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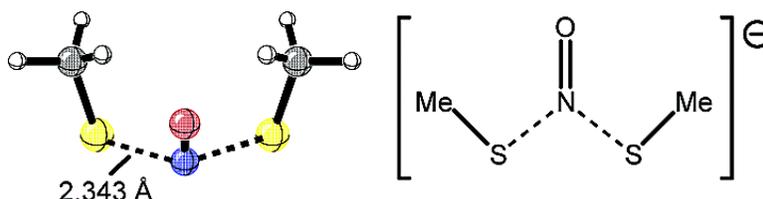
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Nitroxyl Disulfides, Novel Intermediates in Transnitrosation Reactions

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Abstract: A novel anionic RSN(O)SR species, the intermediate in transnitrosation reactions, was explored computationally with B3LYP and CBS-QB3 methods. The species resembles a nitroxyl coordinated to a highly distorted disulfide, and it differs significantly from intermediates in nucleophilic acyl substitution. Reactions of the following species were computed for comparison: $MeS^- + MeSNO$; $MeO^- + MeONO$; $MeS^- + MeSCHO$; $MeO^- + MeOCHO$. The last two have very different intermediates from the first two. Mass spectrometric experimental evidence is presented that is consistent with the formation of a nitroxyl disulfide in the gas phase. The calculated proton affinity and redox potentials of the intermediate are also reported.

Nitric oxide, NO, has many biological functions, including the regulation of blood pressure, action as a neurotransmitter, and involvement in the immune response. The involvement of *S*-nitrosothiols (RSNOs, thionitrites) in such processes has attracted considerable attention.[1–7] S-Nitrosylated cysteines, particularly in albumin[1] and hemoglobin,[2] may be in vivo reservoirs of NO. RSNOs can react with thiols[3] and with other RSNOs[4] to produce free NO and disulfides, but mechanisms for these processes are unclear. NO transport can be effected by transnitrosation—the direct transfer of the NO group from one thiol to another without the generation of free NO (Scheme 1).[5] One possible reaction of this type is protein modification by cysteine S-nitrosation, an important mechanism for the regulation of protein function.[6]

Scheme 1

$$RSH + R'SNO \longrightarrow RSNO + R'SH$$

Scheme 2

Transnitrosation between thiols is generally facile under physiological conditions.[7] The reaction is first order in both thiol and nitrosothiol and does not involve free NO as an intermediate; the rate is diminished by steric hindrance at the thiol and increased by an increase of thiol acidity, elevated pH, and enhanced electrophilicity of the nitrosothiol. These observations are consistent with the attack of a nucleophilic thiolate anion on an electrophilic RSNO (Scheme 2).

We report the first theoretical examination of this reaction, including the description of a novel intermediate with an unprecedented structure, differing significantly in structure from the tetrahedral intermediates so well known in the nucleophilic acyl substitutions involving carboxylic acid derivatives. This species is of potential importance for a variety of processes

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(1) (a) Stamler, J. S.; Jaraki, O.; Osborne, J.; Simon, D. I.; Keaney, J.; Vita, J.; Singel, D.; Valeri, C. R.; Loscalzo, J. *Proc. Natl. Acad. Sci.* **1992**, *89*, 7674–7677. (b) Jourd'heuil, D.; Hallén, K.; Feelisch, M.; Grisham, M. B. *Free Radical Biol. Med.* **2000**, *28*, 409–417.

(2) Stamler, J. S.; Jia, L.; Eu, J. P.; McMahon, T. J.; Demchenko, I. T.; Bonaventura, J.; Gernert, K.; Piantadosi, C. A. *Science* **1997**, *276*, 2034–2037.

(3) (a) Gow, A. J.; Burek, D. G.; Ischiropoulos, H. *J. Biol Chem.* **1997**, *272*, 2841–2845. (b) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14428–14433. (c) Konorev, E. A.; Kalyanaraman, B.; Hogg, N. *Free Radical Biol. Med.* **2000**, *28*, 1671–1678.

(4) Choi, B. Y.; Voyksner, R. M.; Toone, E. J. Manuscript in preparation.

(5) Arnelle, D. R.; Stamler, J. S. *Arch. Biochem. Biophys.* **1995**, *318*, 279–285.

(6) (a) Wolosker, H.; Panizzutti, R.; Engelender, S. *FEBS Lett.* **1996**, *392*, 274–276. (b) Arstall, M. A.; Bailey, C.; Gross, W. L.; Bak, M.; Balligand, J.-L.; Kelly, R. A. *J. Mol. Cell. Cardiol.* **1998**, *30*, 979–988. (c) Percival, M. D.; Ouellet, M.; Campagnolo, C.; Claveau, D.; Li, C. *Biochemistry* **1999**, *38*, 13574–13583. (d) Bonaventura, C.; Ferruzzi, G.; Tesh, S.; Stevens, R. D. *J. Biol. Chem.* **1999**, *274*, 24742–24748. (e) Patel, R. P.; Hogg, N.; Spencer, N. Y.; Kalyanaraman, B.; Matalon, S.; Darley-Usmar, V. M. *J. Biol. Chem.* **1999**, *274*, 15487–15492. (f) Wolzt, M.; MacAllister, R. J.; Davis, D.; Feelisch, M.; Moncada, S.; Vallance, P.; Hobbs, A. J. *J. Biol. Chem.* **1999**, *274*, 28983–28990. (g) Li, J.; Billiar, T. R.; Talanian, R. V.; Kim, Y. M. *Biochem. Biophys. Res. Commun.* **1997**, *240*, 419–424. (h) Xu, L.; Eu, J. P.; Meissner, G.; Stamler, J. S. *Science* **1998**, *279*, 234–237. (i) Park, J.-W. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 916–920.

Figure 1. Geometries for transnitrosation stationary points (selected bond lengths in Å).

Figure 2. CBS-QB3 relative energies (ΔH_{298}, ΔG_{298}) in the gas and aqueous phases (in kcal/mol) for transnitrosation stationary points.

involving nitrosothiols. Gas-phase studies described here show the existence of an ion consistent with the proposed intermediate.

Initial geometry optimizations were performed with density functional theory, B3LYP/6-311+G*, implemented in the Gaussian 98 suite of programs,[8] followed by further optimization and energetic evaluation using the CBS-QB3 method.[9] Each stationary point in the gas phase was characterized with a frequency analysis. Single point calculations and optimizations were performed using Tomasi's PCM model[10] to model chloroform or aqueous solvation, with B3LYP/6-311+G*. Minima in solvent were verified by reoptimizing these structures after distorting one of the S−N bonds by about 0.1 Å. These optimizations led to minima, not to reactants or products.

Methanethiolate (**1**) and *S*-nitrosomethanethiol (**2**) combine in the gas phase to form an ion−molecule complex (**3**) (Figure 1). A transition state (**4**) for nucleophilic attack at the nitrogen of **2** leads to a novel intermediate (**5**). A second transition state and dissociation completes the thiolate exchange.

The energetic profile of this reaction is depicted in Figure 2. In the gas phase, formation of the anionic intermediate, **5**, from separated thiolate, **1**, and nitrosothiol, **2**, is exothermic, proceeding with a ΔH_{298} of −13.9 kcal/mol. The ion−molecule complex, **3**, has an energy of −12.3 kcal/mol relative to separated reactants, and there is a barrier of 5.7 kcal/mol for conversion of this complex to **5**. In aqueous solution, thiolate

anion **1** is more tightly solvated than intermediate **5**, so that the energies of starting materials and products are lowered relative to the intermediate. In an aqueous medium, the formation of intermediate **5** is endothermic, proceeding with a $\Delta G_{298,aq}$ of +15.2 kcal/mol and a $\Delta G_{298,aq}^{\ddagger}$ of +17.4 kcal/mol. While the intermediate is barely bound in water, it should be a longer-lived species in a less polar environment. The computed $\Delta G^{\ddagger}$ in water is in reasonable agreement with experimental rates of NO transfer from *S*-nitrosothiols to thiolates of similar molecular weight in aqueous solution:[7a,c,f,h] rate constants of 0.9−1016 M^{-1} s^{-1} corresponding to a $\Delta G^{\ddagger}$ of 13−18 kcal/mol.

To confirm that the nitroxyl disulfide, **5**, is a true minimum in solution, reoptimizations of **5** were performed for CHCl₃ (ϵ = 4) and H₂O (ϵ = 80.1) with B3LYP/6-311+G*. The reoptimized structures are nearly the same as structure **5** in Figure 1, except for a contraction of the SN bond lengths from 2.32 Å (gas phase) to 2.30 Å (CHCl₃) to 2.28 Å (H₂O) in the B3LYP/6-311+G* optimizations.[10c]

Transition state **4** is relatively early, with a forming S−N distance of 3.761 Å. There is a lengthening (0.076 Å) of the nitrosothiol S−N bond in transition state **3**, and almost no change (0.005 Å) in the length of the NO bond (Figure 1). The most striking aspect of this study is the structure of intermediate **5** (Figure 1). This species has long S−N bonds of 2.343 Å, nearly 0.5 Å longer than the S−N single bond length in nitrosothiol **2**. While such long bonds are often characteristic of transition states, frequency analysis confirms that this is a true energy minimum. The 1.175 Å N−O bond length of **5** is essentially identical to the 1.177 Å bond length of nitrosothiol **2** and somewhat shorter than the 1.273 Å N−O bond length in nitroxyl anion ³NO⁻ calculated at the same level of theory. The lowest energy conformation (**5a**) is the C_S, syn−syn structure shown in Figure 1, with O−N−S−C dihedral angles of 9°. A syn−anti conformation (**5b**), with dihedrals of 19° and 180°, is only 0.4 kcal/mol higher in energy. An anti−anti conformation (**5c**), with dihedrals of −111° and 176°, is 2.8 kcal/mol higher than **5a**. Reoptimization of **5** in a PCM aqueous solvation model, or MP2 reoptimization, caused only minor variations in geometry. We propose the term nitroxyl disulfide to describe species such as **5**.

(7) (a) Meyer, D. J.; Kramer, H.; Özer, N.; Coles, B.; Ketterer, B. *FEBS Lett.* **1994**, *345*, 177−180. (b) Barnett, D. J.; McAninly, J.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1131−1133. (c) Barnett, D. J.; Rios, A.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1279−1282. (d) Zhang, H.; Means, G. E. *Anal. Biochem.* **1996**, *237*, 141−144. (e) Rossi, R.; Lusini, L.; Giannerini, F.; Giustarini, D.; Lungarella, G.; Di Simplicio, P. *Anal. Biochem.* **1997**, *254*, 215−220. (f) Hogg, N. *Anal. Biochem.* **1999**, *272*, 257−262. (g) Wang, K.; Wen, Z.; Zhang, W.; Xian, M.; Cheng, J.-P.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 433−436. (h) Munro, A. P.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1794−1797.

(8) Frisch, M. J.; et al. *Gaussian 98, Revision A.6,* Gaussian, Inc., Pittsburgh, PA, 1998. See Supporting Information for remaining 56 authors.

(9) Montgomery, J. A.; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822−2827.

(10) (a) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117−129. (b) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239−245. (c) The Gaussian 98 default convergence criteria were met, except for a maximum force of 1.3×10^{-3} versus default criterion of 4.5×10^{-4} in the CHCl3 optimization and 5.5×10^{-4} versus 4.5×10^{-4} for the H2O optimization.
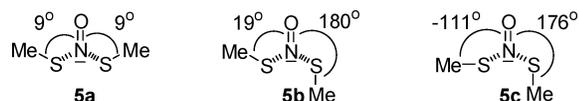
Experimental evidence was obtained for the existence of species analogous to **5** in the gas phase. *S*-Nitroso-*N*-acetyl-penicillamine (SNAP, 10 mM) in acetonitrile was infused through an electrospray nebulizer into an Agilent 1100 LC/MSD ion trap, and ions were detected in negative ion mode. The total ion spectrum[11] shows a strong peak characteristic of the SNAP carboxylate anion (m/*e* 219). An ion of m/*e* 439 is assigned to the SNAP carboxylate hydrogen-bond complex with SNAP. When the ion with mass 219 was isolated in the ion trap, a prominent ion at m/*e* 410, the predicted mass to charge for monoanionic nitroxyl disulfide, was also observed. The location of the charge on this species is unclear, since this particular nitroxyl disulfide incorporates three ionizable residues. No multiply charged ions were observed. To better establish the identity of the ion at m/*e* 410, fragmentation studies were conducted. MS/MS of the m/*e* 410 ion yielded ions at m/*e* 219 and 190, characteristic of SNAP carboxylate and *N*-acetylpeni-cillamine carboxylate, respectively.

Further computational studies gave insights into the electronic structure of **5** and the surprising contrast between nucleophilic substitution at nitroso groups and that at acyl groups. The structure of **5** can be depicted as a hybrid of the three resonance structures (Scheme 3). ChelpG charge calculations[12] reveal that the negative charge is primarily borne by the sulfur atoms; 40% of the negative charge is borne by each sulfur atom, with the remainder on oxygen. The N−O bond length and charge distribution suggest that the central resonance structure, with an N−O single bond and negative charge localized on the oxygen, is no more important than the zwitterionic resonance structure of the parent nitrosothiol.[13]

Scheme 3
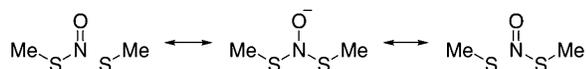
The transition structure for the S_N2 reaction between dimethyl sulfide and methyl sulfide (Figure 3) has partial S···C bonds of 2.445 Å, surprisingly close to the 2.343 Å MeS−N bonds of intermediate **5**. The N···S bonds of the nitroxyl disulfide and the C···S bonds of the S_N2 transition state may all be considered to have bond orders of one-half.

Analogous reactions of nitrites, esters, and thioesters were explored for comparison. Addition/elimination reactions were examined, and the results are summarized in Table 1 and Figure 4. The transnitrosation between methyl nitrite **6** and the methoxide ion (Scheme 4) is very similar to that between **1** and **2**. An initial gas-phase complex (**7**) is formed, and a transition state (**8**) leads to a symmetric intermediate (**9**). As with intermediate **5**, the MeO−N bonds of intermediate **9** are

(11) Provided in the Supporting Information.
(12) (a) Chirlian, L. E.; Francl, M. M. *J. Comput. Chem.* **1987**, *8*, 894−905. (b) Breneman, C. M.; Wiberg, K. B. *J. Comput. Chem.* **1990**, *11*, 361−373.
(13) The minimal change in N−O bond length upon thiolate **1** addition to nitrosothiol **2** is comparable to the minimal change in amide C−O bond length upon rotation about the C−N bond. (a) Wiberg, K. B.; Breneman, C. M.; LePage, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 61−72. (b) Wiberg, K. B.; Breneman, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 831−840. (c) Wiberg, K. B.; Rush, D. J. *J. Am. Chem. Soc.* **2001**, *123*, 2038−2046.

Figure 3. Transition structure for the S_N2 reaction between dimethyl sulfide and methyl sulfide.

Table 1. Energetics for Addition−Elimination Reactions of Transesterification, Transthioesterification, and Transnitrosation Reactions[a]

reaction	complex 1	TS 1	intermediate	TS 2	complex 2
MeSNO + MeS⊖	−11.6	−7.1	−15.3	−7.1[b]	−11.6[b]
MeONO + MeO⊖	−15.7	−10.6	−15.3	−10.6[b]	−15.7[b]
MeSCHO + MeS⊖	−10.2	−5.5	−10.7	−7.6	−10.5
MeOCHO + MeO⊖	−14.6	−10.6	−22.4	−11.2	−15.6

[a] All energies are in kcal/mol relative to infinitely separated starting materials. [b] This is a symmetric reaction, and therefore the bond-breaking process is the mirror image of the bond-forming process.

significantly longer (0.48 Å) than the MeO−N bond of starting material **6**. The analogous MeS−N bonds of intermediate **5** are 0.47 Å longer than the MeS−N bond of nitrosothiol **2**. Neither NO bond stretches at all in the intermediate structures.

Transthioesterification and transesterification intermediates are significantly different. The intermediates **13** and **19** (Figure 3) do not possess the mirror symmetry of **5** and **9**. Two gas-phase complexes were found for each reaction, with the nucleophile either near the methyl group (**11** and **17**) or near the formyl (or thioformyl) proton (**15** and **21**). There were also two transition states, leading from either complex to the intermediate. Whereas **5** and **9** have partial bond lengths 0.5 Å larger than single bonds, the tetrahedral intermediates, **13** and **19**, have partial bonds only 0.1 and 0.2−0.3 Å looser than single bonds; furthermore, the carbonyl stretches by a significant 0.1 Å, about one-third of the 0.28 Å difference between a double and single CO bond length. The energetics (gas-phase B3LYP/6-311+G*) for all of these addition/elimination reactions are compared in Table 1. The unsymmetrical structures of **13** and **19** arise from anomeric effects in the tetrahedral intermediate.[14] Both MeX groups are aligned for anomeric effects with the alkoxide, but one is a donor while the other is an acceptor with respect to each other, accounting for the C−O bond length differences.

Nitroxyl disulfide **5** has a gas-phase proton affinity of −342.6 kcal/mol (ΔH_{298}), corresponding to an aqueous phase pK_a of approximately 13.[15] This implies that species **22** should be

(14) Delongschamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983.
(15) Bartberger, M. D.; Fukuto, J. M.; Houk, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 2194−2198.

MeONO + MeO⊖

MeSCHO + MeS⊖

MeOCHO + MeO⊖

Figure 4. Geometries of stationary points in the transnitrosation, transthioesterification, and transesterification reactions (selected bond lengths in Å).

Scheme 4

readily formed in aqueous solution. Protonated species **22**, widely invoked to account for some nitrosothiol chemistry,[16] can also be formed in the gas phase through the combination of methanethiol **23** and nitrosothiol **2** with a computed ΔH_{298} of -1.2 kcal/mol, or $\Delta G_{298} = +10.4$ kcal/mol. In water, this process has a $\Delta G_{298,aq} = +5.4$ kcal/mol.

The aqueous oxidation potential of **5** is estimated to be $+0.31$ eV versus the standard hydrogen electrode. This value was determined as previously described[17] using the reduction potential of *S*-nitrosothioacetate as a reference.[18] The oxidation

(16) (a) Singh, S. P.; Wishnok, J. S.; Keshive, M.; Deen, W. M.; Tannenbaum, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14428−14433. (b) Wong, P. S.-Y.; Hyun, J.; Fukuto, J. M.; Shirota, F. N.; DeMaster, E. G.; Shoeman, D. W.; Nagasawa, H. T. *Biochemistry* **1998**, *37*, 5362−5371; **1998**, *37*, 18129.

(17) Bartberger, M. D.; Liu, W.; Ford, E.; Miranda, K. M.; Switzer, C.; Fukuto, J. M.; Farmer, P. J.; Wink, D. A.; Houk, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10958−10963.

potential of **5** allows facile reactions with physiologically relevant oxidants (e.g., O_2, with $E_{1/2} = -0.33$ eV).[19] Depending on lifetime and conditions, **5** may act as a one-electron donor followed by radical chemistry of species **24**. Radical **24** can dissociate readily to form a disulfide and free NO.
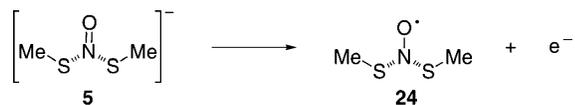
In the aqueous phase, anionic species such as **5** should be short-lived intermediates in transnitrosation reactions. In non-polar media, including nonpolar protein binding sites, these anionic species will be somewhat longer lived than those in water and may be able to undergo other chemical processes, such as proton or electron transfer. By altering the substituents on sulfur, it may be possible to develop anionic nitroxyl disulfides that can act as reversible NO donors or NO sensors, complementary to NO donors such as the NONOates and related electronically to Kochi et al.'s cation bis-arene-NO$^+$ com-

plexes.[20] These processes are currently under investigation in our laboratories.

Supporting Information Available: Mass spectral data, computed geometries and energies for all reported structures, and the full reference to Gaussian are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

JA029655L

(18) Hou, Y.; Wang, J.; Arias, F.; Echegoyen, L.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3065−3070.
(19) Wood, P. M. *Trends Biochem. Sci.* **1987**, *12*, 250−251.
(20) Rosokha, S. V.; Kochi, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 5620−5621.