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Oxidative Reactivity of *S*-Nitrosoglutathione with Hantzsch 1,4-Dihydropyridine

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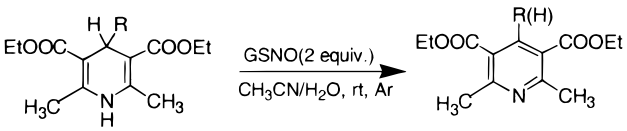
ABSTRACT



***S*-Nitrosoglutathione oxidized 4-substituted Hantzsch 1,4-dihydropyridines in CH_3CN/H_2O or CH_3CN/phosphate aqueous buffer solution to give aromatic products in various yields.**

Nitric oxide (NO) is an endogenous compound of the body involved in a variety of biological processes,[1] including immunity-regulating functions, physiological control of blood pressure, and neurotransmission. Authentic NO in vivo has only a 0.1 s half-life.[2] Nitrosothiols (RSNO), which are formed in vivo from the reaction of NO with protein or nonprotein thiols, can increase the effective tissue half-life of NO.[3] RSNO is commonly referred to as a NO-donor and is found in various biological systems, such as in human plasma, airways, white blood cells, and rat cerebellum.[4] Nitrosoglutathione (GSNO) has been shown to be one of the most aboundant RSNOs,[4c,5,6] e.g., 0.3 μM in bronchial lavage fluid and 0.7 μM in brain, and to exhibit depressor action.[7]

4-Substituted Hantzsch 1,4-dihydropyridines (DHP) are analogues of NADH coenzymes[8] and an important class of drugs which are potent blockers of calcium (Ca^{2+}) currents.[9] Therefore, the oxidation of DHP has recently attracted more attention from chemists.[10] To explore the reaction between GSNO and DHP is certainly of biological significance.

In a representative experiment, an anaerobic CH_3CN/H_2O solution of 0.02 mmol of DHP[11] (**1**) and 0.04 mmol of GSNO[12] was stirred for about 10 h at room temperature under Ar, giving an aromatic product, Hantzsch pyridine (**2**) (Scheme 1). GSNO was converted to the disulfide (GSSG),

Scheme 1

the oxidized species of glutathione. In most cases, the peak for DHP on GC disappeared completely at the end of the reaction. The experimental results are summarized in Table

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Table 1. Oxidation of DHP with GSNO in CH_3CN/H_2O under an Ar Atmosphere

substrate	R	ratio[a]	time (h)	product[b]	yield (%)[c]
Ia	H	2:1	6	IIa	100
Ib	CH_3	2:1	5	IIb	100
Ic	$(CH_3)_2CH$	2:1	10	IIa	50
Id	CH=CHPh	2:1	10	IId	60
Ig	Ph	2:1	10	IIe	70
If	F−Ph	2:1	10	IIf	60
Ig	*p*-methoxy-Ph	2:1	8	IIg	100
Ih	*m*-hydroxy-Ph	2:1	10	IIh	60
Ii	3,4-dihydroxy-Ph	2:1	13	IIi	0

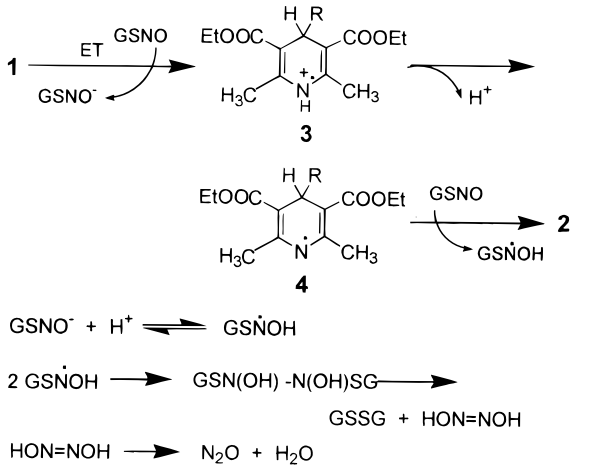
[a] Molar ratio of GSNO to DHP. [b] Determined by ^{1}H NMR. [c] Compared to authentic samples by GC.

1. Under the indicated conditions, the corresponding 4-substituted Hantzsch pyridine (**2**) was the uniquely identifiable oxidation product derived from the substrate, with the exception of **Ic** where only the dealkylation product was obtained. The products were easily separated by extraction.

Under other conditions where oxygen was present or water was replaced by phosphate buffer at pH 7.4 with 10 mM EDTA to eliminate the decomposition of GSNO catalyzed by metal ions such as Cu^+, Fe^{2+}, or Hg^{2+},[13] results as similar to those shown in Table 1 were obtained. Increased molar ratios of GSNO to DHP in the CH_3CN/buffer solution affected the oxidation product yield and significantly shortened the reaction time. This observation ruled out oxidation of DHP by NO, which was generated from the decomposition of RSNO in aqueous solution. Therefore, we reasoned that GSNO might participate as the oxidant in the reaction, although we considered an alternative heterolytic mechanism where GSNO homolytic cleavage[13] of the S−N bond proceeded with release of NO.[3,14] Reactions of GSNO with DHP were also performed in sodium dodecyl sulfate (SDS) micelles (0.5 M), prepared in water containing 10 mM EDTA. Results similar to those shown in Table 1 were obtained.

To our knowledge, two mechanisms for the oxidation of NADH analogues, hydride transfer[8b,15] or electron transfer,[16] are possible. On the basis of our experimental results, we believe that the reaction between GSNO and DHP occurred via an electron-transfer mechanism as shown in Scheme 2.

Scheme 2



The reaction might commence with a one-electron transfer from dihydropyridine to GSNO, yielding the radical cation (**3**) of DHP and the anion of GSNO, $GSNO^-$. The subsequent radical coupling upon protonation of $GSNO^-$ [17]would give an unstable dihydroxyhydrazine that could eliminate hyponitrous acid, HON=NOH, to form the disulfide, GSSG. HON=NOH then decomposes into N_2O.[3,18] N_2O was detected by GC-MS. It is reasonable that radical **3** lost a proton to give aminyl radical (**4**), which then reacted with GSNO to form pyridine (**2**) and GSN(˙)OH. The later species proceeded by the same reaction pathway shown in Scheme 2. The stoichiometry of reactions showed there was an approximately equal molar relationship between dihydropyridine (**1**), GSNO, and pyridine (**2**). This implies that N_2O or hyponitrous acid might react with DHP and N_2 might be the final gaseous product, although further work will be necessary to confirm this proposal.

The present results might be of both biological and chemical significance. First, because of the mild oxidative reactivity of GSNO which enhances selectivity, this reaction could be used for a biommetic model for reduction of NADH. Second, our results reveal an example of the oxidation of DHP by a *S*-nitrosothiol, which is pertinent to the chemical behavior of GSNO and the metabolism of 1,4-dihydripyridine-based drugs. We believe that the present work will stimulate investigations of the chemical features of GSNO and its role in biological and medicinal chemistry.

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