A Mutagen from Histidine Reacted with Nitrite

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Mutagenicity of the reaction products of L-histidine with sodium nitrite at pH 4.0 were studied. One of the five reaction products obtained showed the mutagenicity in *Salmonella typhimurium his* reversion assay. The mutagen was purified by crystallization and identified by instrumental analyses to be (1-nitroso-1*H*-imidazol-4-yl)acetohydroxamic acid (NIAH), which was the product of histidine undergoing nitrosation at the imidazolic secondary amine and oxidation of the primary amine at the side chain. NIAH showed a lethal mutation on TA98 strain without S9 mix and then was submitted to the induced mutation frequency (IMF) test. NIAH gave a dose-dependent IMF value on TA98, and the value at the concentration of 10 μg/plate was 4.29×10^{-6}/cell. NIAH exhibited weak mutagenicity on TA100 strain and did not have a DNA-damaging activity on H17 Rec$^+$ and M45 Rec$^-$ of *Bacillus subtilis*. Therefore, NIAH was considered to be a frame-shift-type mutagen.

INTRODUCTION

Various food components have a risk to be converted into mutagenic nitroso compounds through the reaction with nitrite in a mammalian digestive system or during cooking (Havery and Fazio, 1985). An appreciable amount of nitrite is produced from nitrate in our bodies, since nitrate contained in vegetables is absorbed through the intestines, transferred to saliva, and then reduced to nitrite by salivary microorganisms (Tannenbaum et al., 1979). Nitrite also occurs in cured meat products as a food additive and forms nitrosamines during cooking (Hotchkiss and Vecchio, 1985). Because we are in danger of exposure to the nitroso compounds, it is important to know the toxic products formed by the reaction with nitrite. Many workers identified the nitroso compounds produced by nitrite under acidic conditions near gastric pH through reaction with phenols (Knowles et al., 1974), sorbic acid (Namiki et al., 1981), citrulline and arginine (Ishibashi and Kawabata, 1981), methylaminopyridine (Kalatzis and Papadopoulos, 1981), proline (Kubacka, 1984), and so forth.

On the other hand, nitrite forms the mutagens by both the potencies of nitrosation and oxidation. For example, butylated hydroxyanisole (a food additive) was easily oxidized by nitrite to mutagenic 2-*tert*-butyl-*p*-quinone (Mizuno et al., 1987). When casein and albumin were exposed to nitrite under acidic pH, tryptophan, histidine, tyrosine, cysteine, and lysine residues were oxidatively injured (Natake and Ueda, 1982). Tryptophan has been found to form mutagenic 2-hydroxy-1-(*N*-nitrosoindolyl)-propionic acid by nitrite, which underwent both nitrosation and oxidation (Ohara et al., 1988). Products from the other amino acids have not yet been examined.

In this study, we exposed histidine to nitrite at pH 4.0 and examined the mutagenic products. It was found that a mutagen from histidine was the oxidation and nitrosation product by nitrite.

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MATERIALS AND METHODS

Materials. L-Histidine and sodium nitrite were obtained from Nacalai Tesque, Inc. Agar and extracts from beef and yeast for the cultivation of microorganisms were purchased from Difco Laboratory (Detroit, MI). Water and organic solvent were distilled twice. All other chemicals were commercially available in high grade.

Detection of the Mutagens from the Reaction Mixture of Histidine with Nitrite. Histidine (50 mmol) was reacted with sodium nitrite (50 mmol) in 100 mL of water at pH 4.0 at 37 °C. The reaction products were extracted with 1-butanol three times and submitted to a thin-layer chromatography (TLC) of cellulose (Avicel SF, 5 × 20 cm, purchased from Asahikasei Co. Ltd.). The TLC was developed with the mixed solvent of 1-butanol/acetic acid/water (4:1:5). The products separated on TLC were quantified by the following chemical analyses. The amounts of histidine and nitrite in the reaction mixture decreased with time and reached a minimum 2 h after the reaction in which 65% of histidine was converted to five products. Then, the reaction products were subjected to the Ames test using *Salmonella typhimurium* TA98 and TA100 (Ames et al., 1975), to the induced mutation frequency (IMF) test (Nagao et al., 1977), and to the Rec assay (Kada et al., 1972).

Chemical Analyses. Functional groups of the reaction products from histidine were detected by Pauly's reagent for the imidazole ring (Feigl, 1960), by the Griess reagent for the *N*-nitroso group (Takeda and Kanayama, 1982), and by the ninhydrin reagent for the amino group.

IMF Test. The IMF test used S9 mix, which was prepared by S9 fraction obtained from the liver of Sprague-Dawley rats given 500 mg/kg of body weight polychlorinated biphenyl 5 days before they were sacrificed (Mizuno et al., 1987). *S. typhimurium* TA98 and TA100 were grown overnight in liquid broth medium at 37 °C. A part of the bacterial suspension (0.4 mL) was incubated with the above reaction products (0.1 mL each of various concentrations) at 37 °C for 30 min in the presence or in the absence of 0.5 mL of S9 mix. The bacteria were washed with saline solution by centrifugation at 3500 rpm for 10 min three times and resuspended in 0.4 mL of 0.1 M sodium phosphate buffer (pH 7.4). The resuspension was mixed with 2 mL of molten top agar, poured onto minimal-glucose agar medium, and cultured at 37 °C for 2 days. To detect surviving cells, a part of the bacterial resuspension was diluted simultaneously to 10^5-fold with saline solution, and the 0.1-mL portion was cultured on the minimal medium together with top agar containing 5 mM excess histidine. After the cultivation, the numbers of induced His$^+$ colonies and surviving colonies (His$^-$) were counted. The IMF value was estimated from the number of revertant colonies divided by the number of surviving colonies.

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Table I. Characterization of the Products from the Reaction of L-Histidine with Sodium Nitrite at pH 4.0

compd	R_f value[a]	functional groups	mutagenicity[b]
I	0.19	histidine[c]	
II	0.31	imidazole, amine	nonactive
III	0.38	imidazole, amine	nonactive
IV	0.44	imidazole	weak
V	0.58	imidazole, amine, nitroso	lethal
VI	0.68	not detected	nonactive

[a] On TLC of Avicel SF. [b] The mutagenicities on TA98 and TA100 strains were determined with the Ames test (1975) in the presence or in the absence of S9 mix. "Weak" means that the compound gave 2-fold the number of spontaneous revertants of TA100 without S9. "Lethal" means that the compound exhibited a lethal mutation on TA100 without S9. [c] Identified as histidine by the analyses on TLC and HPLC and the chemical determinations for the functional groups.

Rec Assay. DNA-damaging activity of the reaction products from histidine was measured with the Rec assay. *Bacillus subtilis* H17 Rec$^+$ (wild) and M45 Rec$^-$ (recombinationless) were used in the direct streaking procedure.

High-Performance Liquid Chromatography (HPLC). The reaction products from histidine were analyzed on the HPLC: column, Unisil C18 (0.46 × 25 cm); solvent system, acetonitrile/potassium phosphate solution (49 mM, pH 4.8) (5:95) or acetonitrile/methanol/water/acetic acid (35:20:45:0.1); flow rate, 1.0 mL/min; detection, 220 nm.

Purification of the Mutagen. To obtain a large amount of the mutagenic product, a method of Bonnet and Holleyhead (1974) was used, because they showed nitrite more easily reacted with amino acids in organic solvent than in water. Histidine (310 mg) and sodium nitrite (320 mg) were stirred in the mixed solvent of 10 mL of chloroform, 1 mL of water, and 0.3 mL of acetic acid at 40 °C for 3 min. The mixture was transferred on ice and was further stirred for 2 h, and then a yellow plate crystal occurred. The crystal was collected by filtration (62.6 mg) and dissolved in 5 mL of dimethyl sulfoxide. The solution was allowed to stand at 4 °C after the addition of 6 mL of water. After three recrystallizations, 50.1 mg of pure yellow crystal was obtained.

Hydrolysis of the Yellow Crystal. For structural elucidation, the yellow crystal was hydrolyzed in 1 N HCl at 50 °C for 30 min. A white crystal was obtained as the major product.

Instrumental Analysis. The yellow crystal and its hydrolyzed product (white crystal) were analyzed by infrared, ultraviolet, nuclear magnetic resonance (NMR), fast atom bombardment mass (FAB/MS), and electron ionization mass (EI/MS) spectra. The NMR spectrum was measured with a JEOL PS-100 NMR spectrometer. FAB/MS and EI/MS spectra were obtained using a JEOL JMS-HX100.

RESULTS

Reaction Products of Histidine with Nitrite. When histidine was reacted with nitrite at pH 4.0 and 37 °C for 2 h, six compounds were detected on the TLC. Table I shows their R_f values, functional groups, and mutagenicities. Compound I was idenified as the intact histidine. Compounds II–V kept the imidazole rings which were positive to the Pauly's reagent. Compounds II, III, and V had amino or imino groups responding to the ninhydrin reagent. Vivid yellow compound V possessed a nitroso group. Compound VI was negative to these reagents.

Compound V showed strong inhibition on the growth of TA98 and TA100 strains such as due to lethal mutation (Table I). The other compounds did not exhibit mutagenicity on TA98 and TA100 both in the presence and in the absence of S9, except compound IV showed weak activity on TA100 in the presence of S9. Thus, among the five reaction products, compound V had mutagenicity and then was purified and submitted to structural elucidation.

Identification of the Mutagen. An additional amount of mutagenic compound V was prepared and isolated as described earlier. The crystal had a melting point at 140 °C (decomposition) and gave the same R_f value on the TLC as did compound V and also the same retention times of a single peak on the HPLC: 6.0 min with the mobile solvent of acetonitrile/potassium phosphate solution and 28.0 min with acetonitrile/methanol/water/acetic acid. The crystal was positive to Pauly's, Griess, and ninhydrin reagents as was compound V. Therefore, the yellow crystal was identified with compound V.

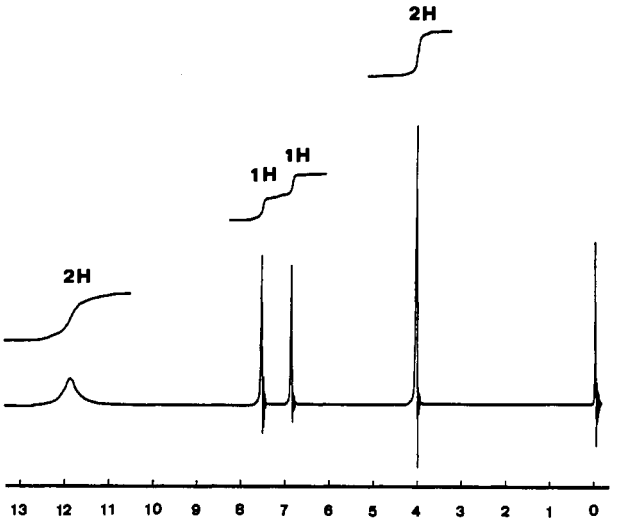

Figure 1. ^{1}H NMR spectrum of a mutagen from the reaction of histidine with nitrite in dimethyl-d_6 sulfoxide.

In the elucidation of chemical structure, the yellow crystal gave a molecular ion peak at m/z 171 $(M + H)^+$ in FAB/MS. Elemental analysis found: C, 35.10; H, 3.75; N, 32.89; O, 28.26. Calcd for $C_5H_6N_4O_3$: C, 35.30; H, 3.55; N, 32.93; O, 28.22. The infrared spectrum in KBr exhibited the presence of a nitroso group (ν_{max} 1530 cm^{-1}) and a hydroxy group (ν_{max} 3350 cm^{-1}) but not carbonyl groups. The ultraviolet spectrum in methanol gave λ_{max} at 208 nm (ϵ = 8740) and at 240 nm (ϵ = 4690), which are due to imidazole or nitrosoimidazole. On the EI/MS spectrum of crystal, m/z 107 as a base ion peak and m/z 81 as the secondary major peak were detected, but the parent ion was not. The base and secondary peaks were assigned to the fragment ions of imidazole ring having $-CH_2C{\equiv}N$ and $-CH_2$ at the 4-position, respectively.

Figure 1 shows the ^{1}H NMR spectrum of yellow crystal in dimethyl-d_6 sulfoxide. The signal at δ 4.0 (2H, s) was assigned to methylene protons. The signals at δ 6.9 (1H, s) and 7.5 (1H, s) were assigned to imidazole protons on carbon atoms, whose chemical shifts are the same as that of L-histidine in NaOD solution (Pouchert, 1983). The imino proton in the imidazole ring was not seen. The signal at δ 11.9 (2H, s) disappeared after the addition of D_2O and then was assigned to two hydroxyl protons. These data indicated that the imino proton in the imidazole ring was substituted for a nitroso group as known generally (Gough, 1978; Ohara et al., 1988) and that the alanyl side chain was converted to an acetohydroxamic acid.

Since it is well-known that *N*-nitrosoamine is easily reduced (Lee and Field, 1987) and hydroxamic acid is converted to carboxylic acid by the hydrolysis, the yellow crystal was hydrolyzed as described earlier and the obtained white crystal was analyzed with ^{1}H NMR. The spectrum coincided with that of imidazole-4-acetic acid (data not shown).

On the basis of these analysis data, the structure of the mutagen from histidine reacted with nitrite (compound V) was determined to be (1-nitroso-1*H*-imidazol-4-yl)-acetohydroxamic acid (Figure 2), which is abbreviated to NIAH.

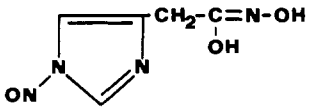

Figure 2. Structure of (1-nitroso-1*H*-imidazol-4-yl)acetohydroxamic acid (NIAH), a mutagen from the reaction of histidine with nitrite.

Table II. IMF Value of NIAH on TA98 Strain without S9

amt of NIAH, μg/plate	viable cells/plate (×10⁵)	no. of His⁺ colony/plate	mutatn freq (×10⁻⁸)
0	516	14	
2.5	124	20	48.4
5.0	78	27	167
7.5	59	29	254
10	35	29	429
12.5	25	26	480
17	6	24	1670

Table III. IMF Value of NIAH on TA100 Strain without S9

amt of NIAH, μg/plate	viable cells/plate (×10⁵)	no. of His⁺ colony/plate	mutatn freq (×10⁻⁸)
0	479	90	
25	388	186	247
50	274	213	449
100	257	199	480

Mutagenicity of NIAH. The mutagenicity of NIAH was determined using TA98 and TA100 strains in the presence or the absence of S9. NIAH did not exhibit toxicity in the presence of S9, but in the absence of S9 NIAH showed strong inhibition on the bacterial growth such as due to lethal mutation (Tables II and III). NIAH decreased the viable numbers of TA98 at the concentration of more than 2.5 μg/plate and of TA100 at more than 25 μg/plate. When the mutagen inhibits the bacterial growth, the orthodox test to detect the mutagenicity is not available. Therefore, NIAH was submitted to the IMF test estimating the mutated number in surviving bacteria. The IMF value of 10 μg/plate NIAH toward TA98 was 429 × 10^{-8}, and that of 100 μg/plate NIAH toward TA100 was 480 × 10^{-8}. Particularly, NIAH gave the dose-dependent IMF value on TA98 (Table II). Thus, NIAH showed greater activity on TA98 than on TA100 and hereby was considered to cause a frame-shift-type mutation.

DNA-damaging effect of NIAH was also measured with Rec assay (data not shown). NIAH did not inhibit the growth of Rec⁺ and Rec⁻ at concentrations of lower than 0.5 mg/plate. NIAH was considered to have neither DNA-damaging nor lethal effects on Rec bacteria.

Cause of the Mutagenicity. To examine whether the mutagenicity of NIAH was caused by the nitroso group, the hydroxamic group, or both, the mutagenicities of reaction products from imidazole, alanine, and L-histidine methyl ester with nitrite were determined by the Ames test (the reaction products from histidine methyl ester were submitted to the test after the intact histidine was removed on the TLC). Table IV shows the mutagenicities of these reaction products on TA98 and TA100 with or without S9. The products from imidazole without S9 showed mutagenicity on TA98 and weak activity on TA100 but did not show lethal activity. The products from imidazole had an *N*-nitroso group positive to the Griess reagent, and the mutagenicity was markedly lowered in the presence of S9. Because the *N*-nitroso group is detoxified by S9 as reported by Potter and Reed (1982), the toxicity of the products from imidazole was considered to be due to the *N*-nitroso group.

Table IV. Mutagenicities of Reaction Products from Histidine-Related Compounds with Nitrite

	revertant colony number/plate			
	TA98		TA100	
products from[a]	with S9	without S9	with S9	without S9
water (as control)	20	23	127	110
imidazole	35	3683	211	676
alanine	67	37	40	38
histidine methyl ester	27	20	146	149

[a] Ten micrograms/plate of the reaction products by dry weight was subjected. These products gave 510–515 × 10^5 viable cells/plate of TA98 bacteria, near to 516 × 10^5 in control, and 468–475 × 10^5 of TA100 bacteria, near to 479 × 10^5 in control, with and without S9.

DISCUSSION

This study demonstrates that histidine formed a frame-shift-type mutagen, NIAH (Figure 2), by the reaction with nitrite under acidic pH.

The mutagenicity of NIAH is compared with the IMF value of 4.29 × 10^{-6} when 10 μg/plate of NIAH was dosed to TA98 and with those of the other mutagens as follows: 10 μg/plate of hydrogen peroxide with the same determination, 9 × 10^{-8}; 50 ng/plate of Trp-P-2, 5.3 × 10^{-5} (Mizuno et al., 1989); 50 μg/plate of *tert*-butylquinone, 1.88 × 10^{-4} (Mizuno et al., 1987); and 100 μg/plate of nitroso and hydroxy derivative of tryptophan, 1.22 × 10^{-6} (Ohara et al., 1988). Thus, the mutagenicity of NIAH is stronger than that of hydrogen peroxide and is on the same level with that of the tryptophan derivative but is weaker than those of Trp-P-2 and *tert*-butylquinone.

In the formation of NIAH from histidine by the reaction with nitrite, both the imino group in the imidazole ring and the carboxyl group at the alanyl side chain were considered to participate, because imidazole produced the mutagenic *N*-nitroso derivative but the histidine methyl ester whose carboxyl group was masked did not (Table IV). Also, both functional groups of nitroso and hydroxamic were considered to contribute to the exhibition of mutagenicity of NIAH. It is known that the *N*-nitroso group is detoxified by P-450 enzymes (Potter and Reed, 1982). Since the mutagenicity of NIAH was reduced in the presence of S9 as was that of *N*-nitrosoimidazole (Tables II and IV), the mutagenicity of NIAH seems to be caused mainly by the imidazolic nitroso group. However, NIAH gave the lethal mutation on the bacteria, but *N*-nitrosoimidazole did not. It was suggested that the hydroxamic group of NIAH facilitated the expression of the mutagenicity of the imidazolic nitroso group.

The *N*-nitroso group induces mutagenicity through alkylation of DNA by alkyl cation from the nitroso compounds as exemplified by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Magee and Barnes, 1956) and *N*,*N*-dimethylnitrosamine (Lijinsky et al., 1968). The former compound generates alkyl cation after spontaneous decomposition, and the latter needs S9 for the generation. NIAH may follow the former mechanism, because the mutagenicity was expressed in the absence of S9. On the other hand, arylhydroxamic acid is reported to be activated by S9 to the ultimate mutagenic form (King, 1974). NIAH lost the mutagenicity in the presence of S9 (Tables II and III). We speculate that the hydroxamic group in NIAH interacts stereospecifically with the imidazolic nitroso group, and hereby the hydroxamic group facilitates the generation of alkyl cation by nitroso group and increases the mutagenicity to lethal activity but is resistant to metabolic activation by S9.

Thus, to form mutagenic NIAH from histidine, a free carboxyl group at the side chain was needed. Because the

amount of free histidine in our diet is very small, NIAH is suggested to be refractory to form in our bodies.

LITERATURE CITED

Ames, B. N.; McCann, J.; Yamasaki, E. Method for Detecting Carcinogens and Mutagens with Salmonella/Mammalian Microsome Mutagenicity Test. *Mutat. Res.* **1975**, *31*, 347–364.

Bonnet, R.; Holleyhead, R. Reaction of Tryptophan Derivatives with Nitrite. *J. Chem. Soc., Perkin Trans. 1* **1974**, 962–964.

Feigl, F. Ehrlich Diazo Test for Compounds Capable of Coupling. In *Spot Tests in Organic Analysis*; Feigl, F., Ed.; Elsevier: Amsterdam, 1960; pp 140–143.

Gough, T. A. Determination of *N*-Nitroso Compounds by Mass Spectrometry. *Analyst* **1978**, *103*, 785–806.

Havery, D. C.; Fazio, T. Human Exposure to Nitrosamines from Foods. *Food Technol.* **1985**, *39*, 80–83.

Hotchkiss, J. H.; Vecchio, A. J. Nitrosamines in Fried-out Bacon Fat and Its Use as a Cooking Oil. *Food Technol.* **1985**, *39*, 67–73.

Ishibashi, T.; Kawabata, T. Formation of *N*-Nitrosoproline by Reacting Nitrite with L-Citrulline and L-Arginine. *J. Agric. Food Chem.* **1981**, *29*, 1098–1099.

Kada, T.; Tsutikawa, K.; Seino, Y. In Vitro and Hostmediated Rec-assay Procedures for Screening Chemical Mutagens; and Phloxine, a Mutagenic Red Dye Detected. *Mutat. Res.* **1972**, *16*, 165–174.

Kalatzis, E.; Papadopoulos, P. Reactions of *N*-Heteroaromatic Bases with Nitrous Acid: Part 6, Kinetics of the Nitrosation of 2- and 4-Methylaminopyridine and Their 1-Oxide Derivatives. *J. Chem. Soc., Perkin Trans. 2* **1981**, 239–247.

King, C. M. Mechanism of Reaction, Tissue Distribution, and Inhibition of Arylhydroxamic Acid Acyltransferase. *Cancer Res.* **1974**, *34*, 1503–1515.

Knowles, M. E.; Gilbert, J.; McWeeny, D. J. Nitrosation of Phenols in Smoked Bacon. *Nature* **1974**, *249*, 672–673.

Kubacka, W.; Libbey, L. M.; Scanlan, R. A. Formation and Chemical Characterization of Some Nitroso Dipeptides N Terminal in Proline. *J. Agric. Food Chem.* **1984**, *32*, 401–404.

Lee, S. H.; Field, L. R. Fluorescence Detection of Some Nitrosoamines in High-Performance Liquid Chromatography after Post-Column Reaction. *J. Chromatogr.* **1987**, *386*, 137–148.

Lijinsky, W.; Loo, J.; Ross, A. E. Mechanism of Alkylation of Nucleic Acids by Nitrosodimethylamine. *Nature* **1968**, *218*, 1174–1175.

Magee, P. N.; Barnes, J. M. The Production of Malignant Primary Hepatic Tumours in the Rat by Feeding Dimethylnitrosamine. *Br. J. Cancer* **1956**, *10*, 114–122.

Mizuno, M.; Ohara, A.; Danno, G.; Kanazawa, K.; Natake, M. Mutagens Formed from Butylated Hydroxyanisole Treated with Nitrite under Acidic Conditions. *Mutat. Res.* **1987**, *176*, 179–184.

Mizuno, M.; Toda, M.; Ueno, N.; Danno, G.; Kanazawa, K.; Natake, M. Desmutagenicity of a Dibenzofuran-Quinone Derivative toward the Mutagenicity of Trp-P-2. *Agric. Biol. Chem.* **1989**, *53*, 959–964.

Nagao, M.; Yahagi, T.; Seino, Y.; Sugimura, T.; Ito, N. Mutagenicities of Quinoline and Its Derivatives. *Mutat. Res.* **1977**, *42*, 335–341.

Namiki, M.; Osawa, T.; Ishibashi, H.; Namiki, K.; Tsuji, K. Chemical Aspects of Mutagen Formation by Sorbic Acid-Sodium Nitrite Reaction. *J. Agric. Food Chem.* **1981**, *29*, 407–411.

Natake, M.; Ueda, M. Changes in Food Proteins Reacted with Nitrite at Gastric pH. *Nutr. Cancer* **1986**, *8*, 41–45.

Ohara, A.; Mizuno, M.; Danno, G.; Kanazawa, K.; Yoshioka, T.; Natake, M. Mutagen Formed from Tryptophan Reacted with Sodium Nitrite in Acidic Solution. *Mutat. Res.* **1988**, *206*, 65–71.

Potter, D. W.; Reed, D. J. Denitrosation of Carcinostatic Nitrosoureas by Purified NADPH Cytochrome *P*-450 Reductase and Rat Liver Microsomes to Yield Nitric Oxide under Anaerobic Conditions. *Arch. Biochem. Biophys.* **1982**, *216*, 158–169.

Pouchert, C. J. 5-Membered Aromatic Heterocycles. In *The Aldrich Library of NMR Spectra*; Pouchert, C. J., Ed.; Aldrich Chemical: Milwaukee, WI, 1983, Vol. 2, p 493.

Takeda, Y.; Kanayama, H. A Screening Procedure for the Formation of Nitroso Derivatives and Mutagens by Drug-Nitrite Interaction. *Chem. Pharm. Bull.* **1982**, *30*, 3399–3404.

Tannenbaum, S. R. Endogenous Formation of Nitrite and *N*-Nitroso Compounds. In *Naturally Occurring Carcinogens—Mutagens and Modulators of Carcinogenesis*; Miller, E. C., Miller, J. A., Hirono, I., Sugimura, T., Takayama, S., Eds.; Japan Scientific Society Press: Tokyo, 1979; pp 211–220.

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