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Characterization of Endoperoxide and Hydroperoxide Intermediates in the Reaction of Pyridoxine with Singlet Oxygen

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Singlet oxygen is a reactive form of dioxygen that can cause damage to lipids, amino acids, DNA, and other biologically important molecules.[1] The phytotoxic fungus *Cercospora keidi* secretes cercosporin, a perylenequinone singlet oxygen photosensitizer, into the extracellular matrix during plant parasitism.[2] In the presence of light and oxygen, singlet oxygen is generated, causing lipid peroxidation and ultimately plant cell lysis.[3] Efforts to understand how the fungus protects itself against its own toxic mechanism demonstrated that genes coding for proteins in the biosynthesis of vitamin B6 are essential for singlet oxygen resistance.[4] Subsequently, the ability of pyridoxine and the biologically interconvertible forms of vitamin B6 to quench singlet oxygen was demonstrated.[5] While the reaction of singlet oxygen with other vitamins has been extensively investigated,[6] the reaction of pyridoxine with singlet oxygen is essentially unknown. We report the results of kinetic and product analyses of the reaction of singlet oxygen with pyridoxine.

Singlet oxygen is generated by photoexcitation of a sensitizer that ultimately transfers energy to ground-state triplet oxygen. The fate of singlet oxygen in the absence of a quencher is a first-order decay to the ground state, k_d. In the presence of a quencher, singlet oxygen can irreversibly react with quencher, k_r, or be quenched directly to the ground-state triplet oxygen, k_q. The latter two processes are first-order with respect to quencher. A plot of observed singlet oxygen decay rate versus quencher concentration should be linear with a slope equal to the total quenching rate constant ($k_r + k_q$).[7]

Singlet oxygen decay rates, measured by time-resolved laser flash generation of singlet oxygen followed by monitoring singlet oxygen phosphorescence decay, confirm previous observations[5] that pyridoxine is a moderate quencher. The total rate constants in deuterated methanol for pyridoxine (**1a**), 3-hydroxypyridine (**2**), and 3-methoxypyridine (**3**) are 1.0×10^7, 3.2×10^6, and 3.5×10^4 M^{-1} s^{-1}, respectively. The data for **1a** and **2** were nonlinear at high concentrations (Figure 1). To avoid pyridoxine consumption during the quenching measurement, new solutions were prepared at each concentration point, and irradiation of the solutions was minimized. There is no evidence for aggregation, as both **1a** and **2** obey Beer's Law over the concentration range of the experiment. The cause of the downward curvature is unknown, but probably results from consumption of the substrate. The bimolecular quenching rate constants reported are fitted from the first four points and represent a lower bound to the true values. The total quenching rate in methanol for **1a** is comparable to previously reported values in ethanol and aqueous buffers.[5]

Quenching rate constants indicate that the phenoxy group is essential for the quenching ability of **1a**. Singlet oxygen is long-lived in pyridine, whereas phenols are efficient quenchers.[8] The quenching rate constant for **2** is approximately 100 times larger than that of the methylated compound, **3**; a similar rate difference is observed between phenol and anisole.[9]

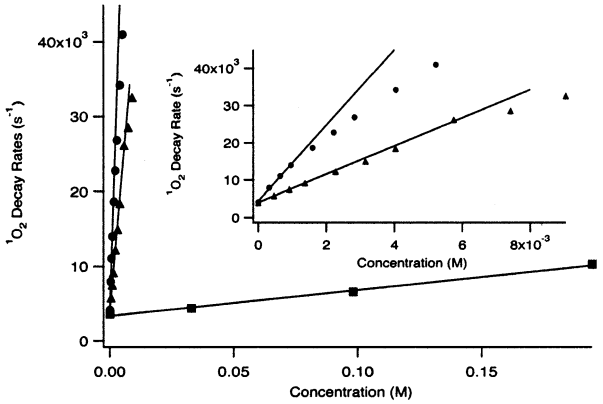

Figure 1. Observed rate constant for luminescence decay of singlet oxygen for **1** (●), **2** (▲), and **3** (■). Inset is expanded plots for **1** and **2**.

The chemical quenching rate constant, k_r, for **1a** was estimated by comparison with a known singlet oxygen reaction (k_r for tetramethylethylene, TME, is 3×10^7 M^{-1} s^{-1}).[10] The relative rate of reaction was estimated by comparing NMR peak heights for mixtures of pyridoxine and TME in deuterated methanol before and after photooxidation.[11] Results showed that degradation of TME is 1.8 times faster than that of **1a**. This result indicates that the chemical quenching rate of **1a** (1.7×10^7 M^{-1} s^{-1}) dominates the total quenching. Because this is larger than the observed value for ($k_r + k_q$), the actual value of the latter may be somewhat higher. Alternatively, the estimate of k_r may be higher than the actual value due to decomposition processes of **1a** which do not involve singlet oxygen.

The major product (>90% by NMR) of a preparative reaction of pyridoxine with singlet oxygen in methanol was isolated and characterized by NMR and MS. The results are consistent with a methanol adduct of substituted 2,5-pyridinedione **4a**. Pulsed gradient HMBC analysis shows correlation of the H4′ methylene (4.56 ppm) with the C5 carbonyl (195.2 ppm). The methoxy group of **4a** is labile and exchanges with the deuterated solvent. Compound **4a** is not stable at high concentrations.

Because of the insolubility of **1** in nonpolar solvents, the compound was derivatized with TBDMSCl. The di-protected compound (**1b**, R = TBDMS) was readily soluble in methylene chloride-d_2 and was partially photooxidized (50% degradation by NMR) at −80 °C. The sample was analyzed by NMR and contained two products in a ratio of 3:2.

The major product is a substituted 6,7-dioxa-8-aza-bicyclo[3.2.1]-octenone (**5b**). The NMR data are summarized in Figure 2. Two characteristic carbons at 96.8 and 86.6 ppm correspond to sp^3

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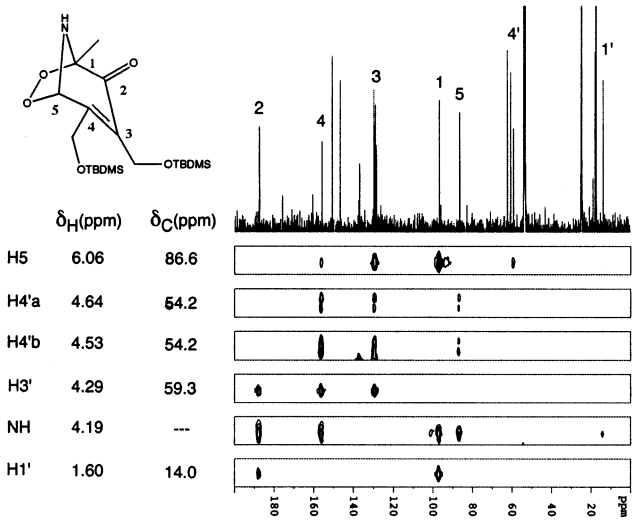


Figure 2. Summary of NMR data for **5**. Below the ^{13}C NMR spectrum, 0.1 ppm slices of the 2D HMBC about proton resonances (δ_H) are represented. HMQC correlations are presented in column δ_C.

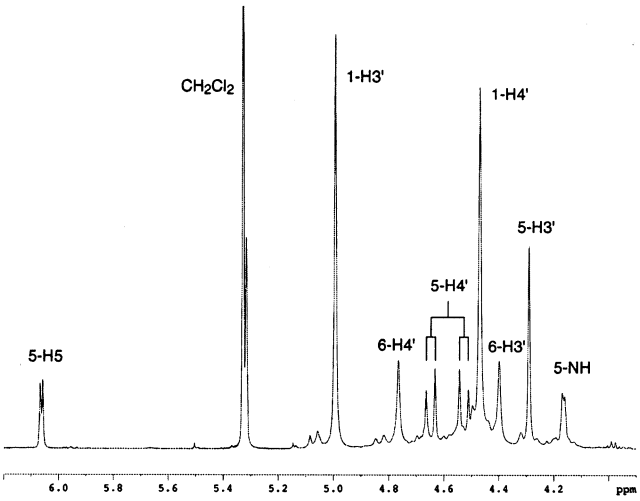


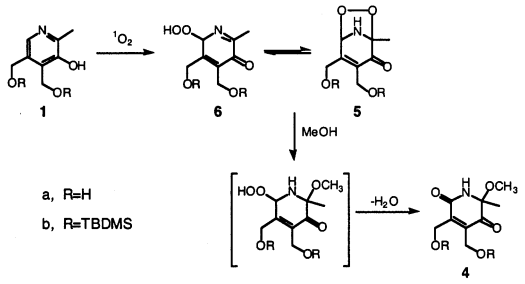
Figure 3. Expanded ^1H NMR spectrum of a photooxidized mixture in CD_2Cl_2 at −80 °C.

hybridized carbons attached to two heteroatoms, C1 and C5. The HMBC data show cross-peaks between both carbons and the NH proton signal at 4.19 ppm and confirm that these carbons reside on the same molecule. The 3′ and 4′ methylene protons are diastereotopic, but only the 4′ protons appear as a doublet of doublets (Figure 3). The highly deshielded H5 proton chemical shift at 6.06 ppm is similar to that in an analogous 1,3-endoperoxide formed from photosensitized oxidation of 13-hydroxyberberinium phenolbetaine.[12]

The minor product is assigned as hydroperoxide **6b**. HMBC analysis indicates that the proton signals at 4.76, 4.40, and 2.16 ppm are correlated with corresponding carbon signals. Two characteristic carbon signals at 160.7 and 96.1 ppm correspond to the imine C1 and hydroperoxide C5 carbons, respectively. HMBC data show correlation between the H4′ methylene (4.76 ppm) and C5, which indicates that the hydroperoxide is not on C1 even though the chemical shift is similar to that of the tertiary endoperoxide carbon in **5b**. The ^{13}C chemical shifts for **5b** and **6b** were consistent with B3LYP/6-311+G** GIAO calculations performed on optimized structures of **5a** and **6a**.

The relative amounts of **5b** and **6b** did not change over the course of the experiment (12 h), which indicates that **6b** is not slowly converting to **5b** but that **5b** and **6b** are probably in equilibrium. When heated to room temperature, the reaction produces a complex mixture of products.

Scheme 1



Reactions performed in water and DMSO are analogous to those in methanol and methylene chloride, respectively. The overall reaction is shown in Scheme 1. At low temperature, the OH proton signal of **1b** at 9.51 ppm shows multiple bond couplings to carbons C1, C2, and C3; no correlation is observed to C4 and C5, which would be expected for any zwitterion. Attack of singlet oxygen leads to addition at C5 and proton transfer to give **6**. Addition para to the hydroxy group is analogous to products formed in the reaction of singlet oxygen with para alkylated phenols, but these have never been observed with 4-unsubstituted phenols.[13] In protic solvents, solvolysis of **5** or direct solvent addition to **6** leads to hydrogen migration and elimination of water to form the final product.

Vitamin B6 is an effective quencher of singlet oxygen and could be used as a protective mechanism against photosensitized oxidation. However, efficient chemical quenching means that it will be rapidly consumed. Also, insolubility of pyridoxine in nonpolar solvents makes it questionable as a protective mechanism against lipid peroxidation.

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Supporting Information Available: Experimental procedures, chemical shift calculations, ^1H, ^{13}C, and HMBC NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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