Stereoelectronic Control in the Aqueous Decomposition of Novel Nitrosothioureas

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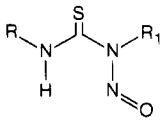
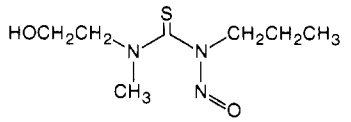
The products and pathways of decomposition of novel nitrosothioureas have been studied under physiological conditions by GC/MS, CIMS, and product isolation. The application of ^{13}C and ^{15}N NMR in conjunction with specifically enriched nitrosothioureas permitted the identification of transient species including thionitrosyl intermediates and carbodiimides. The intermediacy of the postulated tetrahedral species resulting from nucleophilic attack of water at the thiocarbonyl bond is supported by observed ^{18}O exchange into the carbonyl group of isolated ureas and nitrosoureas in ^{18}O-enriched water. The products of reaction of 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosothiourea (**9**) and the isomer **10** provided evidence for intramolecular hydroxyl participation leading to a tetrahedral species while specific deuterium labeling confirmed the implicit molecular rearrangement. Reaction of 3-cyclohexyl-1-(2-fluoroethyl)-1-nitrosothiourea (**8**) in aqueous buffer leads inter alia to the isolation of 2-(cyclohexylimino)-3-nitrosothiazolidine, a direct counterpart of a previously postulated intermediate in 1-(2-chloroethyl)-1-nitrosourea decomposition. Application of cyclic structures with the attendant slow ring nitrogen inversion in the corresponding tetrahedral intermediates permits a critical test for the operation of stereoelectronic control (both primary and secondary) and rationalizes the selection of pathways leading to the observed products. The operation of torsional strain as it relates to the Edwards–Lemieux effect, orbital approach control, and conformational factors to these reactions is also discussed.

Introduction

The clinically useful antitumor activity of (2-chloroethyl)nitrosoureas (CENUs) has been correlated with their capacity to generate electrophiles upon decomposition under physiological conditions. These intermediates alkylate and cross-link critical cellular macromolecules including DNA and proteins.[1-4] The alkyl isocyanate, which is produced simultaneously causes carbamoylation of proteins, which lesion tends to correlate with toxic side effects.[1,2] Attempts to obviate the latter problem have included the synthesis of CENUs designed to trap the isocyanate intramolecularly.[3] In an alternative approach to this problem we recently described the synthesis of novel alkylnitrosothioureas (**1–10**),[5,6] since alkyl isothiocyanates

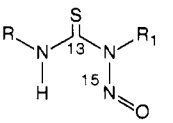




10

1 R = R_1 = CH_3
2 R = R_1 = CH_2CH_3
3 R = R_1 = $CH_2CH_2CH_3$
4 R = R_1 = CH_2CH_2F
5 R = R_1 = $CH_2CH_2OCH_3$
6 R = C_6H_{11}, R_1 = CH_2CH_3
7 R = C_6H_{11}, R_1 = $CH_2CH_2OCH_3$
8 R = C_6H_{11}, R_1 = CH_2CH_2F
9 R = C_6H_{11}, R_1 = CH_2CH_2OH
9a R = C_6H_{11}, R_1 = CH_2CD_2OH

are less reactive than isocyanates and some isothiocyanates exhibit antitumor properties in their own right. We now report an examination of their different modes of aqueous decomposition. The synthesis of specifically ^{13}C- and ^{15}N-labeled nitrosothioureas (**1a–3a** and **6a**) permitted an



1a R = R_1 = CH_3
2a R = R_1 = CH_2CH_3
3a R = R_1 = $CH_2CH_2CH_3$
6a R = C_6H_{11}, R_1 = CH_2CH_3

assessment of the bonding characteristics involved in orbital approach control in the formation of tetrahedral intermediates. Examination of cyclic structures with the attendant slow ring nitrogen inversion permitted a critical test of the operation of stereoelectronic control (both primary and secondary) and rationalized the selection of pathways leading to the observed products.

Results

Nature of the Intermediates and Products. Nitrosothioureas **1–10** were allowed to decompose in aqueous phosphate buffers at different pH at either 25 °C or 37 °C, and the nature of the volatile products and their relative yields were examined by GC and GC/MS at intervals. The identified products together with their corresponding GC and MS data are given in Table I. The identity and the relative yields of the nonvolatile products were established by MS, CIMS, and ^{13}C NMR spectroscopy.

The major products of decomposition of the nitrosothioureas in the pH range 1.5–4.5 are the ureas (50–80% yields) and rather less (10–30% yields) of the alkyl isothiocyanates (Table I). At pH 7.2 the major decomposition products are the alkyl isothiocyanate and the alcohols and smaller amounts of ureas (Table I). In the higher pH range 8.4–12.5 the alkyl isothiocyanates were generally the major products, while in the particular case of nitrosothioureas **8**, 1-cycylohexyl-3-nitrosoimidazolidine-2-thione (**14**) was the major product isolated at pH 12.5 and 25 °C (Scheme I).

The decomposition of the nitrosothiourea **9** in phosphate buffer pH 7.2 led to the formation of acetaldehyde in the volatile fraction, the identity of which was confirmed by GC and GC/MS. The nonvolatile products were the ni-

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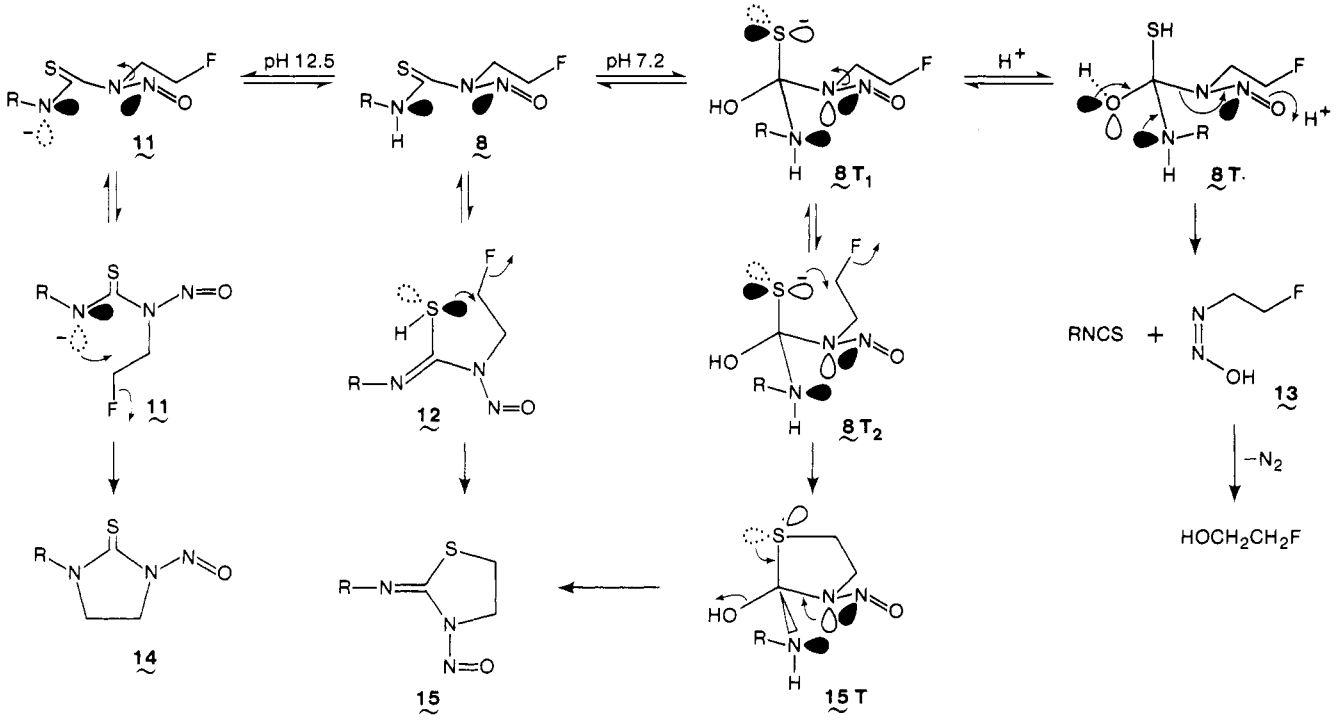
Table I. Mass Spectral Data of the Volatile Decomposition Products of 1,3-Dialkyl-1-nitrosothioureas in Phosphate Buffer at 37 °C and pH 7.2

compd	decomposition products	yields, %[a]	retention time, min[b]	m/e (relative intensity)
1	methanol	trace	0.32	
	methyl isothiocyanate[c]	50	1.89	73 (100.0, M⁺), 72 (42.5, M⁺ - 1), 45 (25.3)
2	ethanol	trace	0.49	
	ethyl isothiocyanate[d]	34	4.26	87 (100.0, M⁺), 72 (28.3), 59 (64.8)
3	*n*-propanol	34	1.45	60 (5.7, M⁺), 59 (12.6, M⁺ - 1), 31 (100.0, $^+CH_2OH$)
	1-propyl isothiocyanate[e]	40	6.15	101 (100.0, M⁺), 73 (49.9), 43 (69.0)
4	2-fluoroethanol	trace	2.13	64 (16.2, M⁺), 45 (2.9, M⁺ - F), 31 (100.0, $^+CH_2OH$)
	2-fluoroethyl isothiocyanate	trace	8.32	105 (100.0, M⁺), 85 (3.2), 71 (10.5)
5	2-methoxyethanol	trace	3.16	76 (5.6, M⁺), 69 (9.1), 45 (100.0, $^+CH_2OH$)
	2-methoxyethyl isothiocyanate	33	14.25	117 (5.3, M⁺), 45 (100.0)
	methoxyethylene	1	1.54	58 (63.1, M⁺), 29 (100.0)
6	ethanol	45	0.51	
	cyclohexyl isothiocyanate	40	20.5	142 (100.0, M⁺), 100 (27.9)
7	2-methoxyethanol	40	3.20	76 (5.7), 45 (100.0)
	cyclohexyl isothiocyanate	43	20.6	142 (100.0, M⁺)
8	2-fluoroethanol	20	2.13	64 (15.6, M⁺), 31 (100.0, $^+CH_2OH$)
	cyclohexyl isothiocyanate	25	20.8	142 (100.0, M⁺)
9	ethylene glycol	25	5.50	
	cyclohexyl isothiocyanate	35	20.90	142 (100.0, M⁺)
10	1-propanol	50	1.45	60 (6.5, M⁺), 59 (12.6, M⁺ - 1), 31 (100.0, $^+CH_2OH$)
	3-methyl-2-oxazolone	60	6.54	101 (100.0, M⁺), 72 (55.1)

[a] Yields were not critically optimized. [b] Hewlett-Packard 5840A gas chromatograph with 10% Carbowax column, helium flow rate of 22 mL/min at 60 °C. [c] ^{13}C NMR ($CDCl_3$) δ 129.60 (C=S), 30.50 (C_1). [d] ^{13}C NMR ($CDCl_3$) δ 130.0 (C=S), 40.60 (C_1), 15.70 (C_2). [e] ^{13}C NMR ($CDCl_3$) δ 130.50 (C=S), 47.20 (C_1), 23.90 (C_2), 11.30 (C_3).

Scheme I



trosothiazolidine **15**, the 2-thiazolidine **21**, 2-(cyclohexylimino)-3-nitrosooxazolidine (**18**), 2-(cyclohexylimino)-2-oxazoline (**20**), (2-hydroxyethyl) *N*-cyclohexyl carbamate (**22**), cyclohexyl isocyanate, ethylene glycol, and a trace of dicyclohexylurea and were confirmed by CIMS and by comparison with authentic samples[7] (Scheme II).

Aqueous decomposition of nitrosothiourea **10** at pH 7.2 gives propanol, 3-methyl-2-oxazolidinone[8] (**28**), and a trace of 3-methyl-2-oxazolidinethione[9] (**23**) (Scheme III).

The decompositions of the specifically ^{13}C=S enriched nitrosothiourea (**1a–3a** and **6a**) in aqueous buffer were examined in order to detect transient species. When the thiourea **3a** was dissolved in acetone and 1.0 N hydrochloric acid and the ^{13}C NMR spectrum was examined, the original absorptions due to the nitrosothiocarbonyl at δ 180.80 decreased with time and new peaks appeared at δ 171.66 (br), 162.45 (br), 154.79, 146.85, and 128.88. The broad peak at δ 171.66 is tentatively ascribed to the 1-protonated nitrosothiourea since the peak position is similar to that reported for this type of carbon.[10,11] The

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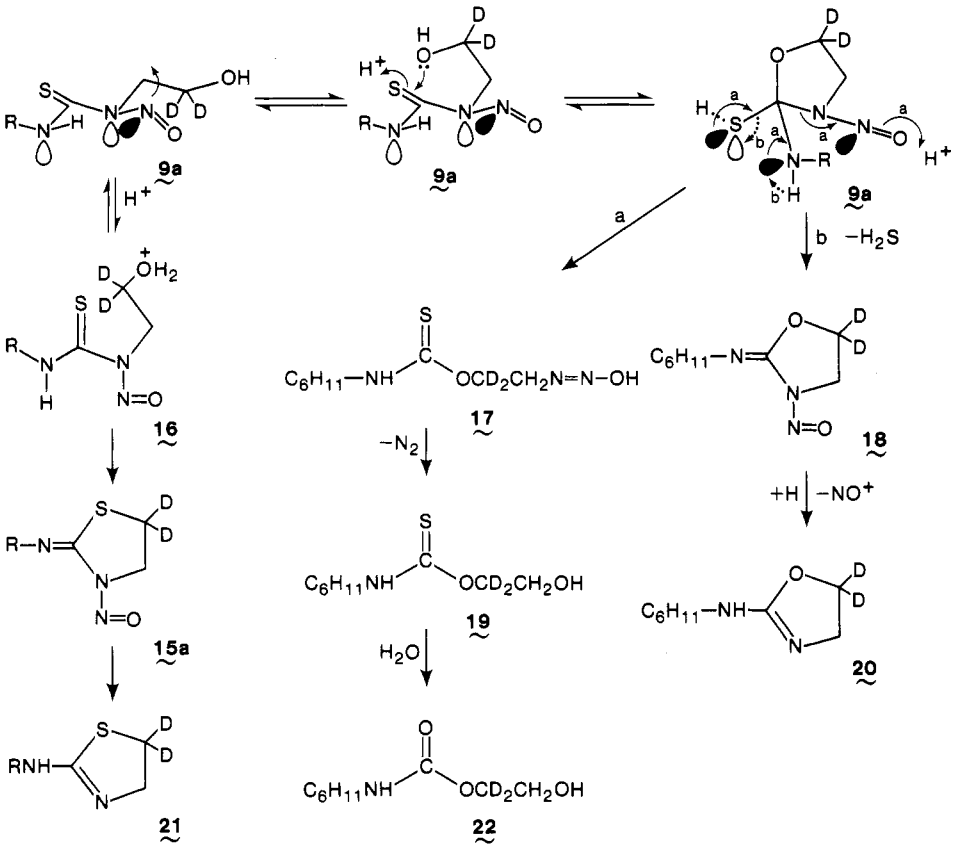
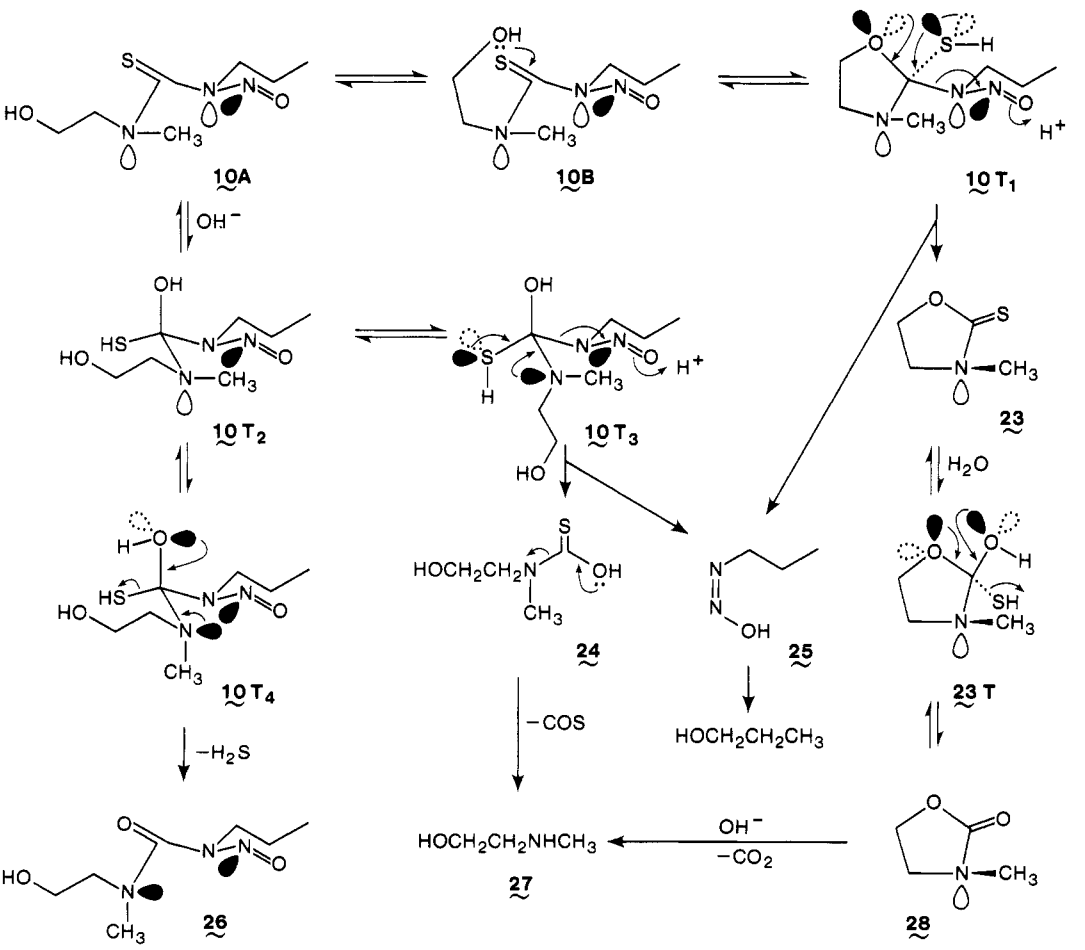
Scheme II



Scheme III



broadening of this resonance may be due to the participation of different conformers.[6] The additional peaks at δ 162.45 and 154.79 were due to the corresponding urea and nitrosourea[24] at confirmed by comparison with authentic samples. The absorptions at δ 146.85 and 128.88 are assigned to the carbodiimide[12] and isothiocyanate,[13] respectively.

When the nitrosothiourea **3a** was allowed to decompose in aqueous acetone buffer at pH 7.2 and 31 °C the ^{13}C NMR absorption due to the $^{13}C{=}S$ appeared initially at δ 180.56 ($^2J_{^{15}N-^{13}C}$ = 2.3 Hz). After 1 h a new absorption assignable to another conformation of the nitrosothiourea appeared at δ 181.54 ($^2J_{^{15}N-^{13}C}$ = 2.4 Hz) together with peaks at δ 154.36 ($^2J_{^{15}N-^{13}C}$ = 4.5 Hz), due to the corresponding nitrosourea,[24] and a broad peak at δ 128.38, ascribed to the isothiocyanate.[13] After 6 h the signals at δ 181.54, 154.36, and 128.38 became stronger at the expense of the original nitrosothiourea peak at δ 180.56. After 12 h, the resonances characteristic of ureas (δ 160.56) and carbamate (δ 158.27) began to appear.

The suggested intermediacy of tetrahedral species such as T_1 in Scheme I was supported by the formation of ^{18}O-labeled ureas from the decomposition of nitrosothiourea **3a** in $H_2{}^{18}O$, as has been demonstrated in the case of nitrosoureas.[14,15]

Formation of Products. Solutions of nitrosothioureas in chloroform or methanol are quite stable at low temperatures (−50 °C) and even at 25 °C for several hours without appreciable decomposition. However decomposition proceeds rapidly in more polar and protic solvents and depends on the pH of the solution.

Acid-catalyzed hydrolysis of nitrosothioureas to nitrosoureas and ureas may plausibly involve the formation and controlled decomposition of tetrahedral intermediates or oxidation pathways such as have been suggested for thioureas.[16,17] Consequently care was taken to exclude oxygen rigorously during these reactions. Protonation of N_1 leads to the formation of a thionitrosyl intermediate[16,18] by nitroso group migration, which can then form the corresponding urea either by nucleophilic displacement by water or alternatively by elimination of HSNO to give the carbodiimide and subsequent hydration.[18] ^{15}N and ^{13}C NMR evidence for the intermediacy of both species[5,6] supports this latter reaction pathway.

The products of hydrolysis under neutral conditions may be rationalized by formation of a neutral tetrahedral intermediate, which by elimination of hydrogen sulfide affords the nitrosourea, subsequent denitrosation of which gives the unsymmetrical urea. Decomposition of the nitrosourea gives the symmetrical urea and carbamate. Similar types of neutral tetrahedral intermediates (formed by slow addition of water) have been invoked to explain the rapid cleavage of the C–S bond during the hydrolysis of thioacetanilides,[19] thiobenzamides,[20] thioimidates,[21,22] and thioesters.[23-25]

The aqueous decomposition of nitrosothiourea **8** at pH 7.2 afforded cyclohexyl isothiocyanate, 2-fluoroethanol (via **13**, Scheme I), and 2-(cyclohexylimino)-3-nitrosothiazolidine (**15**), the formation of which is compatible with either a tetrahedral intermediate T (**15**) (Scheme I) or an intramolecular nucleophilic attack of the sulfur atom and displacement of fluoride ion (**12**) (Scheme I). The formation of **15** is analogous to the suggested intermediacy of 2-(alkylimino)-3-nitrosooxazolidines in the aqueous decomposition of CENUs including BCNU, CCNU, and MeCCNU.[26]

The (2-fluoroethyl)nitrosothiourea **8** gives rise to 1-cyclohexyl-3-nitroso-1,3-imidazolidine-2-thione (**14**) under strongly basic conditions, i.e., pH 12.5. A similar formation of 3-nitroso-1-phenyl-1,3-imidazolidone has been reported in the reaction of 1-(2-chloroethyl)-3-phenyl-1-nitrosourea with triethylamine.[27]

The products obtained from **9** and **9a** include 2-(cyclohexylimino)-3-nitrosooxazolidine (**18**) and 2-(cyclohexylamino)-2-oxazoline (**20**). Decomposition of the tetrahedral intermediate **9a** T (Scheme II, pathway a) affords the thionocarbamate diazohydroxide **17**, thence by hydrolysis the thionocarbamate **19**, and then the carbamate **22**. The rearrangement implicit in this conversion of **9** to **22** was confirmed by specific deuterium labeling of compound **9a** as shown in Scheme II. Similarly formation of 3-methyl-2-oxazolidine (**28**) from the aqueous decomposition of **10** at pH 7.2 implicates the tetrahedral intermediates **10** T and **23** T (Scheme III) formed intramolecularly. Stereoelectronically controlled cleavage of the C–N_1 bond of the latter leads to propanol and 3-methyl-2-oxazolidine-2-thione (**23**). Nucleophilic attack of OH^- and the C=S bond of the latter leads via a second tetrahedral intermediate (**23** T) to 3-methyl-2-oxazolidinone (**28**) by cleavage of the C–S bond. There is precedent for the hydrolytic conversion of 1,3-oxazolidine-2-thione to a 1,3-oxazolidin-2-one rather than by an oxidative pathway.[28] It is conceivable but unlikely that these products arise by the initial hydrolysis of the nitrosothiourea to the corresponding nitrosourea and subsequent conversion to the observed products. For example, the formation of 3-methyl-1,3-oxazolidin-2-one has been observed from N_3-(hydroxyethyl)-3-methyl-1-alkyl-1-nitrosoureas.[29]

Discussion

Primary and Secondary Stereoelectronic Control in the Aqueous Decomposition of Nitrosothioureas. Formation of the different products obtained from the aqueous decomposition of nitrosothioureas at different pH's and temperatures may be rationalized by invoking the stereoelectronic theories (both primary and secondary) of Deslongchamps for control of the preferred direction of decomposition of tetrahedral intermediates.[30,31] Theoretical calculations of the relative energies of different tetrahedral intermediates by Lehn and Wipff[32,33] indicated that the C–N bond of $CH(OH)_2NH_2$ is most prone to cleavage in those conformations where the C–N bond has

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an antiperiplanar orientation with respect to the two oxygen lone pairs and in which the nitrogen lone pair is not antiperiplanar to the C–O bond but rather to the C–H bond, which conclusion is in accord with the primary stereoelectronic effect.

The secondary stereoelectronic effect (Edward–Lemieux effect)[34,35] controls the relative stabilities of the different tetrahedral intermediates arising respectively from *Z* and *E* isomers of, for example, esters or imidate esters.[30] The result of the secondary effect is to predict easier decomposition of the *Z* than the *E* form and may be invoked to discriminate between otherwise similar tetrahedral species.[30]

Recently an analysis of the factors controlling the orientation of nucleophilic attack at the carbonyl group of amide bonds has been formulated by Mock on the basis of torsional strain considerations.[36] The Mock treatment predicts that anti distortion of *Z*-substituted amides results in rehybridization of amide orbitals such that anti addition of a nucleophilic attacking the C=O bond may be anticipated (relative to the amide nitrogen lone pair).[36,37] Conversely syn distortion of *E*-substituted amides predicts the corresponding nucleophilic attack to occur syn relative to the amide lone pair. An analysis of the system represented by the tetrahedral intermediates arising from nitrosothioureas is more complex since it involves five heteroatoms. The relative magnitudes of the $^{1}J_{^{15}N_3\text{-}C(C=S)}$ = 17.5 Hz and $^{1}J_{^{15}N_1\text{-}^{13}C(C=S)}$ = 12.7 Hz indicates that the N_3–C bond has the greater amide-like character, and the lower electronegativity of S compared with oxygen ensures that the N_3 nitrogen will be close to pyramidal in its hybridization. In contrast the cross-conjugation of N_1 into the nitroso group (reflected in the magnitude of $^{1}J_{^{15}N_1\text{-}^{15}N(N=O)}$ = 22.4 Hz) ensures that the N_1 lone pair will be extensively delocalized.

It seems likely therefore from these considerations and those of approach vector analysis[38] that the alignment of the incoming nucleophile to the C=S bond relative to the N_3 lone pair will be the deciding factor in determining orientation, other factors such as steric hindrance effects being equal.

Analysis of Stereoelectronic Control in Cyclic Tetrahedral Intermediates from Nitrosothiourea Aqueous Decomposition. It is difficult to obtain experimental evidence for stereoelectronic control in acyclic systems owing to facile interconversion of conformers via bond rotations, rapid proton exchange, and heteroatom (nitrogen) inversion.[30,31] However it has been demonstrated recently that there is relatively slow nitrogen inversion in five- and six-membered heterocycles, and this offers the opportunity for the experimental test of the operation of stereoelectronic control.[39] An example is provided by the tetrahedral species **10** T_1 in Scheme III. Orbital approach control in the formation of **10** T_1 from **10B** requires the incoming oxygen to approach antiperiplanar to the lone pairs on N_1 and N_3. While the alignment of the lone pair on the ring nitrogen is dictated by the slow inversion, the freely rotating SH group and the ring oxygen permit participation to expel the propyl diazohydroxide **25**. Orbital approach control dictates attack by the incoming oxygen nucleophile on **23** to give **23** T as shown. Subsequent decomposition of **23** T is similarly dictated by the antiperiplanar orbitals on the oxygens to expel SH and not by the ring nitrogen, whose orbital is inappropriately aligned, so that **28** results.

The initial products resulting from either intramolecular or intermolecular nucleophilic addition are subject to stereoelectronic control in their subsequent decomposition. Tetrahedral species **9a** T (Scheme II) may give rise to the thionocarbamate diazohydroxide intermediate **17** by operation of the two lone-pair orbitals on sulfur and N_3 aligned antiperiplanar to the breaking C–N bond (pathway a). Loss of nitrogen from **17** affords the isolated thionocarbamate **19**, subsequent stereoelectronically controlled hydrolysis of which via another tetrahedral species gives the known carbamate **22**. Species **9a** T is subject to an alternative mode of decomposition (pathway b Scheme II) leading to the identified 2-(cyclohexylimino)-3-nitroso-2-oxazoline (**18**) and 2-(cyclohexylimino)oxazoline (**20**).

The effects of conformational constraints in ring systems in controlling the alignment of heteroatom lone pairs may be seen in the case of **10** T_1 species (Scheme III). The thermodynamically favored and stereoelectronically controlled cleavage of the C–N bond of CNN=O in **10** T_1 affords the 3-methyloxazolidine-2-thione (**23**). Subsequent hydrolysis of the latter requires the incoming hydroxyl ion to attack the C=S bond anti to the N_3 lone pair to give species **23** T. If the subsequent expulsion of SH^- from the latter were to involve the nitrogen atom, an atomic inversion would be required. The annular constraints on the latter process compared with the facile C–OH bond rotation plausibly suggest instead that the antiperiplanar aligned oxygen lone pairs are decisive here in the expulsion of SH^-. The alternative decompositions of the tetrahedral species **10** T_2 and **10** T_3 formed from **10** by intermolecular attack leading to the identifiable products shown in Scheme III may similarly be rationalized by participation of lone pair orbitals on sulfur and nitrogen or oxygen and nitrogen, respectively.

The behavior of **8**, representing the first reported example of a thiocarbonyl analogue of a clinically useful nitrosourea, deserves special comment. The initial conformation (Scheme I) indicated is favored by the operation of the Edward–Lemieux effect.[34,35] The dominance of the positioning of the N_3 lone pair as we have seen dictates that intramolecular attack of OH^- at pH 7.2 occurs antiperiplanar to this N_3 orbital to give **8** T_1 as shown. Concomitant rotation about the side-chain N–C bond brings the normally poorly reactive fluoride group in proximity to the strongly nucleophilic S^- group in **8** T_2 so that an intramolecular nucleophilic displacement ensues, resulting in the formation of another tetrahedral intermediate **15** T. Owing to the aforementioned restrictions on the rate of inversion of the ring nitrogen it appears likely that subsequent stereoelectronically controlled expulsion of OH^- from the latter place at the demand of the antiperiplanar alignment of the sulfur and exocyclic nitrogen orbitals to give **15**.

In conclusion it may be seen that nitrosothioureas exhibit significantly different chemistry in their decomposition under physiological conditions from the nitrosoureas. In particular the greater nucleophilicity of sulfur renders them more susceptible to intramolecular cyclization, permitting the isolation of 2-(alkylimino)-3-nitrosothiazolidines analogous to previously postulated intermediates in nitrosourea decomposition. In addition the prevalance of cyclic intermediates in nitrosothiourea chemistry affords a more critical test of the operation of

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stereoelectronic control in their aqueous decomposition.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometric, and only the principal, sharply defined peaks are reported. The ^1H NMR spectra of the compounds were recorded on Perkin-Elmer, Varian HA-100, Bruker WH-200, and WH-400 spectrometers. Most of the ^{13}C spectra were recorded on Varian HA-60 and Bruker HEX-90 spectrometers and the spectra of specifically ^{15}N- and ^{13}C-labeled compounds were recorded on a Bruker WH-200 spectrometer.

GC analysis were performed on a Hewlett-Packard 5840A gas chromatograph equipped with flame ionization detector. GC/MS analysis was done on an AEI-MS12 spectrometer. Most of the gas chromatography was performed on Porapak R and T (80–100 mesh) columns under isothermal conditions at 200 °C with helium flow rate 40 mL/min.

Materials. Ammonium-^{15}N chloride (95–100%), sodium nitrite-^{15}N (95%), potassium phthalimide-^{15}N (99%), carbon-^{13}C disulfide (90%), methylamine-^{15}N hydrochloride (95%), and ethylamine-^{15}N hydrochloride were obtained from Merck, Sharp and Dohme. H_2 ^{18}O (97%) was obtained from KOR Isotopes. 2-Fluoroethanol and cyclohexyl isothiocyanate were obtained from Aldrich. Additional alkyl isothiocyanates such as methyl-, ethyl-, *n*-propyl, and 2-methoxy isothiocyanate were obtained from Trans World Chemicals.

Most of the unlabeled nitrosothioureas were prepared by following the general procedure.

General Methods for the Preparation of Nitrosothioureas.[6] A dilute solution of HCl (0.07–0.1 N, 200–500 mL) was added dropwise to a suspension of the thiourea (20–50 mmol) and sodium nitrite (20–50 mmol) in dichloromethane (100–250 mL) with mechanical stirring and cooling (−10 to −5 °C) under a nitrogen atmosphere during 1–2 h. After the addition was completed, the reaction mixture was allowed to warm up to 5 °C with continuous stirring. The organic layer was removed, washed with water and dried (Na_2SO_4), and solvent was removed under reduced pressure to afford the crude nitrosothioureas which were purified either by crystallization from ether–petroleum ether or by rapid chromatography on silica gel or Florisil.

The following nitrosothioureas were prepared in this way: N_1,N_3-dimethyl-N_1-nitrosothiourea (**1**), mp 46 °C[6]; 1,3-diethyl-N_1-nitrosothiourea (**2**) as a heavy oil; N_1,N_3-dipropyl-1-nitrosothiourea (**3**) as a low-melting solid 1,3-bis(2-fluoroethyl)-1-nitrosothiourea (**4**), mp 23 °C; 1,3-bis(2-methoxyethyl)-1-nitrosothiourea (**5**) as an oil; 3-cyclohexyl-1-ethyl-1-nitrosothiourea (**6**), mp 42 °C; 3-cyclohexyl-1-(2-methoxyethyl)-1-nitrosothiourea (**7**) as a heavy oil; 3-cyclohexyl-1-(2-fluoroethyl)-1-nitrosothiourea (**8**), mp 51–52 °C; and 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosothiourea (**9**), mp 55–57 °C. The characterization of all the above compounds is given in ref 6. Additional new nitrosothioureas were prepared as follows.

3-Cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)-1-nitrosothiourea (9a). The corresponding 3-cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)thiourea (1.02 g, 5 mmol), which was prepared by following the procedure described in the literature,[6] was nitrosated with $NaNO_2$ (420 mg, 6 mmol) and 0.1 HCl (75 mL) at 0.5 °C in the usual way. Crystallization of the residue from ether–petroleum ether afforded 712 mg (70%) of 3-cyclohexyl-1-(2,2-dideuterio-2-hydroxyethyl)-1-nitrosothiourea; mp 55 °C; ^1H NMR (CD_2Cl_2) 1.20–2.20 (m, 10 H, CH_2), 2.25 (br m, 1 H, OH exchangeable), 4.40 (br m, 1 H, CH), 4.45 (s, 2 H, CH_2), 8.85 (br m, NH exchangeable); MS, *m/e* (relative intensity) 232.1078 (2.42, $C_9H_{14}N_3O_2SD_2$, 232.1089), 203.1183 (24.09, $C_9H_{15}N_2OSD_2$, 203.1187), 83.0861 (100.00, C_6H_{11}, 83.0860).

3-(2-Hydroxyethyl)-3-methyl-1-propyl-1-nitrosothiourea (10). A solution of *n*-propyl isothiocyanate (5.05 g, 50 mmol) in ether (20 mL) was added dropwise to a solution of 2-(methylamino)ethanol (3.75 g, 50 mmol) in ether (30 mL), and the reaction mixture was stirred for 12 h at ambient temperature. After the usual workup, 3-(2-hydroxyethyl)-3-methyl-1-propylthiourea (7.5 g, 91%) was obtained: ^1H NMR ($CDCl_3$) 0.98 (t, 3 H, $CH_2CH_2CH_3$), 1.65 (m 2 H, $CH_2CH_2CH_3$), 3.25 (s, 3 H, CH_3), 3.45 (br s, 1 H, OH exchangeable), 3.50 (q, 2 H, $CH_2CH_2CH_3$), 4.85 (br s, 4 H, CH_2CH_2OH), 6.75 (br m, 1 H, NH exchangeable); ^{13}C NMR ($CDCl_3$) 11.48 (C_3), 2.26 (C_2), 39.42 (C_3), 48.00 (CH_3), 55.49 (C'_1), 61.09 (C'_2), 183.30 (C=S); MS, *m/e* (relative intensity) 176.0982 (100.00, M^+, $C_7H_{17}N_2OS$), 176.0984.

A solution of 0.1 N HCl (200 mL) was added dropwise to a solution of the above thiourea (3.29 g, 20 mmol) and sodium nitrite (1.6 g, 22 mmol) at −5 to −0 °C, and the reaction mixture stirred for 1 h at this temperature. The organic layer was removed, washed with water, and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was triturated with petroleum ether/ether (95:5) to afford 2.6 g (61% yield) of nitrosothiourea (**10**) as a heavy oil: IR ($CDCl_3$) 3387 (NH, OH), 1510, 1445, 1400, 1310, 1220, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) 1.00 (t, 3 H, $CH_2CH_2CH_3$), 1.60 (m, 2 H, $CH_2CH_2CH_3$), 3.25 (s, 3 H, CH_3), 3.85 (br m, 3 H, CH_2OH), 4.50 (t, 2 H, $CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$) 11.48 (C_3), 22.50 (C_2), 39.44 (C_1), 47.95 (CH_3), 50.09 (C'_1), 60.98 (C'_2), 183.20 (C=S); MS, *m/e* (relative intensity) 205.0854 (1.12, $C_7H_{15}N_3O_2S$, 205.0885), 204.0800 (3.79, $C_7H_{15}N_3O_2S$, 204.0807), 175.0903 (100.00, $C_7H_{15}N_2OS$, 175.0906). The specifically ^{13}C=S and ^{15}N=O labeled nitrosoureas (**1a**, **2a**, **3a**, and **6a**) were prepared from the corresponding ^{13}C=S thioureas by treatment with $Na^{15}NO_2$ following the above procedures for the unlabeled compounds.

General Method for the Decomposition of 3-Alkyl-1-alkyl-1-nitrosothioureas in Phosphate Buffer. (a) Identification of Volatile Products. A sample of nitrosothiourea (0.1 mmol), suspended in potassium phosphate buffer (0.1 M, 4 mL) at pH 7.2, in a 1-mL capacity screw-capped Reactivial was allowed to decompose for 12 h at 37 °C. An aliquot of the gaseous products (1 mL) was injected by a Pressure-lok syringe into the GC apparatus for analysis of the volatile products. In addition an aliquot of the aqueous solution (1 μL) was injected into the GC for further analysis of the volatile products, the identities of which were confirmed by their retention times by comparison with authentic samples and by their masses by GC/MS analysis (Table I).

(b) Identification of Nonvolatile Products. The solvent was removed from the above decomposition products at low temperature in vacuo, and the different products were identified by MS, CIMS, and TLC.

Aqueous Decomposition of 3-Cyclohexyl-1-(2-fluoroethyl)-1-nitrosothiourea (8) at pH 12.5 and Formation of 1-Cyclohexyl-3-nitroso-1,3-imidazolidine-2-thione (14). The nitrosothiourea (466 mg, 2 mmol) was treated with potassium phosphate buffer (40 mmol), pH 12.5, and stirred for 2 h. The yellow solid which resulted was collected and purified by recrystallization to afford 1-cyclohexyl-3-nitroso-1,3-imidazolidin-2-thione (**14**): 350 mg (82% yield); mp 48 °C; IR ($CHCl_3$) 1507, 1452, 1431, 1324, 1167, 1141 cm^{-1}; NMR ($CDCl_3$) 1.20–2.10 (m, 10 H, CH_2), 3.80 (m, 2 H, CH_2), 3.95 (m, 2 H, CH_2), 4.85 (m, 1 H, H_1); MS, *m/e* (relative intensity) 213.0934 (31.04, $C_9H_{15}N_3OS$, 213.0935), 183.0957 (100.00, $C_9H_{15}N_2S$, 183.0957), 103.0328 (45.37, $C_3H_7N_2S$, 103.0330).

Aqueous Decomposition of 3-Cyclohexyl-1-(2-fluoroethyl)-1-nitrosothiourea (8) at pH 7.2 and Formation of 2-(Cyclohexylimino)-3-nitrosothiazolidine (15), 2-Fluoroethanol, and Cyclohexyl Isothiocyanate as Major Products. A sample of nitrosothiourea (**8**) (0.1 mmol) was suspended in potassium phosphate buffer, pH 7.2, at 37 °C for 12 h as described in the above general method, and the volatile products were detected and identified by GC/MS. Their retention times are given in Table I.

The nonvolatile products were shown by mass spectral analysis and chemical ionization mass spectroscopy to contain 2-(cyclohexylimino)-3-nitrosothiazolidine (**15**) and 2-(cyclohexylimino)-2thiazolidine (**21**); MS, *m/e* (relative intensity) 213.0933 (1.02, $C_9H_{15}N_3OS$, 213.0936), 184.1032 (68.45, $C_9H_{16}N_2S$, 184.1034), 103.0330 (100.00, $C_3H_7N_2S$, 103.0330); CIMS (isobutane) 214 (5.0), 185 (100.0), 103 (1.8). The identities of these two products were further confirmed by chromatography on reverse-phase precoated silica gel plates with a water/acetonitrile (1:4) eluant and by comparison with authentic samples. A similar aqueous decomposition of nitrosothiourea (**8**) at pH 5.0 afforded, in addition to the above heterocyclic products, some dicyclohexylurea.

Aqueous Decomposition of 3-Cyclohexyl-1-(2-hydroxyethyl)-1-nitrosothiourea (9) at pH 7.2 in Phosphate Buffer at 37 °C. The aqueous decomposition was performed as described

above, and acetaldehyde and ethylene glycol were confirmed among the products by GC/MS.

Additional products were analyzed by MS and CIMS. MS showed the presence of strong peaks: *m/e* (relative intensity) 224.1867 (1.0, $C_{13}H_{24}N_2O$, 224.1888); CIMS peak 225.0, which corresponds to dicyclohexylurea: *m/e* 215.1270 (4.26, $C_9H_{17}N_3O_3$, 125.1270); CIMS 216.0 (13.2) corresponds to 3-cyclohexyl-1-(2-hydroxyethyl)-1-nitrosourea; *m/e* 203.0979 (1.01, $C_9H_{17}NO_2S$, 203.0979): CIMS, 204.0 (100.0) corresponds to 2-hydroxyethyl*N*-cyclohexylthionocarbamate (**19**): *m/e* 197.1157 (0.18, $C_9H_{15}N_3O_2$): CIMS 198.0 (1.0) corresponds to 2-(cyclohexylimino)-3-nitrosooxazolidine (**18**); *m/e* 187.1212 (0.24, $C_9H_{17}NO_3$, 187.0209); CIMS 188 (12.0) corresponds to 2-hydroxyethyl *N*-cyclohexylcarbamate (**22**): ^{13}C NMR ($CDCl_3$) 156.41 (C=O), 66.64 (C_1), 61.76 (C_2), 50.64 (C_1'), 33.36 (C_2'), 24.82 (C_3'), 25.50 (C_4'); ^{13}C NMR (acetone-d_6) 157.37 (C=O), 64.14 (C_1), 24.88 (C_2), 11.49 (C_3), 43.19 (C_1'), 23.14 (C_2'), 10.59 (C_3'); *m/e* 186.1368 (7.35, $C_9H_{18}N_2O_2$, 186.1368); CIMS 187.0 (53.6) corresponds to 3-cyclohexyl-1-(2-hydroxyethyl) urea. A minor amount of 2-cyclohexyliminooxazoline (**20**) was also detected.

The authentic samples of 3-cyclohexyl-1-(2-hydroxyethyl)-N_1-nitrosourea-2-cyclohexylimino-3-nitrosooxazolidine (**18**), 2-hydroxyethyl *N*-cyclohexylcarbamate (**22**), and N_3-cyclohexyl-*N*-(2-hydroxyethyl) urea required for identification purposes were prepared by literature procedures.[7,23]

Detection of Intermediates in the Aqueous Decomposition of Nitrosothioureas by ^{13}C NMR of Specifically ^{13}C- and ^{15}N-Enriched Compounds. (a) Acidic Decomposition of 3a. A sample of nitrosothiourea (**3a**) (0.1 mmol) was dissolved in acetone (5 mL) and 1.0 N hydrochloric acid (1 mL), and the ^{13}C NMR spectrum was examined at 31 °C. Initially the ^{13}C=S absorption appeared at δ 180.80 together with a δ 175.66 (br) peak ascribed to the N_1-protonated species[10,11] (relative intensities 8:2). After 1 h additional peaks appeared and with the following intensities relative to the ^{13}C=S peak: δ 162.45 (br) (dipropylurea, 20%), δ 154.79 (nitrosoureas, 11%), δ 146.84 (dipropylcarbodiimide,[14] 3%), and δ 128.88 (propylisothiocyanate, 18%).

(b) Decomposition of 3a under Neutral Conditions. A sample of **3a** (1 mmol) was allowed to decompose in (1:1) aqueous potassium phosphate buffer (pH 7.1) and acetone (total volume 6 mL) at 31 °C and the ^{13}C NMR spectrum was examined. Initially the ^{13}C=S appeared at δ 180.56 ($^2J_{^{15}N-^{13}C}$ =2.3 Hz). After 1 h an additional absorption assigned to a second conformation of **3a** appeared at δ 181.54 ($^2J_{^{15}N-^{13}C}$ = 2.4 Hz) (18% relative to **3a**) together with peaks at δ 154.36 ($^2J_{^{15}N-^{13}C}$ = 4.5 Hz, dipropylnitrosoureas, 9%) and δ 128.38 (propyl isothiocyanate, 16%). After 6 h the signals at δ 181.54, 154.36, and 128.38 had intensities of 38%, 27%, and 130% relative to the original nitrosothiourea **3a** peak at δ 180.56. After 12 h the resonances characteristic of dipropylurea, δ 160.56 (65%), and propyl carbamate, δ 158.27 (12%), had appeared.

Preparation of Authentic Samples of Isolated Products from the Aqueous Decomposition of Nitrosothioureas. 2-(Cyclohexylimino)-3-nitrosothiazolidine (15). A solution of cyclohexyl isothiocyanate (7.6 g, 50 mmol) in 2 mL of ether was added dropwise over a period of 1 h to a stirred solution of 2-chloroethylamine (4.0 g, 50 mmol; prepared by basification of 2-chloroethylamine hydrochloride (5.8 g, 50 mmol) with sodium hydroxide (2.9 g, 50 mmol) in 20 mL of water) with stirring for 3 h at 0 °C and extraction with ether (3 × 50 mL). The ether solution was dried (Na_2SO_4), and the solution was stirred for a further 4–5 h at 0–5 °C during which time a solid separated which was collected and identified as 3-cyclohexyl-1-(2-chloroethyl)-thiourea: 2.5 g (21% yield); mp 155–156 °C; IR ($CHCl_3$) 3334 (NH), 1625, 1590, 1420, 1210, and 1080 cm^{-1}; ^1H NMR ($CDCl_3$) 1.10–2.20 (m, 10 H, CH_2), 3.40 (br m, 1 H, CH), 3.45 (m, 2 H, CH_2CH_2Cl), 5.50 (br d, 2 H, NH exchangeable, J = 8.0 Hz). The above sample on standing at room temperature for 3 h was converted into 2-(cyclohexylamino)thiazoline hydrochloride: mp 224–225 °C; ^1H NMR ($CDCl_3$) 1.10–2.10 (m, 10 H, CH_2), 3.25 (t, 2 H, SCH_2), 3.35 (m, 1 H, CH), 4.00 (t, 2 H, N–CH_2), 6.60 (br m, 2 H, NH exchangeable); MS, *m/e* (relative intensity) 184.1035 (15.43, $C_9H_{16}N_2S$, M^+ – HCl, 184.1035), 103.0332 (21.73, $C_3H_7N_2S$, 103.0330), 56.0520 (100.00, C_3H_6N, 56.0500): ^{13}C NMR (Me_2SO-d_6) 23.86 (C_3, C_5), 24.57 (C_4), 30.54 (C_5), 31.53 (C_2, C_6), 48.99 (C_4), 54.71 (C'_1), 168.27 (C_2).

An ethereal suspension of the solid was treated with sodium hydroxide and extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and concentrated to afford the thiazolidine: 7.5 g (81% yield); mp 166 °C (lit.[50] mp 165 °C); IR (KBr disk) 3200, 1620, 1610, 1525, 1310, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) 1.00–2.20 (m, 10 H, CH_2), 3.30 (t, 2 H, SCH_2), 3.50 (m, 1 H, CH), 4.08 (t, 2 H, NCH_2), 6.50 (br m, 1 H, NH, exchangeable); MS, *m/e* (relative intensity) 184.1029 (72.75, $C_9H_{16}N_2S$, M^+, 184.1034), 103.0326 (100.00, $C_3H_7N_2S$, 103.0285); ^{13}C NMR 24.91 (C_3', C_5'), 25.67 (C_4'), 33.70 (C_2', C_6') 35.00 (C_5), 53.78 (C_4), 60.36 (C_1'), 160.70 (C_2).

The above thiazolidine (1.84 g, 10 mmol) was nitrosated with excess of *n*-butyl nitrite (5.1 g, 50 mmol) and sodium methoxide (0.7 g, 13 mmol) in ether and the product crystallized from ether/dichloromethane to give 1.65 g (77% yield) of the nitrosothiazolidine **25**: mp 68 °C; IR ($CHCl_3$) 1643 (C=N), 1475 (N=O) cm^{-1}; ^1H NMR ($CDCl_3$) 1.20–2.20 (m, 10 H, CH_2), 3.18 (m, 1 H, H'_1), 3.20 (t, 2 H, SCH_2), 4.10 (t, 2 H, NCH_2); ^{13}C NMR ($CDCl_3$) 24.80 (C_3', C_5'), 25.47 (C_4'), 24.80 (C_5), 33.32 (C_2', C_6'), 44.70 (C_4'), 66.08 (C_1'), 148.51 (C_2); MS, *m/e* (relative intensity) 213.0937 (8.28, M^+, $C_9H_{15}N_3OS$, 213.0935), 183.0967 (100.00, $C_9H_{15}N_2S$, 183.0955), 131.0156 (4.43, $C_3H_5N_3OS$, 131.0154), 101.0174 (36.77, $C_3H_5N_2S$, 101.0173). Anal. Calcd for $C_9H_{15}N_3OS$: C, 50.79; H, 7.16; N, 19.86; S, 14.69. Found: C, 50.70; H, 7.04; N, 19.71; S, 15.02.

2-(Cyclohexylimino)-5,5-dideuterio-3-nitrosothiazolidine (15a). A solution of 3-cyclohexyl-2,2-dideuterio-1-(2-hydroxyethyl)thiourea (2.04 g, 10 mmol) was dissolved in 98% formic acid (20 mL) and the reaction mixture was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was basified with dilute sodium hydroxide solution to precipitate the solid oxazolidine which was extracted with chloroform and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was crystallized from chloroform–petroleum ether to afford 1.60 g (86% yield) of 2-(cyclohexylimino)-5,5-dideuteriothiazolidine (**21**): mp 159–160 °C; ^1H NMR ($CDCl_3$) 1.10–2.20 (m, 10 H, CH_2), 3.50 (m, 1 H, CH), 4.08 (s, 2 H, NCH_2), 6.50 (br m, 1 H, NH exchangeable); ^{13}C NMR ($CDCl_3$) 24.89 (C_3', C_5'), 25.69 (C_4'), 33.71 (C_2', C_6'), 53.73 (C_4), 60.49 (C_1'), 160.49 (C_2); MS, *m/e* (relative intensity) 186.1153 (65.78, $C_9H_{14}N_2D_2S$, 186.1160), 105.0453 (100.00, $C_3H_5N_2D_2S$, 105.0455).

The above deuterated thiazolidine (930 mg, 5 mmol) was nitrosated by stirring with *n*-butyl nitrite (5.1 g, 50 mmol) and sodium methoxide (350 mg, 6.5 mmol) in ether (150 mL) for 12 h at room temperature. The solid was collected and the solvent was removed under reduced pressure. The residue was crystallized from ether/petroleum ether to afford 400 mg (26%) of nitrosothiazolidine (**15a**), mp 66–67 °C; ^1H NMR ($CDCl_3$) 1.20–2.20 (m, 10 H, CH_2), 3.18 (m, 1 H, ax H_1'), 4.12 (s, 2 H, NCH_2); ^{13}C NMR ($CDCl_3$) 24.83 (C_3', C_5'), 25.56 (C_4'), 33.34 (C_2', C_6'), 44.59 (C_4), 66.14 (C_1), 148.56 (C_2); MS, *m/e* (relative intensity) 215.1058 (29.89, $C_9H_{13}D_2N_3OS$, 215.1061), 185.10850 (90.30, $C_9H_{13}D_2N_2S$, 185.1081), 133.0277 (17.35, $C_3H_3D_2N_3OS$, 133.0279), 103.0299 (93.88, $C_3H_3D_2N_2S$, 103.0299), 55.0564 (100, C_4H_7, 55.0548).

***N*$_3$-(2-Hydroxyethyl)-*N*$_3$-methyl-*N*$_1$-propyl-*N*$_1$-nitrosourea (26).** A solution of *n*-propyl isocyanate (4.25, 50 mmol) in ether (20 mL) was added dropwise to a solution of 2-(methylamino)-ethanol (3.75 g, 50 mmol) in ether (30 mL) and the reaction mixture was stirred for 12 h at ambient temperature. After the usual workup N_3-(2-hydroxyethyl)-N_3-methyl-N_1-propylurea was obtained in almost quantitative yield and was used for subsequent reaction without further purification: ^1H NMR ($CDCl_3$) 0.95 (t, 3 H, $CH_2CH_2CH_3$), 1.55 (m, 2 H, $CH_2CH_2CH_3$), 3.00 (s, 3 H, CH_3), 3.25 (q, 2 H, $CH_2CH_2CH_3$, 3.45 (t, 2 H, MCH_2), 3.80 (br t, 2 H, CH_2OH), 4.25 (br m, 1 H, OH exchangeable), 5.65 (br m, 1 H, NH exchangeable); ^{13}C NMR ($CDCl_3$) 11.38 (C_3), 23.42 (C_2), 35 (CH_3), 42.78 (C_1), 52.00 (C_1'), 61.47 (C_2'), 160.25 (C=O); MS, *m/e* (relative intensity) 160.1212 (55.61, $C_7H_{16}N_2O_2$, 160.1212), 130.1101 (100.00, $C_6H_{14}N_2O$, 130.1106), 102.0556 (44.85, $C_4H_8NO_2$, 102.0555).

The above urea (3.20 g, 20 mmol) was nitrosated with sodium nitrite (7.00 g, 100 mmol) in concentrated HCl (25 mL) at 0 °C. The reaction mixture was extracted with ether, the extract was washed with water several times and dried (Na_2SO_4), and the solvent was removed in vacuo to obtain 3.10 g (82% yield) of 3-(2-hydroxylethyl)-3-methyl-1-propyl-1-nitrosourea (**26**) as a viscous oil; ^1H NMR ($CDCl_3$) 0.95 (t, 3 H, $CH_2CH_2CH_3$), 1.55 (m,

2 H, $CH_2CH_2CH_3$), 3.2 (s, 3 H, CH_3), 3.80 (m, 6 H, NCH_2, CH_2OH), 5.10 (br s, 1 H, OH exchangeable); ^{13}C NMR ($CDCl_3$) 11.17 (C_3), 20.32 (C_2), 38.78 (CH_3), 42.53 (C_1) 51.45 (C_1'), 65.42 (C_2'), 156.58 (C=O); MS, *m/e* (relative intensity) 158.0933 (6.42, $M^+ - H_3O^+$, $C_6H_{12}N_3O_2$, 158.0929), 131.0455 (58.04, $C_4H_7N_2O_3$, 131.0457), 102.0556 (100.00, $C_4H_8NO_2$, 102.0556).

Preparation of 2-Hydroxyethyl *N*-Cyclohexylthionocarbamate (19). Cyclohexyl isothiocyanate (6.25 g, 50 mmol) was added dropwise to a stirred suspension of ethylene glycol (3.90 g, 50 mmol) in dry benzene and the reaction mixture was stirred at ambient temperature for 12 h. The solvent was removed in vacuo and the residue was triturated with petroleum ether to afford 8.5 g (84% yield) of 2-hydroxyethyl *N*-cyclohexylthionocarbamate (**19**): mp 55 °C (ether/petroleum ether); IR ($CDCl_3$) 3220 (br, NH, OH), 1665 (C=S), 1525, 1212 cm^{-1}; ^1H NMR ($CDCl_3$) 1.2–2.20 (m, 10 H, CH_2), 3.10 (t, 2 H), 3.75 (br s, 1 H, OH), 3.85 (t, 2 H, OCH_2), 5.75 (br m, 1 H, NH); ^{13}C NMR (acetone-d_6) 24.50 (C_3', C_5'), 25.66 (C_4'), 32.63 (C_1), 33.52 (C_2', C_6'), 38.73 (C_2), 51.33 (C_1'), 166.25 (C=S); MS, *m/e* (relative intensity) 203.0972 (2.06, $C_7H_{17}NO_2S$, 203.0964), 159.0712 (2.60, $C_7H_{13}NOS$, 159.0708), 144.1020 (24.57, $C_7H_{14}NO_2$, 144.1024), 83.0861 (100.00, C_6H_{11}, 83.0860).

3-Methyl-2-oxazolidinone (28). Carbon monoxide was passed slowly into a suspension of selenium (7.8 g, 100 mmol), 2-(methylamino)ethanol (7.5 g, 100 mmol), triethylamine (70 mL), and dimethylformamide (400 mL) for 3 h until the solution became clear. A slow stream of oxygen was passed into this solution to precipitate the selenium. Removal of the precipitated selenium by filtration and solvent in vacuo and then further distillation of the residual oil at reduced pressure gave 8.5 g (85% yield) of N_3-methyl-2-oxazolidinone (**28**): bp 75 °C 0.3 mm (lit.[8] bp 120 °C 1 mm); ^1H NMR ($CDCl_3$) 2.75 (s, 3 H, CH_3), 3.55 (t, 2 H, NCH_2), 4.42 (t, 2 H, OCH_2); ^{13}C NMR ($CDCl_3$) 31.0 (CH_3), 46.87 (C_4), 61.65 (C_5), 158.97 (C_2); MS, *m/e* (relative intensity) 101.0475 (100.0, $C_4H_7NO_2$, 101.0476), 56.0520 (32.53, C_3H_6N, 56.0500).

3-Methyl-2-oxazolidine-2-thione (23). This compound was prepared by following the method of Sakai et al.[9] starting from 2-(methylamino)ethanol (2.25 g, 30 mmol) and tributyltin diethylamide (27.7 g, 60 mmol) and carbon disulfide (4.5 g, 60 mmol) at room temperature. Distillation of the reaction mixture at 120 °C (0.3 mm) [lit.[9] bp 127 °C (0.4 mm)] gave 1.7 g (50% yield) of 3-methyl-2-oxazolidine-2-thione (**23**); ^1H NMR ($CDCl_3$) 3.25 (s, 3 H, CH_3), 3.90 (t, 2 H, NCH_2), 4.52 (t, 2 H, OCH_2; MS, *m/e* (relative intensity) 117.0248 (100.0, C_4H_7NOS, 117.0249).

***N*$_1$,*N*$_3$-Dipropyl-*N*$_1$-nitrosourea (R = R$_1$ = Propyl).** 1,3-Dipropylurea (2.88 g, 20 mmol) was nitrosated with sodium nitrite (2.80 g, 40 mmol) in formic acid (21 mL) at 0 °C and after the usual workup it afforded 3.09 g (86% yield) of the nitrosourea: mp 31 °C; IR ($CHCl_3$) 3385, 1715, 1530, 1485, 1166, and 1022 cm^{-1}; ^1H NMR ($CDCl_3$) 0.85 (t, 3 H, CH_3, J = 6.5 Hz), 0.95 (t, 3 H, CH_3), 1.50 (m, 2 H, CH_2), 1.62 (m, 2 H CH_2), 3.45 (q, 2 H, CH_2), 8.10 (t, 2 H, CH_2), 7.00 (br m, 1 H, NH exchangeable); MS, *m/e* (relative intensity) 173.1162 (11.64, M^+, $C_7H_{15}N_3O_2$, 173.1164), 88.0639 (100.00, $C_3H_8N_2O$, 88.0637), 86.0608 (18.26, C_4H_8NO, 86.0605); ^{13}C NMR ($CDCl_3$) 11.26 (C_3, C_3'), 20.40 (C_2), 23.00 (C_2'), 41.20 (C_1), 42.48 (C_2'), 153.48 (C=O); ^{13}C NMR (acetone-d_6) 11.49 (C_3, C_3'), 21.05 (C_2), 23.60 (C_2'), 41.49 (C_1), 42.48 (C_1'), 154.23 (C=O).

^{18}O Exchange at the Thiocarbonyl Group of Nitrosothiourea (3). A degassed solution of **3** (0.1 mmol) in a mixture of acetonitrile (0.2 mL) and $H_2{}^{18}O$ (97% enriched 1.5 mL) with 40 mm potassium phosphate buffer, pH 7.1, was allowed to react for 12 h at 25 °C. The reaction mixture was extracted with ether (4 × 10 mL), the extract was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was analyzed by mass spectrometry. The carbonyl fragment in the corresponding urea which is the major product under these conditions containing the ^{18}O was $CH_3CH_2CH_2NHC{\equiv}{}^{18}O$ 88.0639 (6.35, $C_4H_8N^{18}O$, 88.0630).

Caution. All *N*-nitrosothioureas should be handled with extreme care owing to their potential mutagenicity.

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Mechanism of the Fe(III)-Catalyzed Peracetic Acid Oxidation of Catechol. A Biomimetic Reaction for Pyrocatechase[28]

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The Fe(III)-catalyzed peracetic acid (HOOAc) oxidation of catechol to *cis,cis*-muconic acid (MA) is proposed as a model for the action of the Fe(III)-containing dioxygenase pyrocatechase (catechol 1,2-dioxygenase). The yield of MA is a function of the [Fe(III)] reaching a maximum of 75% when the ratio [catechol]/[Fe(III)] is 1000. No appreciable quantity of MA is formed in the absence of Fe(III). Evidence is presented that implicates peracetic acid and hydrogen peroxide as the active oxidants and *o*-benzoquinone as an intermediate in the reaction. Substrate binding to Fe(III) represents an important part of the reaction. The proposed mechanism for the model involves formation of an Fe(III)–catechol complex which is oxidized to an Fe(III)–*o*-benzoquinone species. The Fe(III)–quinone complex then undergoes nucleophilic attack at carbonyl by H_2O_2 to give a peroxide addition product which undergoes intramolecular nucleophilic addition at the adjacent carbonyl to give a dioxetane intermediate. Spontaneous opening of the dioxetane gives MA.

Since Hayaishi's discovery of the first oxygenase, pyrocatechase (catechol 1,2-dioxygenase), there has been considerable interest in the mechanism of pyrocatechase activity and that of oxygenases in general.[1-9] However, no

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