Comparative nitrosation of etintidine and cimetidine

THOMAS A. MONTZKA,[1] PETER F. JUBY, JOHN D. MATISKELLA, HENRY M. HOLAVA, AND R. R. CRENSHAW

Pharmaceutical Research and Development Division, Bristol-Myers Company, Syracuse, NY 13221–4755, U.S.A.

Received December 16, 1982

THOMAS A. MONTZKA, PETER F. JUBY, JOHN D. MATISKELLA, HENRY M. HOLAVA, and R. R. CRENSHAW. Can. J. Chem. **61**, 1771 (1983).

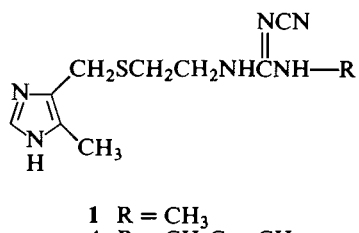
Etintidine (**4**), a new histamine H_2-receptor antagonist, was compared with cimetidine (**1**) for susceptibility to in vitro nitrosation at pH 1 and pH 3. Each agent formed two mono-*N*-nitrosoguanidine derivatives: *N*-cyano-*N'*-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-*N''*-nitroso-*N''*-(2-propynyl)guanidine (**5**) and *N*-cyano-*N'*-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-*N'*-nitroso-*N''*-(2-propynyl)guanidine (**6**) from etintidine and *N*-cyano-*N'*-methyl-*N''*-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-*N'*-nitrosoguanidine (**2**) and *N*-cyano-*N'*-methyl-*N''*-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-*N''*-nitrosoguanidine (**11**) from cimetidine. The *N*-nitroso derivative **5** from etintidine cyclized to 2-cyanoimino-3-{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]ethyl}-4-methylene-1-nitroso-imidazoline (**7**) at neutral or basic pH's. Both agents were nitrosated less at pH 3 than at pH 1, and at both pH's nitrosation of etintidine was considerably less than that of cimetidine. At pH 1, with a nitrite concentration about 150–500 times that expected in fasting human gastric juice, formation of **5** and **6** from etintidine was barely detectable (each <0.5%). Comments are made on the standard WHO conditions for investigating nitrosation of drugs.

THOMAS A. MONTZKA, PETER F. JUBY, JOHN D. MATISKELLA, HENRY M. HOLAVA et R. R. CRENSHAW. Can. J. Chem. **61**, 1771 (1983).

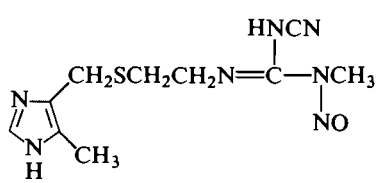
On compare l'étintidine (**4**), un nouvel antagoniste récepteur de H_2 de l'histamine, avec la cimétidine (**1**) du point de vue de la sensibilité à la nitrosation in vitro à des pH de 1 et de 3. Chaque agent donne deux dérivés de mono-*N*-nitrosoguanidine: la *N*-cyano-*N'*{[(méthyl-5 imidazol 1*H*-yl-4)méthylthio]-2 éthyl}-*N''*-nitroso-*N''* (propynyl-2) guanidine (**5**) et la *N*-cyano-*N'*{[(méthyl-5 imidazol 1*H*-yl-4)méthylthio]-2 éthyl}-*N'*-nitroso-*N''* (propynyl-2) guanidine (**6**) à partir de l'étintidine et la *N*-cyano-*N'*-méthyl-*N''* {[(méthyl-5 imidazol 1*H*-yl-4)méthylthio]-2 éthyl}-*N'*-nitrosoguanidine (**2**) et la *N*-cyano-*N'*-méthyl-*N''*{[(méthyl-5 imidazol 1*H*-yl-4)méthylthio]-2 éthyl}-*N''*-nitrosoguanidine (**11**) à partir de la cimétidine. Les dérivés nitroso **5** provenant de l'étintidine se cyclisent en cyanoimino-2{[(méthyl-5 imidazol 1*H*-yl-4)méthylthio]-2 éthyl}-3 méthylène-4 nitroso-1 imidazoline (**7**) à pH neutre ou basique. La nitrosation des deux agents est plus faible à un pH de 3 qu'à un pH de 1 et aux deux pH la nitrosation de l'étintidine est beaucoup plus faible que celle de la cimétidine. On décèle rarement la formation des produits **5** et **6** (chacun <0,5%) à partir de l'étintidine pH de 1 et pour une concentration en nitrite d'environ 150 à 500 fois plus grande que celle du suc gastrique humain à jeûn. On formule des commentaires sur les conditions normales WHO d'étude de la nitrosation des médicaments.

[Traduit par le journal]

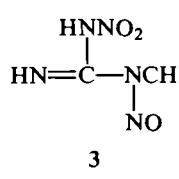
The question has arisen as to whether long-term treatment of disorders of the esophagus, stomach, and duodenum with cimetidine (**1**) might lead to the eventual development of gastric cancer (1). At the root of this concern are a number of factors which include the susceptibility of cimetidine to *N*-nitrosation in vitro (2, 3), the mutagenicity of *N*-nitrosocimetidine (4), the carcinogenic properties of many *N*-nitroso compounds in animals (5), the structural similarities between *N*-nitrosocimetidine (**2**) and the known animal carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG, **3**) (1, 2), the presence of nitrosating species in the stomach (6), and the possibility for levels of nitrosating species and total extractable gastric *N*-nitroso compounds to rise under conditions of lowered gastric acidity (7, 8). Speculation continues in spite of the findings that long-term studies of cimetidine in rats and dogs have revealed no signs of carcinogenesis (9), in vivo formation of *N*-nitrosocimetidine has not been reported, and there is no direct evidence to associate the development of cancer in man with exposure to *N*-nitroso compounds (6).



1 R = CH_3
4 R = $CH_2C{\equiv}CH$



2



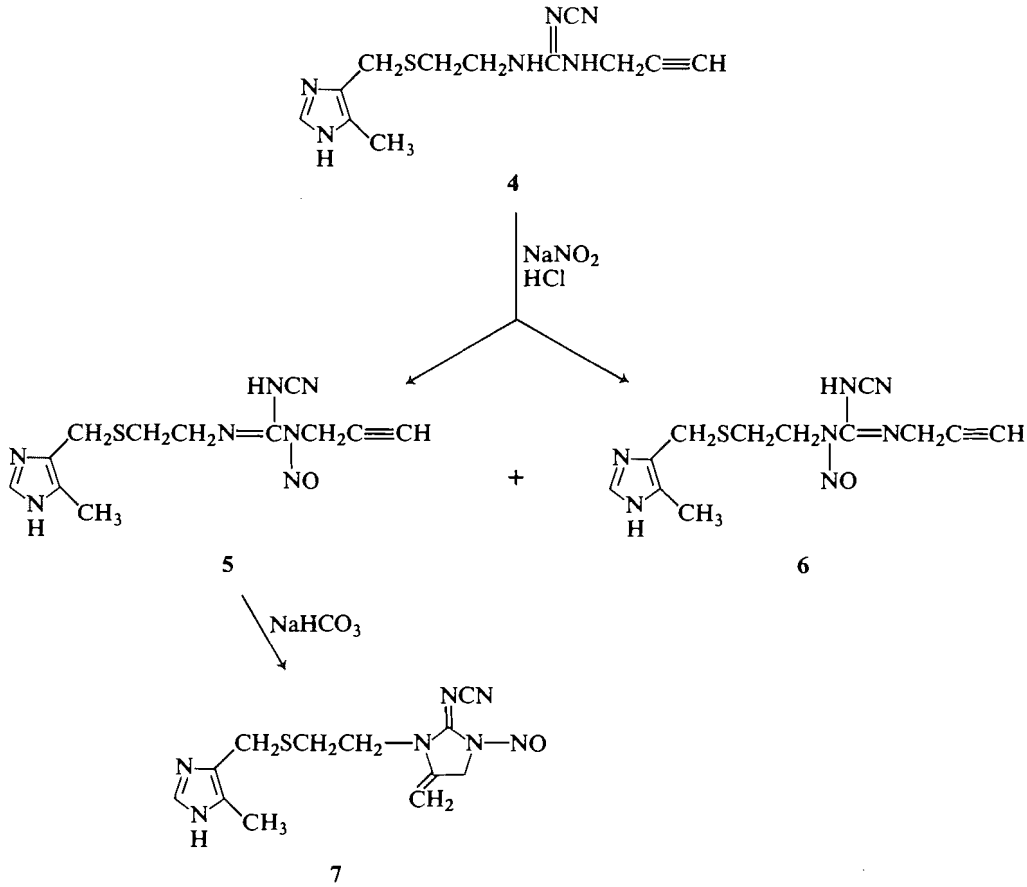
3

A new histamine H_2-receptor antagonist etintidine (BL-5641, **4**), with a potency approximately twice that of cimetidine, was recently reported from these laboratories (10). Because of the structural similarity between cimetidine (**1**) and etintidine (**4**), and because of a regulatory concern in the nitrosation issue, we have compared etintidine with cimetidine for their susceptibilities to *N*-nitrosation in vitro. This paper describes the results and our conclusions from this study.

Chemistry

The nitrosation of etintidine with excess sodium nitrite (4–10 equivalents) in 2 *N* hydrochloric acid gave three products (**5**, **6**, **7**) after neutralization and extraction as outlined in Scheme 1. The three products were separated and purified by

[1]Author to whom inquiries may be addressed.

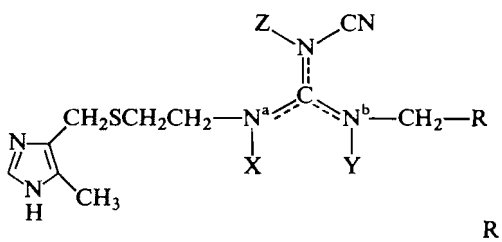


SCHEME 1. Nitrosation of etintidine

semipreparative hplc. The N^b-nitroso[2] compound **5** could not be obtained free from the imidazoline **7** except in acidic solution owing to its facile cyclization to **7** at neutral or basic pH.

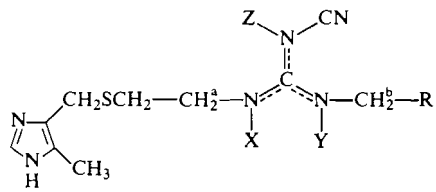
The structural assignment for **6** is supported by elemental analysis, weak maxima in the 380–420 nm region of the uv spectrum (N—NO, $n \rightarrow \pi^*$) (11, 12), and consistent ^1H and ^{13}C nmr spectral data. In the ^1H nmr spectrum (see Table 1), the chemical shifts of the protons of both methylene groups adjacent to the guanidine nitrogens are shifted downfield compared to etintidine, with the methylene protons next to the N^a-NO (CH_2^a in Table 1) shifting further (0.73 ppm) than the propynyl methylene protons (CH_2^b in Table 1) (0.41 ppm). The propynyl methylene protons show as a sharp doublet resulting from coupling to the acetylenic hydrogen. No evidence of coupling to an N—H is seen, therefore we have assigned the tautomer as indicated by **6**. In the case of etintidine, both the CH_2^a and CH_2^b methylenes are coupled to adjacent NH's supporting the tautomer designated in **4**.

[2]For consistency and ease of specifying related products, we have labeled the nitrogens N, N^a, N^b as indicated below:



R = H, —C≡CH
X,Y,Z, see Table 1

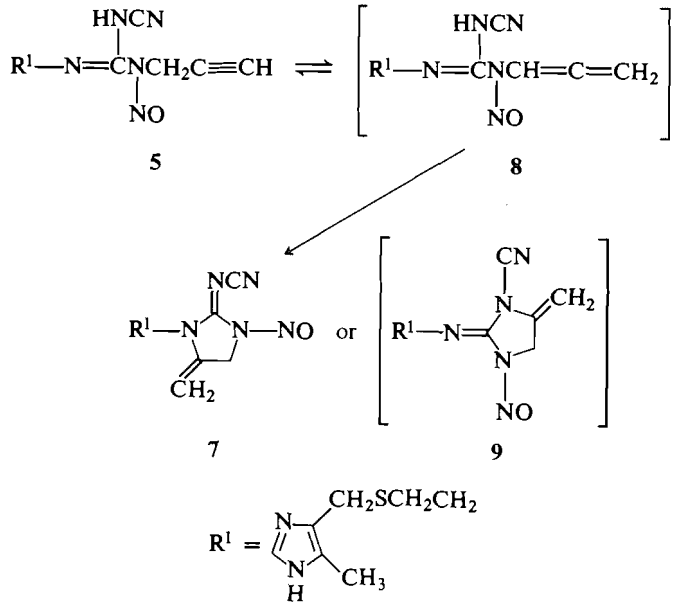
TABLE 1. ^1H nmr chemical shifts



	R	X	Y	Z	^1H nmr chemical shifts[a] CH_2^a	CH_2^b
4 (Etintidine)	C≡CH	H	H	—	3.36	4.00
5	C≡CH	—	NO	H	3.84	4.65
6	C≡CH	NO	—	H	4.09	4.41
1 (Cimetidine)	H	H	H	—	3.32	2.76
2	H	—	NO	H	3.72	3.25
11	H	NO	—	H	3.99	3.17

[a]The nmr spectra were run in DMSO-d_6. Chemical shifts are recorded in ppm relative to Me_4Si.

The structural assignment of **7** is based on ^1H nmr data (discussed below), elemental analysis, and ^{13}C nmr and uv spectra. The structure of **5** is supported by ^1H nmr data (see below) on a mixture of **5** and **7**, and by the ease of conversion of **5** to **7**. The ^1H nmr (360 MHz) spectrum of **7** in DMSO-d_6 shows the absence of the acetylenic proton (3.3–3.5 ppm region) and the presence of two exocylic olefinic protons at 4.82 ppm (1H) and 4.56 ppm (1H). On scale expansion these

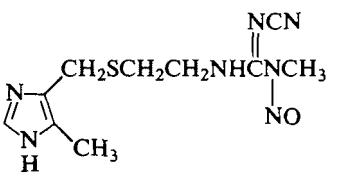


SCHEME 2

appear as narrow multiplets with small couplings to each other and very small long-range coupling to the endocyclic methylene protons. The endocyclic methylene protons appear as a singlet at 4.54 ppm (2H) which on scale expansion show as a very narrow multiplet due to long-range coupling to the olefinic protons. In the ^{1}H nmr spectrum of the mixture of **5** and **7**, the protons of both methylene groups adjacent to the guanidine group in **5** are shifted downfield compared to etintidine (see Table 1) with the methylene protons next to the N^b-NO (CH_2^b in Table 1) shifting further (0.65 ppm) than the methylene protons on the N^a-nitrogen (0.48 ppm).

The cyclization of **5** to **7** was demonstrated by hplc isolation and spiking experiments. A pure fraction of **5** in solution was obtained from reverse phase hplc using an acetonitrile–water–phosphate buffered (pH 4.0) mobile phase. In this solution (pH 4) no evidence of cyclization of **5** to **7** was observed (up to 1.5 hours). When this solution was treated with sodium bicarbonate, the conversion of **5** to **7** was complete in less than one hour. This cyclization may occur via the allene **8** (Scheme 2) (13). The nitrosation of the N^b-guanidine nitrogen activates the propynyl methylene protons so that under neutral or basic conditions the reversible isomerization of **5** to **8** occurs. Cyclization of **8** to **7** follows. Although we have indicated the cyclization to have occurred on the N^a-nitrogen (**7**), our data do not rule out the possibility of cyclization on the N—CN nitrogen to give **9**. We have not observed an analogous cyclization of either etintidine or **6**.

Previously, only one mono-*N*-nitrosated product of cimetidine, an N^b-nitroso derivative, appears to have been isolated and characterized (2, 3). This was indicated to have the tautomeric structure **10** (2). Because the nitrosation of etintidine



10

gave two mono-*N*-nitroso derivatives, we reexamined the nitrosation of cimetidine.

When cimetidine was nitrosated under similar conditions used for etintidine, two mono-*N*-nitroso products were isolated as indicated in Scheme 3. These products **2** and **11** were isolated in approximately 7:1 ratio with the major product apparently the same as the previously described N^b-nitrosated compound **10** (2). Compound **10** has been shown to be a methylating agent, as would be expected if nitrosation had occurred on the N^b-nitrogen (2). The ^{1}H nmr spectra of both **2** and **11** show a downfield shift for the protons of the methyl and methylene groups attached to the cyanoguanidine nitrogens compared to cimetidine (see Table 1). In the case of the N^b-nitroso compound **2**, the methyl protons are shifted further (0.49 ppm) than the N^a-methylene protons (CH_2^a in Table 1) (0.40 ppm), and in the N^a-nitroso compound **11** the methylene protons (CH_2^a in Table 1) are shifted further (0.67 ppm) than the methyl protons (0.41 ppm) relative to cimetidine. Again, our tautomeric assignments are based on ^{1}H nmr. In **2** the CH_2^a is a sharp triplet not coupled to an NH and in **11** the N^b-methyl is a singlet. In the ^{1}H nmr of cimetidine, both the CH_2^a and the N^b-CH_3 are coupled to NH's, which is consistent with the tautomeric structure indicated by **1**.

We have not seen any evidence of a dinitrosated product, although recently a dinitrosated product has been reported when approximately 25 equivalents of $NaNO_2$ were used (14).

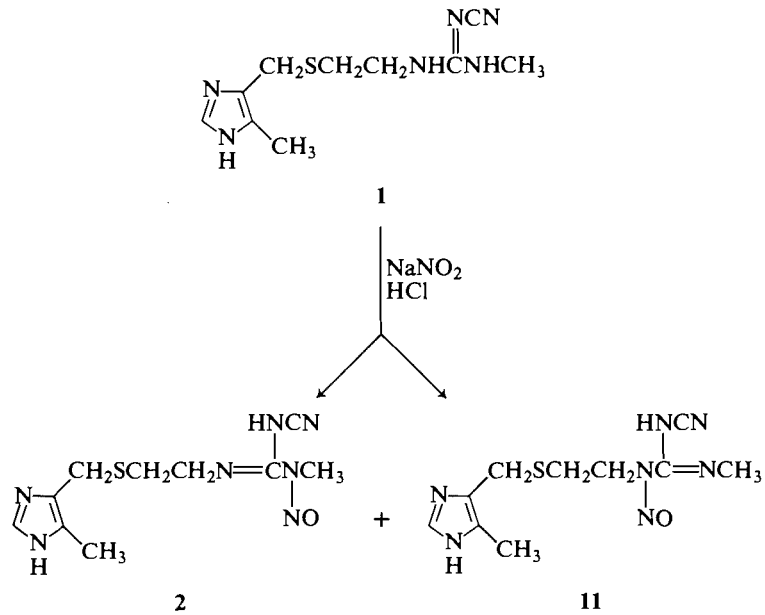
Comparative nitrosation of cimetidine and etintidine

It has been reported (3) that cimetidine was nitrosated <0.5% at pH 4 and 6% at pH 3 under the experimental conditions recommended (15) by a WHO study group for comparing nitrosatable drugs (10 mmol/L compound; 40 mmol $NaNO_2$; pH 3–4; 37°C; one and 4 hour reaction times). When the nitrosation was carried out at pH 1, 39–43% nitrosation occurred (3).

Our initial comparative nitrosation experiments were done in open reaction vessels at pH 1 using WHO recommended concentrations and temperature. The products were analyzed by hplc using a variable wavelength detector set at 220 nm (see Table 2). We found cimetidine was nitrosated rapidly, with peak nitrosation occurring in less than one hour (38% at 1/2 hour). Analysis of the reaction at 4 hours showed a significant loss of the nitrosated cimetidines (4% compared to 38% at 1/2 hour) and a corresponding increase in cimetidine (96% compared to 62% at 1/2 hour). Apparently the nitrosation of cimetidine is a reversible reaction, reversal occurring as nitrosation species are lost to the atmosphere from an open vessel (16). Consequently all subsequent comparative nitrosations were carried out in closed vials.

The nitrosation of cimetidine and etintidine was compared in closed vials, first using WHO recommended concentrations (10 mmol/L drug, 40 mmol/L $NaNO_2$), temperature, and time intervals at both pH 1 and pH 3. The results are summarized in Tables 3 and 4.

At pH 1 cimetidine was nitrosated 51–54% (Table 3) to give both N-nitrosated products **2** and **11** with the N^b-nitroso compound **2** predominant by a factor of 12 at one hour and 6 after 4 hours. Etintidine at pH 1 was nitrosated 6–13% (Table 3) to give both **5** and **6**. About equal amounts of **5** and **6** were present after 4 hours. None of the cyclic material **7** was observed at this pH. The major difference was the amount of the N^b-nitrosoetintidine **5** formed (4–8%) as opposed to the amount of N^b-nitrosocimetidine **2** formed (45–48%). The N^a-nitrosated



SCHEME 3. Nitrosation of cimetidine

TABLE 2. Nitrosation of cimetidine at pH 1 and 37–38°C (open reaction vessel)[a]

Time, h	% Cimetidine unreacted[c]	Analysis of reaction[b]		
		% **1**	% **2**	% **11**
0	100	100	—	—
0.5	65	62	34	4
1	71	70	26	4
4	94	96	2.4	1.5

[a]10 mmol/L cimetidine; 40 mmol/L $NaNO_2$.
[b]Relative percentage of products in reaction mixture.
[c]Compared to peak height of zero time hplc.

derivatives (**6**, **11**) appeared to form about equally though again etintidine may possibly form less N^a-nitroso product.

At pH 3 (Table 4) both cimetidine and etintidine were nitrosated considerably less than at pH 1. Only 4% of the major nitrosation product **2** of cimetidine was formed while the minor isomer **11** was barely detectable (0.6%) at 4 hours. Both *N*-nitroso products of etintidine were barely detectable (<0.5%).

When, however, the concentration of nitrite at pH 1 was reduced to 2 mmol/L and that of cimetidine or etintidine to 0.5 mmol/L, thus retaining the recommended 4:1 drug/nitrite molar ratio, the mononitroso products **2** and **11** of cimetidine were formed in only 4.5 and 0.9% yields, respectively (Table 3). The mononitroso products **5** and **6** of etintidine were barely detectable (each <0.5%, Table 3) at this lower concentration.

Discussion and conclusions

At pH 1 and 3 etintidine and cimetidine each formed two mono-*N*-nitrosoguanidine derivatives in vitro. Our results confirm that nitrosation of cimetidine decreases as the pH is raised (3) and show a similar effect of pH on nitrosation of etintidine.

At both pH 1 and 3 etintidine was nitrosated considerably less than cimetidine, and the extent of nitrosation of both compounds was dependent on the concentration of nitrite. Thus at pH 1, with the WHO recommended concentration of nitrite (40 mmol/L), nitrosation of etintidine was 6–13% compared to 51–54% for cimetidine. This nitrite concentration, however, is approximately 3 000–10 000 times that to be expected in fasting normal human gastric juice (17, 18) and about 140 times that after a nitrite rich meal (17). Reducing the nitrite concentration at pH 1 to a more reasonable level of 2 mmol/L, which is still about 150–500 times that to be expected in fasting normal human gastric juice (17, 18) and about 7 times that after a nitrite rich meal (17), total nitrosation of etintidine was less than 1% (barely detectable) compared to 5% for cimetidine. At pH 3 with 40 mmol/L nitrite, total nitrosation of etintidine was less than 1% compared to 1–5% cimetidine. Clearly, the extent of in vitro nitrosation of etintidine at both pH 1 and 3 under normal physiological nitrite concentrations would be small indeed and beyond our present limits of detection.

At pH 1 and 3 both etintidine and cimetidine were nitrosated on the N^a-cyanoguanidine nitrogen to a similar (small) extent, as might be predicted based on the similarity of environment. Etintidine was nitrosated on the N^b-cyanoguanidine nitrogen considerably less than cimetidine, probably the result of the greater electronic withdrawing and steric hindrance properties of the propynyl compared to the methyl group.

Standard WHO conditions for comparing nitrosatable drugs in vitro include a recommended pH range of 3–4, which, it is suggested, is optimal for most nitrosation reactions (15). This may be the case for secondary amines, but is not the case for cimetidine and etintidine, and may well not be the case for other amide, urea, or cyanoguanidine type compounds (6, 12). The pH range for testing should be extended down to 1, the lower limit being within the pH range of the human stomach (19).

Since N-nitrosation can be a readily reversible reaction (6) as has been shown for cimetidine, standardization (or statement) of reaction vessel conditions (open or closed) is important. Judging from the reasonably comparable percentage conversions of cimetidine to *N*-nitrosocimetidine attained by Bavin *et al.* (3) and ourselves, the former workers' experiments presumably were conducted in closed vessels/cells. Finally, perhaps the difficulties which have apparently been experienced in

TABLE 3. Comparative nitrosation at pH 1 and 37–38°C

Compound	Time, h	N^b-NO product	%	N^a-NO product	%
40 mmol/L $NaNO_2$					
Cimetidine (10 mmol/L)	1	**2**	47.5	**11**	4
	4	**2**	45.3	**11**	8
Etintidine (10 mmol/L)	1	**5**	6(4–8)	**6**	2
	4	**5**	6(4–8)	**6**	5
2 mmol/L $NaNO_2$					
Cimetidine (0.5 mmol/L)	1	**2**	5.0	**11**	~0.7
	4	**2**	4.5	**11**	~0.9
Etintidine (0.5 mmol/L)	1	**5**	<0.5	**6**	<0.5
	4	**5**	<0.5	**6**	<0.5

TABLE 4. Comparative nitrosation at pH 3 and 37–38°C

Compound	Time, h	N^b-NO product	%	N^a-NO product	%
40 mmol/L $NaNO_2$					
Cimetidine (10 mmol/L)	1	**2**	1.3	**11**	<0.5
	4	**2**	4.2	**11**	<0.6
Etintidine (10 mmol/L)	1	**5**	<0.5	**6**	<0.5
	4	**5**	<0.5	**6**	<0.5

attempts to isolate or identify *N*-nitrosocimetidine from the gastric juices of subjects receiving cimetidine (20) may have been compounded by this reversibility potential.

Experimental

Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Varian–Cary model 219 spectrophotometer over the range of 350–450 nm using 95% EtOH as solvent. The ^1H and ^{13}C nmr spectra were recorded on a Varian XL100 instrument or, when indicated, on a Bruker 360 MHz instrument. Chemical shifts were recorded in parts per million (δ) relative to Me_4Si as internal standard.

High pressure liquid chromatography (hplc) was performed using a Waters Associates M6000 pump and U6K loop injector and a LDC variable wavelength uv detector. The following columns, mobile phases, and wavelengths were used: (A) Alltech RSil $C_{18}HL$ (10 μ) (250 × 4.6 mm), 3:2 H_2O–MeCN, 220 nm; (B) Whatman Mag 9 Partisil-10 silica gel (500 × 9 mm), 20:1 MeCN – 95% EtOH, 254 nm; (C) Alltech RSil $C_{18}HL$ (10 μ) (250 × 4.6 mm), 2:1 H_2O–MeCN, 0.01 molar in H_3PO_4 adjusted to pH 4.0 with 20% NaOH, 220 nm; (D) Waters μ-Porasil silica gel (300 × 3.9 mm), 200:10:1 CH_2Cl_2 – 95% EtOH – conc. NH_4OH, 235 nm; (E) Waters μ-Bondapak C_{18} (10 μ) (300 × 3.9 mm), 8:1 H_2O–MeCN, 0.01 molar in H_3PO_4 adjusted to pH 4.0 with 20% NaOH, 220 nm.

For the comparative nitrosation studies, the solutions were analyzed by hplc (5.0–10.0 μL injections) using peak height comparisons to known mixtures of starting materials and products. The data in Tables 3 and 4 are the average of 3 or more hplc determinations. Because the N^b-nitrosoetintidine (**5**) was not obtained pure, the value for **5** was determined assuming a similar uv response (220 nm) for **5** and **6** and this accounts for the ranges (estimated) given in Table 3.

*Nitrosation of etintidine (BL-5641) (**4**)*

A solution of **4** (1.6 g, 5.8 mmol) in 50 mL H_2O and 10 mL concentrated HCl was treated in portions with $NaNO_2$ (3.5 g, 50 mmol) over a period of 10 min. This solution was stirred 30 min at 20°C and then added to a stirred mixture of EtOAc (100 mL), $NaHCO_3$ (15 g), and H_2O (60 mL). The two layers were separated and the aqueous layer extracted further with EtOAc (2 × 75 mL). The dried (Na_2SO_4) extracts were concentrated to leave an amorphous foam (1.8 g). The hplc (A) analysis of this material indicated three products, **5** (*k'* 4.3), **6** (*k'* 2.7), **7** (*k'* 3.6), and starting material **4** (*k'* 2.6). These materials were separated by semi-preparative hplc (B) and characterized as indicated below.

*N-Cyano-N'-{2-[(5-methyl-1H-imidazol-4-yl)methylthio]ethyl}-N'-nitroso-N''-(2-propynyl)guanidine (**6**)*

The first material off the hplc was identified as **6** and was obtained as a viscous oil containing MeCN (0.25 mol): uv λ: 390 nm (shoulder), λ_{max}: 402 (ε 155), λ 420 (shoulder); ^1H nmr (Me_2SO-d_6) δ: 7.57 (s, 1H, imidazole H), 4.41 (d, 2H, N—CH_2—C≡), 4.09 (t, 2H, CH_2—CH_2—NNO), 3.73 (s, 2H, imidazole CH_2—S), 3.44 (t, 1H, ≡CH), 2.60 (appears to be triplet for S—CH_2—CH_2 under Me_2SO peaks), 2.24 (s, 3H, imidazole CH_3), 2.17 (s, 0.75 H, CH_3CN); ^{13}C nmr (Me_2SO-d_6) δ: 155.5 (N—C(=N)—N), 133.2 (C-2 imidazole), 129.3 and 124.8 (C-4 and C-5 imidazole), 113.7 (C≡N), 78.7 (CH_2—C≡C), 74.8 (C≡CH), 42.2 (imidazole CH_2—S), 32.0, 26.8, 25.9 (N—CH_2CH_2S and N—CH_2—C≡), 9.6 (imidazole CH_3). *Anal.* calcd. for $C_{12}H_{15}N_7OS \cdot 0.25(C_2H_3N)$: C 47.46, H 5.03, N 32.18; found: C 47.13, H 5.40, N 31.89.

*N-Cyano-N'-{2-[(5-methyl-1H-imidazol-4-yl)methylthio]ethyl}-N''-nitroso-N''-(2-propynyl)guanidine (**5**)*

The second material off the hplc was identified as **5**. Very careful fraction cutting was required to separate **5** from the starting material **4** which came off the hplc immediately after **5**. Material **5** was obtained free of starting material, but was not obtained free from **7** since concentration of the hplc fractions of **5** always resulted in partial cyclization of **5** to **7** (see below); ^1H nmr (on mixture of **5** and **7**) (Me_2SO-d_6) δ: 7.47 (s, imidazole H (**5** and **7**)), 4.83 (m, one of =CH_2 (**7**)), 4.65 (d, N(NO)CH_2C≡ (**5**)), 4.55 (d, other =CH_2 and endocyclic CH_2 (**7**)), 3.84 (2 overlapping t, CH_2—CH_2—N (**5** and **7**)), 3.75 (s, imidazole CH_2—S (**7**)), 3.76 (s, imidazole CH_2S (**5**)), 3.32 (t, ≡CH (**5**)), 2.81 and 2.73 (2 overlapping t, S—CH_2—CH_2 (**5** and **7**)), 2.18 and 2.19 (ss, imidazole CH_3 (**5** and **7**)).

*2-Cyanoimino-3-{2-[(5-methyl-1H-imidazol-4-yl)methylthio]ethyl}-4-methylene-1-nitrosoimidazoline (**7**)*

The fourth material isolated from the semi-preparative hplc was an amorphous solid identified as **7**; uv λ_{max}: 400 nm (ε 252), λ_{max}: 418 (ε 178); ^1H nmr (pyridine-d_5) δ: 7.88 (s, 1H, imidazole H), 5.02 (q, 1H, one of =CH_2), 4.59 (d, 3H, other =CH_2 and endocyclic CH_2),

4.06 (s) and 4.02 (t) (4H, imidazole CH_2—S and CH_2CH_2-N), 2.98 (t, 2H, S—CH_2—CH_2), 2.38 (s, 3H, imidazole CH_3); ^{1}H nmr (Bruker; Me_2SO-d_6) δ: 7.52 (s, 1H, imidazole H), 4.82 (m, 1H, one of =CH_2), 4.56 (m, 1H, other =CH_2), 4.54 (s, 2H (narrow multiplet on scale expansion), endocyclic CH_2), 3.85 (t, 2H, CH_2CH_2N), 3.74 (s, 2H, imidazole CH_2S), 2.72 (t, 2H, S—CH_2—CH_2), 2.18 (s, 3H, imidazole CH_3); ^{13}C nmr (Bruker; Me_2SO-d_6) δ: 151.7 (s, N—C(=N)—N), 136.1 (s, N—C(=CH_2)—), 133.2 (d, C-2 imidazole), 129.7 and 126.0 (ss, C-4 and C-5 imidazole), 113.0 (C≡N), 88.0 (t, C=CH_2), 47.0 (t, endocyclic CH_2), 41.5 (t, imidazole CH_2—S), 26.7 (tt, S—CH_2CH_2—N), 9.76 (q, CH_3). *Anal.* calcd. for $C_{12}H_{15}N_7OS$: C 47.20, H 4.95, N 32.11; found: C 46.84, H 4.90, N 32.34.

Cyclization of ***5*** *and* ***7*** (analytical hplc detection)

A. The mixture of **5** and **7** from above was dissolved in Me_2SO + MeCN and treated with 2 drops of dilute $NaHCO_3$. The reaction was followed by hplc (C). The hplc indicated complete conversion of the **5** to **7** within 1 h.

B. A pure fraction (in solution) of **5** was isolated from the analytical hplc (C). Reanalysis of a portion of this solution both immediately and after 1.5 h showed only **5**. Treatment of this pure fraction with a few drops of dilute $NaHCO_3$ converted **5** completely to **7** within 1 h (identity by hplc spiking).

*Nitrosation of cimetidine (**1**)*

A solution of **1** (1.26 g, 5 mmol) in water (50 mL) and concentrated HCl (10 mL) was treated in portions with $NaNO_2$ (3.5 g, 50 mmol) over 10 min. The reaction was stirred for 0.5 h at room temperature (20°C). The reaction was diluted with water (60 mL), layered with EtOAc (100 mL), and neutralized with $NaHCO_3$ (15 g). The layers were separated and the aqueous layer was extracted with additional EtOAc (2 × 75 mL). The EtOAc extracts were dried (Na_2SO_4) and concentrated to a yellow gum (1.3 g). The hplc (D) analysis indicated two products (**2** (k' 1.0) and **11** (k' 1.43); 7:1 ratio) and some starting material (k' 3.43). These products were separated by preparative hplc (Waters Associates Prep LC 500A; one PrepPAK-500/silica column; elution with 200:2:1 CH_2Cl_2 – 95% EtOH – conc. NH_4OH; 200 mL/minute) and characterized as indicated below.

N-*Cyano*-N′-*methyl*-N″-*{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]-ethyl}*-N′-*nitrosoguanidine (**2**)*

The first material eluted was the major product, identified as **2**, mp 112–114°C dec. (from MeCN) (lit. (2) mp 112–113°C); uv λ: 385 nm (shoulder), λ_{max}: 397 (ε 200), λ: 414 (shoulder). ^{1}H nmr (Me_2SO-d_6) δ: 7.42 (s, 1H, imidazole H), 3.72 (s and t, 4H, imidazole CH_2—S and CH_2CH_2N), 3.25 (s, 3H, N—CH_3), 2.78 (t, 2H, S—CH_2—CH_2), 2.14 (s, 3H, imidazole CH_3); ^{13}C nmr (Me_2SO-d_6) δ: 156.1 (N—C(=N)—N), 133.2 (C-2 imidazole), 129.4 and 125.0 (C-4 and C-5 imidazole), 114.2 (C≡N), 42.3 (imidazole CH_2—S), 31.0 (N—CH_3), 29.7 and 26.3 (S—CH_2CH_2—N), 9.7 (imidazole CH_3). *Anal.* calcd. for $C_{10}H_{15}N_7OS$: C 42.69, H 5.37, N 34.85; found: C 42.68, H 5.36, N 34.73.

N-*Cyano*-N′-*methyl*-N″-*{2-[(5-methyl-1*H*-imidazol-4-yl)methylthio]-ethyl}*-N″-*nitrosoguanidine (**11**)*

The second material eluted was the minor product, identified as **11**, mp 128–131°C dec. (from MeCN); uv λ: 385 nm (shoulder), λ_{max}: 402 (ε 163), λ: 418 (shoulder); ^{1}H nmr (Me_2SO-d_6) δ: 7.60 (s, 1H, imidazole H), 3.99 (t, 2H, CH_2CH_2—NNO), 3.68 (s, 2H, imidazole CH_2—S), 3.17 (s, 3H, N—CH_3), 2.5 (appears to be triplet for S—CH_2—CH_2 under Me_2SO peaks), 2.18 (s, 3H, imidazole CH_3); ^{13}C nmr (Me_2SO-d_6) δ: 155.9 (N—C(=N)N), 133.2 (C-2 imidazole), 129.2 and 124.8 (C-4 and C-5 imidazole), 114.2 (C≡N), 41.9 (imidazole CH_2—S), 29.4 (N—CH_3), 26.9 and 26.0 (S—CH_2CH_2—N), 9.7 (imidazole CH_3). *Anal.* calcd. for $C_{10}H_{15}N_7OS$: C 42.69, H 5.37, N 34.85; found: C 42.86, H 5.08, N 34.95.

Nitrosation of cimetidine at pH 1 (open reaction vessel)

A suspension of **1** (139 mg, 0.55 mmol) in 50 mL H_2O was adjusted to pH 1.0 with concentrated HCl. The final volume was adjusted to 55 mL with H_2O. This solution was warmed to 36°C and 5.0 mL withdrawn for reference hplc sample. The remaining solution was treated with $NaNO_2$ (138 mg, 2.0 mmol). The temperature was held constant at 36–37°C. The pH fell to 0.93 during the first hour of reaction and was readjusted to 1.0 with 20% NaOH. No further adjustment was needed (pH range 0.98–1.0). The reaction was analyzed by hplc (E) at 0.5 h, 1 h, and 4 h (5.0 mL reaction solution diluted to 50.0 mL with H_2O for analysis). The results are summarized in Table 2.

Comparative nitrosation of etintidine and cimetidine at pH 1

*Etintidine (**4**) (40 mmol/L $NaNO_2$)*

A solution of **4** hydrochloride (172 mg, 0.55 mmol) in 50 mL H_2O was adjusted to pH 1.0 with 3 *N* HCl (about 4.5 mL required). Additional H_2O was added to obtain a volume of 55 mL. This solution was warmed to 35°C and 5.6 mL was withdrawn for reference hplc samples.[3] The remaining solution was treated with $NaNO_2$ (138 mg, 2.0 mmol). The pH remained at 1.0 so that no adjustment was necessary. Two sample vials were filled, sealed, and heated in an oil bath maintained at 37–38°C. At 1 h, 2.6 mL of one of the vials was diluted to 25.0 mL with H_2O and analyzed by hplc (C). At 4 h, 2.6 mL of the other vial was diluted to 25.0 mL with H_2O and analyzed by hplc. The results are summarized in Table 3.

*Cimetidine (**1**) (40 mmol/L $NaNO_2$)*

A suspension of **1** (141 mg, 0.55 mmol) in 50 mL H_2O was adjusted to pH 1.0 with 3 *N* HCl (about 5 mL required). This solution was warmed to 35°C and 5.6 mL was withdrawn for reference hplc samples.[3] The remaining solution was treated with $NaNO_2$ (139 mg, 2.0 mmol). The pH remained at 1.0 so that no adjustment was required. Two sample vials were filled, sealed, and heated in an oil bath maintained at 37–38°C. At 1 h, 2.6 mL of one vial was diluted to 25.0 mL with H_2O and analyzed by hplc (E). At 4 h, 2.6 mL of the other vial was diluted to 25.0 mL and analyzed by hplc. The results are summarized in Table 3.

*Etintidine (**4**) (2 mmol/L $NaNO_2$)*

A solution of **4** hydrochloride (156 mg, 0.5 mmol) in 990 mL H_2O was adjusted to pH 1.0 with concentrated HCl.[3] To this solution was added $NaNO_2$ (138 mg, 2.0 mmol). The pH remained at 1.0. Two sample vials were filled, sealed, and heated in an oil bath maintained at 37–38°C. At 1 h, the solution in one of the vials was analyzed by hplc (C). At 4 h, the solution in the other vial was analyzed by hplc. The results are summarized in Table 3.

*Cimetidine (**1**) (2 mmol/L $NaNO_2$)*

A suspension of **1** (126 mg, 0.5 mmol) in 990 mL H_2O was adjusted to pH 1.0 with concentrated HCl.[3] To this solution was added $NaNO_2$ (138 mg, 2.0 mmol). The pH remained at 1.0. Two sample vials were filled, sealed, and heated in an oil bath maintained at 37–38°C. At 1 h, the solution in one of the vials was analyzed by hplc (E). At 4 h, the solution in the other vial was analyzed. The results are summarized in Table 3.

Comparative nitrosation of etintidine and cimetidine at pH 3

*Etintidine (**4**)*

A solution of **4** hydrochloride (174 mg, 0.56 mmol) and NaCl (128 mg, 2.19 mmol) in 55 mL 0.05 *M* H_3PO_4 was adjusted to pH 3.0 with 20% NaOH. This solution was warmed to 35°C and 5.6 mL was withdrawn for reference hplc samples.[3] The remaining solution was treated with $NaNO_2$ (140 mg, 2.0 mmol). The pH was readjusted to 3.0 with 40% H_3PO_4. Two sample vials were filled, sealed, and heated in a oil bath maintained at 37–38°C. At 1 h, 2.6 mL of one of the vials was diluted to 25.0 mL with H_2O and analyzed by hplc (C). At 4 h, 2.6 mL of the other vial was diluted to 25.0 mL with H_2O and analyzed by hplc. The results are summarized in Table 4.

*Cimetidine (**1**)*

A solution of **1** (140 mg, 0.55 mmol) and NaCl (164 mg, 2.8 mmol)

[3]In all cases the starting solution was analyzed at zero time and after heating in a sealed vial at 37°C for 4 h. Cimetidine was stable at both pH 1 and pH 3. Etintidine was stable at pH 3 but decomposed slightly (~5%) after 4 h at pH 1 and 37°C.

in 55 mL 0.05 *M* H_3PO_4 was adjusted to pH 3.0 with 20% NaOH. This solution was warmed to 35°C and 5.6 mL was withdrawn for reference hplc samples.[3] The remaining solution was treated with $NaNO_2$ (140 mg, 2.0 mmol). The pH was readjusted to 3.0 with 40% H_3PO_4. Two sample vials were filled, sealed, and heated in an oil bath maintained at 37–38°C. At 1 h, 2.6 mL of one of the vials was diluted to 25.0 mL with H_2O and analyzed by hplc (E). At 4 h, 2.6 mL of the other vial was diluted to 25.0 mL with H_2O and analyzed by hplc. The results are summarized in Table 4.

Acknowledgments

We thank the Analytical Research Department for their services and Dr. A. A. Algieri for valuable discussions concerning structure **7**.

1. J. B. Elder, P. C. Ganguli, and I. E. Gillespie. Lancet, **1**, 1005 (1979).
2. A. B. Foster, M. Jarman, D. Manson, and H.-R. Schulten. Cancer Lett. **9**, 47 (1980).
3. P. M. G. Bavin, G. J. Durant, P. D. Miles, R. C. Mitchell, and E. S. Pepper. J. Chem. Res. 212 (1980).
4. B. L. Pool, G. Eisenbrand, and D. Schmähl. Toxicology, **15**, 69 (1979).
5. W. Lijinsky. *In* N-Nitrosamines. *Edited by* J.-P. Anselme. ACS Symposium Series No. 101, ACS, Washington, DC. 1979. pp. 165–173.
6. G. A. Digenis and C. H. Issidorides. Bioorg. Chem. **8**, 97 (1979).
7. P. I. Reed, K. Haines, P. L. R. Smith, F. R. House, and C. L. Walters. Lancet, **2**, 553 (1981), and references cited therein.
8. R. Brimblecombe. Lancet, **2**, 686 (1981).
9. G. P. Crean, G. B. Leslie, and F. J. C. Roe. Lancet, **2**, 797 (1979).
10. R. L. Cavanagh, J. J. Usakewicz, and J. P. Buyniski. Fed. Proc. Fed. Am. Soc. Exp. Biol. **39**, 768 (1980).
11. C. N. R. Rao and K. R. Bhaskar. *In* The chemistry of the nitro and nitroso groups. Pt. 1. *Edited by* H. Feuer. Interscience Publishers, New York. 1969. pp. 137–163.
12. S. J. Mirvish. Toxicol. Appl. Pharmacol. **31**, 325 (1975).
13. M. C. Caserio. *In* Selective organic transformations. Vol. I. *Edited by* B. S. Thyagarajan. Wiley Interscience, New York. 1970. pp. 239–299.
14. D. Ichinotsubo, E. A. MacKinnon, C. Liu, S. Rice, and H. F. Mower. Carcinogenesis, **2**, 261 (1981).
15. WHO Drug Information, **2** (April–June), 4 (1978).
16. T. A. Turney and G. A. Wright. Chem. Rev. **59**, 497 (1959).
17. C. L. Walters, C. S. Dyke, M. J. Saxby, and R. Walker. IARC Sci. Publ. 187 (1976).
18. S. DeFlora and A. Picciotto. Carcinogenesis, **1**, 925 (1980).
19. F. W. Sunderman and F. Boener. *In* Normal values in clinical medicine. W. B. Landers Company, Philadelphia. 1950. p. 230.
20. Minutes, FDA Advisory Committee on Gastrointestinal Drugs. Vol. I. Rockville, Maryland. June 2, 1980.