

Vibrational Spectra, Assignments, and Valence Force Field for S-nitrosocysteine and Isotopic Analogs

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S-nitrosocysteine and the isotopically-substituted S¹⁵NO and N-d₃ analogs have been prepared. FT-IR spectra have been obtained on the normal molecule and the ¹⁵N isotopomer. Spectra of the N-d₃ analog were obtained with a dispersive instrument. The fundamental frequencies of the three isotopomers were calculated by using force constants previously obtained through overlay calculations on amino acids and from a normal coordinate analysis of methyl thionitrite. The average computational error between the observed and calculated frequencies using the transferred force constants was 11 cm⁻¹. This difference was decreased to 7 cm⁻¹ by slightly modifying some of the force constant values by a least-squares refinement. Vibrational assignments are made for all three isotopic analogs by utilizing the potential energy distribution. Out of a total of 96 fundamentals occurring above 300 cm⁻¹, 65 may be classified as group vibrations by the potential energy criterion. Index Headings: Infrared; Force constants; S-nitrosocysteine; Thionitrite.

INTRODUCTION

Over the past few years we have carried out vibrational studies involving a total of eleven isotopically-substituted species (isotopomers) of the amino acids alanine,^{1,2} cysteine, serine, and β-chloroalanine.³ During the course of these studies a transferable symmetry valence force field for amino acids was established by overlay calculations. For complex molecules of low symmetry, such calculations provide the best available approach for obtaining computationally meaningful force fields and frequency assignments.^{3-6,8} In a parallel study, three isotopomers of methyl thionitrite were investigated, and force constants for the CSNO group were established.⁷ The present study applies these results^{3,7} to interpret the vibrational spectra of three isotopomers of S-nitrosocysteine (cysteine thionitrite): O¹⁴NS-CH₂-CH(NH₃⁺)COO⁻, O¹⁵NS-CH₂-CH(NH₃⁺)COO⁻, and O¹⁴NS-CH₂-CH(ND₃⁺)COO⁻.

S-nitrosocysteine is of considerable importance in food safety investigations. The nitrosocysteine grouping may result when sulfhydryl groups in meat proteins and bacterial proteins are treated with nitrite.⁹⁻¹¹ Nitrite is a widely used antibacterial agent and color enhancer for meat products.⁹ A detailed interpretation of the vibrational spectra of S-nitrosocysteine provides a basis for the use of FT-IR and Raman spectroscopy in studies involving nitrite action, via S-nitrosocysteine groups, on foods and on bacteria.

By applying transferred force constants from amino acids¹⁻³ and methyl thionitrite,⁷ the frequencies of three S-nitrosocysteine isotopomers were calculated with an average absolute error of merely 11 cm⁻¹ (~1.2%). Slight

adjustments, based on a least-squares refinement of a few force constants, decreased this error by 40%, to ~7 cm⁻¹ (~0.7%). These results appear to confirm further the reliability of our previous computations.

EXPERIMENTAL

S-nitrosocysteine was prepared following a slight modification of previously reported procedures.⁹ 0.348 g (5.04 mmol) NaNO₂ (Baker Analyzed[†]) was dissolved in ca. 4 ml H₂O. 0.886 g (0.504 mmol) L-cysteine hydrochloride monohydrate (Fisher Scientific Co.) was dissolved in about 6 ml H₂O. The two solutions were cooled to less than 2°C in an ice bath. Then the nitrite solution was added to the cysteine hydrochloride solution. The solution immediately became a characteristic bright red due to the 543 nm absorption band of the -SNO moiety.^{9,11} One ml acetone was added to the red solution to precipitate the S-nitrosocysteine. The red-pink solid was filtered, washed several times with minimal quantities of acetone, and finally dried. The N-deuterated species was prepared in a similar manner with D₂O (99 atom %) from Aldrich as a solvent. Preparation of the ¹⁵N-labeled compound was carried out with sodium nitrite—¹⁵N (95.7 atom %) from Merck, Sharp, and Dohme, Canada.

The reaction between nitrous acid and cysteine is strongly pH dependent.¹¹ Use of the acid chloride of cysteine, instead of the free base, provided a sufficiently low initial pH for the reaction to proceed rapidly without the need for any additional hydrochloric acid. Although the toxicity of S-nitrosocysteine is unknown, this compound should be handled with care. Reaction mixtures containing nitrite and cysteine have recently been reported to exhibit both DNA-damaging and mutagenic activity towards *Salmonella typhimurium* TA1535 and TA98.¹²

Infrared spectra of the three isotopomers were obtained by the KBr pellet technique, and by using Nujol and halocarbon mulls on CsI and NaCl plates for different frequency ranges. Initially, spectra of the normal molecule and the N-deuterated analog were obtained on a Perkin-Elmer Model 457 infrared spectrophotometer. Later, spectra of the normal species and of the ¹⁵N isotopomer were obtained from 4000–400 cm⁻¹ on a Nicolet 7199 Fourier transform infrared spectrometer.

Normal coordinate calculations were carried out by the Wilson GF-matrix formulation,¹³ and by employing previously used mathematical procedures and computer

† Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE I. Force constants for S-nitrosocysteine.

Force constant	Local symmetry coordinate ^{a,b}	Value ^{c,d}	Dispersion ^e
1	ν_a NH ₃ ⁺	5.334 (5.369)	0.016
2	ν_s NH ₃ ⁺	5.399 (5.432)	0.021
3	δ_a NH ₃ ⁺	0.596 (0.618)	0.003
4	δ_s NH ₃ ⁺	0.648 (0.654)	0.006
5	ρ_1 NH ₃ ⁺	0.755	...
6	ρ_\perp NH ₃ ⁺	0.724	...
7	τ NH ₃ ⁺	0.049	...
8	ν_a CH ₂	4.774	...
9	ν_s CH ₂	4.962	...
10	δ CH ₂	0.527 (0.545)	0.006
11	γ CH ₂	0.637 (0.612)	0.006
12	t CH ₂	0.676 (0.664)	0.010
13	ρ CH ₂	0.474 (0.507)	0.016
14	τ CC-CS	0.106	...
15	ν CH	4.741	...
16	δ CCH; δ NCH	0.648	...
17	ν_a COO ⁻	8.500	...
18	ν_s COO ⁻	11.100	...
19	δ COO ⁻	1.147	...
20	ρ COO ⁻	1.430	...
21	γ COO ⁻	0.607	...
22	τ COO ⁻	0.070	...
23	ν C(3)C(8)	4.460	...
24	ν C(2)C(3)	4.390	...
25	ν CN	3.890	...
26	δ NC(3)C(8)	1.345	...
27	δ NC(3)C(2)	1.470	...
28	δ CCC	1.086	...
29	ν NO	9.992 (10.230*)	0.073
30	ν CS	3.240*	...
31	δ CCS	0.581	...
32	δ CSN	1.160*	...
33	τ CC-SN	0.013	...
34	τ CS-NO
35	ν SN	2.599 (2.900*)	0.051
36	δ SNO	0.939 (0.810*)	0.043
37	ν CN, δ_s NH ₃ ⁺	-0.376	...
38	ν_s COO ⁻ , δ COO ⁻	1.773	...
39	δ COO ⁻ , ν C(2)C(3)	-0.320	...
40	ν_a COO ⁻ , ρ COO ⁻	1.289	...
41	δ COO ⁻ , γ COO ⁻	0.036	...
42	ν CS, ν C(3)C(8)	0.227	...
43	ν CS, δ CH ₂	-0.238	...
44	ν CS, γ CH ₂	0.370	...
45	ν CS, δ CCS	0.364	...
46	ν C(3)C(8), δ CH ₂	-0.197	...
47	ν C(3)C(8), γ CH ₂	-0.198	...
48	ν skel, δ CH	0.254	...
49	ν SN, δ SNO	0.500*	...
50	ν NO, δ SNO	0.500*	...

^a ν_a , antisym. str.; ν_s , sym. str.; δ_a , antisym. bend; δ_s , sym. bend; ρ , rocking; γ , wagging; τ , torsion; t , twisting.

^b Atom numbering as in Fig. 2, ref. 3.

^c Units: stretching, mdyne·Å⁻¹; bending and torsion, mdyne·Å·rad⁻²; stretch-stretch, mdyne·Å⁻¹; stretch-bend, mdyne·rad⁻¹; bend-bend, mdyne·Å·rad⁻².

^d Transferred from amino acids (Ref. 3) except as follows: newly refined values are given with starting value in parentheses; values with asterisk are transferred from methyl thionitrite (Ref. 7).

^e Estimated from the standard error in frequency parameters.

programs.¹⁴ The geometry of the molecule was assumed to be that of L-cysteine¹⁵ for the amino acid part. Structural data for the CSNO skeleton were taken from an X-ray study of the unusually stable thionitrite of N-acetyl-D,L-penicillimine,¹⁶ as in previous studies on methyl thionitrite.⁷ Symmetry coordinates were constructed as previously described for L-cysteine.³

Zero-order calculations were carried out with 43 force

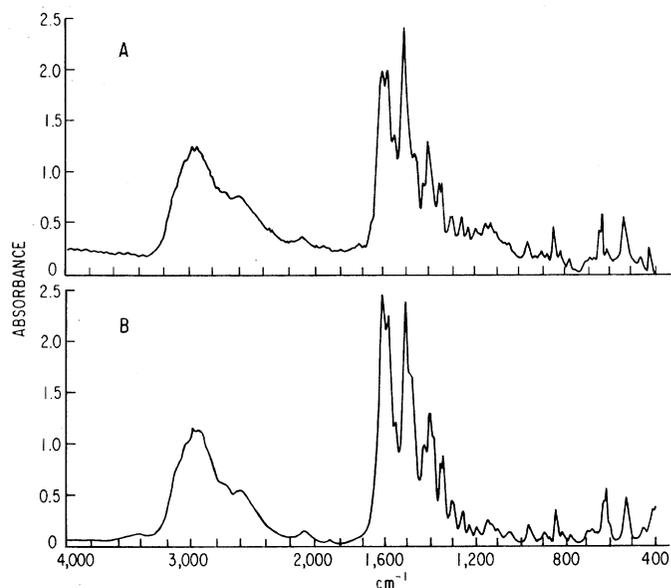


FIG. 1. Fourier transform infrared spectra of S-nitrosocysteine (A) and S-nitrosocysteine-¹⁵N (B), obtained by the KBr pellet technique. (Frequency scale changes at 1800 cm⁻¹.)

constants transferred from the overlay calculations on amino acids,³ and seven from methyl thionitrite.⁷ Subsequently, force constants involving the NH₃ grouping, the CH₂ grouping, and the CSNO skeleton were refined, and the average absolute frequency error was thereby reduced from ca. 11 cm⁻¹ to ca. 7 cm⁻¹. The specific reasons for choosing these particular force constants (11 out of 50) for a least-squares refinement are explained in the next section.

RESULTS AND DISCUSSION

Force Constants. Table I lists the 50 force constants used to calculate a total of 117 frequencies of the three S-nitrosocysteine isotopomers. The force constants transferred from the methyl thionitrite study⁷ are marked with an asterisk. The remaining unrefined force constants were transferred from overlay calculations on amino acids.³ The 11 refined constants are given along with the error estimated from the standard error in frequency parameters. The starting force constants are given in parentheses in cases where refined values were used for the final computations.

A few words should be said concerning the choice of force constants which were refined: (a) The crystal structures of the previously studied amino acids differ from each other,³ and the crystal structure for S-nitrosocysteine is not known. Vibrational frequencies associated with the strongly hydrogen-bonded NH₃ groups thus differ from one amino acid to another depending on the particular scheme of hydrogen bonding in a given crystal. NH₃ stretching and bending force constants [F(1) to F(4)] were therefore chosen for refinement. (b) The CH₂ group force constants depend, to a degree, on the atom next to the methylene group,³ a sulfur atom in the case of S-nitrosocysteine. The CH₂ bending, wagging, twisting, and rocking force constants [F(10) to F(13)] were, therefore, also refined. (c) Finally, three force constants associated with the SNO grouping, F(29) (NO

TABLE II. FT-IR spectra of S-nitrosocysteine and S-nitrosocysteine-¹⁵N, 300–3200 cm⁻¹ region.

	¹⁴ N isotopomer		¹⁵ N isotopomer		Potential energy distribution (PED) ^{c,d}	Approx. descr. ^e
	ν^a	$\Delta\nu^b$	ν^a	$\Delta\nu^b$		
1	3140	5	3150	-5	1 (99)	$\nu_a\text{NH}_3^{+\ddagger}$
2	3140	5	3150	-5	1 (99)	$\nu_a\text{NH}_3^{+\ddagger}$
3	3045	10	3046	9	2 (100)	$\nu_a\text{NH}_3^{+\ddagger}$
4	2982	14	2986	10	8 (100)	$\nu_a\text{CH}_2$
5	(2981) ^f	...	(2981) ^f	...	9 (100)	$\nu_a\text{CH}_2$
6	2945	9	2939	15	15 (99)	νCH
7	1610	9	1613	6	3 (90)	$\delta_a\text{NH}_3^{+\ddagger}$
8	1610	5	1613	2	3 (92)	$\delta_a\text{NH}_3^{+\ddagger}$
9	1587	1	1588	0	17 (108)	$\nu_a\text{COO}^-$
10	1513	5	1512	6	4 (100)	$\delta_s\text{NH}_3^{+\ddagger}$
11	1513	2	1487	0	29 (98)	νNO
12	1422	2	1428	-4	10 (42), 24 (18), 16 (15)	...
13	1406	-4	1404	-2	10 (56), 24 (15), 16 (13)	δCH_2
14	1355	0	1355	0	16 (65), 23 (12), 25 (12)	$\delta\text{CCH}; \delta\text{NCH}$
15	1343	-19	1344	-20	16 (28), 12 (27), 18 (23)	...
16	1298	3	1299	1	11 (83), 23 (15)	γCH_2
17	1255	9	1256	8	12 (50), 18 (40)	tCH_2
18	1194	-12	1195	-13	16 (26), 5 (22), 18 (10)	...
19	1148	-2	1148	-2	6 (48), 5 (14), 11 (10)	$\rho_{-1}\text{NH}_3^{+\ddagger}$
20	1102	-1	1101	2	25 (21), 5 (18), 12 (11)	...
21	(1008) ^f	...	(1008) ^f	...	5 (21), 24 (19), 6 (16)	...
22	924	-4	924	-4	21 (14), 25 (14), 6 (13)	...
23	846	11	846	11	13 (28), 21 (19), 19 (14)	...
24	816	-10	816	-10	21 (34), 13 (30)	...
25	(722) ^f	...	(722) ^f	...	30 (85), 45 (-14)	νCS
26	643	-1	634	8	19 (52), 27 (15), 20 (14)	δCOO^-
27	632	1	623	-3	35 (98), 36 (20), 49 (-28)	νSN
28	536	-1	534	3	25 (28), 20 (27), 26 (13)	...
29	(490) ^f	...	(490) ^f	...	7 (73)	$\tau\text{NH}_3^{+\ddagger}$
30	457	-7	455	-6	26 (23), 20 (16), 31 (13)	...
31	391	-4	389	-4	36 (62)	δSNO
32	342	14	(356) ^f	...	28 (26), 19 (14), 14 (13)	...

^a Observed frequency in cm⁻¹.

^b $\nu(\text{calc.}) - \nu(\text{observed})$.

^c The first number designates the force constant; the second number (in parentheses), its percent PED.

^d The PED is given for the ¹⁴N isotopomer. The PED is very similar for the ¹⁵N isotopomer.

^e Symbols as in Table I.

^f Calculated frequency. No IR band observed.

[‡] Nearly degenerate mode (Type E for local C_{3v} symmetry).

stretching), F(35) (SN stretching), and F(36) (SNO bending) were refined because these three transferred force constants were obtained on gaseous methyl thionitrite whereas the present study is concerned with crystalline S-nitrosocysteine. (Frequency shifts of 20 cm⁻¹ to 25 cm⁻¹ are observed for the NO stretching and SNO bending modes of methyl thionitrite on going from the vapor phase to the solid state.)

A total of 11 force constants (out of 50) were therefore refined. The differences between the initial and final calculated force constant values associated with the amino acid residues are small, as seen in Table I. These changes in value range from 0.6% for F(2) to 6% for F(13), with

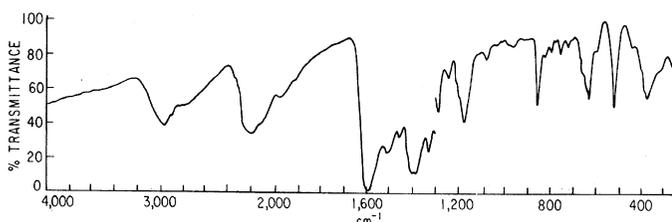


FIG. 2. Infrared spectrum of S-nitrosocysteine-N-d₃. Halocarbon mull 3500–1300 cm⁻¹. Nujol mull 1300–250 cm⁻¹.

an average deviation of 2.6%. For the SNO force constants F(29), F(35), and F(36) the refinement yields final values which differ from the initial ones by 2%, 10%, and 16%, respectively. The largest difference is associated with the SNO bending force constant. All these changes appear reasonable and indicate, in general, a good transferability of the previously obtained values. (The only exception is the SNO bending constant which appears to change noticeably when comparing the gaseous to the solid state.) Transferability of the seven force constants giving rise to vibrations below ca. 250 cm⁻¹ [F(14, 22, 27, 31–34)], i.e., constants associated with low frequency bending and torsional modes, could not be ascertained because no low frequency data were available. (The limit was ~250 cm⁻¹ for the N-d₃ isotopomer and ~380 cm⁻¹ for the other two isotopomers. Raman spectra could not be obtained because of sample decomposition even with the use of the red 6471 Ångstrom line of a krypton ion laser.)

Frequencies and Assignments. Figure 1 shows the Fourier transform infrared spectra of the normal and the ¹⁵N isotopomer. The observed frequencies, errors of calculation, and assignments according to the potential energy distribution¹⁷ (PED) are given in Table II. To avoid confusion, only the three largest PED terms with con-

TABLE III. Infrared spectra of S-nitrosocysteine-N- d_3 from 300 to 3000 cm^{-1} .

	Frequency (cm^{-1})		Potential energy distribution (PED) ^d	Approx. descr. ^c
	Obs.	Calc.		
1	2991	2996	8 (100)	$\nu_a\text{CH}_2$
2	2981	2981	9 (100)	$\nu_s\text{CH}_2$
3	2954	2954	15 (99)	νCH
4	(~2360) ^b	2333	1 (98)	$\nu_a\text{ND}_3^{+d}$
5	(~2360) ^b	2332	1 (98)	$\nu_s\text{ND}_3^{+d}$
6	2220	2193	2 (99)	$\nu_s\text{ND}_3^+$
7	1587	1588	17 (109), 40 (-27), 20 (13)	$\nu_a\text{COO}^-$
8	1517	1515	29 (98)	νNO
9	1412	1418	10 (71), 24 (10)	δCH_2
10	1393	1396	24 (27), 10 (27), 16 (24)	...
11	1360	1356	16 (67), 23 (12), 48 (-11)	$\delta\text{CCH}; \delta\text{NCH}$
12	1338	1323	16 (30), 12 (27), 18 (20)	...
13	1297	1298	11 (86), 23 (18)	γCH_2
14	1250	1263	12 (50), 18 (38), 16 (10)	τCH_2
15	1183	1174	3 (26), 4 (19), 25 (14)	...
16	1174	1161	3 (93)	$\delta_a\text{ND}_3^{+d}$
17	1174	1155	3 (65), 4 (19)	$\delta_s\text{ND}_3^{+d}$
18	1150	1137	4 (59), 16 (10)	$\delta_s\text{ND}_3^+$
19	1079	1062	18 (15), 24 (13), 25 (13)	...
20	1029	1020	6 (15), 5 (10)	...
21	857	859	13 (30), 21 (24), 14 (10)	...
22	823	836	5 (56), 19 (21), 24 (11)	$\rho_{\parallel}\text{ND}_3^+$
23	...	808	23 (18), 21 (18), 6 (17)	...
24	795	787	6 (45), 21 (21), 13 (13)	$\rho_{\perp}\text{ND}_3^+$
25	...	718	30 (86), 45 (-14)	νCS
26	629	631	35 (99), 36 (19), 49 (-28)	νSN
27	605	614	19 (47), 20 (15), 38 (-21)	δCOO^-
28	518	513	20 (31), 25 (29), 19 (15)	...
29	444	441	36 (26), 26 (23), 31 (17)	...
30	370	385	36 (46), 26 (11)	δSNO
31	...	355	28 (20), 7 (17), 19 (12)	...
32	335	346	7 (66)	τND_3^+

^a The first number designates the force constant, the second (in parentheses), the percent PED.

^b Very weak shoulder. Not used in refinement.

^c Symbols as in Table I.

^d Nearly degenerate mode (Type E for local C_{3v} symmetry).

tributions higher than 10% are listed. An approximate group frequency description is given if one term contributes more than ca. 45% to the potential energy, as in previous related work.^{1-3,7} Figure 2 gives the infrared spectrum of the N- d_3 isotopomer, and Table III supplies the corresponding frequency data and assignments. Because the calculation errors are somewhat larger for this molecule than for the other two isotopomers, both observed and calculated frequencies are explicitly given. The overall frequency error, as stated before, is $\sim 7 \text{ cm}^{-1}$ or 0.7%, which appears satisfactory considering the large number of transferred force constants and the very small adjustments in the 11 constants refined.

Assignments for the high frequency NH_3 , ND_3 , CH_2 , and CH stretching modes must be regarded as approximate. In the case of the protonated isotopomers, NH and CH stretching modes overlap as in all amino acids.¹⁻³ This entire spectral region is further distorted

by very strong Fermi resonance, rendering the determination of precise fundamental frequencies very difficult. The overlap is eliminated in the N- d_3 isotopomer, but obvious Fermi resonance distortion is now observed in the ND_3 stretching region, around 2000–2400 cm^{-1} , as well as in the CH stretching region around 3000 cm^{-1} . Only one obvious CH_2 stretching band is observed for each isotopomer. This band is assigned to the CH_2 asymmetric stretching vibration in analogy with earlier studies on the amino acids cysteine^{3,18} and serine.^{3,19} An attempt to describe all modes of a molecule as complex as S-nitrosocysteine as localized group vibrations would not be meaningful. The vibrations above ca. 1500 cm^{-1} can, nevertheless, be so designated in all three isotopomers, as seen in the PED column of Tables II and III. (More than 90% of the potential energy is associated with a single force constant.) Of the lower frequency modes a considerable number can also be called group vibrations by the PED criterion. Such a designation holds primarily for XH deformation modes where X is a nitrogen or a carbon atom. The CS, SN, and NO stretching modes and the asymmetric COO^- stretching vibration can also be called local group vibrations. The CC or CN stretching modes, and the symmetric COO^- stretching vibration, cannot be so designated. Attempts to interpret the vibrational spectra of molecules of this size entirely by a group frequency approach are obviously unjustified oversimplifications. Characteristic group force constants, on the other hand, if based on a broad enough selection of molecules treated by overlay calculations, appear to retain their validity for a considerable variety of structurally related molecules.

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