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Kinetic and Mechanistic Studies on the Reactions of Peroxynitrite with Estrone and Phenols[1]

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Abstract—Reaction of peroxynitrite with estrone, a female sex hormone, was carried out in tetrahydrofuran (THF)/H_2O (8 : 2) basic solutions. The major products are the corresponding *o*-quinone, nitroestrone and 2,2'-biphenol. The reaction of phenols with peroxynitrite under the same conditions leads also to the formation of quinones, nitrophenols and biphenols. The major mechanistic pathways take place via a one-electron oxidation of the phenolic group leading to the formation of a phenoxyl radical intermediate which is further oxidized by peroxynitrite (or by intermediates generated from peroxynitrite) to give the final products. A Hammett correlation of the rate constants for the oxidation of meta substituted phenols support a radical mechanism. The kinetic isotope factors rule out the involvement of a C–H bond cleavage in the rate-determining step. A multistep mechanism showing major intermediates involved in the reaction and the final products has been proposed.

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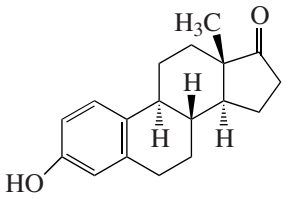
INTRODUCTION

Nitric oxide (NO) is produced by many diverse cell types, and can interact with a variety of molecules and functionalities [1]. The interaction of NO with superoxide to form peroxynitrite has received a great deal attention since reactions occur at a rate near the diffusion-controlled limit (Eq. (I)) [2, 3] (the rate constant is (4–7) $\times 10^2$ l mol^{-1} s^{-1}):

$$NO + O_2^- \longrightarrow ONOO^-. \quad (I)$$

The peroxynitrite anion ($ONOO^-$) is protonated at near-physiological pH values (p$K_a \approx 6.8$), forming peroxynitrous acid (ONOOH), which decays rapidly ($t_{1/2} < 1$ s) [4]. The anion can be preserved for several weeks in strongly basic media at –18°C [5]. Peroxynitrite is produced in biosystems [6], and it has been found to be cytotoxic [7]. It has also been proposed as an oxidant in reactions associated with vascular and ischemic injuries and as agent in development of atherosclerotic lesions [8–10]. Peroxynitrite is a powerful oxidant. A variety of pathways for oxidations with peroxynitrite are conceivable involving heterolytic and homolytic cleavage of O–O and N–O bonds [11–13]. Homolytic cleavage of O–O bond produces the highly reactive $^{\bullet}OH$ radical. It has been reported that this path is only feasible in the presence of a reductant, such as metal ion or organic substrate, which is oxidized rapidly by $^{\bullet}OH$ radical [14]. Many organic, inorganic and biological substrates, such as trolox (Vitamin E), ascorbic acid (Vitamin C), phenols, carbonyl compounds, phosphines, and heme protein, have been oxidized with peroxynitrite, and their reactions were investigated under different conditions [8, 15–20]. In addition to oxidation, peroxynitrite has been involved in nitration of aromatic compounds, such as tyrosine [21]. Nitration of tyrosine by peroxynitrite in the presence of CO_2 (as a catalyst) leads to the formation of 3-nitrotyrosine [22]. Formation of 3-Nitrotyrosine was taken as an evidence for peroxynitrite-mediate damage in a variety of diseases states, including Alzheimer's and Parkinson's diseases [23, 24]. A recent study [25] reported that oxidation of phenol by peroxynitrite as catalyzed by CO_2 at pH ≥ 10 leads to a diamagnetic intermediate which reacts with phenol to form 2,2'-biphenol, nitrophenol and benzoquinone.

Estrone, estradiol, and estriol are all estrogens that are important to the human body. They belong to the steroid hormones, and are primarily responsible for the growth of female characteristics in puberty, and regulating the menstrual cycle. Estrone (**I**) is a naturally occurring weak estrogenic hormone secreted by the mammalian ovary which is synthesized and used to treat estrogen deficiency [26].



I

The steroid hormones are chemically very stable and are excreted by humans and animals in the free form or as conjugates [27]. Primary steroid hormones,

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such as estrone and estradiol, are lipophilic and slightly soluble or insoluble in water. It has been reported that sex hormones, notably estrogenes, utilize nitric oxide to dilate pelvic blood vessels and engorge genital erectile tissues [28a]. In addition, female sex steroids enhance the synthesis and release of nitric oxide (NO) and neuropeptides [28b]. A study on the effect of sex hormones on the vasoconstrictor response to electrical field stimulation as well as neuronal NO modulation of this response found that superoxide and peroxynitrite are generated in situ [29]. These findings show a possibility of interaction between estrogens and peroxynitrite, and have motivated us to study the reaction of estrone with peroxynitrite.

Estrone has three different functionalities that may react with peroxynitrite; the aromatic, the phenol and the ketone. In this work, we studied the reaction of peroxynitrite with estrone, in mixed organic/aqueous basic solutions, to determine the reaction products (oxidation versus nitration) and perform kinetic studies on the reaction of estrone with peroxynitrite. In order to propose suitable mechanism(s) for this reaction, the kinetics of the reactions of different phenols with peroxynitrite were also investigated under the same conditions.

EXPERIMENTAL

Material and Methods

Water was purified by a Milipore-Q deionization system. Carbon tetrachloride, THF, diethyl ether and CH_2Cl_2 (HPLC grade, Aldrich) were used without further purifications. Commercial solution of Bu_4NOH, Aldrich (1.52 M) in water was used. The following chemicals were used as received without any further purification: Estrone (99% Aldrich), 3-chlorophenol (Scharlau), 3-nitrophenol (Loba Chemie), *m*-cresol (Scharlau), *p*-cresol (Scharlau), 2,6-di-tert-butyl-4-methylphenol (Lancaster), phenol (GCC), KOH and NaOH (99% Fisher), D_2O (99% Aldrich) and C_6D_5OH (99% Aldrich). Stock solutions of estrone and each phenol of 0.1–0.2 M were prepared in THF.

FT-IR measurements were carried out using a Nicolet-Impact 410 FT-IR spectrometer. The NMR spectra were obtained on a Bruker DXP-400 spectrometer in $CDCl_3$ solutions. The UV-vis spectra were recorded using Shimadzu UV-2401-PC spectrometer. Quantitative GC measurements were carried out using a Hewlett-Packard Model 5890 gas chromatograph equipped with a FID, a Supelco column Alphadex 120 and a Hewlett-Packard integration unit Model HP 3396.

Peroxynitrite Preparation

Peroxynitrite was prepared using slight modification to literature methods [2, 15–18]:

Acidified hydrogen peroxide (10 mL, 1.2 M) solution was added to a nitrite (10 mL, 1.0 M) solution at 0°C, and the reaction was immediately quenched with 5 mL strong basic solution (either 6 M NaOH or 1.52 M Bu_4NOH). The excess hydrogen peroxide was decomposed by MnO_2 (0.5–1.0 g), and the yellow solution was filtered and kept in strong alkaline media at −18°C. The concentration of the stock solution was determined daily, prior to each experiment, by spectrophotometric method using $\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$ in 0.1 M NaOH [18].

Stoichiometric Studies

Stoichiometrics of the peroxynitrite reactions, each with the oxidant (peroxynitrite) in excess were determined in THF/H_2O (8 : 2) solution with 0.02 M KOH by adding deficient quantities of the reductant (0.2–0.4 mM) (estrone or phenol) to a known concentration of the peroxynitrite (1.0 mM), in a spectrophotometric cell (optical path is 1.0 cm and total volume is 3.0 mL) waiting 2–5 min, and then measuring the decrease in the absorbance at 302 nm. The change in peroxynitrite concentration was calculated from the absorbance change using $\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$. These changes were compared to those occurring when peroxynitrite was treated with excess reductant and corrected to the absorbance changes due to the self-decomposition of peroxynitrite under the same conditions.

Reaction Products from the Reaction of Estrone

Estrone (0.27 g, 1.0 mmol) was mixed with peroxynitrite (10 ml, 2.0 mmol) solution in THF/H_2O (8 : 2) with KOH (0.02 M). The yellow solution was stirred for ~10 min. The solution was immediately acidified with 6.0 M HC1 to pH ~ 1, and THF was removed at 30°C under vacuum. The organic products were extracted from the aqueous solution with diethyl ether (5 × 10 mL), and dried over $MgSO_4$. After a quantitative GC analysis of the product mixture, the ether was removed completely at ~25°C. Major products were isolated by column chromatography (silica gel, 70–230 mesh) using CH_2Cl_2, and characterized by UV-visible, IR, and NMR spectroscopy.

Quantitative GC Analysis

Phenol or estrone (0.5–1.0 mM), 0.05 mM *p*-dichlorobenzene (internal standard) and 0.2 mM peroxynitrite were mixed in 5 mL THF/H_2O (8 : 2) solution with KOH (0.02 M). After the reaction was completed (5–10 min), the solution was acidified to pH ~ 1 with 6.0 M HCl. The organic solvent (THF) was removed under vacuum and the organic products were extracted with diethyl ether (5 × 3 mL), dried over $MgSO_4$, and run neat in a GC column. The products' yields were calculated from the integration of the peaks relative to the integration of the standard (*p*-dichlorobenzene). A blank experiment with *p*-dachlorobenzene and peroxynitrite (without estrone or phenol) was carried out under the same conditions and was used as a reference.

Kinetic Studies

The reactions were carried out in a mixed THF/H_2O (8 : 2) solvent. Kinetic data were collected by following the absorbance change due to the decrease in peroxynitrite at 302 nm or due to the formation of a biphenoquinone intermediate at 400 nm using a Shimadzu UV-2401 spectrophotometer. Quartz cuvettes with optical path of 1.0 cm (V_T = 3.0 mL) were used. The temperature was kept constant at 25.0 ± 0.5°C throughout the entire series of experiments. The solution pH was regulated by adding measured quantities of KOH or $(Bu)_4NOH$, and was checked with a pH-meter, and the ionic strength was maintained by sodium nitrate. All experiments were carried out with 0.1 M $NaNO_3$, with the exception of the ionic strength studies. For kinetic runs, reaction mixtures were prepared in the spectrophotometer cell with the last reagent added being peroxynitrite in basic solution, thus minimizing the loss of peroxynitrite due to the self-decomposition path. All data analysis were carried out with the KaleidaGraph computer program.

RESULTS AND DISCUSSION

Estrone and most phenols are insoluble or slightly soluble in aqueous solution. In contrast, peroxynitrite, is not stable in organic solvents, such as acetonitrile and methanol [7]. The half-life is too short (less than 1 min) to carry out reactions or kinetic studies by conventional methods involving peroxynitrite in organic solvents at room temperature. In addition, methanol may be oxidized by radicals generated throughout peroxynitrite reduction. The reaction of peroxynitrite with organic substrates which are insoluble in aqueous solution can be carried out in mixed organic/aqueous media, such as solution mixtures of THF/H_2O. A basic solution was used to insure a minimum involvement of peroxynitrous acid in these reactions, which decomposes rapidly in aqueous solutions (half-life for its self-decomposition at 25°C is about 1 s) [5].

Self-Decomposition of Peroxynitrite in Basic THF/H_2O Solutions

Since this is the first report on studying peroxynitrite reactions in this media, the self-decomposition was investigated under different basic conditions. The reactions were followed at 302 nm with fixed concentration of peroxynitrite and varying the base (KOH) concentration in the range 0.005–0.1 M. The changes in the absorbance with time at 302 nm due to the loss in peroxynitrite, Fig. 1, fit very well to exponential decay equation, Eq. (1), indicating that the rate of decomposition in this range of pH is first-order in peroxynitrite.

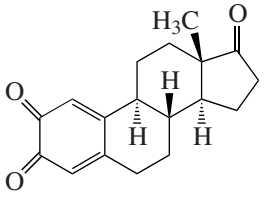
$$A_t = A_\infty + (A_0 - A_\infty)\exp(-k_d t), \quad (1)$$

where A_t, A_0, and A_∞ are the absorbance at anytime, initial and final absorbance, respectively, and k_d is the first-order rate constant [30].

The first-order rate constants, k_d, for the decomposition of peroxynitrite in THF/H_2O (8 : 2) at different concentration of the base were determined by using Eq. (1). The variation of $\log k_d$ with pOH is linear in the pH range 11–13, and the decomposition rate decreases with increasing [OH^-], Fig. 2.

Reaction of Estrone with Peroxynitrite. The Reaction Products

The reaction of estrone with peroxynitrite leads to the formation of many products. The major products were identified by UV-vis, IR and NMR spectroscopic methods. The products overall yield of ~50% was calculated based on peroxynitrite concentration. The major product was the corresponding *o*-quinone (**II**) (25–30%). Nitroestrones (~10%), and the corresponding 2,2'-biphenol (~6%), were also formed, see Table 1. In addition, other minor products were observed in very low yields (<3% each), and were difficult to be identified. The ratio of 1-nitroestrone to 3-nitroesrtone was about 3 : 2. The NMR data for the major product (**II**) showed significant peaks at 6.1 and 5.9 ppm as expected for the protons at C1 and C4 respectively. In addition, the phenolic proton disappears and the newly formed ^{13}C peaks at 185 ppm proved the formation of the ketone functionality at C2 and C4. These data was in good agreement with the literature values [31].



II

The relative yields (Table 1) of the products are sensitive to the reaction conditions including the estrone/peroxynitrite ratio. The yield of the *o*-quinone, **II**, increases with increasing the pH and with the estrone/peroxynitrite ratio, and its decomposition increases with the solution pH and the concentration of H_2O in the solution. Therefore, the solution was immediately acidified with HCl after the reaction is completed so as to minimize the product decomposition. The highest yield of the *o*-quinone (**II**) was obtained in 9 : 1 (THF : H_2O) solution at pH 12.3.

The oxidation of phenols, such as phenol and *m*-cresol, gives quinones, nitrophenols, and biphenols as major products as shown in Table 1.

The Reaction Stoichiometry

Stoichiometric studies, Table 2, have shown that each mole of estrone consumes ~1.3–1.4 mol of peroxynitrite. Peroxynitrite is a two-electron oxidant when converted to nitrite. Estrone is oxidized by 4 electrons when the quinone (**II**) is formed and by 2 electrons

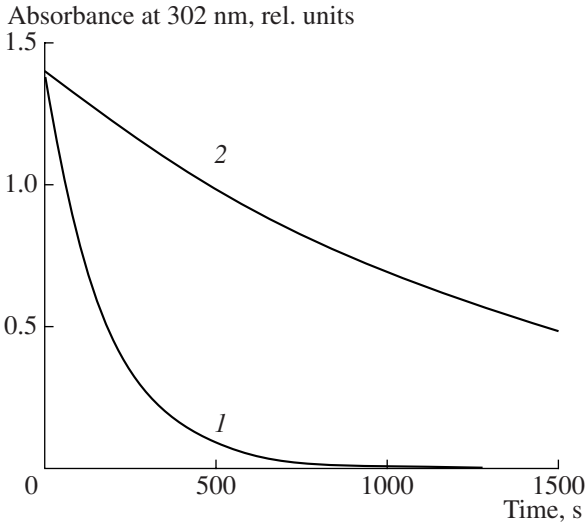


Fig. 1. The absorbance-time curves for the decomposition of peroxynitrite (0.85 mM) in basic solutions; [KOH] = 0.007 (*1*), 0.1 M (*2*). The change in absorbance was recorded at 302 nm due to the loss of peroxynitrite (ε_{302} = 1670 M^{-1} cm^{-1}) in THF/H_2O (8 : 2) at 25°C.

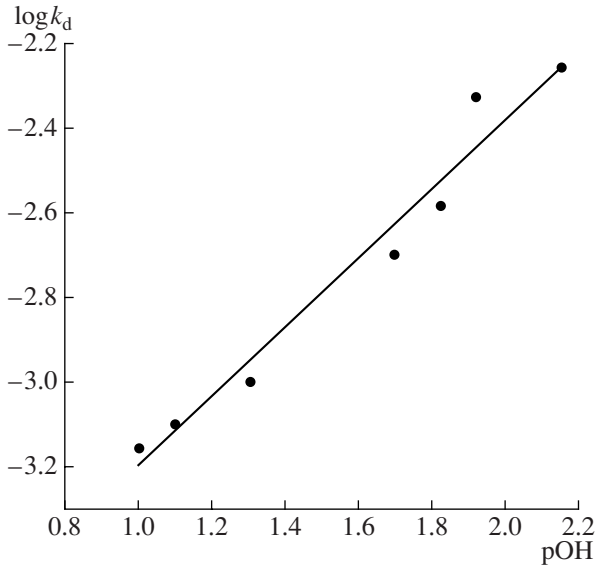
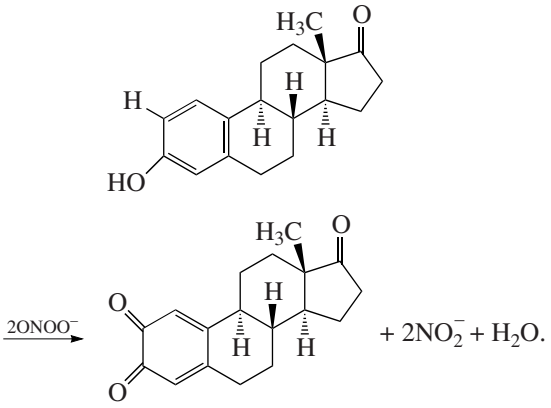


Fig. 2. Variation of the self-decomposition rate constant (k_d) of peroxynitrite with pOH in THF/H_2O (8 : 2) at 25°C.

when the biphenol and the nitroestrone are formed. Therefore, the average stoichiometry for this reaction depends on the relative yields of the products, and should be between 1–2. For the major path, the overall reaction can be written as follow:



(II)

$$+ 2NO_2^- + H_2O.$$

Radical Formation

Phenolic compounds have been oxidized by one- (hydrogen-atom transfer) and two-electron (hydride transfer) processes [32]. The one-electron oxidation leads to phenoxyl radicals which are usually very reactive. Radicals generated from 2,4,6-trialkyl-substituted phenols, such as 2,6-di(tert-butyl)-4-methylphenol, are relatively stable and can be used to detect a one-electron oxidation of phenols. The oxidation of 2,6-di-tert-butyl-4-methylphenol leads to the formation of a relatively stable yellow-greenish phenoxyl radical, Eq. (III), which was identified by its characteristic UV-visible spectrum (Fig. 3, λ_{max} = 377 and 398 nm) [33]. Formation of phenoxyl radical may indicate that estrone and phenols were oxidized initially by a one-electron step. This is also supported by the correlation of the rate constants of oxida-

Table 1. The percentage yields of the products from the reactions of estrone and phenol with peroxynitrite in THF/H_2O (8 : 2) in 0.02 M [OH^-] at 25°C

Reductant, mM	All products, %*	Quinones, %*	Nitration, %*	Biphenols, %*	Others, %*
Estrone					
0.5	53	25**	11	6	11
1.0	49	28**	8	5	8
Phenol					
0.5	57	18***	17	9	13
1.0	59	19***	15	11	14

Note: *Calculated based on the initial concentration of peroxynitrite.

**Only the corresponding *o*-quinone was obtained.

***Mixture of *p*-quinone and *o*-quinone.

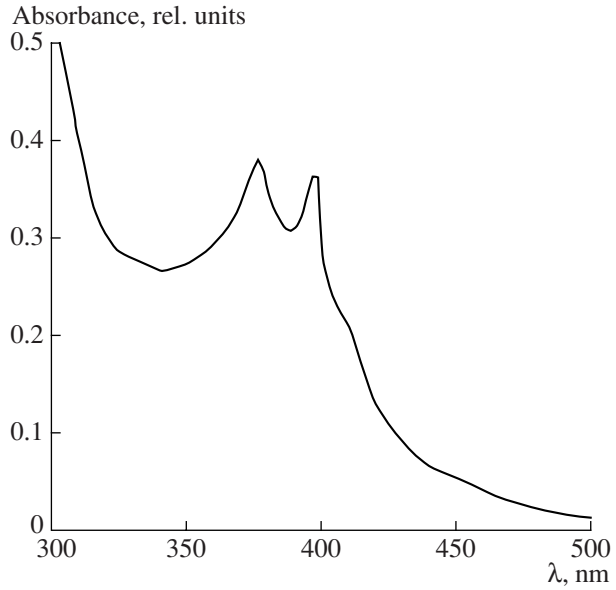
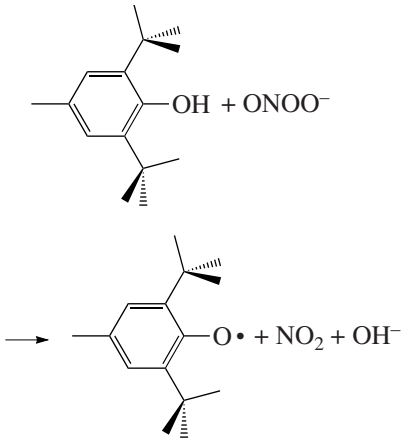


Fig. 3. UV-vis spectrum of the phenoxyl radical (λ_{max} = 377 and 398 nm) from the reaction of peroxynitrite (0.2 mM) with 2,6-di-tert-butyl-4-methylphenol (0.5 mM) in THF/H_2O (8 : 2) with 0.02 M [OH] at 25°C.

tion of different meta substituted phenols with σ according to Hammett equation (as shown later).



(III)

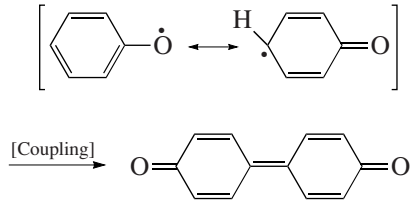
Table 2. Stoichiometry of the reaction of estrone with peroxynitrite. The reactions were carried out in the presence of 0.02 M [OH^-] in THF/H_2O (8 : 2) at 25°C

$[ONOO^-]_o$, mM	[estrone], mM	$[ONOO^-]^a_{reacted}$, mM	Δ[$ONOO^-$]/Δ[estrone]
1.0	0.20	0.26	1.3
1.0	0.30	0.41	1.4
1.0	0.40	0.55	1.4

Note: [a] Corrected to the loss of peroxynitrite due to the self-decomposition.

Phenoxyl Radicals Coupling

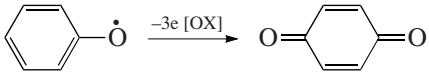
If the phenol is unsubstituted at the ortho or at the para position, a yellow intermediate (that has a maximum absorption close to ≈400 nm characterized by a high molar absorptivity) was observed. This absorption band is typical for 4,4'-biphenoquinone, **III**, which can be formed by the coupling of phenoxyl radicals followed by oxidation, Eq. (IV), [34].



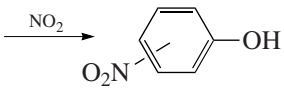
(IV)

III

This intermediate, **III**, was observed because of its very high molar absorbtivity $\varepsilon_{400} \approx 5 \times 10^4$ M^{-1} cm^{-1} and only minor amounts produced [34]. The analysis of the products showed that most of the phenoxyl radicals react with peroxynitrite (or NO_2 radical intermediate) to yield quinones and nitrophenols (Eqs. (V)).



(Va)



(Vb)

Kinetics. Oxidation of Estrone

The change in absorbance with time (at 400 nm) for a solution initially contained 0.2 mM peroxynitrite and excess concentration of estrone (1.0 mM) in THF/H_2O (8 : 2) with 0.02 M n-$(C_4H_9)_4NOH$ at 25°C is shown in Fig. 4. The absorbance-time curves consist of two stages, Fig. 4. First, an increase in the absorbance due to the formation of an intermediate (probably similar to 4,4'-biphenoquinone intermediate, (**III**), and then a decay due to its reaction or decomposition. Since estrone was present in excess with respect to peroxynitrite, the rate of formation of the intermediate (first stage) followed first-order kinetics, meaning that the reaction is first-order in the peroxynitrite concentration. The pseudo-first order rate constants for the two stages were obtained by fitting the data to a bi-exponential equation (Eq. (2)).

$$A_t = A_\infty + \alpha\exp(-k_f t) + \beta\exp(-k_s t), \qquad (2)$$

where A_t, and A_∞ are the absorbance at a given time t and the final absorbance, respectively. α and β are constants related to the molar absorptivities of reactants, products and intermediates [30]. The rate constants k_f and k_s are the first-order rate constants for the first and second stages, respectively.

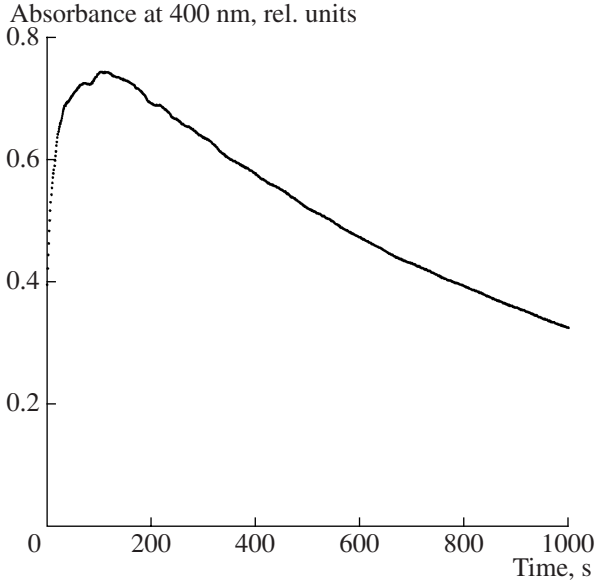


Fig. 4. The absorbance changes with time at 400 nm during the oxidation of estrone (1.0 mM) by peroxynitrite (0.2 mM) in THF/H_2O (8 : 2) with 0.02 M [OH^-] at 25°C.

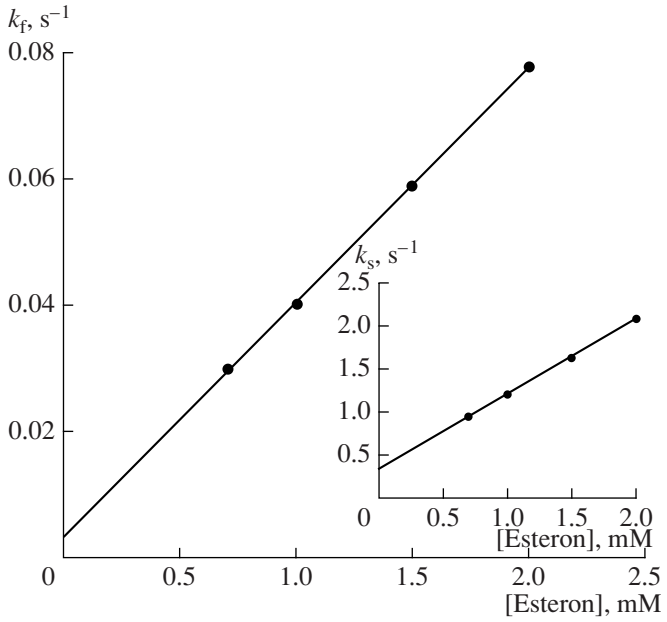


Fig. 5. A plot of the observed-first-order rate constant of the first stage (k_f) for the reaction of estrone and peroxynitrite (0.2 mM) against the concentration of estrone in THF/H_2O (8 : 2) with 0.02 M [OH^-] at 25°C. The inset shows the variation of the observed-first-order rate constant of the second stage (k_s) with the [estrone].

The dependence of the reaction rates on estrone concentration was studied by varying the concentration (in the range 0.7–2.0 mM) in the presence of limiting concentration of peroxynitrite (0.2 mM). Figure 5 shows variation of the pseudo-first-order rate constant (of the first stage) with the concentration of estrone. The reaction was found to be first-order in [estrone] as the plot of the rate constants against the concentration of estrone is linear (Fig. 5). The slope in Fig. 5 is the second-order rate constant which equals 37.1 ± 0.7 M^{-1} s^{-1}.

The Decay of the Intermediate

The subsequent absorbance decrease (second stage) was also dependent on the concentration of estrone. The pseudo-first-order rate constants (k_s) obtained by fitting the biphasic curves to Eq. (2) vary linearly with the concentration of estrone, Fig. 5 inset. The slope is the second-order rate constant for the decay of the intermediate in the second stage and was found to be 0.9 ± 0.1 M^{-1} s^{-1} at 25°C in THF/H_2O with [OH^-] = 0.02 M.

Oxidation of Phenols

The second-order rate constants for the oxidation of different phenols with peroxynitrite were determined by studying the variation of the pseudo-first order rate constants with the phenol concentration, as described previously for the reaction of estrone with peroxynitrite. The pseudo-first order constants of both stages, first and second stages of the absorbance-time curves, were found to increase linearly with the phenol concentration. The first stage (k_f) represents the formation of the biphenoquinone intermediate (**III**), and the second stage (k_s) for its decomposition. Table 3 summarizes the values of k_f and k_s for the reaction of different phenols with peroxynitrite.

The Substituent Effect

The electronic effects of a meta substituent (using phenol, *m*-cresol, *m*-chlorophenol and *m*-nitrophenol) on the rate constants have been correlated by the Hammett equation, $\log(k_X/k_H) = \rho\sigma$ [35]. The plot of $\log(k_X/k_H)$ against σ is linear (Fig. 6), with a slope $\rho = -1.9 \pm 0.2$. This value is consistent with the values obtained for the oxidation of phenols by one-electron processes in the rate-determining step yielding the phenoxyl radical intermediates [32]. Reactions in which a two-electron transfer is involved in the rate-determining step are characterized by more negative ρ values ($\rho < -4$) [36]. Estrone is meta and para dialkylsubstituted phenol, therefore, the rate constant for the oxidation of estrone by peroxynitrite is larger than that for the oxidation of *m*-cresol (Table 3). The electron-donating groups in the meta and para positions in the case of estrone stabilize the phenoxyl radical intermediate in the rate-determining step leading to a smaller energy of activation, and therefore, a faster reaction.

Kinetic Isotope Effects

A slight kinetic isotope effect (k_H/k_D = 1.1) was observed upon changing the solvent from HH_2/THF to

Table 3. Rate constants for the oxidation of estrone and phenols by peroxynitrite (k_f) and for the decomposition of the biphenoquinone intermediate (k_s). In the presence of 0.02 M [OH^-] in THF/H_2O (8 : 2) at 25°C

Reductant	k_f, M^{-1} s^{-1}	k_s, M^{-1} s^{-1}
Estrone	37.1 ± 0.7	0.9 ± 0.1
Phenol	15.0 ± 0.2	0.6 ± 0.2
o-Cresol	32.4 ± 0.6	0.8 ± 0.2
m-Cresol	23.2 ± 0.2	0.8 ± 0.1
m-Chlorophenol	5.1 ± 0.3	0.5 ± 0.1
m-Nitrophenol	0.7 ± 0.1	0.4 ± 0.1

D_2O/THF (8 : 2) in 0.05 M KOH solution. They relate the rate constants for the reaction of *m*$CH_3C_6H_5OH$ with $ONOO^-$ in H_2O/THF to the reaction of *m*$CH_3C_6H_5OD$ with $ONOO^-$ in D_2O/THF. An increase of the kinetic isotope effect was observed when the base concentration was decreased (k_H/k_D = 1.2 when [OH^-] = 0.005 M, and k_H/k_D = 1.4 when [OH^-] = 0.002 M). This may indicate that the reaction involves O–H bond breaking in a rate-determining step. However, at this range of pH, most *m*-$CH_3C_6H_5OH$ (pK_a = 10.08) [37] is in the deprotonated form, *m*-$CH_3C_6H_5O^-$, and only small fraction of *m*-$CH_3C_6H_5OH$ is present in the solution. At the lowest pH, [OH^-] = 0.002 M, the amount of the protonated form (*m*-$CH_3C_6H_5OH$) in the solution is only ~6%, indicating that the phenolate anion is the major reductant. Indeed, this has been confirmed by the effect of the solution ionic strength on the reaction rates which suggests the involvement of similar charge species (phenolate and $ONOO^-$) in the rate-controlling step. The oxidation of C_6D_5OH and C_6H_5OH with peroxynitrite in basic solution showed no kinetic isotope effect, $k_H/k_D \approx 1.0$. This result provides direct evidence that the oxidation of phenols by peroxynitrite does not involve a C–H bond cleavage in the rate-determining step.

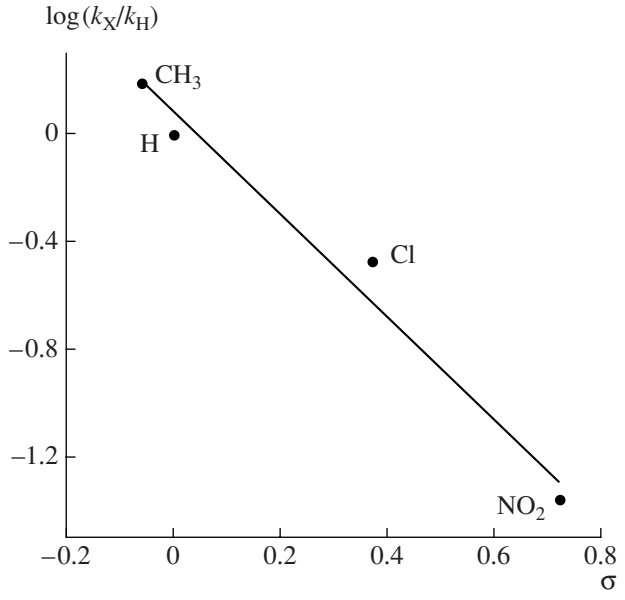


Fig. 6. A plot of $\log(k_X/k_H)$ against σ for the reaction of meta-substituted phenols with peroxynitrite in THF/H_2O (8 : 2) with 0.02 M [OH^-] at 25°C. The slop ρ = –1.9 ± 0.2.

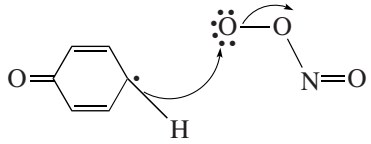
Ionic Strength Effect

Kinetic experiments on the ionic strength effect were carried out for the oxidation of estrone by peroxynitrite in THF/H_2O (8 : 2 vol %) solutions with 0.02 M [OH^-] at 25°C. The ionic strength was varied in the range 0.01–1.0 M using $NaNO_3$. The kinetic profiles were all biphasic (similar to the one shown in Fig. 4). The rate constant k_f (first stage) was increased with increasing the ionic strength. This is as expected from the charge types involved. In basic solutions (pH >12), estrone exists mainly in the deprotonated form (pK_a ≈ 10.5) [38], and therefore, the reaction is assumed to occur between two negatively charged species ($ONOO^-$ and the phenolate form of estrone).

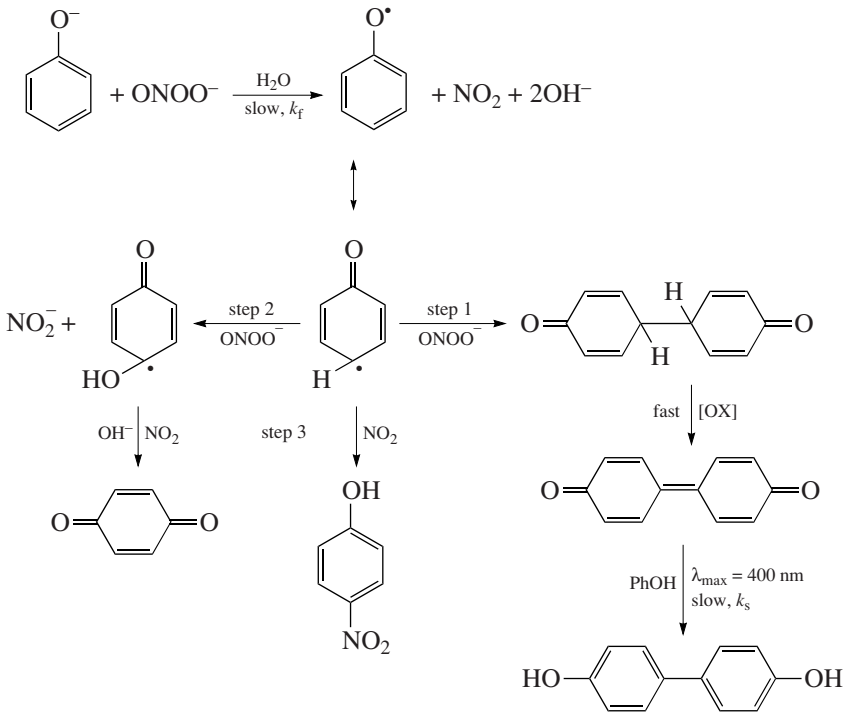
The Proposed Mechanism

Considering the reaction products, intermediates, stoichiometry, and the kinetic results, the mechanism shown in Scheme 1 has been proposed.

The mechanism involves a one-electron transfer from the phenolate anion to peroxynitrite as the rate-determining step leading to a phenoxyl radical, which is rapidly coupled with another one (Path 1), oxidized with peroxynitrite (Path 2), or reacts with nitrogen dioxide intermediate (Path 3). Based on the products' relative yields, Path 2 and Path 3 must be the major paths for the radical consumption. In Path 2, the phenoxyl radical is probably oxidized by peroxynitrite in two-electron process (oxygen-atom transfer or hydride transfer) to yield nitrite and a semiquinone intermediate as follow:



A one-electron oxidation of the semiquinone (by nitrogen dioxide) leads to the quinone. The kinetic results have confirmed the formation of the phenoxyl radical in a rate-determining step, and the major product were identified as the quinones (*ortho* and *para*), in addition to nitrophenols and biphenols. The rapid coupling of the phenoxyl radicals which is followed by oxidation to yield biphenoquinone intermediate (Path 1) accounted for 10–15% of the overall reaction of the phenol. In the case of estrone, this path contributed to



Scheme 1. A proposed mechanism for the reaction of phenol with $ONOO^-$ in basic solution.

less than 10% of estrone conversion presumably due to steric hindrance. The biphenoquinone intermediate is not stable in aqueous solutions. It reacts with the phenol (present in excess) to give biphenols or hydrolyzes (catalyzed by the base) to give other organic products. We were not able to identify them because they exist in very low yield. Table 3 shows that the rate of decomposition of biphenoquinone depends on the nature of the phenol. Biphenoquinones with electron-donating groups, such as CH_3, are expected to be less stable due to steric and electronic factors [38].

Nitration versus Oxidation of Estrone

It has been reported that under acidic and neutral conditions, the predominant products from the reaction of aromatic compounds, including phenols, with peroxynitrous acid/peroxynitrite correspond to the ring nitration [21, 22]. In basic solutions, peroxynitrite also acts as a nucleophilic oxidant leading to oxidation products [39]. In this study, both nitration and oxidation products are obtained from the reaction of estrone with peroxynitrite in basic solutions. The carbonyl group of estrone may have additional effect on this reaction. It seems the nitration path is suppressed and the oxidation path enhanced to form the corresponding orthoquinone. The data in Table 1 shows the effect of estrone concentration on the oxidation/nitration ratio. When comparing estrone with phenol, the ratio is higher in the case of estrone, and it increases with the [estrone]. In addition, estrone seems to enhance the decomposition of peroxynitrite and reduce the total yield. A previous study [40] has reported that ketones catalyze decomposition of peroxynitrite through the formation of dioxirane intermediate. The carbonyl group of the estrone may do the same, and enhance the decomposition of peroxynitrite. However, we did not observe the dioxirane intermediate or have any evidence that supports its involvement in this reaction.

CONCLUSIONS

The results obtained from the reaction of estrone with peroxynitrite, including the products, stoichiometry and kinetics, are similar to those observed in the reactions of phenols with peroxynitrite. Therefore, we propose that estrone was acting simply as a dialkylphenol, and its oxidation by peroxynitrite follows a mechanism similar to the one shown in Scheme 1. The reaction leads to many products because it involves radical organic species and peroxynitrite can act as oxidizing or nitration agent. Since there is a possibility that estrone and peroxynitrite can interact in biological systems, it would be interested to carry out similar study on the reaction of peroxynitrous acid/ peroxynitrite with estrone at physiological pH ~ 7. However, the reaction in neutral or acidic solutions is very fast and requires fast techniques, such as stopped-flow.

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REFERENCES

1. Bove, P.F. and van der Vliet, A., *Free Radical Biol. Med.*, 2006, vol. 15, p. 515.
2. Kissner, R., Nauser, T., Bugnon, P., Lye, P.G., and Koppenol, W.H., *Chem. Res. Texicol.*, 1997, vol. 10, p. 1285.
3. Radi, R., Cosgrove, T.P., Beckman, J.S., and Freeman, B.A., *Biochem. J.*, 1993, vol. 290, p. 51.
4. Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H., and Beckman, J.S., *Chem. Res. Texicol.*, 1992, vol. 5, p. 834.
5. Beckman, J.S., Chen, J., Ischiropoulos, H., and Crow, J.P., *Methods Enzymol.*, 1994, vol. 233, p. 229.
6. Blough, N.V. and Zifarov, D.C., *Inorg. Chem.*, 1985, vol. 24, p. 3504.
7. Szabo, C. and Ohshima, H., *Nitric Oxide*, 1997, vol. 1, p. 373.
8. Hogg, N., Joseph, J., and Kalyanaraman, A., *Arch. Biochem. Biophys.*, 1994, vol. 314, p. 153.
9. Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., and Freeman, B.A., *Proc. Natl. Acad. Sci. U. S. A.*, 1990, vol. 87, p. 1620.
10. Collier, J. and Vallance, P., *Br. Med. J.*, 1991, vol. 302, p. 1289.
11. Hughes, M.N. and Nicklin, H.G., *J. Chem. Soc.*, 1970, p. 925.
12. Pryor, W.A., Jim, X., and Squadrito, G.L., *Proc. Natl. Acad. Sci. U. S. A.*, 1994, vol. 91, p. 11173.
13. Yang, G., Candy, T.E., Boaro, M., Wilkin, H.E., Jones, P., Nazhat, N.B., Saadalla-Nazhat, R.A., and Blake, D., *Free Radical Biol. Med.*, 1992, vol. 12, p. 327.
14. van der Viliet, A., O'Neill, C.A., Halliwell, B., Cross, C.E., and Kaur, H., *FEBS Lett.*, 1994, vol. 339, p. 89.
15. Priyadarsini, I.K., Kapoor, S., and Naik, D.B., *Chem. Res. Texicol.*, 2001, vol. 14, p. 567.
16. Tibi, S. and Koppenol, W.H., *Helv. Chim. Acta*, 2000, vol. 83, p. 2412.
17. Bartlett, D., Churuch, D.F., Bounds, P.L., and Koppenol, W.H., *Free Radical Biol. Med.*, 1995, vol. 18, p. 85.
18. Trostchansky, A., O'Donnell, V.B., Goodwin, D.C., Landino, L.M., Marnett, L.J., Radi, R., and Rubbo, H., *Free Radical Biol. Med.*, 2007, vol. 42, p. 1029; Goldstein, S. and Czapski, G., *Inorg. Chem.*, 1995, vol. 34, p. 4041.
19. Al-Ajlouni, A.M. and Gould, E.S., *Inorg. Chem.*, 1996, vol. 35, p. 7892.
20. Radi, R., Beckman, J.S., Bush, K.M., and Freeman, B.A., *J. Biol. Chem.*, 1991, vol. 266, p. 4244.
21. Lymar, S.V., Jiang, Q., and Hurst, J.K., *Biochemistry*, 1996, vol. 35, p. 7855.
22. Santos, C.X.C., Bonini, M.G., and Augusto, O., *Arch. Biochem. Biophys.*, 2000, vol. 377, p. 146.
23. Pryor, W.A. and Squadrito, G.L., *Am. J. Physiol.*, 1995, vol. 268, p. L699.
24. Shigenaga, M.K., Lee, H.H., Blount, B.C., Christen, S., Shigeno, E.T., Yip, H., and Ames, B.N., *Proc. Natl. Acad. Sci. U. S. A.*, 1997, vol. 94, p. 3211.
25. Papina, A.A. and Koppenol, W.H., *Chem Res. Texicol.*, 2006, vol. 19, p. 382.
26. William, M.B. and Duax, L., *Molecular Structure and Biological Activity of Steroids*, Boca Raton, Fla.: CRC, 1992.
27. Shore, L.S. and Shemesh, M., *Pure Appl. Chem.*, 2003, vol. 75, p. 1859.
28. Mendelsohn, M.E. and Karas, R.H., *New Engl. J. Med.*, 1999, vol. 340, p. 1801; Gupta, S., Mehrotra, S., Villalon, C.M., Perusquia, M., Saxena, P.R., and van den Brink, A.M., *Pharmacol. Ther.*, 2007, vol. 113, p. 321.
29. Martin, M.C., Balfagon, G., Minoves, N., Blanco-Rivero, J., and Ferrer, M., *Nitric Oxide*, 2005, vol. 12, no. 3, p. 163.
30. Espenson, J.H., *Chemical Kinetics and Reaction Mechanisms*, New York: McGraw-Hill, 1995, p. 70.
31. Kirk, D.N., Toms, H.C., Douglas, C.D., and White, K.A., *J. Chem. Soc., Perkin Trans. 2*, 1990, p. 1567; Blunt, J.W. and Stothers, J.B., *Org. Magn. Reson.*, 1977, vol. 9, p. 439.
32. Yamamura, S., in *The Chemistry of Phenols*, 2003, vol. 2, p. 1153.
33. Al-Ajlouni, A.M., Bakac, A., and Espenson, J.H., *Inorg. Chem.*, 1993, vol. 32, p. 5792.
34. Hay, A.S., *J. Org. Chem.*, 1969, vol. 34, p. 1160.
35. Carey, F.A. and Sundberg, R., *Advanced Organic Chemistry*, New York: Plenum, 1984, part A.
36. Radhakrishnamurt, P.S. and Pat, S.N., *Indian J. Chem., Sect. A: Inorg., Bioinorg., Phys., Theor. Anal. Chem.*, 1978, vol. 16, p. 139.
37. Sims, P., *J. Chem. Soc.*, 1959, p. 3648.
38. Hurwitz, A.R. and Liu, S.T., *J. Pharm. Sci.*, 1977, vol. 66, p. 624.
39. Bohle, D.S., Glassbrenner, P.A., and Hansert, B., *Tetrahedron Lett.*, 1997, vol. 38, p. 2425.
40. Yang, D., Tang, Y.-C., Chen, J., Wang, X.-C., Bartberger, M.D., Houk, K.N., and Olson, L., *J. Am. Chem. Soc.*, 1999, vol. 121, p. 11976.