Origin of the Oxygen Atom in C−H Bond Oxidations Catalyzed by a Water-Soluble Metalloporphyrin

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The monopersulfate oxidation of 4-isopropylbenzoic acid performed in $H_2{}^{18}O$ and catalyzed by a water-soluble metalloporphyrin indicated that half of the oxygen atoms incorporated in 4-(1-hydroxy-1-methylethyl)benzoic acid, the primary hydroxylation product, came from water. A redox tautomerism of the active high-valent hydroxo−metal−oxo porphyrin intermediate coupled with an oxygen rebound mechanism explained this result. Under similar conditions, ketoprofen was directly oxidized to 3-benzoylacetophenone, via at least two different reaction pathways. Trapping of radical intermediates by molecular oxygen competed with the oxygen rebound mechanism.

Introduction

The hydroxylation of saturated carbon−hydrogen bonds of water-soluble substrates is performed by cytochrome P-450 monooxygenases by incorporation of an oxygen atom from molecular oxygen, not from water.[1] The key steps of these enzyme-mediated hydroxylations involve (i) the formation of a high-valent iron−oxo species resulting from the heterolytic splitting of an intermediate metal peroxide and (ii) the abstraction of the hydrogen atom of a C−H bond by the electrophilic metal−oxo complex followed by the fast recombination of the carbon radical with the iron(IV)−OH species (oxygen rebound mechanism),[2] leading to the formation of the alcohol function with high retention of configuration at the carbon center.[3] In epoxidation reactions catalyzed by hydrophobic manganese− or iron−porphyrin complexes used as cytochrome P-450 models, the oxygen atom which is incorporated into the olefinic substrate comes entirely from the oxidant when the reaction is performed in organic solvents.[4] However, when the reaction is performed in water with water-soluble olefins and manganese porphyrin catalysts, the epoxide oxygen atom comes from the primary oxidant ($KHSO_5$, potassium monopersulfate) and from water in a 50/50 ratio due to a "redox tautomerism" of the active hydroxo−metal−oxo complex.[5] Using the same catalytic system, we recently observed the same behavior in the course of the oxidative cleavage of DNA, which was shown to involve hydroxylation of the C−H bond at the 1′ position of deoxyriboses units.[6] Since C1′ is linked to two heteroatoms, the C1′−H bond is not the paradigm for studying the origin of the oxygen atom in hydroxylations of nonactivated aliphatic C−H bonds performed in water. Metalloporphyrin catalysts were also used for oxidation of drugs and xenobiotics in water solutions.[7] So, in order to propose a mechanism for the origin of the oxygen atom in metalloporphyrin-catalyzed hydroxylations performed in water, we decided to study the oxidation of 4-isopropylbenzoic acid (**BA**) and ketoprofen (**KP**), a widely used nonsteroidal anti-inflammatory drug,[8] by using the Mn-TMPyP/$KHS^{16}O_5$/$H_2{}^{18}O$ system (see Scheme 1 for structures of **BA** and **KP**).[9]

Experimental Section

General Procedures. Potassium monopersulfate (the triple salt $[KHSO_5]_2[KHSO_4][K_2SO_4]$, Curox) was a gift from Interox. $H_2{}^{18}O$ (97 atom %) and $^{18}O_2$ (99 atom %) were supplied by Eurisotop (Gif-sur-Yvette, France). Mn-TMPyP[9] was prepared according to ref 12. 4-Isopropylbenzoic acid and 4-acetylbenzoic acid were purchased from Aldrich, and ketoprofen was obtained from Sigma. All reactions were performed at room temperature under air or, in some cases, under $^{18}O_2$ atmosphere (see below). HPLC analyses of diluted aliquots were performed on a Waters Millipore chromatograph equipped with a diode-array detector with a Nucleosil C18 column 10 μm (Interchrom). The eluent was a mixture of methanol/water 4/6 or 1/1 (v/v) (for analyses of **BA** or **KP** derivatives, respectively) and contained 5 mM ammonium

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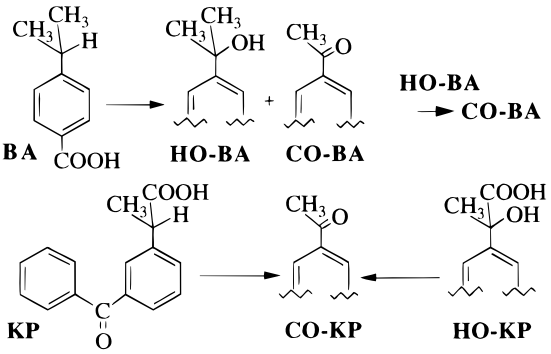
Scheme 1. Products Obtained in the Metalloporphyrin-Catalyzed $KHSO_5$ Oxidation of 4-Isopropylbenzoic Acid (**BA**) and the Hydroxylated Derivative **HO-BA** (Top) and of Ketoprofen (**KP**) and the Hydroxylated Derivative **HO-KP** (Bottom)



acetate and 17 mM acetic acid. Detection was performed at 234 or 260 nm for **BA** or **KP** derivatives, respectively. The retention times were 47, 6.2, and 4.7 min for **BA**, **HO-BA**, and **CO-BA**, respectively, and 15, 21, and 6−12 min for **KP**, **CO-KP**, and **HO-KP**, respectively (see below for a comment on the particular HPLC behavior of **HO-KP**). Oxidation products were identified by ^{1}H NMR (Bruker 200 MHz) and GC-MS analyses (Hewlett-Packard 5890 instrument using electron impact ionization at 70 eV). The GC column used was a nonpolar capillary column (12 m × 0.2 mm HL-1, cross-linked methylsilicone gum), and the carrier gas was helium. The injector temperature was 250 °C, and analyses were performed at 80 °C for 2 min and then up to 200 °C (10 °C/min). **BA**, **HO-BA**, **CO-BA**, and **HO-KP** were converted to methyl esters with diazomethane before GC-MS analysis. The retention times were 7.2, 8.0, and 8.4 min for methyl esters of **BA**, **HO-BA**, and **CO-BA**, respectively, and 16.5, 15.5, and 12.3 min for **KP**, **HO-KP** (methyl ester), and **CO-KP**, respectively. Reported experimental errors in labeling studies were obtained from two independent experiments and three GC-MS determinations on each independent experiment.

Preparation of Reference Compounds. 4-(1-Hydroxy-1-methylethyl)benzoic Acid, HO-BA (Procedure Adapted from Ref 13). To a stirred slurry of 1.0 g of **BA** in 2 mL of CCl_4 was added 0.5 mL of a solution of 1 g of bromine in 5 mL of CCl_4. The solution was then irradiated with a 200 W tungsten lamp. The deep-red bromine color disappeared almost immediately. The remaining bromine solution was added over a period of 30 min, and the color was continuously dissipated. The mixture was allowed to stir, in the dark, for a further 1.5 h, and then filtered. The solid was washed with several portions of CCl_4 and air-dried. Approximately 1 g of crude product was obtained. The product (0.5 g) was dissolved in 25 mL of H_2O at 80 °C, and the mixture was stirred for 15 min, after which it was cooled and the resulting precipitate was filtered off, washed with water, and air-dried. About 120 mg of white crystalline **HO-BA** was obtained. ^{1}H NMR (DMSO-d_6; δ ppm): 1.55 (s, 3 H, CH_3), 5.28 (s, 1 H, OH), 7.69 (d, 2 H, J = 8.4 Hz, H arom), 7.99 (d, 2 H, J = 8.4 Hz, H arom), 12.9 (s, 1 H, COOH). GC-MS of methyl ester, *m/z* [assignment, relative intensity]: 194 [M^+, 2], 179 [$(M - CH_3)^+$, 100), 176 [$(M - H_2O)^+$, 12], 163 [$(M - OCH_3)^+$, 12], 145 [$(M - OCH_3 - H_2O)^+$, 24].

4-Acetylbenzoic Acid, CO-BA. To a solution of 3.07 g of $KHSO_5$ (10 mmol) in 500 mL of 66 mM phosphate buffer, pH 7, was added a solution of 66 mg of **BA** (0.40 mmol) in 10 mL of acetonitrile. The solution was stirred for 24 h at room temperature to allow the conversion of 95% of the starting material as indicated by HPLC. The reaction mixture was reduced to a volume of 40 mL under vacuum, and then the solution was acidified with acetic acid and extracted with two 50-mL portions of dichloromethane. The organic phase was evaporated to dryness, and the solid was recrystallized from water, giving pure **CO-BA** (yield = 75%). ^{1}H NMR (DMSO-d_6; δ, ppm): 2.74 (s, 3 H, CH_3), 8.17 (s, 4 H, H arom). GC-MS of methyl ester, *m/z* [assignment, relative intensity]: 178 [M^+, 18], 163 [$(M - CH_3)^+$, 100], 147 [$(M - OCH_3)^+$, 16], 135 [$(M - CO - CH_3)^+$, 25].

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2-(3-Benzoylphenyl)-2-hydroxypropionic Acid, HO-KP. To a slurry of 0.5 g of ketoprofen in 1 mL of CCl_4 was added a solution containing 0.3 g of bromine in 0.25 mL of CCl_4. The mixture was stirred and irradiated with a 200-W tungsten lamp for 2 h. At this point, all the solid had dissolved and the solution was clear red. A further 0.3 g of bromine in 0.5 mL of CCl_4 was added, and the solution was stirred for another 2 h, followed by the addition of 0.15 g of bromine. The mixture was then stirred for 24 h without irradiation. Since HPLC indicated the presence of starting material, two more portions of 0.15 g of bromine in 0.25 mL of CCl_4 were added over a 12 h period with irradiation. The resulting dark red oil dissolved in a minimum amount of dichloromethane was added slowly to water at 85 °C. The solution was stirred rapidly for 4 h. After cooling, a colloidal precipitate settled out of the solution. The entire mixture was extracted with three 25-mL portions of diethyl ether, and the ether was then slowly removed. This resulted in a pale yellow oil. All attempts to obtain crystalline material failed. ^{1}H NMR showed that the oil was approximately 88% **HO-KP**, 8% **KP**, and less than 2% **CO-KP**. **HO-KP** data are as follows. ^{1}H NMR ($CDCl_3$; δ, ppm): 1.81 (s, 3 H, CH_3), 7.4−8.1 (m, 9 H, H arom). GC-MS of methyl ester, *m/z* [assignment, relative intensity]: 269 [$(M - CH_3)^+$, 76], 252 [$(M - CH_3 - OH)^+$, 8], 239 [$(M - OCH_3 - CH_2)^+$, 100]. On HPLC profiles, two bands corresponded to **HO-KP** with relative intensities highly dependent on concentration. At a concentration of 5 mM, a major wide band with a retention time of 6.2 min and a relatively minor band with a retention time of 12 min were obtained. As the concentration was decreased, the retention time of the major band increased toward 12 min. The minor band retention time did not depend on the concentration. Both peaks showed an apparent decrease in intensity with dilution and gave identical UV spectra. This unexpected behavour might be due to inter- or intramolecular H-bonding interactions between the carboxylato and hydroxy substituents.

3-Benzoylacetophenone, CO-KP. A reference sample of **CO-KP** was obtained from a metalloporphyrin-catalyzed reaction (see experiment below with conditions D).

General Catalytic Procedures. Oxidation of 4-Isopropylbenzoic Acid. Conditions A. All reactions were carried out at room temperature (25 °C) according to the following standard procedure: The reaction mixture (1 mL) contained 66 mM phosphate buffer, pH 5, and 500 μM **BA** (introduced as a 47 mM solution in acetonitrile). The $KHSO_5$ concentration was either 1.0 or 10 mM, and the Mn-TMPyP concentration was 10 μM. The latter compound was introduced in five consecutive 2 nmol additions, every 2 min. The first addition initiated the reaction. Reactions were monitored by HPLC. After 10 min and at low $KHSO_5$ concentration (1.0 mM), the main product was the hydroxylated derivative **HO-BA** (**BA**, **HO-BA**, and **CO-BA** molar ratios determined by HPLC were 0.35, 0.45, and 0.20, respectively), whereas at high concentration of $KHSO_5$ (10 mM) **CO-BA** accounted for 90% of the products. With **HO-BA** as starting material and under experimental conditions identical to those above (with 6.25−12.5 mM $KHSO_5$), the yields of **CO-BA** were 70−80% after 10 min of reaction.

Oxidation of 4-Isopropylbenzoic Acid. Conditions B. To 120 mL of 66 mM phosphate buffer, pH 5, containing 420 μM **BA** was added 6 mM potassium monopersulfate. To this stirred solution was added 12 μM Mn-TMPyP introduced in seven consecutive 0.2 μmol portions, every 5 min. After 1.5 h, the volume of the solution was reduced to about 20 mL by evaporation under vacuum, and the concentrate was extracted with two 25-mL portions of diethyl ether. The solid remaining after ether removal contained the three compounds **BA**, **HO-BA**, and **CO-BA**, which were present in the following molar ratio (determined by NMR): 0.38/0.31/0.31, respectively.

Oxidation of Ketoprofen. Conditions C. KP was oxidized as under conditions A, except that the catalyst was added at 5 min intervals. Using 10 mM $KHSO_5$, the conversion was 66% after 25 min, and the yield of **CO-KP**, which was the sole product detected, was 60% (estimated from HPLC analysis using $A^{260}_{CO-KP}/A^{260}_{KP} = 2.7$). Under the same experimental conditions, a reference sample of **HO-KP** was oxidized to **CO-KP** with 80% conversion and 75% yield in 20 min. In the absence of catalyst, at room temperature and after 2 h, **HO-KP** was not oxidized at all.

Oxidation of Ketoprofen. Conditions D. To 50 mL of 66 mM phosphate buffer, pH 5, containing 500 μM **KP** was added 16 mM

$KHSO_5$. To this stirred solution was added 20 μM Mn-TMPyP introduced in 10 consecutive 0.1-μmol portions, every 5 min. After 1.5 h, the volume of the solution was reduced to about 20 mL by evaporation under vacuum, the concentrate was extracted with two 20-mL portions of diethyl ether, and the ether was removed. The remaining solid was the only product and was shown by NMR to be **CO-KP**. ^{1}H NMR (DMSO-d_6; δ, ppm): 2.76 (s, 3 H, CH_3), 7.7−8.4 (m, 9 H, H arom). GC-MS, *m/z* [assignment, relative intensity]: 224 [(M^+, 59)], 209 [($M - CH_3$)$^+$, 100], 181 [($M - CO - CH_3$)$^+$, 17], 147 [($M - C_6H_5$)$^+$, 19], 119 [($M - CO - C_6H_5$)$^+$, 5], 105 [(C_6H_5CO)$^+$, 82], 77 [$C_6H_5^+$, 57].

$H_2{}^{18}O$ Experiments. Reactions were carried out in $H_2{}^{18}O$ (97 atom %), all experiments being performed in duplicate. Solutions of buffer, substrate, $KHSO_5$, and metalloporphyrin of the required concentration and volume were prepared separately and taken to dryness using a Speed-Vac. Appropriate volumes of $H_2{}^{18}O$ water were added, and the reactions were carried out as described above for catalytic procedures under analytical conditions, but in this case, the total volume was 500 μL and the $KHSO_5$ concentration was 10 mM. In all cases, blank experiments using this procedure were carried out with normal water and gave the same HPLC chromatograms as above. Reactions were allowed to proceed for 25 min, and then the mixtures were extracted with three 1-mL portions of diethyl ether. After concentration, the samples were used for GC-MS analysis.

The ^{16}O and ^{18}O compositions in **CO-KP** were determined by the relative abundances of molecular peaks at *m/z* = 224 for ^{16}O and *m/z* = 226 for ^{18}O. The GC-MS analysis of **HO-BA** and **CO-BA** was performed after methylation of the carboxylic group with diazomethane (*m/z* = 179 [($M - CH_3$)$^+$] and 178 [M^+], respectively). The mass spectrum of the **HO-BA** methyl ester showed a very weak molecular peak, the relative abundance being only 2%, which did not allow us to perform correct analyses. However, the most abundant peak was due to the loss of one methyl with retention of the ^{16}O and ^{18}O contents, which were determined by the relative abundances at *m/z* = 179 and 181, respectively.

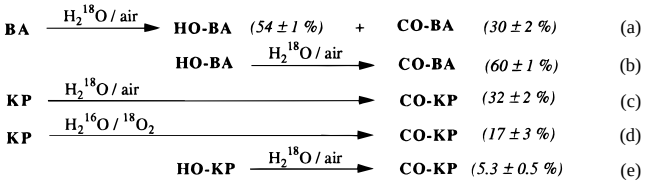
$^{18}O_2$ Experiments. Reactions on **KP** were carried out in $H_2{}^{16}O$ as described above for catalytic procedures under analytical conditions (total volume was 500 μL, and $KHSO_5$ concentration was 10 mM). Before the addition of the catalyst, the solution was placed in a small flask equipped with a septum-stoppered side arm on a vacuum line and was degassed by three consecutive freeze−thaw cycles under vacuum and then repressurized with $^{18}O_2$ from a reservoir. The required amounts of Mn-TMPyP solution (previously degassed and stored under argon) were then added via a gastight syringe to initiate the reaction. The reaction was allowed to proceed for 1.5 h, and then the mixtures were extracted with three 1-mL portions of diethyl ether. After concentration, the samples were used for GC-MS analysis.

Results

Oxidation of 4-Isopropylbenzoic Acid. The oxidation of **BA** by potassium monopersulfate without a metalloporphyrin catalyst can be performed in a mixture of acetonitrile/phosphate buffer, pH 7 (20/1, v/v), at room temperature in 24 h by using a large excess of the oxidant (25 equiv). After complete conversion of **BA**, the only identified product was 4-acetylbenzoic acid (**CO-BA**), an oxidation product resulting from the loss of one methyl group. No hydroxylation product, namely 4-(1-hydroxy-1-methylethyl)benzoic acid (**HO-BA**), resulting from hydroxylation of a benzylic C−H bond, was detected during this noncatalyzed oxidation. In contrast, the $KHSO_5$ oxidation of **BA** catalyzed by Mn-TMPyP (2 mol % of catalyst with respect to the starting material, only 2 equiv of $KHSO_5$) in phosphate buffer, pH 5, within 30 min resulted in a mixture of **HO-BA**, **CO-BA**, and remaining **BA** in the molar ratio 0.45/0.20/0.35, respectively (see conditions A in the Experimental Section). Both products only resulted from the catalytic reaction, since no substrate conversion was observed after 2 h in the absence of metalloporphyrin (even with 10 mM $KHSO_5$). We also verified that an authentic sample of **HO-BA** could be oxidized by Mn-TMPyP/$KHSO_5$ to **CO-BA** in 10 min under similar reaction conditions (yield was 80%; no other product was detected).

Oxidation of Ketoprofen. KP was not oxidized by a large excess of $KHSO_5$ alone (32 equiv) after 2 h at room temperature, whereas in the presence of 2 mol % of Mn-TMPyP, it was directly oxidized in 25 min to the ketone derivative **CO-KP** (Experimental Section, conditions C). No intermediate hydroxylated compound (**HO-KP**) could be detected during the reaction course, although this alcohol derivative was the major product in the PhIO oxidation of **KP** catalyzed by iron(III) *meso*-tetrakis(pentafluorophenyl)porphyrin in dichloromethane.[8b] Since a reference sample of **HO-KP** was quickly oxidized to **CO-KP** in our catalytic system, it remains reasonable to assume that **KP** was converted into **CO-KP** through the formation of **HO-KP** as an intermediate. It should be noted that, under the same conditions but in the absence of catalyst, **HO-KP** was not oxidized by $KHSO_5$ after 2 h at room temperature.

In eqs a−e are shown the catalytic oxidations of **BA**, **HO-BA**, **KP**, and **HO-KP** by the Mn-TMPyP/$KHSO_5$ system under different conditions (in italics: molar percent of ^{18}O introduced in the oxidation products).

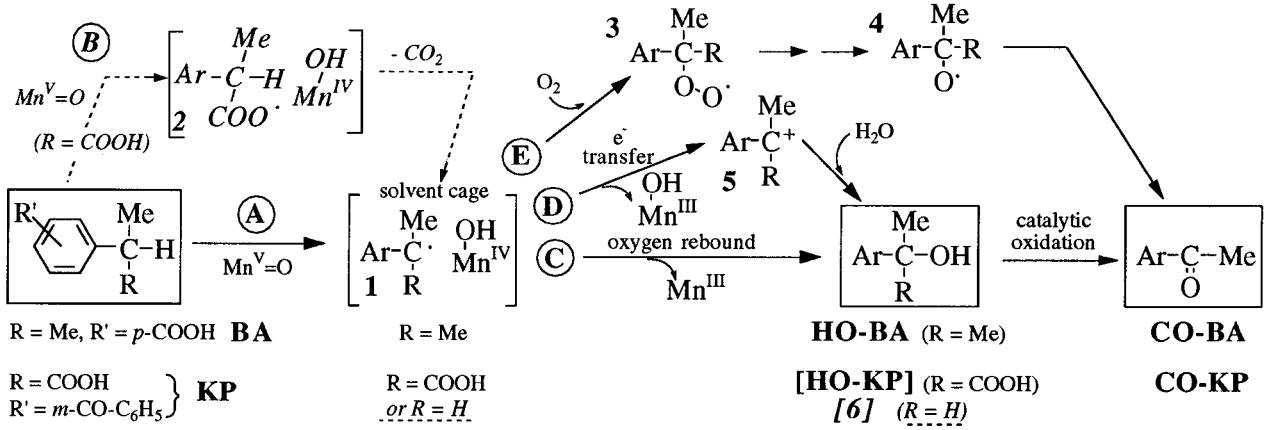


Studies Using ^{18}O-Labeled H_2O or O_2 (Respective Labels: 97 and 99 atom %). The present biomimetic oxidation of the aliphatic moiety of the water-soluble substrates **BA** and **KP** led to the formation of the alcohol **HO-BA** and the ketone derivatives **CO-BA** and **CO-KP**. Using ^{18}O-labeling experiments performed in $H_2{}^{18}O$, we investigated the origin of the oxygen atom introduced into the hydroxylated products as well as into the ketone derivatives. It should be noted that potassium monopersulfate does not exchange oxygen atoms with water in oxygenation reactions catalyzed by metalloporphyrins.[4c,d,5]

Within experimental errors, the GC-MS analyses of the isotopic composition of **HO-BA** obtained in the Mn-TMPyP-catalyzed oxidation of **BA** indicated that *54%, nearly half, of the oxygen atoms incorporated into the alcohol came from the water* (eq a). Under the same conditions, GC-MS analyses of the remaining **HO-BA** in the Mn-TMPyP-catalyzed oxidation of a reference sample of **HO-BA** showed no exchange of the hydroxyl oxygen with $H_2{}^{18}O$. Additionally, we found 30 ± 2% ^{18}O incorporated in **CO-BA**, which was coproduced in the metalloporphyrin-catalyzed oxidation of **BA** after 25 min at room temperature. We verified that this ketone was also generated from **HO-BA** under the same catalytic conditions used for the oxidation of **BA**, and we found that, in this case, 60 ± 1% ^{18}O was incorporated after 25 min (eq b). Unfortunately, ^{18}O incorporation was also observed in control experiments where the oxygen atom of the ketone group of **CO-BA** was found to exchange with the oxygen of labeled water (67 ± 1% ^{18}O was incorporated when a reference sample of **CO-BA** was incubated at pH 5 with $H_2{}^{18}O$ for 1.5 h at room temperature). Consequently, such high levels of O exchange in **CO-BA** with water did not allow a detailed discussion on the ^{18}O incorporation in this compound.

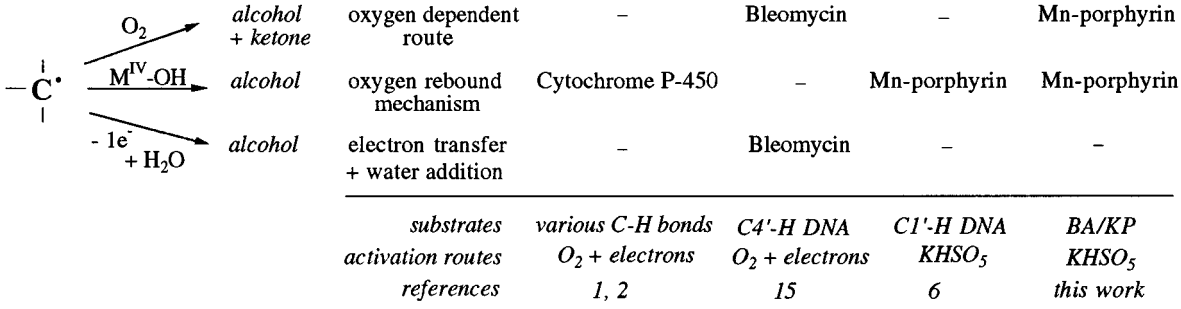
When **KP** was oxidized by Mn-TMPyP/$KHSO_5$ in $H_2{}^{18}O$, 32 ± 2% ^{18}O was incorporated into the corresponding ketone derivative **CO-KP** after a reaction time of 25 min at room temperature (eq c). This ^{18}O incorporation was clearly mediated

Scheme 2. Various Possible Reaction Pathways in Metalloporphyrin-Catalyzed $KHSO_5$ Oxidation of **BA** or **KP** To Produce **HO-BA** and **CO-BA** or **CO-KP**, Respectively[a]



[a] In italics is indicated the alternative decarboxylation pathway B in **KP** oxidation, giving the radical **1** ($R = H$) with further evolution according to C/D/E.

Scheme 3. Various Reaction Pathways Observed from a Carbon-Centered Radical Produced by Abstraction of a Hydrogen Atom from a C−H Bond of a Substrate in an Aqueous Medium When the Oxidation is Mediated by the Putative Metal−Oxo Species of Cytochrome P-450, Activated Bleomycin, or a Water-Soluble Manganese Porphyrin



by the manganese−porphyrin catalyst since the ^{18}O incorporation into an unlabeled sample of **CO-KP** via a possible oxygen atom exchange of the methyl ketone group with labeled water was only 13 ± 1% when **CO-KP** was incubated with $H_2{}^{18}O$ for 90 min (the diphenyl ketone group did not significantly exchange under these conditions, less than 2% based on the PhCO fragment at *m/z* 105). A low ^{18}O content (5.3 ± 0.5%) was found in **CO-KP** formed during the oxidation of a reference **HO-KP** sample by Mn-TMPyP/$KHSO_5$ in $H_2{}^{18}O$ for 30 min at room temperature (eq e). In addition, it must be noted that molecular oxygen was not innocent in the metalloporphyrin-mediated oxidation of **KP**, since 17 ± 3% ^{18}O was found in **CO-KP** when the catalytic oxidation was run under an $^{18}O_2$ atmosphere (eq d).

Discussion

The metalloporphyrin-catalyzed oxidation of **BA** showing 54% ^{18}O incorporation in **HO-BA** allowed us to discuss the validity of the redox tautomerism of a high-valent manganese(V)−oxo−hydroxo species in the hydroxylation of a nonactivated C−H bond by the MnTMPyP/$KHSO_5$ system using labeled water. Recently, using the same water-soluble metalloporphyrin activated by $KHSO_5$ in olefin epoxidation[5] or hydroxylation of DNA deoxyriboses,[6] we reported that the active hydroxo−metal−oxo porphyrin complex undergoes a "redox tautomerism", *i.e.* a tautomeric equilibrium which localizes the oxidizing entity on either one or the other face of the high-valent metalloporphyrin.

Redox tautomerism: $H\bullet - M^{V}=O \rightleftharpoons \bullet = M^{V}-OH$

Therefore, only one oxygen atom of the two comes from the oxidant and the other one originates from water. The 54% ^{18}O incorporation presently observed supports such a redox tautomerism mechanism and also indicates that hydroxylation of **BA** occurred via an oxygen rebound mechanism as proposed for hydroxylations catalyzed by cytochrome P-450: **HO-BA** resulted from the intermediate tertiary-carbon-centered radical **1** (R = Me, Scheme 2, pathways A + C) that did not escape from the solvent cage after the hydrogen atom abstraction step.

The relatively fast exchange with water of the carbonyl oxygen atom of **CO-BA** precluded a complete discussion on the mechanism of further oxidation of **HO-BA** to **CO-BA**. However, since the experimental ^{18}O label was lower in **CO-BA** than in **HO-BA**, we must consider the possibility of an oxygen-dependent pathway (route E in Scheme 2). The trapping of radical **1** (R = Me) by $^{16}O_2$, competing with the oxygen rebound mechanism, should lower the ^{18}O labeling of **CO-BA** under the expected 50% label resulting from a redox tautomerism (one-third of the intermediate radical generated from isopropylbenzene by Fe(TPP)Cl/PhIO escaped from the solvent cage when the hydroxylation was performed in benzene[14]). This hypothesis was supported by experiments performed with $^{18}O_2$ in studies on **KP** oxidation (see below). The possibility of an electron transfer, leading to a 100% ^{18}O label in **HO-BA** (route D in Scheme 2), can be excluded as the major route, although such a mechanism with addition of water to a carbocation was previously observed in C−H bond activation of the 4′-positions

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of deoxyribose units of DNA by the so-called activated bleomycin.[15]

The metalloporphyrin-catalyzed oxidation of ketoprofen only afforded **CO-KP** (Scheme 1). Under identical experimental conditions, a reference sample of **HO-KP** was oxidized quickly to **CO-KP** by the catalytic system. The low O exchange of the methyl carbonyl group of **CO-KP** with labeled water allowed us to discuss the different pathways involved in the oxidation of ketoprofen and the possible role of **HO-BA** as a key intermediate. Pathway A (Scheme 2) corresponds to the abstraction of the benzylic hydrogen atom (intermediate **1**, R = COOH) by the high-valent Mn^{V}═O. This pathway should lead to **HO-KP** with 50% ^{18}O incorporation (according to the redox tautomerism discussed above for **BA** oxidation) and then to **CO-KP** after an oxidative decarboxylation without changing the ^{18}O content of the carbonyl group, since the O exchange with water is slow. The second route, pathway B, involves an oxidative decarboxylation via a Mn^{V}═O complex.[8b] The intermediate carboxyl radical **2** loses carbon dioxide quickly to give rise to the carbon-centered radical **1** (R = H in this particular case; see footnote *a*, of Scheme 2).[16] Such a reactive radical might be able to produce **CO-KP** via three different routes. Pathway C corresponds to an oxygen rebound mechanism with the ^{18}O-labeled Mn^{IV}−OH species resulting in the alcohol **6**, which is oxidized to **CO-KP** with preservation of the label. Alternatively, in pathway E, intermediate **1** (with R = H) could be trapped by molecular oxygen to produce **CO-KP** via radicals **3** and **4**. About 17%-^{18}O-labeled **CO-KP** could be obtained via such a route as shown when the catalytic oxidation was conducted under $^{18}O_2$ atmosphere. This pathway could explain the reduction of ^{18}O incorporation in **CO-KP** when ketoprofen was oxidized by the Mn-TMPyP/$KHSO_5$/$H_2{}^{18}O$ system (50% ^{18}O incorporation was expected according to redox tautomerism involving either A + C or B + C routes; the found value was 32%). As mentioned in the case of **BA** oxidation, the electron transfer pathway D can be excluded since it should produce a 100% ^{18}O incorporation in alcohol **6** (R = H) and consequently in **CO-KP** where such a high isotopic label was not observed.

The sole production of **CO-KP** when the metalloporphyrin-catalyzed ketoprofen oxidation was conducted in water (this work) as opposed to the formation of both alcohol and ketone derivatives when the reaction was performed in an aprotic solvent[14] suggests that, in a water solution, either the oxidation of the postulated intermediate **HO-KP** to the end product **CO-KP** is faster than the initial oxidation of **KP** to **HO-KP** or the decarboxylation process (pathway B) is predominant over the C−H activation and consequently the formation of **HO-KP** is bypassed. It should be noted that **HO-KP** was detected as a ketoprofen metabolite *in vivo*, suggesting that pathway A is clearly mediated by cytochrome P-450 monooxygenases.[17]

The present work allowed us to form three main conclusions: (i) the redox tautomerism can be considered to be valid in the hydroxylation of C−H benzylic bonds by the MnTMPyP/$KHSO_5$ system in aqueous solution and appears now to be a more general concept when the catalytic activity of such biomimetic oxygenation systems is described, (ii) the first step of the catalytic oxidation reaction consists of a H atom abstraction at the tertiary aliphatic C−H bond of the substrate to give a carbon-centered radical, and (iii) this carbon radical mainly recombines with the OH ligand of Por−Mn^{IV}−OH in the case of an efficient oxygen rebound mechanism or, in a competitive way, could react with molecular oxygen when it escapes from the solvent cage. An overview of the fate of such a carbon-centered radical intermediate is proposed in Scheme 3 in the case of the activation of a saturated C−H bond by cytochrome P-450, activated bleomycin,[18] or a water-soluble metalloporphyrin catalyst. With Mn-TMPyP as catalyst, the main reaction pathway corresponds to the oxygen rebound mechanism with a minor oxygen-dependent route (the latter pathway being the main one in the case of activated bleomycin). The evolution of the carbon-centered radical is controlled by the kinetic parameters within the solvent cage (or the active site of a heme monooxygenase) where it has been generated by H atom abstraction.

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