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Amidine Nitrosation

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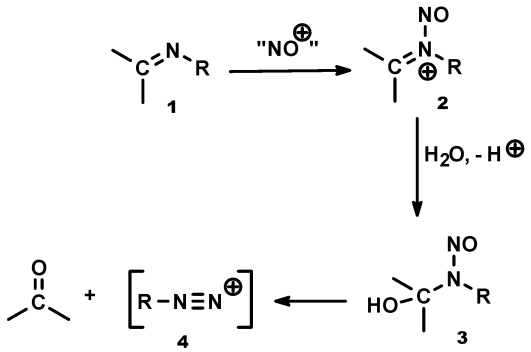
The acidic nitrosation chemistry of nine acyclic secondary and tertiary amidines (Ph-N=C(R_1)-NR_2R_3; R_1 = H, CH_3, Ph; R_2, R_3 = H, Ph or $(CH_3)_2$ or $C(CH_2)_4$) and several *N*-acylamidines was investigated. The principal nitrosation products were amides derived from the amino moiety and compounds derived from the benzenediazonium ion, which was independently trapped for quantitation in several cases. Tertiary amidines also produce nitrosamines in minor, but significant, yields. The benzamidines did not react, and the *N*-acylamidines hydrolyzed much more rapidly than they nitrosated. The data support the hypothesis that the reaction occurs by nitrosation on the imino nitrogen, followed by the addition of H_2O to give a tetrahedral intermediate (α-hydroxynitrosamine) for which the main decomposition pathway generates an amide and a diazonium ion. In the case of the pyrrolidine-derived amidines, about 25% of the decomposition results in cleavage of the amine moiety, which nitrosates to give *N*-nitrosopyrrolidine. Pseudo-first-order rate constants for amidine nitrosation in aqueous acetic acid with excess nitrite at 25 °C ranged from (3 to 106) × 10^{-5} s^{-1}, while the amidine basicity ranged over 5 pK_a units. Rate constants corrected for amidine basicity showed the pyrrolidine derived amidines to be most reactive. The lack of benzamidine nitrosative reactivity is attributed to a very slow rate of H_2O additon to the *N*-nitrosoamidinium ion and reversible nitrosation.

Introduction

The chemistry literature reveals little about the nitrosation of the carbon−nitrogen double bond **1** → **2**. Reference to Scheme 1 shows why this might be true. In aqueous solution, the resulting *N*-nitrosoiminium ion **2** will rapidly react with water to give the α-hydroxynitrosamine **3**. This compound, of course, resembles intermediates in the hydrolysis of imines and is expected to decompose to the corresponding carbonyl compound and the diazonium ion **4**. α-Hydroxynitrosamines **3** are well-known in the literature relating to the carcinogenesis of nitrosamines. They are formed from the metabolic oxidation of these compounds.[1] They have been prepared, and their chemistry has been studied.[2−7] α-Hydroxynitrosamines are unstable in aqueous solution and decompose as shown. Thus, the nitrosation of an imine in aqueous solution is expected to ultimately give the same products that would be derived from the nitrosation of the corresponding primary amine. Moreover, with the exception

SCHEME 1



of those cases where the nitrosoiminium ion **2** can be trapped either by intramolecular or intermolecular nucleophilic addition,[7−13] the transformation is expected to be of little synthetic value.

From another perspective, however, that of chemical carcinogenesis, transformations such as those shown in Scheme 1 could be of significance. Many drugs contain carbon−nitrogen double bonds. The focus of this paper is on the nitrosation chemistry of the amidine functional

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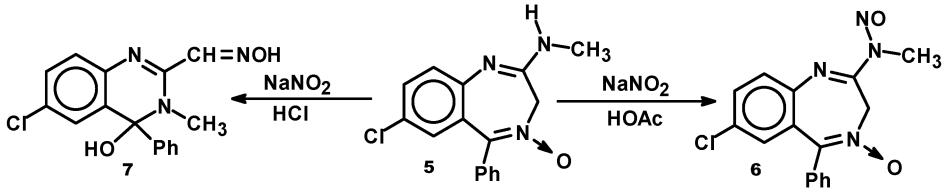
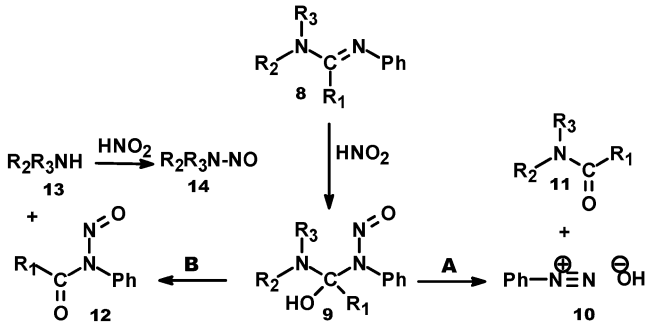
SCHEME 2



group, which can be found in many drugs, as well as in other commercial compounds. Nitrosation occurs in the human body at variable levels.[14−17] All humans excrete the nitrosated amino acid *N*-nitrosoproline in their urine,[14] a significant portion of which arises from endogenous nitrosation and dietary consumption of this *N*-nitrosoamino acid.[15] There are three principle sources of endogenous nitrosating agents:[18] nitrite, nitrate, and products of the oxidation of NO, which is formed in various cells from arginine.[19] The human stomach, because of its acidity, is an ideal place for the formation of nitrous acid from dietary nitrite.[17,20−26] Dietary nitrate, through its bacterially mediated temporal biphasic reduction to nitrite in the saliva, significantly contributes to endogenous nitrosation, because the major nitrite pulse does not occur while eating (phase 1), but from blood-circulating nitrate secreted into the saliva glands.[27−31] Other nitrate-reducing bacteria, such as may exist in an infected stomach,[23−26] or another organ, are capable of mediating the nitrosation of nitrogen compounds, such as amines through processes which both do or do not involve direct production of nitrite. Because of these well-documented processes, it behooves the chemist to better understand the reactivity of nitrogen substrates toward nitrosation.

Although several *N*-nitrosoamidines derived from secondary amidines have been reported in the literature, little appears to be known about the nitrosation chemistry of amidines. One exception involves the nitrosation

SCHEME 3



chemistry of the common drug chlordiazepoxide **5** and related derivatives (Scheme 2).[32] The reaction of **5** with slightly more than one equivalent of sodium nitrite in glacial acetic acid generates the *N*-nitrosoamidine **6**. This compound has interesting chemistry and has been utilized in the synthesis of numerous derivatives.[32−34] On the other hand, the nitrosation of **5** in hydrochloric acid takes another course and results in the formation of the oximino quinoxaline **7**. The *N*-nitrosoamidine **6** forms under gastric nitrosation conditions in animals. It possesses genotoxic activity but is not carcinogenic toward mice.[35−38]

N-Nitroso derivatives of 2-arylimidazolines have been prepared by the reaction of cyclic secondary amidines with N_2O_4.[39] Another group of *N*-nitrosoamidines have been prepared in order to study the migration of the *N*-nitroso group from one nitrogen to another by an intramolecular process, but the nitrosation chemistry of the parent compounds has not been discussed.[40]

We have previously shown that three tertiary amidines reacted with nitrous acid under acidic conditions to give nitrosamines, *N*-nitrosoamides, and an array of products derived from the decomposition of diazonium ions (see Scheme 3).[41] At the time that research was done, our attention was principally focused on the deleterious biological action which could result from the formation of nitrosamines or other *N*-nitroso compounds. We imag-

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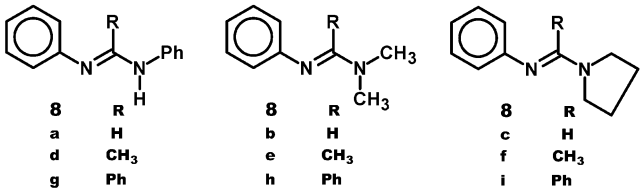
CHART 1



ined the diazonium ion products to arise mainly from the decomposition of *N*-nitrosoamides. In reconsideration of that research, and with new insight gained from our nitrosation studies on oxazolines and *N*-alkylimidazolines (cyclic tertiary amidines),[42] we simplified and reformulated our hypotheses to that shown in Scheme 3. We postulate that amidines react by effective addition of nitrous acid across the C=N− bond to give a tetrahedral intermediate, which is also an α-hydroxynitrosamine. This intermediate is proposed to undergo competitive decomposition to generate a less stable diazonium ion **10** (path A) or an *N*-nitrosoamide **12** and a nitrosatable amine (path B). A goal of the research reported here has been to test these postulates, to quantify as much as possible the partitioning between paths A and B, and to determine how these transformations are affected by structure, with emphasis being paid to the role of the carbon substituent R_1. Here, we demonstrate that path A, which gives diazonium ions, is the predominant one and that as R_1 is changed from H to CH_3, to Ph, the amidines become progressively less reactive. Benzamidines do not nitrosate at all. We also show that *N*- or *N*′-acyl acyclic amidines hydrolyze much more rapidly than they nitrosate.

Results and Discussion

Synthesis of Amidines. The nine amidines **8a**−**i** (Chart 1) used in this work are all known compounds and were either procured or synthesized by literature procedures as documented in the Experimental Section. Their physical and spectroscopic properties are given in the Supporting Information.

Nitrosation Reactions. In our prior experiments with amidines, significant consumption of nitrous acid was observed.[41] As a result, only partial reaction of the substrate occurred, which could be bolstered by the addition of NO_2^-. Accordingly, in this work we used a large excess of nitrous acid in all of the transformations that we studied. In a typical experiment, the amidine of interest was dissolved in glacial acetic acid or buffered acetic acid, and a 5−20 × excess of nitrite was added to the stirring mixture. Transformations were allowed to go for variable times depending upon the reactivity of the amidine and/or the goal of the experiment. The reactions were quenched by dilution with water followed by bicarbonate basification. Following extraction into an organic solvent and concentration, the reaction products were separated by flash chromatography. In some cases, the reaction mixtures were analyzed by GCMS or HPLC after extraction. In addition to the ancillary information from MS, all products were characterized by chromatographic and spectroscopic comparison with authentic compounds, which were also used as standards in the quantitative determination of product yields by GC or HPLC. Reaction conditions and product yields are summarized in Table 1. The transformations result in scission of the amidine into two sets of products derived from the two nitrogen containing moieties as minimally depicted in Scheme 3. For example, the nitrosation of the *N*,*N*′-diphenylformamidine **8a** (run 1) at 0 °C gives formanilide (97%) and the benzenediazonium ion derived products 4-azophenylphenol (23%) and 2,4-bis(4-azophenyl)phenol (47%), giving material balances for the two fragments of 97% and 90%, respectively. In general, material balances were quite good for all runs. The exceptions were for those runs where a highly water-soluble amide (DMF) or dimethylnitrosamine was a product. In runs 10, 12, and 13, we modified our analytical procedure to detect these compounds as effectively as possible in order to make valid comparisons. In some cases, our emphasis was on establishing the nature of the products, e.g., runs 6−9. Even at long reaction times, the more basic amidines (**8b**,**c**,**e**,**f**) reacted incompletely (substrate recoveries are given in Table 1). Regardless of basicity, the benzamidines **8g**−**i** did not nitrosate. As a control, hydrolyses of all amidines were examined under conditions similar to those for nitrosations, and in no case was hydrolysis observed on the time scale and conditions of the attempted transformation.

The products of two typical nitrosation reactions, runs 2 and 10, are illustrated in Scheme 4. Substrates **8a**, **8d**, and **8g** are secondary amidines and could produce *N*-nitrosoamidines as products, but none were observed. Prior work[39] and our own experience[43] suggest that *N*-nitrosoamidines derived from **8a** and **8d** will not survive the acidic reaction conditions due to their rapid decomposition. In other work,[43] we have shown that *N*-nitrosoamidines readily nitrosate and decompose, but our product analyses and material balances indicate that this transformation is occurring to only a very minor extent at best because *N*-nitrosoamides are expected products (see below). In the two illustrated cases of Scheme 4, and in all runs where nitrosative reactivity was observed, products characteristic of the intermediacy of diazonium ions were observed. Most significantly, the nitrosation of **8a** at 37 °C gives 4-hydroxyazobenzene **19**. This product is formed by the hydrolysis of the benzenediazonium ion to phenol which then undergoes azo coupling with the diazonium ion. As noted above, the reaction of **8a** at 0 °C, which prolongs the lifetime of the benzenediazonium ion, produces 2,4-bis(phenylazo)phenol in 46% yield. In the case of **8e** and related tertiary amidines, the benzenediazonium ion derived reaction products phenyl acetate **18**, phenol **17**, and nitrophenols **22** and **23** are observed. Similar products were observed in all other amidine (**8a**−**f**) nitrosation transformations. In all cases, the major fragment from the amino moiety of the amidine was an amide **11** (specifically **16** from **8e**), which for the secondary amidines is a formanilide (here **11** = **15**).

One of the goals of this research was to determine whether diazonium ions are primary products of amidine nitrosation and to see if we could quantitate the diazonium ion formation. Naturally, this required the use of substrates which would generate a stable aryl diazonium ion that could be trapped. Accordingly, we performed a

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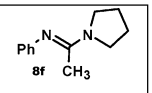
TABLE 1. Product Yields from the Nitrosation of Amidines[m]

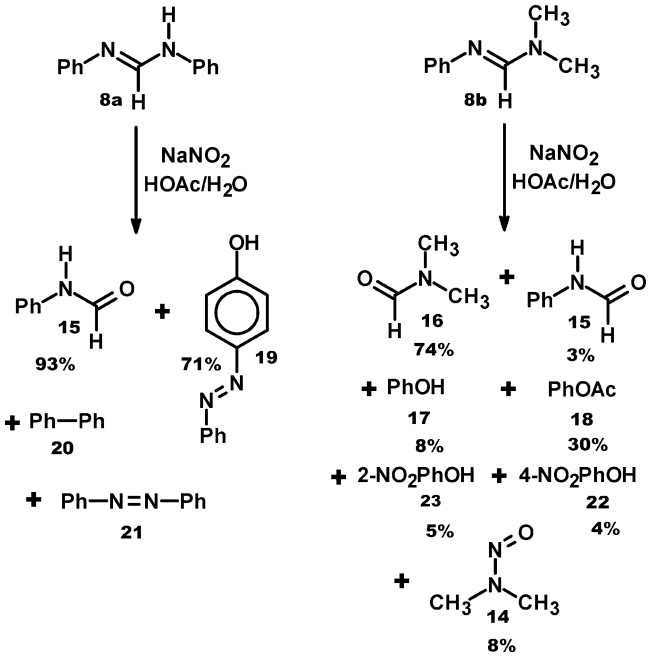
Substrate		 8a					 8d		 8b			 8e		 8f
Run		1	2	3[a]	4[b]	5[b]	6	7	8	9	10[c]	11	12[c]	13[c]
Product	**No.**	Percent Yields (%)[d]												
Amide[e]	**11**	na	na	na	na	na	75	88	8	nd	74	52	82	62
Anilide[f]	**15**	97	93	91	94	89	na	na	nd	+	3	7	2	0.7
Nitrosamine[g]	**14**	na	na	na	na	na	na	na	nd	nd	8	nd	1.8	26
PhOH	**17**	nd	nd	na	-	-	9	nd	6	7	8	12	34	37
PhOAc	**18**	nd	nd	na	-	-	48	nd	28	27	30	12	23	18
4-PhN=NAr[h]	**19**	24[j]	71	93	91	89	-	10	8	5	-	-	-	-
2-NO_2PhOH	**23**	nd	nd	nd	-	-	16	nd	27	26	5	-	-	+
4- NO_2PhOH	**22**	nd	nd	nd	-	-	+	nd	+	-	4	10	-	-
PhN=NPh	**21**	+	+	+	-	-	-	nd	-	-	-	-	-	+
Ph-Ph	**20**	+	+	+	-	-	-	nd	-	-	-	-	-	-
Subst. Recvd.[i]	**8**	-	-	-	-	-	54	67	54	59	29	72	85	90
NO_2^- Eq.[k]		5	5	5	5	5	16.3	6.2	10	5	10	10	10	10
Rxn. Time (h)		1	1	2	1	1	17	3	22	5	69	16	48	70[l]
Temp. °C		0	37	30	30	30	25	25	25	25	25	25	25	25

[a] Two equivalents of phenol were added, and 4-nitrosophenol was also a product. [b] Two equivalents of *o*-cresol were added, and *p*-nitroso-*o*-cresol was also a product. [c] The analytical method used for product quantitation in each of these runs was identical to ensure valid comparison of yields of both more and less water-soluble products. [d] Yields corrected for unreacted substrate. [e] Amide structure that of amidine where O replaces Ph-N (imino). [f] Either formanilide or acetanilide as indicated by R_1. [g] Dimethylnitrosamine for **8b** and **8e** or *N*-nitrosopyrrolidine for **8f**. [h] Ar = 4-HOPh except for runs 4 and 5 where Ar = 4-hydroxy-2-methylphenyl. [i] % unreacted substrate recovered. [j] 2,4-Bis(phenylazo)phenol (46%, based on the reaction of three moles of substrate to yield 1 mole of product) was also isolated. [k] NO_2^- molar equivalents relative to substrate. [l] In a separate run with a duration of 45 h, >92% of **8f** was recovered but products were not quantitated. [m] General abbreviations: na, not applicable; nd, not determined; +, trace; −, not observed.

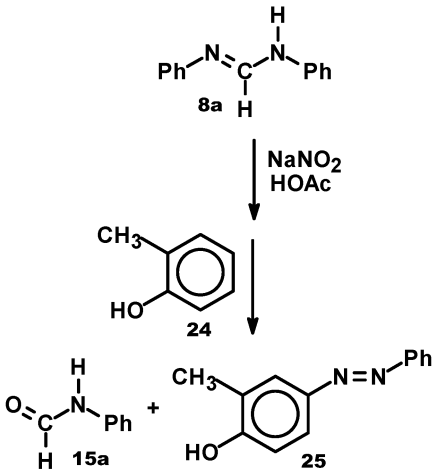
set of experiments employing the *N*,*N*′-diphenylformamidine **8a** where the benzenediazonium ion was trapped by azo coupling to either phenol or *o*-cresol. Data from these experiments are given Table 1 (runs 3−5). The experiments with *o*-cresol were most meaningful because phenol is a decomposition product of the benzenediazonium ion. In a typical trapping experiment, the nitrosation reaction was allowed to proceed for 15 min at 30 °C, at which time a 2-fold excess of the trapping phenol was added and stirring was continued for 1 h. Workup, product identification, and quantitation were performed in the same manner as described above with the use of authentic standards. The major products are shown in Scheme 5 for *o*-cresol. In all runs (3−5), the benzenediazonium ion was trapped as the corresponding azo compound in yields ranging from 89 to 93%. Similarly, the yields of formanilide **15a**, which is derived from the other amidine molecular fragment, were 89−94%. As we discuss below, these data argue for, but do not require, the formation of the diazonium ion by path A of Scheme 3.

In the discussion which follows, we reasonably assume that the nitrosation of the amidine free base at either nitrogen atom is fast and, in the case of the tertiary amidines, reversible (see below). Nitrosation at either nitrogen of the secondary amidines examined will ultimately give the same products, even though nitrosation of the imino nitrogen for all amidines will give rise to the more stable cation due to the delocalization of the charge over the two nitrogens and carbon which make up the amidine moiety. The additon of water to the central carbon followed by deprotonation then gives the α-hydroxynitrosamine **9** (tetrahedral intermediate) depicted in Scheme 3. A key point supporting the argument that the decomposition of this intermediate in the case of the secondary amidines is occurring almost exclusively by path A is the near equality of the yields of azo compound and formanilide in each run (3−5). In our preliminary work on amidine nitrosation, we isolated *N*-nitrosopyrrolidine and *N*-benzyl-*N*-nitrosoformamide from the nitrosation of the tertiary amidine *N*-benzylformimidoylpyrrolidine.[41] Because *N*-nitrosoamides decompose to diazonium ions with facility, it was unclear as to which path was being followed. *N*-Aryl-*N*-nitrosoamides are much less stable than their *N*-alkyl analogues. The kinetics of decomposition of *N*-nitrosoformanilide, *N*-nitrosoacetanilide, and related *N*-nitroso amides have been examined in both protic and aprotic solvents.

SCHEME 4



SCHEME 5



Half-lives are typically less than 10 min at 25 °C.[44] These facts explain why we were unable to detect *N*-nitrosoamides in this study compared to or prior work where were able to detect *N*-benzyl-*N*-nitrosoformamide. In the case of the nitrosation of **8a**, decomposition by path A of Scheme 3 gives the benzenediazonium ion and formanilide, but the benzenediazonium ion would also form readily in the medium by the diazotization of aniline generated by decomposition of the tetrahedral intermediate **9** by path B. The latter transformation, however, would produce *N*-nitrosoformanilide **12** ($R_1 = H$). In order for **12** to give formanilide, the *N*-nitrosoamide would have to undergo nearly quantitative denitrosation, a known reaction in the presence of nucleophilic anions such as chloride, in competition with the well-known decomposition of these intermediates to yield diazonium ions. The weaker nucleophilicity of the acetate ion, and most importantly, the near equivalent yields of the azo compound and formanilide make this unlikely.

Further evidence on the partitioning of paths A and B can be obtained from the data from the nitrosation of tertiary amidines **8b**, **8e**, and **8f**, as well as our prior published data from the nitrosation of **8c**, its *p*-chlorophenyl derivative, and *N*-benzylformimidoylpyrrolidine.[41] In these cases, the respective yields of the nitrosamine **14** and the dialkylamide **11** (**16** for **8e**) give minimal markers of the percent of the transformation which is occurring by each path, A or B. The nitrosamine arises from path B and the amide from path A. Material balances for these dialkyl products range from 82 to 88% (runs 10, 12, and 13). Because of their complete water miscibility, dimethylnitrosamine and DMF are more difficult to accurately quantitate in these assays than are their pyrrolidine analogues, yet the material balances are good. The greatest yield of nitrosamine (26%) was observed for **8f**, where the dialkylamine moiety is pyrrolidine. Thus, assuming that the transformations occur as shown in Scheme 3, at least 26% of the reaction of **8f** must occur by path B. We previously reported that *N*-nitrosopyrrolidine was obtained in 23% yield from the nitrosation of *N*-benzylformimidoylpyrrolidine,[41] which agrees well with what we see for **8f**. On the other hand, the dimethylamino amidines **8b** and **8e** give less nitrosamine, 8% and 2%, respectively, and correspondingly larger yields of DMF and *N*,*N*-dimethylacetamide (Table 1, runs 10 and 12). The large relative yields of the amides require that at least 74% and 82% of the nitrosative transformations of **8b** and **8e**, respectively, are giving the diazonium ion by path A. Thus path A, appears to be the major transformation route for all compounds **8a**−**f**.

The participation of path B increases as the amino portion is changed in the order aniline < dimethylamine < pyrrolidine. These limited data suggest that path B becomes more prominent as the amino portion of the amidine becomes more basic. Decomposition of the tetrahedral intermediate **9** by path B almost certainly involves prior protonation of the amine nitrogen to convert it into a better leaving group, as is observed in both acid-catalyzed amidine and amide hydrolysis.[45,46] The electron withdrawing PhNNO and OH substituents attached to the central carbon will significantly reduce the basicity of adjacent amino moiety of **9**, but the basicity of the amino nitrogen will still be proportional to that of the related amine. The relative proportion of the amino nitrogen protonated species, required for transformation by path B, will then be greatest for the pyrrolidine-derived amidines that show the largest yields of nitrosamine.

It is, of course, possible that the observed transformations occur by pathways other than those depicted in Scheme 3. The key steps of several possibilities are given in Scheme 6. Nitrosation, rather than protonation of the dialkylamino moiety of **9** (path C) could lead to the nitrosamine directly by decomposition of the tetrahedral intermediate **26**. It is also possible, as shown in path D, that nitrosation of the tetrahedral intermediate **27** at either nitrogen atom, following reversible addition of H_2O

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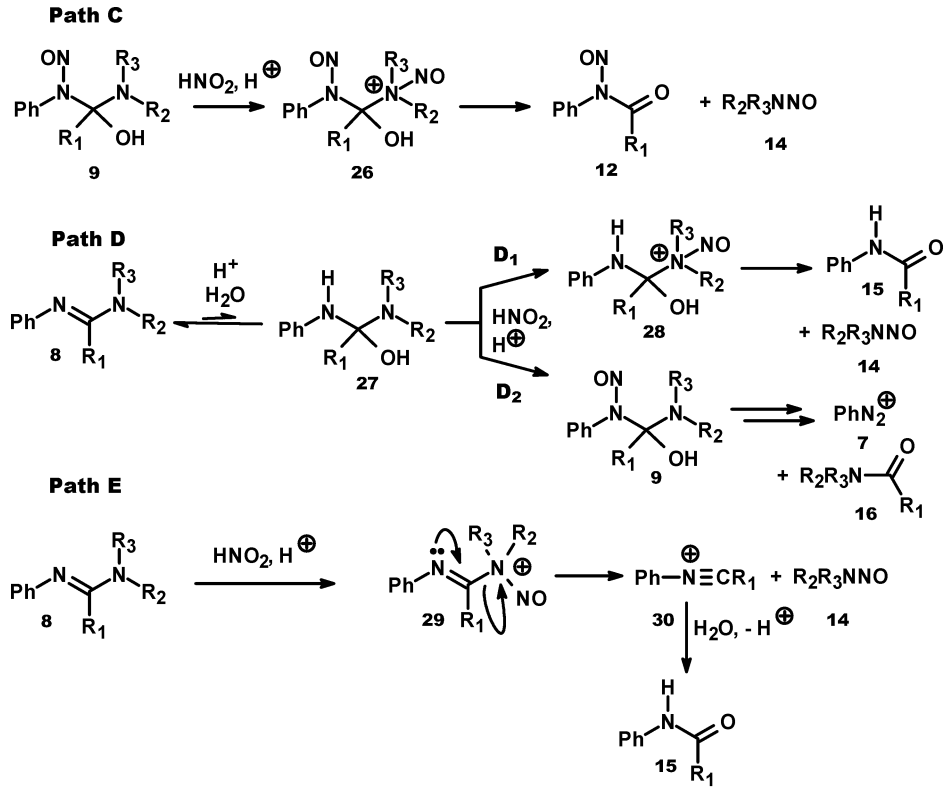
(44) Hey, D. H.; Stuart-Webb, J.; Williams, G. H. *J. Chem. Soc.* **1952**, 4657−4665.
(45) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H. *Acc. Chem. Res.* **1992**, *25*, 481−488.
(46) DeWolfe, R. H.; Keefe, J. R. *J. Org. Chem.* **1961**, *27*, 493−496.

SCHEME 6



to the protonated amidine, could lead to products. We may expect path D_1 to predominate because of the greater nucleophilicity of the dialklyamino N, which may give more **28** than **9**. This would lead to large amounts of nitrosamine being formed, which we do not observe. The pathway has the virtue of generating the amide as a coproduct without the necessity of being formed by denitrosation of an *N*-nitrosoamide as is foreseen in path B. In our prior publication,[41] we postulated that the chemistry shown in path E was operative. This process also avoids *N*-nitrosoamide formation, but the yields of the amides **15** are much smaller than those of the nitrosamines, suggesting that the pathways in Scheme 3 are operative, rather than those of paths D_1 and E.

While we have shown that an increase in the basicity of the amino moiety of the amidine increases the participation of path B in the competitive decomposition of **9**, we cannot state that these intermediates will always preferentially decompose by path A. The factors which control decomposition by path A vs path B are subtle and sensitive to structure. We have previously shown that 1-methyl-2-phenylimidazoline, a five membered cyclic amidine, nitrosates and decomposes by path B.[42] We invoked stereoelectronic arguments, which are not applicable here, to explain this observation, but the basicity of the nitrogen is also enhanced by the methyl substitution, favoring decomposition by path B through its protonation. On the other hand, the acid-catalyzed decomposition of both 2-benzyl- and 2-phenyl-*N*-nitrosoimidazoline occurs by path A. An examination of the 1,2,4-triazole-catalyzed decomposition of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine shows that the amine catalyst departs as its anion from the tetrahedral intermediate at a rate greater than the less basic methyldiazotate (basic media).[47] In the reactions reported here, the preferred leaving group from the tetrahedral intermediate **9** is phenyldiazohydroxide and the acidic medium likely ensures some form of acid catalysis for this process. Conjugative interaction between the benzene ring and the forming N=N bond may enhance the rate and thus the "leaving group ability" of this moiety.

Reactivity and Kinetics. Reaction rates for the loss of the starting amidine upon nitrosation, as determined by HPLC or GC, were measured at 25 °C in glacial acetic acid employing a 10- or 20-fold excess of sodium nitrite so as to ensure pseudo-first-order conditions. Plots of ln([amidine]) vs time showed good linearity in all cases, and are the result of two−three independent determinations. The rate constants for the nitrosation of amidines **8a**−**f** are reported in Table 2 in two ways. In the first case the observed rate constant (k_{obs}), as determined from linear regression (slope) of the plotted data, is given. In the second entry, k has been determined by calculation from k_{obs} by correction for the amidine basicity (free amidine concentration). The values of amidine pK_a's used were taken from the literature[48,49] and are also given in Table 2. Although pK_a data for some amidines in aqueous solution are available, for the sake of consistency we used data where pK_a's were determined in 95% ethanol, which have been reported for all of the compounds studied except **8i**. As a control we also made cursory determinations of the approximate hydrolysis rates (data not given) for amidines **8a**−**i** under the acidic conditions used for

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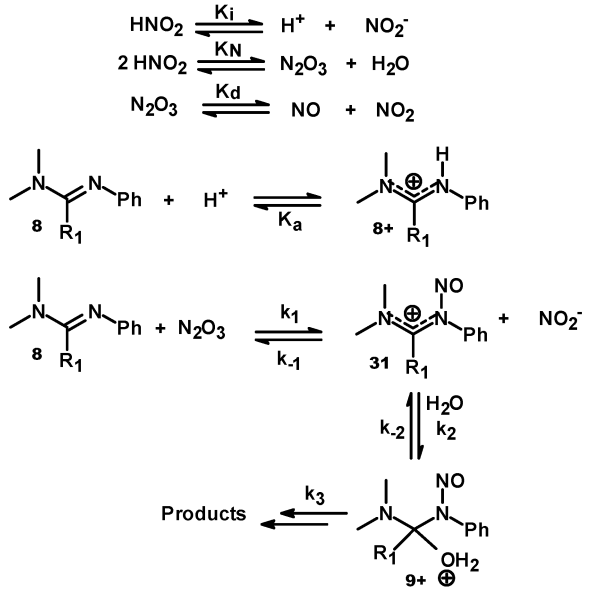
(48) Oszczapowicz, J.; Ciszkowski, K. *J. Chem. Soc., Perkin Trans. 2* **1987**, 663−668.
(49) Oszczapowicz, J.; Krawczyk, W.; Lyzwinski, P. *J. Chem. Soc., Perkin Trans. 2* **1990**, 311−314.

TABLE 2. Amidine Nitrosation Rate Constants

compd	R_1	R_2	R_3	pK_a[a]	$k_{obs} \times 10^5$ [b] (s^{-1})	$\pm \times 10^5$	$k \times 10$[c]	$\pm \times 10$
8a	H	Ph	H	5.38	1061[d]	28	5.2	0.1
8b	H	CH_3	CH_3	7.45	13	1	7.4	0.8
8c	H	$(-CH_2-)_4$		8.12	13.6	0.8	36	2
8d	CH_3	Ph	H	6.97	12.0[d]	0.8	2.2	0.1
8f	CH_3	$(-CH_2-)_4$		10.6	6.8	0.7	5425	556
8f	CH_3	$(-CH_2-)_4$		10.6	6.8	0.7	5425	556
8g	Ph	Ph	H	6.15	no reaction			
8h	Ph	CH_3	CH_3	7.8	no reaction			
8i	Ph	$(-CH_2-)_4$		no reaction				

[a] Literature values, measured in 95% ethanol, see text. [b] [amidine] = 0.0363 M, $[NO_2^-]$ = 0.726 M, 25 °C in acetic acid/H_2O, pH = 3.7. [c] $k_{obs} \times [H^+]/K_a$. [d] Because of the high reaction rate of **8a**, k_{obs} was determined at [amidine] = 0.038 M and $[NO_2^-]$ = 0.414 M (10.9 equiv of NO_2^-) and found to be 376 ± 10 × 10^{-5} s^{-1}. k_{obs} = 4.3 ± 0.3 × 10^{-5} s^{-1} for 8 d was determined at [amidine] = 0.0363 M and $[NO_2^-]$ = 0.392 M as well as the table entry. The ratio of these rate constants is 2.82 and k_{obs} for **8a** (entry 1) at the higher $[NO_2^-]$ was estimated by multiplying by 2.82 × 376 × 10^{-5} s^{-1}.

SCHEME 7



the nitrosation. In all cases, hydrolysis did not compete with nitrosation.

The completely inert character of the benzamidines **8g**−**i** toward nitrosation is the most striking result to emerge from this study. The nitrosation rates of many nitrogen compounds in acid have been shown to be inversely proportional to their basicity.[22] *N*-Nitrosation requires an unshared electron pair on an N-atom. Coordination of this electron pair with a proton prevents nitrosation until it is removed by some, even weakly basic species. While *N*-nitrosation of many amines and some other nitrogen compounds has been shown to effectively be encounter controlled,[50] the high basicity of these compounds effectively makes the nitrosation of these compounds rate limiting. The data of Table 2 show that this is not the case for the benzamidines. The benzamidines **8g**−**h** are less basic than their respective acetamidine analogues **8d**,**e** which do nitrosate, so amidine basicity cannot be the sole determinant of nitrosation rate and reactivity.

In Scheme 7, and in the rate equations (1 and 2) derived from it, we have sketched what we believe are the important steps in amidine nitrosation. Step 1 involves *N*-nitrosation of the amidine free base by N_2O_3[50,51] to give the more thermodynamically stable *N*-nitrosoamidinium ion **31**.[52] In the case of trialkyl tertiary amines, the initial nitrosation step is reversible,[53] and it seems reasonable that that some of our substrates will exhibit this property as well.

While the hydration of *N*-nitrosoiminium ions (**2** → **3**, Scheme 1, where the C substituents are H or C) has been shown to be very fast,[9] depending upon R_1, this may or may not be the case for the analogous hydration of **31** shown as step 2 of Scheme 7, which is akin to the acid-catalyzed hydration of a carboxylic acid derivative or an amidine. The effective reversibility of this step, which in the case of carboxylic acid derivatives can be measured using ^{18}O exchange, depends on the nature of the leaving group, or in kinetic terms the relative magnitudes of k_3 and k_{-2}.[45] Step 3 likely proceeds by either prior proton loss from O and/or transfer to the O-atom of the NO of the incipient diazohydroxide or, as discussed above, to the amino nitrogen.

In the case of the tertiary benzamidines (R_1 = Ph), we propose that their lack of their nitrosative reactivity is due to a very small value of k_2 in comparison to k_{-1} and k_{-2}. In other words, the *N*-nitrosoiminium ion **31** forms reversibly but does not hydrate at an appreciable rate. At analysis time, any **31** present will be converted back to **8**. This supposition is well supported by the low reactivity to benzoic acid derivatives toward nucleophilic acyl substitution reactions, which was discovered relatively early in the history of physical organic chemistry.[54,55] There are many examples. Benzamides and benzamidines undergo acid-catalyzed hydrolysis slowly in comparison to their aliphatic analogues.[56] There is significant evidence that this low reactivity stems from

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(51) Casado, J.; Castro, A.; Mosquera, M.; Rodriguez-Prieto, M. *Monatsh. Chem.* **1984**, *115*, 669−682.

(52) Numerous studies in similar acid media strongly support our assumption that the nitrosating agent is N_2O_3. At modest to high $[NO_2^-]$ relative to the [substrate], ONOAc is converted to N_2O_3. The reverse step need not involve NO_2^- directly but may involve reaction with H_2O or another nucleophile. Nevertheless, the nature of nitrous acid chemistry is such that the result will be the same, that is, the regeneration of N_2O_3 and the amidine.

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(56) Smith, C. R.; Yates, K. *J. Am. Chem. Soc.* **1972**, *94*, 8811−8817.

the conjugative stabilization of the carbonyl, protonated carbonyl, or their imino analogues by the aromatic ring. The transformation may also be slowed by steric compression in the tetrahedral intermediate.

Pairwise comparison of the data of Table 2 for the formamidines (**8a**−**c**) with their structurally analogous acetamidines (**8d**−**f**) shows in each case that the formamidines are less basic and have higher values of k_{obs} for nitrosation than the corresponding acetamidines, as may be expected. On the other hand, when the rate data are corrected for the basicity of the amidine, so as to give the rate constant k for the nitrosation of free amidine (the intrinsic reactivity), the picture is less clear. De Wolfe and Keefe showed that the hydrolysis of **8a** is 1548 times more rapid than its acetamidine analogue **8d** in 20/80 dioxane-H_2O (0.42 M HCl) at 86 °C.[46] Under these conditions, the amidines are completely protonated and the rate depends on the rate constant for the attack of H_2O on the amidinium ion. Both the inductive stabilization of the cation by the methyl group and its greater steric bulk retard the reaction of the acetamidine. We might expect these structural differences to influence amidine nitrosation rates in a similar way, but this is not observed. Amidine nitrosation, however, is more complicated and involves, at least, the three steps shown in Scheme 7, and the competitive protonation equilibrium of the amidine, which prevents nitrosation, also plays a role. Different kinetic and mechanistic scenarios arise depending upon how the equilibrium and rate constants change with structure.

$$\frac{dP}{dt} = \frac{k_1k_2k_3[\mathrm{Am}][\mathrm{N_2O_3}][\mathrm{H_2O}]}{k_{-1}(k_{-2} + k_3)[\mathrm{NO_2^-}] + k_2k_3[\mathrm{H_2O}]} \quad (1)$$

$$\frac{dP}{dt} = \frac{k_1k_2k_3[\mathrm{H^+}]^2[\mathrm{Am}][\mathrm{NO_2^-}]^2}{K_i^2(k_{-1}(k_{-2} + k_3)[\mathrm{NO_2^-}] + k_2k_3[\mathrm{H_2O}])} \quad (2)$$

For discussion we assume the operation of rate eq 1 for the rate of product formation from the tertiary amidines, where [Am] is the amidine concentration and the equilibrium and rate constants are as defined in Scheme 7. The nitrosative decomposition of the reactive secondary amidines, even though they may form *N*-nitrosoamidines, is presumed to obey a very similar rate law since their protonation will generate a species **9**. Substitution for the equilibrium concentration of N_2O_3 ($[N_2O_3] = K_N[H^+]^2[NO_2^-]^2/K_i^2$), ignoring its homolytic decomposition, gives eq 2. We have assumed that the nitrosation is rapid and reversible and that steady-state concentrations can be defined for both the *N*-nitrosoamidinium ion **31** and the tetrahedral intermediate **9**. Without more extensive experimentation and detailed kinetic data, we can only make some pertinent observations. Because $[NO_2^-]$ appears both in the numerator and denominator of eq 2, the reaction order in NO_2^- may change from substrate to substrate and mask intrinsic reactivity. For example, if $k_3 \gg k_{-2}$ and $k_{-1}[NO_2^-] \gg k_2[H_2O]$ then the rate will be first order in NO_2^- and hydration of **31** will be rate limiting (see the Supporting Information).

The similar magnitudes of k most plausibly result from cases where k is dominated by k_2k_3. Literature precedent suggests that these two rate constants will respond differently to structural change of R_1. From the hydrolysis data we see that replacement of H by CH_3 decreases the rate of that reaction which is very similar to step 2 of our transformation.[46] On the other hand, CH_3 substitution for H in α-hydroxynitrosamines, analogous to **31**, significantly increases the rate of their decomposition to diazonium and carbonyl products.[57] In addition to the diazohydroxide, the immediate precursor to the diazonium ion, this transformation generates a carbonyl compound, which in our case is an amide. The thermodynamic stability of the carbonyl compound is experimentally observed to influence the rate of decomposition of the α-hydroxynitrosamine from which it is derived.[57] The more stable the incipient carbonyl compound is, the more rapid the decomposition is, suggesting that these transformations are thermodynamically neutral or have some endothermic character. The transition state structure appears to be influenced byproduct structure. Thus, CH_3 for H substitution at the central carbon of the amidine will retard the rate of H_2O attack (k_2) but is expected to enhance the rate of **9** decomposition (k_3). While the individual rate constants may change significantly with structure, their product may not.

The pyrrolidine-derived amidines **8c** and **8f** are unusually reactive as measured by their corrected rate constants k. The acetamidine **8f** is 150 times more reactive than the formamidine **8c**. These reactivity differences are unexpected.[58] While the diversity of substrates examined here do not permit a complete understanding of the reactivity differences, we offer the following rationalization. As discussed above, these amidines react by path B, producing *N*-nitrosopyrrolidine, to the extent of at least 25%. But path B participation by itself cannot be the sole reason for the rate enhancement or it would out compete path A. It is probable that the more rapid decomposition of the tetrahedral intermediate **9** leads to the rate enhancement by both paths A and B. This could result from the release of steric compression and "I strain" in the conversion of the tetrahedral intermediate to products of trigonal geometry. Reactions involving the pyrrolidine ring system have been observed to be accelerated when the nitrogen goes from tetrahedral to trigonal geometry as in the case of path A where it is converted from amine N to amide N, or in path B where it is converted from protonated amine N to free amine N.[59] This is due to the ring size enforced preference for atoms to have a more trigonal geometry in the five-membered ring.[59]

We also investigated the nitrosation of several imino-*N*-acylamidines and amino-*N*-acylamidines. These compounds hydrolyze much more rapidly than they nitrosate, however. Details are given in the Supporting Information.

Conclusions

Both secondary and tertiary amidines are modestly reactive toward nitrosation. Nitrosation rates are only

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(58) While an error in, or the inappropriate use of, pK_a values determined in 95% ethanol to correct our k_{obs} values for amidine protonation could be responsible, the pK_a values were determined by the same group using the same methodology.

(59) Kresge, A. J.; Fitzgearld, P. H.; Chiang, Y. *J. Am. Chem. Soc.* **1974**, *96*, 4698−4699.

roughly correlated with the basicity of the amidine. The values of k_{obs} varied at most by a factor of 100 where the amidine K_a values differed by as much as 10^5, where the more basic amidines reacted more rapidly than expected in comparison to a variety of other nitrogen compounds where *N*-protonation effectively reduces the nitrosation rate. Diazonium ions are the primary products of amidine nitrosation for all substrates examined here and are formed by path A of Scheme 3. The tertiary amidines also form nitrosamines by path B where the pyrrolidine derived amidines show a greater tendency to produce products by this route. The factors which control decomposition by path A vs path B are subtle and sensitive to structure. The benzamidines examined are unreactive toward nitrosation. This must be due to a greatly reduced rate of addition of H_2O to the *N*-nitrosoamidinium ion **31**. Formamidine and acetamidine nitrosation rates are comparable, in contrast to their acid-catalyzed hydrolysis rates. We attribute this phenomenon to the counteraction of substituents at R_1 on the rate of H_2O addition (k_2) compared to the rate of tetrahedral intermediate decomposition (k_3). *N*-Acylamidines hydrolyze more rapidly than they nitrosate, but some of the hydrolysis products are easily nitrosated. Although all of the work presented here was done at high nitrite concentrations, it is clear that most *N*-arylamidines react with nitrosating agents to produce electrophilic diazonium ions, while nitrosamines are generated in lesser yields. We have also shown previously that *N*-nitrosoamides are products of these transformations. Thus, amidines must be viewed as nitrosatable compounds capable of generating mutagens and possible carcinogens.

Experimental Section

Caution: Nitrosamines, *N*-nitrosoamides, *N*-nitrosoamidines, and nitrosation reaction mixtures which produce them should be considered carcinogenic and appropriate care taken in their handling. We performed all operations in well-ventilated hoods. Nitrosamines are effectively destroyed by treatment with 30% HBr-glacial acetic and we routinely treat all of our glassware with this solution prior to further cleansing. Dilute aqueous solutions of nitrosamines can be destroyed by bring the solution to pH 12−13 and reaction with Raney-nickel.

Synthesis of Amidines 8b−i and *N*-Acylamidines 32b and 33a−c. All of the amidines and *N*-acylamidines used in this work are known compounds. These substrates, as individually cited, were synthesized for use in this work by literature methods or slight variations thereof. In all cases the physical and spectral properties were consistent with those reported in the literature. Additional spectral data for each compound are given in the Supporting Informaiton. The compounds are as follows: *N*,*N*′-diphenylformamidine, **8a** (commercially available), *N*,*N*-dimethyl-*N*′-phenylformamidine,[60] **8b**; 1-[1-(phenylimino)methyl]-pyrrolidine,[61] **8c**; *N*,*N*′-diphenylacetamidine,[62] **8d**; *N*,*N*-dimethyl-*N*′-phenylacetamidine,[60] **8e**; 1-[1-(phenylimino)ethyl]-pyrrolidine[63] **8f**; *N*,*N*′-diphenylbenzamidine,[64] **8g**; *N*,*N*-dimethyl-*N*′-phenylbenzamidine,[65] **8h**; 1-[phenyl(phenylimino)methyl]-pyrrolidine,[66] **8i**; and *N*,*N*-dimethyl-*N*′-benzoylformamidine.[67]

Amidine Nitrosation. All amidines **8a−i** were nitrosated under similar conditions in glacial acetic acid with an excess of aqueous sodium nitrite. The precise molar equivalents of sodium nitrite, the reaction temperature, and the reaction time are given for each run along with the product yields in Table 1. The reaction products are all known, well-characterized commonly available compounds. 2,4-(Bis)phenylazophenol[68] and 2-methyl-4-azophenylphenol **25**[69] were prepared by the literature methods as cited. In every case, the compounds were obtained and their ^1H, ^{13}C NMR spectra and mass spectra examined to ensure conformity with literature values. These authentic compounds were used as chromatographic standards. In all cases, the reaction mixtures were submitted to flash column chromatography following extraction to isolate and characterize the major components. Depending upon the goal of the particular run, the products were also submitted to GC−MS analysis and/or HPLC RP chromatography. Quantitation was done through the use of external standards.

Typical Nitrosation. *N*,*N*-Dimethyl-*N*′-phenylformamidine **8b** (0.57 g, 3.8 mmol) was dissolved in 10 mL of glacial acetic acid. To the solution was added dropwise 7 mL of 5.5 M aqueous sodium nitrite solution (38.0 mmol) over a 3 min. The mixture was shielded from light by aluminum foil and stirred at room temperature for 69 h. The solution was neutralized to pH 7 with 6 g of potassium carbonate. The aqueous phase was extracted with methylene chloride (3 × 50 mL). The combined organic layers were washed with brine and dried ($MgSO_4$). The filtered solution was diluted to 250 mL in a 250 mL volumetric flask (extract 1). The aqueous phase was basified with 30 g of potassium carbonate and 2 g of sodium hydroxide. This mixture was extracted with methylene chloride (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and diluted to 250 mL in a 250 mL volumetric flask (extract 2). To determine the amount of phenyl acetate and DMF in extract 1, the solution was diluted five times. To determine the amount of unreacted starting material in extract 2, the solution was diluted 10 times. The amounts of dimethylnitrosamine **14b** (0.2 mmol, 8%), formanilide **15** (0.08 mmol, 3%), DMF **16** (2 mmol, 74%), phenol **17** (0.2 mmol, 8%), phenyl acetate **18** (0.8 mmol, 30%), 2-nitrophenol **23** (0.14 mmol, 5%), and 4-nitrophenol **22** (0.1 mmol, 4%) were determined by GC−FID (40 °C, 5 °C/min to 250 °C). Retention times (min): **14b**, 2.8; **16** (DMF), 3.4; **17**, 7.8; **18**, 10.2; **23**, 12.2; **15** (formanilide), 17.7; **8b**, 19.4; and **22**, 23.3 min). After vacuum-assisted removal of the solvent, the resulting oil was subjected to flash column chromatography on silica gel.

Lack of Benzamidine 8g−i Nitrosative Reactivity. The benzamidines **8g−i** were nitrosated under the conditions described above for at least 4 h. In each case, the parent amidine was recovered in near-quantitative yield and no products could be observed in the extracts.

Diazonium Ion-Trapping Experiments. In runs 3−5 (Table 1), *N*,*N*′-diphenylformamidine **8a** was subjected to nitrosation under conditions where the benzenediazonium ion could be trapped as quantitatively as possible by azo coupling with either added phenol or *o*-cresol as described. Phenol (2 equiv), which is also a reaction product, was used in run 3, and *o*-cresol was utilized in runs 4 and 5. The conditions for run 4 were as follows. Compound **8a** (1.0 g, 5.1 mmol) was dissolved in 7.5 mL of acetic acid. The solution was stirred in

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an oil bath at 30.5 °C. To the solution sodium nitrite (1.8 g, 26.1 mmol) aqueous solution (3.0 mL water) was added all at once. After the addition of sodium nitrite, the mixture was stirred for 15 min. To the mixture was added *o*-cresol (1.1 g, 10.2 mmol). The resulting mixture was stirred for 1 h. To the mixture was added 20 mL of ice water. The resulting mixture was extracted with benzene (4 × 20 mL). The combined organic layers were washed with brine and dried ($MgSO_4$). An aliquot of the benzene extract was injected in GC−MS. 4-(Phenylazo)-2-cresol **25**, formanilide **15**, and 4-nitroso-2-cresol were identified in the chromatogram. Benzene was evaporated in vacuo. The resulting oil was purified by column chromatography (ethyl acetate−hexane, 1:6) to give 0.98 g of **25** (91%), 0.58 g of **15** (94%), and 0.65 g of 4-nitroso-2-cresol.

Kinetics of the Nitrosation the Amidines 8a−f with a Large Excess of Sodium Nitrite. Typical kinetics procedure: The amidine was dissolved in 12.0 mL of glacial acetic acid and 10.0 mL of water. The pH of the solution was adjusted to 3.7 by 5 N NaOH solution (the concentration of the amidine was 0.036 M). The amidine solution was stirred in an oil bath at 25.0 °C. To the solution 5 mL of sodium nitrite aqueous solution (20 equiv) was added all at once. During the reaction course, 10−12 aliquots (∼500 μL) were taken from the bulk solution at specific times (12 min intervals for **8a** and 1 h intervals for the other amidines). Each sample solution was quenched by base (for **8a**, sodium carbonate; for other amidines, sodium hydroxide). The resulting basic aqueous solution was extracted with methylene chloride (3 × 15 mL). The combined organic layers were washed with brine and dried ($MgSO_4$). Methylene chloride was evaporated in vacuo. The residue was dissolved in acetonitrile in a 50 mL volumetric flask (for **8a** the methylene chloride extract was diluted to 50 mL with methylene chloride in a 50 mL volumetric flask). The concentration of the amidine was determined by HPLC (for **8a** by GC−FID) with external standards of the amidine (mobile phase: acetonitrile & 0.025 M sodium phosphate buffer; flow rate: 1 mL/min). Four standard solutions of the amidine were used to make a calibration curve. The concentration of the amidine in each sample solution was determined by the calibration curve. The rate constants reported in Table 2 were obtained from the slopes of linear regressions of ln([amidine]) vs time and are the averages of several runs for each substrate.

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Supporting Information Available: Details of *N*-acylamidine preparation, attempted nitrosation, and hydrolysis; additional spectral data for the compounds used in this research; control hydrolysis experiment; abbreviated derivation of eq 1 and presentation of limiting cases. This material is available free of charge via the Internet at http://pubs.acs.org.

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