Is the Hypothiocyanite Anion $(OSCN)^-$ the Major Product in the Peroxidase Catalyzed Oxidation of the Thiocyanate Anion $(SCN)^-$? A Joint Experimental and Theoretical Study

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The hypothiocyanate anion $(OSCN)^-$ is reported to be a major product of the lactoperoxidase/H_2O_2/$(SCN)^-$ system, and this anion is proposed to have significant antimicrobial properties. The collision induced (CID) negative ion mass spectrum of "$(OSCN)^-$" has been reported: there is a pronounced parent anion at *m*/*z* 74, together with fragment anions at *m*/*z* 58 $(SCN)^-$ and 26 $(CN)^-$. These fragment anions are consistent with structure $(OSCN)^-$. However there is also a lesser peak at *m*/*z* 42 (OCN^- or CNO^-) in this spectrum which is either formed by rearrangement of $(OSCN)^-$ or from an isomer of this anion. The current theoretical investigation of $(OSCN)^-$ and related isomers, together with the study of possible rearrangements of these anions, indicates that ground-state singlet $(OSCN)^-$ is a stable species and that isomerization is unlikely. The three anions $(OSCN)^-$, $(SCNO)^-$, and $(SNCO)^-$ have been synthesized (in the ion source of a mass spectrometer) by unequivocal routes, and their structures have been confirmed by a consideration of their collision induced (negative ion) and charge reversal (positive ion) mass spectra. The CID mass spectrum of $(SCNO)^-$ shows formation of *m*/*z* 42 (CNO^-), but the corresponding spectra of $(OSCN)^-$ or $(SNCO)^-$ lack peaks at *m*/*z* 42. Combined theoretical and experimental data support earlier evidence that the hypothiocyanite anion is a major oxidation product of the H_2O_2/$(SCN)^-$ system. However, the formation of *m*/*z* 42 in the reported CID spectrum of "$(OSCN)^-$" does not originate from $(OSCN)^-$ but from another isomer, possibly $(SCNO)^-$.

Introduction

It has been known for more than 40 years that a mixture of a particular peroxidase, hydrogen peroxide and the thiocyanate anion $(NCS)^-$ produce a relatively stable chemical species with antimicrobial activity.[1−3] It was later proposed that the active species was the hypothiocyanite anion $(OSCN)^-$ formed either (i) by oxidation of $(SCN)^-$ to $(SCN)_2$, followed by hydrolysis, or (ii) by direct oxidation of $(SCN)^-$.[4] There has been considerable literature concerning the antimicrobial activity of this reaction mixture [peroxidase/H_2O_2/$(SCN)^-$] in various biological systems, but little is known quantitatively of its cytotoxicity. The reaction mixture is active against a number of Gram positive and negative organisms (e.g., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Eschericia coli* and *Candida albicans*) apparently at the micromolar concentration,[5] but further oxidation of the system causes tissue damage.[6] Antibacterial activity is also noted in the absence of the peroxidase,[7] for example an aqueous solution containing $(SCN)^-$ (0.5%) and H_2O_2 (0.1%) is effective against gingivitis.[8]

The products of the oxidation sequence of the lactoperoxidase/H_2O_2/$(SCN)^-$ system were poorly defined until they were recently probed using nuclear magnetic resonance and electrospray mass spectrometry. The results were interpreted in terms of the initial oxidation products being cyanate $(OCN)^-$ and hypothiocyanite $(OSCN)^-$ in essentially a 1:1 ratio.[9] The collision induced (CID) negative ion mass spectrum of "$(OSCN)^-$" [measured on a Micromass Quatro 2 triple quadrupole mass spectrometer; Ar collision gas in second quadrupole (1.7×10^{-3} Torr)] is particularly informative: there is a pronounced parent anion at *m*/*z* 74, together with fragment anions at *m*/*z* 58 $(SCN)^-$ and 26 $(CN)^-$. These fragment anions are consistent with structure $(OSCN)^-$. However there is also a lesser peak at *m*/*z* 42 (OCN^- or CNO^-) which either has to be formed (i) from a parent anion isomeric with $(OSCN)^-$ or (ii) by rearrangement of $(OSCN)^-$ prior to or during decomposition. The aim of this paper is to determine the origin of *m*/*z* 42.

Our interest in the structure of this antimicrobial anion is 2-fold: first, as a followup of our earlier work on vertical one electron oxidation of $(OCN)^-$ [10] and $(SCN)^-$,[11] and second, because of our particular interest in interstellar chemistry. In the latter context, the parent acids HONC and HSCN are known interstellar molecules,[12−14] and $(OCN)^-$ is likely to be a component of interstellar ice (particularly in comets).[15] It would not be surprising if $(SCN)^-$ is present in interstellar ice, and if so, the oxidation product of $(SCN)^-$ may have been one of the first antimicrobial agents formed on primeval earth and elsewhere.

Experimental Section

A. **Mass Spectrometric Methods.** For a detailed description of the experiment and the instrument used, see ref 16. In brief, the experiments were performed using a two-sector modified VG ZAB 2HF mass spectrometer with BE configuration, where B and E represent magnetic and electric sectors, respectively. The anions $(OSCN)^-$, $(SCNO)^-$, and $(SNCO)^-$ were formed in the chemical ionization ion source by the reactions shown in Scheme 3. Typical source conditions were as follows: source temperature 200 °C, repeller voltage −0.5 V, ion extraction

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voltage 7 kV, and mass resolution $m/\Delta m \geq 1500$. Each neutral precursor was inserted into the ion source through the direct probe, which was heated to 100 °C to give a measured pressure of ca. 10^{-5} Torr inside the source housing. In the cases of $(SCNO)^-$ and $(SNCO)^-$, the source also contained HO^- (from H_2O) to give a measured source pressure of 10^{-4} Torr (estimated total source pressure is 10^{-1} Torr). Collision induced (CID) spectra were determined using B to select m/z 74 in each case, and utilizing argon as the target gas in the first collision cell following B. The pressure of argon in the first cell was maintained such that 80% of the parent ion beam was transmitted through the cell. This corresponds to an average of 1.1–1.2 collisions per ion.[17] Product anion peaks resulting from CID processes were recorded by scanning E.

Charge reversal ($^-CR^+$) spectra[18,19] were recorded using single collision conditions in collision cell 1 (O_2, 80% transmission of main beam).

B. Precursor Molecules. The precursor molecules NCS(O)-CN,[20] 1-thiomethoxy-2-cyano-3-phenylisoxazole,[21] 3-hydroxyisothiazole,[22] and 1-(*N*-methoxythiocarbamoyl)imidazole[23] were made by literature methods.

C. Theoretical Methods. Geometry optimizations were carried out with the Becke 3LYP method[24,25] using the 6-31+G(d) level of theory within the GAUSSIAN 98 suite of programs.[26] Stationary points were characterized as either minima (no imaginary frequencies) or transition structures (one imaginary frequency) by calculation of the frequencies using analytical gradient procedures. The minima connected by a given transition structure were confirmed by intrinsic reaction coordinate (IRC) calculations. The calculated frequencies were also used to determine zero-point vibrational energies which were used as a zero-point correction for the electronic energies. We have previously reported the success of the B3LYP method in predicting geometries of unsaturated chain structures, and that this method produces optimized structures, at low computational cost, that compare favorably with higher level calculations.[27] Higher level single point energies were calculated using the CCSD(T) method[28,29] together with the Dunning aug-cc-pVDZ basis set.[30] All calculations were carried out on the Alpha Server at the Australian Partnership for Advanced Computing (APAC) National Facility (Canberra).

Results and Discussion

Theoretical Results. There are many isomers of an anion containing one atom of each of C, N, O and S. However, if the oxidation occurs from an intact thiocyanate anion $(SCN)^-$, the only feasible products are $(OSCN)^-$ and $(SCNO)^-$. If the isothiocyanate anion $(SNC)^-$ is also present in interstellar ice [either in its own right or as a product of a thiocyanate–isothiocyanate rearrangement], oxidation of this anion could form $(OSNC)^-$ and perhaps $(SNCO)^-$. Before attempting to synthesize these anions, molecular modeling at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory was used to determine the structures and energies of these isomeric anions together with others which might be formed by rearrangement of $(OSCN)^-$. Data are listed in Schemes 1 and 2 and Tables 1 and 2.

There are, in principle, singlet and triplet forms of the even-electron anions **1**$^-$ to **5**$^-$. An early ab initio study at the 6-31G(d) level suggested that **1**$^-$ should be unstable.[31] The results of our theoretical study indicate that **1**$^-$ to **5**$^-$ are stable with the exception of ^{3}**5**$^-$ which is unstable with respect to decomposition to SO and NC^-. Singlet structures are the ground states of **1**$^-$

TABLE 1: Structures and Energies of the Isomeric Singlet Anions (Level of Theory Used = CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) with Relative Energies in kcal mol^{-1} with Respect to ^{1}1$^-$ (0 kcal mol^{-1}))

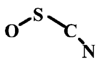
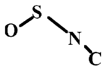
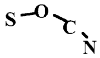
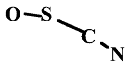
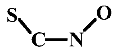
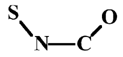
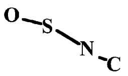
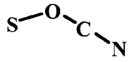
	 ^{1}**1**$^-$	S—C-N—O ^{1}**2**$^-$	S—N—C—O ^{1}**3**$^-$	 ^{1}**4**$^-$	 ^{1}**5**$^-$
state	$^1A_1$	^1SG	^1SG	$^1A_1$	$^1A_1$
symmetry	C_s	$C_{\infty v}$	$C_{\infty v}$	C_1	C_s
energy (hartrees)	−565.38239	−565.36191	−565.41467	−565.3481	−565.38638
energy relative to ^{1}**1**$^-$	0	+12.9	−20.3	+21.5	−2.5
dipole moment (D)	2.4036	0.5686	1.2528	7.6542	1.195
adiabatic electron affinity (neutral, eV)	2.42	2.98	2.62	2.25	3.10
bond lengths (Å)					
OS	1.585			1.561	1.871
SC	1.735	1.673			
CN	1.178	1.173	1.181	1.185	1.177
NO		1.272			
SN			1.704	1.787	
CO			1.221		1.267
bond angles (deg)					
OSC	110.0				
SCN	177.5	180.0			
NCO			180.0		175.0
COS					116.8
CNO		180.0			
OSN				109.2	
SNC			180.0	178.8	
dihedral angle (deg)					
OSCN	180.0				
SCNO		0.0			
SNCO			0.0		
OSNC				−179.8	
SOCN					180.0

TABLE 2: Structures and Energies of the Isomeric Triplet Anions (Level of Theory Used = CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d), with Relative Energies in kcal mol^{-1} with Respect to $^{1}\mathbf{1}^{-}$ (0 kcal mol^{-1}))

	 $^{3}\mathbf{1}^{-}$	 $^{3}\mathbf{2}^{-}$	 $^{3}\mathbf{3}^{-}$	 $^{3}\mathbf{4}^{-}$	 $^{3}\mathbf{5}^{-}$
state	$^{3}A''$	$^{3}A''$			unstable
symmetry	C_s	C_s	C_1	C_1	
energy (hartrees)	−565.35346	−565.28963	−565.34432	−565.35486	
energy relative to $^{1}\mathbf{1}^{-}$	+18.1	+58.2	+23.8	+17.2	
dipole moment (D)	2.1398	1.4687	0.6507	7.6542	
bond lengths (Å)					
OS	1.541			1.537	
SC	2.808	1.654			
CN	1.181	1.322	1.322	1.184	
NO		1.316			
SN			1.741	2.676	
CO			1.222		
bond angles (deg)					
OSC	168.5				
SCN	179.0	140.1			
NCO			141.3		
CNO		118.2			
OSN				179.4	
SNC			117.8	178.9	
dihedral angle (deg)					
OSCN	0.0				
SCNO		0.0			
SNCO			−0.008		
OSNC				0.0	

to $\mathbf{3}^{-}$ and $\mathbf{5}^{-}$ whereas the triplet is the ground state of $\mathbf{4}^{-}$ by 4.2 kcal mol^{-1} at the level of theory used in this investigation. The relative stabilities of the singlet states of the anions are $^{1}\mathbf{3}^{-} > {}^{1}\mathbf{5}^{-} > {}^{1}\mathbf{1}^{-} > {}^{1}\mathbf{2}^{-} > {}^{1}\mathbf{4}^{-}$. Details of the structures of the anions are recorded in Tables 1 and 2. While it is difficult to represent each species by a single valence bond structure, the closest representative structures are illustrated in Scheme 2. The hypothiocyanite anion $^{1}\mathbf{1}^{-}$ is bent as are $^{1}\mathbf{4}^{-}$ [the triplet ground state is linear (Table 2)] and $^{1}\mathbf{5}^{-}$, while $^{1}\mathbf{2}^{-}$ and $^{1}\mathbf{3}^{-}$ are linear.

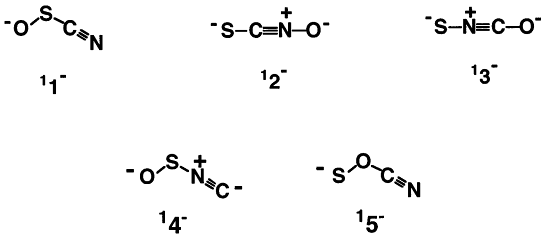
The major questions to be addressed are whether the hypothiocyanite anion is a stable species under the conditions it may be formed in interstellar ice (as well as in the ion source of a mass spectrometer) or can it undergo facile rearrangement to an isomeric anion which may decompose to form NCO^{-} (or CNO^{-}). The singlet state of (OSCN)$^{-}$ ($^{1}\mathbf{1}^{-}$) is 18.1 kcal mol^{-1} more stable than the corresponding triplet, which is an ion−dipole complex formed between SO and $^{-}$CN (Table 2). As a consequence, rearrangements of $^{1}\mathbf{1}^{-}$ are considered first. These have been investigated at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory. The rearrangements are summarized in Figures 1 to 3, with full data provided in Tables 1−3.

The rearrangement of singlet (OSCN)$^{-}$ ($^{1}\mathbf{1}^{-}$) to singlet (SCNO) ($^{1}\mathbf{2}^{-}$) is shown in Figure 1. This rearrangement is exothermic (+12.9 kcal mol^{-1}), but the barrier for dissociation of the cyclic intermediate $^{1}\mathbf{6}^{-}$ is a prohibitive +80 kcal mol^{-1}. The rearrangement $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{4}^{-}$ is endothermic (+21.5 kcal mol^{-1}) with a barrier of 32.3 kcal mol^{-1} (Figure 2).[32] The stepwise rearrangements $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{3}^{-}$ and $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$ (see Figure 2) are exothermic (−20.3 and −2.5 kcal mol^{-1} respectively): both proceed through the intermediacy of $^{1}\mathbf{4}^{-}$. However, the barrier (+57.1 kcal mol^{-1}) for the two reactions is high. The interconversion of $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$ can also occur by a concerted pathway (Figure 3), but again, there is a significant barrier (+ 37.2 kcal mol^{-1}) for this rearrangement.

SCHEME 1

(OSCN)$^{-}$	(SCNO)$^{-}$	(SNCO)$^{-}$	(OSNC)$^{-}$	(SOCN)$^{-}$
$^{1}\mathbf{1}$ (0 kcal mol^{-1})	$^{1}\mathbf{2}$ (12.4)	$^{1}\mathbf{3}$ (-20.3)	$^{1}\mathbf{4}$ (21.5)	$^{1}\mathbf{5}$ (-2.5)
$^{3}\mathbf{1}$ (18.1)	$^{3}\mathbf{2}$ (58.2)	$^{3}\mathbf{3}$ (23.8)	$^{3}\mathbf{4}$ (17.2)	$^{3}\mathbf{5}$ (unstable)

SCHEME 2



$^{1}\mathbf{1}^{-}$ $^{1}\mathbf{2}^{-}$ $^{1}\mathbf{3}^{-}$ $^{1}\mathbf{4}^{-}$ $^{1}\mathbf{5}^{-}$

The energies of the major decomposition pathways of $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$ are shown in Table 4. These are all high energy processes

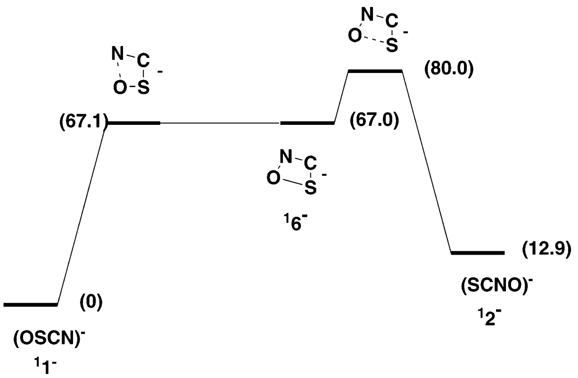


Figure 1. Rearrangement of $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{2}^{-}$. Calculations at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory. Relative energies are given in kcal mol^{-1}.

TABLE 3: Structures and Energies of the Singlet Anion Transition States and Cyclic Minima. Level of Theory Used − CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d). Relative Energies in kcal mol^{-1} with Respect to $^{1}\mathbf{1}^{-}$ (0 kcal mol^{-1})

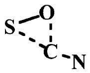
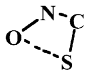
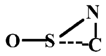
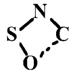
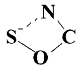
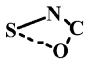
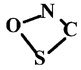
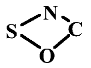
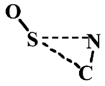
	 **TS $^{1}1^{-}/^{1}5^{-}$**	 **TS $^{1}1^{-}/^{1}6^{-}$**	 **TS $^{1}6^{-}/^{1}2^{-}$**	 **TS $^{1}1^{-}/^{1}4^{-}$**	 **TS $^{1}4^{-}/^{1}7^{-}$**	 **TS $^{1}7^{-}/^{1}5^{-}$**	 **TS $^{1}7^{-}/^{1}3^{-}$**	 **$^{1}6^{-}$**	 **$^{1}7^{-}$**
state	1-A′	1-A′	1-A′	-	1-A′	1-A′	1-A′	1-A′	1-A′
symmetry	C_s	C_s	C_s	C_1	C_s	C_s	C_s	C_s	C_s
energy (hartrees)	−565.32307	−565.27543	−565.25492	−565.33087	−565.29137	−565.30053	−565.29764	−565.27564	−565.30291
energy relative to $^{1}\mathbf{1}^{-}$	+37.2	+67.1	+80.0	+32.3	+57.1	+53.2	+51.4	+67.0	+49.9
dipole moment (D)								3.5066	4.1705
bond lengths (Å)									
OS	1.781	1.750	2.195	1.544	1.673	1.808	2.019	1.761	1.600
SC	1.746	1.740	1.711	2.510				1.825	
CN	1.193	1.277	1.261	1.193	1.271	1.278	1.293	1.304	1.375
CO	1.711				1.935	1.363	1.315		1.664
NO		1.722	1.409					1.514	
SN				2.510	1.691	2.057	1.852		1.690
bond angles (deg)									
OSC	58.0	81.6	66.5	109.9				76.0	
SCN	159.6	97.7	105.0	88.1				92.7	
NCO	138.3				91.0	110.4	112.1		121.4
COS	60.0				79.4	95.0	86.1		63.6
CNO		97.9	109.4					103.0	
NOS		82.7	79.1					88.4	
OSN				115.4	87.9	67.9	67.2		107.5
SNC				63.5	101.7	86.7	94.1		67.5
SCO	62.0			−					
NSC				28.4					
dihedral angle (deg)									
OSCN	180.0	0.0		−106.7					
ONCS			0.0						
OSNC					0.0	0.0	0.0		
SCNO								0.0	
SOCN									0.0

TABLE 4: Dissociations of Single and Triplet Anions. Calculations at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) Level of Theory with Energies in kcal mol^{-1} and EA [Adiabatic Electron Affinity] in kcal mol^{-1} (Energy Required To Remove an Electron from the Anion)[a]

^{1}(OSCN)$^{-}$ ($^{1}1^{-}$)	→	NC^{-} + SO	(+53.7)
(EA, 56.0)	→	O^{-} + NCS	(+99.7)
	→	SCO^{-} + N	(+215.4)
^{1}(SCNO)$^{-}$ ($^{1}2^{-}$)	→	CS^{-} + NO	(+92.4)
(EA, 68.7)	→	O^{-} + NCS	(+86.9)
	→	CNO^{-} + S	(+103.5)
^{1}(SNCO)$^{-}$ ($^{1}3^{-}$)	→	NS^{-} + CO	(+64.3)[b]
(EA, 60.4)	→	O^{-} + CNS	(+150.0)
	→	NCO^{-} + S	(+70.7)[b]
^{1}(OSNC)$^{-}$ ($^{1}4^{-}$)	→	CN^{-} + SO	(+32.2)
(EA, 51.9)	→	O^{-} + CNS	(+108.2)
	→	NSO^{-} + C	(+174.7)
^{1}(SOCN)$^{-}$ ($^{1}5^{-}$)	→	CN^{-} + SO	(+56.2)
(EA, 71.5)	→	NCO− + S	(+52.9)
	→	COS− + N	(+143.9)
^{3}(OSCN)$^{-}$($^{3}1^{-}$)	→	CN^{-} + SO	(+11.9)
^{3}(OSNC)$^{-}$ ($^{3}4^{-}$)	→	CN^{-} + SO	(+12.8)

[a] A reviewer has asked that we compare the electron binding energy of each anion with the dissociation energies. The data listed above show that (i) loss of an electron from $^{1}\mathbf{1}^{-}$ requires 2.3 kcal mol^{-1} more energy than that required for the lowest energy dissociation, (ii) loss of an electron from each of $^{1}\mathbf{2}^{-}$ and $^{1}\mathbf{3}^{-}$ (68.7 and 60.4 kcal mol^{-1} respectively) will compete with the lowest energy dissociation of these anions, and (iii) the electron binding energies of $^{1}\mathbf{4}^{-}$ and $^{1}\mathbf{5}^{-}$ are higher than the lowest energy dissociation of these anions. [b] These values have also been calculated at higher levels of theory (see ref 33).

TABLE 5: Structures and Energies of the Triplet Anion Minima and Transition State (Level of Theory Used = CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d), with Relative Energies in kcal mol^{-1} with Respect to $^{1}\mathbf{1}^{-}$ (0 kcal mol^{-1}))



TS $^{3}1^{-}/^{3}4^{-}$

	TS $^{3}1^{-}/^{3}4^{-}$
state	$^{3}A''$
symmetry	C_S
energy (hartrees)	−565.35222
energy relative to $^{1}1^{-}$	+18.9
bond lengths (Å)	
OS	1.533
SC	3.004
CN	1.183
SN	3.177
bond angles (deg)	
OSC	163.2
SCN	87.3
OSN	175.0
SNC	70.8
CSN	21.8
dihedral angle (deg)	
OSCN	180.0
OSNC	

and indicate that unless significantly energized, the singlet isomers $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$ should be stable to decomposition.

The triplet form of the hypothiocyanite anion ($^{3}\mathbf{1}^{-}$) is an ion−dipole complex (OS···CN)$^{-}$ formed between SO and $^{-}$CN. The S···C distance is 2.81 Å with the angles OSC and SCN being 168 and 179° respectively. The complex $^{3}\mathbf{1}^{-}$ converts to $^{3}\mathbf{4}^{-}$ (OS···NC)$^{-}$ over a barrier of only 0.8 kcal mol^{-1} in a reaction exothermic by 0.9 kcal mol^{-1}. Complex $^{3}\mathbf{4}^{-}$ has an S···N

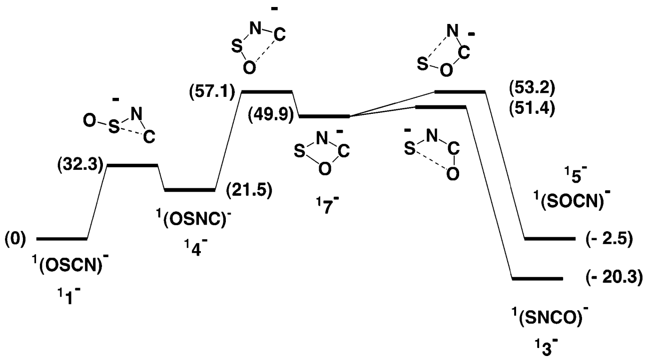


Figure 2. Rearrangements $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{3}^{-}$, $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{4}^{-}$ and $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$. Calculations at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory. Relative energies are given in kcal mol^{-1}.

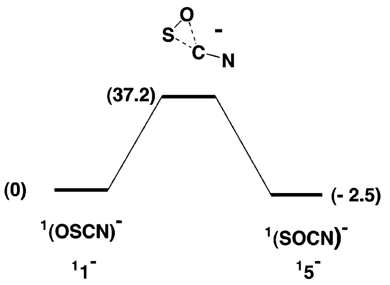
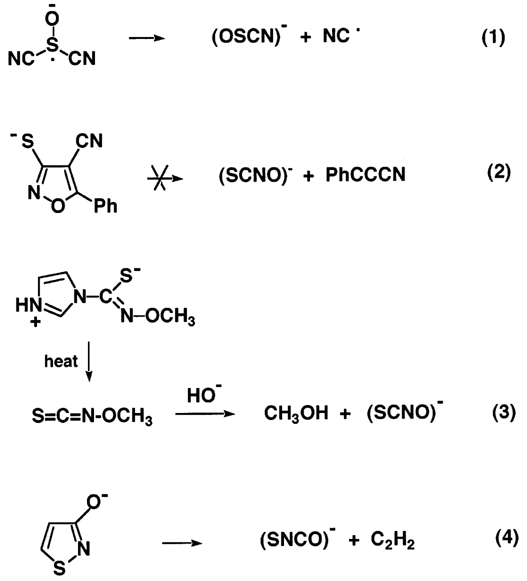


Figure 3. Concerted rearrangement of $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{5}^{-}$. Calculations at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory. Relative energies are given in kcal mol^{-1}.

SCHEME 3



distance of 2.67 Å with the angles OSN and SNC each being 179°. Each of the ion dipole complexes $^{3}\mathbf{1}^{-}$ and $^{3}\mathbf{4}^{-}$ decompose to SO and NC^{-} in facile reactions which require only 11.9 and 12.8 kcal mol^{-1} respectively.

The conclusions from the theoretical part of this study are as follows: (i) the ground-state singlet form of the hypothiocyanite anion is stable, and no facile rearrangement process should produce an isomer which can decompose to either NCO^{-} or CNO^{-}; (ii) the triplet state of (OSCN)$^{-}$ converts readily to triplet (OSNC)$^{-}$, and both triplet anions are complexes of SO and NC^{-} which decompose readily to yield NC^{-} and SO.

Syntheses of the Anions (OSCN)$^{-}$, (SCNO)$^{-}$ and (SNCO)$^{-}$. Theoretical data suggest that the ground states of the isomers $\mathbf{1}^{-}$−$\mathbf{5}^{-}$ should be stable. Having determined this, the next task was to synthesize each of these anions, then to determine their spectra in order to attempt to rationalize the formation of *m/z* 42 [(NCO^{-} or CNO^{-})] in the collision induced spectrum of the oxidation product "OSCN^{-}" reported earlier.[9] The anions $\mathbf{1}^{-}$, $\mathbf{2}^{-}$, and $\mathbf{3}^{-}$ were formed by unequivocal routes: no satisfactory routes to $\mathbf{4}^{-}$ and $\mathbf{5}^{-}$ were found.

The hypothiocyanite anion ($\mathbf{1}^{-}$) was produced (in the source of the mass spectrometer) by electron capture of NCS(O)CN to form the parent radical anion which decomposes by loss of a nitrile radical to form the target anion in high yield (eq 1). To form (SCNO)$^{-}$ ($\mathbf{2}^{-}$) was more challenging. An S$_{N}$2 reaction between HO^{-} and 1-thiomethyl-2-cyano-3-phenylisoxazole in the ion source of the mass spectrometer gave the precursor anion shown in eq 2. This species was expected to undergo retro cleavage (as shown in eq 2) to form (SCNO)$^{-}$. The retro reaction did not occur; instead, the precursor anion lost nitric oxide. The required anion (SCNO)$^{-}$ was formed as follows. Thermolysis of the heterocycle shown in eq 3 gave neutral SCNOCH$_{3}$ as reported by Wentrup et al.[23] The S$_{N}$2 reaction between HO^{-} and SCNOCH$_{3}$ in the ion source of the mass spectrometer gave the required anion as shown in eq 3 (Scheme 3).

The third isomer, (SNCO)$^{-}$ ($\mathbf{3}^{-}$) was made as summarized in eq 4 (Scheme 3). 1-Hydroxyisothiazole was deprotonated in the source of the mass spectrometer using HO^{-} as the base. The collision induced mass spectrum (MS/MS) of this species shows *m/z* 74 [(SNCO)$^{-}$], 88%; 57[(HCCS)$^{-}$], 76%; and 42 [(NCO)$^{-}$], 100%. The required species (SNCO)$^{-}$ (*m/z* 74), is produced by retro cleavage [eq 4 (Scheme 3)]. The other two processes proceed through ion complex [NCO^{-} (HCCHS)] to form (NCO)$^{-}$ (*m/z* 42) and (HC$_{2}$S)$^{-}$ (*m/z* 57).

The Mass Spectra of the Isomers $\mathbf{1}^{-}$, $\mathbf{2}^{-}$, and $\mathbf{3}^{-}$. To confirm the structures of the three isomeric anions synthesized as outlined above, we have measured the collision induced mass spectra (negative ion) and charge reversal ($^{-}$CR^{+}) (positive ion) mass spectra of the three anions. Charge-reversal spectra are obtained by vertical two electron (Franck−Condon) oxidation of an anion in a collision cell following the magnetic sector of the mass spectrometer; the resulting positive ion decompositions (arising from energized parent cations) are measured by scanning the electric sector. A combination of the negative ion and positive ion decompositions of the precursor anion can be used to confirm (or refute) the proposed structure of that anion.

The CID and $^{-}$CR^{+} mass spectra of the hypothiocyanite anion (OSCN)$^{-}$ ($\mathbf{1}^{-}$) are recorded in Figure 4. The corresponding spectra of $\mathbf{2}^{-}$ and $\mathbf{3}^{-}$ are listed in Table 6. The negative ion spectrum of (OSCN)$^{-}$ (Figure 4A) shows fragment anions at *m/z* 58 (SCN)$^{-}$ and 26 (CN)$^{-}$ consistent with bond connectivity OSCN. There is no fragment anion at *m/z* 42 [(CNO)$^{-}$ or (NCO)$^{-}$] in Figure 4A analogous to that reported in the CID spectrum of *m/z* 74 from the oxidation of (SCN)$^{-}$.[cf.9] The $^{-}$CR^{+} spectrum of (OSCN)$^{-}$ (Figure 4B), shows a parent cation at *m/z* 74, with fragment cations 58 (SCN)$^{+}$, 48 (SO$^{+\bullet}$), and 26 (NC)$^{+}$ consistent with a parent cation with connectivity OSCN. These data are consistent with the formation of a stable species (OSCN)$^{-}$ by the process shown in eq 1 (Scheme 3). There are two minor peaks in the $^{-}$CR^{+} spectrum (Figure 4B) which indicate that some of the energized (OSCN)$^{+}$ species can undergo rearrangement to other isomers [e.g. to form *m/z* 42 (CNO)$^{+}$ or (NCO)$^{+}$] and *m/z* 46 (NS)$^{+}$].

The CID spectrum of (SCNO)$^{-}$ ($\mathbf{2}^{-}$) is listed in Table 6. The fragment ions at *m/z* 58 (SCN)$^{-}$, 44 (CS$^{-\bullet}$) and 42 (CNO)$^{-}$ are in accord with fragmentation of an anion with structure (SCNO)$^{-}$. There are no ions in this spectrum that indicate any rearrangement of energized (SCNO)$^{-}$. The $^{-}$CR^{+} spectrum (Table 2) shows major fragment cations at *m/z* 58 (SCN)$^{+}$ and

TABLE 6: CID and $^-$CR$^+$ Spectra of $(SCNO)^-$ and $(SNCO)^-$ [*m/z* (Composition), Relative Abundance]

$(SCNO)^-$	
CID	74 $(SCNO)^-$100 58 $(SCN)^-$ 12; 44 $(CS)^{+\bullet}$ 2, 42 $(CNO)^-$ 9
$^-$CR$^+$	74 $(SCNO)^+$ 95; 58 $(SCN)^+$ 82^a;48 $(SO)^{+\bullet}$ 38; 42 $(CNO)^+$ 100^b, 32 $(S)^{+\bullet}$ 28; 28 $(CO)^{+\bullet}$,6
$(SNCO)^-$	
CID	74 $(SNCO)^-$ 100; 46 $(NS)^-$ 4
$^-$CR$^{+\,(c)}$	74 $(SNCO)^+$ no peak; 58 $(SNC)^+$ 100; 46 $(NS)^{+\bullet}$ 75; 44 $(CS)^{+\bullet}$ 25; 42 $(NCO)^+$ 18; 32 $(S)^{+\bullet}$ 65, 28 $(CO)^{+\bullet}$ 8

[a] This peak is composite, with a large Gaussian peak superimposed on a smaller dish-shaped peak. This means that there are two losses of oxygen from *m/z* 74. This information together with the formation of *m/z* 48 ($SO^{+\bullet}$) and 28 ($CO^{+\bullet}$) indicates some deep seated skeletal reorganization of a minor proportion of energized parent cations. [b] $(CNO)^+$ will rearrange to $(NCO)^+$ under the reaction conditions of this experiment.[10] [c] This spectrum is qualitatively similar to the CID spectrum of $(SNCO)^+$ reported by Flammang et al.[34]

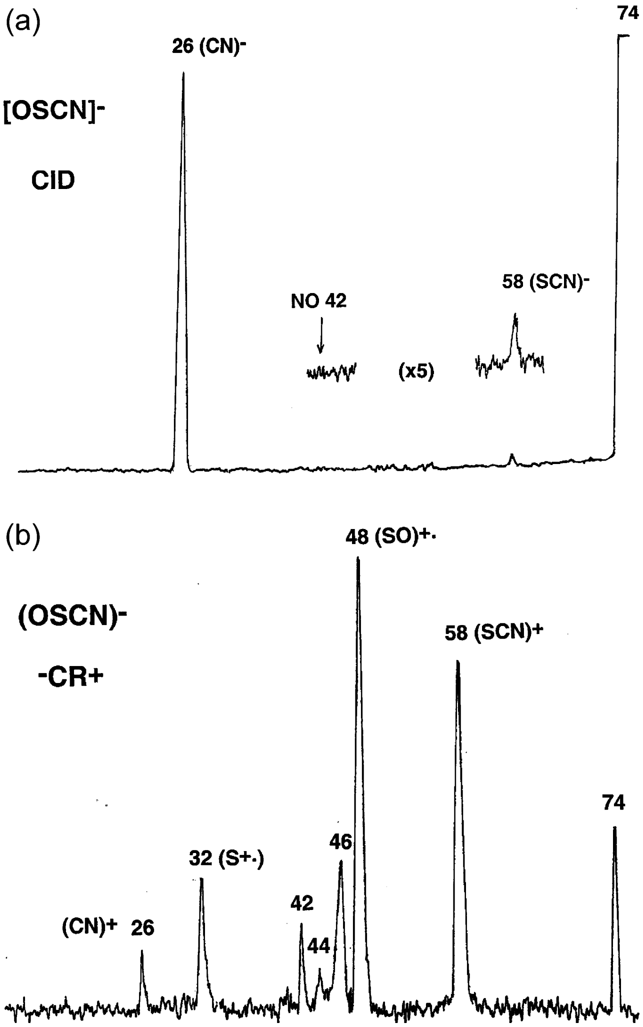


Figure 4. (A) CID spectrum of $(OSCN)^-$. (B) $^-$CR$^+$ spectrum of $(OSCN)^-$. VG ZAB 2HF mass spectrometer. For experimental details, see Experimental Section.

42$(CNO)^+$ consistent with a parent cation of structure $(SCNO)^+$. This spectrum contains smaller peaks at *m/z* 48 $(SO)^{+\bullet}$ and 28 $(CO)^{+\bullet}$ which must be formed following internal rearrangement of energized and decomposing $(SCNO)^+$.

Finally, the CID spectrum of $(SNCO)^-$ (**3**$^-$) shows *m/z* 46 (NS^-) as the sole anion fragment.[34] Since there is no peak at *m/z* 42 (NCO^-) in this spectrum, $(SNCO)^-$ cannot be the precursor of the *m/z* 42 peak in the CID spectrum of oxidized (NCS^-).[9] The $^-$CR$^+$ spectrum shown in Table 6 is consistent with the structure of the precursor anion being $(SNCO)^-$.

In summary:

(i) Theoretical calculations indicate that the singlet forms of the isomeric anions $(OSCN)^-$ (**1**$^-$), $(SCNO)^-$ (**2**$^-$), $(SNCO)^-$ (**3**$^-$) and $(SOCN)^-$ (**5**$^-$) are stable species.

(ii) Three of these anions, **1**$^-$, **2**$^-$ and **3**$^-$ have been produced in the source of the ZAB mass spectrometer by unequivocal synthetic routes.

(iii) A combination of CID and $^-$CR$^+$ spectra confirm that the three anions do not undergo rearrangement of the skeleton when energized.

(iv) The CID spectra of (OSCN) (**1**$^-$) and (SNCO) (**3**$^-$) show no fragment peaks at *m/z* 42 [$(CNO)^-$ or $(NCO)^-$], while that of (SCNO) (**2**$^-$) does exhibit a peak at *m/z* 42 (CNO^-).

From point i, a consideration of the spectroscopic data given previously,[9] and point ii, the theoretical and experimental evidence provided by the present study, it is concluded that oxidation of $(SCN)^-$ by peroxide gives two isomeric product anions. The major component is the hypothiocyanite anion $(OSCN)^-$. The presented data suggest that the minor component could be $(SCNO)^-$.

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(32) Although the process $^{1}\mathbf{1}^{-}$ to $^{1}\mathbf{4}^{-}$ is energetically unfavorable, it has the lowest barrier of the rearrangements shown in Figures 1 to 3. Even if the rearrangement does occur, the product $(OSNC)^{-}$ cannot be the origin of the *m*/*z* 42 anion observed in the spectrum of "$[(OSCN)^{-}]$". Product $^{1}\mathbf{4}^{-}$ is formed with excess energy of 32.3 kcal mol^{-1}, which is very close to that required to effect decomposition to SO and $^{-}$CN (32.2 kcal mol^{-1}, see Table 4). In addition, $^{1}\mathbf{4}^{-}$ is not the ground state of $(OSNC)^{-}$ (Scheme 1). If spin crossing forms $^{3}\mathbf{4}^{-}$, this anion will have enough energy to cause cleavage to SO and $-$CN (12.8 kcal mol^{-1}; Table 4).

(33) The results of calculations shown in Table 4 indicate that the process forming NS^{-} is only 6.4 kcal mol^{-1} lower in energy than that forming (NCO^{-}) [at the CCSD(T)/aug-cc-pVDZ//B3LYP/6-31+G(d) level of theory]. The expectation is that both decomposition processes should be observed, however experimentally, the formation of the lower energy decomposition (formation of NS^{-} and CO) is the only process detected. The two processes have been investigated at other levels of theory and provide results consistent with those recorded in Table 4:- namely, at (A) CCSD(T)/aug-cc-pVTZ//MP2/6-31+G(d,p) and (B) MP4SDTQ/aug-cc-pVDZ//MP2/6-31+G(d,p) levels of theory $^{1}(SNCO)^{-} \rightarrow NS^{-} + CO$ [(A) + 69.3 kcal mol^{-1}; (B) + 68.1 kcal mol^{-1}] $\rightarrow NCO^{-} + S$ [(A) + 76.3 kcal mol^{-1}; (B) + 78.8 kcal mol^{-1}]. There is also an added complication in this system that the energy necessary to remove an electron from $SNCO^{-}$ is 4 kcal mol^{-1} lower than that required to effect dissociation to NS^{-} and CO. Thus electron detachment and the formation of NS^{-} should be competitive processes.

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