A Novel Application of Chloroperoxidase: Preparation of *gem*-Halonitro Compounds

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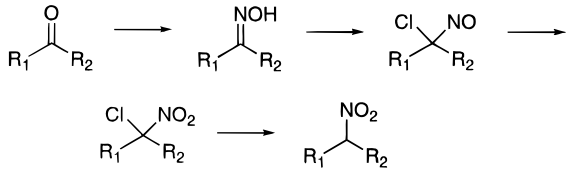
Conversion of carbonyl to nitro compounds is usually carried out in three or four steps via addition of hydroxylamine to ketones to form the corresponding oximes, oxidation of the oximes to halonitroso intermediates followed by ozonolysis, and halogen removal by catalytic hydrogenation (Scheme 1).[1]

The second step of the transformation, chlorination of oximes to chloronitroso compounds, is achieved by a number of reagents including elemental chlorine[2a] and bromine,[2b,e] aqueous hypochlorous acid,[2a] *tert*-butyl hypochlorite,[2c] and *N*-bromosuccinimide.[2d] The resulting chloronitroso intermediate is then oxidized further to chloronitro product with nitric,[2d] trifluoroperoxyacetic,[2e] or *m*-chloroperbenzoic acids,[3b] ozone,[3a] aqueous sodium,[3c] or *n*-butylammonium hypochlorite.[2c] This two-step oxidation method proceeding through the *gem*-chloronitroso species is considered superior to the oxidation of oximes directly to chloronitro compounds. The latter when performed using trifluoroperacetic, pyridinium dichromate, ozone, or lithium hypochlorite usually yields larger amounts of byproducts.[1a] There are reports that some triazine derivatives convert oximes to *gem*-halonitro compounds in good yield;[4] however, the reaction takes up to 48 h to complete and requires approximately 5 equiv of the halogenating reagent.

We report in this paper that chloroperoxidase from the fungus *Caldaromyces fumago* (CPO) is effective in converting oximes to halonitro compounds and ketones in a single step. The reaction is carried out in aqueous media in the presence of halide ions and hydrogen peroxide.

Chloroperoxidase *C. fumago* is a hemoprotein that catalyzes H_2O_2-dependent oxidation of inorganic and organic substrates.[5] It is generally believed that halogenation proceeds via the enzymatic formation of hypohalous acid, which then halogenates substrates in solution without assistance by the enzyme.[5a] Nevertheless, there are indications that direct transfer of a halogen species from a halogenated enzyme intermediate to the substrate also takes place.[6] Chloroperoxidase, in addition to its ability to halogenate a variety of organic substrates, also catalyzes numerous oxidative reactions including oxidation of alcohols to aldehydes and acids,[7a,f] sulfides to sulfoxides,[7b,c] epoxidations of olefins,[7d−f] and benzylic hydroxylations.[7a,f] To the best of our knowledge, oximes have never been identified as substrates for chloroperoxidase.

Scheme 1



In our attempt to oxidize oximes directly to nitro compounds, we incubated the oximes of cyclohexanone (**5**) and butanone (**4**) with CPO in the presence of H_2O_2 in an aqueous buffer pH 5.0 (see Experimental Section). To our surprise, after 4 h of incubation the oximes were converted to the corresponding ketones with no traces of nitro compounds. The nonenzymatic control reaction yielded less than 2% of the corresponding ketones. Since no desired nitro product was produced by the enzymatic reaction, the reaction pathway was altered by introducing a halide ion into the system. We proposed that the presence of halide ions would force the CPO-catalyzed oxidation/halogenation of oximes to proceed through the formation of halonitroso intermediates which would be further oxidized to the desired halonitro products. Indeed, in the presence of KBr the oxidation of oximes **4** and **5** proceeded smoothly leaving no starting material after 4 h. As anticipated, the reaction proceeded beyond the intermediate bromonitroso oxidation level and resulted in *gem*-bromonitro products.

A variety of oximes were converted in a similar fashion in the presence of KCl and KBr to the corresponding halonitro products (Table 1). In all the cases but one (oxime **9** with KBr) most of the substrate was converted to a mixture of the corresponding *gem*-halonitro product and ketone. No enantioselectivity was observed with substrates **1**, **2**, **4**, and **7**. The ratio of the amount of *gem*-halonitro products to that of ketones varies significantly among the substrates. Moreover, under more acidic conditions (pH < 3.5) the nonenzymatic hydrolysis of oximes back to their parent ketones lowers the yield of *gem*-halonitro products. The use of *tert*-butyl hydroperoxide as an oxidant, instead of hydrogen peroxide and lower reaction temperatures (4 °C) have only a minor effect on the reaction yield and product composition. Surprisingly, the addition of water miscible cosolvents (dioxane, acetone) significantly decreases the yield of the halonitro products and increases the yield of the ketones. In fact, **5** is nearly quantitatively converted to cyclohex-

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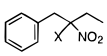
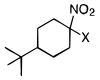
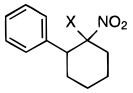
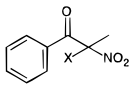
Table 1. Chloroperoxidase *C. fumago*-Catalyzed Synthesis of *gem*-Halonitro Compounds[a]

Oxime	Gem-halonitro product	Yield[b], %	Oxime conversion, %	Ketone, yield %
(±)-norcamphor oxime **1**		**1a** X = Br 82%; **1b** X = Cl 59%	>98% >98%	17% 39%
1-phenyl-2-butanone oxime **2**		**2a** X = Br 66% **2b** X = Cl 56%	85% 96%	16% 37%
tert-butyl-cyclohexanone oxime **3**		**3a** X = Br 58% **3b** X = Cl 54%	92% 97%	30% 41%
2-butanone oxime **4**		**4a** X = Br 55% **4b** X = Cl 29%	>99% >99%	45% 70%
cyclohexanone oxime **5**		**5a** X = Br 46% **5b** X = Cl 29%	>99% >99%	52% 70%
adamantanone oxime **6**		**6a** X = Br 50% **6b** X = Cl 37%	99% 99%	46% 62%
1-phenyl--cyclohexanone oxime **7**		**7a** X = Br 23% **7b** X = Cl 25%	87% 85%	41% 40%
acetophenone oxime **8**		**8a** X = Br 5% **8b** X = Cl 3%	70% 75%	60% 70%
1-phenyl-1,2-propanedione 2-oxime **9**		**9a** X = Br 0% **9b** X = Cl 0%	0% 72%	0% 50%

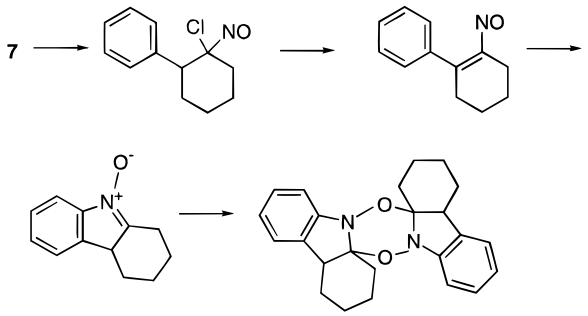
[a] See Experimental Section for details.
[b] Attempt to increase the yields by ketone recycling *in situ* failed due to severe enzyme inhibition by hydroxylamine.

anone in an aqueous buffer containing 25% acetone in the presence of the enzyme (<2% of *gem*-chloronitrohexane is observed). It was found that this significant change in the product composition stems from much higher sensitivity of the halide-dependent activity of the enzyme to the presence of a cosolvent, relative to the halide-independent activity. Indeed, in a separate experiment it was shown that while the rate of chlorination of monochlorodimedon in the presence of chloride ions is reduced 20 fold by the addition of 15% acetone to the reaction medium, the rate of benzyl alcohol oxidation in the absence of halide ions remains virtually unchanged under the same solvent conditions.

Most transformations in Table 1 result in a low amount (<5%) of byproducts (other than ketones), making the product isolation relatively simple. The major byproduct (~20%) of the oxidation of **7** in the presence of KCl and KBr is a dimer formed possibly as a result of elimination of HCl or HBr from the halonitroso intermediate followed by dimerization via 1,3-addition of the unsaturated nitroso compound (Scheme 2).

The oxidation of **9** yields benzoic acid as a major byproduct which is likely formed as a result of oxidative cleavage of the oxime (or the corresponding diketone) by hypohalous acid produced via the enzyme-catalyzed oxidation of halides.

Scheme 2



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Although the above oxidative halogenations are carried out at pH 5.0, which is about 2 pH units above the pH-optimum of the CPO-catalyzed oxidation of halide ion, some hypohalous acid is nevertheless produced during the oxidation. Earlier reports indicate that in the absence of CPO hypochlorous acid converts cyclic oximes to halonitroso compounds in benzene in 71−93% yield, and that a second oxidizing agent, tetra-*n*-butylammonium hypochlorite, is required to continue this oxidation to the chloronitro product.[2c] Unlike nonenzymatic reaction, the CPO-catalyzed oxidations proceed without the accumulation of the chloronitroso intermediates (which

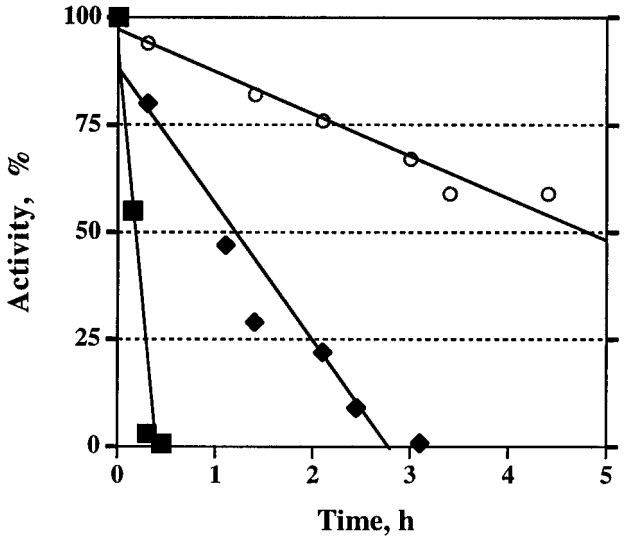

Figure 1. Irreversible inactivation of chloroperoxidase as a function of substrate concentration (see Experimental Section for details): (■) no substrate; (◆) 20 mM cyclohexanone oxime, (○) 20 mM butanone oxime.

are easily identified by their blue color), indicating that the rate of oxidation of chloronitroso compounds in the presence of CPO is much higher than the chlorination of the oximes.

Despite the difference in the outcome of the enzymatic and the chemical transformations, there is no clear evidence whether CPO binds the substrates and catalyzes the chlorination/oxidation of oximes directly, or if the process is carried out by an undefined species produced as a result of CPO-catalyzed oxidation of the halide ion (Scheme 3).

Scheme 3

$$H_2O_2 + Cl^- \leftrightarrow Cl_2 \leftrightarrow ClO^- \leftrightarrow ClO_2^- \leftrightarrow ClO_3^-$$

The following experimental observations shed some light on this problem. Since oximes **4** and **5** are stable in citrate/phosphate buffer at pH 5.0 in the presence of H_2O_2, their conversion to the corresponding ketones in the absence of halide ions by CPO points to the enzyme's ability to bind the substrates and catalyze the oxidation. Another indication that the oximes interact with CPO stems from following inactivation experiments. CPO is known to be rapidly and irreversibly inactivated in the presence of H_2O_2.[5c] Indeed, under the experimental conditions but in the absence of substrate, CPO loses 99% of its activity in less than 1 h (Figure 1). Remarkably, in the presence of oximes (but not ketones) the enzyme remains active for several hours. The degree of stabilization depends on the concentration and the structure of the oxime. After the oxime is converted to halonitro product the enzyme becomes inactivated. It has been suggested that in the absence of substrates the formation of an unstable enzyme-bound ferryl-oxygen species during the catalytic cycle leads to oxidation of the protein and/or deterioration of the heme.[8] Therefore, it is likely that the ability of different oximes to protect the CPO against inactivation stems from their direct interaction with the labile enzyme intermediates which leads to their oxidation before enzyme autooxidation can occur. Following substrate oxidation, the enzyme returns undamaged to its inherently more stable, reduced resting state.

In conclusion, it was found that oximes in the presence of CPO, halide ions and hydrogen peroxide are converted to the corresponding halonitro products. The reaction is accompanied by formation of the corresponding ketones. The CPO-catalyzed oxidation of oximes to ketones and the stabilization of the CPO by oximes against inactivation by H_2O_2 strongly suggest that the enzyme directly participates in the reaction. It is likely, however, that this enzymatic pathway competes with nonenzymatic oxidation/halogenation of oximes by oxidized halogen species. The lack of enantioselectivity suggests that the direct involvement of CPO in the overall process may be limited.

Experimental Section

Materials and Methods. CPO was obtained through Chirazyme (Urbana, IL) as a 12 mg/mL solution with a specific activity of 1400 units/mg enzyme with monochlorodimedon as a substrate. Hydrogen peroxide was purchased from Fisher as a 30% aqueous solution. Substrates **4**, **5** and **9** were obtained from Aldrich and used without further purification. Other oximes were synthesized chemically from the corresponding ketones according to the general procedure.[9] NMR measurements were carried out on standard Varian spectrometers at 200, 300, and 400 MHz.

General Procedure for Oxidation of Oximes. In a typical experiment 4.0 mg of substrate and 0.6 mg of the CPO were added to 2 mL of 100 mM phosphate/citrate buffer pH 5.0 containing 50 mM KBr or KCl. The reaction was initiated by adding 3% aqueous H_2O_2 at 0.05 mL/h.[10] Periodically 0.1 mL samples were withdrawn, extracted with 0.9 mL of CH_2Cl_2, and analyzed by GC or HPLC. After 4−5 h the entire reaction was extracted with CH_2Cl_2, purified by preparative TLC (3% *t*-BME in hexane) or HPLC, and subjected to ^{13}C and ^1H NMR and IR analysis. All calibration curves for quantitative GC and HPLC analysis were performed by using the halonitro derivatives synthesized chemically from the corresponding oximes according to the procedure of Walters *et al.*[4]

Oxidation of 1 on a Millimolar Scale. Solid **1** (125.0 mg, 1 mmol) was placed in a 100 mL round bottom flask containing 62 mL of 100 mM phosphate/citrate buffer pH 5.0 and 50 mM KBr and the formed suspension sonicated for 2 min to facilitate the oxime dissolution. To this mixture was added 1.5 mL of 12 mg/mL solution of CPO, and the reaction was initiated by the addition of 3% H_2O_2 at 1.5 mL/h. After 3 h >99% of the oxime was converted to the mixture of the corresponding *gem*-bromonitro product (90%) and the ketone (10%). The products were extracted with *t*-BME (2 × 60 mL), the solvent was evaporated at reduced pressure, and the residual oil was subjected to preparative TLC (hexane/*t*-BME 97/3). The isolated material was dried *in vacuo* to give 143 mg of **1a** (64% yield, 99.8% pure by GC. Somewhat lower than expected isolated yield resulted most likely from partial evaporation of **1a** under vacuum).

CPO Inactivation Experiments. To 2 mL of 100 mM phosphate/citrate buffer pH 5.0 containing 0.25 mg/mL of CPO, 50 mM KCl, and oxime was added 3% H_2O_2 at a rate of 0.05 mL/h. The mixture was stirred at 23 °C. Periodically, 0.01 mL samples were removed from the reaction mixtures, diluted 50-fold with 100 mM phosphate/citrate buffer pH 3.0, and assayed with monochlorodimedon according to the procedure of Morris and Hager.[11]

(8) Ortiz de Montellano, P. R. *Acc. Chem. Res.* **1987**, *20*, 289−94.

(9) For a review, see Sandler, S. R.; Karo, W. *Organic Functional Group Preparations*; Academic Press: New York, 1972; Vol. 3, pp 372−81.

(10) Under the above conditions this rate of addition of H_2O_2 was found to be close to optimal. The rates of 0.025 and 0.1 mL/min led to lower yield most likely due to faster inactivation of the enzyme. During the scale-up, the buffer volume, the amount of substrates and the enzyme, and the rate of H_2O_2 addition were increased by the same factor.

(11) Morris, D. R.; Hager, L. P. *J. Biol. Chem.* **1966**, *241*, 1763−68.

2-Bromo-2-nitronorbornane (1a). ^{13}C NMR ($CDCl_3$) δ 24.2, 27.0, 37.6, 38.3, 46.5, 51.7, 97.7; ^1H NMR ($CDCl_3$) δ 1.26, cd (2), 1.59−1.70, cm (3), 2.15, cd (1), 2.48, bs (1), 2.57, dq (1), 2.94, dd (1) 3.08, bs (1); IR (thin film) 1553 cm^{-1} GC/EI MS 172 ($M^+ - HNO_2$).

2-Chloro-2-nitronorbornane (1b). ^{13}C NMR ($CDCl_3$) δ 24.0, 26.8, 37.1, 38.1, 45.6, 51.3, 108.1; ^1H NMR ($CDCl_3$) δ 1.12−1.39, cdt (2), 1.51−1.76, cm (3), 2.04, cd (1), 2.31, dq (1), 2.50, bs (1), 2.89, dd (1) 2.94, bs (1); IR (thin film) 1555 cm^{-1}.

2-Bromo-2-nitro-1-phenylbutane (2a). ^{13}C NMR ($CDCl_3$) δ 10.1, 35.1, 48.2, 104.4, 128.2, 128.6 (2), 130.4 (2), 133.2; ^1H NMR ($CDCl_3$) δ 1.14, t (3), 2.25, sx (1), 2.52, sx (1), 3.68, d (1), J = 14.5 (AB), 3.73, d (1), J = 14.5 (AB), 7.21, cm (2), 7.32, cm (3); IR (thin film) 1556 cm^{-1}; GC/EI MS 257 (M^+).

2-Chloro-2-nitro-1-phenylbutane (2b). ^{13}C NMR ($CDCl_3$) δ 8.6, 35.0, 47.8, 110.8, 128.2, 128.5 (2), 130.5 (2), 132.5; ^1H NMR ($CDCl_3$) 1.08, t (3), 2.19, sx (1), 2.56, sx (1), 3.52, d (1), J = 14.2, 3.67, d (1), J = 14.2, 7.21, cm (2), 7.32, cm (3); IR (thin film) 1560 cm^{-1}.

1-Chloro-1-nitro-4-*tert*-butylcyclohexane (3b). ^{13}C NMR ($CDCl_3$) δ isomer 1 (52%): 25.0 (2), 27.4 (3), 32.3, 38.9 (2), 45.8, 105.0, isomer 2 (48%): 23.6 (2), 27.5 (3), 34.5* , 38.5 (2), 46.4, 101.1; ^1H NMR ($CDCl_3$) δ 0.83, s (9), 0.90, s (9), 1.01−1.17, cm (4), 1.52, qd (2), 1.78−1.97, cm (4), 2.04, bt (2), 2.22, td (2), 2.51, dc (2), 3.05, dc (2); IR (thin film) 1560 cm^{-1}. * possibly carbowax impurity; δ 32.3 peak may be correct for both isomers.

2-Bromo-2-nitrobutane (4a). IR (solution in Nujol) 1564 cm^{-1}.

2-Chloro-2-nitrobutane (4b). ^1H NMR ($CDCl_3$) δ 0.99, t (3), 2.02, s (3), 2.23, sx (1), J = 14.5, 7.3, 2.36, sx (1), J = 14.5, 7.3; IR (solution in Nujol) 1561 cm^{-1}.

1-Chloro-1-nitrocyclohexane (5b). ^{13}C NMR ($CDCl_3$) δ 22.9 (2), 24.0, 38.3 (2), 103.7; ^1H NMR ($CDCl_3$) δ 1.40, cm (1), 1.61, cm (1), 1.68−1.86, cm (4), 2.32, dt (2), 2.45, cm (2); IR (micr/NaCl) 1556 cm^{-1}.

1-Chloro-1-nitroadamantane (6b). ^{13}C NMR ($CDCl_3$) δ 25.5, 26.0, 34.2 (2), 34.9 (2), 37.1, 37.4 (2), 108.0; ^1H NMR ($CDCl_3$) δ 1.71−1.86, bcm (5), 1.86−1.99, bcm (5), 2.30, bd (2), 2.84, bs, (2); IR (KBr pellet) 1556 cm^{-1}; GC/EI MS 169 ($M^+ - NO_2$).

1-Bromo-1-nitro-2-phenylcyclohexane (7a). Analysis for major isomer only (∼90%) ^{13}C NMR ($CDCl_3$) δ 22.8, 23.7, 29.7, 40.2, 54.3; ^1H NMR ($CDCl_3$) δ 1.6, cm (1), 1.8−1.9, cm (2), 1.9−2.1, cm (1), 2.1−2.3, cm (2), 2.5−2.6, cm (1), 2.9−3.0, cdt (1), 3.65 dd (1); IR 1556 cm^{-1}

1-Chloro-1-nitro-2-phenylcyclohexane (7b). ^{13}C NMR ($CDCl_3$) δ isomer 1 (80%) 23.1, 23.6, 29.2, 40.1, 54.2, 107.2, 128.0, 128.2 (2), 129.3 (2), 137.7, isomer 2 (20%) 22.7, 25.1, 29.1, 40.9, 51.9, 128.1, 128.2 (2), 128.9 (2), 137.2; ^1H NMR ($CDCl_3$) δ 1.50−1.66, cm (∼1.5), 1.76−2.20, cm (∼5.3), 2.27−2.45, cm (∼2.4), 2.72, dd (0.23), 2.83, dt (1), 3.42, dd (1), 3.70, dd (0.2), 7.13−7.36 (6); IR (KBr pellet) 1550, 1458 cm^{-1}; GC/EI MS 239 (M^+).

Dimer 10. ^{13}C NMR ($CDCl_3$) δ 25.7, 26.8, 32.6, 32.8, 35.3, 113.5, 117.8, 123.1, 126.5, 128.1, 151.1, 158.9; ^1H NMR ($CDCl_3$) δ 1.5−1.8, cm (4), 1.9−2.5, cm (4), 2.65, cd (1), 3.32, dd (1), 6.89 d (1), 6.99, t (1), 7.07, d (1), 7.16, t (1); m/z 375 ($M + H)^+$.

Supporting Information Available: ^1H NMR ($CDCl_3$) spectra of compounds **1a**, **1b**, **2a**, **2b**, **3b**, **4b**, **5b**, **6b**, **7a**, **7b**, and **10** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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