Ab Initio Study of Nuclear Magnetic Shieldings and Ultraviolet Spectra for Hypothiocyanite and Its Isomers. The Molecular Structure of Hypothiocyanite

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Abstract: The ^{13}C, ^{15}N, ^{17}O, and ^{33}S chemical shieldings for three optimized structures of hypothiocyanite have been calculated at the SCF, MP2, and CCSD levels using the gauge-including atomic-orbital approach. The first few excitation energies for the corresponding hypothiocyanous acids have been obtained using the equation-of-motion CCSD and the CI singles methods. Comparison of the calculated ^{15}N chemical shifts and of the excitation spectra with experimental data suggests that hypothiocyanite has a bent $OSCN^-$ structure with C_s symmetry, even though the linear $SNCO^-$ isomer is 86 kJ/mol lower in energy. The linear $ONCS^-$ structure is 146 kJ/mol above $SNCO^-$. No other low-lying structures were found.

1. Introduction

The peroxidase-catalyzed oxidation of SCN^- by H_2O_2 results in the formation of a chemical species with antimicrobial activity.[1-5] Several different species such as cyanide (CN^-),[6] thiocyanogen ($(SCN)_2$),[7] cyanosulfurous acid (HO_2SCN) or cyanosulfuric acid (HO_3SCN),[8] and hypothiocyanite ($OSCN^-$) or hypothiocyanous acid (HOSCN)[2-4] have been suggested to be responsible for the antimicrobial action. The peroxidase-catalyzed oxidation reaction of SCN^- by H_2O_2 has been extensively studied experimentally and several chemical methods to characterizing the product have been reported.[2,3,9-12] The major product of the SCN^- oxidation has been identified to be the hypothiocyanite anion ($OSCN^-$) or the hypothiocyanous acid (HOSCN).[2-4,13,14] The molecule is unstable at higher concentrations and its chemical properties are not well-known. The same oxidation product ($HOSCN/OSCN^-$) is also obtained by the oxidation of $(SCN)_2$ with water.[2] However, the oxidation of KSCN in aqueous solutions by electrolysis or by bromine yields another species which is much more stable than hypothiocyanite and hypothiocyanous acid ($HOSCN/OSCN^-$).[2]

The ultraviolet (UV) spectra for $HOSCN/OSCN^-$ in aqueous solutions has been measured, and the extinction coefficients for $OSCN^-$ and HOSCN are known.[8,14-16] The ^{15}N chemical shift for $HOSCN/OSCN^-$ has recently been measured in a phosphate buffer solution in the presence of lactoperoxidase.[14]

Different linear isomers of the hypothiocyanite anion[17] and of the corresponding hypothiocyanous acid[18] have been studied using Hartree–Fock (SCF) and second-order Møller–Plesset (MP2) calculations. In the *ab initio* study the lowest energy was found for the linear $SNCO^-$ isomer. At the MP2 level, the linear $ONCS^-$ structure was found to be 145 kJ/mol above $SNCO^-$, and no stable structure was found for the linear $OSCN^-$ structure. The MP2 calculations provided two plausible structures for hypothiocyanite, but it was not possible to judge from the calculations which isomer is generated in the peroxidase-catalyzed oxidation reaction of SCN^-. In the *ab initio* study no hypothiocyanite isomers with nonlinear structures were considered.[17]

Since the ^{15}N chemical shift has been measured for $OSCN^-$, in the presence of lactoperoxidase in a phosphate buffer solution,[14] the molecular structure of $OSCN^-$ may be determined by comparing experimental chemical shieldings with the chemical shieldings calculated for a few plausible molecular structures. Comparison of the calculated excitation energies for the acid form of the isomers with experimental data supports the determination of the molecular structure of the oxidation product.

In this work, two linear structures ($SNCO^-$ and $ONCS^-$) and one bent structure ($OSCN^-$ with C_1 symmetry) of the hypothiocyanite anion, and the corresponding structures of the hypothiocyanous acid (HSNCO (C_s), HONCS (C_s), and HOSCN (C_1)) have been studied by means of large scale *ab initio* calculations. The molecular structures have been optimized at the MP2 level using three different basis sets. The ^{13}C, ^{15}N, ^{17}O, and ^{33}S chemical shieldings have been calculated at the SCF, MP2, and

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coupled-cluster (CCSD) levels[19–23] using the gauge-including atomic-orbital method (GIAO). Transition energies to the few lowest excited states have been calculated at the coupled-cluster level using the equation-of-motion coupled-cluster method (EOM-CCSD).[24]

2. Computational Methods

In this study, the fully optimized Gaussian basis sets of references 25 and 26 have been used. The Karlsruhe basis sets which have been optimized at the self-consistent-field (SCF) level were augmented by polarization functions. The employed basis sets are the double-ζ basis set plus one polarization function (DZP), the triple-ζ basis set plus two polarization functions (TZ2P), and the triple-ζ valence basis set plus two d-functions and one f-function for C, N, O, and S, and two p-function and one d-function for H (TZVPP). The exponents of the polarization functions of the DZP basis sets are 0.80 for H, 0.80 for C, 1.00 for N, 1.20 for O, and 0.55 for S. The exponents of the polarization functions of the TZ2P basis sets were obtained by dividing and multiplying the exponent of the polarization function of the DZP basis set by $\sqrt{3}$. The polarization functions of the TZ2P basis sets have the following exponents: 0.46 and 1.39 for H, 0.46 and 1.39 for C, 0.58 and 1.73 for N, 0.69 and 2.08 for O, and 0.32 and 0.95 for S. The polarization functions of the TZVPP basis set are the polarization functions of Dunning's correlation consistent basis sets (cc-p-VTZ).[27,28] Due to program restrictions in the nuclear magnetic resonance (NMR) chemical shift program, six cartesian d-functions have been used in the calculation of the chemical shieldings. The MP2 DZP and MP2 TZ2P geometry optimizations and the CCSD calculations were for consistency also performed using six d-functions. In the MP2 geometry optimizations using the TZVPP basis sets only the true spherical d- and f-components were included in the basis set.

Correlation effects have been considered by using the second-order Møller–Plesset perturbation theory (MP2) method, the coupled-cluster method with singles and doubles excitations (CCSD),[29] and the CCSD method with perturbative triple excitations (CCSD(T)).[30,31] The excitation energies have been obtained using the equation-of-motion CCSD method (EOM-CCSD)[24] and the configuration interaction singles (CIS) method (or the Tamm–Dancoff approximation).[32]

The ^{13}C, ^{15}N, ^{17}O, and ^{33}S chemical shieldings have been calculated at the SCF, MP2,[19–21] and CCSD[22,23] levels using the gauge-including atomic-orbital method (GIAO) and the MP2 equilibrium geometries. The DZP and TZ2P chemical shielding calculations, the coupled-cluster (CCSD and CCSD(T)) calculations, the equation-of-motion coupled-cluster (EOM-CCSD), and the configuration interaction singles (CIS) calculations were performed using the *ACES II* program package.[33] The MP2 TZVPP geometry optimizations and also CIS calculations were performed using the *TURBOMOLE* program package.[34]

Table 1. Optimized MP2 Geometries (Bond Distances, *R*, in pm and Angles, *a*, in deg) for the ABCD Isomers of the Hypothiocyanite Anion and the Hypothiocyanous Acids (HABCD) Using Different Basis Sets

molecule	basis set	*R*(AB)	*R*(BC)	*R*(CD)	*a*(ABC)	*a*(BCD)
OSCN$^-$ [a]	DZP	157.68	173.70	118.87	111.13	176.31
	TZ2P	156.14	172.70	118.03	110.48	177.25
	TZVPP	155.99	171.42	118.34	109.72	177.76
ONCS$^-$ [b]	DZP	124.57	119.01	166.89		
	TZ2P	125.19	118.00	166.66		
	TZVPP	124.83	118.12	165.95		
SNCO$^-$ [b]	DZP	171.72	118.65	121.78		
	TZ2P	170.51	117.96	121.61		
	TZVPP	169.62	118.08	121.67		
HOSCN	DZP[c]	167.92	170.63	117.94	100.45	177.74
	TZ2P[d]	166.52	170.14	117.07	100.97	177.82
	TZVPP[e]	165.84	169.52	117.33	100.97	178.03
HONCS[a]	TZVPP[f]	139.10	122.38	156.26	123.17	171.57
HSNCO[a]	TZVPP[g]	169.74	121.47	117.39	129.98	173.20

[a] The molecule has C_s symmetry. [b] The molecule is linear. [c] The molecule has C_1 symmetry. R(HO) = 97.13 pm, a(HOS) = 106.09 deg, and the two dihedral angles a(HAB,ABC) = 86.15 and a(AB-C,BCD) = −167.45 deg. [d] The molecule has C_1 symmetry. R(HO) = 96.43 pm, a(HOS) = 106.58 deg, and the two dihedral angles a(HAB,ABC) = 85.98 and a(ABC,BCD) = −171.63 deg. [e] The molecule has C_1 symmetry. R(HO) = 96.52 pm, a(HOS) = 106.86 deg, and the two dihedral angles a(HAB,ABC) = 85.87 and a(AB-C,BCD) = −171.09 deg. [f] The molecule has C_s symmetry. R(HO) = 96.27 pm, a(HON) = 101.69 deg. [g] The molecule has C_s symmetry. R(HS) = 133.26 pm, a(HSN) = 94.15 deg.

3. Results and Discussion

3.1. Geometries and Energies. The equilibrium molecular structures have been optimized at the MP2 level using the DZP, TZ2P, and TZVPP basis sets. The optimized geometries are given in Table 1. The ONCS$^-$ and SNCO$^-$ molecules are linear, and the corresponding acids have C_s symmetry. The OSCN$^-$ isomer has C_s symmetry, and its acid form has C_1 symmetry. For the anions, the equilibrium bond lengths obtained in the MP2 TZ2P calculations are 0.23–1.54 pm shorter than those of the MP2 DZP structures. The only exception is R(NO) in ONCS$^-$ which is 0.62 pm longer at the MP2 TZ2P level. The largest difference in the bond lengths of the optimized MP2 DZP and MP2 TZ2P structures (1.54 pm) was found for R(SO) in OSCN$^-$. The largest difference in the bond angles (1.16 deg) of the DZP and TZ2P calculations was found for a(SCN) in OSCN$^-$. By adding f-functions to the basis set the sulfur bonds become slightly shorter. Comparison of the TZ2P and TZVPP structures shows that the R(SC) bond in bent OSCN$^-$ is 1.28 pm shorter for the TZVPP geometry. For SNCO$^-$, the R(SN) bond is 0.91 pm shorter, and for ONCS$^-$ the R(SC) bond is 0.71 pm shorter in the MP2 TZVPP calculation. The other bonds and angles are not much affected by the addition of f-functions to the basis sets. The R(SO) bond for OSCN$^-$ is about 10 ppm shorter than for HOSCN, and the R(NO) bond in ONCS$^-$ is about 15 pm shorter than for HONCS. This can

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Table 2. Calculated Vibrational Frequencies (in cm^{-1}) for Three Structures of the Hypothiocyanite Anion and for One Structure of the Hypothiocyanous Acid[e]

molecule	ν_1	ν_2	ν_3	ν_4	ν_5	ν_6
$OSCN^-$ [a]	183	367	464	595	942	2053
HOSCN[b]	184	329	459	475	693	785
$ONCS^-$ [c]	201	331	622	1272	2230	
$ONCS^-$ [c,d]	264	556	609	1152	2618	
$SNCO^-$ [c]	93	495	534	1349	2444	
$SNCO^-$ [c,d]	123	535	653	1496	2622	

[a] The irreducible representations of the vibrations are A′, A″, A′, A′, A′, and A′, respectively. [b] ν_7 = 1201 cm^{-1}, ν_8 = 2169 cm^{-1}, and ν_9 = 3791 cm^{-1}. [c] The irreducible representations of the vibrations are π, π, σ, σ, and σ, respectively. [d] Vibrational frequencies calculated at HF level using 6-31G* basis set.[17] [e] The vibrational frequencies are obtained at the MP2 level using the DZP basis set.

be explained by the presence of some double-bond character in the R(SO) and R(NO) bonds of the anions.

Since the vibrational frequencies calculated at the MP2 DZP level were all real, the obtained structures are local or global minima. The calculated vibrational frequencies are given in Table 2. The total energies of the three structures for the hypothiocyanite anion and for the corresponding hypothiocyanous acids are given in Table 3, and the relative energies with respect to the linear $SNCO^-$ isomer are given in Table 4. The energies have been calculated for the optimized MP2 geometries given in Table 1. As seen in Table 3, the lowest energy is obtained for the linear $SNCO^-$ structure. Since the $SNCO^-$ isomer has C and N interchanged as compared to SCN^-, one may ask whether lactoperoxidase is able to isomerize thiocyanate to the lowest isomer structure or one of the higher-lying structures is generated. At the CCSD TZ2P level the linear $ONCS^-$ isomer is 158 kJ/mol above $SNCO^-$, and the bent $OSCN^-$ is 101 kJ/mol above $SNCO^-$. The effect of triple excitations is estimated by performing CCSD(T) calculations using the DZP basis set. The corrections for triple excitations are −8 and −3.5 kJ/mol for $ONCS^-$ and $OSCN^-$, respectively. The addition of f-functions to the basis sets decreases the energy differences between the $SNCO^-$ and $OSCN^-$ structures and between the $SNCO^-$ and $ONCS^-$ structures by 12.1 and 4 kJ/mol, respectively. By adding the triple excitation contribution and the f-function contribution to the CCSD TZ2P energies, the extrapolated CCSD(T) TZVPP energies of 86 and 146 kJ/mol relative to $SNCO^-$ are obtained for $OSCN^-$ and $ONCS^-$, respectively. Pyykkö and Runeberg[17] obtained also the lowest energy for the $SNCO^-$ structure. In their MP2 6-31G* calculations, the linear $ONCS^-$ was 145 kJ/mol above $SNCO^-$. However, they did not find any stable structure for $OSCN^-$.

The proton affinity for the bent $OSCN^-$ structure calculated at the MP2 TZ2P level is 1462 kJ/mol. By including f-functions in the basis sets the obtained proton affinity calculated at the MP2 level becomes 1439 kJ/mol. The proton affinities obtained at the MP2 DZP, CCSD DZP, and CCSD(T) DZP levels are 1518, 1530, and 1528 kJ/mol, respectively, yielding an extrapolated CCSD(T) TZVPP value of 1452 kJ/mol for the proton affinity of $OSCN^-$. The proton affinities for $ONCS^-$ (HONCS) and $SNCO^-$ (HSNCO) calculated at the MP2 TZVPP level are 1381 and 1417 kJ/mol, respectively. At the SCF level SNCOH is about 50 kJ/mol above HSNCO.

The dissociation limit for $SO(^3\Sigma) + CN^-(^1\Sigma)$ obtained at the MP2 TZ2P level is 258 kJ/mol above $SNCO^-$, and the dissociation to $SO(^1\Sigma) + CN^-(^1\Sigma)$ is further about 100 kJ/mol higher up in energy. The bent $OSCN^-$ is thus about 150 and 250 kJ/mol below the dissociation limits.

3.2. The ^{15}N Chemical Shieldings. The experimental values for the ^{15}N chemical shifts in the presence of 0.1 μM lactoperoxidase in a 0.1 M phosphate buffer solution (pH = 6.1) are −128, −170, and −179 ppm for CN^-, SCN^-, and $OSCN^-$, respectively.[14] An external NO_3^- aqueous solution was used as reference. The ^{15}N chemical shift for a pure 0.3 M NO_3^- aqueous solution has been measured to be −3.55 ppm[35] with respect to neat CH_3NO_2. Since the absolute chemical shielding for neat CH_3NO_2 is −135.8 ppm,[36] the experimental ^{15}N chemical shieldings for CN^-, SCN^-, and $OSCN^-$ in the phosphate buffer solution are −4.3, 37.7, and 46.7 ppm, respectively.

Witanowski et al.[35] have measured the ^{15}N chemical shifts for 0.3 M aqueous solutions of KCN and KSCN with CH_3NO_2 as reference. They obtained experimental values of −106.11 ppm for KCN and −174.07 ppm for KSCN which correspond to absolute chemical shieldings of −29.7 and 38.3 ppm for KCN and KSCN, respectively. Howarth et al.[37] and Vaes et al.[38] have reported ^{14}N and ^{15}N chemical shifts of −166 and −159.5 ppm for SCN^- with respect to a NO_3^- reference.

The values for the ^{15}N chemical shielding for SCN^- in the aqueous solution and for SCN^- in the phosphate buffer solution agree rather well. However, the ^{15}N chemical shielding of −29.7 ppm for an aqueous solution of KCN obtained by Witanowski et al.[35] is 25 ppm smaller than the ^{15}N chemical shielding for CN^- in the phosphate buffer solution.[14] Cyanide is known to bind to iron in lactoperoxidase, but the ^{15}N resonance of the lactoperoxidase-cyanide complex has been found at +423 ppm relative to NO_3^-,[39] so the ^{15}N chemical shielding for CN^- must be shifted 25 ppm by the presence of the phosphate buffer. The ^{15}N chemical shielding for SCN^- is almost unaffected by the presence of the phosphate buffer. The shift of the ^{15}N chemical shieldings for $HOSCN/OSCN^-$ introduced by the presence of the phosphate buffer may also be a few tens of ppm.

Table 3. Energies (in au) of the Three Structures of the Hypothiocyanite Anion and of the Corresponding Structure of the Hypothiocyanous Acids as a Function of the Size of the Basis Set and of the Level of Correlation[a]

molecule	basis set	SCF	MP2	CCSD	CCSD(T)
$OSCN^-$	DZP	−564.624 840	−565.380 127	−565.391 914	−565.418408
	TZ2P	−564.739 035	−565.616 238	−565.619 822	
	TZVPP	−564.760 175	−565.667 396		
$ONCS^-$	DZP	−564.611 913	−565.377 509	−565.383 007	−565.411085
	TZ2P	−564.713 466	−565.599 610	−565.598 204	
	TZVPP	−564.731 211	−565.647 667		
$SNCO^-$	DZP	−564.687 587	−565.433 413	−565.444 464	−565.469 627
	TZ2P	−564.787 845	−565.654 187	−565.658 435	
	TZVPP	−564.804 560	−565.700 732		
HOSCN	DZP	−565.197 654	−565.957 854	−565.975 464	−566.000868
	TZ2P	−565.300 247	−566.173 005		
	TZVPP	−565.316 214	−566.215 600		
HONCS	TZVPP	565.267 020	−566.173 774		
HSNCO	TZVPP	−565.347 509	−566.240 297		

[a] The molecular structures are optimized at the MP2 level.

Table 4. Relative Energies (in kJ/mol)[a] of the Three Structures of the Hypothiocyanite Anion and of One Structure of the Hypothiocyanous Acid as a Function of the Size of the Basis Set and of the Level of Correlation[b]

molecule	basis set	SCF	MP2	CCSD	CCSD(T)
$OSCN^-$	DZP	164.7	139.9	138.0	134.5
	TZ2P	128.2	99.6	101.4	
	TZVPP	116.5	87.5		
$ONCS^-$	DZP	198.7	146.8	161.4	153.7
	TZ2P	195.3	143.3	158.1	
	TZVPP	192.6	139.3		
HOSCN	DZP	−1339.2	−1376.9	−1394.1	−1394.8
	TZ2P	−1345.3	−1362.2		
	TZVPP	−1343.3	−1351.8		

[a] 1 au = 2625.5 kJ/mol. [b] The molecular structures are optimized at the MP2 level. The energies of the linear $SNCO^-$ isomer are used as references.

Table 5. Comparison of Calculated and Experimental ^{15}N Chemical Shieldings with Previously Calculated and Experimental Results (in ppm)

molecule	method	$\sigma(^{15}N)$	ref
CN^-	SCF TZ2P[a]	−71.8	PW
	MP2 TZ2P[a]	−16.4	PW
	CCSD TZ2P[a]	−31.0	PW
	experimental[c,d]	−4.3	14
	experimental[c,e]	−29.7	35
HCN	SCF TZ2P[a]	−46.4	PW
	MP2 TZ2P[a]	5.9	PW
	MP2 PZ3D2F[b]	−0.3	19−21
	CCSD PZ3D2F[b]	−16.7	23
	experimental[c]	−20.4	36
NH_3	SCF TZ2P[a]	268.3	PW
	MP2 TZ2P[a]	281.9	PW
	MP2 PZ3S2F[b]	276.5	19−21
	CCSD PZ3D2F[b]	269.7	23
	experimental[c]	264.5	40
SCN^-	SCF TZ2P[a]	10.2	PW
	MP2 TZ2P[a]	56.0	PW
	CCSD TZ2P[a]	39.2	PW
	experimental[c,d]	37.7	14
	experimental[c,e]	38.3	35

[a] The molecular structures are optimized at the MP2 level. [b] The experimental structures are used. [c] Uncorrected for rovibrational motions. For rovibrational corrections see ref 21 and references therein. [d] The chemical shift is measured in a 0.1 M phosphate buffer solution in the presence of 0.1 μM lactoperoxidase at pH = 6.1. [e] Measured in a 0.3 M KCN aqueous solution.

Table 6. Calculated Chemical Shieldings for the Hypothiocyanite Anions and for the Hypothiocyanous Acid as a Function of the Level of Correlation and the Size of the Basis Set (in ppm)

molecule	calculation[a]	$\sigma(^{13}C)$	$\sigma(^{15}N)$	$\sigma(^{17}O)$	$\sigma(^{33}S)$
$OSCN^-$	SCF DZP[b]	59.4	−68.7	447.0	301.8
	MP2 DZP[b]	67.2	34.3	483.1	272.2
	CCSD DZP[b]	70.6	−12.0	453.5	272.2
	SCF TZ2P[b]	53.1	−86.7	468.6	271.2
	MP2 TZ2P[b]	51.3	3.2	516.2	232.5
	CCSD TZ2P[b]	56.9	−39.9	481.1	255.8
	CCSD TZ2P[c]	55.2	−42.0	463.0	244.0
HOSCN	SCF DZP[d]	88.5	−117.1	350.4	178.4
	MP2 DZP[d]	83.8	26.9	369.9	179.3
$ONCS^-$	SCF DZP[e]	123.4	78.5	221.4	797.3
	MP2 DZP[e]	157.3	113.9	247.5	830.6
	SCF TZ2P[e]	118.2	67.9	218.2	809.5
	MP2 TZ2P[e]	149.4	93.6	241.4	840.6
$SNCO^-$	SCF DZP[e]	78.4	232.8	273.3	159.1
	MP2 DZP[e]	98.7	253.2	288.8	255.4
	SCF TZ2P[e]	63.6	234.7	269.6	223.0
	MP2 TZ2P[e]	80.1	252.2	281.9	309.3
$HOSCN/OSCN^-$	experiment[f]		46.7		

[a] The molecular structures are optimized at the MP2 level. [b] The molecule has C_s symmetry. [c] Calculated in the DZP geometry using TZ2P basis set. [d] The proton shieldings are 28.8 and 29.6 ppm as obtained in the SCF DZP and MP2 DZP calculations, respectively. [e] The molecule is linear. [f] The measured chemical shielding for hypothiocyanite and hypothiocyanous acid. See text.

In Table 5, the calculated absolute chemical shieldings for CN^-, HCN, NH_3, and SCN^- are compared to previously calculated[19−21,23] and measured[14,35,36,40] values. The basis set truncation errors are about 6 ppm which can be estimated by comparing the MP2 TZ2P and MP2 PZ3D2F values for the ^{15}N chemical shieldings of HCN and NH_3.[19−21,23] The geometrical and rovibrational corrections to the calculated chemical shieldings are of the same order of magnitude.[21] The triple and higher-order excitations which are neglected in the present calculations may also contribute to the chemical shieldings. However, the agreement between the MP3, MP4, and CCSD values for the ^{15}N chemical shieldings of HCN[21−23] and the close agreement with experiment for the ^{15}N chemical shieldings of the molecules in Table 5 show that no large corrections due to higher order excitations can be expected for the CCSD ^{15}N chemical shielding of $OSCN^-$.

Judging from the data in Tables 5 and 6, the uncertainty in the CCSD ^{15}N chemical shielding for $OSCN^-$ is less than 20 ppm. The corrections due to vibrational motions may be several ppm. The ^{15}N chemical shieldings obtained using the TZ2P basis sets and the DZP and TZ2P structures differ by only 2 ppm. This shows that the ^{15}N chemical shielding for $OSCN^-$ is not very sensitive to nuclear displacements. However, for comparison with experiment the largest uncertainty is introduced by the presence of the buffer solution.

For the molecules studied here, the ^{15}N chemical shieldings obtained at the SCF level are too small, and the correlation corrections are overestimated at the MP2 level. See Tables 5 and 6. An extrapolated value for the ^{15}N chemical shieldings may therefore be obtained by subtracting one-third of the correlation correction obtained at the MP2 level from the MP2 chemical shielding. See, for example, the tables in refs 20 and 22. The extrapolated ^{15}N chemical shieldings for $ONCS^-$ and $SNCO^-$ become 85 and 246 ppm, respectively.

Remarkable is that for the ^{15}N chemical shielding of CN^-, the medium shift is 25 ppm upfield, but for SCN^- there is almost no shift due to the presence of the lactoperoxidase and phosphate solution. The solvent induced shifts of the ^{15}N resonances correlate with the hydrogen-bonding capacity of the nitrogen lone-pair electrons.[41] For hydrogen-bonding solvents, the ^{15}N resonance is shifted upfields.[42] For the bent $OSCN^-$ structure, the difference between the CCSD TZ2P value for the ^{15}N chemical shielding and the experimental result is 86 ppm. Assuming that the hypothiocyanite anion possesses the bent structure, the medium shift would in this case be in the same direction as for CN^- (upfield), and about three times larger than for CN^-.

Assuming a linear $ONCS^-$ structure, the calculated ^{15}N chemical shielding would be about 40 ppm larger than the experimental value, and the shift due to the buffer solution would for $ONCS^-$ be downfield, i.e., in the opposite direction as compared to CN^-.

The extrapolated value for the ^{15}N chemical shielding for $SNCO^-$ is about 200 ppm larger than the experimental result. This discrepancy is too large to be explained by solvent and vibrational effects, and one may conclude that the $SNCO^-$ structure is not produced in the lactoperoxidase oxidation reaction.

The solvent induced shifts of the ^{15}N resonances were estimated at the SCF level using DZP basis set. The structures

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of HSNCO, HONCS, and HOSCN surrounded by six water molecules were optimized at the SCF level. In the optimization all structural parameters were optimized. The solvent induced shifts were estimated as the difference in the chemical shieldings obtained with and without the water molecules but with unrelaxed molecular structure. The obtained solvent shifts for HSNCO, HONCS, and HOSCN were −9, −5, and +27 ppm, respectively. For HOSCN, the obtained solvent induced shift reduces the discrepancy between the calculated and measured chemical shieldings by 27 ppm which is about one-third of the discrepancy between the calculated and experimental values. The shift due to the presence of the phosphate buffer is not considered in the calculation. For HSNCO and HONCS, which have nitrogen in the middle of the molecule, the solvent induced shifts were found to be significantly smaller.

The ^{15}N chemical shieldings for HOSCN and OSCN$^-$ obtained at the MP2 DZP level are quite similar. In the measurement, Modi et al.[14] could not find any difference in the ^{15}N chemical shifts for OSCN$^-$ and HOSCN. Since the pK_s value is 5.3 for the hypothiocyanous acid,[4] both forms are present at pH = 6.1. They did not find any pH dependency of the position of the ^{15}N resonance which shows that the ^{15}N chemical shieldings for the acid and base forms are equal. The nitrogen environment is quite similar for the hypothiocyanite anion and for the hypothiocyanous acid. The protonation of OSCN$^-$ does not significantly affect the SCN structure. This is supported by the present calculations, see Table 1.

3.3. The ^{13}C, ^{17}O, and ^{33}S Chemical Shieldings. The ^{13}C, ^{17}O, and ^{33}S chemical shieldings have been calculated for the three hypothiocyanite structures and for the bent structure of the hypothiocyanous acid at the SCF and MP2 levels using DZP and TZ2P basis sets. The chemical shieldings of the bent hypothiocyanite structure has also been performed at the CCSD level. The results are summarized in Table 6. The ^{13}C, ^{17}O, and ^{33}S chemical shieldings have not been measured for hypothiocyanite.

Comparison of the ^{13}C chemical shieldings shows that at least the ONCS$^-$ structure would be distinguished from the others by measuring the ^{13}C NMR shifts. The ^{13}C chemical shielding for ONCS$^-$ is at the MP2 TZ2P level 149 ppm. The corresponding values for OSCN$^-$ and SNCO$^-$ are 51 and 80 ppm, respectively. The solvent shifts for the ^{13}C shieldings are in general smaller than for the ^{15}N shieldings,[43] and the OSCN$^-$ and SNCO$^-$ structures could probably be identified by ^{13}C NMR spectroscopy. The solvent induced shifts for ^{13}C estimated at the SCF level are −1, +3, and −5 ppm for HSNCO, HONCS, and HOSCN, respectively. The protonation shifts for the ^{13}C chemical shieldings calculated at the MP2 DZP level is 17 ppm, and a pH dependency of the ^{13}C chemical shielding would probably be observed.

The ^{13}C chemical shielding for CN$^-$ (R(CN) = 118.43 pm) calculated at the MP2 TZ2P level is 34.2 ppm, and for the SCN$^-$ (R(CS) = 166.28 pm and R(NC) = 118.40 pm) it is 68.3 ppm. At the CCSD TZ2P level they are 24.9 and 59.4 ppm, respectively.

The relative ^{13}C chemical shifts with respect to tetramethylsilane (TMS) may be obtained by comparing chemical shieldings calculated at the same level of approximation and experimental results. The experimental value for the ^{13}C chemical shielding of TMS is 188.1 ppm.[43] The experimental ^{13}C chemical shift of SCN$^-$ with TMS as reference is 134.0 ppm[44] yielding an experimental ^{13}C chemical shielding of 54.1 ppm for SCN$^-$. The TMS and SCN$^-$ ^{13}C chemical shieldings are not corrected for vibrational motion. The ^{13}C chemical shifts for OSCN$^-$, HOSCN, ONCS$^-$, and SNCO$^-$, with respect to TMS obtained by comparing the ^{13}C chemical shieldings calculated at the MP2 TZ2P and MP2 DZP levels, are 151.0, 134.4, 52.9, and 122.2 ppm, respectively.

The ^{17}O chemical shieldings for OSCN$^-$, ONCS$^-$, and SNCO$^-$ calculated at the MP2 TZ2P level are 516, 241, and 282 ppm, respectively. The OSCN$^-$ structure would be distinguished from the others by measuring the ^{17}O NMR spectra. The ^{17}O chemical shielding for HOSCN calculated at the MP2 DZP level is 113 ppm smaller than the ^{17}O chemical shielding for OSCN$^-$. As seen from the two CCSD calculations of the chemical shieldings, the ^{17}O chemical shielding for OSCN$^-$ is sensitive to changes in the geometry, and one may expect that vibrational corrections are large. However, the difference of 113 ppm is probably larger than solvent and vibrational effects. At the SCF level, the estimated solvent induced ^{17}O shifts are +2, +8, and −8 ppm for HSNCO, HONCS, and HOSCN, respectively. The ^{17}O chemical shielding of HOSCN/OSCN$^-$ will show a pH dependency from which the individual concentrations of HOSCN and OSCN$^-$ can be deduced.

The ^{33}S chemical shieldings for OSCN$^-$, ONCS$^-$, and SNCO$^-$ calculated at the MP2 TZ2P level are 232, 840, and 308 ppm, respectively. Since all ^{33}S chemical shieldings for these molecules differ, the oxidation product could probably be identified with the aid of ^{33}S NMR spectroscopy. The ^{33}S chemical shielding for HOSCN calculated at the MP2 DZP level is 93 ppm smaller than the ^{33}S chemical shielding for OSCN$^-$, and the ^{33}S NMR spectroscopy is probably also capable of giving a measurement of the individual concentrations of HOSCN and OSCN$^-$ in mixtures of these two substances. At the SCF level, the estimated solvent induced ^{33}S shifts are +31, +14, and +31 ppm for HSNCO, HONCS, and HOSCN, respectively. The ^{33}S chemical shieldings for SCN$^-$ (R(CS) = 166.28 pm and R(NC) = 118.40 pm) calculated at the MP2 TZ2P level is 824.8 ppm, and at the CCSD TZ2P level it is 806.3 ppm.

3.4. Excitation Energies. The ultraviolet (UV) spectra of HOSCN/OSCN$^-$ have been measured at pH = 8 and 3 by Tenovuo et al.[16] The hypothiocyanite anion and the hypothiocyanous acid have significantly different UV spectra. The hypothiocyanite spectrum shows a maximum at about 220 nm. This strong band has a maximum extinction coefficient of 3870 M^{-1} cm^{-1} at 220 nm, and the oscillator strength estimated from the spectrum is 0.11. For the hypothiocyanous acid, the ultraviolet absorption in the range 200−300 nm is much weaker. The ultraviolet spectrum for the hypothiocyanous acid has its maximum at about 240 nm. The extinction coefficient at 240 nm is 95 M^{-1} cm^{-1}, and the oscillator strength is estimated from the spectrum to be 0.14×10^{-2}.

Since the excited states of the free anions are unstable, it is not possible to calculate reliable excitation energies for the anions. However, the UV spectrum for the hypothiocyanous acid has been measured, and a comparison of calculated and measured UV spectra for the acids supports the determination of the structure of HOSCN/OSCN$^-$. In Table 7, the calculated excitation energies for the acid forms are given and compared

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Table 7. Calculated Excitation Energies (in eV) and the Corresponding Wavelengths (in nm), for Three Structures of the Hypothiocyanous Acid Using the EOM-CCSD Method and DZP Basis Sets as Compared to the Experimental Results[b]

molecule	excitation	excitation energy	wavelength
HOSCN	1A − 2A	4.57	271
	1A − 3A	6.49	191
HONCS	1A′ − 1A″	3.05	406
	1A′ − 2A′	4.84	256
	1A′ − 2A″	6.25	198
HSNCO	1A′ − 1A″	4.46	278
	1A′ − 2A″	5.96	207
	1A′ − 2A′	6.74	184
HX[a]	experiment	5.17	240

[a] Hypothiocyanous acid. [b] The molecular structures are optimized at the MP2 level.

to the experimental value. The excitation energies have been calculated using the EOM-CCSD method[24] and DZP basis set. The EOM-CCSD method is able to provide a good description of excited states dominated by single excitations.[45] The approximate excitation level (AEL)[45] is about 1.1 for all excited states considered in this work.

For HONCS, the excitation energy for the first dipole transition is calculated to be 3.05 eV (406 nm) which is 2 eV smaller than the maximum of the experimental spectrum. The shift of the energy levels due to the solvent is much smaller than the energy difference of 2 eV between the experimental and calculated excitation energies, and we may conclude that the HONCS/ONCS$^-$ is not the generated product of the lactoperoxidase oxidation.

The first dipole transition for HSNCO is obtained at 4.46 eV (278 nm). This excitation energy is only 0.7 eV smaller than the experimental value, but as we saw previously, the ^{15}N chemical shielding for the SNCO$^-$ anion is about 200 ppm larger than the experimental result, and we concluded that linear SNCO$^-$ is not generated in the peroxidase reaction. However, the stable oxidation product obtained by the oxidation of aqueous solutions of KSCN by bromine or generated by electrolysis[2] may be linear SNCO$^-$ which was found to be the energetically lowest structure. The first excitation energy for HOSCN of 4.57 eV (271 nm) agrees qualitatively with the experimental value of 5.17 eV (240 nm), which indicate that HOSCN/OSCN$^-$ structure is produced in the lactoperoxidase oxidation reaction. For the acids all studied transitions are weak, and the oscillator strengths do not provide any additional information.

The effect of diffuse functions on the excitation energies was estimated at the CIS level by adding diffuse functions to the DZP basis set. For three acid structures, the excitation energies for the first excited state of each symmetry was almost unaffected (0.01−0.09 eV) by the addition of diffuse functions to the basis set. The excitation energies of the second excited states of each symmetry decreased by about 0.3 eV due to the addition of diffuse basis functions.

The solvent effects on the excitation energies are somewhat more important for the first excitation energies than addition of diffuse functions. The solvent shifts of the excitation energies were estimated as follows: The acids were surrounded by six water molecules, and all structural parameters were optimized at the SCF level. The solvent shifts were obtained as the difference in the CIS excitation energies with water and without water but with unrelaxed molecular structure. The solvent effects were found to stabilize the excited states by 0.1−0.3 eV.

4. Conclusions

Three molecular structures for hypothiocyanite and the corresponding structures for the hypothiocyanous acid have been optimized at the MP2 level using different basis sets. The ^{13}C, ^{15}N, ^{17}O, and ^{33}S chemical shieldings have been calculated at the SCF, MP2, and CCSD levels for two linear and one bent structures of hypothiocyanite using the gauge-including atomic-orbital approach.[19−23]

The linear SNCO$^-$ structure is found to be the energetically most stable isomer. The bent OSCN$^-$ and linear ONCS$^-$ are calculated to be 86 and 146 kJ/mol above SNCO$^-$, respectively. This is in agreement with previous calculations by Pyykkö and Runeberg[17] who in MP2 6-31G* calculations also found that SNCO$^-$ is the lowest structure of hypothiocyanite and that ONCS$^-$ is 145 kJ/mol above it. However, they did not find any stable structure for OSCN$^-$. In the present calculations, no other low-lying structures for hypothiocyanite have been found.

Comparison of calculated and measured ^{15}N chemical shieldings shows that the linear SNCO$^-$ structure is not generated in the lactoperoxidase oxidation reaction of SCN$^-$ by H_2O_2. The calculated ^{15}N chemical shielding for linear SNCO$^-$ differs by about 200 ppm from the experimental result, and for linear ONCS$^-$ and bent OSCN$^-$ the difference between the calculated and measured shieldings are 40 and −86 ppm, respectively. Medium and vibrational corrections are estimated to be several tens of ppm, and the oxidation product cannot be uniquely identified from the ^{15}N chemical shielding data. At the MP2 level, HOSCN and OSCN$^-$ have about the same ^{15}N chemical shieldings which also is supported by the experiment.[14]

The solvent shifts are in general smaller for ^{13}C chemical shieldings than for ^{15}N chemical shieldings, and all three isomers would probably be identified by ^{13}C NMR spectroscopy. The pH dependency of the ^{17}O and ^{33}S chemical shieldings for mixtures of HOSCN and OSCN$^-$ would provide a measurement of the individual concentrations of the two substances.

The first few excitation energies have been calculated for three structures of the hypothiocyanous acid using the EOM-CCSD method.[24] The lowest excitation energy for HONCS is found to be 2 eV (165 nm) too low in energy. The linear ONCS$^-$ isomer is also 60 kJ/mol above bent OSCN$^-$, and we may conclude that HONCS/ONCS$^-$ is not the oxidation product of lactoperoxidase oxidation reaction of SCN$^-$ by H_2O_2.

The lowest dipole allowed excitation for HSNCO is calculated to be 4.57 eV which is in qualitative agreement with experiment. However, the chemical shift calculations on the linear SNCO$^-$ structure showed that HSNCO/SNCO$^-$ is not generated in the enzymatic oxidation reaction. Since no other low-lying structures for hypothiocyanite have been found, the only remaining structure for hypothiocyanite is the HOSCN/OSCN$^-$ isomer. For HOSCN, both the calculated UV spectrum and the ^{15}N chemical shift are in qualitative agreement with experiment.

The stable oxidation product obtained by oxidation of aqueous solutions of KSCN by bromine or by electrolysis[2] may be linear SNCO$^-$ which was found to be the energetically lowest structure.

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