Kinetics and Mechanism for Pyridinecarboxaldehyde Phenylhydrazone Formation[1]

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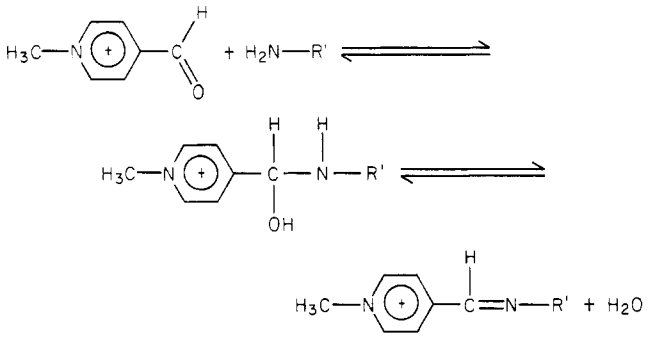
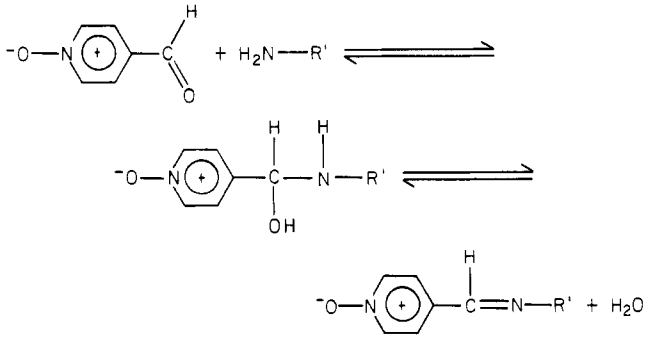
Phenylhydrazone formation from 4- and 3-pyridinecarboxaldehyde occurs with rate-determining carbinolamine dehydration under acidic, neutral, and basic conditions. Plots of first-order rate constants against amine concentration are nonlinear for these substrates in the range of pH studied. This nonlinearity reflects carbinolamine accumulation. The subsequent dehydration of the carbinolamine exhibits both specific-acid catalysis and general-acid catalysis, with values of the Brønsted exponent ranging from 0.8 to 0.6. In contrast, phenylhydrazone formation from 2-pyridinecarboxaldehyde exhibits a transition in the rate-determining step from carbinolamine dehydration to carbinolamine formation under mildly acidic conditions. General acid–base catalysis for the formation or dehydration of the carbinolamine intermediate is not detectable.

We have begun to explore in some detail the kinetics and mechanism for addition of amines to a particular set of carbonyl substrates, pyridine aldehydes, and their derivatives. An earlier investigation examined the addition of hydroxylamine, phenylhydrazine, and semicarbazide to the three isomeric formyl-1-methylpyridinium ions.[2]



It was established that, contrary to most other cases, carbinolamine dehydration is rate determining under acidic, neutral, and basic conditions, reflecting the influence of the electron-withdrawing power of the cationic nitrogen function.

In continuation of this work, we reported the kinetics for addition of phenylhydrazine to 4-, 3-, and 2-pyridine *N*-oxide carboxaldehyde.[3]



The *N*-oxides possess a less potent electron-withdrawing ring nitrogen atom, and this is reflected in the kinetics and mechanism for amine addition. Phenylhydrazone formation from the three isomeric pyridine *N*-oxide carboxaldehydes occurs with two breaks in the pH–rate profile. Under alkaline conditions, carbinolamine dehydration is rate determining, while under more acidic conditions, stepwise formation of carbinolamine becomes the rate-determining step. The second break in the pH profile was interpreted as reflecting a transition to concerted rate-determining formation of the carbinolamine. In the present work, we report the kinetics of addition of phenylhydrazine to 4-, 3-, and 2-pyridinecarboxaldehydes. In addition to their intrinsic interest, these studies are of importance for understanding the chemistry and biochemistry of piridoxal phosphate.

Experimental Section

Materials. All reagents employed were obtained commercially and, with the exception of reagent grade inorganic salts, were either redistilled or recrystallized before use. Solutions of phenylhydrazine were prepared just prior to use. Solutions of carboxylic acids in 20% aqueous-ethanol (v/v) were prepared just prior to use to avoid esterification.

Kinetics measurements[4–6] were carried out spectrophotometrically at 25.0 °C with the aid of a Zeiss PMQ II spectrophotometer equipped with a thermostated cell holder. The reaction of phenylhydrazine with the aldehydes was followed by observing the appearance of product at the following wavelengths: 4-isomer, 420 nm; 3-isomer, 396 nm; 2-isomer, 420 nm. The initial aldehyde concentration was 2.0×10^{-5} M. In all cases, a sufficient excess of nucleophilic reagent was employed so that pseudo-first-order rate behavior was observed. First-order rate constants were evaluated from slopes of plots of log $(OD_\infty - OD_t)$ against time in the usual manner. Second-order rate constants were obtained by dividing the first-order rate constants by the concentration of nucleophilic reagent in the reactive, free-base form. Catalytic (third-order) rate constants were evaluated from the slopes of plots of second-order rate constants against the concentration of the catalyst.

All kinetic experiments were carried out at 25.0 ± 0.1 °C in 20% aqueous ethanol (v/v) at an ionic strength of 0.50 maintained with KCl and in the presence of 2.0×10^{-4} M EDTA. Values of apparent pH were recorded with a Radiometer Model PHM 4d meter equipped with a glass electrode. Calculations of the concentration of phenylhydrazine free base and undissociated carboxylic acids were made by employing the Henderson–Hasselbalch equation and pK_a values determined earlier[7,8] (pK_a of phenylhydrazine = 5.26).

Results and Discussion

As is developed in detail below, phenylhydrazone formation from 4- and 3-pyridinecarboxaldehydes proceeds

(1) Supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo.

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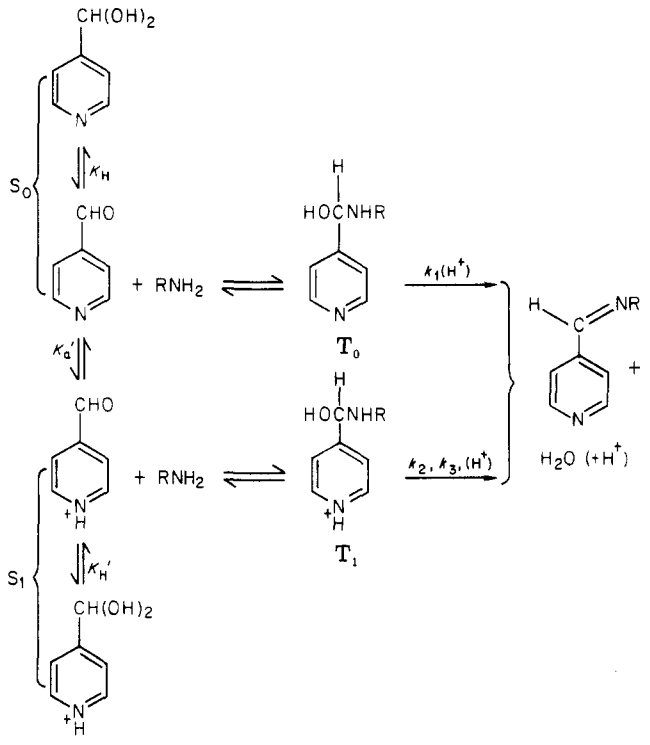
Scheme I[a]



[a] $K_1 = [T_0]/[S_0][RNH_2]$; $K_1' = [T_0]/[Ald_0][RNH_2]$; $K_2 = [T_1]/[S_1][RNH_2]$; $K_2' = [T_1]/[Ald_0][RNH_2]$; $K_a' = [S_0][H^+]/[S_1]$; $K_H = [hydrate]_0/[Ald_0]$; $K_{H^+} = [hydrate]_1/[Ald_1]$.

with rate-determiing carbinolamine dehydration over the entire pH range investigated. The kinetics for these reactions are complicated by the hydration equilibrium of the substrates. Prior to analysis of experimental data, it will prove helpful to have rate laws for these reactions in hand. In Scheme I is provided a summary of the pathways for reaction of 4-pyridinecarboxaldehyde with phenylhydrazine, assuming rate-determining carbinolamine dehydration.

The neutral carbinolamine is considered to require acid catalysis for dehydration, and the cationic carbinolamine is viewed as dehydrating both with and without acid catalysis. The rate law is then given by eq 1.

$$dp/dt = k_1[H^+][T_0] + k_2[T_1] + k_3[H^+][T_1] \quad (1)$$

Expressing $[T_0]$ and $[T_1]$ in terms of the equilibrium constants defined in Scheme I, eq 1 may be rewritten as eq 2.

$$\frac{dp}{dt} = [S_0][RNH_2]\left(k_1K_1[H^+] + \frac{k_2K_2[H^+]}{K_a'} + \frac{k_3K_2[H^+]^2}{K_a'}\right) \quad (2)$$

Recognizing that the total substrate concentration S_t, is given by $S_t = [S_0] + [S_1] + [T_0] + [T_1]$ and employing the equilibrium constants of Scheme I as before, we can finally derive the expression for the observed first-order rate constant, k_{obsd} (eq 3).

$$k_{obsd} = \frac{k_1K_1K_a'[H^+] + k_2K_2[H^+] + k_3K_2[H^+]^2}{[H^+] + K_a' + K_1K_a'[RNH_2] + K_2[RNH_2][H^+]} \quad (3)$$

In Figure 1, first-order rate constants for phenylhydrazone formation from 4-pyridinecarboxaldehyde at pH 3.82 are plotted as a function of the concentration of amine. Rate constants increase less rapidly than amine concentration and approach a plateau level. Such behavior has been observed on many previous occasions and is interpreted as reflecting carbinolamine accumulation with rate-determining dehydration of this intermediate. Similar behavior was observed with the other substrates employed in this study at the same pH.

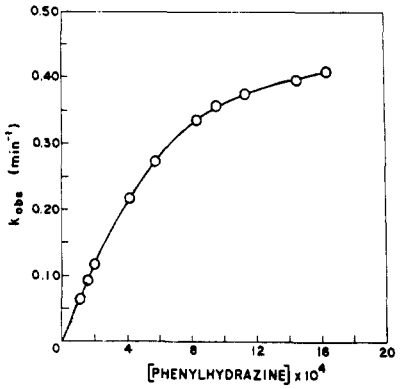


Figure 1. First-order rate constants for 4-pyridinecarboxaldehyde phenylhydrazone formation plotted as a function of the concentration of phenylhydrazine free base. Kinetic measurements were carried out in 20% aqueous ethanol at 25.0 °C, an ionic strength of 0.50 and a pH of 3.82.

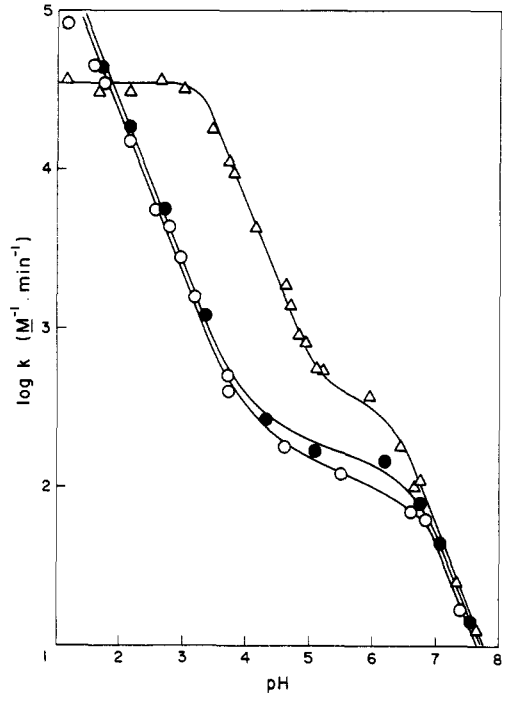


Figure 2. Logarithms of second-order rate constants for phenylhydrazone formation for 4-pyridinecarboxaldehyde (solid circles), 3-pyridinecarboxaldehyde (open circles), and 2-pyridinecarboxaldehyde (triangles) plotted as a function of pH. Measurements were made in 20% aqueous ethanol at 25.0 °C and an ionic strength of 0.50. All rate constants have been extrapolated to zero buffer concentration.

In Figure 2, logarithms of second-order rate constants for phenylhydrazone formation for 4-, 3-, and 2-pyridinecarboxaldehyde phenylhydrazone formation are plotted against pH. Second-order rate constants were calculated by dividing first-order constants by the concentration of phenylhydrazine free base. For avoidance of errors resulting from accumulation of carbinolamine, the total concentrations of phenylhydrazine were maintained below 1×10^{-3} M in all cases. For the three isomeric aldehydes, pyridinecarboxaldehyde phenylhydrazone formation was

Table I. Summary of Rate and Equilibrium Constants for Pyridinecarboxaldehyde and Formylmethylpyridinium Ion Phenylhydrazone Formation at 25.0 °C in 20% Aqueous Ethanol at an Ionic Strength of 0.50[a]

substrate	k_3K_2, M^{-2} min^{-1}	$10^{-8}k_1K_1$, M^{-2} min^{-1}	pK_a'	α[b]	ref
4-formyl-1-methylpyridinium ion	3.0×10^6			0.80	2
3-formyl-1-methylpyridinium ion	3.5×10^6			0.65	2
2-formyl-1-methylpyridinium ion	7.0×10^4			0.70	2
4-pyridinecarboxaldehyde	2.6×10^6	5.0	6.5	0.82	this work
3-pyridinecarboxaldehyde	2.2×10^6	4.3	6.5	0.65	this work
2-pyridinecarboxaldehyde	6.0×10^7	6.0	6.5	c	this work

[a] All constants are defined in Scheme I. [b] The Brønsted exponent for general-acid catalysis. [c] General catalysis is not detectable.

Table II. Third-order Rate Constants for General-Acid Catalysis of 4- and 3-Pyridinecarboxaldehyde Phenylhydrazone Formation at 25.0 °C in 20% Aqueous Ethanol and at a Ionic Strength 0.50[a]

	catalysts				
phenylhydrazone	CNAcOH	ClAcOH	HCOOH	$BrCH_2AcOH$	AcOH
4-pyridinecarboxaldehyde	1.3×10^5	6.1×10^4	1.8×10^4	8.8×10^3	2.1×10^3
3-pyridinecarboxaldehyde	1.8×10^5	8.8×10^4	3.2×10^4	2.6×10^4	5.4×10^3

[a] All rate constants have units of M^{-2} min^{-1}.

shown to be first-order in amine concentration up to 5.0×10^{-3} M at pH 6. On the basis of the data of Cabani et al.[9] concerning rates of hydration and dehydration of pyridine aldehydes, one can estimate that the rate constant for aldehyde dehydration is near 3 min^{-1}. Since our first-order rate constants never exceeded 0.3 min^{-1}, dehydration of the aldehyde hydrates cannot significantly influence the measured rate constants reported here.

For the 4- and 3-pyridinecarboxaldehydes, rate constants are correlated with pH by two straight lines with slopes of −1 with a transition between them. This is interpreted as reflecting rate-determining carbinolamine dehydration over the entire pH range studied coupled with substrate ionization as depicted in Scheme I. The solid lines drawn in Figure 2 are calculated on the basis of the rate law in eq 3 by assuming that k_2 in Scheme I is zero. The rate and equilibrium constants are taken from Table I.

The behavior of 2-pyridinecarboxaldehyde is somewhat more complicated. Above pH 3.5, the pH–rate profile resembles those for the 4- and 3-aldehydes. The curve from pH 3.5 to pH 7.5 is calculated on the basis of the rate law in eq 3 and the values of rate and equilibrium constants from Table I. Below pH 3.5 rate constants become independent of pH. Since first-order rate constants measured in this pH region were at least 1 order of magnitude less than those for aldehyde hydrate dehydration,[9] this cannot reflect the influence of dehydration on the rate of phenylhydrazone formation. The prefered interpretation is that the break at pH 3–3.5 reflects a transition to rate-determining attack of phenylhydrazine on the protonated substrate, or the kinetic equivalent, in accord with the behavior commonly observed for these reactions.[10,11]

Rate and equilibrium constants for phenylhydrazone formation from the pyridinecarboxaldehydes and the formyl-1-methylpyridinium ions are collected in Table I.

Note that the third-order rate constants, k_3K_2, for acid-catalyzed carbinolamine decomposition of 4- and 3-formyl-1-methylpyridinium ions are similar to the same rate constants for the corresponding reactions of the protonated pyridinecarboxaldehydes. Since the polar effects of the methyl group and the proton are expected to influence rate and equilibrium constants for these reactions in a similar way, this result is both anticipated and corroborative of the identitiy of mechanism for the two classes of substrates.

A surprising finding is that values of pK_a' for the pyridinecarboxaldehydes, near 6.5, required to fit the rate law (eq 3) to the experimental data are 2–3 pH units greater than related values reported earlier.[12] The lower values were measured in aqueous solution and accord with expectations based on the pK_a of pyridine itself and the modest electron-withdrawing capacity of the dihydroxymethyl function. Nonetheless, assignment of the mechanism seems secure, and the pH–rate profiles require the higher values of pK_a.

The behavior of 2-pyridinecarboxaldehyde is quite unusual in this reaction. At high values of pH, the observed reactivity in phenylhydrazone formation is similar to that found with the 3- and 4-pyridinecarboxaldehyde, as expected. However, under conditions more acidic than the break point in the pH–rate profile, this compound is unexpectedly reactive. The value of k_3K_2 is larger than those for the other aldehydes and, most surprisingly, this value is about 3 orders of magnitude greater than that for 2-formyl-1-methylpyridinium ion (Table I). Why this should be true is not clear. The greater reactivity might reflect a smaller conversion of the protonated aldehyde to the hydrate (although this factor cannot be a large one), a greater equilibrium constant for addition of phenylhydrazine to form the carbinolamine (possibly by favorable hydrogen bond formation), or an increased rate constant for dehydration. Like the other substrates, the nature of the pH–rate profile requires a higher than expected value of pK_a for the conjugate acid of this substrate. Finally, the break in the pH–rate profile near pH 3, interpreted to reflect a transition to rate-determining amine attack, requires that this substrate be less reactive in this respect than the 4- and 3-isomers, contrary to what one might have expected.

Second-order rate constants for 4- and 3-pyridinecarboxaldehyde phenylhydrazone formation were measured as a function of the concentration of carboxylic acid buffers. Rate constants were observed to increase linearly with increasing buffer concentration. Studies of buffer catalysis conducted with varying ratios of carboxylic acid to carboxylate revealed that the buffer catalysis is of the general-acid type. Third-order rate constants for general-acid catalysis were calculated and are collected in Table

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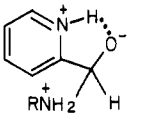
II. The catalytic rate constants yield satisfactory Brønsted plots from which values of 0.82 and 0.65 were obtained for the 4- and 3-aldehydes, respectively. These values are similar to those previously determined for general-acid catalysis of the same reaction of 4- and 3-formyl-1-methylpyridinium ions.[2]

General-acid catalysis for 2-pyridinecarboxaldehyde phenylhydrazone formation could not be detected in cyanoacetate, chloroacetate, formate, β-bromopropionate, and acetate buffers (pH range 1.9–4.7) up to a concentration of the acidic form of the buffer of 0.1 M. This means that neither amine addition or carbinolamine dehydration is subject to detectable general-acid catalysis. In this respect also, phenylhydrazone formation from 2-pyridinecarboxaldehyde is distinct from that with 2-formyl-1-methylpyridinium ion, for which general-acid catalysis of carbinolamine dehydration ($\alpha = 0.7$) is observed.[2] The general-acid catalysis of most addition reactions of nitrogen nucleophiles to the carbonyl group involves trapping of the zwitterionic intermediate, $T^{\pm}$, by diffusion-controlled proton transfer.[10,11,13] For the intermediate $T^{\pm}$ from this aldehyde, the necessary proton transfer could probably occur intramolecularly, as shown below, or through a water molecule, with a rate that is even



faster than diffusion together of $T^{\pm}$ and an external catalyst, thus avoiding the need for general-acid catalysis, as observed.

Registry No. CNAcOH, 372-09-8; ClAcOH, 79-11-8; HCOOH, 64-18-6; $BrCH_2AcOH$, 590-92-1; AcOH, 64-19-7; 4-pyridinecarboxaldehyde, 872-85-5; 3-pyridinecarboxaldehyde, 500-22-1; 2-pyridinecarboxaldehyde, 1121-60-4; phenylhydrazine, 100-63-0; 4-pyridinecarboxaldehyde phenylhydrazone, 7757-39-3; 3-pyridinecarboxaldehyde phenylhydrazone, 57023-37-7; 2-pyridinecarboxaldehyde phenylhydrazone, 7727-07-3.

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Monobactams. Preparation of (*S*)-3-Amino-2-oxoazetidine-1-sulfonic Acids from L-α-Amino-β-hydroxy Acids via Their Hydroxamic Esters

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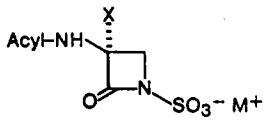
The cyclization of the *O*-methylhydroxamates **13–15** afforded excellent yields of the 1-methoxyazetidinones **16–18**. Reductive cleavage of the methoxy group gave N-1-unsubstituted azetidinones **9**, **19**, and **20** which were sulfonated and deprotected, yielding zwitterions **29–31**. These materials serve as general intermediates for the preparation of a large variety of monobactam antimicrobial agents.

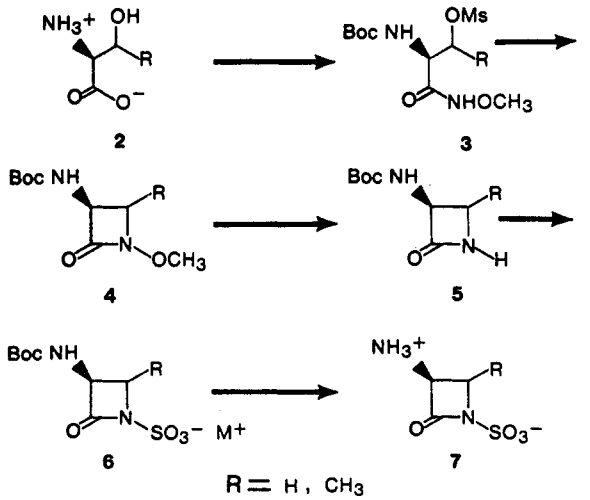
In previous papers we have described the isolation and structure determination of several members of a new class of β-lactam antibiotics, the monobactams **1**.[2] We have also



1 X = H, OCH_3

communicated synthetic routes to these N-1-sulfonated monocyclic azetidinone antimicrobials employing as the starting materials either 6-aminopenicillanic acid (6-APA)[3] or L-α-amino-β-hydroxy acids.[4] In this paper we describe an additional route to these systems from L-α-amino-β-hydroxy acids which proceeds through an N-1-unsubstituted azetidinone. Since our interest in monobactam analogues containing alkyl substituents at C-4 precluded the use of 6-APA degradation,[3] we have concentrated on totally synthetic routes from α-amino acids. On the basis of our initial 6-APA derived route,[3] the problem reduced

Scheme I



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