studies. Even dimeric species may be expected to yield negative NOE's at 270 MHz. For example, the cyclic 12-residue depsipeptide valinomycin exhibits negative NOE's at 250 MHz.[28] In experiments with the D-Ala peptide **2**, only very weak NOE's (~1%) could be detected. Since all other spectroscopic characteristics of the two peptides are clearly very similar, the nonobservation of appreciable NOE's in **2** could result from correlation times very close to the region of zero NOE. These results suggest that at high frequencies, observation of NOE's in oligopeptides of moderate size, may be rendered difficult in certain cases by unfavorable correlation times.[29]

The spectroscopic results described above provide compelling evidence for the antiparallel β-sheet conformation of the bis(cystine peptides) **1** and **2**. The stability of the structure is undoubtedly influenced by the presence of two disulfide cross-links. The similarity of the spectral properties of **1** and **2** suggests that the conformational angles (ϕ,ψ) of the central L- and D-Ala residues may be similar, reflecting the ability of the two covalent S-S cross-links to impose intrinsically unfavorable conformations on the neighboring residues. While the NMR evidence suggests association of peptides in $CDCl_3$ at the concentrations studied, disaggregation is likely in $(CD_3)_2SO$ with little alteration of backbone conformation. In CD studies of the disulfide $n \rightarrow \sigma^*$ transitions (340-250 nm),[30] in $CHCl_3$ and $(CH_3)_2SO$ the position and sign of the S-S cotton effect ($CHCl_3$, 270 nm, **1** 12 900, **2** 17 620 deg cm^2 dmol^{-1}, $(CH_3)_2SO$, 270 nm, **1** 8120, **2** 12 100 deg cm^2 dmol^{-1}) remain unaltered in the two solvents. This suggests that no major change occurs in disulfide conformations on changing solvent polarity in both peptides. The reduced ellipticity value in $(CH_3)_2SO$ is consistent with enhanced flexibility about the S-S bond in this solvent.

The conformations of **1** and **2** involve transannular hydrogen-bond formation, between the CO groups of the residue preceding Cys and the NH group of the succeeding residue. Such structures are likely to be a general feature of cystine residues, in peptides, unconstrained by other factors. Studies of acyclic cystine peptides containing a single S-S bridge suggest that this is indeed the case[31,32] (Antony Raj, P.; Balaram, P., unpublished results). The results of the present study supporting the occurrence of disulfide bridges across an antiparallel β-sheet structure are at variance with conclusions based on analysis of protein crystal structure data, where the observed S-S conformations have generally precluded interstrand bridging in β-sheets.[2] The cyclic bis(cystine peptide) skeleton provides a relatively rigid structural model for further investigations of interactions between S-S linkages and functional side chains. Studies of peptides with different residues in the central position may yield valuable information.

Acknowledgment. We are grateful to Dr. T. M. Balasubramaniam, Washington State University, for providing the FAB mass spectra. This research was supported by a grant from the Department of Science and Technology, India. The use of the WH-270 NMR spectrometer at the Sophisticated Instruments Facility, Indian Institute of Science, is gratefully acknowledged.

Registry No. **1**, 93629-01-7; **2**, 93713-45-2; H-Cys(*S*-Bzl)-OH, 3054-01-1; H-Cys(*S*-Bzl)-OCH_3, 22728-88-7; Boc-L-Ala-OH, 15761-38-3; Boc-D-Ala-OH, 7764-95-6; Boc-L-Ala-Cys(*S*-Bzl)-OCH_3, 98688-00-7; Boc-D-Ala-Cys(*S*-Bzl)-OCH_3, 98688-01-8; Boc-L-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-02-9; Boc-D-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-03-0; H-L-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-04-1; H-D-Ala-Cys(*S*-Bzl)-$NHCH_3$, 98688-05-2; Boc-Cys(*S*-Bzl)-OH, 5068-28-0; Boc-Cys(*S*-Bzl)-L-Ala-Cys(*S*-Bzl)-NHMe, 98688-06-3; Boc-Cys(*S*-Bzl)-D-Ala-Cys(*S*-Bzl)-NHMe, 98688-07-4.

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Structure Elucidation of Nitrosocimetidine, a Mutagenic Charge-Transfer System

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Abstract: The antiulcer drug cimetidine is nitrosated by using $NaNO_2$ and aqueous HCl to generate a mononitroso product which is isolated in up to 80% yield as either the free base or nitrate salt. The structures of both the free base and nitrate salt were elucidated by using spectral methods. It was found that nitrosation of cimetidine causes a shift in the guanidine π-electron distribution from predominantely conjugated cyanimino-type bonding to a more delocalized arrangement typically observed for unsubstituted guanidines. The free base of nitrosocimetidine in $CHCl_3$ or Me_2SO forms a hydrogen bond between the uncharged imidazole and guanidine moieties. The hydrogen bond can be intramolecular, and its formation is followed by the gradual appearance of charge transfer (CT) between the hydrogen-bonded moieties. Similar CT complexes are formed in $CHCl_3$ from equal molar amounts of 1-methylimidazole and *N*,*N*'-dimethyl-*N*''-cyano-*N*-nitrosoguanidine (DCNG) or *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG). The charge-transfer transitions for these systems are similar to those of the $n \rightarrow \pi^*$ transitions normally observed for the nitrosoguanidines, suggesting that nearly equivalent orbital energies are occupied by the π electrons of the guanidine and imidazole moieties in the charge-transfer complex. The greater mutagenic activity reported for nitrosocimetidine as the free base compared to its nitrate salt may be related to the presence of an intramolecular complex in the neutral compound.

A. Introduction

Cimetidine (**1**) is a widely prescribed drug for the control of various disorders of the esophagus, stomach, and duodenum.[1,2] The drug blocks the histamine H_2-receptor controlling gastric acid secretion, so it is frequently used to treat peptic ulcer disease. Although it is quite effective in the management of this disease,[3] a possible association of cimetidine therapy with gastric cancer has led to questioning the safety of this drug. Elder and co-

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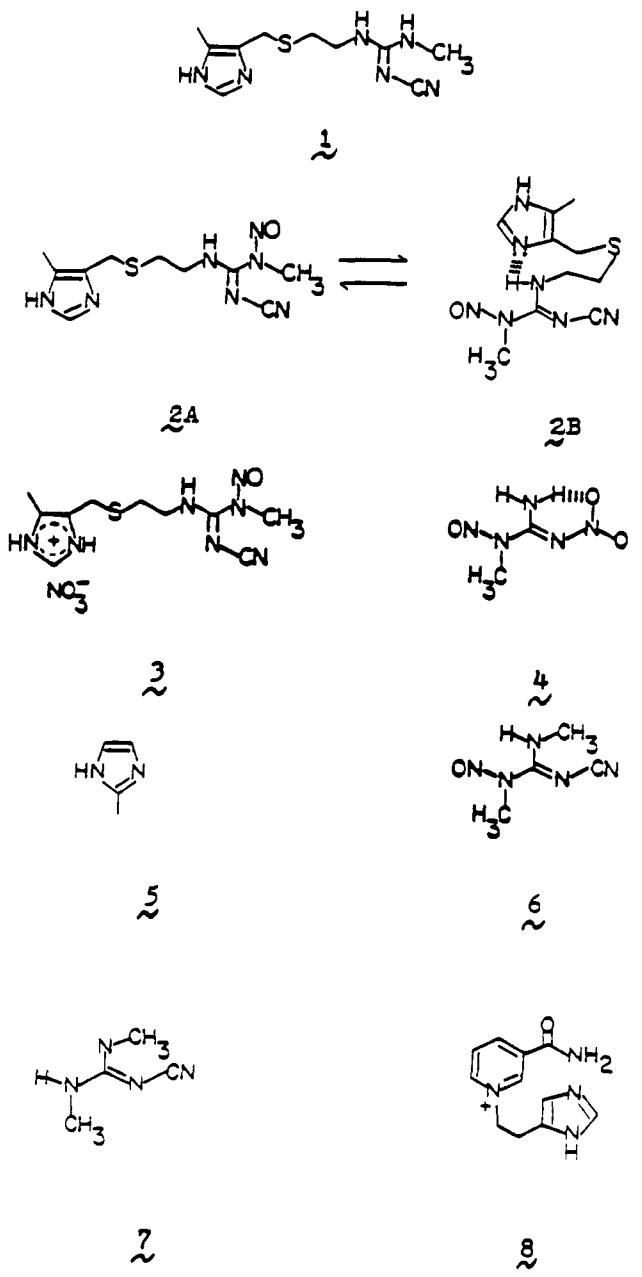
0002-7863/85/1507-8023$01.50/0

Chart I



workers[4] first reported cases of gastric carcinoma detected after cimetidine treatment and expressed concern that this drug may be nitrosated in the human stomach to form a nitrosoguanidine like structure **2A**.

Two different procedures using $NaNO_2$ in acidic aqueous solution nitrosate cimetidine to generate mononitroso derivatives in good yield. However, the product is isolated as the nitrate salt **3** in one procedure[5] and as the neutral form **2** in the other procedure.[6] The major product of the procedure used in this report was initially assigned a dinitroso structure,[7] but additional data have confirmed that it is the neutral mononitroso derivative **2**. However, structure **2A** does not provide sufficient detail to satisfy certain of the chemical and physical data for this compound. The neutral product also demonstrates significantly greater mutagenic

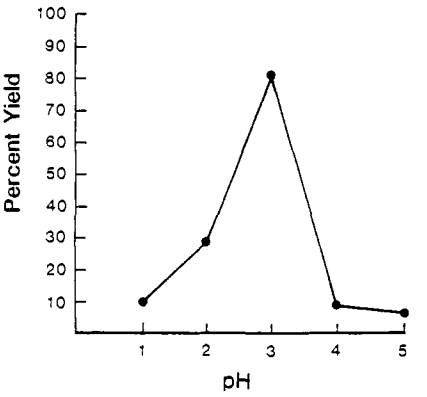


Figure 1. Percent yield of nitrosocimetidine free base vs. pH.

activity than the nitrate salt **3** in both mammalian and bacterial mutagen tests,[8,9] with the neutral form having activity similar to that of the well-known animal carcinogen, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG, **4**), whereas cimetidine itself is not mutagenic or carcinogenic.[10]

We report here evidence of hydrogen bonding between the guanidine and uncharged imidazole moieties of nitrosocimetidine. The hydrogen bonding initially observed for nitrosocimetidine in $CHCl_3$ or Me_2SO changes character as these solutions gradually change color from pale yellow to bright red. This color change represents the formation of a charge-transfer complex between the two hydrogen-bonded moieties. Similar charge-transfer character is observed in $CHCl_3$ solutions containing equimolar amounts of 2-methylimidazole (**5**) and *N*-*N'*-dimethyl-*N''*-cyano-*N*-nitrosoguanidine (DCNG, **6**). The DCNG was readily formed from *N*,*N'*-dimethyl-*N''*-cyanoguanidine (**7**) by using the same conditions for nitrosating cimetidine to the neutral form **2**.

B. Experimental Section

1. General. All melting points were determined by using a Fisher 335 Digital Melting Point Analyzer and the Griess reagent was used to detect the *N*-nitroso moiety. The UV–vis absorption properties were measured in methanol by using a Beckman Acta 25 spectrophotometer, and IR spectra were determined on a Beckman IR-10 spectrophotometer. High-resolution mass spectrometry with electron impact ionization was performed by using a Varian Mat 311 spectrometer, and Field Desorption mass spectra were supplied by the Biomedical Mass Spectrometry Resource, at the University of California, Berkeley. The ^1H NMR spectra at 100 MHz were acquired by using a Varian XL-100 spectrometer interfaced with a Nicolet 1180 data system. Proton chemical shifts are reported on the δ scale and are calculated relative to the solvent offset value of 725 ppm for $CHCl_3$ and 2.49 ppm for Me_2SO. Proton-coupled and -decoupled natural-abundance ^{13}C NMR spectra were measured at 125 MHz by using a Bruker WM-500 spectrometer. Carbon-13 chemical shifts are reported on the δ scale and are calculated relative to the offset value of 39.5 ppm for the solvent, Me_2SO-d_6. Proton-coupled and -decoupled natural-abundance ^{15}N spectra were obtained at 50.68 MHz by using a Bruker WM-500 spectrometer. Nitrogen-15 chemical shifts are derived values in parts per million downfield of liquid ammonia at 25 °C with a possible error of ±1 ppm.

2. Materials. Cimetidine and *N*,*N'*-dimethyl-*N''*-cyanoguanidine were obtained from Smith, Kline & French Co. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co., and 2-methylimidazole was purchased from Pfaltz-Bauer, Inc.

3. Nitrosocimetidine (2). The formation of **2** at different pH values in the range 0–5 began by dissolving 0.25 mL (1.0 nmol) of **1** in 10 mL of 0.11 M H_2KPO_4 buffer and adjusting the pH to the desired value with 12 N HCl. The solution was then cooled to 5 °C in an ice bath, and 2.10 g (30.4 nmol) of $NaNO_2$ was added slowly with mixing. This final reaction mixture was allowed to stand at 5 °C for 30 min. The pH was then increased to 8.5 with 6 N NaOH, followed by extraction of the aqueous solution with 3 × 10 mL portions of $CHCl_3$. The combined $CHCl_3$ layers were dried over anhydrous sodium sulfate and filtered, and

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Table I. ^{1}H NMR 100 MHz Data for Neutral Nitrosocimetidine (**2**), Nitrosocimetidine Nitrate (**3**), *N,N'*-Dimethyl-*N''*-cyanoguanidine (**7**), and *N,N'*-Dimethyl-*N''*-cyano-*N*-nitrosoguanidine (**6**) in Me_2SO-d_6

	chem shift, ppm (multiplicity; coupling const)			
proton	**2**	**3**	**6**	**7**
CH_3—C=	2.15 (s)	2.29 (s)		
—S—CH_2—C—	2.77 (t, J = 7 Hz)	2.77 (t, J = 7 Hz)		
CH_3—N(NO)—	3.25 (s)	3.24 (s)	3.25 (s)	
—C—CH_2—N—	3.72 (t, J = 7 Hz)	3.67 (d, t, J = 7 and 5 Hz)		
=C—CH_2—S—	3.66 (s)	3.89 (s)		
—N—CH=N—	7.38 (s)	8.95 (s)		
—C—N(H)—C=	7.28 (s)	9.61 (t, J = 5 Hz)	9.45 (s)	6.9
>N—H	7.28 (s)	13.5 (s, br)		
H_3C—N			3.10	2.7

Table II. ^{13}C NMR Data (125 MHz) for Neutral Nitrosocimetidine (**2**), Nitrosocimetidine Nitrate (**3**), *N,N'*-Dimethyl-*N''*-cyano-*N*-Nitrosoguanidine (**6**), and *N,N'*-Dimethyl-*N''*-cyanoguanidine (**7**) in Me_2SO-d_6

	chem shift, ppm (multiplicity with off-resonance decoupling)			
carbon	**2**	**3**	**6**	**7**
—N—C(N)—N—	156.3 (s)	156.6 (s)	156.6 (s)	160.9 (s)
—N—C(H)=N—	133.3 (d)	133.3 (d)		
—C=C(N)—CH_2—	129.2 (s)	126.4 (s)		
CH_3—C(N)=C	125.4 (s)	125.8 (s)		
—C≡N	114.4 (s)	114.5 (s)	114.4	118.8
—C—CH_2—S—	42.3 (t)	42.1 (t)		
—C—CH_2—N—	28.3 (t)	30.0 (t)		
CH_3—N(NO)—	30.1 (q)	31.3 (q)	30.8 (q)	
CH_3—N—			29.4 (q)	28.3 (q)
—S—CH_2—C—	26.0 (t)	23.4 (t)		
CH_3—C=C—	9.8 (q)	8.8 (q)		

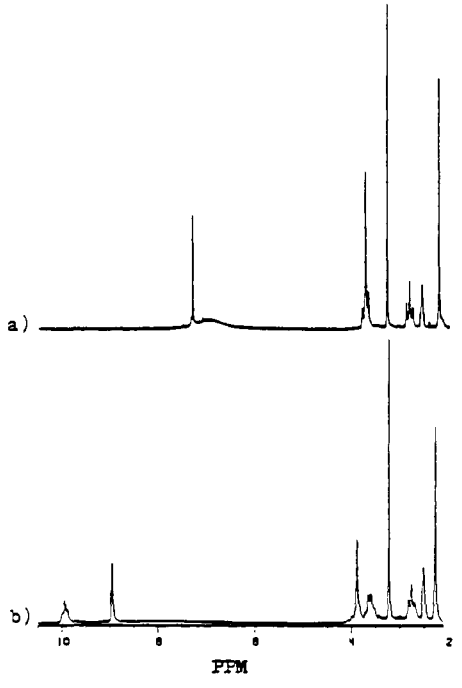

Figure 2. ^{1}H NMR spectra (100 MHz) of (a) nitrosocimetidine free base and (b) nitrosocimetidine nitrate in Me_2SO-d_6.

the solvent was removed under vacuum to afford impure **2** as a yellow viscous oil. This product was purified by column chromatography by using Whatman DC-2 silica gel with 5% acetone in hexane as the solvent and 394-nm detection for nitrosoguanidines. The purity of the eluting product was checked by TLC using Whatman DC-2 silica gel plates developed in 5% acetone in hexane solution and by HPLC using both an Altex 254-69 Lichrosorb RP C_{18} 10-μm column with 40% methanol in water as the solvent and an Altex 254-40 Partisil 10 PAC 10-μm column with 25% 2-propanol in chloroform as the solvent. The Griess reagent was used to detect *N*-nitroso groups in eluting solutions and on developed TLC plates. Purified **2**, obtained as a yellow-to-red oil, was weighed and

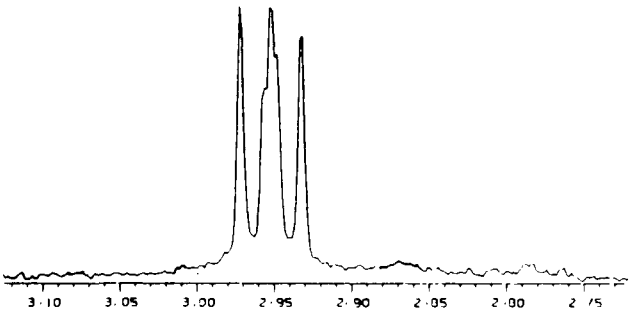

Figure 3. ^{1}H NMR signal (300 MHz) for the methylene-bearing nitrogen of nitrosocimetidine free base in $CHCl_3$.

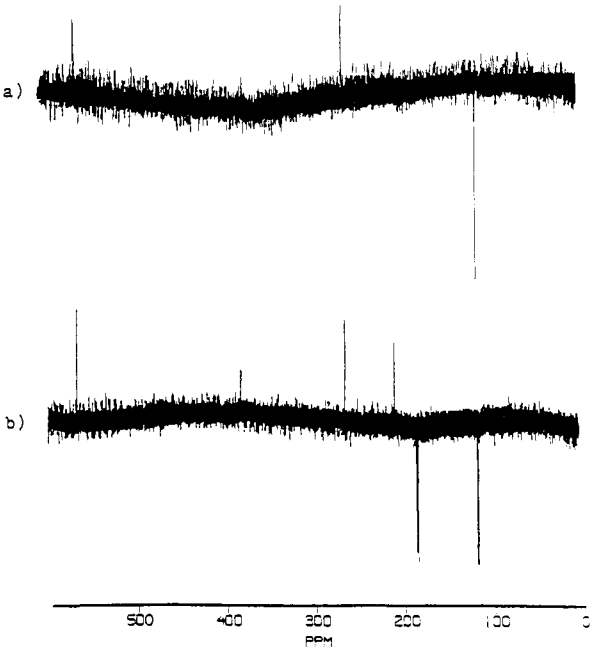

Figure 4. ^{15}N NMR decoupled spectra (52 MHz) of (a) nitrosocimetidine free base and (b) nitrosocimetidine nitrate in Me_2SO-d_6.

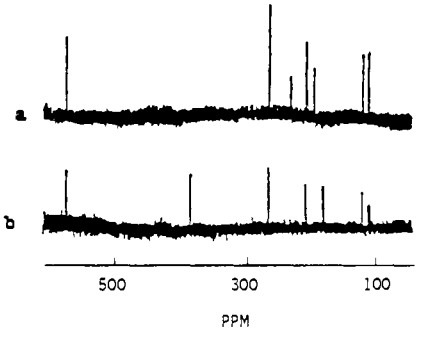

Figure 5. ^{15}N NMR proton-coupled spectra (52 MHz) of (a) nitrosocimetidine free base and (b) nitrosocimetidine nitrate in Me_2SO-d_6.

Table III. ^{15}N NMR Data for Cimetidine (1), Nitrosocimetidine Free Base (2), Nitrosocimetidine Nitrate (3), and *N,N'*-Dimethyl-*N''*-cyano-*N*-nitrosoguanidine (6)

	^{15}N resonance shift, ppm (coupling const)			
nitrogen atom	1	2	3	6
NO		566.4	566.4	564.0
NO_3			380.2	
N–CH_3 (NO)		263.4	263.4	261.6
N≡C–	196.7	207.7	207.9	205.5
N3 (imidazole)		230.5	182.9	
N1 (imidazole)		197.0	(J = 103 Hz)	
	93.6	123.7	124.1	121.3
	91.9 (J = 90 Hz)	115.0	115.1 (J = 90 Hz)	100.4
	79.6 (J = 90 Hz)			

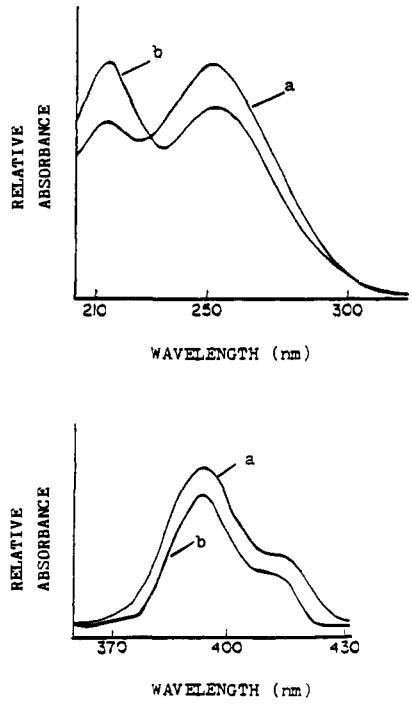

Figure 6. UV–vis absorption spectra for (a) nitrosocimetidine free base and (b) nitrosocimetidine nitrate in MeOH. Visible spectrum (bottom) plotted ~100 times the intensity of UV spectrum (top).

the yield calculated for each reaction (Figure 1). Spectral data were then acquired for pure **2**: UV (MeOH, initial) λ_{max} 215 nm (ϵ 15 934), 254 (26 211), 395 (>200); IR (chloroform) ν 3440 (NH free), 3230 (NH bonded), 2920 (CH), 2160 (C≡N), 1620 (1st NO, C=N), 1480 (NN-O), 1200 (2nd NO), 1010 (C—NO), 890 (NN), 720 (NNO), 645 cm^{-1} (NNO); MS (FD), m/z 281 (M^+, 100%); ^1H NMR (see Figures 2 and 3 and Table I); ^{13}C NMR (see Table II); ^{15}N NMR (see Figures 4 and 5 and Table III).

4. Nitrosocimetidine Nitrate (3). This compound was prepared by using a modification of a published procedure.[5] A solution of 1.25 g (5.0 mmol) of **1** in 15 mL of 1.25 N HCl was maintained at 0 °C in an ice bath while adjusting the pH to a value in the range 0–5 using 1 N NaOH. This was followed by the addition of 1.40 g (20.0 mmol) of $NaNO_2$ with stirring. After 2 h at 0 °C, a pale-yellow crystalline precipitate was filtered from the cold solution, washed with 3 × 5 mL portions of cold distilled water and dried under reduced pressure to yield 1.31 g (4.0 mmol, 80%) of **3**: mp 145–146 °C; UV (MeOH) (Figure 6) λ_{max} 214 nm (ϵ 31 552), 254 (20 719), 395 (192); IR (KBr) 3200, 2180, 1645, 1475, 1350 cm^{-1}; MS (EI), m/z 281 (M^+, 3%), 251 (M – NO, 5%); MS (FD), m/z 281 (M^+, 100%); ^1H NMR (see Figure 2 and Table I); ^{13}C NMR (see Table II); ^{15}N NMR (see Figures 4 and 5 and Table III).

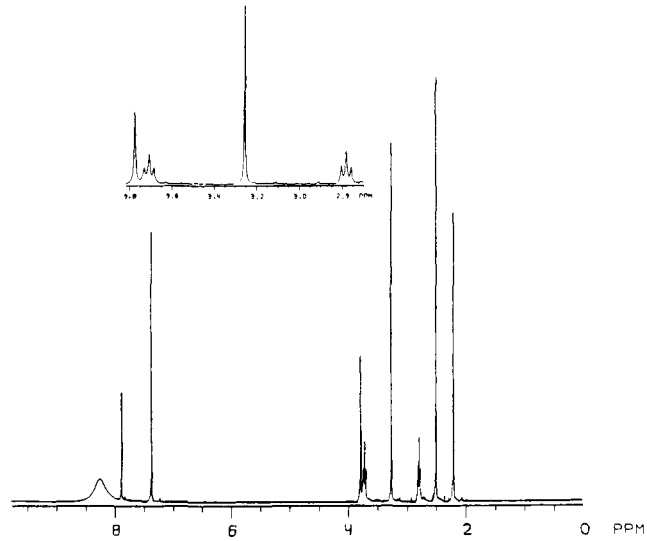

Figure 7. ^1H NMR spectrum (300 MHz) for equal molar amounts of 2-methylimidazole and nitrosocimetidine nitrate in Me_2SO-d_6.

5. *N,N'*-Dimethyl-*N''*-cyano-*N*-nitrosoguanidine (DCNG 6). Compound **6** was formed from **7** by using a method similar to that used to prepare **2**. A solution of 1.12 g (10.0 mmol) of **7** in 100 mL of 1.25 N HCl was adjusted to pH 3.0 with 1 N NaOH and cooled to 5 °C in an ice bath. Then 2.10 g (300 mmol) of $NaNO_2$ was added slowly with mixing, and the final solution was allowed to stand in an ice bath. After 2 h, a yellow precipitate was filtered from this solution, washed with 3 × 5 mL of cold water, and recrystallized from hot ethanol to yield 1.22 g (87%) of **6** as bright yellow crystals: mp 132–133 °C; UV (MeOH) λ_{max} 215 nm (ϵ 9640), 254 (15 864), 395 (199); MS (EI), m/z 141 (M^+, $C_4H_7N_5O$, 14%), 112 (M – NO + H, $C_4H_8N_4$, 19%), 82 (M– $ONNCH_3$, 100%); ^1H NMR (see Table I); ^{15}N (see Table III).

6. *N,N'*-Dimethyl-*N''*-cyano-*N*-nitrosguanidine (6) plus 2-Methylimidazole (5). To 1 mL of Me_2SO-d_6 was added 14.1 mg of **6**, followed by 8.2 mg of **5**. The resulting solution was then analyzed by using ^1H NMR at 300 MHz (see Figure 7): δ 2.09 (3 H, S), 2.38 (3 H, S), 2.67 (2 H, t, J = 2.3 Hz), 3.14 (3 H, S), 3.60 (2 H, t, J = 2.3 Hz), 3.69 (2 H, S), 7.24 (2 H, S), 7.75 (1 H, S), and 7.80 (3 H, br s).

C. Results and Discussion

1. Nitrosation of Cimetidine. A single neutral *N*-nitroso derivative was isolated when cimetidine was reacted with a 30 mol excess of $NaNO_2$ in aqueous HCl at pH 1–5. This product was isolated in good yield over a narrow pH range by using these conditions (Figure 1). A maximum yield of 81% was achieved at pH 3.0 but rapidly declined with decreasing pH to 19% at pH 2.0 and 10% at pH 1.0. The optimum pH of 3.0 for this procedure generating nitrosocimetidine in good yield is unusually high since low yields of nitrosguanidines are generally expected for nitrosation at pH 2.0.[12] Also, it has been reported that the rate of nitrosation of cimetidine diminishes markedly with increasing pH above 1.0; only 1.7% conversion to nitrosocimetidine was detected after 1 h at pH 3.0 and 37 °C.[5] However, those results for the rate of cimetidine nitrosation were obtained by a procedure using only a 4 mol excess of $NaNO_2$, and the procedure using a 30 mol excess of $NaNO_2$ may rely on different mechanisms to nitrosate cimetidine.

2. ^1H NMR. The spins of the guanidino proton and the adjacent methylene protons are strongly coupled in Me_2SO-d_6 solutions of the nitrate salt **3**, but not in similar solutions of nitrosocimetide free base (Figure 2 and Table I). Also, proton spectra of **2** show a single resonance signal for the imidazole and guanidine protons of nitrogen. This uncoupled signal is observed near 9 ppm in freshly prepared Me_2SO-d_6 solutions, with slow upfield migration of the signal to values as high as 6.5 ppm after 24 h or more at room temperature. When $CHCl_3$ is the solvent,

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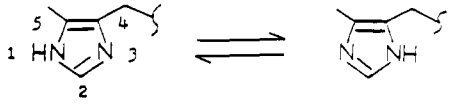
the signal is initially near 7 ppm and can be observed as far upfield as 4.5 ppm after standing at room temperature for 24 h. The broadness of this averaged proton signal suggests that the imidazole and guanidine protons are exchanging at an intermediate rate in these solvents. The upfield migration of this signal is probably due to increased shielding by π electrons or averaging with the signal for water.[13] Shielding appears to be a more favorable explanation since the integration for this signal does not increase with migration and the addition of water to **2** in Me_2SO-d_6 initially results in separate signals for the water and proton on nitrogen.

The signal for protons of the two adjacent methylene groups in **2** show second-order coupling of an AA'BB' spin system (Figure 3), indicating that a preferred conformation exists for this molecule in solution. The same second-order coupling is observed for cimetidine, which is known to form an intramolecular hydrogen bond in crystalline form,[14] but there is no spectral evidence of a charge-transfer complex. This hydrogen bond in cimetidine crystals involves the imidazole N3 imino nitrogen and the proton on the nitrogen bearing methyl to form a 10-membered cyclic structure, but nitrosocimetidine free base can only form a intramolecular bond involving the proton on nitrogen-bearing methylene. No uncoupling or averaging of proton signals is observed for cimetidine in solution, suggesting that hydrogen bonding is probably stronger in **2**.

The 2-H, 5-methyl, and 4-methylene protons of the imidazole system in the nitrate salt **3** produce resonance signals that are noticeably downfield of the corresponding signals for **1** and **2**, which is consistent with the assignment of a protonated imidazole to **3** only.[15]

3. ^{13}C NMR. Nitrosation of cimetidine to form either the free base **2** or the nitrate salt **3** results in an upfield shift of nearly 4 ppm in the central guanidino carbon and the nitrile carbon resonance signals as well as a 2 ppm downfield shift in the *N*-methyl carbon (Table II). Similar changes in carbon-13 chemical shifts are also observed with the nitrosation of **7** to form **6**. Thus, formation of the mononitroso derivative of *N,N*-dialkyl-*N''*-cyanoguanidines appears to shift the guanidine π-bonding character from predominantely conjugated iminonitrile to a system having greater contribution from other resonance forms typical for guanidines.[16]

The imidazole carbon-13 resonances for the nitrate salt **3** compared to the free base forms **1** and **2** are shifted downfield by 0.14 ppm for C5, upfield by 2.8 ppm for C4, and nearly 1 ppm for the 5-methyl carbon. These differences are very similar to those found in the neutral vs. protonated forms of the imidazole in histidine.[17] A larger shift for the C4 than C5 signal with imidazole protonation suggests that the neutral imidazole of **1** and **2** exists primarily as tautomer X in which N1 possesses amino-type



X Y

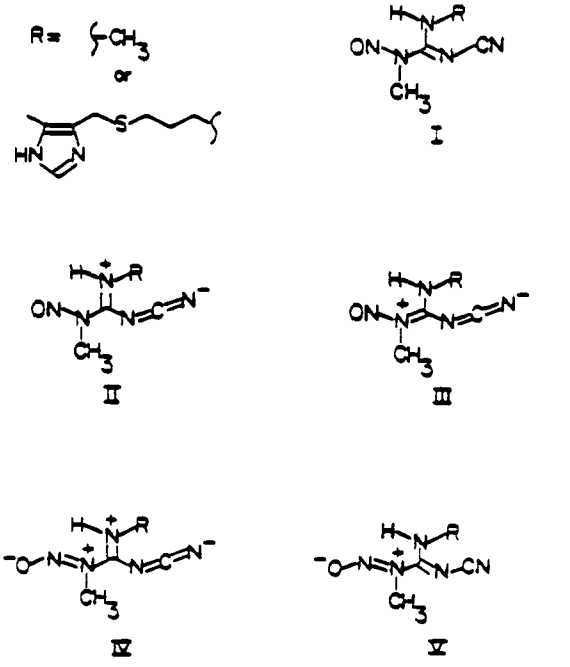
bonding and N3 possesses the imino-type bonding. Thus, an intramolecular hydrogen bond in nitrosocimetidine must involve the N3 imidazole nitrogen and the proton on nitrogen bearing methylene to form an eight-membered structure similar to **2B**.

4. ^{15}N NMR. A comparison of the 51.6-MHz coupled ^{15}N NMR spectra of **2** and **3** in Me_2SO-d_6 is shown in Figure 4, and the data from these spectra and the coupled spectrum for **1** are presented in Table III. The ^{15}N data for the uncoupled spectrum of **6** in Me_2SO-d_6 are also presented in Table III, and a comparison of the coupled ^{15}N spectra for **2** and **3** is shown in Figure 5. The assignment of ^{15}N resonance signals was accomplished by correlation with the chemical shifts reported for similar nitrogen atoms in other molecules.[18]

Nitrosation of cimetidine (**1**) results in a 184 ppm downfield shift in the ^{15}N signal representing the methyl-bearing nitrogen, and a single signal for *N*-nitroso nitrogen is observed in the spectra of **2** and **3**, indicating that nitrosation occurs at the methyl-bearing nitrogen to form the isolated products. Nitrosation of **1** also produces downfield shifts in ^{15}N signals of 11 ppm for the nitrile nitrogen and 23 ppm for the nitrogen-bearing nitrile. These shifts in ^{15}N signals for guanidino nitrogens not substituted during nitrosation show that a different electronic configuration exists for the guanidine moiety when nitrosated. It can be seen in the coupled spectrum for **2** that negative nOe experienced by three of the four guanidino nitrogens results in the loss of their signals in the noise. The signals for the guanidino nitrogen-bearing methylene in **2** and **3** experience similar strong nOe in the coupled spectra, indicating that a hydrogen atom is strongly bonded to this hydrogen at a similar distance in all these compounds. The guanidino ^{15}N–^{1}H bond in **3**, however, allows strong coupling of the spins of these nuclei ($J_{^{15}N-^{1}H}$ = 92.4 Hz), while the spins of these nuclei in **2** and **6** are uncoupled as a result of proton exchange. When the uncoupled signal in the proton spectrum of **2** is selectively irradiated, the ^{15}N resonances of the imidazole nitrogens and the guanidino nitrogen-bearing methylene experience a significant nOe. This demonstrates that the broad uncoupled proton signal for **2** represents two protons on nitrogen, and these protons appear to be exchanging only between the imidazole nitrogens and the methylene-bearing guanidino nitrogen. Thus, only one tautomer of the guanidino moiety was detected for **2** in ^{15}N NMR studies.

There is only one ^{15}N resonance signal for the imidazole nitrogens of the nitrate salt **3**. This signal is strongly coupled (90 Hz) to the proton signal at 3.67 ppm and has a chemical shift in the range for imidazole cations. These imidazole nitrogen data, together with a characteristic nitrate anion (NO_3^-) resonance at 380.2 ppm, confirm that **3** is a nitrate salt.

The N1 and N3 signals for neutral imidazoles assume chemical shifts which differ in proportion to changes in K_T, the equilibrium constant for the tautomerization of the imidazole ring between forms X and Y.[21] Thus, the greater the difference in N1 and



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N3 signals, the greater the deviation of K_T from unity. The N1 signal for the free base **2** is 13.5 ppm further downfield than for the nitrate salt **3**, while the N3 signal is observed 49.9 ppm further downfield and is much broader than the corresponding N1 signal. The 36.4 ppm separation between N1 and N3 signals for **2** corresponds to a major deviation in K_T from unity such that N1 is predominately an imino-type nitrogen in this neutral imidazole. This shift is K_T is not explainable entirely on the basis of inductive effects by the asymmetric imidazole substitution since much smaller differences in the N1 and N3 shifts are observed for similarly substituted imidazoles in the absence of other effects.[15] The N1 and N3 chemical shift differences for **2** are close to those measured for imidazoles with side chains in the 3-position which establish bonding selectively with N3, as is the case in histamine and histidine.[19] Thus, the best explanation for the predominately tautomer X character of **2** is stabilization of this form by intramolecular hydrogen bonding.

5. UV–Vis Spectroscopy. The superimposed UV–vis spectra for freshly prepared **2** and **3** in methanol (Figure 6) have the same three absorption maxima between 200 and 450 nm. For **3**, λ_{max} 214 nm is more intense than λ_{max} 254 nm and λ_{max} 395 nm is relatively weak. For **2**, λ_{max} 254 nm is the most intense absorption. The absorption at λ_{max} 214 nm is more intense in **3**, but absorptions at λ_{max} 395 nm are stronger for **2**. These different intensities for similar peaks in the two absorption curves indicate differences in the contribution of the absorbing species to the structures. The absorption at λ_{max} 214 nm probably results from $\pi \rightarrow \pi^*$ transitions involving the isolated cyanimino and nitrosamino chromophores, while absorptions at λ_{max} 254 nm and λ_{max} 395 nm are $\pi \rightarrow \pi^*$ and $\eta \rightarrow \pi^*$ transitions, respectively, which are characteristic for *N*-nitrosoguanidines.[20] This explanation for the absorption differences, however, may be too simple since the intensity of the λ_{max} 395 nm absorption for **2** increases with time, while the absorption curve for **3** does not change. The rate of this increase in absorption is enhanced by certain solvents; it requires at least 24 h for a solution of **2** in chloroform at room temperature to be stabilized in coloration and many days for the color change to be completed in Me_2SO. The final color of **2** is most intense in $CHCl_3$, and the solubility of **2** in this solvent decreases as the coloration deepens to result in the deposition of an oily layer on the walls of the containers holding $CHCl_3$ solutions of this compound. Compound **6** in MeOH has a UV–vis absorption curve very similar to that of freshly prepared **2**, but no increase in UV–vis absorption with time has been observed for $CHCl_3$ solutions of **6**. Thus, the neutral imidazole is necessary for the gradual increase in nitrosoguanidine absorption at λ_{max} 395 nm demonstrated by solutions of **2**.

6. Model Systems. The addition of an equimolar amount of 2-methylimidazole (**5**) to the nitrate salt (**3**) in Me_2SO-d_6 results in uncoupling of the ^1H NMR signals for the guanidino proton on nitrogen and the adjacent methylene protons, with a single signal appearing for the two protons on nitrogen (Figure 7). There is also a 0.04 ppm downfield shift in the resonance of the *N*-methylene protons with no second-order coupling evident in this signal. Thus, the second-order coupling of *N*-methylene protons in nitrosocimetidine free base (**2**) is probably due to intramolecular rather than intermolecular interactions.

The addition of an equimolar amount of 2-methylimidazole to *N*,*N'*-dimethyl-*N''*-cyano-*N*-nitrosoguanidine (DCNG, **6**) in $CHCl_3$ results in uncoupling of the proton on nitrogen and the adjacent methyl proton resonances of this model guanidine with a 0.02 ppm downfield shift in the uncoupled signal for the methyl protons. A 1:1 complex of the two starting molecules is formed that is less soluble in $CHCl_3$ than the individual components, and some of this complex crystallizes from concentrated solutions of the mixed components. The crystal structure of this complex is presently under investigation.

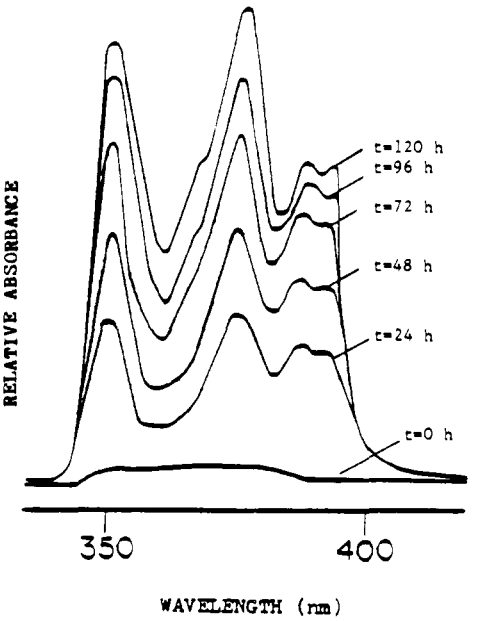

Figure 8. Change in UV–vis absorption with time (t) for a 1:1 mixture of 2-methylimidazole and DCNG in $CHCl_3$.

The difference in UV–vis absorption with time between a 1:1 mixture of 2-methylimidazole (**5**) plus DCNG in $CHCl_3$ (sample cell) and an equivalent amount of DCNG alone in the $CHCl_3$ (reference cell) is shown for the 335–420-nm range in Figure 8. Since imidazole **5** does not absorb above 200 nm at the experimental concentration, only absorption by the nitrosoguanidine **6** is subtracted to isolate absorption due to interaction between the two compounds. There is initially no difference in absorption for the two solutions, but after 24 h, a complex absorption pattern with maxima at λ_{max} 332, 358 (shoulder), 383, 402, and 415 nm is evident. The relative intensities of these absorptions remain constant, while their molar absorptivities (ϵ) continue to increase with time until 120 h. After that time, only a small increase in absorbance is detected.

The appearance of new UV–vis absorption in the solutions containing a neutral imidazole and a nitrosoguanidine probably denotes charge transfer (CT) between the two components following the formation of electron-donor–acceptor (EDA) complexes.[21] The choice of CT over polarization bonding to explain the absorptions is made on the basis that little change from the initial bonding in the complexes was observed by NMR measurements as the absorptions increased in intensity. Only the resonances of protons on nitrogen change as CT absorptions increase, and the upfield migration of their averaged signal with increasing CT absorptions suggest that π-shielding of the exchanging protons increases with complex formation.

A charge-transfer band at λ_{max} 294.5 nm for *N*-(β-(4'-imidazolyl)ethyl) 3-carbamoylpyridinium chloride (**8**) in methanol has been attributed to an intramolecular complex between the pyridinium and imidazole rings.[22] This complex may also involve intramolecular hydrogen bonding between an imidazole nitrogen atom and a proton on the nitrogen atom of the carbamoyl substituent, resulting in an eight-membered cyclic conformation similar to that proposed for **2**. The CT absorption observed for the 1:1 complexes and nitrosoguanidines probably represent $\eta \rightarrow \pi^*$ transitions of a single η electron from an imidazole nitrogen atom to a π^* orbital of the nitrosoguanidines since the values of absorption maxima and general curve shapes are similar for the CT absorptions and the $\eta \rightarrow \pi^*$ absorptions normally present in the visible spectrum of the nitrosoguanidines alone. If this is the CT transition, the energy of the imidazole η electrons must be similar to the energy of the η electrons of the nitrosoguanidine system in these complexes.

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D. Conclusions

The treatment of cimetidine with $NaNO_2$ and aqueous HCl affords a *N*-nitroso derivative which can be readily isolated in good yield (~80%) as either the neutral form using pH 3.0 and 30 mol excess of $NaNO_2$ or as the nitrate salt using pH ≤1.0 and 4 mol excess of $NaNO_2$. This nitrosation of cimetidine causes a shift in the guanidine π-electron distribution from predominantly conjugated cyanimino-type bonding to a more delocalized arrangement typically observed for unsubstituted guanidines. Nitrosocimetidine free base forms an intramolecular complex involving the imidazole and nitrosoguanidine moieties, with rapid initial hydrogen bonding leading to slow equilibration to a relatively stable complex. The final complex in neutral nitrosocimetidine exhibits increased absorption in the visible range, probably as a result of charge transfer involving promotion of an imidazole η electron to a π^* orbital of the nitrosoguanidine. The character of this CT transition is similar to that of the η–π^* transition normally found in nitrosoguanidines, suggesting that nearly equivalent orbital energies are occupied by the η electrons of the nitrosoguanidine and imidazole in the complex.

Although cimetidine is not mutagenic in the short term bacterial and mammalian mutagen assays, both the neutral nitrosocimetidine and its nitrate salt are mutagenic in these assays with the neutral compound having a much greater mutagenicity. The *N*-nitroso moiety is certainly a contributing factor to the mutagenicity, but the difference in mutagenicity between the neutral nitrosocimetidine and its nitrate salt may be the result of the physical properties of the compounds and the biological systems in which they were tested. These would include pharmacological properties such as solubilities and membrane transport as well as inherent chemical reactivity. The significance of charge transfer to the mutagenicity of nitrosocimetidine is unknown. Neutral nitrosocimetidine exhibits mutagenic and nuclear fragmentation activity similar to that measured for the known animal carcinogen MNNG, and MNNG also forms an intramolecular complex with rapid proton exchange.[23] The nitrate salt does not form this intramolecular complex and does not appear to readily convert in aqueous solution at physiological pH to the charge-transfer complex.

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A Theoretical Study of the Phosphinonitrene (H_2P=N)–Iminophosphane (HP=NH) Rearrangement

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Abstract: The interconversion of phosphinonitrene, H_2PN, and iminophosphane, HP=NH, is studied in both the singlet and triplet electronic states using ab initio methods. At the MP4SDQ/6-31++G*//HF/3-21G* level plus zero-point energy contributions, the energy barrier for the rearrangement to iminophosphane in the singlet state is calculated to be 33.1 kcal/mol. In the triplet state, the barrier is 30.2 kcal/mol. The triplet–singlet energy separation in H_2PN is predicted to be small (7 kcal/mol). There is thus no intersystem crossing. In both states, the predicted energy barriers appear to be sufficient to guarantee the existence of the H_2PN species, if formed during the decomposition of iminophosphanes in an inert matrix at low temperature. The vibrational frequencies of the species studied are also calculated, and the P–N stretching frequency of H_2PN is predicted to lie in the region of 1250–1300 cm^{-1}. Finally, the *Z–E* isomerization in iminophosphane and the mechanism of the 1,2-hydrogen shift (from a LMO analysis) are considered.

The question of whether or not nitrenes (RN) exist as discrete intermediates during the thermolysis and photolysis of azides (RN_3) has long attracted the attention of both experimentalists and theoreticians.[1] It now appears that their existence strongly depends upon the nature of the substituents R. In the case of azidophosphines (R_2PN_3), evidence for the formation of phosphinonitrenes (R_2PN) has recently been reported by Bertrand, Majoral, and collaborators[2,3]. These authors have shown that the products **1** obtained by photolysis of an azidophosphine at room temperature in the presence of various trapping agents AX can be considered as 1,2-adducts on the P–N bond of intermediate phosphinonitrenes, and no insertion products of type **2** were observed in their experiments. In the absence of trapping agents, phosphinonitrenes undergo dimerization, yielding stable cyclodiphosphazenes **3** in quantitative yield.[3] Furthermore, in contrast to tetra- or pentacoordinated phosphorus azides,[4,5] a Curtius-type rearrangement leading to iminophosphanes **4** (or phospha(III)-azenes) does not take place. Hence the reported experimental data[2,3] clearly demonstrate the stabilization of nitrenes by phosphino (R_2P) groups.

The present theoretical study aims to quantitatively assess this stabilization by ab initio calculations on the interconversion pathway between two model species: phosphinonitrene (H_2PN) and iminophosphane (HP=NH) via a 1,2-hydrogen shift. This

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