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COMMUNICATION

Novel ring chemistry of vitamin B_6 with singlet oxygen and an activated ene: isolated products and identified intermediates suggesting an operable [3 + 2] cycloaddition†

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Pyridoxine reaction with $^1O_2$ in aqueous solution at neutral pH resulted in oxidation at the 2- and 6-positions of the pyridine ring and unprecedented ring contraction. Kinetic and low temperature studies provided observable intermediates by NMR spectroscopy. In addition, novel cycloaddition between pyridoxine and *N*-methylmaleimide, without *N*-alkylation and in water, suggest a common [3 + 2] cycloaddition with the 3-hydroxypyridine ring.

Introduction

Vitamin B_6 (Fig. 1), in its active form pyridoxal-5′-phosphate (PLP), is a well known cofactor necessary for primary metabolism in both eukaryotic and prokaryotic organisms.[1] It has been estimated that 1.5% of the genes in a typical *E. coli* bacterium encode PLP-dependent enzymes.[2] These enzymes function in a variety of settings including amino acid metabolism, hormone biosynthesis, neurotransmitter biosynthesis, glycogen degradation, and lipid biosynthesis.[3] Much of the chemistry of PLP relates to its ability to form a Schiff base with a potential substrate and utilize the 3-hydroxypyridine ring as an electron sink to catalyze a select group of chemical reactions including decarboxylation, transamination, racemization, and deamination.[4]

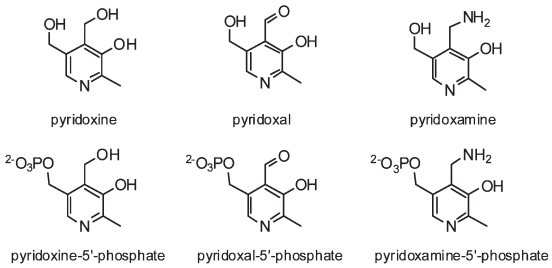
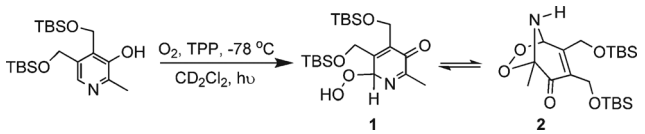


Fig. 1 All forms of vitamin B_6.

In recent studies, vitamin B_6 has been implicated as an antioxidant, countering the known effects of various reactive oxygen species (ROS).[5,6] For example, pyridoxamine has been shown to inhibit the formation of 4-hydroxynonenal (4-HNE) and malonaldehyde (MDA), markers for lipid peroxidation and protein glycosylation in red blood cells.[7,8] *A. thaliana* and the fungus *C. keidi* have been shown to upregulate vitamin B_6 expression under singlet oxygen ($^1O_2$) induced stress.[9,10]

Specifically looking at singlet oxygen-related stress, pyridoxine has been shown to be an efficient quencher of $^1O_2$ with a second order rate constant of 10^7 to 10^8 M^{-1} s^{-1} under high intensity light stress.[6,11] Of particular interest, endoperoxide **2** (Scheme 1) was observed when a di-TBS analog of pyridoxine was oxidized at reduced temperatures in CD_2Cl_2 and shown to be in equilibrium with the hydroperoxide **1**.[11]



Scheme 1 Products by NMR of singlet oxygen induced oxidation of a di-TBS protected pyridoxine analog.[11]

Results and discussion

We report novel products of reaction between pyridoxine and singlet oxygen in protic solvents at room and reduced temperatures. The isolated final product is an unprecedented ring contracted α-hydroxyketone. Probing the reaction mechanism by intermediate identification uncovered a six-membered ring keto-lactam through time-dependent NMR studies. Temperature-dependent experiments also revealed a transient endoperoxide bicycle in methanol. Pyridoxine was also found to undergo a previously undescribed reaction with *N*-methylmaleimide under similar conditions providing an analogous bicyclic product. We

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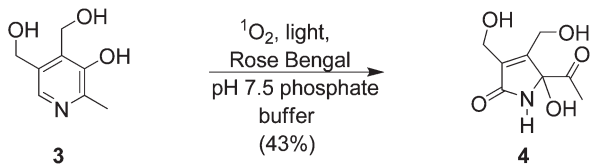
propose that both reactions proceed through a formal or concerted [3 + 2] dipolar cycloaddition.

Singlet oxygen addition to pyridoxine

Pyridoxine was subjected to $^1O_2$ through the photosensitization of rose bengal (RB) in aqueous solution.[12] Reactions performed in phosphate buffer, NH_4OAc buffer, and in unbuffered water, all at near neutral pH, produced the same product shown in Scheme 2. These results suggest that the salt content of the aqueous solution is not important to the reaction outcome. The crystalline compound **4**, produced as the major product in 43% yield, was characterized by 1D and 2D NMR and then further confirmed by X-ray crystallography (Fig. 2). When the reaction was run without RB or light, no reaction was observed over a 24 h time period when monitored by TLC or ^1H NMR.[13] The ring contracted product shows additional oxygenation at the original 2- and 6-positions. This unusual ring contraction led to an



Scheme 2 Isolated ring contraction product of $^1O_2$ treated pyridoxine in buffered water in the presence of 510 nm filtered light for 4 h.

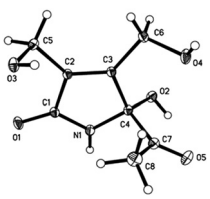


Fig. 2 ORTEP plot of lactam product **4**.

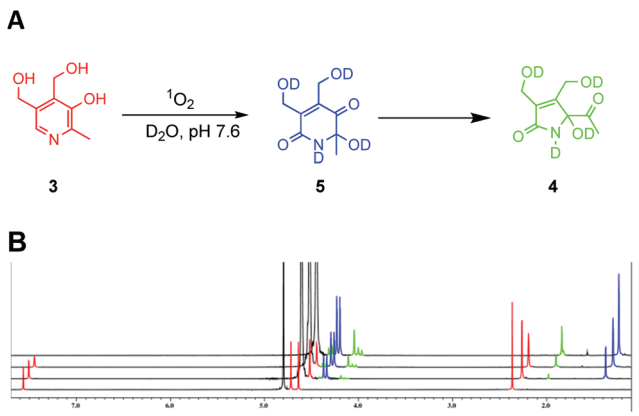


Fig. 3 ^1H NMR kinetic study of pyridoxine in the presence of singlet oxygen shown in 1 h intervals. Signals are labelled according to the reaction scheme in (A). After one hour, the main product is compound **5**. Over three hours, **3** is converting to **4** (green) and **5** (blue).

investigation of the underlying pathway associated with this particular transformation.

In order to determine if product **4** was an artifact of purification, we observed its formation directly by NMR. The experiment involved a time course run in one hour intervals in D_2O under the same conditions as in Scheme 2. Fig. 3 illustrates the reaction progression over a total of three hours. Pyridoxine is converted to **4**, with an additional intermediate formed. 2D NMR analysis of this compound supports lactam **5** (Fig. 4). The data obtained through HMBC analysis shows long range coupling between the 2-position carbonyl (162.7 ppm) and the 3′-methylene as well as coupling between the 5-position carbonyl (196.2 ppm) and the 6′-methyl at 1.59 ppm. Integration of corresponding peaks indicate a lag in the production of **4** relative to **5** suggesting the initial formation of **5** with subsequent conversion to **4**.

When the reaction solvent was changed to CH_3OH, product **6** was isolated; identical to that described by Foote (Scheme 3A).[11] The similarity between compounds **5** in water and **6** from methanol is noteworthy, as is the inability of **6** to contract to compound **4** in CH_3OH. With analogous products of singlet oxygen addition formed in CH_3OH and aqueous buffer, reduced temperature NMR studies were carried out in methanol-d_4 to observe the initial adduct between $^1O_2$ and pyridoxine (Scheme 3B).

When the reaction was run at −78 °C in CD_3OD, a single product was seen by NMR. The endoperoxide **7** matches the obtained NMR data. Fig. 5 illustrates the ^{13}C and pertinent 2D data. The pyridoxine derived bicycle **7** is analogous to compound **2** observed by Foote in CD_2Cl_2 and closely matches the reported NMR spectra.[11] Of particular note is the peak at 5.93 ppm representing the C1 proton. This signal is coupled to both alkene carbons as well as the opposite bridgehead carbon. No analog to the reported hydroperoxide **1** was found in this experiment indicating no observable equilibrium between an open hydroperoxide adduct and the observed endoperoxide bicycle **7**.

An analogous NMR experiment replacing CD_3OD with CH_3OH did not support the incorporation of an OCH_3 from solvent into the intermediate, further supporting **7** (ESI†). A temperature-dependent experiment was conducted to observe the stability of **7** (Fig. 6). ^1H NMR reveals that as the reaction is warmed, **7** is stable to −40 °C. It then converts to a set of compounds from −30 to −10 °C. Eventually the transient intermediate converts to one major product, **8**, at room temperature, which, again matches the final product previously described.[11]

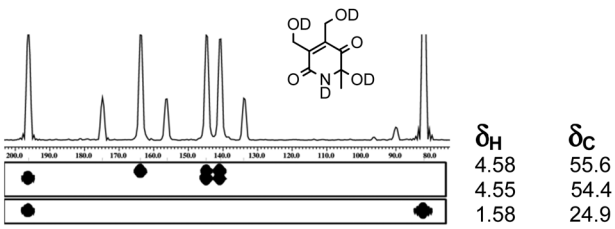
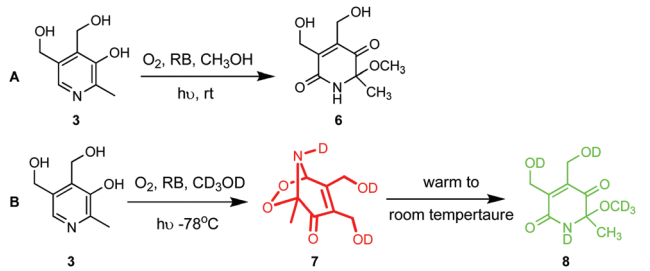


Fig. 4 Summary of data for compound **5**. ^{13}C NMR of mixture of **4** and **5** and summary of HMBC data for **5** presented in 2D spectra. Summary of ^1H NMR and HMQC data for **5** listed in columns.



Scheme 3 (A) Reaction of pyridoxine in methanol. (B) In CD_3OD the bicyclic adduct **7** represents the product of the clean and specific conversion at reduced temperatures after 4 h.

Addition of an electron deficient ene to unactivated pyridoxine

Imagining a mechanism for endoperoxide formation with $^1O_2$, pyridoxine's calculated and experimentally determined zwitterionic nature in pH 7 aqueous solvent (Fig. 7) presents an electronic arrangement which could both donate and accept electronic density in the aromatic ring.[14,15] This electronic arrangement is analogous to *N*-alkylated 3-hydroxypyridines under basic conditions, which are utilized in the [3 + 2] dipolar cycloaddition of

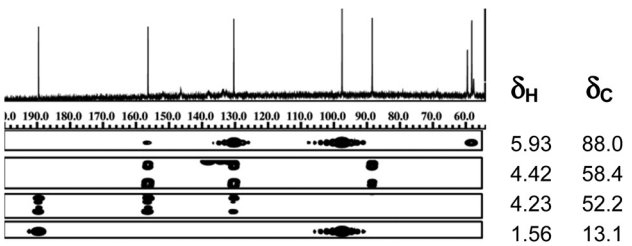


Fig. 5 Summary of NMR data for endoperoxide **7** at −78 °C in CD_3OD. ^1H and HMQC data for **7** listed in right columns. ^{13}C and HMBC data for **7** presented in 2D spectral cutouts.

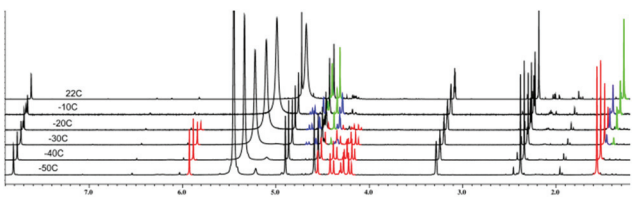


Fig. 6 Intermediate **7** (peaks associated shown in red) appear stable to temperatures of −40 °C. An unidentified intermediate (blue) appears at approximately −30 °C but disappears at room temperature. The final product, **8** (green), appears at −30 °C and grows until it is the major product at room temperature.

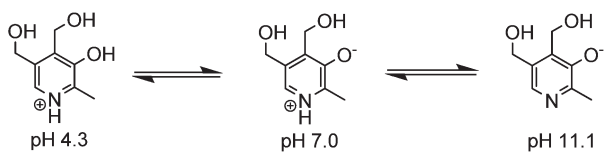
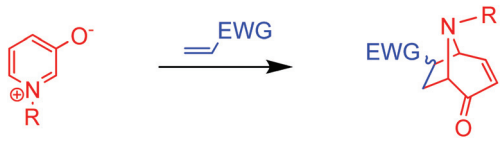
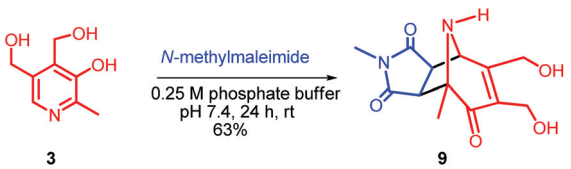


Fig. 7 Experimentally and computationally determined charge state of pyridoxine at differing pH values.



Scheme 4 3,2-Dipolarcycloaddition of 3-hydroxypyridinium betaines with electron poor ene.



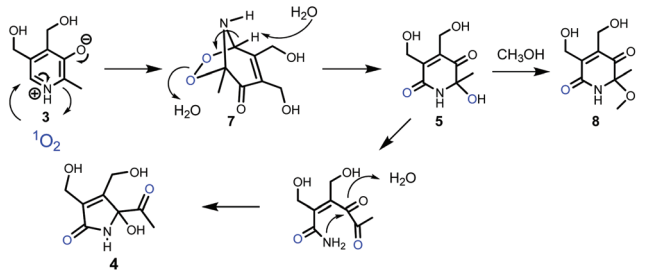
Scheme 5 Successful [3 + 2] cycloaddition with pyridoxine in aqueous buffer.

electron deficient enes (Scheme 4).[16] This zwitterionic structure, along with the identification of structure **7** provides mechanistic clues suggesting a possible formal or concerted [3 + 2] cycloaddition reaction between pyridoxine and singlet oxygen at physiological pH. The endoperoxide **7** would be the product of such a reaction.

Cycloadditions with singlet oxygen are well known[17] and a mechanistic hypothesis has been suggested in a previous computational study on singlet oxygen addition to pyridoxine, but not investigated.[18] Similar bicyclic endoperoxides, through this hypothesized mechanism, have been reported though in the $^1O_2$ oxidation of pyrroles and furans.[19]

To further validate a proposed [3 + 2] cycloaddition mechanism, pyridoxine was treated with *N*-methylmaleimide at room temperature in pH 7.5 aqueous phosphate buffer (Scheme 5). Surprisingly, a single diastereomer was isolated in 63% yield and determined by X-ray crystallography to be the exo product **9**. The hypothesized zwitterionic nature of pyridoxine in neutral water electronically allows the observed cycloaddition, supporting the ability of vitamin B_6 to react specifically at the 2- and 6-position. When the reaction was extended to 3-hydroxypyridine, no product was observed.[20] The apparent superiority of the pyridoxine structure will warrant additional investigation of the pyridine substitution necessary for robust reaction. It is noteworthy that this is the first described cycloaddition reaction between a 3-hydroxypyridine and ene in water *and* the first without prior nitrogen alkylation.

Given the intermediates and products formed, a mechanistic hypothesis is proposed for $^1O_2$ quenching by pyridoxine (Scheme 6) consistent with the Kornblum–DeLaMare rearrangement.[21] Initial cycloaddition with the pyridoxine zwitterion provides the endoperoxide bicycle and is followed by proton removal from the bridgehead with concomitant peroxide cleavage and carbonyl formation. The resulting lactam (capable of exchanging in methanol to provide product **8**) then opens and recloses on the 1,2-diketone to produce the ring contracted product. The nature of ring contraction in aqueous solutions is uncertain and may proceed through a stepwise or concerted mechanism. Additional work has begun looking at similar reactions with the other forms of vitamin B_6 and in determining the source of oxygen atoms in the final product.



Scheme 6 Hypothesized [3 + 2] cycloaddition of singlet oxygen to pyridoxine in protic solvents.

Conclusions

Pyridoxine has been shown to undergo a hypothesized [3 + 2] cycloaddition reaction to give bridged bicycles with singlet oxygen and *N*-methylmaleimide. The reactivity, in aqueous buffer, is believed to proceed through the pyridoxine zwitterion leading to unprecedented reaction with the 3-hydroxypyridine ring. The described reactions are the first reported products of $^1O_2$ reaction in aqueous systems with production of a novel ring-contracted lactam. We also describe a novel product of reaction between pyridoxine and maleimide as the first dipolar cycloaddition with a 3-hydroxypyridine in water and without prior nitrogen alkylation.

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