Spontaneous and Selective Formation of HSNO, a Crucial Intermediate Linking HS and Nitroso Chemistries

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Abstract: Thionitrous acid (HSNO), a potential key intermediate in biological signaling pathways, has been proposed to link NO and H$_2$S biochemistries, but its existence and stability in vivo remains controversial. We establish that HSNO is spontaneously formed in high concentration when NO and H$_2$S gases are mixed at room temperature in the presence of metallic surfaces. Our measurements reveal that HSNO is formed by the reaction H$_2$S + N$_2$O$_3$ → HSNO + HNO$_3$, where N$_2$O$_3$ is a product of NO disproportionation. These studies also suggest that further reaction of HSNO with H$_2$S may form HNO and HSSH. The length of the S-N bond has been derived to high precision, and is found to be unusually long: 1.84 Å — the longest S-N bond reported to date for an R-SNO compound. The present structural and, particularly, reactivity investigations of this elusive molecule provide a firm foundation to better understand its potential physiological chemistry and propensity to undergo S-N bond cleavage in vivo.

The intriguing similarities between the biological profiles of NO and H$_2$S, two important gasotransmitters acting notably as blood pressure mediators, have raised questions about the possibility of ‘cross-talk’ between the two species. Such an interaction could explain the surprisingly beneficial effects of these signaling molecules as it may allow H$_2$S to modulate the availability of NO within the body. The vast majority of NO in vivo is bound as S-nitrosothiols (RSNO), a family of molecules well known for their crucial role in response to oxygen deprivation as well as for being important biological reservoir for NO. RSNOs are typically formed through the reaction of nitrosylating agents with thiols (RSH). Once formed, RSNOs can also act as nitrosylating agents thus highlighting their role as NO shuttles at the cellular level.

The simplest RSNO, thionitrous acid (HSNO), has been proposed to be the product of the reaction between NO and H$_2$S; other possible species have been implicated in this ‘cross-talk’, including nitrosopropylene (SSNO) and N-nitrosodihydroxyamine-N-sulfonate. Unlike larger RSNOs, HSNO is thought to be able to freely diffuse through cell membranes suggesting that it has the ability to transmit these proteins removed from the sites where NO is synthesized, and therefore could play a key role in cellular redox regulation. Understanding the role of small molecules such as NO and HNO in biological signal transduction has resulted in important advances in medicine. To shed light on the role of HSNO in vivo, sensors are currently being developed to image this elusive molecule. However, the biochemistry (formation, transport, decomposition) of HSNO remains poorly understood and the structure of the molecule has not been determined to date. HSNO has been spectroscopically characterized by infrared measurements in low temperature argon matrices. The molecule was produced from photolysis of HSNO, a low-lying stable structural isomer of HSNO (ΔH ≥ 10 kcal/mol, see Supplementary Information). Recently, HSNO has been detected under physiologically relevant conditions through protonation of [SNO]$^-$ or treatment of S-nitrosoglutathione with sodium sulfide by infrared, $^{15}$N NMR and mass spectroscopy. Additionally, coordinated HSNO has been transiently observed by mass spectroscopy and very recently a crystal structure was obtained of an RSNO coordinated to a copper complex. However, these measurements provided only limited insight into the structure and subsequent reactivity of HSNO.

The length of the S=N bond in HSNO is used to infer the stability of this molecule under physiological conditions, ostensibly because a long, weak S-N bond would allow HSNO to undergo facile homolysis to the NO and SH radicals, thus rationalizing its ability to serve as a NO carrier. Theoretical studies of this bond length, remain controversial because, as with larger RSNOs, the length and strength of this central bond appear to be highly dependent on the method and basis set used. Calculated S-N bond lengths in HSNO range from as short as 1.78 Å to as long as 1.93 Å. From theoretical calculations, however, it is well-established that HSNO has two conformers very close in energy (the trans is 0.8 kcal/mol more stable than the cis) but separated by a relatively high torsional barrier (6.7 kcal/mol, see figure S1), a finding which is qualitatively consistent with a partial double bond character of the S=N bond. In contrast, the NO donor character of this class of molecules is likely to arise from the weak strength of the S=N bond (25-31 kcal/mol), an indication of a partial ionic character. To explain these paradoxical bonding properties, theoretical models of the electronic structure of RSNOs include contributions from conventional (R−S=N=O), zwit-
In a serendipitous discovery, we found that when a mixture of H$_2$S and NO heavily diluted in an inert buffer gas (Ne) is expanded into a vacuum chamber, intense rotational lines of a previously unknown molecule, now unambiguously identified as HSNO, are observed by Fourier-transform microwave (FTMW) spectroscopy, a highly sensitive and selective technique which has been used to detect many molecules, both familiar and exotic.\textsuperscript{9,27} Rotational transitions (Figure 1) have been measured for both the cis and trans conformers of the main isotopeologue of HSNO and four singly-substituted isotopic species (D$_2$NO, H$_2^{15}$NO, HS$_{15}$NO, HSN$^{18}$O). From these data, highly accurate molecular parameters (rotational, centrifugal distortion, and hyperfine constants) have been derived (Table S1). A precise geometrical structure has been obtained for both conformers from a combination of isotopic substitution and vibrational corrections calculated quantum-chemically (see Figure 2 and Tables S2-S3). Most significantly, this analysis yields a S–N bond length of 1.834 ± 0.002 Å for cis-HSNO and 1.852 ± 0.002 Å for trans-HSNO, both of which are very long in comparison to other RSNOs where the S–N bond is typically 1.75 Å (see figures S2 and S3, and references therein). The S–N bond length in HSNO is in fact the longest experimentally reported for an RSNO compound and has significant consequences on the stability of HSNO with respect to homolytic or heterolytic cleavage, particularly in vivo. With respect to calculated S–N bond lengths of HSNO, the precise values reported here should serve as a useful benchmark for future quantum chemical studies. These values are in good agreement with high level quantum chemical calculations [CCSD(T)] from the literature\textsuperscript{21,22} and undertaken in this study (Table S2). Natural resonance theory analysis performed in this work also indicates that there is only a single intrinsic resonance structure for HSNO, H–S–N–O. Additional experimental details, theoretical calculations, measured frequencies, rotational constants, and structural parameters (experimental and calculated) can be found in the supplementary material.

![Figure 1](image1.png)

**Figure 1.** (Top) FTMW spectrum of the two Doppler components of the $v_{10} - v_{00}$ transition of trans-HS$^{15}$NO [integration time: $\sim$ 1 min]. (Bottom) DR spectrum of the $v_{10} - v_{01}$ transition of trans-HS$^{15}$NO, obtained while monitoring the signal of the $v_{10} - v_{00}$ transition [integration time: $\sim$ 5 min].

![Figure 2](image2.png)

**Figure 2.** Structure of cis- (top panel) and trans-HSNO (bottom panel) determined in this study [semi-experimental equilibrium structure, see Table S1]. Bond lengths are in Å and angles in degrees.

Our study also shows that although both trans and cis forms of HSNO are readily produced, trans-HSNO is roughly 5 times more abundant than the cis form. Since the conformational change cis-HSNO $\rightleftharpoons$ trans-HSNO faces a high barrier of 6.7 kcal/mol (see Supplementary Information), another formation pathway must be operative under our experimental conditions. We have thus undertaken experiments and calculations to understand the formation chemistry of HSNO and its energetics.

Figure 3 shows how the abundance of several NO-bearing species changes upon addition of H$_2$S. NO is known to slowly disproportionate on metallic surfaces\textsuperscript{26-30} into N$_2$O$_3$ and N$_2$O ($\Delta G(1+2) = -24.4$ kcal/mol\textsuperscript{31}) via the reactions:

\[
\begin{align*}
3 \text{NO} & \rightarrow \text{N}_2\text{O} + \text{NO}_2 \\
\text{NO} + \text{NO}_2 & \rightarrow \text{N}_2\text{O}_3
\end{align*}
\]

which results in the presence of these species in our experiment. N$_2$O$_3$ also appears to be a competent nitrosylating agent in vitro since its reaction with RSH has been demonstrated to occur at a rate exceeding that of its hydrolysis\textsuperscript{19,32} and has been shown to be produced in vivo.\textsuperscript{35} From Fig. 3, it is clear that the production of HSNO is strongly correlated with a decrease in the concentration of N$_2$O$_3$ and a commensurate increase in HNO$_2$, while the concentration of N$_2$O remains relatively constant. Under the range of the experimental conditions studied here, up to 0.25 % of NO is converted into trans-HSNO, an abundance presumably limited by the availability of N$_2$O$_3$. 
Energy calculations performed in this study conclude that \( \text{N}_2\text{O}_3 \) can react exothermically with \( \text{H}_2\text{S} \) (\( \Delta H = -7.5 \text{kcal/mol at 298 K} \)) to form HSNO via the reaction:

\[
\text{H}_2\text{S} + \text{N}_2\text{O}_3 \rightarrow \text{HSNO} + \text{HNO}_2
\] (3)

Reaction 3 is analogous to the well-known nitrosation of thiols by \( \text{N}_2\text{O}_3 \) in aqueous solution (\( \text{RSH} + \text{N}_2\text{O}_3 \rightarrow \text{RSNO} + \text{NO}_2^- + \text{H}^+ \)).\(^\text{31}\) However, this reaction is kinetically improbable under our experimental conditions due to a high activation energy (\( \Delta H^\ddagger = 15.9 \text{kcal/mol at 298 K} \), see Figure S4). Instead, a surface reaction is almost certainly yielding HSNO. Crucial confirmation that reaction (3) occurs in our experiment is the nearly perfect anti-correlation between the abundances of \( \text{HNO}_2 \) and \( \text{N}_2\text{O}_3 \) upon addition of \( \text{H}_2\text{S} \) (Figure 3). Furthermore, the maximum concentration of HSNO is reached when the \( \text{N}_2\text{O}_3 \) concentration is close to zero, ostensibly because the steady-state supply of \( \text{N}_2\text{O}_3 \) has been exhausted. Finally, although the production of \textit{trans}-HSNO is remarkably high, it is an order of magnitude smaller than \( \text{HNO}_2 \), a possible indication of the chemical instability of HSNO once formed. Weak signal attributed to \textit{cis}-HSNO, the ground state isomer of \textit{trans}-HSNO (see Supplementary Information), has been detected, although an order of magnitude weaker than lines of its metastable isomer. None of the other structural isomers of the \([\text{H},\text{S},\text{N},\text{O}]\) system were observed, suggesting that structural reorganization is relatively unimportant in the disappearance of HSNO.

The reactivity of HSNO can be inferred from figure 3, which illustrates that the addition of \( \text{H}_2\text{S} \) beyond the amount resulting in the maximum concentration of HSNO also induces its decay. This results implies that HSNO is unstable with respect to \( \text{H}_2\text{S} \) which would be consistent with the reaction:

\[
\text{HSNO} + \text{H}_2\text{S} \rightarrow \text{HNO} + \text{HSSH}
\] (4)

Support for this reaction in solution is by the treatment of \( \text{S}-\text{nitrosglutathione (RSH) with an excess of glutathione (RSH) which is known to result in the observation of nitrous oxide, a known decomposition product of HNO}_3\).\(^\text{33}\) Furthermore, it has been demonstrated that colocalized \( \text{H}_2\text{S} \) and NO result in the generation of HNO which triggers a neuroendocrine signalling cascade.\(^\text{34}\) Unfortunately, neither product, HNO or HSSH, can be monitored in our apparatus (rotational transitions for the aforementioned molecules cannot be probed in the accessible spectral region) and thus this reaction cannot be directly confirmed.

Addition of a single drop of \( \text{H}_2\text{O}^{18}\text{O} \) to the gas manifold containing NO resulted in \( \text{O}^{18}\text{O} \) incorporation into \( \text{N}_2\text{O}_3 \) and \( \text{HNO}_2 \), but not \( \text{N}_2\text{O} \) (see Table S4), which is consistent with the reactions:

\[
\begin{align*}
2 \text{NO}_2^- + \text{H}_2\text{O}^+ &\rightarrow \text{HNO}_2^- + \text{HNO}_3^- \quad (5) \\
2 \text{HNO}_2^- &\rightarrow \text{H}_2\text{O}^+ + \text{NO}^+ + \text{NO}_2^+ \quad (6) \\
\text{NO}^+ + \text{NO}_2^- &\rightleftharpoons \text{N}_2\text{O}_3^- \quad (7)
\end{align*}
\]

where asterisks indicate possible \( ^{18}\text{O} \)-labeled positions. Once \( \text{H}_2\text{S} \) was mixed with this gas mixture, HSN\textsuperscript{18}O and, interestingly, \( \text{N}_2\text{O}^{18} \) were detected in significant amounts. The latter suggests that \( \text{N}_2\text{O}^{18} \) was formed through decomposition of HN\textsuperscript{18}O, which would be derived from the decomposition of HSNO:

\[
\begin{align*}
\text{N}_2\text{O}^*_3 + \text{H}_2\text{S} &\rightarrow \text{HSNO}^* \\
\text{HSNO}^* + \text{H}_2\text{S} &\rightarrow \text{HNO}^* + \text{HSSH} \quad (9) \\
2 \text{HNO}^* &\rightarrow \text{N}_2\text{O}^* + \text{H}_2\text{O}^* \quad (10)
\end{align*}
\]

A detailed scheme of reactions (5–10) is presented in the supplementary material. The observation of \( \text{N}_2\text{O}^{18} \) is thus an indirect confirmation of reaction (4).

To determine the role of metallic surfaces in the formation of HSNO, high surface area stainless steel mesh has been inserted in the nozzle source, directly before the gas is expanded into the vacuum chamber, resulting in an enhancement in the production of HSNO (by more than a factor of two). In contrast, placement of this mesh farther upstream in the gas line resulted in significant reduction of the HSNO signal (to near depletion), indicating that the mesh, or metallic surfaces, are crucial for forming HSNO, but also that HSNO is only able to diffuse roughly 1–5 centimeters at near atmospheric pressure before most of the molecules have decomposed. Realizing that HSNO has a limited lifetime under our experimental conditions, a new nozzle assembly was designed such that the two gas streams, one containing NO (and \( \text{N}_2\text{O}_3 \)) and the other \( \text{H}_2\text{S} \), could be mixed just prior to expansion. This modified valve design combined with the stainless steel mesh resulted in a 10-fold signal enhancement of HSNO in comparison to the original experimental setup (up to a concentration of 3% relative to NO, see Figure S5). As a side note, under these experimental conditions \textit{trans}-HSNO was not detected which suggests the isomerization from HSNO to HNSO is a slow process.

\( \text{N}_2\text{O}_3^- \) and \( \text{H}_2\text{S} \) to the gas manifold containing NO resulted in \( ^{18}\text{O} \) incorporation into \( \text{N}_2\text{O}_3 \) and \( \text{HNO}_2 \), but not \( \text{N}_2\text{O} \) (see Table S4), which is consistent with the reactions:

\[
\begin{align*}
2 \text{NO}_2^- + \text{H}_2\text{O}^+ &\rightarrow \text{HNO}_2^- + \text{HNO}_3^- \quad (5) \\
2 \text{HNO}_2^- &\rightarrow \text{H}_2\text{O}^+ + \text{NO}^+ + \text{NO}_2^+ \quad (6) \\
\text{NO}^+ + \text{NO}_2^- &\rightleftharpoons \text{N}_2\text{O}_3^- \quad (7)
\end{align*}
\]

where asterisks indicate possible \( ^{18}\text{O} \)-labeled positions. Once \( \text{H}_2\text{S} \) was mixed with this gas mixture, HSN\textsuperscript{18}O and, interestingly, \( \text{N}_2\text{O}^{18} \) were detected in significant amounts. The latter suggests that \( \text{N}_2\text{O}^{18} \) was formed through decomposition of HN\textsuperscript{18}O, which would be derived from the decomposition of HSNO:

\[
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\text{HSNO}^* + \text{H}_2\text{S} &\rightarrow \text{HNO}^* + \text{HSSH} \quad (9) \\
2 \text{HNO}^* &\rightarrow \text{N}_2\text{O}^* + \text{H}_2\text{O}^* \quad (10)
\end{align*}
\]

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![Figure 3](image-url)

**Figure 3.** Change in the concentration of several NO containing molecules upon addition of \( \text{H}_2\text{S} \) to the NO gas stream.

Since \( \text{N}_2\text{O}_3 \) appears to be the limiting reagent in HSNO formation, means were taken to increase its concentration prior to mixing with \( \text{H}_2\text{S} \). The use of a 1:1 mixture of NO and \( \text{N}_2\text{O}_3 \) resulted in a five-fold enhancement in the amount of \( \text{N}_2\text{O}_3 \), by analogy with reaction (7), which correspondingly increased the amount of HSNO. More efficiently, ad-
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studying this system for R = CH

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K. N. C. w ere supp orted b y a CfA P ostdo ctoral F ellowship

is based up on w ork supp orted b y the National Sci-

Iv ano vi¢-Burmazo vi¢ for an insigh-tful and helpful discus-

In summary , w e nd that when dilute samples of H

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RSH + N

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→

RSNO + HNO

(13)

2

(11)

(12)

RSH + N

2

→

RSNO + HNO

(13)

A direct confirmation of reaction (4) could be obtained by

this system for R = CH

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(3) Lipton, A. J.; Johnson, M. A.; Macdonald, T.; Lieber-


(5) Berger, P. J.; Skura, E. M.; Brodelevy, V.; Wilkinson, M. H.


(6) van Faassen, E.; Vanin, A. F. In Radicals for Life; Faassen, E. V.,


(7) Prong, Y.; Localzio, J. In Radicals for Life; Faassen, E. V.,


(9) Whitelam, M.; Li, L.; Kootetsui, I.; Chiu, S. H.; Siau, J. L.;


(11) Filipovic, M. R.; Eberhardt, M.; Prokopenovic, P.; Mijuskovic,


Chem. 2013, 56, 1499–1508.


Rivera-Fuentes, P. J. Am. Chem. Soc. 2014, 136, 4007–4008,

PMID: 24564324.


12, 2315–2323.


5200–5209.


(17) Miljkovic, J. L.; Kenkel, I.; Ivanovic-Burazovic, I.; Filipovic,


(18) Zhang, S.; Melzer, M. M.; Sen, S. N.; Celebi Öğüzım, N.; War-


(19) Cortese-Krotli, M. M.; Fernandez, B. O.; Kelm, M.; Butler,


9952.


(22) Ivanova, L. V.; Anton, B. J.; Timereghazin, Q. K. Phys. Chem.


(23) Barberger, M. D.; Houk, K. N.; Powell, S. C.; Mannion, J. D.;

Lo, K. Y.; Stanier, J. S.; Toone, E. J. Am. Chem. Soc. 2000,

122, 5889–5890.

(24) Barberger, M. D.; Mannion, J. D.; Powell, S. C.; Stanier, J. S.;


8869.


2013, 343, 1354–1357.


(31) Wagon, D. D.; Evans, W. H.; Parker, V. B.; Schuman, R. H.;


(33) Wong, P. S. Y.; Hyun, J.; Fukuto, J. M.; Shiota, F. K.; De-

Master, E. G.; Shoeman, D. W.; Nagasawa, H. T. Biochemistry


Graphical TOC Entry

H₂S + N₂O₃ → HSNO + HONO