate product. Cleavage of diphenylpropanetrione **9** resulted in the formation of **4** and benzoylformic acid (Table 2, entry 4). ^{1}H NMR spectroscopy and LC-MS analysis revealed that **10** is cleaved at both of the two potentially labile C–C bonds, to give **4** and benzoylformic acid in a ratio of approximately 6:4 (Table 2, entry 5; Figure S8 in the Supporting Information).

Diketones were then screened for potential C–C fragmentation. The analogue of **1** that lacks a carbonyl group at C2 (**11**), the 1,3-dione **12** (which can potentially chelate iron through two oxygen atoms to form a six-membered ring), and diethyl oxomalonate **13** were not cleaved (Table 2, entries 6–8). The need for a tricarbonyl-containing substrate was supported by the lack of conversion observed for the vicinal dicarbonyl compounds benzil (**14**) and 2,2′-pyridyl (**15**; Table 2, entries 9 and 10). Importantly, the dicarbonyl compounds phenylglyoxal (**16**) and the trifluoromethyl derivative **17**, both existing predominantly in their hydrated forms in water, were not fragmented (Table 2, entries 11 and 12). Also, the reaction of 2-oxoglutaric acid (**18**) in the presence of 2 equivalents of Fe^{III} did not result in cleavage (Table 2, entry 13). Overall, these results imply that the reaction requires a tricarbonyl motif with a vicinal dicarbonyl arrangement; the third carbonyl group can come from an ester, amide, or ketone functionality.

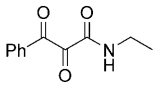
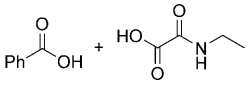
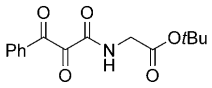
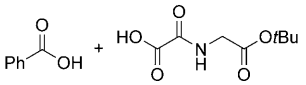
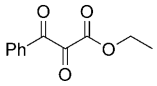
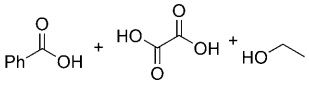
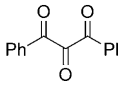
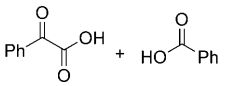
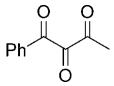
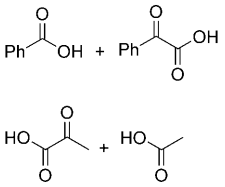
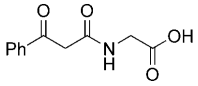
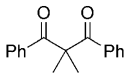
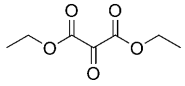
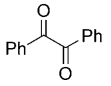
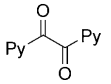
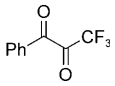
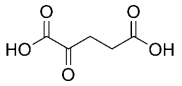
Next we studied ninhydrin and dehydro-L-ascorbate (**21**), which are tricarbonyl compounds of biological interest. Ninhydrin, which is widely used for the detection of amino acids,[15] was cleaved to give α-ketoacid **19** and phthalic acid (**20**); substituting Fe^{III} with $KMnO_4$ or $KRuO_4$ also gave **19** and **20** (Scheme 2; Figures S9 and S10 in the Supporting Information). Consistent with the observed iron(III)-catalyzed fragmentation of ninhydrin, D'Aniello et al. have reported that the standard ninhydrin test for amino acids fails in the presence of Fe^{III}.[16]

Dehydro-L-ascorbate (**21**), the oxidized form of L-ascorbic acid (**22**; vitamin C) is probably the most important naturally occurring tricarbonyl-containing compound (Scheme 3). A biologically and commercially important reducing agent, **22** is degraded by both non-oxidative and oxidative pathways—potentially via more than 100 different intermediates.[17,18] Oxidative degradation of **22** proceeds via **21**,[19,20] which can undergo further oxidation and hydrolytic lactone ring-opening to L-threonic acid (**23**) and oxalic acid (**24**).[21]

Compound **21** was cleaved to give **23** and **24** under standard reaction conditions after 18 h. However, the cleavage did not proceed in the presence of other noncomplexed biologically relevant transition-metal ions (Zn^{II}, Cu^{II}, Co^{II}, Ni^{II}, Mn^{II}), even at higher temperatures (37 °C, 50 °C; Figure S11 in the Supporting Information). As compounds **21**–**24** had similar LC retention times, 5,6-isopropylidene-L-ascorbic acid was used in further studies. 5,6-Isopropylidene-L-ascorbic acid was oxidized to 5,6-isopropylidene-L-dehydroascorbate when 4 equivalents of Fe^{III} was used, and cleavage occurred to give 3,4-isopropylidene-L-threonic acid and **24** (ca. 50% conversion after 6 h; Figure S12 in the Supporting Information).

Despite the biological importance of ascorbate, pathways for its degradation are not fully elucidated. In some plants L-ascorbic acid (**22**) is degraded to L-tartrate via L-idonate. However, in most plants, degradation occurs via **21** to yield **23** and **24**. Recent work has shown that the pathway occurs in plant extracts and involves both enzymatic and nonenzymatic

Table 2: Scope of the iron-catalyzed cleavage of carbonyl compounds in water at room temperature.

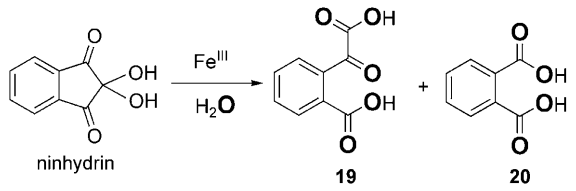
Entry		Starting material	Products	*t* [h]	Conversion [%][a]
1	**6**			2	100
2	**7**			2	100
3	**8**			9	100
4	**9**			2	100
5	**10**			2	100
6	**11**		–	5	n.d.
7	**12**		–	5	n.d.
8	**13**		–	5	n.d.
9	**14**		–	5	n.d.
10	**15**		–	24	n.d.
11	**16**[b]		–	24	n.d.
12	**17**[b]		–	5	n.d.
13	**18**		–	24	n.d.

[a] Conversion was determined by ^{1}H NMR spectroscopy and LC-MS analysis. [b] Shown to be predominantly hydrated in water.

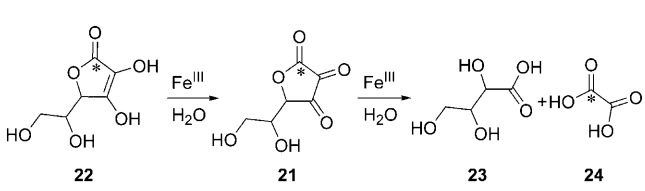
steps.[21,22] Interestingly, the in vivo labeling studies showed that C1 of **22** is incorporated into **24**.[21] Our work has implications in this regard as the demonstration that Fe^{III} can catalyze the conversion of **21** into **23** and **24** reveals the potential for non-enzymatic iron(III)-catalyzed degradation of **22** (there is also evidence for oxygenase involvement in vivo[23]), and may in part account for loss of vitamin C in food preparation.

To investigate the mechanism of cleavage of the tricarbonyl compound, we then performed labeling experiments. Consistent with the observed cleavage under anaerobic conditions, when **1** was reacted in the presence of Fe^{III} in H_2O under $^{18}O_2$, no (<5%) ^{18}O was incorporated into either benzoic acid (**4**) or NOG **3** according to LC-MS analysis. In contrast, when the reaction was performed in $H_2{}^{18}O$, two ^{18}O atoms were incorporated into both **4** and **3** (Figure 2). When **1** was reacted without Fe^{III} in $H_2{}^{18}O$, only one ^{18}O atom was incorporated, likely into its central carbonyl group, which is consistent with the observation that the central keto group of tricarbonyl compounds exists predominantly in a hydrated form in water (Figure S13 in the Supporting Information);[13] ^{18}O incorporation into either **4** or **3** was not observed in $H_2{}^{18}O$.

When ninhydrin was treated with Fe^{III} in $H_2{}^{18}O$, five ^{18}O atoms were incorporated into α-keto acid **19** and four ^{18}O atoms into phthalic acid (**20**; Figure S14 in the Supporting Information). With dehydro-L-ascorbic acid (**21**), the ^{18}O labeling experiments led to the incorporation of two ^{18}O atoms from $H_2{}^{18}O$ into threonic acid (**23**; Figure S15 in



Scheme 2. C–C cleavage of ninhydrin (2 equiv of Fe^{III}) to give α-keto acid **19** and phthalic acid (**20**). The major products of the reaction in $H_2{}^{18}O$ are shown.



Scheme 3. Iron(III)-mediated cleavage of the C–C bond in dehydro-L-ascorbate (**21**) to give threonic acid (**23**) and oxalate **24**. The result of a ^{13}C labeling experiment is shown.

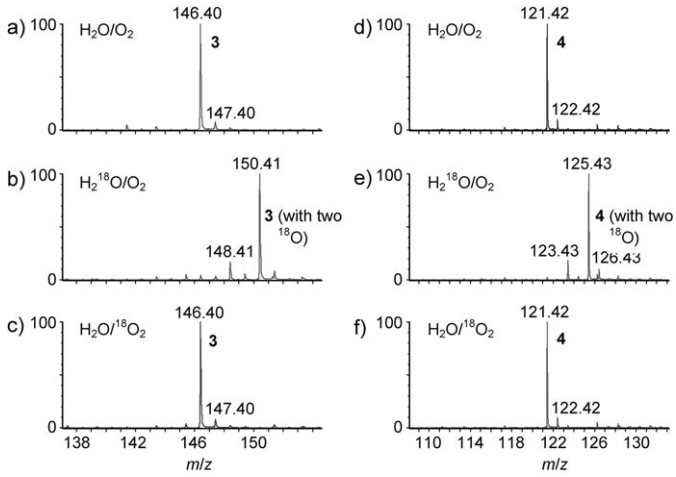
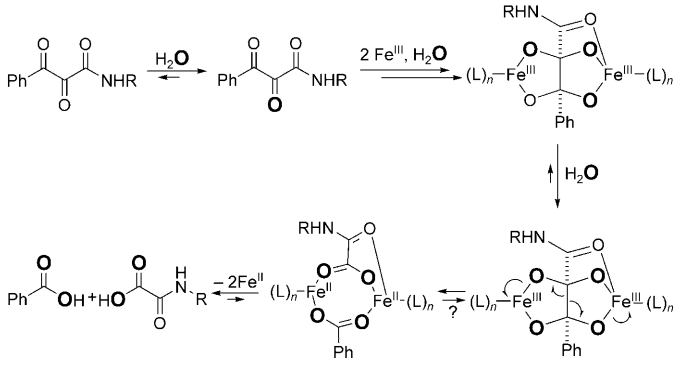


Figure 2. Mass spectra from the oxygen incorporation studies for the cleavage reaction of **1** (2 equiv of Fe^{III} for 90 min). The two oxygen atoms, incorporated into NOG **3** (left) and benzoic acid (**4**; right) come from water; a) non-labeled; b) only $H_2{}^{18}O$ was labeled; c) only $^{18}O_2$ was labeled; d) non-labeled, e) only $H_2{}^{18}O$ was labeled, f) only $^{18}O_2$ was labeled.

the Supporting Information). The reaction of [^{13}C-1]-labeled L-ascorbic acid with Fe^{III} revealed ^{13}C incorporation into **24**, but not into **23** (Scheme 3; Figure S16 in the Supporting Information).

The overall process for the fragmentation of tricarbonyl compounds involves a two-electron oxidation; that is, it requires reduction of two Fe^{III} to two Fe^{II} ions. Any proposed mechanisms must account for the labeling results, the requirement of a tricarbonyl moiety, the range of substrates, and the likely involvement of a dihydrated intermediate. Tricarbonyl compounds can potentially form a six-membered chelate ring involving their C1 and C3 carbonyl groups, form a five-membered ring, or chelate through all three carbonyl groups. We propose a "minimal" mechanism involving the initial formation of a dihydrated tricarbonyl compound, which complexes with two Fe^{III} ions (Scheme 4), one of which is complexed by the "third" carbonyl oxygen. Concomitant with C–C cleavage, each of the ferric ions can then accept an electron to give a complex formed from two ferrous ions, benzoic acid, and an α-keto acid (it is also possible that the



Scheme 4. A proposed mechanism for the iron(III)-catalyzed cleavage of tricarbonyl compounds.

reaction involves a complex stoichiometry of two Fe^{III} ions and two tricarbonyl compounds with a single C–C cleavage occurring per cycle).

Overall, we have shown that aqueous ferric ions in the absence of added ligands mediate the cleavage of vicinal tricarbonyl compounds. The reaction has relevance for the detection of amino acids by ninhydrin and for the metabolism of vitamin C. We note that if the reverse reaction; that is, ligation of a 2-oxocarboxylate and a carboxylate to give a carbonyl compound, could be achieved under mild aqueous conditions it would be of interest—perhaps even from a prebiotic perspective. Such a process may have precedent in the acyloin reaction, which requires much harsher non-aqueous conditions.[24]

Experimental Section

Solutions of tricarbonyls/dicarbonyls (10 mM, deionized water) and ferric sulfate (10 mM, deionized water) were mixed in a 1:1 ratio (final concentration of tricarbonyl/dicarbonyl 500 μM, Fe^{III} 1 mM, pH 4) and then reacted at room temperature prior to LC-MS analysis; samples were analyzed by using a Waters LCT Classic MS Machine). For ^{1}H NMR analyses samples were prepared in the same manner as for LC-MS, except that ^2H_2O was used as the solvent; samples were analyzed by using a Bruker AVIII 700 MHz spectrometer with an ^{1}H inverse TCI cryoprobe. For ^{18}O labeling experiments, reactions were carried out under the standard reaction conditions, except that $^{18}O_2$ or $H_2{}^{18}O$ were used. Anaerobic experiments were carried out in a Belle Technology glove box (< 0.1 ppm O_2).

Received: December 23, 2008
Published online: March 11, 2009

Keywords: C–C cleavage · homogeneous catalysis · iron · redox chemistry · tricarbonyl compounds

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