Ketone-Catalyzed Decomposition of Peroxynitrite via Dioxirane Intermediates

Dan Yang,*,† Yeung-Chiu Tang,† Jian Chen,† Xue-Chao Wang,† Michael D. Bartberger,‡ K. N. Houk,*,‡ and Leif Olson§

Contribution from the Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, and Eastman Kodak Co., 1999 Lake Ave., Rochester, New York 14650-2102

Received December 21, 1998. Revised Manuscript Received October 25, 1999

Abstract: Ketones are found to be catalysts for the decomposition of peroxynitrite. Kinetics, product studies, and B3LYP transition-state calculations together provide consistent evidence for a mechanism involving the formation of dioxirane intermediates.

Introduction

Peroxynitrite[1] ($ONOO^-$) is a potent oxidant generated in cells from the nearly diffusion-controlled reaction between nitric oxide (NO) and superoxide ($O_2^{\bullet-}$) [$k = (4.3-6.7) \times 10^9$ M^{-1} s^{-1}].[2] It has been shown to contribute to tissue injury in a number of human diseases such as rheumatoid arthritis,[3] heart disease,[4] septic shock,[5] atherosclerosis,[6] and stroke.[7]

$$^{\bullet}NO + O_2^{\bullet-} \rightarrow ONOO^-$$

Under physiological conditions, $ONOO^-$ can oxidize biological substrates such as sulfhydryls,[8] sulfides,[9] and lipids,[10] and it decomposes through the formation of peroxynitrous acid which can isomerize to form nitrate (NO_3^-) spontaneously, or more rapidly upon the reaction with CO_2[11] (Scheme 1). These pathways produce reactive intermediates that can nitrate and hydroxylate tyrosine residues of proteins, interfering with signaling processes.[12,13] Therefore it is important to find reagents that scavenge peroxynitrite in harmless pathways and thus reduce its toxicity in vivo.

Some organic selenium-containing compounds such as ebselen [2-phenyl-1,2-benzisoselenazol-3(2H)-one] react very fast with peroxynitrite stoichiometrically and preempt the formation of oxidizing species from peroxynitrite.[14] Recently, several iron and manganese porphyrin complexes were reported to decompose $ONOO^-$ at a rate up to 2.2×10^6 M^{-1} s^{-1}.[15a−d] In particular, the iron porphyrin complexes are efficient catalysts for the decomposition of peroxynitrite,[15d] and they can protect culture cells from damage by exogenously added peroxynitrite and reduce the carrageenan-induced inflammation in rat paws.[15e] Some short-chain aliphatic aldehydes were found to decompose $ONOO^-$ catalytically, although not efficiently, and a radical mechanism was proposed.[16] Now we have found that ketones

† The University of Hong Kong.
‡ University of California.
§ Eastman Kodak Co.

(1) For recent reviews on peroxynitrite chemistry, see: (a) Beckman, J. S.; Koppenol, W. H. *Am. J. Physiol. (Cell Physiol.)* **1996**, *271*, C1424−C1437. (b) Edwards, J. O.; Plumb, R. C. *Prog. Inorg. Chem.* **1994**, *41*, 599−635. (c) Pryor, W. A.; Squadrito, G. L. *Am. J. Physiol. (Lung Cell. Mol. Physiol.)* **1995**, *268*, L699-L722.

(2) (a) Zafiriou, O. G.; Blough, N. V. *Inorg. Chem.* **1985**, *24*, 3502−3504. (b) Huie, R. E.; Padmaja, S. *Free Rad. Res. Commun.* **1993**, *18*, 195−199. (c) Goldstein, S.; Czapski, G. *Free Rad. Biol. Med.* **1995**, *19*, 505−510.

(3) Kaur, H.; Halliwell, B. *FEBS Lett.* **1994**, *350*, 9−12.

(4) Matheis, G.; Sherman, M. P.; Buckberg, G. D.; Haybron D. M.; Young, H. H.; Ignarro, L. J. *Am. J. Physiol. (Heart Circ. Physiol.)* **1992**, *262*, H616−H620.

(5) Szabo, C.; Salzman, A. L.; Ischiropoulos, H. *FEBS Lett.* **1995**, *363*, 235−238.

(6) (a) White, R. C.; Brock, T. A.; Chang, L.-Y.; Crapo, J.; Briscoe, P.; Ku, D.; Bradley, W. A.; Gianturco, S. H.; Gore, J.; Freeman, B. A.; Tarpey, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1044−1048. (b) Hogg, N.; Darley-Usmar, V. M.; Graham, A.; Moncada, S. *Biochem. Soc. Trans.* **1993**, *21*, 358−362.

(7) (a) Oury, T. D.; Piantadosi, C. L.; Crapo, J. D. *J. Biol. Chem.* **1993**, *268*, 15394−15398. (b) Dawson, V. L.; Dawson, T. M.; London, E. D.; Bredt, D. S.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6368−6371.

(8) Radi, R.; Beckman, J. S.; Bush, K. M.; Freeman B. A. *J. Biol. Chem.* **1991**, *266*, 4244−4250.

(9) Pryor, W. A.; Jin, X.; Squadrito, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11173−11177.

(10) (a) Rubbo, H.; Radi, R.; Trujillo, M.; Telleri, R.; Kalyanaraman, B.; Barnes, S.; Kirk, M.; Freeman B. A. *J. Biol. Chem.* **1994**, *269*, 26066−26075. (b) Radi, R.; Beckman, J. S.; Bush, K. M.; Freeman B. A. *Arch. Biochem. Biophys.* **1991**, *288*, 481−487.

(11) Lymar, S. V.; Hurst, J. K. *J. Am. Chem. Soc.* **1995**, *117*, 8867−8868.

(12) (a) Beckman, J. S.; Ischiropoulos, H.; Zhu, L.; Van der Woerd, M.; Smith, C.; Chen, J.; Harrison, J.; Martin, J. C.; Tsai, M. *Arch. Biochem. Biophys.* **1992**, *298*, 438−445. (b) Van der Vliet, A.; O'Neill, C. A.; Halliwell, B.; Cross, C. E.; Kaur, H. *FEBS Lett.* **1994**, *339*, 89−92. (c) Van der Vliet, A.; Eiserich, J. P.; O'Neill, C. A.; Halliwell, B.; Cross, C. E. *Arch. Biochem. Biophys.* **1995**, *319*, 341−349.

(13) (a) Uppu R. M.; Squadrito, G. L.; Pryor, W. A. *Arch. Biochem. Biophys.* **1996**, *327*, 335−343. (b) Lemercier, J.-N.; Padmaja, S.; Cueto, R.; Squadrito, G. L.; Uppu R. M.; Pryor, W. A. *Arch. Biochem. Biophys.* **1997**, *345*, 160−170. (c) Lymar, S. V.; Jiang, Q.; Hurst, J. K. *Biochemistry* **1996**, *35*, 7855−7861. (d) Meli, R.; Nauser, T.; Koppenol, W. H. *Helv. Chim. Acta* **1999**, *82*, 722−725. (e) Bonini, M. G.; Radi, R.; Ferer-Sueta, G.; Ferreira, A. M. D. C.; Augusto, O. *J. Biol. Chem.* **1999**, *274*, 10802−10806.

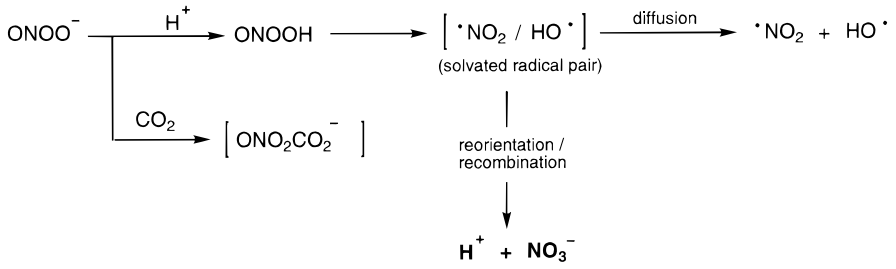
(14) (a) Masumoto, H.; Kissner, R.; Koppenol, W. H.; Sies, H. *FEBS Lett.* **1996**, *398*, 179−182. (b) Briviba, K.; Roussyn, I.; Sharov, V. S.; Sies, H. *Biochem. J.* **1996**, *319*, 13−15.

(15) (a) Groves, J. T.; Marla, S. S. *J. Am. Chem. Soc.* **1995**, *117*, 9578−9579. (b) Hunt, J. A.; Lee, J.; Groves, J. T. *Chem. Biol.* **1997**, *4*, 845−858. (c) Lee, J.; Hunt, J. A.; Groves, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 6053−6061. (d) Stern, M. K.; Jensen, M. P.; Kramer, K. *J. Am. Chem. Soc.* **1996**, *118*, 8735−8736. (e) Salvemini, D.; Wang, Z.-Q.; Stern, M. K.; Currie, M. G.; Misko, T. P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2659−2663.

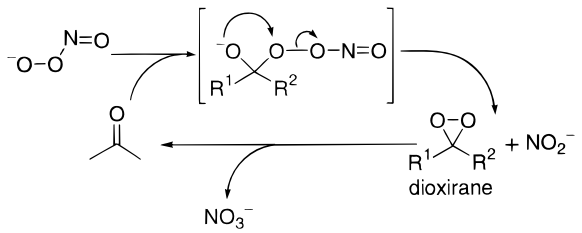
(16) Uppu R. M.; Winston, G. W.; Pryor, W. A. *Chem. Res. Toxicol.* **1997**, *10*, 1331−1337.

10.1021/ja984358v CCC: $18.00 © 1999 American Chemical Society
Published on Web 12/09/1999

Scheme 1



Scheme 2. Hypothetical Catalytic Cycle



also react with peroxynitrite: experiments and quantum mechanical calculations indicate a novel mechanism for the decomposition of peroxynitrite, involving dioxirane intermediates.

Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, a commercial source of peroxymonosulfate) reacts with ketones to form dioxiranes.[17] Given the structural similarity between $ONOO^-$ and HO_3SOO^-, as well as the fact that dioxiranes are excellent reagents for heteroatom oxidation,[18] we hypothesized that $ONOO^-$ might react with ketones to form dioxiranes and NO_2^- (Scheme 2), which could then react to give ketones and NO_3^-, thereby completing a catalytic cycle. Indeed, we have found that ketones can catalyze the decomposition of peroxynitrite.[19]

Results and Discussion

Kinetic Studies. At pH 7.4, addition of ketones accelerated the decomposition of peroxynitrite. As shown in Figures 1 and 2, the pseudo-first-order rate for the decay of peroxynitrite increases linearly with the initial concentration of methyl pyruvate (**1**).[20] This reveals a bimolecular reaction between peroxynitrite and the ketone. Since the ketones in aqueous solutions exist in an equilibrium of free (RCOR′) and hydrated [$RC(OH)_2R'$] forms, the bimolecular reaction may be due to either form. The apparent second-order rate constants $k_{2,app}$ for ketones **1**−**8** were determined at room temperature (Table 1).

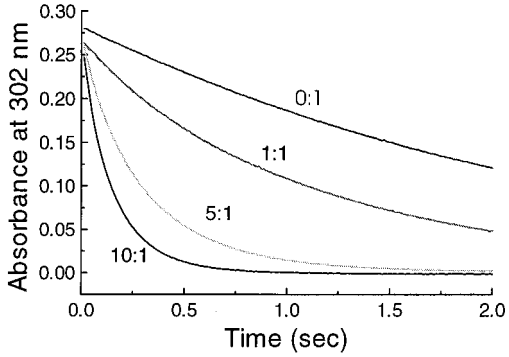


Figure 1. Stopped-flow kinetic traces of peroxynitrite decay at different ratios of methyl pyruvate to $ONOO^-$ in 0.125 M phosphate buffer at pH 7.4 and 22 °C where $[ONOO^-]_0 = 0.26$mM.

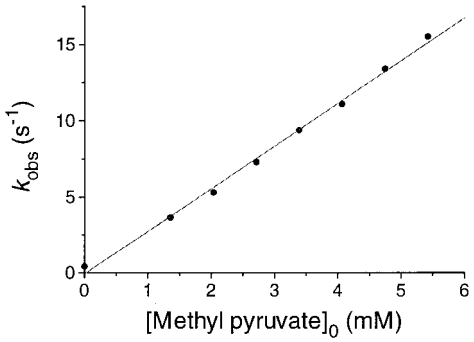


Figure 2. A plot of pseudo-first-order rate constants for the decomposition of peroxynitrite (0.14 mM) in 0.125 M phosphate buffer at pH 7.4 and 22 ± 1 °C, containing 0−5.4 mM methyl pyruvate. Each data point represents the mean of five measurements.

Compared with acetone (**8**), ketones **1**, **2**, and **5** are activated toward nucleophilic addition by the adjacent electron-withdrawing substituents. Ketones **3**−**4** and **6**−**7** are all 4-heterocyclohexanones, and their electrophilicity is enhanced by ring strain and by through-space electrostatic repulsion with the heteroatom at the 1-position.[21] The data shown in Table 1 reveal the importance of electronic factors in accelerating the decomposition of peroxynitrite. On the other hand, steric hindrance at the α-position retards the reaction rate (compare ketone **4** with **6**). Both results indicate that the observed reaction is a nucleophilic addition of peroxynitrite to the electron-deficient carbonyl group of the free ketone. As the hydrated form is unreactive toward peroxynitrite, the effective concentration of the ketone is less than the nominal value. After hydration equilibria of the ketones were taken into account, the second-order rate constants, k_2, were found to be up to half of that between $ONOO^-$ and CO_2 ($k_2 = 2.9 \times 10^4\ M^{-1}\ s^{-1}$ at 24 °C).[11]

Figure 3 shows the pH-rate profiles for ketones **2** and **4**, which give an unsymmetrical bell-shaped curve with a maximum

(17) For excellent reviews on dioxirane chemistry, see: (a) Murray, R. W. *Chem. Rev.* **1989**, *89*, 1187−1201. (b) Curci, R. In *Advances in Oxygenated Processes*; Baumstark, A. L., Ed.; JAI Press: Greenwich, CT, 1990; Vol. 2, Chapter 1. (c) Adam, W.; Curci, R.; Edwards, J. O. *Acc. Chem. Res.* **1989**, *22*, 205−211. (d) Adam, W.; Curci, R.; D'Accolti, L.; Dinoi, A.; Fusco, C.; Gasparrini, F.; Kluge, R.; Paredes, R.; Schulz, M.; Smerz, A. K.; Veloza, L. A.; Weinkotz, S.; Winde, R. *Chem. Eur. J.* **1997**, *3*, 105.

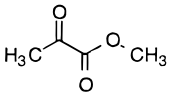
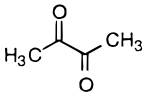
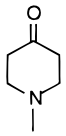
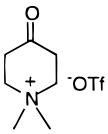
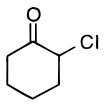
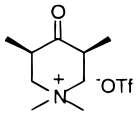
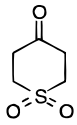
(18) (a) Wittman, M. D.; Halcomb, R. L.; Danishefsky, S. J. *J. Org. Chem.* **1990**, *55*, 1981−1983. (b) Baboul, A. G.; Schlegel, H. B.; Glukhovtsev, M. N.; Bach, R. D.; *J. Comput. Chem.* **1998**, *19*, 1353 and references therein.

(19) Pyruvate anion was reported to react with peroxynitrite to give acetate, carbon dioxide, and carbon dioxide radical anion: Vásquez-Vivar, J.; Denicola, A.; Radi, R.; Augusto, O. *Chem. Res. Toxicol.* **1997**, *10*, 786−794.

(20) Atmospheric carbon dioxide CO_2 (from air-equilibrated solutions) was not excluded from the reaction mixtures, but the presence of CO_2 does not affect the kinetic results for the reactions of peroxynitrite with ketones, nor the conclusion that dioxirane intermediates are involved in these reactions.

(21) Conroy, J. L.; Sanders, T. C.; Seto, C. T. *J. Am. Chem. Soc.* **1997**, *119*, 4285−4291.

Table 1. Rate Constants for Peroxynitrite Decomposition Catalyzed by Ketones[a]

Ketone		$k_{2,app}$ (M^{-1} s^{-1})	hydrate/ketone[b]	k_2 (M^{-1} s^{-1})[c]
	**1**	2.8×10^3	3.5	1.3×10^4
	**2**	3.3×10^3	3.3	1.4×10^4
	**3**[d]	1.5×10^3	7.0	1.2×10^4
	**4**	1.2×10^3	11.7	1.6×10^4
	**5**	59	0.63	96
	**6**	46	0.47	68
	**7**	540	12.8	7.5×10^3
	**8**	4.2	0	4.2

[a] The experiments were carried out by mixing peroxynitrite solution with phosphate buffer in equal volumes. the conditions after mixing were: 0.12−0.3 mM $ONOO^-$ and 0.6−6 mM 1-7 or 0.08−1.4 M acetone in a mixture of CH_3CN and 0.125 M phosphate buffer (v/v 1:19) at pH 7.4.[b] The hydrate/ketone ratio was measured by ^1H NMR under the conditions used for the kinetic experiments before mixing (0.25 M phosphate buffer in CD_3CN/D_2O v/v 1:9). [c] $k_2 = k_{2,app} \times$ (1 + hydrate/ketone). [d] Ketone **3** was mostly protonated in the above conditions.

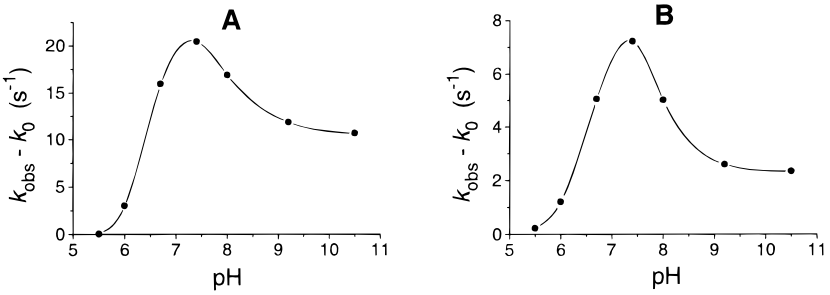
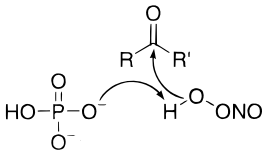


Figure 3. pH dependence of the difference between the pseudo-first-order rate constants in the presence (k_{obs}) and absence (k_0) of (A) ketone **2** (6 mM) or (B) ketone **4** (6 mM). Peroxynitrite (A: 0.45 mM; B: 0.37 mM) was allowed to react at (A) 24 °C or (B) 22 °C, and pH 5.5−10.5 in 0.125 M phosphate buffer.

between pH 6.8 and 7.5. Two possible rate laws (eqs 1 and 2), together with the corresponding mechanisms (Schemes 3 and 4), can be proposed to explain the bell shape. Both rate laws will give the same symmetrical bell-shaped pH-rate profile with a maximum at about pH 7 because the pK_a values of HOONO and $H_2PO_4^-$ are 6.8 and 7.2, respectively. However, the observed curve is unsymmetrical, and the rate at high pH is much higher than that at low pH, which indicates a pathway that occurs under basic conditions; this must be the direct addition of $ONOO^-$ to the carbonyl group. As a result, the general-acid-catalyzed

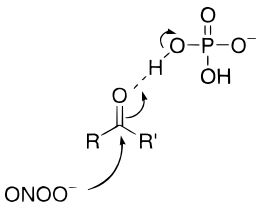
Scheme 3



Specific-acid general-base catalyzed pathway

$$\text{Rate} = k_3\,[\text{ketone}]\,[\text{HOONO}]\,[\text{HPO}_4^{2-}] \qquad \text{(eq 1)}$$

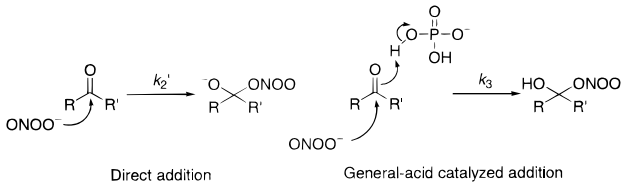
Scheme 4



Specific-base general-acid catalyzed pathway

$$\text{Rate} = k_3\,[\text{ketone}]\,[\text{ONOO}^-]\,[\text{H}_2\text{PO}_4^-] \qquad \text{(eq 2)}$$

Scheme 5



Direct addition General-acid catalyzed addition

$$\text{Rate} = k_2'\,[\text{ketone}][\text{ONOO}^-] + k_3\,[\text{ketone}][\text{H}_2\text{PO}_4^-][\text{ONOO}^-] \qquad \text{(eq 3)}$$

$$k_{\text{obs}} - k_0 = k_2'\frac{K_a}{K_a + [\text{H}^+]}\,[\text{ketone}] + k_3\,\frac{K_a}{K_a + [\text{H}^+]}\,[\text{ketone}][\text{H}_2\text{PO}_4^-] \qquad \text{(eq 4)}$$

where K_a is the acid dissociation constant of HOONO

addition of $ONOO^-$ shown in Scheme 4 is more likely than the pathway in Scheme 3.

The two parallel pathways are summarized in Scheme 5, and the pH-rate profile can be approximated by eq 4.

Below pH 6.8, the concentration of $ONOO^-$ decreases, giving a low reaction rate. Above pH 7.5, the general-acid-catalyzed addition becomes less important as the concentration of $H_2PO_4^-$ decreases, and thus the rate declines, finally leveling off above pH 9, where only uncatalyzed addition of $ONOO^-$ occurs.

Eq 4 shows a linear relationship between $k_{obs} - k_0$ and $[H_2PO_4^-]$. At a fixed pH, $[H_2PO_4^-]$ is proportional to the total buffer concentration. Therefore, a plot of $k_{obs} - k_0$ against buffer concentration at a constant ketone concentration should give a straight line. Figure 4 shows this relationship for ketones **2** and **4**, which further confirms the general-acid catalysis.

Dioxirane Formation. Dioxiranes are mild and versatile oxidants under neutral conditions.[17] A wide variety of olefins can be epoxidized by dioxiranes generated in situ from Oxone and ketones.[22] Dioxiranes usually give diastereoselectivities different from those given by peracids, and different dioxiranes give distinct stereoselectivities due to steric and electronic effects.[23] The evidence for the formation of dioxiranes from peroxynitrite and ketones is summarized in Table 2. In the absence of ketone, 1,3-dimethylcyclohexene and $ONOO^-$ did not yield a detectable amount of epoxide. Upon addition of **1** or **2**, a significant amount of epoxide products was formed. Either Oxone[24] or $ONOO^-$ in combination with **1** or **2** gave the identical *trans*/*cis* ratios of epoxides, that is, the same diastereoselectivity was obtained when the same ketone was employed, irrespective of the terminal oxidant. This implicates a common dioxirane intermediate.

Regeneration of Ketones. As shown in Scheme 2, the addition of peroxynitrite to ketone generates dioxirane intermediate, which reacts further with nitrite to give back ketone as the only carbon-containing product; that is, ketone should be regenerated in the reaction process. Indeed, ketones **2** and **3** were recovered almost quantitatively in their reactions with peroxynitrite, where a 4.5-fold amount of ketone was employed to ensure that more than 90% of peroxynitrite was consumed by the ketone (see Table 3).

Analysis of Nitrite/Nitrate Ratio. At neutral pH, nitrite and nitrate are the nitrogen-containing products from self-decomposition of peroxynitrite.[25] The data in Table 4 reveal that nitrate and nitrite are the only nitrogen-containing products in the reactions of peroxynitrite with ketones **1** and **2**, and the nitrite/nitrate ratio increases with the amount of ketone added. According to Scheme 2, the adduct of peroxynitrite and ketone collapses to nitrite and dioxirane, and the oxidation of nitrite by dioxirane leads to nitrate. Meanwhile, the formation of nitrite can be explained by proposing another pathway shown in Scheme 6, where dioxirane reacts with peroxynitrite to give ketone, nitrite, and oxygen. Kinetic studies on the reaction of dioxirane with peroxynitrite or nitrite may provide some evidence for the above proposed pathways.

Reaction of Dimethyldioxirane with Nitrite or Peroxynitrite. Due to the difficulty in obtaining the corresponding isolated dioxiranes derived from ketones **1**−**7**, dimethyldioxirane (DMD) was used as a model to examine the reactions of dioxiranes with nitrite or peroxynitrite. Nitrite was found to be oxidized quantitatively (see Table 5) to nitrate by DMD with a rate constant of $3.2 \times 10^3\ M^{-1}\ s^{-1}$ (at 25.6 ± 0.1 °C and pH 7.4), and DMD decomposed peroxynitrite efficiently at a rate of $2.5 \times 10^4\ M^{-1}\ s^{-1}$ (at 25.6 ± 0.1 °C and pH 7.4), about 10-fold faster than that for the former reaction. These kinetic studies suggest that dioxirane generated in situ from ketone and peroxynitrite may react further with peroxynitrite and nitrite, giving nitrite and nitrate respectively, and nitrite formation may compete with nitrate formation in these processes.

Nitration of Phenolic Compounds by Peroxynitrite. One potentially damaging biochemical reaction of peroxynitrite is the nitration of phenolic compounds,[12] which has been shown to be enhanced by the presence of CO_2.[13] However, in the presence of excess amounts of ketones **1**-**4**, the nitration yield of 4-methylphenol was found to decrease significantly to 4−6% while the yield was 10% in the absence of ketone (Table 6).

Figure 5 shows the yields of nitration of 4-hydroxyphenylacetate (4-HPA) by peroxynitrite with 0−15 mM ketones **3** and **4** at pH 7.4 and room temperature. In the absence of ketone,

(22) (a) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887−3889. (b) Yang, D.; Yip, Y.-C.; Tang, M.-W.; Wong, M.-K.; Zheng, J.-H.; Cheung K.-K. *J. Am. Chem. Soc.* **1996**, *118*, 491−492. (c) Denmark, S. E.; Wu. Z. *Synlett* **1999**, 847−859.

(23) (a) Murray, R. W.; Singh, M.; Williams, B. L.; Moncrieff, H. M. *J. Org. Chem.* **1996**, *61*, 1830. (b) Yang, D.; Jiao, G.-S.; Yip, Y.-C.; Wong, M.-K. *J. Org. Chem.* **1999**, *64*, 1635−1639.

(24) As shown in Table 2, Oxone gave a lower epoxidation yield in the presence of ketone **1** or **2**, which is because the dioxiranes formed from these ketones also consumed Oxone and the amount of Oxone was limited. See: Montgomery, R. E. *J. Am. Chem. Soc.* **1974**, *96*, 7820−7821.

(25) Pfeiffer, S.; Gorren, A. C. F.; Schmidt, K.; Werner, E. R.; Hansert, B.; Bohle, D. S.; Mayer. B. *J. Biol. Chem.* **1997**, *272*, 3465−3470.

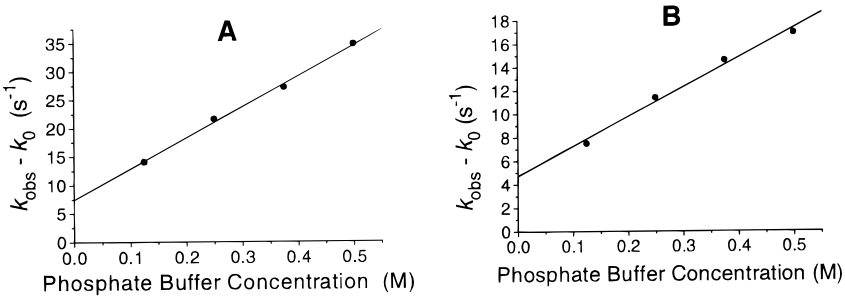
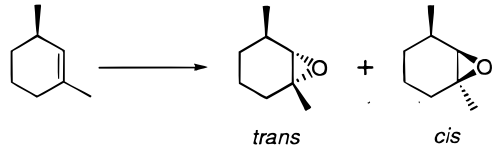


Figure 4. Effect of phosphate buffer concentration on $k_{obs} - k_0$ for the reaction between peroxynitrite (A: 0.35 mM; B: 0.22 mM) and (A) ketone **2** (4 mM) or (B) ketone **4** (6 mM) in 0.125−0.5 M phosphate buffer at 24 °C and pH 7.4.

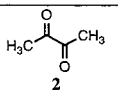
Table 2. Epoxidation of 1,3-Dimethylcyclohexene[a]



oxidant	% conv	*trans*/*cis*	oxidant	% conv	*trans*/*cis*
oxone only	77	2.1:1	$ONOO^-$ only	0	-
Oxone + **1**	42	4.6:1	$ONOO^-$ + **1**	16	4.8:1
Oxone + **2**	15	only *trans*[b]	$ONOO^-$ + **2**	23	only *trans*[b]

[a] Conditions: 1.24 mM of olefin, 1.24 mM of NaOONO or Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) and 6.19 mM of ketone **1** or **2** in a total volume of 33 mL phosphate buffer ($CH_3CN:H_2O$ = 1:10 v/v). Phosphate buffer concentration is 0.16−0.17 M at pH 7.4. The % conversion and *trans*/*cis* ratios were determined by GC after extraction with CH_2Cl_2. [b] No detectable amount of the *cis*-epoxide was found in the gas chromatogram.

Table 3. Recovery of Ketones **2** and **3**[a]

Ketone	 **2**	 **3**
	mM	
$[ONOO^-]_0$	0.322	0.644
$[ketone]_0$	1.61	2.91
[recovered ketone][b]	1.49 ± 0.02	2.65 ± 0.04
[recovered ketone][b,c]	1.50 ± 0.02	2.71 ± 0.01

[a] Decomposition of $ONOO^-$ in the absence or presence of ketone was conducted at room temperature in 0.160 M phosphate buffer at pH 7.4 in a total volume of 11 mL. [b] Determined by GC analysis after the extraction of ketone with CH_2Cl_2. [c] The control experiment was carried out under similar conditions except adding ketone 10 min after mixing peroxynitrite with buffer alone.

the yield of 3-nitro-4-hydroxyphenylacetate (3-NO_2-4-HPA) was 8−10 mol % based on the concentration of peroxynitrite. In the presence of ketone, the yield progressively decreased to a steady value, at which the ketone reached saturating conditions, that is, all peroxynitrite present reacted with the ketone. The maximum possible inhibitions of 4-HPA nitration by ketones **3** and **4**, calculated using Scatchard-like plots, are 69 and 42%, respectively.

These results show that the nitration of phenolic compounds by peroxynitrite can be partially suppressed by ketones. On the other hand, propionaldehyde was reported to inhibit 90% of 4-HPA nitration.[16] Similar to the case of CO_2 and aldehydes, the protonated tetrahedral adduct of ketones and peroxynitrite [RR′C(OH)OONO] may undergo homolytic cleavage, yielding a radical pair RR′C(OH)O$^\bullet$ and $^\bullet NO_2$. The residual yield of

Table 4. Shifts in $[NO_2^-]/[No_3^-]$ Ratios by Ketones **1** and **2** at 25 °C[a]

ketone	[ketone]:[$ONOO^-]_0$[b]	mM $[NO_2^-]$[c]	mM $[NO_3^-]$[d]	$[NO_2^-]/[NO_3^-]$ ratio
	0:1	0.080	0.254	0.32
	1:1	0.108	0.237	0.46
1	2.5:1	0.125	0.211	0.59
	5:1	0.144	0.198	0.72
	10:1	0.153	0.193	0.79
	0:1	0.070	0.325	0.22
	1:1	0.151	0.235	0.64
2	2.5:1	0.182	0.211	0.85
	5:1	0.201	0.195	1.03
	10:1	0.240	0.146	1.64

[a] Reaction was conducted in 0.160 M phosphate buffer at pH 7.4 in a total volume of 11 mL. All data are the mean values of three experiments. [b] $[ONOO^-]_0$ = 0.322 mM. [c] After subtracting $[NO_2^-]_0$. [d] $[NO_3^-]_0$ was found negligible.

Scheme 6

Table 5. HPLC[a]Analysis of NO_2^- and NO_3^-

reaction[b]	$[NO_2^-]$ (mM)	$[NO_3^-]$ (mM)
NO_2^- + acetone	5.96 ± 0.03	0
NO_2^- + DMD	0	5.87 ± 0.02

[a] HPLC conditions: Hamilton PRP-X100 anion-exchange column, aqueous potassium hydrogen phthalate (2.0 mM) at pH 5.0 as the mobile phase. [b] Reaction conditions: $[NO_2^-]_0$ = 6.00 mM, 5 equiv of DMD, room temperature, and 0.5 h. All data are the mean values of two experiments.

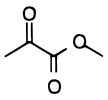
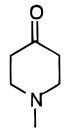
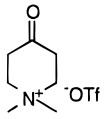
nitration in the case of ketones may be due to the oxidation of the phenols by RR′C(OH)O$^\bullet$.

Theoretical Studies. Theoretical evidence for this mechanism was obtained from density functional theory (DFT) calculations (B3LYP/6-31G*).[26] Transition structures were found for concerted oxygen atom transfer to acetone from peroxynitrite (Figure 6) and peroxynitrous acid. The B3LYP/6-31G* transition structures for epoxidations of ethylene by $ONOO^-$ and ONOOH were reported earlier and are quite similar.[27−29] The transition structure for $ONOO^-$ oxidation of acetone has considerable

(26) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94* (Revision A.1); Gaussian, Inc., Pittsburgh, PA, 1995.

(27) Houk, K. N.; Condroski, K. R.; Pryor, W. A. *J. Am. Chem. Soc.* **1996**, *118*, 13002−13006.

Table 6. Nitration of 4-Methylphenol by Peroxynitrite and Ketones[a]

Entry	Ketone[b]		% Nitration
1	No ketone		10 ± 2
2		**1**	4.0 ± 0.1
3		**2**	5.6 ± 0.2
4		**3**	4.2 ± 0.3
5		**4**	5 ± 1

[a] Nitration of 4-methylphenol (0.9−1.5 mM) by peroxynitrite (1 equiv) was performed in 0.45 M phosphate buffer at pH 7.4 and room temperature. The data shown are the mean of the results from two experiments. [b] Ketone **1** was in 5-fold excess; the others were in 10-fold excess.

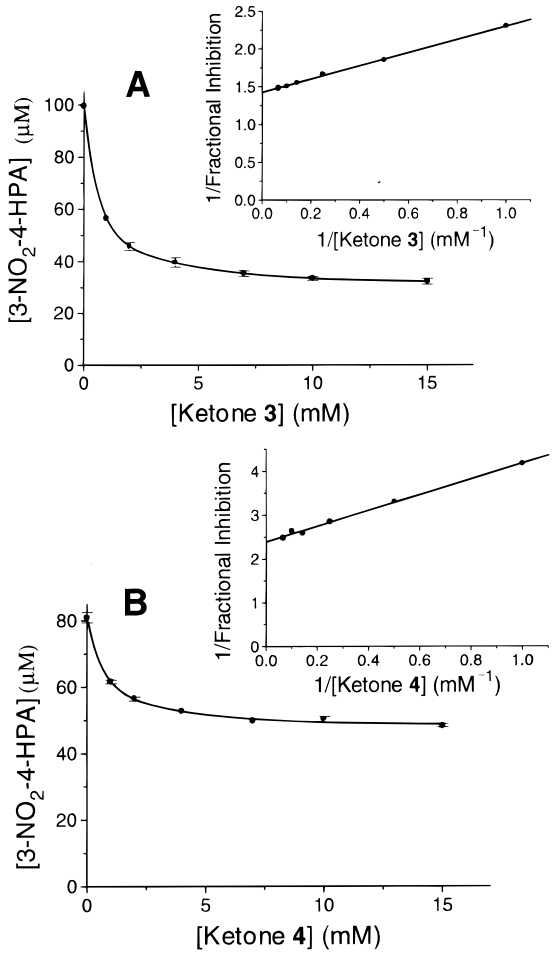


Figure 5. Nitration of 4-HPA (4 mM) by peroxynitrite (1 mM) in the presence of 0−15 mM (A) ketone **3** or (B) ketone **4** in 4 mL of 0.17 M phosphate buffer at pH 7.4 and room temperature. Each data point represents the mean (±SD) of the results from two experiments. Inset is a double-reciprocal plot of fractional inhibition of 4-HPA nitration against the concentration of (A) ketone **3** or (B) ketone **4**.

nucleophilic character, but gives NO_2^- and dimethyldioxirane directly. While acetone (used for convenience in the calculations) is a relatively electron-rich ketone, a nucleophilic attack transition state is consistent with the observation that electron-deficient ketones react most readily with $ONOO^-$. This reaction is predicted to be especially facile; peroxynitrite and acetone form an ion−molecule complex followed by a transition structure which is 1.1 kcal/mol lower in energy than the separated reactants in the gas phase, an energetic profile similar to the reactions of other anionic nucleophiles with aldehydes and ketones.[30] In solution, activation barriers are increased due to solvation of the nucleophile.[31]

The second step in the catalytic process occurs with no barrier at all: if NO_2^- and dimethyldioxirane are juxtaposed in a reasonable reacting conformation, oxygen atom transfer is spontaneous, yielding NO_3^- and acetone. Our calculations indicate that dioxiranes might also react with unconsumed $ONOO^-$. Oxygen atom transfer from dimethyldioxirane to the terminal peroxide oxygen of $ONOO^-$ to give acetone, NO_2^- and singlet O_2 (predicted on the basis of spin conservation) occurs with no barrier by DFT. Brauer et al. have demonstrated via direct spectrophotometric detection the quantitative liberation of singlet oxygen in the ketone-catalyzed decomposition of peroxymonosulfuric acid;[32,33] dioxiranes have also been shown to release singlet oxygen in the reaction of amine *N*-oxides.[34] Experiments to detect singlet O_2 in the ketone-catalyzed decomposition of $ONOO^-$ are currently underway.

The alternative N-atom oxidation of $ONOO^-$ by dimethyldioxirane to give O_2NOO^- (peroxynitrate) should also be facile, and the oxygen transfer transition state is 2.8 kcal/mol more stable than the separated reactants in the gas phase (similar to the situation with $ONOO^-$ + acetone). Thus, our DFT calculations indicate that O_2NOO^- should, like $ONOO^-$, react readily with ketones and dioxiranes. The B3LYP/6-31G* structures and energies of O_2NOO^- reactions with acetone (to give NO_3^- and dimethyldioxirane) and with dimethyldioxirane (to give NO_3^-, acetone, and singlet O_2) are nearly perfect analogues of the $ONOO^-$ reactions, suggesting that ketones should catalyze the decomposition of peroxynitrate. The experimentally observed NO_3^- may arise partly from these peroxynitrate decomposition pathways and partly from oxidation of NO_2^- by dioxirane.

The general-acid-catalyzed reaction of peroxynitrite with ketones at neutral pH leads to formation of the peroxynitrous acid adduct $ONOOCR_2OH$. The tetrahedral intermediate shown in Figure 7 could form a dioxirane. However, we find no energetically accessible pathway for an intramolecular nucleophilic displacement that would lead to dioxirane formation from

(28) Bartberger, M. D.; Olson, L. P.; Houk, K. N. *Chem. Res. Toxicol.* **1998**, *11*, 710−711.

(29) The validity of B3LYP results has been confirmed by comparisons with higher level calculations of epoxidations: see ref 27 and Glukhovtsev, M. N.; Canepa, C.; Bach, R. D. *J. Am. Chem. Soc.* **1998**, *120*, 10528−10533.

(30) Pranata, J. *J. Phys. Chem.* **1994**, *98*, 1180−1184 and references therein.

(31) Yu, H. A.; Karplus M. *J. Am. Chem. Soc.* **1990**, *112*, 5706−5716 and references therein.

(32) Lange, A.; Hild, M.; Brauer, H.-D. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1343−1350.

(33) Lange, A.; Brauer, H.-D. *J. Chem. Soc., Perkin Trans. 2* **1996**, 805−811.

(34) Ferrer, M.; Sanchez-Baeza, F.; Messenguer, A.; Adam, W.; Golsch, D.; Gorth, F.; Kiefer, W.; Nagel, V. *Eur. J. Org. Chem.* **1998**, 2527−2532.

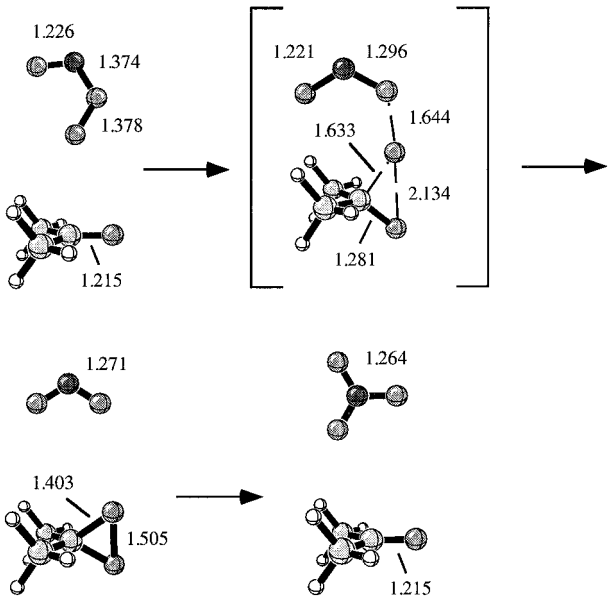


Figure 6. B3LYP/6-31G* structures of peroxynitrite plus acetone, the transition state for O transfer, and the structures of NO_2^- plus dimethyldioxirane, and nitrate plus acetone.

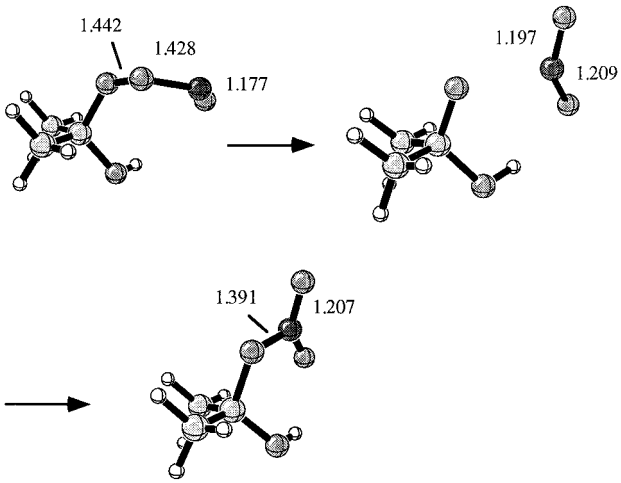


Figure 7. B3LYP/6-31G structures of the peroxynitrous acid adduct with acetone, the radical pair, and the nitric acid adduct with acetone.

such an intermediate. Simple O−O bond homolysis to form ONO$^\bullet$ and $^\bullet$OC(CH$_3$)$_2$OH (Figure 7), requires only 10.1 kcal/mol. O−O recombination of the radicals to regenerate starting material would occur, or the radicals could recombine at N. N−O bond formation gives the adduct of nitric acid and acetone, a process calculated to be exothermic by 37.3 kcal/mol. This latter species will be unstable at moderate pH, since its conjugate base readily reverts to NO_3^- and acetone. DFT found no barrier for this reversion. Therefore, the general-acid-catalyzed portion of the pathway may share similar features with uncatalyzed decomposition of ONOOH, or $ONOO^-$ decomposition mediated by CO_2.[28] That is, covalent bonding of the terminal peroxide oxygen of $ONOO^-$ to an electrophile such as H^+ or CO_2 (or, in this case, a protonated ketone) facilitates O−O bond homolysis and, ultimately, formation of NO_3^-.[28]

In summary, we have identified a new mechanism for catalytic decomposition of $ONOO^-$ by ketones, involving dioxirane intermediates under neutral conditions. In addition, the general-acid-catalyzed component is likely to occur by the O−O bond homolysis pathway, similar to the one suggested earlier by Pryor and co-workers.[16] These pathways avoid the damaging hydroxyl radical which arises from self-decomposition of peroxynitrite, or the highly reactive carbonate radical anion arising from the CO_2-mediated decomposition of peroxynitrite,[13d,e] and might promote a quite different biochemistry in vivo.

Experimental Section

General. All kinetic studies were performed on the Applied Photophysics SX.18MV stopped-flow spectrophotometer with a dead time less than 2 ms. NMR spectra were recorded at ambient temperature on a Bruker AVANCE DPX 300 Fourier transform spectrometer. Gas chromatograms were recorded on a Hewlett-Packard 5890A gas chromatograph using a flame ionization detector (FID), and a capillary column [either a 30 m × 0.32 mm × 0.50 μm HP-INNOWAX (cross-linked poly(ethylene glycol)) polar one, or a 25 m × 0.32 mm × 0.52 μm HP-Ultra 1 (cross-linked methyl silicone gum) nonpolar one].

All chemicals were used as received; 2-chlorocyclohexanone was redistilled before use. Acetonitrile was HPLC or AR grade. All aqueous solutions were prepared in doubly distilled water. Dry dichloromethane was distilled from calcium hydride. Ketones **4** and **7** were prepared according to the literature procedures.[35,36] 1,3-Dimethylcyclohexene was prepared by the dehydration of 2,6-dimethylcyclohexanol in HMPA at 230−240 °C.[37]

Synthesis of Peroxynitrite. Peroxynitrite was synthesized either by the reaction between acidified hydrogen peroxide and sodium nitrite followed by rapid mixing with sodium hydroxide in a quenched flow reactor[38] or by the nitrosation of hydroperoxide anion with 2-ethoxyethyl nitrite in basic medium.[39] The yellow peroxynitrite solution was filtered through a layer of manganese dioxide to remove excess hydrogen peroxide. (EDTA)Na_2 was added to chelate any adventitious metal contaminants. The concentrations obtained by these two methods were 24−70 mM and 12−40 mM, respectively, which were determined spectrophotometrically at 302 nm (ϵ = 1670 M^{-1} cm^{-1}).[40]

Synthesis of Ketone 6 (1,1,3,5-Tetramethyl-4-oxopiperidinium Trifluoromethanesulfonate). 1,3,5-Trimethyl-4-piperidone (1.37 g, 9.7 mmol) was dissolved in dry CH_2Cl_2 (40 mL) under N_2 atmosphere and cooled to 0 °C. MeOTf (1.6 mL, 13.7 mmol, 1.4 equiv) was added via a syringe. The reaction mixture was allowed to warm to room temperature slowly and stirred for 21 h. The white precipitate was filtered and recrystallized (EtOAc/CH_3CN) to provide 2.14 g (72%) of **6** as white plates: mp 183−187 °C (EtOAc/CH_3CN); ^{1}H NMR (300 MHz, acetone-d_6) δ 4.04 (dd, J = 11.2, 6.0 Hz, 2H), 3.78 (t, J = 13.3 Hz, 2H), 3.73 (s, 3H), 3.49 (s, 3H), 3.35 (dqn, J = 12.9, 6.4 Hz, 2H), 1.06 (d, J = 6.5 Hz, 6H); ^{13}C NMR (75.47 MHz, acetone-d_6) δ 204.8, 122.2 (q, J = 321 Hz), 68.2, 56.9, 49.3, 39.2, 10.9; FAB-MS (+ve) m/z 156 (M^+, 100); FAB-MS (−ve) m/z149 ($^-$OTf, 100); HRMS for $C_9H_{18}NO$ (FAB, +ve) m/z calcd 156.1383, found 156.1381; IR (KBr disk) 1733, 1263, 1166, 1031 cm^{-1}. Anal. Calcd for $C_{10}H_{18}F_3NO_4S$: C, 39.34%; H, 5.94%; N, 4.59%. Found: C, 39.22%; H, 5.99%; N, 4.57%.

Kinetic Studies on Peroxynitrite Decomposition. The rate of peroxynitrite decomposition was followed at 302 nm or 265

(35) Denmark, S. E.; Wu, Z. *J. Org. Chem.* **1997**, *62*, 8964−8965.
(36) Yang, D.; Yip, Y.-C.; Jiao, G.-S.; Wong, M.-K. *J. Org. Chem.* **1998**, *63*, 8952−8956.
(37) Monson, R. S. *Tetrahedron Lett.* **1971**, *12*, 567−570.
(38) Beckman, J. S.; Chen, J.; Ischiropoulos, H.; Crow, J. P. *Methods Enzymol.* **1994**, *233*, 229−240.
(39) Leis, J. R.; Peña, M. E.; Ríos, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1298−1299.
(40) Hughes, M. N.; Nicklin, H. G. *J. Chem. Soc. (A)* **1968**, 450−452.

nm at pH below 6. Peroxynitrite (0.25−0.6 mM) in 15 mM NaOH solution was mixed in equal volume with a mixture of CH_3CN and potassium phosphate buffer (v/v 1:9) (final pH = 5.5−10.5) at 20−24 °C. Stock solutions of ketones in CH_3CN were mixed with the phosphate buffer at least 5 min before the addition of peroxynitrite to allow the ketones to reach the hydration equilibria.

Pseudo-first-order rates, $k_{obs}(s^{-1})$, were determined by nonlinear least-squares fitting of stopped-flow data to a single-exponential function with a nonzero offset. Results from five measurements were averaged to obtain each rate constant. Apparent second-order rate constants ($k_{2,app}$) for the reactions of peroxynitrite with ketones were calculated from the slopes of the plots of k_{obs} versus the concentration of the ketone. The self-decomposition rate of peroxynitrite, k_0, was subtracted from k_{obs} before plotting against pH or buffer concentration in the relevant experiments. Data fitting was performed using Origin 3.5 (Microcal Software, Inc.).

Epoxidation of 1,3-Dimethylcyclohexene by Oxone or Peroxynitrite. Oxone (25.1 mg, 41 μmol) or peroxynitrite (41 μmol) was added to a solution of 1,3-dimethylcyclohexene (41 μmol) in 0.18 M phosphate buffer at pH 7.4, containing 3 mL of CH_3CN and 0.204 mmol of ketone **1**or **2** in a total volume of 33 mL. The solution was stirred for 30 min at room temperature, and then it was extracted with CH_2Cl_2 (4 × 5 mL). After drying over anhydrous magnesium sulfate and filtration, the solution was subjected to GC analysis (HP-Ultra 1 column; carrier gas, helium).

GC Analysis of Ketone Recovery. Peroxynitrite was decomposed by incubation with 0.16 M phosphate buffer, containing 1 mL of CH_3CN and ketone in a total volume of 11 mL, for an hour at pH 7.4 and room temperature. Extraction of the reaction mixture with dichloromethane was followed by GC analysis using toluene and 2-methylcyclohexanone as internal standard references for ketone **2** and **3** respectively (HP-INNOWAX column; carrier gas, helium).

Analysis of Nitrite/Nitrate Ratio. Peroxynitrite (0.32 mM) was decomposed by incubation with 0.16 M phosphate buffer, containing 1 mL of CH_3CN and ketone (0−3.22 mM) in a total volume of 11 mL, for an hour at pH 7.4 and room temperature. Measurement of nitrite and nitrate was performed following the ASTM methods.[41] Nitrate was reduced to nitrite by passing nitrate through a copper-coated cadmium column, and nitrite was determined through UV measurement at 543 nm of an azo complex produced from the diazotization and subsequent coupling of sulfanilamide to *N*-(1-naphthyl)ethylenediamine. $[NO_2^-]_0$ and $[NO_3^-]_0$ were determined by decomposing peroxynitrite under acidic or basic/copper(II) conditions following literature methods.[42]

Oxidation of Nitrite to Nitrate by Dimethyldioxirane (DMD). DMD was prepared from the reaction of acetone with Oxone following the reported procedure.[43] The oxidation of nitrite (6.00 mM) to nitrate by DMD (30.00 mM) in acetone was conducted at room temperature. The control experiment was carried out under the same conditions except using acetone only. Nitrite and nitrate were analyzed quantitatively by anion HPLC (Hamilton PRP-X100 anion-exchange column, serial number PRP-X100 2857, and aqueous potassium hydrogen phthalate (2.0 mM) at pH 5.0 as the mobile phase).

Kinetic Studies on the reaction of DMD with Peroxynitrite. Peroxynitrite (0.544 mM) in 0.15 mM NaOH solution was mixed in equal volume with a solution of DMD (0−11 mM) in a mixture of acetone and potassium phosphate at pH 7.4 and 25.6 °C. The pseudo-first-order rates were corrected by subtracting the rates of peroxynitrite/acetone reaction. The reported value of k_2 is the mean of the results from three experiments.

Kinetic Studies on the reaction of DMD with Nitrite. The rate of nitrite oxidation was followed at 356 nm. A solution of DMD (5−80 mM) in a mixture of acetone and potassium phosphate was mixed in equal volume with a nitrite solution (5 mM) at pH 7.4 and 25.6 °C. The reported value of k_2 is the mean of the results from two experiments.

Nitration of 4-Methylphenol. Peroxynitrite (0.9−1.5 mM) was allowed to react with 1 equiv of 4-methylphenol in 0.45 M phosphate buffer at pH 7.4, containing 0−10 equivs of ketone. The reaction mixture was stirred for an hour at room temperature. Then it was extracted with diethyl ether (five times). The extract was dried over anhydrous magnesium sulfate and evaporated to dryness to give a yellow residue. The residue was analyzed by ^1H NMR in $CDCl_3$. The percentage nitration was estimated by the integration ratio of the signals of 2-nitro-4-methylphenol to that of 4-methylphenol in the aromatic region.

Nitration of 4-Hydroxyphenylacetate (4-HPA). An aliquot of peroxynitrite solution (4 μmol) was mixed rapidly with 0.17 M phosphate buffer at pH 7.4, containing 0.4 mL of CH_3CN, 16 μmol of 4-HPA, and 0−15 mM ketone in a total volume of 4 mL. Throughout the course of addition of peroxynitrite, the contents of the reaction mixture were vigorously shaken by using a vortex mixer, and shaking was continued for an additional 10 s at room temperature. The concentration of 3-nitro-4-hydroxyphenylacetate (3-NO_2-4-HPA) was estimated spectrophotometrically at 430 nm (ϵ = 4400 M^{-1} cm^{-1})[12a] after the addition of 1 mL of 1.2 M NaOH solution.

Acknowledgment. We are grateful to the U.S. National Science Foundation and the National Institutes of Health, and the Hong Kong Research Grants Council for financial support of this research. X.-C.W. is a recipient of the University Postdoctoral Fellowship, and J.C. is a recipient of the Hui Pun Hing Scholarship for Postgraduate Research from The University of Hong Kong. We are grateful to Professor William A. Pryor for extensive discussions and to Professor C.-M. Che for the use of the stopped-flow UV spectrometer.

Supporting Information Available: Typical procedure for determination of hydrate/ketone ratios for ketones **1**−**7** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA984358V

(41) *Annual Book of ASTM Standards*; International Library Service: Provo, Utah, 1989; Vol. 11.01, pp 438−446.

(42) Plumb R. C.; Edwards, J. O.; Herman, M. A. *Analyst* **1992**, *117*, 1639−1641.

(43) Murray, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847−2853.