Found: C, 51.07; H, 6.48; N, 14.71; S, 13.45.

4ba$_i$ (R^1 = Ph and R^2 = *i*-C_5H_{11}): mp 153.5-154.5 °C (CH_2Cl_2); ^1H NMR δ 0.84 (d, J = 6 Hz, 6 H), 1.35-1.57 (m, 3 H), 2.27 (t, J = 7 Hz, 2 H, $COCH_2CH_2$), 2.82-2.94 (m, 2 H, SCH_2CH_2N), 3.44-3.52 (m, 2 H, SCH_2CH_2N), 3.71-3.80 (m, 2 H, SCH_2CO), 7.06-7.12 (m, 1 H), 7.29-7.36 (m, 2 H), 7.49-7.55 (m, 2 H), 8.57 (s, 1 H), 10.31 (s, 2 H), 10.82 (s, 1 H); MS, *m/e* 426. Anal. Calcd for $C_{18}H_{26}N_4O_4S_2$: C, 50.70; H, 6.15; N, 13.14; S, 15.01. Found: C, 50.89; H, 6.01; N, 12.85; S, 14.84.

4c$_n$c (R^1 = *n*-C_5H_{11} and R^2 = cyclo-C_5H_9): mp 146.0-147.5 °C (CH_2Cl_2); ^1H NMR δ 0.86 (t, J = 7 Hz, 3 H), 1.17-1.86 (m, 14 H), 2.71-2.90 (m, 3 H, COCH and SCH_2CH_2N), 3.12-3.18 (m, 2 H, $NCH_2CH_2CH_2$), 3.42-3.49 (m, 2 H, SCH_2CH_2N), 3.59-3.65 (m, 2 H, SCH_2CO), 8.18 (s, 1 H), 8.58 (s, 1 H), 10.31 (s, 1 H), 10.44 (s, 1 H). Exact mass for $^{12}C_{17}{}^1H_{30}{}^{14}N_4{}^{16}O_4{}^{32}S_2$: calcd, 418.1709; found, 418.1720.

4c$_i$c (R^1 = *i*-C_5H_{11} and R^2 = cyclo-C_5H_9): mp 150.0-151.5 °C (CH_2Cl_2); ^1H NMR δ 0.87 (d, J = 7 Hz, 6 H), 1.31-1.84 (m, 11 H), 2.70-2.90 (m, 3 H, COCH and SCH_2CH_2N), 3.14-3.21 (m, 2 H, NCH_2CH_2CH), 3.43-3.50 (m, 2 H, SCH_2CH_2N), 3.59-3.64 (m, 2 H, SCH_2CO), 8.17 (s, 1 H), 8.57 (s, 1 H), 10.30 (s, 1 H), 10.42 (s, 1 H); MS, *m/e* 418. Anal. Calcd for $C_{17}H_{30}N_4O_4S_2$: C, 48.77; H, 7.24; N, 13.39; S, 15.30. Found: C, 48.63; H, 7.23; N, 13.40; S, 15.35.

Oxidation of a Pair of Thiols. A mixture of **1** (0.50 mmol) and **2** (0.50 mmol) in 12.5 mL of a solvent was stirred vigorously under oxygen for 15 min in a well-stirred water bath which was thermostated to ±0.1 °C for 20-50 °C and to ±0.5 °C for 70 °C. To this mixture was added Et_3N (0.05 mmol), and vigorous stirring was continued for the time required to complete the oxidation (the oxidation was performed at least twice under the same conditions). When the oxidation was completed, the reaction mixture was evaporated to dryness. The yields of **3** and **4** were determined by use of their absorption at 280 (system A) and 254 nm (systems B-D) after separation of disulfides **3-5** in the mixture by HPLC using μ-Bondapak-CN (system A) and LiChrosorb CN (systems B-D) with *n*-hexane-*i*-PrOH [85:15 (system A), 95:5 (systems B and C), and 94:6 (system D)] as an eluent. The r values given in Figures 1-5 and Tables I-V represent the mean values of two or more experiments and were reproducible within the errors shown therein. The errors in r values are far smaller for systems A_i and A_n because of the larger molar extinction coefficients (ϵ)[25] of the corresponding disulfides.

Acknowledgment. We express our sincere thanks to Professor Teruaki Mukaiyama for encouragement with this work. This work was partly supported by Grant-in-Aids for Scientific Research 61226012 and 62216019 from the Ministry of Education, Science and Culture, Japan. We thank Mr. Tsukasa Ishiodori for helpful suggestion, Mr. Takashi Suzuki for valuable assistance, and Mr. Akihiko Kusai, JEOL, for measuring high-resolution mass spectra.

Kinetics and Mechanism of the Decomposition in Aqueous Solutions of 2-(Hydroxyamino)imidazoles

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Abstract: A kinetic study is reported of the reaction in aqueous solution whereby 1-X-2-(hydroxyamino)imidazoles **2** are converted into 1-X-2-amino-4,5-dihydro-4,5-dihydroxyimidazolium ions, with substituents X = H (**2a**), CH_3 (**2b**), CH_2CH_2Br (**2c**), $CH_2CHOHCH_2OCH_3$ (**2d**), $CH_2CONHCH_2CH_2OH$ (**2e**), and $CH_2CHOHCH_2NC_5H_{10}$ (**2f**). A mechanism is proposed with the neutral form of the imidazole as the kinetically active species, undergoing rate-limiting cleavage of the N-O bond with no catalysis (OH^- as leaving group) and with catalysis by the hydronium ion and by buffer acids. These reactions produce a resonance-stabilized imidazolenitrenium ion **7**, which reacts with water and added nucleophiles leading to products. Observations consistent with the mechanism include the following: (i) The 1,3-dimethyl-2-(hydroxyamino)imidazolium ion, a model of protonated **2** that cannot be converted to the reactive neutral form, is unreactive. (ii) Under conditions where 98% of the products are due to reaction with the added nucleophile glutathione (GSH), there is no change in rate constant. (iii) The rate-pH profiles for **2b-2e** have regions at high pH and low pH where the rate constants are independent of pH, as required by the mechanism. (iv) The acidity constant for **2bH$^+$** obtained through kinetic analysis is the same as that obtained by NMR spectroscopy. (v) Effects of the N-1 substituents are consistent with the formation of an electron-deficient intermediate. (vi) Decreasing solvent polarity results in a decrease in the rate constant of the uncatalyzed N-O heterolysis, with an m value of ~0.5. The proposed mechanism is a heterocyclic analog of the Bamberger rearrangement of *N*-phenylhydroxyamine to *p*-aminophenol. The 2-imidazole system is however more reactive, a feature shown to be predictable on the basis of the σ^+ value for this group. Through analogy with acetal hydrolysis, the production of a stabilized cationic intermediate is suggested to be responsible for the presence of general acid catalysis. The ratio k_{GS}:k_w for reactions of the nitrenium ion **7b** with glutathione anion and water is 5×10^5 M^{-1}. This implies that k_w must be less than 10^4 s^{-1}, since the reaction with the thiol anion cannot occur faster than diffusion. A comparison with k_w values for other nitrenium ions and carbenium ions shows that the imidazolenitrenium ion is an exceptionally long-lived species. Metabolic reduction of 2-nitroimidazole drugs is known to result in covalent binding to DNA as well as in depletion of intracellular glutathione; the possibility that the nitrenium ion is responsible is considered in the context of the results of this investigation.

Nitroimidazoles see use against a variety of anaerobic bacterial and protozoal infections[1] and are currently in clinical trials as radiation sensitizers of hypoxic (oxygen-deficient) tumor cells.[2] Biological investigations of 2-nitroimidazoles **1** (Scheme I), the derivatives more commonly employed in the latter studies, have demonstrated that there are a number of effects that appear to correlate with reductive metabolism, with the implication that some product or intermediate of reduction of the nitro group is the biologically active species.[3]

To evaluate the origins of these effects, we have been examining the chemistry of the 2-(hydroxyamino)imidazoles **2** which result from reduction with four-electron equivalents. Such species can

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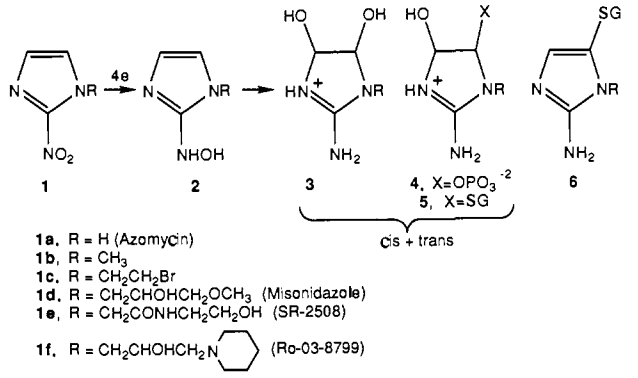
Scheme I



be chemically prepared but are unstable under physiological conditions.[4] The product in the absence of added nucleophiles (and oxygen) is a 2-amino-4,5-dihydro-4,5-dihydroxyimidazolium ion **3**, obtained as a cis:trans mixture.[4b,d,e,5] With phosphate present (from the buffer), monophosphate **4** is also observed,[4d] while with thiols two products **5** and **6** are found.[4b,e,6,7] Through preliminary kinetic investigations with 1-methyl-2-(hydroxyamino)imidazole (**2b**) we have proposed a mechanism for the formation of these products based upon an electrophilic nitrenium ion intermediate.[4b] In this paper we report detailed kinetic studies that provide convincing evidence in favor of this mechanism. The hydroxylamines investigated (Scheme I) include the derivatives of misonidazole (**1d**), the drug most extensively investigated as a radiation sensitizer,[8] of SR-2508 (**1e**) and Ro-03-8799 (**1f**), misonidazole analogues currently under clinical study,[9] and of azomycin (**1a**), the nitroimidazole whose antibiotic properties were first recognized.[10] A comparison with benzenoid hydroxylamines, which react in a similar manner, is presented. Also reported are the results of competition experiments involving partitioning of the nitrenium ion between water and the added thiol glutathione, γ-L-glutamyl-L-cysteinylglycine (GSH), the latter chosen because of its biological importance (vide infra).

Experimental Section

Materials. 2-Nitroimidazoles. Misonidazole (**1d**), SR-2508 (**1e**), and Ro-03-8799 (**1f**) were supplied by Dr. A. M. Rauth of the Ontario Cancer Institute. Azomycin (**1a**),[11] 1-methyl-2-nitroimidazole (**1b**),[11] and 1-(2′-bromoethyl)-2-nitroimidazole (**1c**)[12] were prepared following literature methods. 1,3-Dimethyl-2-nitroimidazolium trifluoromethanesulfonate (**1g**·$CF_3SO_3^-$) was prepared by the reaction of **1b** with an equivalent amount of methyl trifluoromethanesulfonate in anhydrous dichloromethane. After standing overnight, dry ether was added resulting in the precipitation of the salt. This was purified by dissolving in dichloromethane, followed by precipitation with ether. ^{1}H NMR (D_2O) δ 4.2 (3 H, s), 7.9 (1 H, s). Anal. Calcd for $C_5H_8N_3O_2{\cdot}CF_3SO_3$: C, 24.75; H, 2.76; N, 14.44. Found: C, 24.43; H, 2.99; N, 14.16.

2-(Hydroxyamino)imidazoles were prepared as hydrochloride salts by electrochemical reduction of the 2-nitroimidazole at pH 3–4.[4a,d,13] Purification involved lyophilization of the aqueous solution after reduction, addition of a minimum quantity (10–20 mL) of methanol, filtration to remove undissolved inorganic salts, and addition of excess anhydrous ether (100–200 mL) to precipitate the product. The purity of the resulting material was determined by analysis of the NMR spectra recorded in acidic D_2O, from the integration of the signals near 7 ppm due to H4 and H5 of the imidazole relative to the methyl signal of *tert*-butyl alcohol, which had been added quantitatively as an internal standard. The isolated solids were in general about 60% by weight 2-(hydroxyamino)imidazole hydrochloride, with about 20% decomposition products such as **3**, which had formed during the time required to prepare and isolate the material. The remainder of the solid was presumably sodium chloride, the electrolyte employed in the reaction. Attempts to purify the material further by recrystallization or by ion-exchange chromatography failed, presumably since the hydroxylamine was unstable in protic solvents. The parent **2a** was also produced from the reaction of 2-fluoroimidazole[14] with hydroxylamine, as previously described.[4a] Kinetic experiments with this material and with samples obtained by reduction of azomycin showed no difference. The hydroxylamines **2a**,[4a,d] **2b**,[4a,b,d,13] **2d**,[4a] and **2e**[4c,e] have been characterized previously; **2c**, **2f**, and the 1,3-dimethyl-2-(hydroxyamino)imidazolium ion (**2g**) were new compounds. The identification of all products as (hydroxyamino)imidazoles was based on several arguments, which have been presented previously.[4] The essential observations were the stoichiometry of reduction, 4.0 ± 0.2 electrons per nitroimidazole, coupled with the signals in the NMR spectrum near 7 ppm, a pair of closely spaced doublets ($J \sim 2$ Hz) for N1-substituted derivatives and a singlet for the symmetrical **2a** and **2g**. Another important indicator was that these peaks disappeared, on standing for long periods in acid solutions and very quickly in neutral D_2O, being replaced by a complex set of resonances at 5.0–5.5 ppm due to the decomposition products.[4d] The exception was **2g**, which was stable (see later).

Buffers and solvents were the best commercial grade and were used without further preparation. Trichloromethylphosphonic acid was prepared following the literature procedure.[15]

Kinetic Methods. Kinetic experiments were performed with a Varian 2300 spectrophotometer equipped with an automatic cell changer and a constant-temperature circulating bath operating at 25.0 ± 0.1 °C. In general 3.0 mL of the appropriate buffer solution was placed in a standard 1-cm UV cuvette, which was then sealed with a rubber septum. Argon gas was then bubbled through the solution for at least 10 min, after which time the cuvette was placed in the spectrophotometer and allowed to stand for 10 min to come to the thermostat temperature. At this point 10 μL of a 10–100 mM solution of the (hydroxyamino)imidazole in 0.01 M HCl was introduced by syringe through the rubber septum, and the cuvette shaken and placed in the UV instrument. The reaction was monitored by measuring the disappearance of the hydroxylamine at 230–240 nm over a period of 3–5 half-lives. Absorbance–time data were collected with an Apple IIe Microcomputer that was interfaced with the spectrophotometer. First-order rate constants were calculated by using a Varian Associates PLUS kinetics calculations program, which fits the data to the exponential equation through a reiterative procedure. In general rate constants obtained from replicate measurements agreed within ±3%. (Initially kinetic studies were performed in open systems, but deviations from exponential behavior and problems with reproducibility were encountered. These were eliminated by saturating the solutions with argon prior to introduction of the hydroxylamine. Oxygen dissolved in water is known to react with 2-(hydroxyamino)imidazoles to produce hydrogen peroxide and dimeric azoxy compounds.[4c,e])

The faster kinetic runs that occurred with **2a** at higher pH were performed with a Durrum-Gibson stopped-flow spectrophotometer operating at 25.0 ± 0.1 °C, by mixing a pH 2 solution of the hydroxylamine (~0.2 mM) with the appropriate buffer. This apparatus was interfaced

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(7) (a) Isolated samples of **6** have been found to consist of two isomers, assigned the structures of the 5-substituted substance as shown in Scheme I and a form with the glutathione attached through carbon 4 of the imidazole ring.[6] However, in our experience, ^{1}H NMR spectra recorded immediately upon completion of the reaction of the hydroxylamine have shown only one isomer, with the second appearing after a considerable time has elapsed.[7b] 2D NMR experiments have conclusively established the structure of the initially formed product as the 5-substituted isomer.[7c] (b) Panicucci, R. Ph.D. Thesis, University of Toronto, 1987. (c) Farah, S., unpublished observations.

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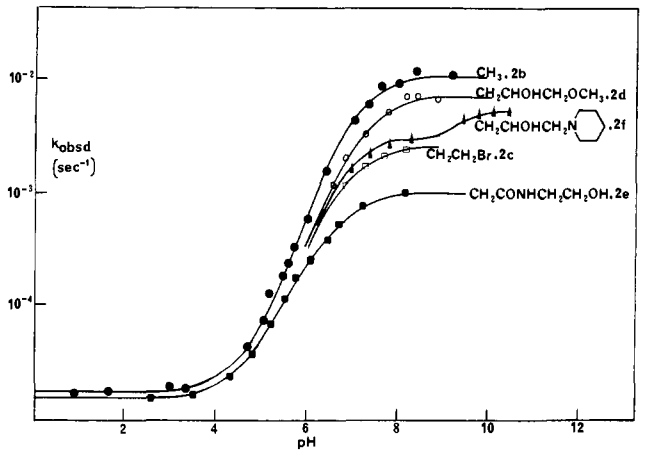

Figure 1. First-order rate constants for 1-substituted 2-(hydroxyamino)imidazoles. Rate constants were obtained by extrapolation to zero buffer concentration, in argon saturated solutions at 25 °C and an ionic strength of 1.00 maintained with $NaClO_4$. Rate constants for **2c**, **2d**, and **2f** at pH < 6 are not shown; these cluster between the rate constants for **2b** and **2e**.

to a Tektronix Minicomputer for data analysis.

Product Analysis. These experiments were carried out essentially as previously described,[4d] by the addition of solid hydroxylamine to solutions containing the appropriate buffer and added reagents, followed by lyophilization of the solution after 15–30 min of standing under argon. After addition of D_2O, spectra were recorded with a Varian XL-400 NMR spectrometer.

Determination of the Acidity Constant of 2b. The dissociation constant of the conjugate acid of 2-(hydroxyamino)-1-methylimidazole was determined by recording 400-MHz ^1H NMR spectra of 0.01 M solutions of the compound in D_2O. A series of buffers in D_2O of Na_2DPO_4:NaD_2PO_4 were prepared at ionic strength 1.0 with the ratios of the components ranging from 1:10 to 100:1. These solutions were added to an NMR tube, followed by the solid hydroxylamine. NMR spectra were recorded as quickly as possible, within 2–3 min of the preparation of the solution. The solutions were removed, and the pH meter reading was obtained with a microcombination electrode. The pD values of these solutions were calculated from the relationship[16] pD = pH(meter) + 0.40. Spectra in 0.001 M DCl and in a pD 11 NaOD solution were also recorded, to establish the chemical shifts of the conjugate acid (δ_{BH}) and conjugate base (δ_B) forms.

Analysis for Unreacted Glutathione. Phosphate buffers (1.5 mL) of pH 6.2–7.7 and ionic strength 1.0 were saturated with argon in a sealed UV cuvette, as described previously, and 5–25 μL of a freshly prepared solution in 0.1 M HCl of 0.012 M reduced glutathione and 0.01 M 1-methyl-2-(hydroxyamino)imidazole hydrochloride was added by syringe. After 20 min of standing to allow for complete reaction of the hydroxylamine, 1.00 mL of an argon-saturated solution of 3.7×10^{-4} M Ellman's reagent in 0.33 M Na_2HPO_4 was added, and the absorbance at 412 nm was recorded as quickly as possible. For each reaction a control experiment was performed by adding the equivalent volume (5–25 μL) of 0.012 M reduced glutathione in 0.1 M HCl to the same phosphate buffer, followed by introduction of the Ellman's solution and measurement of the absorbance at 412 nm following the same procedure.

Results

Rate–pH Profiles. 2-(Hydroxyamino)imidazoles absorb in the region 210–240 nm, with a significant decrease in absorbance upon reaction to the 4,5-substituted products. This decrease obeyed good exponential kinetics when followed in argon-saturated solutions, with the first-order rate constants, which will be defined as k_{obsd}, being dependent upon pH and buffer concentration. Rate–pH profiles are shown in Figures 1 and 2. The general pattern for the substrates with a 1-substituent (Figure 1) consists of a plateau in acid corresponding to a relatively long half-life for the hydroxylamine, with the rate constants increasing between pH 5 and pH 7 to a second plateau at high pH where there is a significantly shorter half-life. The piperidine-substituted **2f** has a similar profile, but there is a slight kink at high pH. With **2a**

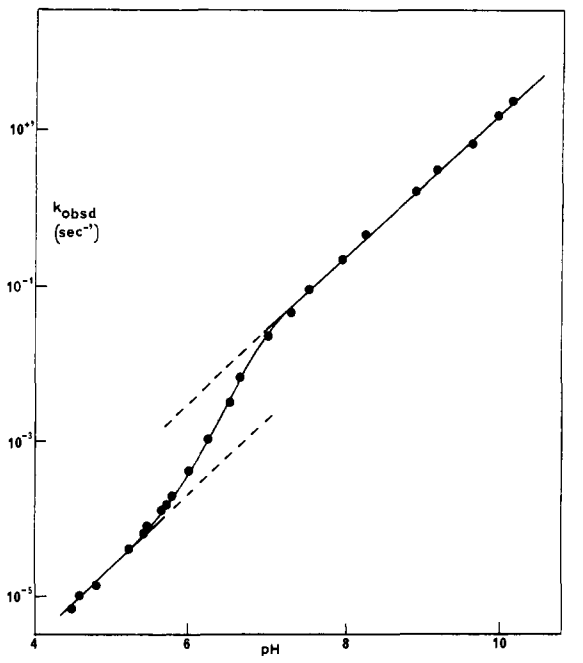

Figure 2. First-order rate constants for 2-(hydroxyamino)imidazole (**2a**). For conditions see Figure 1. The two linear portions have been drawn with a slope of unity for a plot of log k_{obsd} versus pH.

(Figure 2) the rate constants do not level at high pH (the behavior in solutions with pH < 4 was not investigated). Product studies have been carried out with **2a**, **2b**, **2d**, and **2e**,[4b,d,e,5] and in each case **3** (Scheme I) is the principal species formed in the absence of added nucleophiles. Moreover there is no difference in the products between neutral (pH 6–8) and acid (pH 1–3) solutions,[4d] other than the rate of the reaction.

Buffer Catalysis. Rate constants measured at pH $<\sim 7$ were found to depend upon the buffer concentration; a detailed study of this effect was conducted with **2b**. For experiments at constant pH with a given buffer ratio, k_{obsd} increased in a linear fashion with increasing total buffer concentration. The slopes of these lines were then plotted against the fraction of the buffer in the base form at each pH (α). Such plots were linear, with zero intercepts at $\alpha = 0$, within experimental uncertainty. This situation corresponds to apparent catalysis by the base component of the buffer. Values of the catalytic coefficients obtained as the slopes of the k_{cat}–α plots for a series of catalysts are given in Table IV.

Spectroscopic Acidity Constant. Although the compound is unstable, particularly at neutral pH, it proved possible with ^1H NMR spectroscopy to measure a value for the acidity constant of the conjugate acid of **2b**. This necessitated the acquisition of spectra immediately after preparation of the solution, and although a significant amount of products did form, their signals were well removed from the region of interest and thus did not interfere. There did appear to be a difference in the UV spectra of protonated and neutral (hydroxyamino)imidazole. However, quantitative analysis of these changes required both an extrapolation to zero time and precise knowledge of the initial concentration, and this created large uncertainties. With the NMR method the mere observation of a signal was all that was needed. There are three nonequivalent hydrogens in **2b**, and all exhibited upfield shifts at higher pH. To determine the acidity constant, we analyzed the signal which underwent the greatest change, that near 7 ppm due to one of the ring hydrogens. The chemical shifts, which are shown in Figure 3, follow the equation for a titration curve:

$$\delta = \frac{K_a\delta_B + [D^+]\delta_{BH}}{K_a + [D^+]} \quad (1)$$

where δ_B and δ_{BH} are the chemical shifts of the neutral and protonated forms respectively. A least-squares fit of δ versus $[D^+]$ resulted in a pK_a of 7.65 ± 0.09. This number refers to D_2O, the

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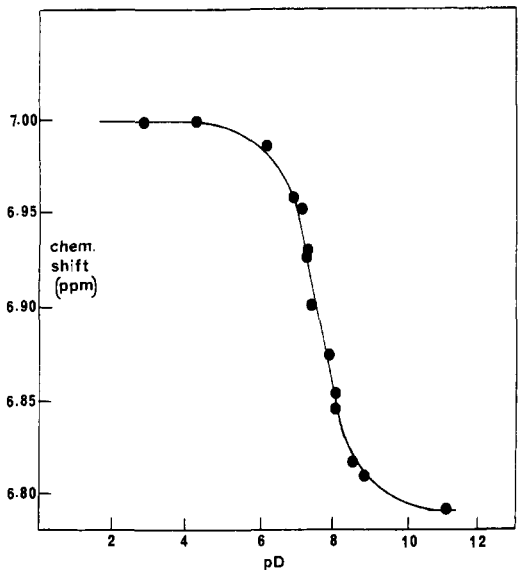


Figure 3. Variation with pD of the chemical shift of one of the ring protons of 1-methyl-2-(hydroxyamino)imidazole (**2b**). The points are experimental; the curve is the best fit to eq 1.

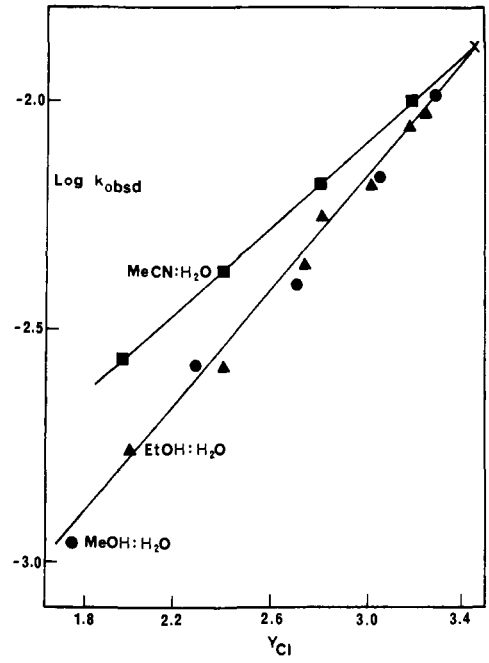
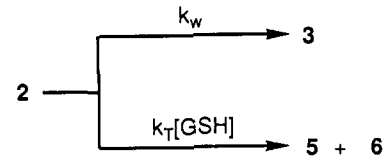


Figure 4. Rate constants for **2b** in mixed aqueous solutions containing borate buffer with pH ~ 9 (in 100% water). The solvent ranges are 0–40% (v/v) MeCN in water and 0–50% ROH in water. The point X represents the 100% aqueous solution.

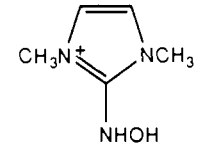
solvent employed for the NMR experiments. For a direct comparison with the kinetic results in H_2O a value of 7.1 ± 0.2 can be estimated by subtracting 0.55. This is the numerical difference in the pK_a values obtained for imidazole itself in D_2O and H_2O,[17] with the additional error because of the uncertainty in the exact correction factor for the system in question.

Effect of Solvent. Kinetic measurements were made with **2b** in mixed aqueous solutions, with the pH ~ 9 so that the rate constants refer to the plateau region of Figure 2 at high pH, a point which was verified in several cases by conducting experiments with slightly different pH. As shown in Figure 4 addition of organic cosolvent resulted in a decrease in k_{obsd}. Within the individual solvent systems, the behavior adhered reasonably well to the Grunwald–Winstein equation, using the Y_{Cl} scale obtained for the solvolysis of *tert*-butyl chloride as a measure of the ionizing power of the solvent.[18] Data for the two alcohol systems produced a common line, with $m = 0.60 \pm 0.02$, while aqueous acetonitrile had a slightly less steep behavior, with $m = 0.46 \pm 0.02$.

Scheme II



1,3-Dimethyl-2-(hydroxyamino)imidazolium Ion (2g). This was prepared as a model for a protonated (hydroxyamino)imidazole which cannot deprotonate from the ring nitrogen.[19] The ion was



2g

obtained by the electrochemical reduction of the 1,3-dimethyl-2-nitroimidazolium ion, which in turn had been prepared by methylation of 1-methyl-2-nitroimidazole with methyl trifluoromethanesulfonate. Identification as the hydroxylamine derivative was based upon a four-electron stoichiometry for the reduction and the appropriate ^1H NMR spectrum, which consisted of two singlets at 3.5 and 7.0 ppm. Solutions of this substance in DCl/D_2O with pD ~ 2 and in phosphate buffered D_2O with pD ~ 7 were examined for stability with NMR spectroscopy. On standing for 1 day at pD 7 and for 3 days at pD 2, there was no significant change in the spectra. There was a reaction at much longer times resulting in colored solutions which appeared complex judging from their NMR spectra, but these products were not examined further. They however did not have the characteristic[4] NMR patterns of the dihydroimidazolium ions **3** found as products with the other (hydroxyamino)imidazoles.

Reactions in the Presence of Glutathione. The question that was addressed here concerned the competition between the water solvent and added glutathione in the reactions of the hydroxylamine **2b**, with the objective being to measure the rate constant ratio k_T/k_w of Scheme II. Initial experiments were conducted in the manner normally employed in these studies, by reacting the substrate in aqueous solutions containing excess trapping nucleophile, with the intention being to quantitatively measure the product ratio ([**5**] + [**6**])/[**3**]. Analysis with NMR spectroscopy indicated that **5** and **6** accounted for virtually all the hydroxylamine, even at low concentrations (~1 mM) of the thiol. However there was an experimental problem preventing accurate measurements, since **3** was an impurity in the hydroxylamine sample, and a precise measurement of the concentration of this species actually produced as a product in the reaction in the presence of the thiol proved impossible. In consequence a second approach was developed, involving an assay for unreacted thiol in reactions carried out with close to a stoichiometric ratio of the two reagents. The measurement was accomplished through the addition of excess Ellman's reagent, 5,5′-dithiobis(2-nitrobenzoic acid), a disulfide that rapidly reacts with free thiols in a thiol exchange, quantitatively releasing 1 equiv of the colored 4-

(17) Li, N. C.; Tang, P.; Mathur, R. *J. Phys. Chem.* **1961**, *65*, 1074–1076.

(18) (a) Grunwald, E.; Winstein, S. *J. Am. Chem. Soc.* **1948**, *70*, 846–854. (b) Fainberg, A. H.; Winstein, S. *Ibid.* **1956**, *78*, 2770–2777. (c) Values of Y_{Cl} for aqueous acetonitrile were taken from: Bunton, C. A.; Mhala, M. M.; Moffatt, J. R. *J. Org. Chem.* **1984**, *49*, 3637–3639.

(19) In principle **2g** can lose a proton from the NH of the hydroxylamine group to give a neutral species that is an oxime derivative. Although this point was not investigated in detail, there appeared to be no difference in the ^1H NMR spectra of this substance in aqueous solutions of pD 2 and pD 7, indicative that deprotonation had not occurred in the latter solution.

Table I. Loss of Glutathione in the Reaction of 1-Methyl-2-(hydroxyamino)imidazole at pH 7.04[a]

$[GSH_0]$[b]	A_0[c]	A[d]	$10^{-3}k_T/k_w$,[e]
0.000 08	0.65	0.48	8.5
0.000 12	0.88	0.59	7.1
0.000 16	1.27	0.77	7.7

[a] Phosphate buffer, concentration = 0.2 M; ionic strength = 1.0. [b] Initial concentration of glutathione. The ratio [hydroxylamine]:[glutathione] was 0.83 throughout. [c] Absorbance at 412 nm obtained for control solution (no hydroxylamine). [d] Absorbance at 412 nm obtained for reaction solution. [e] Calculated according to eq 2. Units are m^{-1}.

Table II. Trapping Ratios for the Reactions of 1-Methyl-2-(hydroxyamino)imidazole in Aqueous Solutions in the Presence of Glutathione[a]

pH	k_T/k_w, M^{-1}	k_{GS}/k_w,[b] M^{-1}
6.24	$(1.8 \pm 0.3) \times 10^3$	6.7×10^5
6.44	$(3.1 \pm 0.3) \times 10^3$	7.0×10^5
6.63	$(3.5 \pm 0.5) \times 10^3$	5.1×10^5
6.78	$(4.0 \pm 0.9) \times 10^3$	4.4×10^5
6.85	$(5.8 \pm 1.2) \times 10^3$	5.2×10^5
6.90	$(5.3 \pm 0.3) \times 10^3$	4.3×10^5
7.04	$(7.8 \pm 0.7) \times 10^3$	4.6×10^5
7.28	$(1.3 \pm 0.3) \times 10^4$	4.5×10^5
7.45	$(2.4 \pm 0.8) \times 10^4$	5.6×10^5

[a] At ionic strength = 1.0. [b] $k_T/k_w(K_a(GSH) + [H^+])/K_a(GSH)$ with $pK_a(GSH) = 8.8$.

nitro-2-carboxybenzenethiol anion (λ_{max} = 412 nm).[20] The equation that applies to the competition of Scheme II is as follows (see Appendix for derivation):

$$\frac{k_T}{k_w} = \frac{\ln A/A_0}{[GSH_0](1 - A/A_0 - [\mathbf{2b}_0]/[GSH_0])} \quad (2)$$

where k_T/k_w is the ratio of rate constants with k_w expressed in s^{-1} units, $[\mathbf{2b}_0]$ and $[GSH_0]$ are initial concentrations, and A and A_0 are the optical densities at 412 nm obtained in the assays of the reaction mixture and a control solution treated in an identical manner but without the hydroxylamine. Typical data for three experiments at one particular pH are summarized in Table I. Since the calculation required knowledge or measurement of several quantities, the precision of replicate measurements was low, with a standard deviation of the order of ±20%. What is clear is that even at 10^{-4} M thiol a significant fraction of the hydroxylamine reacts to give thiol-incorporated products, or, in other words, the thiol is highly competitive with the solvent. This is the same conclusion reached qualitatively in the experiments involving direct product analysis.

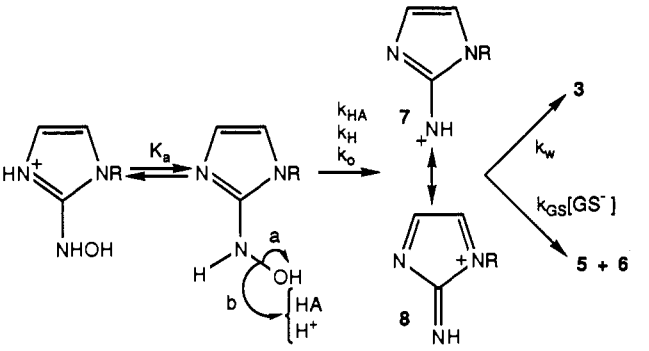
Table II shows that over the region studied the trapping ratio increases with increasing pH, indicative that it is the thiol anion that is the reactive species. The acidity constant of the SH group in glutathione has been determined,[21] although not at the ionic strength 1.0 used in these experiments. With use of a pK_a of 8.8, the ratio k_{GS}/k_w for the anion versus water is 5×10^5 M^{-1} (Table II).

The effect of glutathione on the rate of decay of **2b** was examined in pH 7.4 phosphate buffer. Under the reaction conditions, [GSH] = 0.001 and 0.002, the k_T/k_w ratios, calculated from data just discussed, indicated that 95% and 98% respectively of the products were derived from the thiol. There was however no effect on the rate constant: k_{obsd} = 0.009 07, 0.009 77, and 0.009 04 s^{-1} for [GSH] = 0.0, 0.001, and 0.002.

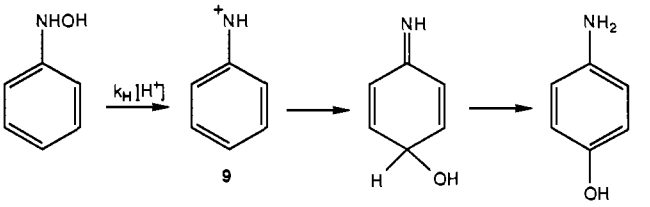
Discussion

Mechanism (N1-Substituted Imidazoles). The proposed mechanism for the 1-substituted compounds is given in Scheme III. This has the neutral imidazole as the reactive species, undergoing rate-limiting cleavage of the nitrogen–oxygen bond. This cleavage can occur (a) with hydroxide ion as a leaving group (rate constant k_0) and (b) with acid catalysis involving the hydronium ion (k_H) and added general acids (k_{HA}). These reactions result in an electron-deficient intermediate that in one of its resonance forms, **7**, is a nitrenium ion. This cation reacts with the solvent and other nucleophiles in fast steps leading to the final products.

Scheme III



Scheme IV



As will be discussed in the sections below, there are a number of indicators of this reaction mechanism. Two key observations are the stability of the analogue **2g**, a 2-(hydroxyamino)-imidazolium ion that cannot form the reactive neutral species of Scheme III, and the independence of the rate constant on added glutathione, in spite of the products being >95% derived from this nucleophile. This latter observation requires an intermediate that is formed in a rate-limiting step by a reaction not involving the added reagent.

The mechanism of Scheme III is analogous to that of the Bamberger rearrangement of *N*-phenylhydroxylamines to *p*-aminophenols (Scheme IV).[22] The difference in the nature of the products obtained with the two systems (aromatic versus 4,5-saturated) has been discussed.[4b,d] An important difference from a kinetic point of view is that the phenylhydroxylamines require acid conditions, and the reaction is H^+-catalyzed (specific acid catalysis) only.[23] The *N*-imidazolylhydroxylamines, on the other hand, are more stable in acid but reactive at neutral pH, with no catalysis.

Rate–pH Profiles. For the mechanism of Scheme III, the variation in the solvent-catalyzed rate constants obtained by extrapolation to zero buffer concentration is given by the following equation, which predicts a rate–pH profile of the type experimentally observed (Figure 1):

$$k_{obsd} = \frac{(k_0 + k_H[H^+])K_a}{K_a + [H^+]} \quad (3)$$

The plateau region at high pH occurs where the imidazole is predominantly in its reactive neutral form ($K_a > [H^+]$), with reaction by the noncatalyzed route, so that k_{obsd} is simply equal

(20) Ellman, G. L. *Arch. Biochem. Biophys.* **1958**, *74*, 443–450; *Ibid.* **1959**, *82*, 70–77.

(21) (a) Huckey, T. N.; Tudor, A. J.; Dawber, J. G. *J. Chem. Soc., Perkin Trans. 2* **1985**, 759–763. (b) Cheesman, B. V.; Arnold, A. P.; Rabenstein, D. L. *J. Am. Chem. Soc.* **1988**, *110*, 6359–6364.

(22) For recent references see: (a) Sone, T.; Tokudo, Y.; Sakai, T.; Shinkai, S.; Manabe, O. *J. Chem. Soc., Perkin Trans. 2* **1981**, 298–302. (b) Sone, T.; Hamamoto, K.; Seiji, Y.; Shinkai, S.; Manabe, O. *Ibid.* **1981**, 1596–1598. (c) Kohnstam, G.; Petch, W. A.; Williams, D. L. H. *Ibid.* **1984**, 423–427.

(23) For the neutral phenylhydroxylamine. Rate constants do level in very acidic solutions (pH 1–2) due to N-protonation.[22]

Table III. Rate and Acidity Constants for 1-Substituted 2-(Hydroxyamino)imidazoles

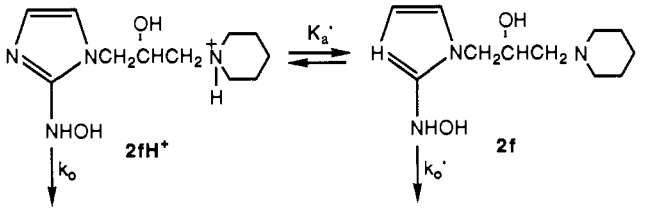
substituent	pK_a	k_0, s^{-1}	k_H, M^{-1} s^{-1}
CH_3	7.34 ± 0.10	$(1.5 \pm 0.3) \times 10^{-2}$	$(4.0 \pm 0.8) \times 10^2$
$CH_2CHOHCH_2CHOCH_3$	7.28 ± 0.08	$(8.3 \pm 1.4) \times 10^{-3}$	$(2.6 \pm 0.5) \times 10^2$
$CH_2CHOHCH_2NH^+C_5H_{10}$[a]	6.76 ± 0.06	$(3.3 \pm 0.5) \times 10^{-3}$	$(1.2 \pm 0.4) \times 10^2$
CH_2CH_2Br	6.77 ± 0.03	$(2.8 \pm 0.2) \times 10^{-3}$	$(1.4 \pm 0.2) \times 10^2$
$CH_2CONHCH_2CH_2OH$	6.45 ± 0.07	$(9.7 \pm 0.1) \times 10^{-4}$	$(4.7 \pm 0.2) \times 10^1$

[a] Parameters based on the rate–pH profile with pH < 8. At pH > 10, $k_0' = 7.9 \times 10^{-3}$ s^{-1}.

to k_0. As the pH is lowered, protonation of the imidazole ring results in a decrease in the relative concentration of the reactive form and k_{obsd} decreases. There is a short region around pH 5–6 where k_{obsd} is approximately inversely proportional to $[H^+]$ concentration; this arises since the cleavage continues to occur by the k_0 route and a proton must be removed to get the reactive form (thus, $k_{obsd} \sim k_0K_a/[H^+]$). In more acidic solutions the H^+-catalyzed process dominates. However k_{obsd} is independent of pH since the requirement that the proton be removed to form the reactive species cancels the H^+ dependency of the rate-limiting step ($k_{obsd} = k_HK_a$). As discussed below, values of k_H are high, being considerably larger, for example, that those observed in the reactions with the phenylhydroxylamines. The imidazole systems owe their comparative stability in acid solutions to their conversion into the unreactive protonated forms.

The experimental k_{obsd}–$[H^+]$ data were fit to eq 3 with nonlinear least-squares with the three parameters k_0, k_H, and K_a as adjustable parameters. The values providing the best fit are given in Table III. Further justification for the general mechanism can be seen in the data for **2b**, for which there is reasonable agreement in the acidity constants from the kinetic analysis (pK_a = 7.34 ± 0.10) and from the analysis of the changes in the NMR spectra (pK_a = 7.1 ± 0.2).

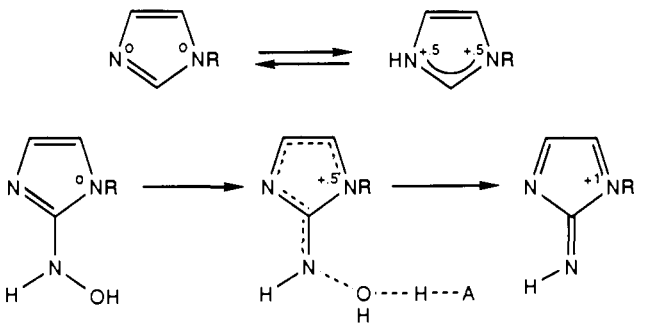
The hydroxylamine **2f** has a piperidine substituent in its side chain, and this adds a complication to the rate–pH behavior, since there are now two forms, **2f**H^+ and **2f**, which are in principle reactive. The piperidine nitrogen is expected to be protonated



in solutions more acidic than pH 9. Therefore only data below pH 8 were included in fitting the rate–pH profile, so that the parameters obtained (Table III) were the rate constants for **2f**H^+ and the acidity constant for its imidazole-protonated conjugate acid. The further slight increase in k_{obsd} that is observed at pH 9–10 must reflect conversion of **2f**H^+ to **2f**, with the final plateau at high pH representing k_0', the pH-independent rate constant for the reaction of **2f**. The change in k_{obsd} is however small, and although k_0' could be determined with reasonable precision it proved difficult to accurately define the acidity constant K_a'. The value for pK_a' is approximately 10, as is expected for this type of system.[24] The rate constant k_0' for **2f** is 2.4 times k_0 for **2f**H^+; this reflects the polar effects of the N1 substituents in the two compounds, as discussed in the next section.

Substituent Effects. The effects of the N1 substituents on the two rate constants provide further evidence for the presence of the cationic intermediate. An ab initio theoretical investigation has shown that the majority of the positive charge in this type of ion resides in the ring rather than at the external nitrogen and that the structure is better represented as that of the resonance contributor **8** in Scheme III.[25] Thus the formation of the ion

Scheme V



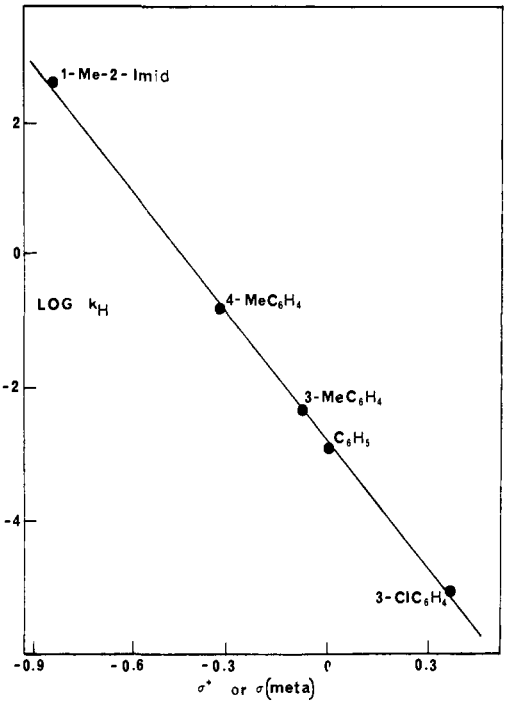


Figure 5. Hammett plot for H^+-catalyzed N–O heterolysis of hydroxylamines R–NHOH. The rate constants for 3-MeC_6H_4, C_6H_5, and 3-ClC_6H_4 are those at 25 °C from ref 22b. The authors of this work reported rate constants k_N for the conversion of the N-protonated hydroxylamines to products, and we have converted to k_H values by dividing k_N by K_a. The k_H value for 4-MeC_6H_4 is an estimate at 25 °C based upon data in ref 22c. These authors reported a rate constant ratio for 4-MeC_6H_4 versus C_6H_5 of 108 in 0.005 M H_2SO_4 at 31 °C; we have assumed that the same ratio applies to k_H at 25 °C. The k_H for the imidazole is from this work, with σ^+(group) taken from ref 27a.

results in positive charge buildup at N1, and the reaction is faster for imidazoles bearing electron-donating N1 substituents. In terms of a linear free energy correlation the most appropriate reference system is the equilibrium protonation of the same compounds, and in fact such plots, log k_0 and log k_H versus pK_a, are reasonably linear, with slopes of 1.14 and 1.01, respectively. If the argument[26] is made that the protonation places 0.5 units of positive charge on each nitrogen of the imidazole ring, then these approximately

(24) (a) The piperidinium ion has pK_a = 11.2. A 2-hydroxyalkyl substituent, as is present in **2f**H^+ should decrease this by approximately 1 log unit, as is seen in a comparison of the ethyl- and 2-(hydroxyethyl)ammonium ions.[24b] (b) Fife, T. H.; DeMark, B. R. *J. Am. Chem. Soc.* **1977**, *99*, 3075–3080.

(25) Bolton, J. L.; McClelland, R. A. *THEOCHEM* **1988**, *165*, 379–389.

(26) Williams, A. *Acc. Chem. Res.* **1984**, *17*, 425–430.

Table IV. Catalytic Coefficients for N–O Heterolysis of 2-(Hydroxyamino)-1-methylimidazole (**2b**, 25 °C, ionic strength = 1.0)

acid catalyst	pK_{Ha}	k_A(exp),[a] M^{-1} s^{-1}	k_{HA}, M^{-1} s^{-1}
H_3O^+	−1.7		4.0×10^{2} [b]
HCOOH	3.6	6.4×10^{-5}	3.5×10^{-1} [c]
$CCl_3PO_3H^-$	4.2	1.3×10^{-4}	1.8×10^{-1} [c]
CH_3COOH	4.6	7.5×10^{-5}	4.1×10^{-2} [c]
CH_3CH_2COOH	4.8	1.3×10^{-4}	4.8×10^{-2} [c]
$H_2PO_4^-$	6.5	1.2×10^{-3}	8.6×10^{-3} [c]
H_2O	15.7		2.7×10^{-4} [d]

[a] Catalytic coefficients for general base catalysis obtained from analysis of experimental kinetic data, as described in results section. [b] k_H from Table III. [c] $k_A(\text{exp})K_{HA}/K_a$. [d] k_0 from Table III divided by 55.5.

unit slopes can be taken as an indicator that in the transition states for the N–O heterolyses, the substituents interact with a similar 0.5 units of charge at N1, or in other words, the transition state occurs approximately 50% along the reaction coordinate (Scheme V).

Noyce has determined σ^+ constants defining the accelerating effect of heterocyclic ring systems in reactions that result in the formation of a carbenium ion center adjacent to the ring.[27] This was accomplished by generating a normal Hammett plot for the solvolysis of substituted 1-phenylethyl derivatives and then placing onto the line rate constants for heterocyclic systems obtained under identical conditions. The σ^+ so obtained for the 1-methyl-2-imidazole group was −0.82.[27a] Figure 5 shows the result of using this value in a Hammett plot for H^+-catalyzed N–O cleavage of hydroxylamines, constructed with literature data for *N*-aryl-hydroxylamines and the single point for **2b**. An excellent overall correlation is observed (correlation coefficient 0.9991), with a large negative slope of −6.5. The latter is typical of Bamberger-type reactions proceeding via *N*-arylnitrenium ions.[22b,28] The conclusion is that the imidazole system behaves no differently from benzene analogues, once the cation-stabilizing ability of the heterocycle is taken into account. As noted above, pH-independent Bamberger rearrangements have not been observed with simple phenylhydroxylamines. If the ρ of −6.5 is used along with k_0 for **2b**, *N*-phenylhydroxylamine is calculated to have a k_0 of 7×10^{-8} s^{-1}. This corresponds to a half-life of 114 days in neutral water, which should be regarded as a lower limit, since the actual ρ could be more negative with the lack of catalysis requiring a greater interaction with substituents in the transition state. Whatever the ρ value, the failure to observe the k_0 process is not surprising, particularly since other reactions of an oxidation–reduction nature occur at neutral pH.[22c] One interesting prediction of this work is that *N*-(*p*-methoxyphenyl)hydroxylamine (σ^+(*p*-MeO) = −0.78) should have a reactivity similar to that of to **2b** and should therefore undergo a neutral Bamberger rearrangement.

Buffer Catalysis. We interpret the buffer catalysis in terms of general acid catalysis of N–O bond breaking, again with the neutral substrate as the reactive form. This appeared experimentally as general base catalysis, since the imidazole ring was substantially protonated in the solutions where the buffer effects were studied in detail. (Specific base catalysis + general acid catalysis = general base catalysis). At higher pH where the imidazole is in the neutral form, true general acid catalysis would be observed. However such solutions can be prepared only with buffers that either have weak conjugate acids or very low concentrations of their conjugate acids and the buffer catalysis is swamped by the pH independent reaction.[29]

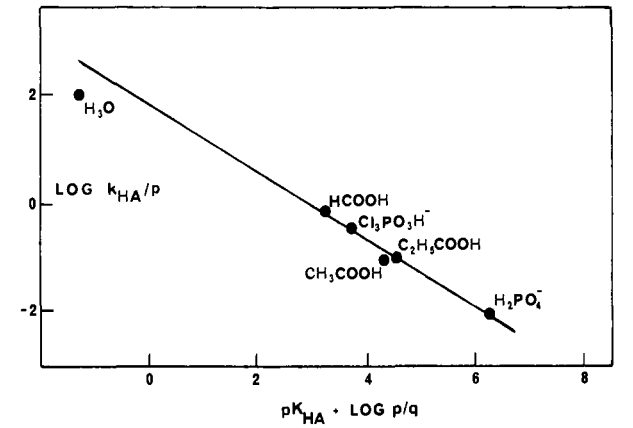


Figure 6. Brønsted plot for general acid catalysis of N–O cleavage of 1-methyl-2-(hydroxyamino)imidazole (**2b**). The line drawn has a slope of 0.62 and is the linear regression line ignoring the point for H_3O^+.

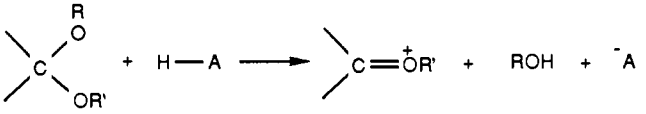
The experimentally determined rate constants for general base catalysis are related to the acid rate constants k_{HA} by eq 4, where

$$k_A(\text{exp}) = k_{HA}K_a/K_{HA} \quad (4)$$

K_{HA} is the acidity constant for the buffer acid. Values of k_{HA} calculated through this equation are given in Table IV, and Figure 6 shows a Brønsted plot. A reasonably linear relationship with slope $\alpha = 0.62$ is obtained. The line includes both the neutral carboxylic acids and the anionic phosphate derivatives, although the point for the proton exhibits a slight negative deviation, a not uncommon occurrence normally attributed to specific solvation effects.[30]

The observation of general acid catalysis raises the possibility that the pH-independent process represents the solvent acting as an acid catalyst. However, even on converting k_0 into second-order units by dividing by the water concentration (55 M), the point shows a 4 log unit positive deviation from the Brønsted plot extrapolated for the other catalysts. This implies that the mechanism is different, and we suggest that it is better represented as in Scheme III with N–O heterolysis forming the cation and hydroxide ion.

This study represents the first demonstration of general acid catalysis of N–O cleavage, specific acid catalysis being the general rule with phenylhydroxylamines.[31] The relationship between the nature of acid catalysis and structure has been examined extensively in the hydrolysis of acetals and ortho esters, where the normal rate-limiting step is a C–O cleavage producing an oxocarbonium ion:[32a,33]



(27) (a) Noyce, D. S.; Stowe, G. T. *J. Org. Chem.* **1973**, *38*, 3762–3766. (b) Noyce, D. S.; Sandel, B. B. *Ibid.* **1976**, *41*, 3640–3642.

(28) (a) Gassman, P. G.; Campbell, G. *J. Am. Chem. Soc.* **1971**, *93*, 2567–2569; *Ibid.* **1972**, *94*, 3891–3896. (b) Gassman, P. G.; Granrud, J. E. *Ibid.* **1984**, *106*, 1498–1499. (c) Novak, M.; Pelecanou, M.; Roy, A. K.; Andronico, A. F.; Plourde, F. M.; Olefirowicz, T. M.; Curtin, T. J. *Ibid.* **1984**, *106*, 5623–5631. (d) Novak, M.; Pelecanou, M.; Pollack, L. *Ibid.* **1986**, *108*, 112–120. (e) Novak, M.; Laberman, R. K. *J. Org. Chem.* **1988**, *53*, 4762–4769.

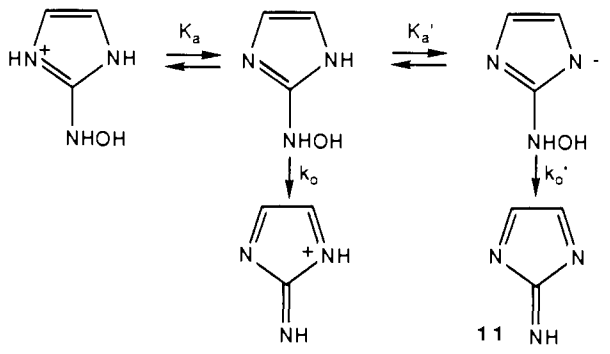
(29) (a) Fife has discussed this aspect of acid catalysis in an analysis of the similar reactions of acetals, demonstrating that there is a window of buffer pK_a values for which significant buffer effects are seen.[29b] With the various data that have been calculated for **2b** (Tables III and IV) it can be shown that in a phosphate buffer of pH 5.5, where **2b** is mainly protonated, k_{obsd} measured at 1 M total buffer concentration is 45% larger than the value at the same pH with no buffer present. However in a phosphate buffer with pH 8.0, where there is mainly neutral **2b**, the same change in total buffer concentration results in an increase of only 3%. (b) Fife, T. H.; Anderson, E. *J. Org. Chem.* **1971**, *36*, 2357–2361.

(30) Kresge, A. J. *J. Chem. Soc. Rev.* **1973**, *2*, 475–503.

(31) (a) Admittedly, the question of the nature of the acid catalysis has in most studies not been addressed. After the findings with the imidazoles, we examined the kinetics of the Bamberger rearrangement of *N*-(2,6-dimethylphenyl)hydroxylamine in acetic acid solutions but could find no indication for buffer catalysis even at 1 M buffer concentration.[31b] (b) Fishbein, J. C.; McClelland, R. A., unpublished results.

(32) (a) Fife, T. H. *Acc. Chem. Res.* **1972**, *5*, 264–272. (b) Fife, T. H.; Jao, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 4081–4085. (c) Fife, T. H.; Brod, L. H. *Ibid.* **1970**, *92*, 1681–1684.

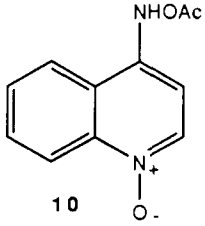
Scheme VI



One general conclusion is that for poor leaving groups such as ethanol catalysis by general acids is observed only if a relatively stable cationic intermediate is formed, such as an oxotropylium ion.[32a] The N–O cleavage would appear to be following the same pattern, with the *N*-imidazolylhydroxylamines falling into the "stable cation" category and showing general catalysis. As further evidence of the analogy between the N–O and C–O reactions, it can be noted that where general acid catalysis has been observed with the latter, there is also a pH-independent reaction involving a unimolecular decomposition.[32b,c]

General acid catalyzed acetal and ortho ester hydrolyses are interpreted in terms of a concerted mechanism, in which proton transfer to the catalyzing acid occurs at the same time as C–O bond breaking.[33] By analogy we propose that a similar mechanism applies here (Scheme V). It is interesting to note that the Brønsted α implies that the proton is approximately 60% transferred in the transition state. This is a similar conclusion about the extent of reaction as reached in the analysis of N1 substituent effects.

Effects of Solvent. These effects were studied at high pH where uncatalyzed heterolysis of the N–O bond is proposed to be occurring, and, although not definitive on its own, the pattern of a decreased rate with decreased solvent polarity is consistent with this mechanism. The *m* values from the Grunwald–Winstein treatment are relatively low, being around 0.5. The solvolysis of *tert*-butyl chloride is however a poor model for the reaction in question, in terms of both the leaving group and the cation that is being produced, and thus it is not surprising that slopes are not closer to unity. Suggestions have been made that C–X solvolyses that involve highly delocalized cations are less sensitive to solvent polarity,[34] and this could be part of the explanation. There has been a previous study of solvent effects in a solvolysis involving a nitrenium ion with the quinoline *N*-oxide derivative **10**; the data also correlated with Y_{Cl}, but the sensitivity was again low, with an *m* value of only 0.26.[35]



Rate–pH Profile: N1-Unsubstituted Imidazole. A mechanism consistent with the kinetic data for the parent system is shown in Scheme VI. This has the neutral hydroxylamine as the reactive species, as with the 1-substituted derivatives, with an additional pathway involving the anion that generates the intermediate **11**. The mechanism is written only with noncatalyzed N–O cleavage; reactions involving H^+ and general acids likely exist but are not necessary to explain the rate–pH profile, which was not investigated in the acid region. Based upon this mechanism, the following equation is derived:

$$k_{obsd} = \frac{(k_0 + k_0'K_a'/[H^+])K_a}{K_a + [H^+]} \quad (5)$$

The observed kinetic data were fit to this equation, providing values for the parameters of $pK_a = 6.96 \pm 0.13$, $k_0 = (2.2 \pm 0.4) \times 10^{-3}$ s^{-1}, and $k_0'K_a' = (1.80 \pm 0.16) \times 10^{-9}$ M^{-1} s^{-1}. The first two are of similar magnitudes to the constants obtained with the 1-substituted derivatives, as expected. The constants k_0' and K_a' are unique to N1-unsubstituted systems. These cannot be separated with the available data, since kinetic measurements in solutions where the concentration of the anion is significant could not be carried out because the reaction is too fast. The anionic form of the hydroxylamine is obviously highly reactive. An estimate for k_0' of 10^5 s^{-1} is obtained by using a pK_a' of 14 typical of the deprotonation of a simple imidazole. In terms of the rate–pH profile (Figure 2), three regions of behavior can be defined. Around pH 5 reaction occurs with the neutral hydroxylamine and k_{obsd} is approximately equal to $k_0K_a/[H^+]$, the inverse $[H^+]$ dependency arising from the need to remove a proton to achieve a reactive state. As the pH is increased, there is a short region around pH 6–7 where k_{obsd} increases at a greater rate than $1/[H^+]$. Reaction via the anionic form has now become important, and the steep acidity dependence $k_0K_a'K_a/[H^+]^2$). since two protons must be removed ($k_{obsd} \sim k_0'K_\alpha'K_a/[H^+]^2$). Above pH 7.5 the neutral hydroxylamine becomes the predominant species in the equilibrium, and the dependence returns to $1/[H^+]$ ($k_{obsd} = k_0'K_a'/[H^+]$).

Intermediate Lifetime. The ratio k_{GS}/k_w (Table II) must represent the ratio of rate constants for the reactions of the intermediate cation with the two nucleophiles. Trapping ratios obtained for carbocation intermediates have recently been used to estimate the absolute values for the rate constants for solvent capture.[36] The assumption that is made is that the combination with the added nucleophile is diffusion limited, and thus k_{Nuc} can be assigned an absolute value, taken as 5×10^9 M^{-1} s^{-1}.[37] With this assumption for k_{GS} in the presence case, k_w is calculated as 10^4 s^{-1}. This is best regarded as an upper limit, since with such

Table V. Rate Constants for the Reactions of Cations with Water

cation	k_w, s^{-1}
p-methoxyphenethyl (**12**)[a]	1.4×10^8
phenethyl (**13**)[b]	$\sim 10^{11}$
1-methyl-2-imidazoylmethyl (**14**)[c]	6×10^7
p-(methoxyphenyl)phenylmethyl (**15**)[d]	2.0×10^6
1-methyl-2-imidazoylphenylmethyl (**16**)[e]	1.2×10^5
2,6-dimethylphenylnitrenium (**17**)[f]	7×10^8
phenylnitrenium (**9**)[g]	5×10^9
1-methyl-2-imidazoylnitrenium (**7b**)[h]	$<10^4$

[a] Reference 36g, azide:water ratio. [b] Reference 36g, estimated by extrapolation of data for more stabilized cations. [c] Bolton, J. L.; McClelland, R. A. *Can. J. Chem.*, in press, azide:water ratio. [d] Rerference 37b, directly measured. [e] McClelland, R. A.; Steenken, S., unpublished observation, directly measured. [f] Fishbein, J. C.; McClelland, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 2824–2825, azide:water ratio. [g] Fishbein, J. C.; McClelland, R. A., unpublished observation, halide:water ratio. [h] This work.

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a large trapping ratio there is a question as to whether the assumption is justified.[36g] The ratio is smaller than that observed for activation limited reactions involving very stable carbocations, where $k_{RS}/k_w \sim 10^8$ M^{-1},[38] and this has been taken as evidence to support diffusion control for the nucleophile.[36] However, the ratio is not that much smaller, and moreover a carbenium ion may be a bad model for the nitrenium ion.

The conclusion regardless is that the imidazole nitrenium ion **7b** is a remarkably long-lived intermediate in the absence of thiols. This is put into perspective in Table V, which summarizes selected k_w values for carbenium ions and nitrenium ions. Two trends can be identified, the combination of which result in the relative kinetic stability of **7b**. Imidazole-stabilized cations are much longer lived than benzene analogues (compare **14** and **13**—a difference in lifetime of $>10^3$, and **7b** and **9**, a difference of $>10^5$). This is obviously a reflection of the relative cation-stabilizing effects of these two groups, as has been discussed. The comparisons **16** versus **15** and **14** versus **12** also show that the imidazole cations are longer lived than *p*-methoxyphenyl analogues, although the differences are not so great. (One prediction is that the *p*-methoxyphenylnitrenium ion should be relatively long lived in water.) The second conclusion is that nitrenium ions RNH^+ are longer lived than analogous carbenium ions RCH_2^+ (compare **7b** and **14**, a difference of at least 10^4, and **9** and **13**, a factor of 20, which must be a lower limit since **13** is a secondary cation). This difference is perhaps unexpected in terms of the electronegativities of the atoms formally bearing the positive charge. However the carbenium ions combine with water at the carbon external to the ring without loss of aromaticity, while the reactions of the nitrenium ions occur at a ring carbon so that the initial products are nonaromatic. This may create a larger intrinsic barrier for the latter process. A second and perhaps related explanation is based upon indications that the positive charge of arylnitrenium ions is highly delocalized in the aromatic ring.[22c,25,39] There is an indication from recent experiments involving trifluoromethyl substitution that carbenium ions with this feature are exceptionally long lived.[40]

Biological Implications. 2-Nitroimidazoles deplete intracellular glutathione[41] and covalently bind to DNA and other cellular macromolecules,[42] both effects seen selectively in hypoxic cells where nitroreduction is promoted. A model would be reduction to the hydroxylamine level followed by formation of the nitrenium ion which reacts with the various cellular nucleophiles. There is some evidence for the intermediacy of the (hydroxyamino)-imidazole in cells, based upon observations of its products of further reaction. For example, the dihydroimidazole **3** fragments[4d] under certain conditions to give glyoxal, which is observed as a derivative,[4d,43] and a monosubstituted guanidinium ion, and, with misonidazole, both of these have been observed in cells,[44,45] and the glyoxal derivative in vivo in man.[46] More direct evidence is the detection in cells treated with misonidazole of the product **6** derived from glutathione.[47] The formation of this product obviously accounts for the cellular depletion observed with this thiol, which must represent covalent modification since the procedure employed for the assay of cellular glutathione measured the total of the reduced (GSH) and oxidized (GSSG) forms.[41] One important finding of the present study is that glutathione and presumably other thiols are highly competitive scavengers of the nitrenium ion intermediate. Glutathione is normally found in cells at concentrations of 0.5–50 mM,[48] and under these conditions most of the nitrenium ion will react with the thiol, certainly in competition with other weak oxygen nucleophiles such as the solvent. There is a correlation between the cytotoxic effects of nitroimidazoles and the amount of glutathione in the cell, toxicity being observed only after depletion of GSH to relatively low levels[41] and the addition of exogenous thiol having a protecting effect.[49] This may be related to the ability of glutathione to remove the electrophilic intermediate.

To date there has been no chemical structural assignments relating to the DNA binding of 2-nitroimidazoles. There is however a recent report characterizing a guanosine adduct obtained upon dithionite reduction of the 5-nitroimidazole metronidazole,[50] and the structure of this adduct is consistent with it being derived from a nitrenium ion intermediate. Hydroxylamines obtained from oxidative metabolism of aromatic amines such as 2-aminofluorene have seen extensive investigation,[51] and in several cases covalent adducts of DNA attributable to a nitrenium ion intermediate have been chemically characterized. With the benzenoid hydroxylamines there is a further activation through esterification of the OH group, a better leaving group being required for the nitrenium ion to form under physiological conditions.[51] Such activation is not necessary with the 2-imidazole derivatives, the hydroxylamine itself, once formed, readily losing hydroxide ion without further assistance. A similar situation is also likely to hold with hydroxylamines derived from reduction of some other five-atom nitroheterocycles of clinical importance,[1] in particular 5-nitroimidazoles, such as metronidazole and 2-nitrofurans. This conclusion is based upon the Noyce σ^+ values[27a] for these ring systems (1-methyl-5-imidazoyl, $\sigma^+ = -1.04$; 2-furyl, −0.90), which indicate that they are also excellent stabilizers of adjacent cationic centers. This ability of certain heterocycles to stabilize an adjacent nitrenium ion center has been noted qualitatively, being suggested as a possible explanation as to why the nitro compounds are mutagenic in bacterial assays, while nitro derivatives of less cation-stabilizing aromatic groups are not.[52]

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Appendix

Derivation of Equation Relating Unreacted Thiol to Trapping Ratio (Scheme II). Defining

$$[T_0] = \text{initial thiol concentration} \quad \text{(A1)}$$

$$[T_\infty] = \text{final concentration} \quad \text{(A2)}$$

$$[RX_0] = \text{initial substrate concentration} \quad \text{(A3)}$$

$$[RX_\infty] = \text{final substrate concentration} = 0 \text{ since substrate will entirely react} \quad \text{(A4)}$$

$$[RT_\infty] = \text{final concentration of thiol-incorporated products} \quad \text{(A5)}$$

$$[ROH_\infty] = \text{final concentration of water-incorporated products} \quad \text{(A6)}$$

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At the completion of the reaction

$$[RT_\infty] + [ROH_\infty] = [RX_0] \quad (A7)$$

$$[RT_\infty] + [T_\infty] = [T_0] \quad (A8)$$

At intermediate time t

$$[RT] + [ROH] + [RX] = [RX_0] \quad (A9)$$

$$[RT] + [T] = [T_0] \quad (A10)$$

and therefore

$$[T_0] - [T] + [ROH] + [RX] = [RX_0] \quad (A11)$$

For the rate equations

$$-d[T]/dt = k_T[T][RX] \quad (A12)$$

$$d[ROH]/dt = k_w[RX] \quad (A13)$$

Divide eq A12 by eq A13

$$-d[T]/d[ROH] = k_T[T]/k_w = t[T] \quad (A14)$$

where $t = k_T/k_w$ = trapping ratio. From eq A11

$$d[ROH] = d[T] - d[RX] \quad (A15)$$

Substituting into eq A14 and rearranging yield

$$d[T]/[T] = td[RX] - td[T] \quad (A16)$$

Integration gives

$$\ln [T] = t([RX] - [T]) + C \quad (A17)$$

At start of reaction, $[T] = [T_0]$ and $[RX] = [RX_0]$ (eq A1 and A3), therefore

$$C = \ln [T_0] - t([RX_0] - [T_0]) \quad (A18)$$

$$\ln [T]/[T_0] = t([RX] - [T]) - t([RX_0] - [T_0]) \quad (A19)$$

Measurement of thiol is made at completion of reaction, where $[T] = [T_\infty]$ and $[RX] = [RX_\infty]$:

$$\ln [T_\infty]/[T_0] = t([T_0] - [T_\infty] - [RX_0]) \quad (A20)$$

Rearranging gives

$$t = (\ln [T_\infty]/[T_0])/([T_0](1 - [T_\infty]/[T_0] - [RX_0]/[T_0])) \quad (A21)$$

Define

A_0 = absorbance produced by thiol no substrate = $\epsilon[T_0]$ (A22)

A = absorbance produced by thiol remaining after reaction = $\epsilon[T_\infty]$ (A23)

Therefore

$$t = (\ln A/A_0)/([T_0](1 - A/A_0 - [RX_0]/[T_0])) \quad (A24)$$

Solid-State Structures of 1-Alkyl-2,2-dimesitylethenols. Application of the Principle of Structural Correlation to Ring-Flip Processes in 1,1-Diarylvinyl Systems[1]

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Abstract: The solid-state structures of 1-R-2,2-dimesitylethenols (R = H, Me, Et, *i*-Pr, *t*-Bu (**1a–e**)) and of 1,1-dimesitylethylene (**4**) were determined by X-ray diffraction. Enol **1a** displayed tetramers of four crystallographically independent molecules in the unit cell, and **1b** crystallized with an EtOH molecule. As the bulk of R is increased, the C=C bond length increases, the R—C=C bond angle (α_4) opens from 118.1° (**1a**) to 133.2° (**1e**), the RCO bond angle closes from 118.7° (**1a**) to 107.4° (**1e**), and the torsional angles ϕ_1 (of the Ar group cis to the OH) and ϕ_2 (of the aryl group trans to the OH) increase. α_4 is linear in Taft's E_s steric parameter. These trends are reproduced by MM2(85) calculations. Intermolecular enol–enol and enol–EtOH and intramolecular π (Ar)–HO hydrogen bonding are observed. The potential energy surface for 1,1-diphenylethylene (**8**) was calculated by molecular mechanics, and a propeller conformation with $\phi_1 = \phi_2 = 40°$ is the lowest energy conformer. The calculated enantiomerization barriers for correlated rotations of zero-, one-, and two-ring flips are 12.9, 1.2, and 3.0 kcal mol^{-1}. In the calculated transition state for the zero-ring flip, both rings are puckered. The Cambridge Structural Database gave 116 crystallographically independent molecules with the $Ar_2C{=}CR^1R^2$ subunit. The ϕ_1 vs ϕ_2 angles for the 1,1-diarylethylenes were superimposed on the calculated surface for **8** in a conformational map. Experimental points concentrate around the calculated minimum and are absent in the vicinity of the (0°, 0°) region. When R^1 and R^2 differ much in bulk, the points prefer to concentrate around (0°, 90°) and (90°, 0°) diagonal whereas when the bulk of R^1 resembles that of R^2, many points concentrate along the diagonal between (40°, 40°) and (90°, 90°). By the crystal structure correlation principle, the one- and the two-ring flips are clearly favored over the zero-ring flip. Trimesityl-substituted systems are displaced toward the (90°, 90°) region compared with other triarylvinyl systems. Correlations between the bond angle ArCAr and the sum $\phi_1 + \phi_2$ and between the C—Ar bond length and the bond angle ArC=C were found.

Recent investigations on the structures, the K_{enol} values (=[enol/ketone] at equilibrium), and dynamic behavior of stable simple enols—the crowded tri- and diarylethenols—showed the importance of steric effects on several phenomena.[2] (a) The $\Delta G°$ values for the ketone ⇌ enol equilibria for 1-alkyl-substituted and unsubstituted 2,2-dimesityl ethenols **1a–e** decrease linearly with Taft's E_s values.[3] (b) The K_{enol} values for 1-aryl-2,2-dimesitylethenols **2** increase with the increased steric bulk of the aryl

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