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The Reaction of Oximes with 4-Phenyl-1,2,4-triazoline-3,5-dione to Produce Nitric Oxide – Model Compounds for Nitric Oxide Synthase

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ABSTRACT

Article history: Received Received in revised form Accepted Available online Certain oximes form nitric oxide upon reaction with 4-phenyl-1,2,4-triazole-3,5-dione (PTAD). The oximes appear to undergo an Alder-ene reaction with the PTAD enophile to form a nitroso intermediate capable of dimerization and/or nitric oxide formation. Upon exposure to oxygen, the nitroso compounds eventually form ketones. This reaction may serve as a model for the study of nitric oxide synthase (NOS), the enzyme responsible for physiological production of nitric oxide. NOS is known to produce an oxime intermediate which reacts with oxygen to produce nitric oxide and citrulline.

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Oxidation; Oximes; Nitric Oxide; Ene Reaction

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Nitric oxide synthase (NOS) is a complex enzyme with multiple cofactors that is responsible for forming preparing physiological nitric oxide (NO). The mechanism of formation of nitric oxide is not entirely understood. What is known is that the enzyme forms an intermediate oxime by hydroxylation of a terminal nitrogen of arginine substrate. This oxime, N⁽⁰⁾-hydroxy-L-arginine, reacts enzymatically with oxygen to form nitric oxide and citrulline (see Scheme 1).¹



Scheme 1. Nitric Oxide Synthase Reaction

The NOS oxime intermediate may undergo an enzymatically facilitated Alder-ene reaction with oxygen to form a nitroso intermediate. The Alder-ene reaction (also called the ene reaction), is similar to the Diels-Alder reaction.² An ene reaction with an oxime and a typical enophile would theoretically produce a nitroso compound. Various mechanisms have been proposed for the ene reaction. These include the concerted one shown below (Scheme 2), a radical version, and a polar mechanism. Ene reactions may involve heteroatoms.⁴

Scheme 2. Ene Reaction With Oxime 'Ene,'

In NOS, the oxime intermediate reacts with an oxygen molecule that is coordinated to a heme iron cation. This oxygen molecule could play the role of enophile, in the formation of the nitroso intermediate, through one of the ene mechanisms mentioned above. The nitroso intermediate might then be unstable enough to collapse into citrulline, nitric oxide and hydroxyl radical (Scheme 3).

Scheme 3. Hypothetical NOS Mechanism

To the best of our knowledge, ene reactions with an oxime ene are not known, nor have they been proposed as a potential mechanism for NOS. Therefore, we undertook this investigation to determine 1) whether oxime enes can undergo ene reactions and 2) if the nitroso products could be observed.

An O-alkyl oxime has been reported to undergo an ene reaction as an enophile.⁵ Nitroso compounds⁶ and nitro-like oxoammonium cations⁷ also undergo ene reactions as enophiles.

Nitroso compounds have a characteristic $n \rightarrow \pi^*$ absorption from 630-790 nm, giving them a blue color.⁸ When nitroso compounds form with an alpha hydrogen, they rapidly tautomerize to oximes (Scheme 4a), making it next to impossible to detect the nitroso tautomer. However, nitroso compounds with no alpha hydrogens can be observed and even isolated in some cases. Nitroso compounds are also known to dimerize (Scheme 4b) to form colorless *cis* and *trans* dimers,^{9,10,11} which further complicate their detection. This dimerization is a result of the dual nucleophilic and electrophilic character of the nitroso nitrogen. Its nucleophilic character arises from its lone pair and its electrophilicity from its similarity to an aldehyde carbon.

Scheme 4. (a) Nitroso-Oxime Tautomerization. (b) Nitroso Dimerization



With these considerations in mind we set out to test the reactivity of various oximes with one of the most reactive



enophiles known, 4-phenyl-1,2,4-triazole-3,5-dione (PTAD).



Like the Diels-Alder reaction, ene reactions often require high temperatures, which may not be suitable for observing nitroso compounds. The reactivity of PTAD allows it to undergo ene reactions at or below room temperature.¹² The double bond between the two PTAD nitrogens has a reactivity that is similar to singlet oxygen. Solutions of PTAD are bright red.

PTAD

Acetone oxime reacts with PTAD to produce a somewhat unstable nitroso compound. The reaction is best done in either methylene chloride or chloroform solvent due to the instability/reactivity of PTAD in other solvents. The reaction is confirmed by loss of the red PTAD color and the appearance of a blue solution and corresponding UV-visible maximum at of 670 nm, consistent with nitroso compounds. A new singlet also appears in the ¹H NMR spectrum at 1.79 ppm (similar to the ¹H NMR signal of 2-methyl-2-nitrosopropane).^{8b} Crude ESI-MS spectra of the reaction mixture also showed peaks corresponding



to the nitroso compound. This nitroso compound then enters a complex equilibrium with two competing reactions, 1) dimerization to *cis* and *trans* nitroso dimers, and 2) radical formation of nitric oxide and an organic radical (Scheme 5). When even small amounts of oxygen are present, the nitric oxide reacts with it to form nitrogen dioxide, which then forms an equilibrium with dinitrogen tetroxide.

Scheme 5. Reaction of Acetone Oxime with PTAD

The appearance of two colorless nitroso dimers is confirmed by two additional ¹H NMR signals that appear at about 1.8 ppm (similar to the ¹H NMR shift of the 2-methyl-2-nitrosopropane dimer at 1.6 ppm).^{8b} The formation of nitric oxide is observed by running the reaction in an NO analyzer, which detects the presence of NO by reacting it with ozone, forming exited state NO₂, which emits a photon that can be detected. manifested by the initial formation of a gas (when the reaction is run in a capped NMR tube the cap pops off as the reaction progresses). Furthermore, when the reaction is run in a small vial with a screwed on cap the final solution has a white precipitate and is colorless (nitric oxide and dinitrogen tetroxide are colorless); however, when the cap is opened, more oxygen enters the vial and the equilibrium shifts to yield a yellow brown gas (nitrogen dioxide) which evolves from the vial. Also, when the reaction is run in a closed ESR tube two new radical signals appear (presumably due to the nitric oxide and organic radical). The solution also turns pink indicating that some PTAD is reformed. As the reaction is allowed to run to completion, two additional products are formed, acetone and a precipitate, 4-phenylurazole (confirmed by their ¹H NMR signals). The products are both derived from the organic radical formed from the initial nitroso compound. No other major products are observed in the reaction of acetone oxime with PTAD. A mechanism consistent with these observations is shown below.

Other oximes also react with PTAD. Acetophenone oxime reacts slowly with PTAD, possibly due to steric crowding or conjugation of the ene with aromatic ring. The major product is acetophenone, but other minor products are also formed, consistent with a radical reaction. No nitroso peak is observed in the UV-visible spectrum, possibly because the nitroso rapidly falls apart to NO and a benzyl radical. The reaction of cyclohexanone oxime with PTAD is very fast and similar to the reaction of acetone oxime with PTAD – a nitroso intermediate is observed by UV and the final product is almost exclusively cylcohexanone. Heptanal oxime also reacts rapidly with PTAD, and a fleeting nitroso compound is observed briefly by UV, which most likely tautomerizes to the oxime.

The alpha nitrogen present in all of the nitroso compounds made in this study may have something to do with their instability and radical decomposition. Indeed, similar compounds with an alpha acetate, such as 1-nitrosylcyclohexyl acetate, are much more stable, but form HNO, and cyclohexanone when the acetate is hydrolyzed.^{13,14} An examination of the radical stabilizing ability (radical sigma value)^{15,16} of an amine ($\sigma =$ 0.69), relative to methoxy ($\sigma = 0.24$), or methyl group ($\sigma =$



0.11), indicates that the adjacent nitrogen atom is much better at

stabilizing a radical, and may facilitate loss of nitric oxide from the nitroso.

In conclusion, acetone oxime, acetophenone oxime, cyclohexanone oxime, and heptanal oxime react with PTAD at room temperature. When a benzyl nitroso is formed, it is not detected by UV. Nitric oxide is produced as the nitroso compounds decompose. The reaction of oximes with PTAD at room temperature might be a good model reaction for the formation of nitric oxide by NOS.

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Supplementary Material

Supporting Information is available and includes experimental procedures and spectra (NMR, UV, EPR, NO analysis, and ESI-MS).

Graphical Abstract



Highlights

- Oximes react with PTAD to produce nitric oxide and ketones.
- An intermediate nitroso compound is detected.
- Accepter The mechanism may provide insights into the mechanism of nitric oxide synthase. .