



Supporting Information

for

Angew. Chem. Int. Ed. 200460457

© Wiley-VCH 2004

69451 Weinheim, Germany

Dihydrogen trioxide (HOOOH) is generated during the thermochemical reaction between hydrogen peroxide and ozone**

*Paul T. Nyffeler, Laxman Eltepu, Nicholas A. Boyle, Chi-Huey Wong, Albert Eschenmoser, Richard A. Lerner & Paul Wentworth Jr.**

[*] P. T. Nyffeler, Dr. L. Eltepu, Dr. N. A. Boyle, Prof. C.-H. Wong, Prof. A. Eschenmoser, Prof. R. A. Lerner, Prof. P. Wentworth Jr.

Department of Chemistry & The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd. La Jolla, CA92037, U.S.A.

Fax: (+1)858 784 2590

e-mail: paulw@scripps.edu

Prof. A. Eschenmoser

Laboratorium für organische Chemie, Eidgenössische Technische Hochschule (ETH) Hönggerberg HCl-H309, Universitaetstrasse 16 CH-8093 Zürich, Switzerland.

Prof. P. Wentworth Jr.

Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Rd., Oxford, OX1 3QU, UK

Experimental Section

General Methods. All reagents and solvents were supplied by Aldrich Chemical Company unless otherwise stated. All reactions were carried out under an atmosphere of Ar or N₂ unless specified otherwise. NMR experiments were performed on either a Varian Mercury 300 MHz or a Bruker DRX 600 MHz spectrometer unless otherwise stated. Low temperature cooling of the NMR machines was achieved with liquid nitrogen, and all samples were introduced at -60 °C. All spectra were processed using Varian's VNMR software and phased individually by hand. Chemical shifts are reported in parts per million (ppm) on the δ scale from a PhTMS internal standard. Infrared data was recorded on a Nicolet Avatar FT-IR machine.

Safety Warning: *These experiments utilize ozone and H₂O₂ at high concentrations, both of which are potentially explosive therefore use extreme caution when attempting these reactions.*

Resin-bound azobenzene (3). 4-Phenylazophenol **2** (4.4g, 11 mmol), Merrifield resin (10.9 g, 1% cross-linked chloromethyl-polystyrene, 1.0 mmol/g loading, 100-200 mesh), and cesium carbonate (16.9 g, 55 mmol) were combined into a 250 mL round-bottom flask with a magnetic stir bar and fitted with a rubber septum. The flask was purged with argon, and 75 mL of anhydrous *N,N*-dimethylformamide (DMF) was added *via* syringe. The solution was heated to 40 °C overnight and allowed to cool. The reaction contents were filtered, and the crude resin was washed with DMF, water, acetone, and hexane. The wash cycle was repeated, and the

bright orange resin was dried *in vacuo* to give 11.03g of a bright orange colored resin (98% of theoretical yield). ^1H NMR (400 MHz, CDCl_3) δ 1.20-2.10 (bs, polymer), 4.40-4.50 (bs, unreacted polymer benzylic methylene), 4.75-4.85 (bs, derivatized polymer benzylic methylenes), 6.00-7.20 (bm, polymer), 7.35-7.45 (bm, hydrazone aryl), 7.75-7.85 (bm, hydrazone aryl). Note integral ratio of derivatized/underivatized polymer benzylic methylenes was 9.2:1, suggesting loading was $\sim 92\%$. FTIR (wavenumbers, cm^{-1}) 1139 (N=N).

Polystyrene-bound 1,2-diphenylhydrazine (1). Resin **3** (250 mg, 0.25 mmol) was placed in a 25 mL round-bottom flask with a stir bar. Anhydrous toluene (5 ml) was added, and argon was bubbled through the solution for 10 minutes to exclude oxygen. The flask was fitted with a reflux condenser, and tri-*n*-butyltin hydride (3 mL, 11.153 mmol) was added. The solution was heated to 120 °C for approximately 5 h, at which time the orange resin had turned pale yellow or colorless. The suspension was cooled to room temp.¹ The suspension was then transferred under argon to a Schlenk filter fitted with a Schlenk flask on each end. The resin was washed with CH_2Cl_2 , water, methanol, acetone, diethyl ether, and hexanes using positive argon pressure. All solvents were degassed by bubbling argon through them prior to use except diethyl ether, which had been stored over sodium/fluorenone and was oxygen- and stabilizer-free. The resin was transferred to a

¹ Resin invariably became pale yellow, but would become colorless again if reheated. If resin was still orange after 5 h, additional Bu_3SnH was added.

flame-dried 10 mL Schlaker tube (Aldrich), stoppered with a silicon rubber septum, and evacuated for several hours in the dark.² To remove any impurities and solvents that had been trapped in the polystyrene matrix, the resin was swollen and stirred in *d*₆-acetone (2 x 1 mL ampoules) for 10 minutes, then isolated by filtration over the glass frit in the arm of the Schlaker tube (250 mg, 98% theoretical yield). The resin was stored *in vacuo* for an additional hour and used without further purification. ¹H NMR (400 MHz, CDCl₃) δ 1.20-2.10 (bs, polymer), 4.45-4.80 (bs, derivatized polymer benzylic methylenes and unreacted polymer benzylic methylene), 5.22-5.40 (bs, N-H), 6.20-6.76 (bm, polymer and hydrazine), 6.80-7.15 (bm, hydrazine aryl). FTIR (wavenumbers, cm⁻¹) 3349 (N-H).

Formation of H₂O₃ via Ozone Treatment of Resin 1. Resin 1 (1 g, 1.0 mmol) was suspended in *d*₆-acetone (5 mL) (99.9%-D, Cambridge Isotopes) and cooled to -78 °C in a Schlaker tube. A stream of ozone/oxygen gas (generated by a Polymetrics Ozone generator fed with pure oxygen) was passed through the suspension *via* a long stainless steel needle, punctured through the silicon rubber septum. The solution was bubbled with the ozone/oxygen gas mixture for approximately 2 minutes until the pale yellow resin had turned bright orange, indicating oxidation to **3**. The long needle was removed while simultaneously opening the sidearm of

² Resin **2** was found to slowly oxidize back to **1** after several days under argon, so all resin was made immediately prior to use.

the Schlaker tube to a positive pressure of argon. The septum was then replaced by a glass adapter that contained a fritted glass filter and a syringe tip with a long stainless steel needle. The end of the long needle was placed in a second flame-dried 10 mL Schlaker with a silicon rubber septum that had been precooled to $-78\text{ }^{\circ}\text{C}$. By inserting the vent needle into the septum of the second tube and inverting the first, the resin was rapidly filtered by the frit, and the d_6 -acetone solution containing H_2O_3 was quickly transferred to the second tube by the positive argon pressure.

The solution of H_2O_3 in deuterated acetone was then transferred *via* cannula through a Teflon valve into an NMR tube containing a reference capillary. The tube was closed tightly, carefully freeze/pump/thaw degassed with liquid nitrogen and argon, and stored at $-78\text{ }^{\circ}\text{C}$ until it was placed in the NMR spectrometer for analysis.

This resin-based procedure produced very pure H_2O_3 , with the main contaminants being H_2O_2 and H_2O . While the concentration of HOOH was not measured absolutely, it was assumed to be $\sim 0.2\text{ M}$, based on a loading of 1.0 mmol/g and the use of 1 g of resin in 5 mL of solvent.

Preparation of pure H_2O_2 . As an alternative to vacuum distillation of aqueous hydrogen peroxide (that contains stabilizers) *vide infra*, H_2O_2 was prepared essentially pure *via* reduction of pure oxygen with resin **1**. In a typical procedure the freshly prepared resin **1** (100 mg , $\sim 0.25\text{ mmol}$ hydrazine) was suspended in d_6 -acetone (5 mL) and cooled to $0\text{ }^{\circ}\text{C}$ in an ice bath. A balloon of

pure oxygen with a long needle was added to the solution, and a vent needle was used to purge the flask. After stirring for 4 h, the resin was removed by filtration using the fritted Shlenk tube *vide supra* and the filtrate was used without further purification.

Peroxone Reactions between Ozone and Hydrogen Peroxide. The hydrogen peroxide used in these studies was prepared either *via* the resin method described above, or *via* vacuum distillation of an aqueous H₂O₂ (50 % w/v containing stabilisers) solution to approximately one-third volume. This distillation approach has been reported to yield a 96.3 wt. % H₂O₂ solution.³

An aliquot of the H₂O₂ solution (0.3 mL) was transferred, *via* a Teflon needle, into *d*₆-acetone (99.9% D, 10 mL) (final H₂O₂ concentration 0.28 mmol/mL) or into *d*₁₀-tetrahydrofuran (99.9% D, 10 mL) pre-cooled to -78 °C, and the solution was allowed to warm to room temperature. The solution was then transferred by syringe and Teflon needle into flame-dried argon-filled Schlaker flasks, which were then topped with silicon rubber septa (stable to ozone) and cooled to -78 °C. A gaseous stream of ozone/oxygen (Fischer Model 502 ozone generator fed with pure oxygen) was bubbled through the solution for 20 sec. This mixture was then transferred, by glass pipette, to an NMR tube containing a Teflon valve and reference capillary at -78 °C. The NMR tubes was then frozen in liquid nitrogen, freeze/pump/thaw degassed three times

³ Cofré, P. and Sawyer, D. T. Redox Chemistry of Hydrogen peroxide in Anhydrous Acetonitrile. *Inorg. Chem.* **25**, 2089-2092 (1986).

with argon, and kept at $-78\text{ }^{\circ}\text{C}$ until NMR analysis. Each experiment was repeated in at least duplicate and gave essentially the same amount of HOOOH.

NMR Experiments

Calibration and Relaxation Time. A reference solution of trimethylphenylsilane (PhTMS, chosen for its lower volatility compared to tetramethylsilane) in d_4 -methanol was prepared and rapidly transferred to several 2.0 mm O.D. precision capillaries (Wilmad) that had been sealed previously at one end. The volumes of the solution in the tubes were kept as close as possible to the same height in all capillaries. The tubes were quickly sealed using melted wax, then sealed with a butane torch using a lathe to minimize evaporation and ensure proper sealing. The length of the reference tubes was chosen to allow the constriction at the top of a Teflon-valved NMR (J. Young, Kontes Glass) to hold the reference approximately in the center of the tube.

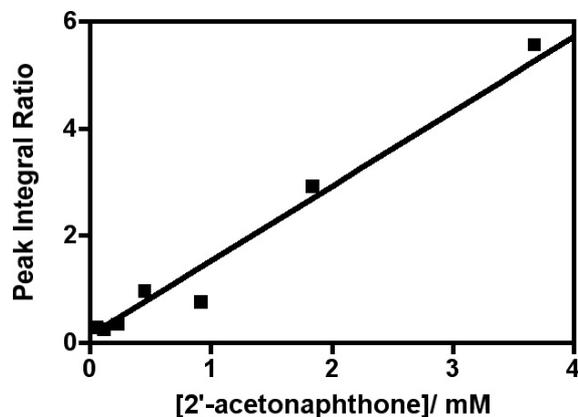
Relaxation values (T_1) were measured using the T_1 inversion-recovery method⁴ at -60 and $25\text{ }^{\circ}\text{C}$ for all proton resonances used in this experiment and are collated in SITable 1. To ensure full proton relaxation and hence facilitate concentration measurements a delay of 20s ($> 5 \times$ the longest measured value of T_1 for any or the components in the NMR of the peroxone mixture) was utilized for all our quantitative NMR experiments of the peroxone mixture.

⁴ Breitmaier, E.; Voelter, W. *Carbon-13 NMR spectroscopy*, 3rd ed.; VCH, New York, 1987.

Compound	-60 °C		25 °C	
	T1	error	T1	error
H ₂ O ₃	2.59	0.23	4.13	0.31
H ₂ O ₂	2.59	0.23	4.13	0.31
H ₂ O	1.07	0.04	4.19	0.05
CD ₃ OH	3.63	0.28	5.81	0.27
CD ₂ HOD	3.95	0.50	7.97	1.00
Acetone	3.53	0.28	7.70	0.36
PhTMS	1.73	0.18	4.21	0.32

Table 1. Relaxation (T1) values at two temperatures

Reference capillaries, containing PhTMS in d_4 -MeOH were placed into NMR tubes that contained known concentrations of 2'-acetonaphthone ($T_1 = 4.2 \pm 0.2$ s) in d_6 -acetone and the ^1H NMR spectra recorded at -60 °C using a relaxation time of 25 s. Acetonaphthone was chosen as an arbitrary calibrant because of its stability. A plot of the concentration of 2'-acetonaphthone versus the ratio of the integral of the methyl peak of 2'-acetonaphthone and the trimethyl peak of PhTMS provided a straight line (slope = with excellent fit (SI Figure 1)). This plot and the calculated slope were used to interpolate the measured proton integral of HOOH (from Figure 1a) to facilitate its absolute quantification.



SIFigure 1 Calibration line of integral ratio versus concentration. Each point is the mean of ten (10) measurements of the integral ratio of the methyl groups of acetone and PhTMS. These mean values were then plotted and analyzed in Graphpad Prism 4.0. Non-linear regression analysis (first order polynomial) with weighting (1/x), performed on PC, gave a gradient value of 1.394 ($r^2 = 0.999$, Sy.x 0.2705)