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Nitration and hydroxylation of substituted phenols by peroxynitrite. Kinetic feature and an alternative mechanistic view

Nobuaki Nonoyama,[a] Kazuhiko Chiba,[a] Kaori Hisatome,[a] Hitomi Suzuki [a,*] and Futoshi Shintani [b]

[a]*Department of Chemistry, School of Science, Kwansei Gakuin University, Uegahara, Nishinomiya 662-8501, Japan*
[b]*Department of Neuropsychiatry, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan*

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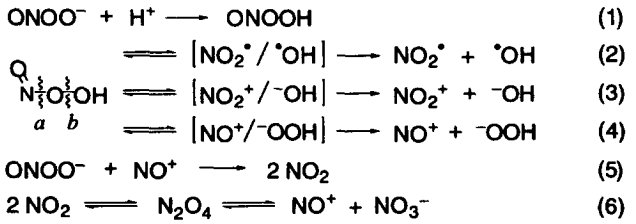
Abstract

The reaction of peroxynitrite ($ONOO^-$) with a series of *para*-substituted phenols has been examined in aqueous phosphate buffer and acetonitrile solutions. Major products were the corresponding 2-nitro derivative and the 4-substituted catechol. Kinetic study showed good correlation with Hammett σ_p^+ parameters and reduction potentials, suggesting the possible one-electron transfer process involving the nitrosoniun ion (NO^+) as initial electrophile generated from peroxynitrous acid.

Keywords: phenol; nitration; mechanism; nitrous acid; nitrous acid derivatives.

Peroxynitrite ($ONOO^-$) is a cytotoxic species that is considered to form from nitric oxide (NO) and superoxide ($O_2^{\cdot -}$) in biological systems.[1] The toxicity of this compound is attributed to its ability to oxidize, nitrate, and hydroxylate biomolecules. For instance, tyrosine is nitrated to form 3-nitrotyrosine,[2,3] phenylalanine is hydroxylated to yield *o*-, *m*- and *p*-tyrosines, cysteine is oxidized to give cystine,[4] glutathione is converted to *S*-nitro or *S*-nitroso derivative,[5] and catecholamines are oxidatively polymerized to melanin.[6] Lipids are also oxidized[7] and DNA can be scissored by peroxynitrite.[8] Despite considerable literature on the various modes of reactions induced by peroxynitrite, the kinetic and mechanistic aspects of these transformations remain to be clarified.



* Corresponding author. E-mail: hsuzuki@kwansei.ac.jp

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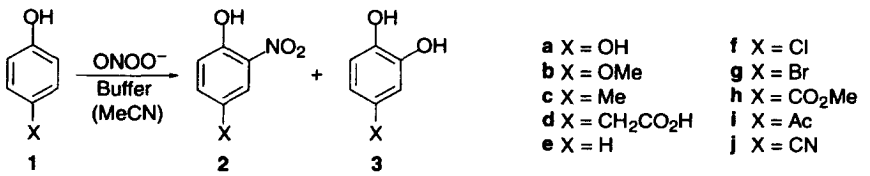
Peroxynitrite is a stable anionic species in alkaline solution. At physiological pH, it is rapidly protonated to form peroxynitrous acid (ONOOH), which triggers oxidation, nitration, and hydroxylation reactions either through the homolytic decomposition to OH and NO_2 radical species (Eqs. 1 and 2), or by the isomerization to nitric acid via a high energy intermediate $ONOOH^*$.[9] As to the former mode of decomposition, there exist contradictory evidences for[10] and against[11] the OH radical generation. In order to get an insight how peroxynitrite attacks activated aromatic substrates at the initial stage of the reaction, we have examined the kinetic features of the reaction of peroxynitrite using a series of *para*-substituted phenols.[12] The results obtained showed good correlation with Hammett σ_p^+ parameters and reduction potentials in both phosphate buffer and acetonitrile solutions.

Peroxynitrite was obtained as 0.78–0.96 M solutions according to the following procedure;[13] to a solution of sodium azide (10 mmol) in water (5 cm^3) at pH 12 was passed ozone at a rate of 15 mmol h^{-1} for 1 h and the concentration of the resulting peroxynitrite was determined by characteristic absorption at 302 nm (ε=1670 M^{-1} cm^{-1}). The typical reaction of peroxynitrite with *p*-cresol **1c** was carried out by adding 1 equiv. of peroxynitrite to a solution of **1c** in phosphate buffer (pH=7.0) and allowing the mixture to stand at 20–25°C. After 1 h, the conversion of cresol reached 16% and one-third of the total conversion was nitration to **2c** (6%). A trace amount (<1%) of hydroxylation product **3c** was also detected (Scheme 1). A similar action of 1 equiv. of peroxynitrite on *p*-chlorophenol **1f** led to 14% consumption and the corresponding nitration and hydroxylation products **2f** and **3f** obtained were 4% and <1%, respectively. Low material balance could be attributed to further degradation of initial hydroxylation products.



Scheme 1.

In order to look closer into the reactivity of peroxynitrite toward phenols, we have carried out competitive reactions for a series of substituted phenols according to the following typical procedure: *p*-cresol **1c** (0.1 mmol), *p*-bromophenol **1g** (0.1 mmol), and *p*-nitrobenzoic acid (0.01 mmol) as an internal standard were dissolved in 0.1 mol dm^{-3} phosphate buffer (10 cm^3) or acetonitrile (10 cm^3), to which was added a total of 2 equiv. of peroxynitrite solution five times at a regular interval of 3 min. The reaction was instantaneous under these conditions. After every addition, an aliquot was drawn from the reaction mixture and analyzed by HPLC. The results obtained are compiled in Table 1. The profiles of the competition between **1c** and **1g** are shown in Fig. 1, where the slope of each line corresponds to the respective consumption rate, i.e., relative rate. The reaction is facilitated by electron-donating substituents and retarded by electron-withdrawing ones in both aqueous and organic media. A logarithmic plot of the relative rates against Hammett constants[14] showed a good linear correlation with σ_p^+, but failed to produce this relationship with σ_m (Fig. 2). The plot of logarithmic relative rates in aqueous media versus the reduction potentials of *p*-substituted phenoxy radicals also showed a good correlation (Fig. 3). A similar correlation between σ_p^+ and one-electron reduction potentials has previously been reported for a series of *para*-substituted phenoxy radicals.[15] Poor correlation between the logarithmic relative rates and σ_m disfavors initial electrophilic attachment of NO_2^+ ion to the position *meta* to the substituent X.

On the basis of the results obtained, we may take that *para*-substituted phenols undergo electrophilic attack by a highly reactive species in situ generated from peroxynitrous acid. The decomposition of peroxynitrous acid has generally been accepted to occur through either homolysis or heterolysis of the

Table 1
Relative rates and Hammett parameters of *p*-substituted phenols **1a–j**

	X	k_X/k_{Me} [a]		Hammett σ value		E^{0} [e]
		Buffer	MeCN	σ_m	σ_p^+	
1a	OH	8.2[b]	11[b]	0.12	-0.92	0.46
1b	OMe	3.7	5.3	0.12	-0.78	0.72
1c	Me	1.0	1.0	-0.07	-0.31	0.87
1d	CH_2CO_2H	0.60			-0.01	
1e	H	0.34		0.00	0.00	
1g	Br	0.30	0.26	0.39	0.15	0.96
1h	CO_2Me	0.077[c]	0.10[c]	0.37	0.49	
1i	Ac	0.090[c]		0.38		1.06
1j	CN	0.056[d]	0.067[c]	0.56	0.66	1.17

[a] Competitive reactions were carried out at least three times in phosphate buffer and acetonitrile. [b] Calculated from competition with **1b**. [c] From competition with **1g**. [d] From competition with **1h**. [e] One-electron reduction potentials of *p*-substituted phenoxy radicals.[15]

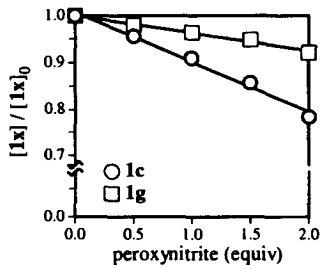


Figure 1. Competitive reaction between **1c** and **1g**

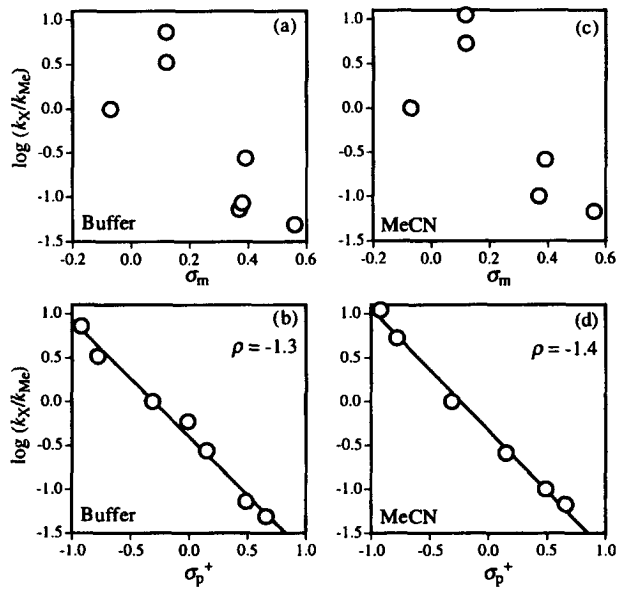


Figure 2. Relation between Hammett constants σ_m and σ_p^+, and logarithmic relative rate in phosphate buffer and MeCN

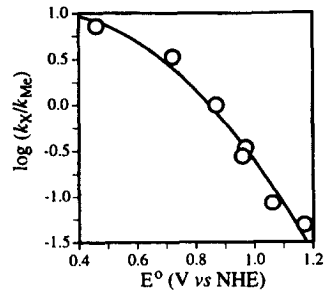
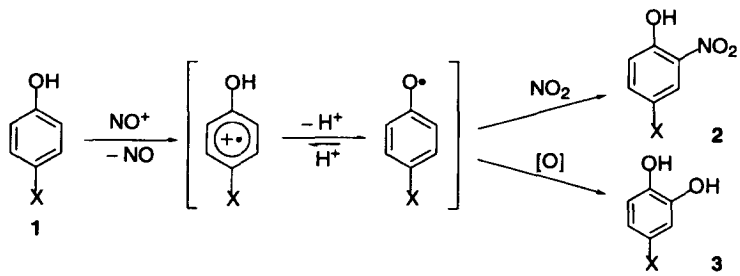


Figure 3. Logarithmic relative rate versus the reduction potential of phenoxy radicals

O–O bond (Eqs. 2 and 3; scission *b*). However, we would like to suggest an additional possibility, in which the N–O bond is heterolytically cleaved to form NO^+ and HOO^- ions (Eq. 4; scission *a*). The resulting NO^+ ion oxidizes phenols to the corresponding radical cations or captures peroxynitrite to form two molecules of NO_2 (Eq. 5). The radical cations are deprotonated to give the phenoxy radicals, which either combine with NO_2 to give nitrophenols **2** or are oxidized to form substituted catechols **3** (Scheme 2). In polar organic media, NO_2 goes into equilibrium with NO^+ and NO_3^- (Eq. 6) and in water it decomposes to nitric acid and NO. In accordance with this mechanistic scheme, we have found that 1,4-naphthoquinone can be converted into the 2,3-epoxide and *N,N*-dimethylaniline can be demethylated under similar conditions, endorsing the probable involvement of HOO^- and NO^+ ions as reactive intermediates.[16] Both the epoxidation of naphthoquinones by an HOO^- ion[17] and the demethylation of *N,N*-dimethyl aromatic amines by an NO^+ ion[18] have been well established.



Scheme 2.

Acknowledgements

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