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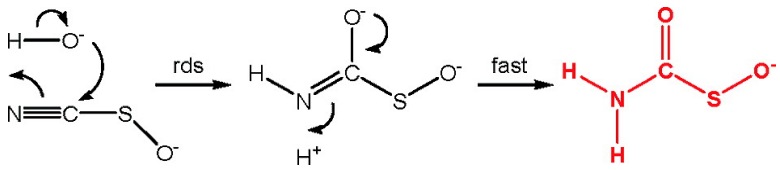

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Reactive Sulfur Species: Hydrolysis of Hypothiocyanite To Give Thiocarbamate-*S*-oxide

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Hypothiocyanite is produced by the reaction of thiocyanate with hydrogen peroxide in a reaction that is catalyzed by human peroxidases (including lactoperoxidase, salivary peroxidase, myeloperoxidase, and eosinophile peroxidase) for the purpose of managing microbial growth, particularly, in the regions of the body that are controlled by the mucosa:[1]

$$SCN^- + H_2O_2 \xrightarrow{\text{peroxidase}} OSCN^- + H_2O \quad (1)$$

In addition, SCN^- serves as a sequestering agent in vivo for the more powerful oxidants hypochlorite[2] (X = Cl) and hypobromite[3] (X = Br) in reactions that also yield $OSCN^-$:[4]

$$SCN^- + HOX \rightarrow OSCN^- + H^+ + X^- \quad (2)$$

The latter reaction is believed to restrict the lifetimes of HOX and thereby their propensities to inflict host tissue damage that can lead to inflammatory diseases.[2,3]

An equilibrium exists between HOSCN and $(SCN)_2$ under acidic conditions,[5,6] and in the presence of excess SCN^-, $(SCN)_3^-$ is formed.[7] However, the properties of $OSCN^-$ under more alkaline conditions remain largely unexplored. While alternative structures for $OSCN^-$ (including $OCNS^-$, $SOCN^-$, $ONCS^-$, and $OSNC^-$) have been ruled out,[4] it has been previously suggested that the antimicrobial properties of the peroxidase/SCN^-/H_2O_2 defensive mechanism could be due to other chemical species that could be derived from $OSCN^-$, including $(SCN)_2$, NCSCN, HO_2SCN, and HO_3SCN.[8−11] Cyanosulfite, $NCSO_2^-$, has been characterized in SO_2 solvent,[12,13] and cyanosulfate, $NCSO_3^-$, has been proposed in HCN solvent,[14] but neither species has been observed in an aqueous environment. In the absence of another reaction partner, solutions of $(SCN)_2$/HOSCN eventually decompose in water to give cyanide or cyanate, depending upon the reaction conditions. The reactions that lead to the decomposition of $OSCN^-$ are generally described as disproportionations:

$$2\ HOSCN \rightarrow HO_2SCN + HSCN \quad (3)$$

$$HO_2SCN + HOSCN \rightarrow HO_3SCN + HSCN \quad (4)$$

Hydrolysis of the C−S bond of HO_3SCN completes the reaction sequence and yields the following net reactions:

$$3\ HOSCN + H_2O \rightarrow H_2SO_4 + HCN + 2\ HSCN \quad (5)$$

and/or

$$H_2SO_3 + HOCN + 2\ HSCN \quad (6)$$

While eqs 3−6 illustrate one possible reaction pathway, through disproportionation,[10] no intermediates have been previously characterized for the decomposition reactions of $OSCN^-$. We report here the unprecedented direct hydrolysis of $OSCN^-$ under alkaline conditions to produce thiocarbamate-*S*-oxide, which illustrates that the intermediate oxidative derivatives of SCN^- are capable of undergoing hydrolysis reactions.

Alkaline solutions of $OSCN^-$ that have been prepared by one of the standard methods (e.g., via eq 1, followed by a pH-jump to more alkaline conditions, or by eq 2) are relatively stable as compared to more acidic conditions. However, alkaline solutions of $OSCN^-$ also eventually decompose via a species that is more stable than $OSCN^-$ itself. The half-life of the decomposition reaction of $OSCN^-$ at pH 13 and 20 °C (a first-order process at constant $[OH^-]$) is ca. 30 min. We have until recently codified the new species "190" to reflect the unusual chemical shift that is observed in the ^{13}C NMR spectrum at pH 13 (190.9 ppm vs internal dioxane at 66.6 ppm) that was correlated with other spectroscopic signatures at pH 13, including a corresponding resonance in the ^{15}N NMR spectrum (−101.6 ppm vs external NO_3^- at 0 ppm), a new band in the UV spectrum (λ_{max} = 250 nm, ϵ_{240} = 3600 M^{-1} cm^{-1} for pH > 8), and the appearance of a signal in the ion chromatogram (Figure 1). Similar chromatograms and spectra were obtained when $OSCN^-$ was generated from the LPO system at pH 7, followed by a pH-jump to pH 13, and by extraction of $(SCN)_2$ from a CCl_4 solution into 0.1 M NaOH (aq). On the basis of the observation that ^{13}C-labeled 190 formed during the hydrolysis of $OS^{13}CN^-$, the reaction exhibited heterogeneous second-order kinetics (first-order each in $[OSCN^-]$ and $[OH^-]$ and independent of $[SCN^-]$), and the overall reaction did not result in a change in $[H^+]$ at pH 11.7 (as determined using an unbuffered solution in the presence of the indicator Tropaeolin O), we propose that the following mechanism produced 190 (note the different charges of the species in eq 3 vs eqs 7 and 8):

$$OSCN^- + OH^- \xrightarrow{\text{rds}} HO_2SCN^{2-} \quad (7)$$

$$HO_2SCN^{2-} + H^+ \xrightarrow{\text{fast}} H_2O_2SCN^- \quad (8)$$

We note that, following the initial synthesis of $OSCN^-$, no additional SCN^-, CN^-, OCN^-, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, or any other common inorganic ion was produced while $OSCN^-$ is converted to 190. The new species with the empirical formula $H_2O_2SCN^-$ remained a mystery until an independent synthesis was devised that involved the oxidation of thiocarbamate:[15]

$$COS + 2\ NH_3 \xrightarrow[-10\ °C]{\text{EtOH/AcOH}} H_2NC(=O)S^-\cdot NH_4^+ \quad (9)$$

$$H_2NC(=O)S^- + OCl^- \xrightarrow{\text{pH 13}} H_2NC(=O)SO^- + Cl^- \quad (10)$$

Both synthetic procedures (eqs 7 and 8 and eqs 9 and 10) produce the same UV spectra and the same IC chromatograms. Thus, we propose the product of the hydrolysis of $OSCN^-$ at pH 13 is thiocarbamate-*S*-oxide (the conjugate base of carbamothioperoxoic acid). To the best of our knowledge, no example of a carbamothioperoxoic acid has been previously characterized, although the

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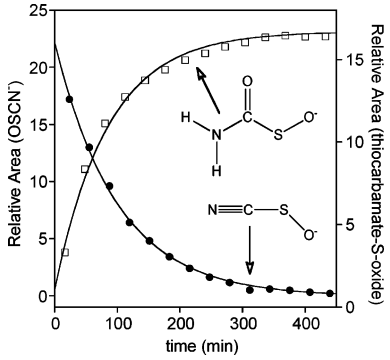

Figure 1. Hydrolysis of $OSCN^-$ (48 μM) at 10 °C as monitored by ion chromatography at pH = 13. $OSCN^-$ was generated by the reaction of OCl^- (2.42 mM) and SCN^- (500 mM) followed by a 50-fold dilution. Two new peaks were assigned to be $OSCN^-$ (solid circles) and thiocarbamate-*S*-oxide (open squares) with single-exponential fits ($k = 1.67(3) \times 10^{-4}$ and $1.96(8) \times 10^{-4}$ s^{-1}, respectively).

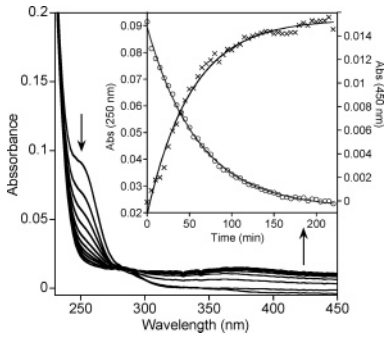

Figure 2. UV−vis spectra for the decomposition of thiocarbamate-*S*-oxide (50 μM, 0.10 M phosphate buffer, $I = 1.0$ M) at pH 6.64 (time interval = 900 s). Inset: Time traces at 250 and 450 nm with single-exponential fits ($k = 2.74(5) \times 10^{-4}$ and $3.2(2) \times 10^{-4}$ s^{-1}, respectively). Note the decrease in absorption at 250 nm due to thiocarbamate-*S*-oxide and the increase in the baseline that is attributed to light scattering by precipitating sulfur.

N-methyl derivative has been proposed as an oxidation product of metam, the soil fumigant *N*-methyldithiocarbamic acid.[16] Infrared studies of the oxidation of thiocarbamic acid *O*-aryl esters have suggested the products exist as tautomeric mixtures of the sulfenic acids and the *S*-oxides,[17] so it is conceivable that thiocarbamate-*S*-oxide also exists in tautomeric forms (with various resonance structures).

Thiocarbamate-*S*-oxide is relatively stable at pH 13 (the half-life for decomposition of a 50 μM solution is 18 days), but it is considerably less stable at neutral pH.[18] The hydrolytic decomposition of thiocarbamate-*S*-oxide at neutral pH is a first-order process (independent of initial concentration) that yields elemental sulfur (mass spectrum) and carbonate (^{13}C NMR) as two of the products (Figure 2). The precipitation of elemental sulfur (as indicated by the increase in light scattering, inset of Figure 2) occurs at the same rate as the decomposition of thiocarbamate-*S*-oxide (under aerobic and anaerobic conditions). These observations suggest an intramolecular rearrangement since a disproportionation reaction might be expected to yield different products with second-order kinetics:[19]

$$H_2NC(=O)SOH + H_2O \rightarrow NH_4^+ + HCO_3^- + S\downarrow \quad (11)$$

To further characterize thiocarbamate-*S*-oxide, we investigated its reaction with various reductants. Sulfite was not an effective reductant for thiocarbamate-*S*-oxide at pH 13, but cysteine reacts with thiocarbamate-*S*-oxide to give thiocarbamate and cystine (as determined by ion chromatography and ^1H NMR). The reaction proceeds through an intermediate that appears to be consistent with the mixed disulfide (eq 12) that eventually reacts with cysteinate to give cystine (eq 13):

$$H_2NC(=O)SOH + CySH \rightarrow H_2NC(=O)SSCy + H_2O \quad (12)$$

$$H_2NC(=O)SSCy + CySH \rightarrow H_2NC(=O)SH + CySSCy \quad (13)$$

By analogy, we have previously observed the formation of cysteine sulfenyl thiocyanates[20] from $(SCN)_2$/HOSCN that yield mixed disulfides upon reaction with thiols.[21] Thus, thiocarbamate-*S*-oxide exhibits reaction properties that are similar to $OSCN^-$ itself.

The possible relevance of thiocarbamate-*S*-oxide with respect to the human defense mechanism remains to be investigated. While an extrapolation of the pH dependency of the formation of thiocarbamate-*S*-oxide from $OSCN^-$ to physiological pH might lead one to conclude that the hydrolysis of $OSCN^-$ to produce thiocarbamate-*S*-oxide is too slow to be of physiologic consequence, we note that we have observed thiocarbamate-*S*-oxide (by ion chromatography and UV−vis) under various conditions that should not be the result of the hydrolysis mechanism of $OSCN^-$ (e.g., during the hydrolysis of $(SCN)_2$ at pH 4.5), thus, alternative reaction pathways may exist to produce thiocarbamate-*S*-oxide.

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Supporting Information Available: Experimental details, quantitative analyses, and time-resolved spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ashby, M. T. Where Old Biocides Meet New: Hypohalite Defense Factors of the Human Oral Cavity. In *New Biocides Development: The Combined Approach of Chemistry and Microbiology;* Zhu, P., Ed.; American Chemical Society: Washington, DC, 2007; pp 269−290.
(2) Ashby, M. T.; Carlson, A. C.; Scott, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15976−15977.
(3) Nagy, P.; Beal, J. L.; Ashby, M. T. *Chem. Res. Toxicol.* **2006**, *19*, 587−593.
(4) Nagy, P.; Alguindigue, S. S.; Ashby, M. T. *Biochemistry* **2006**, *45*, 12610−12616.
(5) Barnett, J. J.; McKee, M. L.; Stanbury, D. M. *Inorg. Chem.* **2004**, *43*, 5021−5033.
(6) Nagy, P.; Lemma, K.; Ashby, M. T. *Inorg. Chem.* **2007**, *46*, 285−292.
(7) Barnett, J. J.; Stanbury, D. M. *Inorg. Chem.* **2002**, *41*, 164−166.
(8) Bjoerck, L.; Claesson, O. *J. Dairy Sci.* **1980**, *63*, 919−922.
(9) Hogg, D. M.; Jago, G. R. *Biochem. J.* **1970**, *117*, 779−790.
(10) Thomas, E. L. Products of Lactoperoxidase-Catalyzed Oxidation of Thiocyanate and Halides. In *The Lactoperoxidase System: Chemistry and Biological Significance*; Pruitt, K. M., Tenovuo, J. O., Eds.; Marcel Dekker: New York, 1985; Vol. 27, pp 31−53.
(11) Pruitt, K. M.; Tenovuo, J.; Andrews, R. W.; McKane, T. *Biochemistry* **1982**, *21*, 562−567.
(12) Kornath, A.; Blecher, O.; Ludwig, R. *J. Am. Chem. Soc.* **1999**, *121*, 4019−4022.
(13) Kornath, A.; Blecher, O. *Z. Anorg. Allg. Chem.* **2002**, *628*, 625−631.
(14) Jander, G.; Gruttner, B.; Scholz, G. *Chem. Ber.* **1947**, *80*, 279−289.
(15) D'Amico, J. J.; Fuhrhop, R. W.; Bollinger, F. G.; Dahl, W. E. *J. Heterocycl. Chem.* **1986**, *23*, 641−645.
(16) Kim, J.-H.; Lam, W.-W.; Quistad, G. B.; Casida, J. E. *J. Agric. Food Chem.* **1994**, *42*, 2019−2024.
(17) Walter, W.; Wohlers, K. *Justus Liebigs Ann. Chem.* **1971**, *752*, 115−135.
(18) The pK_a of thiocarbamate-*S*-oxide is 6.68(3).
(19) (a) Thiocarbamates rapidly hydrolyze at neutral pH with the loss of COS: Ewing, S. P.; Lockshon, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 3072−3084. (b) COS is not readily hydrolyzed at neutral pH: Elliott, S.; Lu, E.; Rowland, F. S. *Environ. Sci. Technol.* **1989**, *23*, 458−461. (c) H_2S is only slowly oxidized by O_2 in the absence of a catalyst: Hoffmann, M. R.; Lim, B. C. *Environ. Sci. Technol.* **1979**, *13*, 1406−1414. (d) Disproportionation followed by oxidation of HS^- by HSO_3^- is not kinetically competent: Siu, T.; Jia, C. Q. *Ind. Eng. Chem. Res.* **1999**, *38*, 1306−1309 and 3812−3816.
(20) Ashby, M. T.; Aneetha, H. *J. Am. Chem. Soc.* **2004**, *126*, 10216−10217.
(21) Nimmo, S. L.; Lemma, K.; Ashby, M. T. *Heteroat. Chem.* **2007**, *18*, 467−471.

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