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Photo-sensitized Oxygenation of Phenethylguanidoxime: a Possible Chemical Model for the Biological Oxidation of N^{ω}-Hydroxy-L-arginine to L-Citrulline

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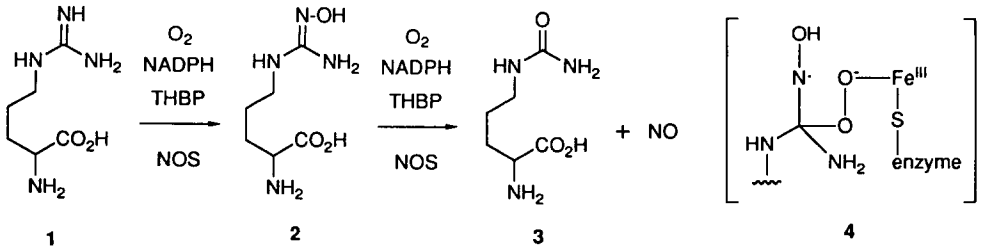
Abstract: Photo-sensitized oxygenation of phenethylguanidoxime led to the effective production of an expected urea derivative along with generation of nitric oxide (NO) or its equivalent. The formation of both products could be reasonably explained by the mechanism based on singlet oxygen ene reaction of olefins. This should give a possible chemical model for the biological oxidation of N^{ω}-hydroxy-L-arginine into L-citrulline and NO.

Nitric oxide (NO) plays important roles in cardiovascular and central and peripheral nervous systems, and modulates immunological responces.[1,2] NO is biosynthesized *in vivo* during the overall conversion of L-arginine (Arg) (**1**) into L-citrulline (Cit) (**3**) through N^{ω}-hydroxy-L-arginine (NOHA) (**2**), which is catalyzed by NO synthases (NOSs). Each step in the oxidation basically requires molecular oxygen (O_2), NADPH and tetrahydrobiopterin (THBP).[3,4] Although the precise mechanism for the oxidative cleavage of the *N*-hydroxyguanidyl (guanidoxime) group of NOHA (**2**) by O_2 has not been established, a plausible mechanism[2,3,5] through a peroxyheme complex[6] like **4** has been proposed.

The singlet oxygen ($^1O_2$) ene reaction[7] has been shown to be a powerful method for allylic oxidation of



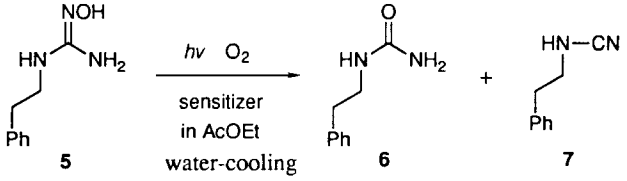
1 **2** **3** **4**

olefins. We assumed that, when a guanidoxime was used as a substrate in the $^1O_2$ ene reaction, the oxime bond (C=N-OH) of a guanidoxime function should act as an ene to give a nitrosohydroperoxide (like **D_1** in **Scheme**), structurally related to the above peroxyheme complex, which may spontaneously degrade into a urea with the evolution of NO or its equivalent. In this communication we present the effective conversion of phenethylguanidoxime (**5**) into the corresponding urea (**6**) along with generation of NO or its equivalent in a photo-sensitized oxygenation and discuss the possible mechanism of the photooxygenation reaction.

A solution of **5**[8] in ethyl acetate (AcOEt)[9] was irradiated for 1 h with a high pressure mercury lamp (400 W) through a Pyrex filter in the presence of rose bengal (RB) as a sensitizer with bubbling O_2 under water-cooling. (run 1 in **Table**) After removal of the RB from the reaction mixture by treatment with a decolorizing carbon the crude product was purified by preparative TLC (SiO_2) to give the expected phenethylurea (**6**)[10] and phenethylcyanamide (**7**)[11] in 58 % and 4 % isolated yields, respectively. The combination of light, O_2 and a sensitizer was necessary for the oxidation because no reaction was observed when any one component among them was omitted. (run 2-5[12]) The use of tetraphenylporphine (TPP) instead of RB as a sensitizer[13] led to nearly the same product distribution. (run 6) Thus, it was clear that the C=N-OH of a guanidoxime was expectedly cleaved by $^1O_2$ to effectively yield a urea derivative.

The concomitant formation of a cyanamide in addition to a urea suggested the possibility of an alternative ene reaction, in which the amidino bond (N=C-NH) in a guanidoxime function could act as an ene. However, no reaction was observed when phenethylguanidine[14] was subjected to the photo-sensitized O_2 oxidation.

Table. Photo-sensitized oxygenation of phenethylguanidoxime (**5**) under various conditions



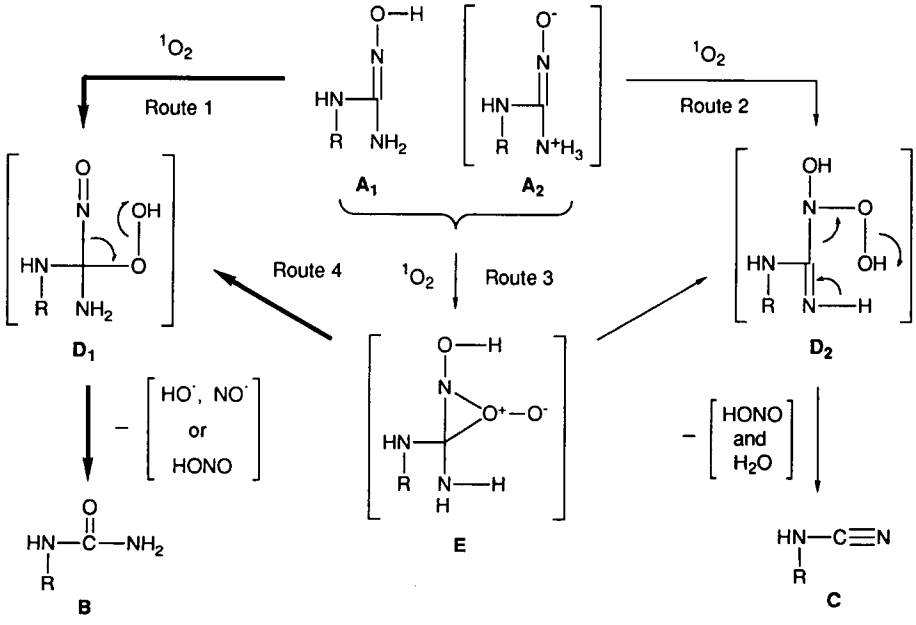
Run	Conditions					Products (%)[a]	
	5 (10^{-3} mol/L)	*hν*	O_2	Sensitizer	Time (h)	**6**	**7**
1	16.6	+	+	RB[b]	1	58	4
2	15.6	-	-	RB[b]	3	N. R.[c]	
3	16.6	-	+	RB[b]	3	N. R.[c]	
4	16.3	+	+	-	3	N. R.[c]	
5[12]	16.3	+	Ar (+)	RB[b]	3	N. R.[c]	
6	16.2	+	+	TPP[d]	1	50	5

[a]Unoptimized, isolated yields. [b]RB (1.8 mg) was suspended in AcOEt (100 ml) and the saturated solution was used as a solvent after removal of the insoluble RB by filtration (The concentration of RB: $<1.8 \times 10^{-5}$ mol/L). [c]No reaction. [d]The concentration was 1.3×10^{-4} mol/L.

Furthermore, application of the O_2 oxidation to benzylaceoxime[15] resulted in the recovery of the starting material,[16] too. These facts indicated that the presence of a whole guanidoxime function [$RNHC(NH_2)$=NOH], not a limited functionality of the guanidoxime such as the C=N-OH or the N=C-NH, was essential for the photooxidative production of not only a urea but also a cyanamide.[17]

It is known that RB[18] and TPP[19] resulted in effective generation of $^1O_2$ in a photo-sensitized O_2 oxidation. Therefore, it would be reasonable to suppose that a guanidoxime **A** was oxygenated by $^1O_2$ to yield a urea **B** and a cyanamide **C** derivatives. (**Scheme**) In the photooxygenation *C*- and *N*- hydroperoxides $\mathbf{D_1}$ and $\mathbf{D_2}$ should play a crucial role because of their spontaneous degradation into **B** and **C** with evolution of NO or its equivalent. $\mathbf{D_1}$ and $\mathbf{D_2}$ could be given by direct ene reaction between **A** and $^1O_2$ (Routes 1 and 2) and/or by a hydrogen transfer triggered by the ring-opening of a perepoxide **E**,[20] derived from 1, 2-cycloaddition of $^1O_2$ to **A**. (Route 3) The major production of **B** compared to **C** (see **Table**) strongly indicates a larger contribution of $\mathbf{D_1}$ than $\mathbf{D_2}$ to the product distribution. If the hydroperoxides $\mathbf{D_1}$ and $\mathbf{D_2}$ are produced by direct ene reaction the reaction may be governed by a potential zwitter-ionic character of a guanidoxime such as $\mathbf{A_2}$, in which electrophilic $^1O_2$[21] should attack on the more nucleophilic carbon atom of the C=N-OH in a guanidoxime function, to afford $\mathbf{D_1}$ preferentially. (Route 1) In the case of the alternative route through a cycloadduct **E**, the easy transfer of the more acidic hydrogen on the hydroxy group rather than that on the amino group could also lead to the predominant formation of $\mathbf{D_1}$. (Route 4)

According to our proposed mechanism NO and/or nitrous acid should generate during the reaction. However, they must be immediately oxidized to NO_2, easily hydrolyzable to NO_2^- and NO_3^-, under the condition used because of their susceptibility to O_2. Thus, analysis of the reaction mixture after photoirradiation for 1.5 h (see run 1 in **Table**) by application of Griess reaction [6,22] led to identification of



Scheme

NO_2^- and NO_3^-, albeit in low 4 % and 9 % yields, respectively.[23]

Photo-sensitized oxygenations often use as chemical mimics for oxygenase-catalyzed biological oxidations.[24] The smooth conversion of a guanidoxime into a urea and NO or its equivalent in the photo-sensitized oxygenation should allow us to consider the oxygenation reaction as one of possible chemical models for the NOS-catalyzed oxidation of NOHA (**2**) into Cit (**3**) and NO.[25]

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