Oxidation

Dihydrogen Trioxide (HOOOH) Is Generated during the Thermal Reaction between Hydrogen Peroxide and Ozone**

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In memory of Bernard (Bernie) M. Babior

The reaction between HOOH and O_3 has fascinated scientists for almost a century.[1–3] The reactivity of O_3 is markedly increased by the presence of HOOH, although a clear mechanistic understanding of this phenomenon has thus far eluded researchers.[4–8] In modern times, the reaction between HOOH and O_3 has received industrial utility and has been named both the peroxone process and an advanced oxidation reaction.[9,10] It is one of the most potent antibacterial processes known and is a powerful chemical remediation tool utilized to treat soil, groundwater, and wastewater contaminated with polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), methyl *tert*-butyl ether (MTBE), benzene, toluene, ethylbenzene and xylene (BTEX), trinitrotoluene (TNT), and other persistent organic pollutants.[10]

Recently, the reaction between HOOH and O_3 has become of biological interest. It has been discovered that all antibodies have the ability to catalyze the oxidation of water by singlet oxygen ($^1\Delta_g$), with the postulated intermediacy of HOOOH, to generate HOOH and detectable amounts of an oxidant with the chemical signature of O_3.[11–13] The proposition of HOOOH as a key intermediate in the antibody-catalyzed reaction has led to an interest in the study of thermally accessible routes to HOOOH that may utilize biologically relevant components.

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A rigorous kinetic examination of the reaction between O_3 and HOOH was first performed by Rothmund and Burgstaller in 1917.[1] Weiss[14] first proposed a free-radical mechanism for this process, and subsequently Bray[3] concluded that either free radicals, $^{\bullet}OH$ and hydroperoxyl ($HO_2^{\bullet}$), or HOOOH were necessary to explain the observed kinetics of the process. Taube and Bray[2] in 1940 then established the role of $^{\bullet}OH$ and $HO_2^{\bullet}$ as chain-initiating intermediates in a complex radical cascade (Table 1, entries 1–4). Subsequent detection of $^{\bullet}OH$, the superoxide anion ($O_2^{\bullet -}$), and the ozonide radical anion ($O_3^{\bullet -}$) as intermediates in this process supported the free-radical mechanism proposed by Weiss.[15–17] Thus, the contemporary view is that a common free-radical mechanism may account for the decomposition of O_3 by HOOH under all conditions, with the potential involvement of HOOOH being largely ignored. Recently however, HOOOH has been thrust into center stage as a plausible intermediate in the photochemical peroxone process. Engdahl and Nelander[18] observed that mixing HOOH and O_3 in an argon matrix results in a complex that, when photolyzed at 266 nm, produces HOOOH. However, there is no report of HOOOH being detected from the strictly thermal peroxone reaction.

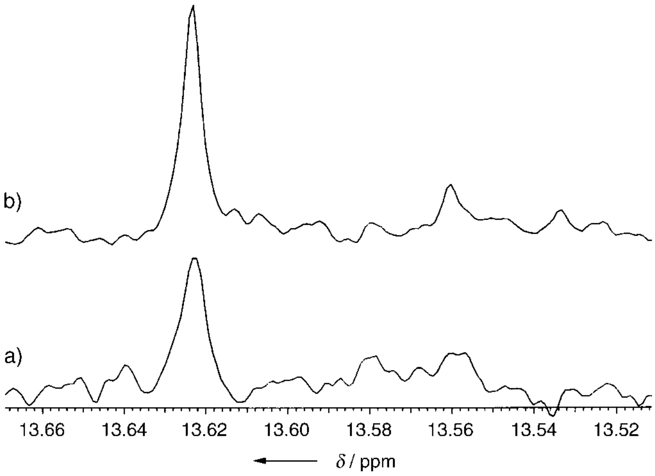


Figure 1. ^{1}H NMR spectra (600 MHz recorded at −60 °C) of the peroxone reaction between $\delta = 13.50$ and 13.70 ppm downfield from phenyltrimethylsilane (PhTMS) in [D$_4$]methanol (99 %, external reference). a) $H_2O_2 + O_3/O_2$ (20 s), b) $H_2O_2 + O_3/O_2$ (20 s) + H_2O_3 (authentic sample). The authentic HOOOH used as an additive in (b) was generated by the ozonation of a resin-supported 1,2-diphenylhydrazine (hydrazobenzene) derivative.[19] An aliquot of this HOOOH solution (100 μL) was added with a glass syringe and needle to the teflon-valved NMR tube at −60 °C. Note that no signal at about 13.60 ppm was present in the following control NMR spectra: 1) After O_3/O_2 was bubbled through [D$_6$]acetone (99.9 %) for 20 s and 2) HOOH (28 M) in [D$_6$]acetone (99.9 %).

Table 1: Selected equations from the reaction between ozone and hydrogen peroxide (in aqueous media unless otherwise stated).

Entry	Equation	Comment
1[a]	$O_3 + H_2O_2 \rightarrow {}^{\bullet}OH + HO_2^{\bullet} + O_2$	$k_1 = 6.5 \times 10^{-2}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$
2[b]	$O_3 + {}^{\bullet}OH \rightarrow HO_2^{\bullet} + O_2$	$k_{2=}1.1 \times 10^{8}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$
3[c]	$O_3 + {}^{-}O_2^{\bullet} + H^+ \rightarrow {}^{\bullet}OH + 2\,O_2$	$k_3 = 1.6 \times 10^{9}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$
4[d]	$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2^{\bullet} + H_2O$	$k_4 = 2.7 \times 10^{7}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$
5[e]	$O_3 + H_2O_2 \rightarrow H_2O_3 + {}^3O_2$	$\Delta H_r = -32.3\,\mathrm{kcal\,mol^{-1}}$
6[f]	$O_3 + HO_2^{-} \rightarrow HO_3^{-} + O_2$	$k_6 = 2.8 \times 10^{6}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$
7[g]	$H_2O_3 \rightarrow {}^1O_2 + H_2O$	$\Delta H_r = -16.2\,\mathrm{kcal\,mol^{-1}}$, $\Delta H_a(0\,H_2O) = 45.1\,\mathrm{kcal\,mol^{-1}}$[h] $\Delta H_a(1\,H_2O) = 12.7\,\mathrm{kcal\,mol^{-1}}$ $\Delta H_a(2\,H_2O) = 0.2\,\mathrm{kcal\,mol^{-1}}$

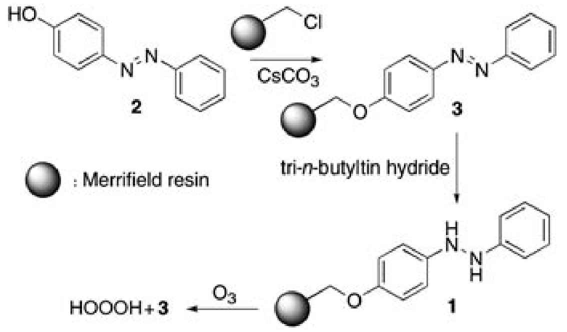
[a] Ref. [2,33]. [b] Ref. [34]. [c] Ref. [35]. [d] Ref. [36,37]. [e] Gas-phase calculation: Ref. [31]. [f] Ref. [32]. [g] Ref. [21,38]. [h] The number of water molecules (in parentheses) signifies the molar equivalents of water participating as a catalyst in the reaction.

Herein we demonstrate the direct observation of HOOOH formed during the thermal reaction between HOOH and O_3. Passage of a stream of ozone/oxygen gas through a solution of aqueous hydrogen peroxide (ca. 96 % w/w), generated either by vacuum distillation of commercially available HOOH (containing stabilizers) or pure HOOH generated by reduction of O_2,[19] in [D$_6$]acetone or [D$_{10}$]tetrahydrofuran at −78 °C produced an intermediate that could be characterized by ^{1}H NMR analysis (−60 °C) as having a typical OOOH resonance at $\delta = 13.6 \pm 0.2$ ppm relative to tetramethylsilane (Figure 1 b).

This signal disappeared upon warming the [D$_6$]acetone and [D$_{10}$]tetrahydrofuran solutions to room temperature and did not reappear upon recooling. The ^{1}H NMR chemical shift and thermal instability of this intermediate are both in line with the wealth of physical data available for HOOOH.[18,20–23]

To add support to the notion that this ^{1}H NMR resonance at $\delta = 13.6 \pm 0.2$ ppm in the peroxone reactions corresponds to HOOOH, an authentic solution of HOOOH in [D$_6$]acetone was required as a control. Neither of the two main chemical routes for the preparation of HOOOH (pulsed radiolysis of air-saturated perchloric acid solutions[24,25] or reduction of ozone by reducing agents such as 2-ethylanthrahydroquinone[20] and 1,2-diphenylhydrazine[20,21,23]) were suitable for this control sample. The lifetime of HOOOH in acid solutions (ca. 200 ms in perchlorate pH 2) precludes the pulsed radiolysis method as being viable for NMR analysis (a single pulse sequence in a typical ^{1}H NMR experiment shown in Figure 1 is > 25 s). Plesnicar et al.[26] have shown that HOOOH is always contaminated with a number of oxidation products of 1,2-diphenylhydrazine and HOOH when the low-temperature ozonation of 1,2-diphenylhydrazine in an organic solvent is employed. Therefore a new methodology was required for the generation of a pure solution of HOOOH.

The development of polymer-supported methodologies to facilitate solution-phase chemistry has exploded over the past decade in response to the stringent requirements of high-throughput solution-phase combinatorial and parallel synthesis.[27–30] We rationalized that HOOOH could be prepared free of the contamination products arising from the oxidation of 1,2-diphenylhydrazine (1,2-DPH) if the 1,2-DPH was on a resin support. Thus, resin-supported 1,2-DPH derivative **1** was prepared (Scheme 1).[19]



Scheme 1. Preparation of resin-bound diphenylhydrazine **1** and its use to prepare HOOOH from O_3.

The resin-supported hydrazine **1** was prepared in two steps by an initial addition of 4-phenylazophenol (**2**) to low cross-linked (1% divinylbenzene) chloromethylpolystyrene followed by reduction of the orange azo resin derivative **3** with tri-*n*-butyltin hydride in refluxing toluene. Low-temperature ozonation (−78 °C) of resin-1,2-DPH **1** in [D$_6$]acetone or [D$_{10}$]tetrahydrofuran with a stream of ozone/oxygen led to the formation of HOOOH in solution, with the only contaminant being HOOH (Figure 2). The oxidized resin turns bright orange during the ozonation, as it becomes oxidized in part to **3**, and can then be removed by filtration.

Addition of the solution of HOOOH in [D$_6$]acetone (generated by ozonation of **1**) to the NMR tube containing the peroxone reaction resulted in an increase in the area of the OOOH resonance (13.6 ± 0.2 ppm, Figure 1 a), thus strengthening the hypothesis that the unknown resonance corresponds to HOOOH.

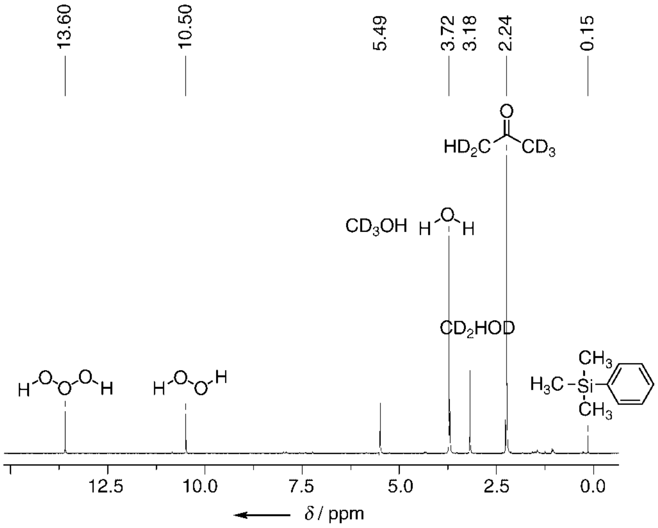


Figure 2. ^{1}H NMR spectrum (600 MHz recorded at −60 °C) of the filtrate following low-temperature ozonation (−78 °C) of resin-**1**. Note PhTMS in [D$_4$]methanol is present as an external standard for quantification of HOOOH.

The amount of HOOOH generated during the peroxone reaction was determined from an analysis of the relationship between the area of the OOOH resonance (13.6 ± 0.2 ppm) and the amount of HOOOH present in the NMR sample. This relationship was determined by a standard method involving interpolation of the ratio of the peak areas of the OOOH resonance at 13.6 ± 0.2 ppm and the methyl protons of an external standard (phenyltrimethylsilane) to a standard calibration curve. This analysis revealed that approximately 29 μmol of HOOOH (corresponding to a concentration of 2.9 mM in the [D$_6$]acetone solution) was formed in this specific peroxone reaction (10 mL reaction volume). This quantity of H_2O_3 was reproducibly generated when the peroxone reaction was repeated under identical conditions of HOOH concentration and O_3 flow. This amount of H_2O_3 generated in the peroxone process is significant, and therefore the chemistry of the peroxone reaction may now have to be revised to not only consider ozone and HOOH chemistry, but also that of H_2O_3.

There are at least two mechanistically viable possibilities for the origin of HOOOH in the peroxone reaction. By using quantum chemical methods Xu and Goddard[31] have calculated that the enthalpy of reaction (ΔH_r) for the thermal reaction of HOOH and O_3 to yield HOOOH and $^3O_2$ in the gas phase is $-32.3\ \text{kcal mol}^{-1}$, with the highest enthalpy of activation (ΔH_a) being $4.8\ \text{kcal mol}^{-1}$ (Table 1, entry 5). The process involves two sequential single electron transfer reactions that is formally the redox reaction between O_3 and HOOH. Xu and Goddard[31] have proposed that the reaction proceeds via a planar seven-membered-ring biradical intermediate (with the spin multiplicity of the triplet and singlet states being almost degenerate) to yield HOOOH and $^3O_2$ (Figure 3 and Table 1, entry 5). More recently, to explain hydrogen isotope effects on the reaction kinetics of the peroxone reaction in water, Lesko et al.[32] have concluded that HO_3^-, the conjugate base of H_2O_3, may be generated from a reaction between the anion of hydrogen peroxide and O_3 (Table 1, entry 6).

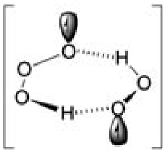


Figure 3. Postulated seven-membered-ring triplet biradical intermediate on the pathway from H_2O_2 and O_3 into H_2O_3 and $^3O_2$.

The observation that HOOOH is generated in the thermal peroxone reaction in low-water-content organic solvents at low temperature brings with it clear questions. These include the as yet, unknown generation of HOOOH under totally aqueous conditions at room temperature of a peroxone mixture. Bielski[24,25] has shown that HOOOH, detected chemically and spectroscopically, is quasistable under totally aqueous conditions ($t_{1/2}$ = ca. 2 s at pH 1.5, 298 K). Koller and Plesnicar[21] and Xu and Goddard[31] have independently calculated that HOOOH decomposes to yield $^1O_2$ and H_2O in a water-catalyzed reaction (Table 1 entry 7). Thus, although not yet reported, $^1O_2$ may well be generated during the peroxone reaction in aqueous systems from the known decomposition of H_2O_3. In addition, further reactions of HOOOH with either itself, HOOH, $^1O_2$, or O_3 to yield, as yet, undefined products will add further complexity to this rich and complex reaction process.

Experimental Section

Reaction between O_3 and HOOH: All samples were prepared as solutions in [D_6]acetone (99.9%) or [D_{10}]tetrahydrofuran (99.9%, 10 mL). The experiments were carried out by using Schlenk techniques. In brief, a stream of O_3 in O_2 was bubbled through an HOOH solution (28 M) in [D_6]acetone (99.9%) or [D_{10}]tetrahydrofuran (99.9%, 10 mL) precooled to −78 °C for 20 s. The hydrogen peroxide used in these studies was prepared either by the resin method described in the Supporting Information or by vacuum distillation of an aqueous H_2O_2 (50% w/v containing stabilizers) solution to approximately one-third volume. These ozonolyzed solutions were then degassed briefly by bubbling argon through the solution of H_2O_2 and transferred to a teflon-valved NMR tube. The samples were then warmed to −60 °C and ^{1}H NMR spectra were measured. Each experiment was repeated at least twice and gave essentially the same amount of HOOOH.

Generation of HOOOH in [D_6]acetone with **1**: Resin-supported 1,2-diphenylhydrazine **1** was suspended in [D_6]acetone (5 mL) and cooled to −78 °C. A stream of ozone/oxygen gas was passed through the suspension for 2 min. The resin was then removed by filtration under argon and the filtrate was quickly transferred to an NMR tube under a positive argon pressure.

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[1] V. Rothmund, A. Burgstaller, *Monatsh. Chem.* **1917**, *38*, 295.
[2] H. Taube, W. C. Bray, *J. Am. Chem. Soc.* **1940**, *62*, 3357.
[3] W. C. Bray, *J. Am. Chem. Soc.* **1938**, *60*, 82.
[4] C.-H. Kuo, L. Zhong, M. E. Zappi, A. P. Hong, *Can. J. Chem. Eng.* **1999**, *77*, 473.
[5] R. J. Spanggord, D. Yao, T. Mill, *Environ. Sci. Technol.* **2000**, *34*, 450.
[6] M. Elovitz, U. von Gunten, *Ozone Sci. Eng.* **1999**, *21*, 239.
[7] M. Elovitz, U. von Gunten, H. Kaiser, *Ozone Sci. Eng.* **2000**, *22*, 123.
[8] J. L. Acero, U. von Gunten, *Ozone Sci. Eng.* **2000**, *22*, 305.
[9] R. Brunet, M. M. Bourbigot, M. Dore, *Ozone Sci. Eng.* **1984**, *6*.
[10] J. Prousek, *Chem. Listy* **1996**, *60*, 229.
[11] P. Wentworth, Jr., L. H. Jones, A. D. Wentworth, X. Zhu, N. A. Larsen, I. A. Wilson, X. Xu, W. A. Goddard III, K. D. Janda, A. Eschenmoser, R. A. Lerner, *Science* **2001**, *293*, 1806.
[12] P. Wentworth, Jr., J. McDunn, A. D. Wentworth, C. Takeuchi, J. Nieva, K. D. Janda, A. Eschenmoser, R. A. Lerner, *Science* **2002**, *298*, 2195.
[13] P. Wentworth, Jr., A. D. Wentworth, X. Zhu, I. A. Wilson, K. D. Janda, A. Eschenmoser, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1490.
[14] J. Weiss, *Trans. Faraday Soc.* **1935**, *31*, 668.
[15] K. Sehested, H. Corfitzen, J. Holcman, E. J. Hart, *J. Phys. Chem. A* **1998**, *102*, 2667.
[16] J. Staehelin, R. E. Buhler, J. Hoigne, *J. Phys. Chem.* **1984**, *88*, 5999.
[17] R. E. Buhler, J. Staehelin, J. Hoigne, *J. Phys. Chem.* **1984**, *88*, 2560.
[18] A. Engdahl, B. Nelander, *Science* **2002**, *295*, 482.
[19] See Supporting Information for full details.
[20] J. Cerkovnik, B. Plesnicar, *J. Am. Chem. Soc.* **1993**, *115*, 12169.
[21] J. Koller, B. Plesnicar, *J. Am. Chem. Soc.* **1996**, *118*, 2470.
[22] B. Plesnicar, J. Cerkovnik, T. Tuttle, E. Kraka, D. Cremer, *J. Am. Chem. Soc.* **2002**, *124*, 11260.
[23] B. Plesnicar, J. Cerkovnik, T. Tekavec, J. Koller, *Chem. Eur. J.* **2000**, *6*, 809.
[24] B. H. J. Bielski, *J. Phys. Chem.* **1970**, *74*, 3213.
[25] B. H. J. Bielski, H. A. Schwartz, *J. Phys. Chem.* **1968**, *72*, 3836.
[26] B. Plesnicar, T. Tuttle, J. Cerkovnik, J. Koller, D. Cremer, *J. Am. Chem. Soc.* **2003**, *125*, 11553.
[27] C. Spanka, P. Wentworth, K. D. Janda, *Comb. Chem. High Throughput Screening* **2002**, *5*, 233.
[28] P. Wentworth, Jr., K. D. Janda, *Curr. Opin. Biotechnol.* **1998**, *9*, 109.
[29] P. Wentworth, Jr., K. D. Janda, *Chem. Commun.* **1999**, 1917.
[30] P. Wentworth, Jr., *Trends Biotechnol.* **1999**, *17*, 448.
[31] X. Xu, W. A. Goddard, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15308.
[32] T. M. Lesko, A. J. Colussi, M. R. Hoffman, *J. Am. Chem. Soc.* **2004**, *126*, 4432.
[33] K. Sehested, H. Corfitzen, J. Holcman, E. J. Hart, *J. Phys. Chem.* **1992**, *96*, 1005.
[34] K. Sehested, J. Holcman, E. Bjergbakke, E. J. Hart, *J. Phys. Chem.* **1984**, *88*, 4144.
[35] NDRL/NIST Solutions Kinetics Database, NIST Standard Reference Database 40, http://kinetics.nist.gov/solution/index.php, **2002**.
[36] H. Christensen, K. Sehested, H. Corfitzen, *J. Phys. Chem.* **1982**, *86*, 1588.
[37] G. V. Buxton, C. L. Greenstock, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.
[38] X. Xu, P. R. Muller, W. A. Goddard III, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3376.