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Reactions of Nitric Oxide with β-Hydroxylimino Esters

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ABSTRACT

Nitric oxide (NO) and its one electron reduction product nitroxyl (HNO) are known to act as vasodilators in mammals, including humans, and are considered a possible cure to cardiovascular diseases. However, their instability and high reactivity with atmospheric oxygen preclude the direct use of them as drugs. Recent work has shown that NO donor molecules can be used as pro-drugs to deliver NO to the appropriate bio-sites. Therefore, there is considerable research interest in the synthesis and studies of donors capable of releasing either of the molecules under physiological conditions. As part of our research effort towards the synthesis of new NO- and HNO-donors, we have synthesized new nitroxy derivatives from the reaction of two β-hydroxylimino esters namely, methyl 3-hydroxyliminobutanoate and methyl 3-hydroxyliminopentanoate, with potassium hydroxide and NO. The isolated products are characterized as potassium 3-methyl- and 3-ethyl-5-isoxazolone-4-diazeniumdiolate, as well as potassium 4-acetyltriazolium-1,2,3-triolate. The new diazeniumdiolates are first examples of diazeniumdiolate-substituted isoxazolone. Under modified conditions, the reactions yield the potassium salts of 5-acetyl- and 5-propionylsydnonate-N-oxides.

Introduction

This experiment was aimed at the synthesis of nitric oxide (NO) donor pro-drugs. Nitric oxide and its one electron reduction product nitroxyl (HNO) are known to have physiological roles in the body, where nitroxyl can act as a deterrent to alcoholism, and nitric oxide can act as a cure to cardiovascular diseases by acting on epithelial tissue. Compounds containing both nitric oxide and nitroxyl are known to be versatile, where they can release NO and HNO under controlled physiological conditions. However, due to the versatility of these compounds, nitric oxide can react with oxygen in air, causing explosive properties. Further study of these decomposition properties can suggest how compounds release nitric oxide and how they will react under physiological conditions.

The addition of nitric oxide (NO) is assumed to be dimeric. This dimeric addition is used for the formation of sydnone-N-oxide and diazeniumdiolate products. The dimeric addition can add in a cis- or trans- configuration, with the majority of products having added cis-. In our synthesis, we assume the possibility of a trans- addition for the sydnone product. Depending on the amount of NO available and added, the biological effect is different. Figure 1 below demonstrates the possible additions of dimeric nitric oxide in each configuration, ultimately creating the same product:
Figure 1: Dimeric addition of nitric oxide for the formation of sydnone-N-oxide and diazeniumdiolate products. Upper path adds in a trans-formation with the condensation and loss of methanol. The cis-formation first rotates on a single bond before condensing.

By creating a reaction between a β-ketoxime ester, such as methyl acetoacetoxime (R = Me), and nitric oxide, products can be created as NO releasing compounds. By using ketoximes with both R groups of methyl and ethyl, the nitric oxide is expected to react with the acidic CH$_2$ group on the ketoxime. Figure 2 shows what the expected products could be under the conditions mentioned in the figure, as well as in the following experimental section:

Figure 2: Under given conditions, this reaction of a ketoxime is expected to produce one or all of these four products with diazeniumdiolate groups attached at the acidic CH$_2$ groups.

Experimental

The required oxime substrate was not commercially available, and was generated in situ without being isolated. The following Figure 3 shows previous research of this substrate being generated and isolated under the condition of pyridine. During this synthesis, hot methanol was used in the place of pyridine.

Figure 3: The synthesis of hydroxylamine hydrochloride and potassium chloride under the conditions of hot methanol to produce the required oxime substrate to use for continued synthesis and addition of nitric oxide.

Two different methods were used for the synthesis of products. The initial steps included the addition of about 150 mL of ethanol, one equivalent of hydroxylamine hydrochloride, and one equivalent of the required substrate (either ethyl acetoacetate (R=Me) or ethyl propionylacetate (R=Et)).

The first method allowed the reaction mixture to sit for 24 hours before adding one equivalent of potassium hydroxide. After the 24 hours at room temperature, the mixture was degassed and treated with NO for six hours. For the second method, one equivalent of potassium hydroxide was added immediately to treat the acidic CH$_2$ group. This was cooled to -10°C, then de-gassed with N$_2$ in order to remove oxygen, as NO is reactive with air. The following contraption was used for degassing the mixture:

![Degassing contraption and NO reactor](image)

**Figure 4:** Degassing contraption and NO reactor.

**Figure 4** also shows the setup for the NO reactor, as the mixture was treated with NO after being de-gassed. This second procedure treated the mixture with NO for six hours as well.

**Results and Discussion**

The β-ketoxime reactions formed two unexpected products under the two different methods. The products from the different methods were found to be different through dissimilar UV-Visible and NMR spectra. A diazeniumdiolate product and a sydnone product were both observed. Mechanisms and spectra are both shown below. The first method that required a 24-hour reaction time created a diazeniumdiolate product. Only the ethyl derivative was used for this synthesis, resulting in the product of potassium 3-ethyl-5-isoxazolone-4-diazeniumdiolate, as shown below in **Figure 5**:
Figure 5: The synthesis of potassium 3-ethyl-5-isoxazolone-4-diazeniumdiolate (R = Et) through a 24-hour reaction mixture time and nitric oxide treatment.

Previous research shows that over an extended period of time, this condensation is possible to create an isoxazolone ring, as shown in Figure 6:

Figure 6: The condensation and creation of an isoxazolone ring under given conditions. Condensation mechanism was used for further analysis of this product.

The following UV-Visible and NMR spectra are for confirmation of the structure of this diazeniumdiolate product, or EtC$_3$HNO$_2$N$_2$O$_2$K. Diazeniumdiolate products will have a single peak at 245 with one addition of a diazeniumdiolate, 258 with two additions, or 265 nm with three additions.

Figure 7: UV-Visible spectra of the ethyl derivative of the diazeniumdiolate product with a single peak at 245 nm.

Figure 7 shows evidence of a diazeniumdiolate product with only one addition, contrary to the original predicted products for this reaction. This is a result of the diluted product for the UV-
Visible spectrum analysis, showing a possible structure for the diazeniumdiolate. Further evidence for the structure can be seen in the following NMR spectra.

**Figure 8:** H1 NMR spectra for the ethyl derivative of the diazeniumdiolate product. Triplet found at 1.1, and quartet found at 2.3-2.4. Single peak at 4.7 is indicative of reference solution D$_2$O/DSS.

The above proton spectra for the product EtC$_3$HNO$_2$N$_2$O$_2$K shows a triplet and a quartet, which is indicative of an ethyl group on the compound. However, no peak is found for the single hydrogen off of the carbon containing the diazeniumdiolate. This can be explained by the C13 NMR spectra in **Figure 9:**
Figure 9: C13 NMR for EtC3HNO2N2O2K, showing two lower peaks from ethyl group, two singlets and high ppm values, and five carbons total with one triplet present at 75.6 ppm.

Figure 9 also shows the presence of a triplet around 75.6 ppm. While these values indicate an isoxasolone ring, the triplet presents the possibility of something else. However, due to the lack of a hydrogen peak in the H1 NMR, it was found that the reference solution, D2O/DSS had interfered with the structure. Previous research shows that deuterium can split a carbon into a triplet. With the deuterium substituting the hydrogen, no peak is found on the proton NMR and a triplet is found on the C13 NMR, further clarifying the structure of this product.

Due to the previously known condensation of the isoxazolone ring and the structure suggested from the spectra, the following mechanism for this diazeniumdiolate can be introduced with the addition of the nitric oxide:
Figure 10: Assumed mechanism for isoxazolone ring diazeniumdiolate product, through condensation over 24-hour reaction time and addition of NO and KOH.

Evidence for the methyl derivative of this product is not shown, as the methyl product has yet to be purified and further tested.

The second method for this synthesis took place immediately, rather than over a 24-hour reaction time before adding NO and KOH. Both methyl and ethyl derivatives were available for analysis, with products assumed to be potassium 4-acetyltriazolium-1,2,3-triolate and potassium 4-propionyltriazolium-1,2,3-triolate:

Figure 11: Synthesis of potassium 4-acetyltriazolium-1,2,3-triolate (R=Me) and potassium 4-acetyltriazolium-1,2,3-triolate (R=Et) sydnone-N-oxime products with immediate additions of KOH and NO.

These products are assumed to be different from the diazeniumdiolate products due to the additional peaks of the UV-Vis spectra as well as differing NMR spectra. These are assumed to be sydnone products, as UV-Vis data is somewhat similar to previously recorded sydnone-oxide UV-Vis, but with one additional peak, as shown below for the methyl derivative (MeCOC$_2$N$_3$O$_3$K$_2$):
This product was assumed to be a sydnone derivative based on previous literature stating that sydnone products have two peaks, one at ~230 nm and one at 300 nm. Further data confirmed the structure of a sydnone derivative, but with an extra nitrogen in the ring, resulting in the third extra peak in the UV-Visible spectrum. In Figure 13, a peak at 2.402 can be seen for the H1 NMR, indicative of the acetyl group on the product:
Figures 13 and 14 show the NMR spectra for the methyl sydnone product, with peaks for an acetyl group and four carbons. The NMR spectra are somewhat consistent with an expected sydnone already known in the literature, but the numbers are different from the literature values. The peak positions of this product are not identical, which leads to the conclusion that this is indeed a sydnone derivative, but with a different structure, given in each of the spectra above. This particular synthesis produced crystals that were used for X-ray crystallography and allowed for an actual structure, as shown below:
Figure 15 allows a look at the overall structure of the compound and the associated ions and water. The bond distances given suggest neither single nor double bond, but rather delocalization around the ring. This compound was not found in the literature, meaning the structure was previously unknown. Based on the delocalized bonds, it can be suggested that aromaticity drives the condensation of this compound. UV-Visible and NMR data for the ethyl derivative of this sydnone compound is also given below:
**Figure 16**: UV-Visible spectra for EtCOC$_2$N$_3$O$_3$K$_2$ with associated structure, with extra peak beyond the basic literature sydnone structure.

**Figure 17**: H1 NMR for EtCOC$_2$N$_3$O$_3$K$_2$ with expected triplet and quartet and 1.1 and 2.75 ppm for ethyl indicator. Spectra also has peaks consistent with methanol impurity.
Figure 18: C13 NMR spectrum for EtCOC₂N₃O₃K₂ with five carbon peaks for the carbons present in the structure.

Figures 16-18 are all associated with the ethyl derivative of the sydnone product, indicating the ethyl groups as well as the added nitrogen in the sydnone ring. Based on these spectra, the associated structure can be confirmed. Due to the dimeric addition of nitric oxide (NO), the mechanism below is associated with the resulting synthesis:

\[
\text{R} = \text{Me, Et} \quad \xrightarrow{\text{MeOH, NH₂OH}} \quad \xrightarrow{\text{NO, -10 °C, -H₂O}} \quad \xrightarrow{\text{+KOH, -H₂O}} \quad \text{R} \quad \xrightarrow{\text{condensation, -H₂O}} \quad \xrightarrow{\text{+KOH, -MeOH}} \quad \text{R}
\]
Figure 19: The synthesis of the sydnone products potassium 4-acetyltriazolium-1,2,3-triolate and potassium 4-propionyltriazolium-1,2,3-triolate under the direct and immediate addition of potassium hydroxide and nitric oxide. Mechanism shows dimeric addition of NO and condensation.

Figure 19 shows the reaction mechanism for immediate addition of potassium hydroxide and nitric oxide. It was concluded that the potassium had no time to react with the acidic CH$_2$ group before the NO reacted with the electron rich nitrogen. After reacting with nitrogen, the reaction condenses at the acidic CH$_2$, and then reacts with the remaining potassium hydroxide to release methanol.

Conclusion

All three current products were formed and characterized by UV-Visible and NMR spectroscopy. Only one of the products allowed the use of X-ray crystallography for characterization purposes given the crystals produced. The synthesis was successful in creating NO containing compounds for possible future physiological purposes. Further research for these compounds should focus on EPR (electron paramagnetic resonance) by creating a nitric oxide trap and testing the release of NO under physiological conditions in order to confirm if these products can be used in a medical application. TGA/DSC (Thermogravimetric and Differential Scanning Calorimetry) should also be included in research in order to test the thermal decomposition properties and produce an acceptable decomposition mechanism in order to determine if these products can be explosive or used in a safe and controlled setting. This synthesis produced 3-4 viable candidates for the use of NO donor pro-drugs, with the products being potassium 3-ethyl-5-isoxazolone-4-diazeniumdiolate (R = Et), potassium 4-acetyltriazolium-1,2,3-triolate (R=Me), and potassium 4-acetyltriazolium-1,2,3-triolate (R=Et). These diazeniumdiolate and sydnone-N-oxide products can be further characterized and tested, as well as the methyl derivative of the diazeniumdiolate. More detailed research can be done on the yield of each of these products under the given conditions, as expected yields were not calculated due to the nature of this synthesis and the goal of simply creating a viable product.

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