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O-atom Transfer versus Aromatic Nitration Reactions of Peroxynitrite

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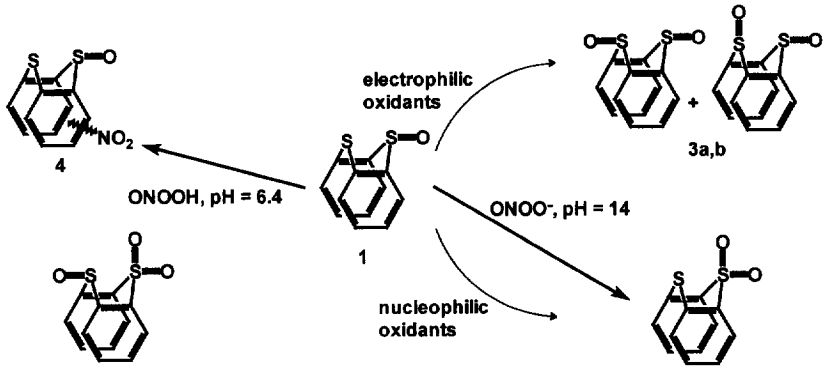
Abstract: The O-atom transfer reactions of the cytotoxin peroxynitrite, $ONOO^-$ has been examined using thianthrene-5-oxide as a probe at various pH. Under basic conditions the product is a sulfide/sulfone derivative indicating strong nucleophilic oxidant character of peroxynitrite. Under acidic conditions the predominant product corresponds to ring nitration of the thianthrene.

Peroxynitrite, $ONOO^-$, was first described by Bayer and Villiger as part of their seminal work on the reactions of hydrogen peroxide.[1] There is widespread recent interest in peroxynitrite stemming from the Beckman hypothesis that it is formed under physiological conditions by cytokine activated macrophages from the diffusion controlled reaction of nitric oxide and superoxide.[2] While direct detection of peroxynitrite has been hampered by its short life time under physiological conditions, $\tau_{½}$ = 1.5 seconds for pernitrous acid HOONO, $pK_a = 6.8$,[3] numerous physiological studies are consistent with its transitory existence in cases of systemic inflammation. Perhaps the most compelling evidence for a distinct physiological role of peroxynitrite is the formation of peptidic 3-nitrotyrosyl moieties, as detected by monoclonal antibodies,[4] and the detection of 3-nitrotyrosine and its metabolites in urine.[5] Unfortunately both the mechanistic and systematic chemistry of peroxynitrite is complicated by the widespread use of peroxynitrite solutions with high concentrations of nitrite and nitrate, and their remain many unresolved questions concerning peroxynitrite's solution structure and conformational dynamics.[6] Nevertheless, the picture which emerges is of a reactive oxidant with both nitrating and oxidizing ability.



Scheme 1

Adam has recently described the use of thianthrene-5-oxide **1** as a mechanistic probe for the electrophilic or nucleophilic character for a range of oxo-transfer agents.[7] Nucleophilic agents such as benzene suspensions of potassium superoxide or hydrogen peroxide in sodium hydroxide result in the formation of the sulfone **2**

while electrophilic agents such as hydrogen peroxide in perchloric acid results in the formation of a bis sulfoxide derivative, **3a,b**, (scheme 1). The product ratio of **2** to **3** is very sensitive to the reagent and conditions for these reactions, with radical-type oxygen transfer agents producing mixtures of **2** and **3** with the disulfoxide **3** often in 50% excess.

When **1** is treated with peroxynitrite under basic conditions[8] the sole organic product is the sulfone **2** with there being no detectable (< 0.1%) bis sulfoxide **3** or the dioxidation product, the sulfone/sulfoxide **5**, by GC-MS. Under these conditions peroxynitrite is clearly a strong *nucleophilic* oxo-transfer agent with little if any nitrating or electrophilic reactivity. On the other hand, when **1** is treated with peroxynitrous acid at pH=6.5 ONOOH is neither an electrophilic nor nucleophilic oxidant with regard to O-atom transfer and instead results in the nitration of one of the aromatic rings in **1** to give nitro-thianthrene-5-oxide.[10]

Under physiological conditions, peroxynitrite, can nitrate a variety of aromatic rings to give 3-nitrotyrosine,[4,5] nitrotryptophan as mixture of isomers,[11] 3-nitrophenylalanine,[12] and 8-nitroguanine.[13] However, it has also been suggested that oxygen atom transfer from peroxynitrite to methionine sulfur under physiological conditions occur in preference to ring nitration of phenylalanine in the small decapeptide MER10, an inhibitor of an α1-proteinase.[14] The absence of products stemming from O-atom transfer to thianthrene-5-oxide, **2** and **3a,b**, when it is treated with peroxynitrite under neutral conditions, suggests that the nitration and O-atom transfer reactivity of this cytotoxin are finely balanced and may vary markedly with substrate and reaction conditions.

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8. For all reactions fresh tetramethylammonium peroxynitrite salts[7] were used, stored, and handled under a nitrogen. Thianthrene-5-oxide and its oxidation by products were prepared by the literature methods[9] and the products separated and quantified by GC-MS. For the reactions under basic conditions, 10 mg $[NMe_4][ONOO]$ was dissolved in 5 mL 1 M NaOH, and the solution diluted with additional alkali while monitoring the UV-vis spectrum to give a final concentration of 10 mM ($\varepsilon_{302\ nm}$ = 1700 $cm^{-1}M^{-1}$).[7] To this solution was added a 20 mg of thianthrene-5-oxide. This heterogeneous mixture was vigorously stirred for 48 hr at room temperature, after which the organic soluble materials were extracted twice with dichloromethane and then washed twice with 10 mL 1 M NaOH, and twice with 10 ml water, dried, and analyzed by GC-MS.
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10. A peroxynitrite solution 10 mL, 75 mM, was prepared from 0.01 M NaOH, and this was added drop-wise to 20 mL of a 1 M pH = 6.4 phosphate buffer, in which 20 mg of thianthrene-5-oxide was suspended. The peroxynitrite solution was added over the course of an hour, during which the pH was monitored and kept constant with additions of 0.1 M phosphoric acid, after which the organic soluble fraction was extracted with three 25 mL washes with dichloromethane, dried, and analyzed by GC-MS. Identification of single peak of product nitrothianthrene-5-oxide based on parent ion *m/e* = 277 AU. Furthermore, when ^{15}N labeled peroxynitrite is the mass of this parent ion peak increases by one unit.
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