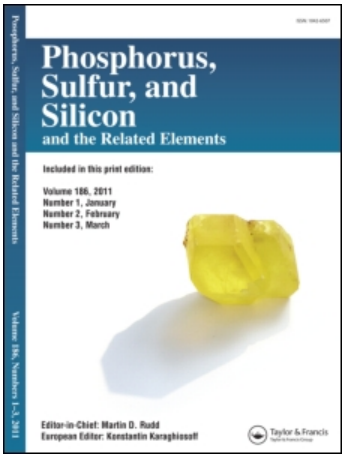


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Bioorganic Chemistry of Hypothiocyanite

Michael T. Ashby[a]; Halikhedkar Aneetha[a]; Amy C. Carlson[a]; M. Jared Scott[a]; Jennifer L. Beal[a]
[a] Department of Chemistry and Biochemistry, University of Oklahoma, Norman, USA

To cite this Article Ashby, Michael T. , Aneetha, Halikhedkar , Carlson, Amy C. , Scott, M. Jared and Beal, Jennifer L.(2005) 'Bioorganic Chemistry of Hypothiocyanite', Phosphorus, Sulfur, and Silicon and the Related Elements, 180: 5, 1369 — 1374
To link to this Article: DOI: 10.1080/10426500590912664
URL: http://dx.doi.org/10.1080/10426500590912664

Phosphorus, Sulfur, and Silicon, 180:1369–1374, 2005

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500590912664

Taylor & Francis
Taylor & Francis Group

Bioorganic Chemistry of Hypothiocyanite

Michael T. Ashby
Halikhedkar Aneetha
Amy C. Carlson
M. Jared Scott
Jennifer L. Beal
Department of Chemistry and Biochemistry, University of Oklahoma, Norman, USA

Hypothiocyanite ($OSCN^-$) plays an important role in the human host defense system as a nonimmunological antimicrobial agent. Although many conjugate reactions of proteins have been attributed to $OSCN^-$, there is little precedence for such reactions in small-molecule chemistry. We will discuss the derivative species that are in equilibrium with $OSCN^-$, including hypothiocyanous acid (HOSCN), thiocyanogen [$(SCN)_2$], and trithiocyanate [$(SCN)_3^-$], the first organic derivatives of this mixture to be fully characterized, and we will describe a new method of synthesizing hypothiocyanite.

Keywords Antimicrobial; hypothiocyanite; reactive sulfur species; sulfenyl thiocyanate

INTRODUCTION

Oxidative stress has been implicated in the pathogenesis of neurodegenerative disease,[1] cancer,[2] and aging.[3] Oxidative stress occurs as a response to increased oxidants, decreased antioxidants, or failure to repair oxidative damage. The constituents of oxidative stress include a variety of transitory compounds such as reactive oxygen species (ROS)[4,5] and reactive nitrogen species (RNS).[6–8] Although sulfur is usually considered to be part of the cellular antioxidant system,[9] there is increasing evidence that reactive sulfur species (RSS)[10,11] are formed during oxidative stress. Thiols and disulfides can be transformed into radicals, reactive conjugates, and compounds with sulfur in higher oxidation states. Well-documented examples of such RSS include thiyl radicals ($RS^{\cdot}$), nitrosothiols (RSNO), and sulfenic acids (RSOH). In addition to these

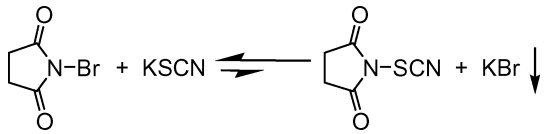
Received July 9, 2004; accepted October 5, 2004.
Address correspondence to Michael T. Ashby, Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Room 208, Norman, OK, USA. E-mail: mashby@ou.edu

better-understood RSS, the very recent literature is sprinkled with citations of more esoteric species including sulfenamides (RSNHR),[12] sulfinic acids (RSO_2H),[13–17] and sulfenyl thiocyanates (RSSCN).[18,19] A major obstacle in the critical evaluation of this emerging field of RSS is a dearth of data concerning the chemistry of such compounds in aqueous media. While most RSS that have been studied to date are organic in nature, there are several neglected inorganic RSS. One such inorganic biological RSS is hypothiocyanite [$OSCN^-$, but actually an equilibrium mixture that includes HOSCN, $(SCN)_2$, and $(SCN)_3^-$]. We discuss here a new reagent for synthesizing $OSCN^-$, N-thiocyanatosuccinimide (NTS).

NTS has been previously proposed, but never isolated, by Still et al.[20] This earlier investigation involved electrophilic thiocyanation reactions of electron-rich aromatic compounds using a reagent that was prepared *in situ* by reaction of N-bromosuccinimide (NBS) with alkali metal salts of thiocyanate.[20] The aryl thiocyanate products were not observed when authentic samples of $(SCN)_2$ were reacted under analogous conditions, and the authors concluded that N-thiocyanatosuccinimide (NTS) was likely formed *in situ*.[20] Our interest in this reagent stems from the possibility that hydrolysis of NTS might produce $OSCN^-$ in the absence of excess SCN^- (*vide infra*). This article describes the synthesis, isolation, and crystal structure of NTS. Reactions of NTS with thiols in water to give sulfenyl thiocyanates are also described. $OSCN^-$ and $(SCN)_2$ are implicated in these latter reaction.

RESULTS

After investigating several solvent systems, NTS was isolated as a pale yellow solid after reaction of NBS with NaSCN in CH_2Cl_2 under Finkelstein conditions.



We note that CH_2Cl_2 has been used previously for Finkelstein reactions of alkyl halides and SCN^-.[21] The crude product can be used without further purification, or it can be recrystallized from CH_2Cl_2/pentane to give colorless crystals.

In addition to characterization by NMR and IR spectroscopy,[22] the structure of NTS was further confirmed by X-ray crystallography (Figure 1). While several structures have been previously determined

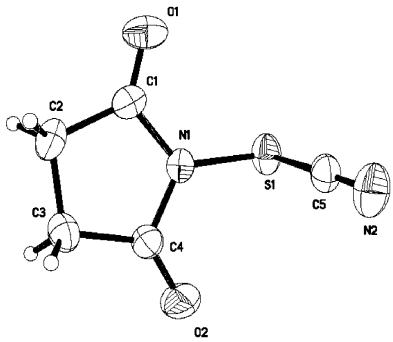
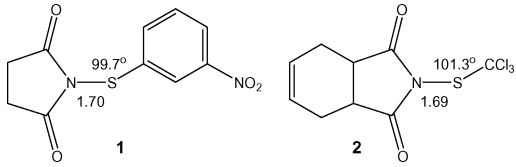


FIGURE 1 Thermal ellipsoid drawing of the crystal structure of NTS at the 50% level with the labeling scheme.

for compounds with N–S bonds, this is the first example of a compound with a N–SCN bond to be structurally characterized. The observed geometries about the N–S moiety of NTS (Table I) may be compared with the related compounds **1**[23] and **2**.[24]



The geometries about the sulphur atoms of NTS, **1**, and **2** are acute, approximately 100 deg, and consistent with incomplete hybridization at S.[25] The geometries about the succinimide N centers of NTS, **1**, and **2** are planar, as indicated by sum of the angles (359.8, 359.4, and 359.2 deg,

TABLE I Selected Bond Lengths (Å) and Angles (Deg) for NTS

S(1)–N(1)	1.6860(14)
S(1)–C(5)	1.696(2)
N(2)–C(5)	1.130(3)
N(1)–S(1)–C(5)	98.93(8)
C(4)–N(1)–C(1)	113.42(14)
C(4)–N(1)–S(1)	121.40(12)
C(1)–N(1)–S(1)	124.96(13)
N(2)–C(5)–S(1)	177.9(2)

respectively). Thus, the N center is highly conjugated with the carbonyl groups of these compounds, and the SCN moiety of NTS apparently does not affect this interaction. The observed N—S bond length of NTS (1.69 Å) is also comparable to those of **1** and **2**. We note that in all three compounds, the C—N—S—C torsional angles are close to 90 deg, a conformation that orientates the S p-type lone pair orthogonal with respect to the N—C=O π system. Accordingly, there appears to be little N—S conjugation. Nonetheless, the SCN moiety of NTS is electrophilic, which is attributed to inductive rather than conjugative effects. Evidence for the electrophilic character of NTS is provided by its implication in electrophilic aromatic substitution reactions[20] and our observation that the reagent readily generates sulfenyl thiocyanates when reacted with thiols in organic solvents.[26]

Sulfenyl thiocyanates are in many respects esoteric compounds, seemingly only of special interest because they are unstable. Nonetheless, despite the fact there exist no *bona fide* examples of such compounds in aqueous medium, sulfenyl thiocyanates are proposed intermediates in the synthesis of polypeptides[27] and nascent biological reactive species (*vide supra*).[18,19,28] It has been suggested that cytotoxic hypothiocyanite ($OSCN^-$), the putative antimicrobial agent that is produced by myeloperoxidase (MPO),[29,30] eosinophil peroxidase (EPO),[28,31–33] lactoperoxidase (LPO),[34] and salivary peroxidase (SPO),[35] may target key protein sulfhydryl groups in pathogens *vis-à-vis* RSSCN derivatives. We have carried out preliminary investigations of the reactions of $(SCN)_2$ and NTS with biologically relevant nucleophiles and have discovered these reagents react with cysteine derivatives at very low pH to yield sulfenyl thiocyantes. These products represent the first water-soluble sulfenyl thiocyanates.[26]

The nature of the electrophilic thiocyanating agent in these reactions is not unambiguous. At pH = 0, HOSCN is in rapid equilibrium with several other species.[36]

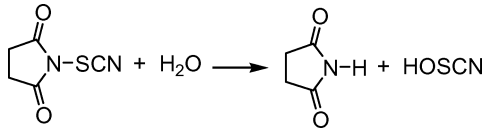
$$HOSCN \underset{}{\overset{pK_a = 5.3}{\rightleftharpoons}} H^+ + OSCN^-$$

$$HOSCN + SCN^- + H^+ \overset{K_{comp} > 1000\ M^{-2}}{\rightleftharpoons} (SCN)_2 + H_2O$$

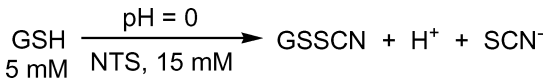
$$(SCN)_2 + SCN^- \overset{K = 0.6\ M^{-1}}{\rightleftharpoons} (SCN)_3^-$$

Although the principal species at pH = 0 is $(SCN)_2$, we cannot rule out the possibility that HOSCN is the thiocyantating electrophile when $(SCN)_2$ is used as a reagent. Accordingly, it is desirable to find a way of synthesizing HOSCN in the absence of excess SCN^- (beacuse even

small amounts of SCN^- will drive the second equilibrium to the right at low pH). NTS rapidly hydrolyzes in water to give succinimide (identified by ^1H NMR) and presumably hypothiocyanous acid.



Despite the instability of NTS in aqueous media, we have employed the reagent to synthesize sulfenyl thiocyanates in aqueous solution, *e.g.*, the glutathione (GSH) derivative.



Given the instability of NTS in water, rather than direct reaction of NTS with GSH, it is possible that the electrophilic thiocyanating agent in this case is an inorganic species that is derived from the hydrolysis [*e.g.*, HOSCN or $(SCN)_2$].[26]

REFERENCES

[1] L. M. Sayre, M. A. Smith, and G. Perry, *Cur. Med. Chem.*, **8,** 721–738 (2001).
[2] S. A. Akman, *Crit. Rev. Oxid. Stress Aging*, **2,** 925–954 (2003).
[3] J. Cai, P. Sternberg Jr., and D. P. Jones, *Crit. Rev. Oxid. Stress Aging*, **2,** 987–998 (2003).
[4] K. Rydzynski and W. Wasowicz, *Comm. Toxicol.*, **9,** 3–4 (2003).
[5] G. Bartosz, *Comm. Toxicol.*, **9,** 5–21 (2003).
[6] R. P. Patel, J. McAndrew, H. Sellak, C. R. White, H. Jo, B. A. Freeman, and V. M. Darley-Usmar, *Biochim. Biophys. Acta*, **1411,** 385–400 (1999).
[7] D. A. Wink and J. B. Mitchell, *Free Radical Biol. Med.*, **34,** 951–954 (2003).
[8] M. G. Espey, K. M. Miranda, D. D. Thomas, S. Xavier, D. Citrin, M. P. Vitek, and D. A. Wink, *Ann. N.Y. Acad. Sci.*, **962,** 195–206 (2002).
[9] K. J. A. Davies, *NATO ASI Series, Series A: Life Sciences*, **296,** 253–266 (1998).
[10] G. I. Giles and C. Jacob, *Biol. Chem.*, **383,** 375–388 (2002).
[11] G. I. Giles, K. M. Tasker, and C. Jacob, *Free Radical Biol. Med.*, **31,** 1279–1283 (2001).
[12] A. Salmeen, J. N. Andersen, M. P. Myers, T.-C. Mong, J. A. Hinks, N. K. Tonks, and D. Bradford, *Nature*, **423,** 769–773 (2003).
[13] K.-S. Yang, S. W. Kang, H. A. Woo, S. C. Hwang, H. Z. Chae, K. Kim, and S. G. Rhee, *J. Biol. Chem.*, **277,** 38029–38036 (2002).
[14] Z. A. Wood, L. B. Poole, and P. A. Karplus, *Science*, **300,** 650–653 (2003).
[15] H. A. Woo, H. Z. Chae, S. C. Hwang, K.-S. Yang, S. W. Kang, K. Kim, and S. G. Rhee, *Science*, **300,** 653–656 (2003).
[16] G. Georgiou and L. Masip, *Science*, **300,** 592–594 (2003).

[17] A. Claiborne, J. I. Yeh, T. C. Mallett, J. Luba, E. J. Crane III, V. Charrier, and D. Parsonage, *Biochem.*, **38,** 15407–15416 (1999).
[18] E. L. Thomas and T. M. Aune, *Infect. Immun.*, **20,** 456–463 (1978).
[19] T. M. Aune and E. L. Thomas, *Biochem.*, **17,** 1005–1010 (1978).
[20] F. D. Toste, V. De Stefano, and I. W. J. Still, *Synth. Commun.*, **25,** 1277–1286 (1995).
[21] S. Bekkevoll, I. Svorstoel, H. Hoeiland, and J. Songstad, *Acta Chem. Scand.*, **B37,** 935–945 (1983).
[22] ^{1}H NMR (CD_2Cl_2, 300 MHz) δ 2.95 (s, 4 H), $^{13}C\{^{1}H\}$ NMR (CD_2Cl_2, 75.5 MHz) δ 173.7, 108.8, 29.2, IR (CH_2Cl_2) 2160 cm^{-1} (ν_{CN}), IR (Nujol) 2173 cm^{-1} (ν_{CN}).
[23] J. N. Low, E. J. Storey, M. Mccarron, J. L. Wardell, G. Ferguson, and C. Glidewell, *Acta Crystallogr.*, **B56,** 58–67 (2000).
[24] L. E. Moss and R. A. Jacobson, *Cryst. Struc. Communic.*, **10,** 1545–1548 (1981).
[25] M. T. Ashby, *Comments Inorg. Chem.*, **10,** 297–313 (1990).
[26] M. T. Ashby and H. Aneetha, *J. Am. Chem. Soc.,* **126,** 10216–10217 (2004).
[27] R. G. Hiskey and B. F. Ward, Jr., *J. Org. Chem.*, **35,** 1118–21 (1970).
[28] M. Arlandson, T. Decker, V. A. Roongta, L. Bonilla, K. H. Mayo, J. C. Macpherson, S. L. Hazen, and A. Slungaard, *J. Biol. Chem.*, **276,** 215–24. (2001).
[29] C. J. Van Dalen, M. W. Whitehouse, C. C. Winterbourn, and A. J. Kettle, *Biochem. J.*, **327,** 487–492 (1997).
[30] A. J. Kettle and C. C. Winterbourn, *Redox Report*, **3,** 3–15 (1997).
[31] P. G. Furtmueller, U. Burner, G. Regelsberger, and C. Obinger, *Biochem.*, **39,** 15578–15584 (2000).
[32] A. Slungaard and J. R. Mahoney Jr., *J. Biol. Chem.*, **266,** 4903–4910 (1991).
[33] C. J. Van Dalen and A. J. Kettle, *Biochem. J.*, **358,** 233–239 (2001).
[34] E. L. Thomas, *Immunol. Series*, **27,** 31–53 (1985).
[35] K. M. Pruitt, B. Mansson-Rahemtulla, D. C. Baldone, and F. Rahemtulla, *Biochem.*, **27,** 240–245 (1988).
[36] J. J. Barnett, M. L. McKee, and D. M. Stanbury, *Inorg. Chem.*, **43,** 5021–5033 (2004).