(pM075),[10] was subjected to two passages (18 and 2.5 h at 37 °C) in NB plus carbenicillin (500 μg/mL) and kanamycin sulfate (20 μg/mL). A 0.5-mL aliquot of the final culture was washed four times with NB and suspended in 0.5 mL of NB. Donor and recipient strain were mixed, pelleted by centrifugation, and resuspended in 50 μL of NB. The drop was placed on a NB agar plate having a dry surface and allowed to dry before incubation at 28 °C for 16 h. Bacteria that had grown at that time were removed, suspended in 1 mL of 0.9% NaCl and screened for Tn*5* mutants on NB agar containing rifamycin and kanamycin (28 °C for 2 days). After a second passage on this selective medium, colonies of Tn*5* mutants were obtained at a frequency of 5.2×10^{-3} per donor cell. The colonies were screened for Tox^- mutants employing the method of Gasson,[11] who found that Tox^+ bacteria produce an inhibition zone on a lawn of *E. coli*. Two Tn*5*-generated Tox^- mutants were isolated, PT039 and PT243. Lack of toxin production was verified using a tobacco leaf response.[12]

Quantitative Analysis of Culture Filtrates. Quantitation of **1**, **3**, and tabtoxins in culture filtrates from diverse *P. syringae* pv. *tabaci* isolates was by ion-exchange, Sephadex LH20, and RP chromatography as described by Thomas et al.[5] Identification of **1** for strains other than PT039 was based on cochromatography.

N^6-Acetyl-5-hydroxy-5-(hydroxymethyl)lysine (1). *P. syringae* pv. *tabaci* PT039 was grown in the chemically defined medium of Wooley et al.[12] (1 L, at 20 °C and 250 rpm on a rotary shaker for 3 days). Cells were removed by centrifugation (40000*g* for 15 min), and **1** was extracted from the culture filtrate by passing the latter over a cation-exchange column (Amberlite CG-120, 2 × 29 cm), from which it was then desorbed with 4% aqueous ammonia. The eluate (30 mL) was immediately concentrated almost to dryness by rotatory evaporation at 15–20 °C, and the concentrate was repeatedly diluted with water and concentrated in vacuo to remove the ammonia. The final concentrate (5 mL) was adjusted to 80% methanol and the resulting precipitate removed by centrifugation (20000*g* for 10 min). The supernatant was concentrated to 200 μL in vacuo and applied to preparative silica gel plates (Brinkman silica gel 60 F254, 20 × 20 cm, 2 mm thick). Upon development with butanol–acetic acid–water (3:1:1), **1** migrated as a band with R_f 0.26, was removed with water, concentrated, and finally purified by isocratic chromatography on a Sephadex LH-20 column (2.5 × 80 cm) using MeOH–water (1:1; 0.61 mL/min) for elution. Ten minute fractions were collected, spotted onto a TLC plate, and analyzed for amino acids using ninhydrin; fraction 23 contained 7 mg of pure **1** and was used for the structure elucidation; less pure **1** was also found in fractions 24 and 25. HRFABMS M + H^+ obsd 235.1328, $C_9H_{18}N_2O_5$, calcd 235.1294. D/H exchange of **1** was studied by FABMS using 1 μL of the ^1H NMR sample.

Deacetylation, Dansylation, and Methylation/Acetylation of 1. All derivatizations were carried out on approximately 200 μg of **1**. For deacetylation, **1** was treated for 20 h at 105 °C with 200 μL of 6 N HCl under N_2 in a sealed vial. Dansylation was achieved by dissolving **1** or deacetylated **1** in 200 μL of 0.2 M $NaHCO_3$ and treating it with a 2–4-fold molar excess of dansyl chloride in acetone (ca. 2 mg/mL) for 1 h at 37 °C. FABMS of the dansyl derivatives was only successful after separation from excess danylOH by crude purification with RP-HPLC.[16] Methylation to **4** was by 1 mL of anhydrous 3 N HCl in MeOH for 4 h at 25 °C. Subsequent acetylation to a mixture of **5** and **6** was achieved by treating **4** with 200 μL of acetanhydride/pyridine (1:1) for 16 h at 25 °C.

Tms_5 Derivative of 1 (8). Approximately 200 μg of **1** was silylated in a sealed vial with 25 μL of neat BSTFA for 3 h at 100 °C. The Tms_5 derivative proved to be unstable. After contact with moisture, only the Tms_4 derivative could be detected. The Tms_5 derivative was, however, repeatedly observed when drying of the reaction mixture was performed en route to mass analysis, namely, in the roughing vacuum of the mass spectrometer: EIMS *m/z* (rel intensity) M^+, $C_9H_{13}N_2O_5Tms_5$, calcd 594.3192, obsd 594.3188 (28), daughter ions 504, 274, 147; $M^+ - AcNTmsCH_2$, $C_6H_8NO_4Tms_4$, calcd 450.2347, obsd 450.2355 (5); $M^+ - AcNTmsCH_2 - TmsOH$, $C_6H_7NO_3Tms_3$, calcd 360.1847, obsd 360.1856 (70), daughter ions 270, 242, 191, 170; $M^+ - AcNTmsCH_2 - 2\ TmsOH - CO$, $C_5H_6NOTms_2$, calcd 242.1396, obsd 242.1371 (5); $M^+ - TmsOH$, $C_9H_{12}N_2O_4Tms_4$, calcd 504.2691, obsd 504.2708 (2); $M^+ - TmsOH - TmsOCH_2$, $C_8H_{10}N_2O_3Tms_3$, calcd 401.2112, obsd 401.2100 (24); $M^+ - CO_2Tms$, $C_8H_{13}N_2O_3Tms_4$, calcd 477.2820, obsd 477.2841 (14), daughter ions 387, 288, 198, 128; obsd 288.1668 (68), daughter ions 272, 198, 170, 147, 129, 103, 94; TmsNH=CHCOOTms, $C_2H_2NO_2Tms_2$, calcd 218.1055, obsd 218.1059 (8).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra, EI-MS spectrum of Tms_5–1, and EI-B/E-linked scan spectrum of *m/z* 477 of Tms_5–1 (6 pages). Ordering information is given on any current masthead page.

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Nucleophilic Substitution at Nitrogen and Carboxyl Carbon of *N*-Aryl-*O*-pivaloylhydroxylamines in Aqueous Solution: Competition with S_N1 Solvolysis of Model Carcinogens

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It has become apparent that S_N2 attack of aromatic and aliphatic amines, and certain carbon nucleophiles, can occur on the nitrogen of ester derivatives of *N*-arylhydroxylamines (eq 1) under conditions in which solvolysis

$$\underset{\perp}{\text{ArNHOY}} + \text{NuH} \longrightarrow \text{ArNHNu} + \text{HOY} \quad (1)$$

via an S_N1 mechanism is suppressed by low solvent polarity.[1,2] Specifically, the reaction of eq 1 has been demonstrated to occur in the neat aliphatic or aromatic amine as solvent,[1a] in THF[1b] or MeOH,[2] or in mixed-solvent systems of $CHCl_3/EtOH/H_2O$.[1c] How well the S_N2 reaction competes with S_N1 solvolysis in H_2O has not been previously reported. This is of interest because the ester derivatives **1** are models for carcinogenic metabolites of polycyclic aromatic amines, and the environment in which the carcinogens are generated in vivo is an aqueous one.[3] The possibility that acyl transfer (eq 2) may occur in an aqueous environment must also be considered.

$$\underset{\perp}{\text{ArNHOY}} + \text{NuH} \longrightarrow \text{ArNHOH} + \text{NuY} \quad (2)$$

(1) (a) Boche, G.; Bosold, F.; Schröder, S. *Angew Chem., Int. Ed. Engl.* **1988**, *27*, 973–974. Famulok, M.; Bosold, F.; Boche, G. *Tetrahedron Lett.* **1989**, *30*, 321–324. (b) Ulbrich, R.; Famulok, M.; Bosold, F.; Boche, G. *Tetrahedron Lett.* **1990**, *31*, 1689–1692. (c) Famulok, M.; Bosold, F.; Boche, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 337–338. Meier, C.; Boche, G. *Tetrahedron Lett.* **1990**, *31*, 1693–1696.

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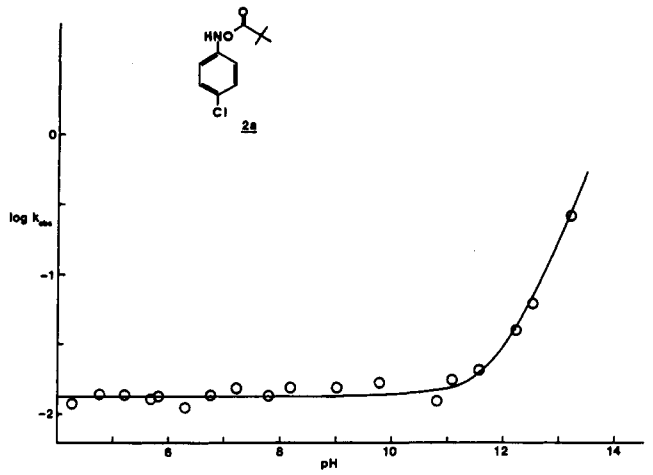
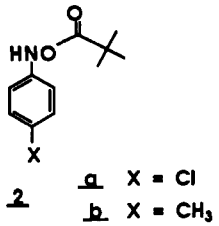


Figure 1. pH–rate profile for the decomposition of **2a** in 5% CH_3CH–H_2O, μ = 0.5 M (KCl), at 40 °C. The data were fit, by nonlinear least-squares methods, to the following equation: $k_{obs} = k_0 + k_{OH^-}[OH^-]$.

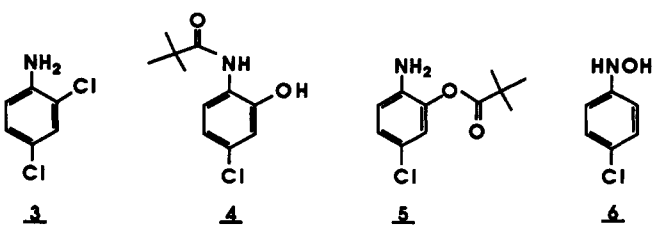
Accordingly, we have investigated the reactions of **2a** with OH^- and $(Et)_2NH$ in an aqueous solvent system in which **2a** has previously been demonstrated to undergo rapid hydrolysis via an S_N1 mechanism.[4] The reaction of the more labile ester **2b**[2b] with $(Et)_2NH$ has also been investigated in an aqueous solvent system.



2 **a** X = Cl
b X = CH_3

Results and Discussion

The pseudo-first-order rate constant for decomposition of **2a** in 5% CH_3CN–H_2O, μ = 0.5 M (KCl), T = 40 °C, is pH dependent (Figure 1) but is not dependent on buffer concentration in phosphate, borate, or carbonate buffers. The pH-independent rate constant, k_0, of (1.36 ± 0.04) × 10^{-2} s^{-1} is comparable to the rate constant of (1.7 ± 0.1) × 10^{-2} s^{-1} previously obtained from only three measurements in the pH range 1.0–7.0.[4] The hydroxide-dependent rate constant, k_{OH^-}, of 0.56 ± 0.03 M^{-1} s^{-1} is associated with a change in product distribution noted in Table I.[5] Under pH conditions in which k_0 governs the rate of decomposition of **2a**, the major reaction products are **3**, apparently generated by nucleophilic attack of Cl^- on a nitrenium ion,[4] and **4**, previously shown to be produced by intramolecular rearrangement of **5**, apparently formed in turn by internal return of a tight ion pair.[4] In the previous study, *p*-benzoquinone was also detected in low yields,[4] but no attempt was made to quantify this product in this study. At pH > 11.0, the hydroxylamine **6** becomes a major reaction product. Quantification of this product was complicated by its rapid oxidation in the presence of O_2 under basic conditions. The reactions were run in solutions extensively outgassed with N_2, but some oxidation of **6** occurred during quenching of the reaction and workup. The yields reported for **6** in Table I are the sums of the yields of **6** and its oxidation products **7** and **8**. The pH measurements of the KOH solutions reported in Table I were made before initiation of the reaction since the reaction mixtures were quenched with 1 M KH_2PO_4 after 10 half-lives to minimize oxidation of **6**. This leads to some uncertainty in the pH of these solutions during reaction. Given the difficulty in quantifying the yield of **6** at a given pH, the agreement between the observed yields and those predicted from the kinetic data is adequate.

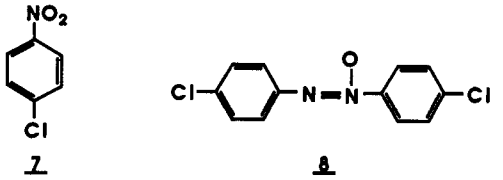


3 **4** **5** **6**

Table I. Yields of Decomposition Products of 2a in 5% CH_3CN–H_2O at 40 °C[a]

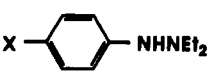
		% yields[b]			
conditions	pH	3	4	6 (obsd)[c]	6 (predicted)[d]
0.05 M phosphate	7.26	57 ● 2	28 ± 1		0
0.05 M borate	8.31	56 ± 1	29 ● 1		0
0.05 M borate	9.36	59 ± 1	30 ± 1		0
KOH	11.28	42 ± 2	19 ± 1	12 ± 1	19 ± 1
KOH	11.55	34 ± 1	14 ● 1	46 ± 6	30 ± 2
KOH	12.22	16 ± 2	6 ● 1	51 ± 5	67 ± 5
KOH	12.51	10 ± 1	*e*	83 ± 5	80 ± 6
KOH	13.20		*e*	85 ± 8	95 ± 8

[a] μ = 0.50 M (KCl). [b] Determined by HPLC peak areas averaged from three injections. Extinction coefficients were determined from the authentic compounds. The runs in KOH were quenched with 1 M KH_2PO_4 to minimize oxidation of **6** during quantification. [c] Since **6** is oxidized, in part, to **7** and **8** during quenching and workup, these are the combined observed yields of **6**, **7**, and **8**. [d] Predicted on the basis of the kinetic data assuming that **6** is the only aromatic product of the k_{OH^-} process. [e] Present, but not quantifiable, due to interference from HPLC peak of **7**.



7 **8**

The decomposition of **2a** in a Et_2NH buffer 2 M in total amine (1/1 $Et_2NH/Et_2NH_2^+$, pH 11.2, μ = 1.0 M, 5% CH_3CN, T = 25 °C) generates **3** (44 ± 1%), **4** (7 ± 1%), **6** (22 ± 2%), and the hydrazine product **9a** (16 ± 1%).



9 **a** X = Cl
b X = CH_3

Under these same conditions, kinetics of the decomposition of **2a** can be shown to follow eq 3, where k_0 is (6.1 ± 0.3)

$$k_{obs} = k_0 + k_{OH^-}[OH^-] + k_{Et_2NH}[Et_2NH] \quad (3)$$

(4) Novak, M.; Lagerman, R. K. *J. Org. Chem.* **1988**, *53*, 4762–4769.
(5) This rate constant was calculated on the basis of an apparent pK_w of 13.52 for this solvent system. See Experimental Section. A table of observed rate constants for the hydrolysis of **2a** as a function of pH is available. See supplementary material.

$\times 10^{-3}$ s^{-1}, $k_{OH^-}[OH^-]$ is $(0.6 \pm 0.2) \times 10^{-3}$ s^{-1}, and k_{Et_2NH} is $(2.5 \pm 0.2) \times 10^{-3}$ M^{-1} s^{-1}.[6] The Et_2NH-dependent term is 27 ± 3% of k_{obs} under the conditions of the product study, but **9a** is only 16 ± 1% of the reaction products. This indicates that a substantial part of the Et_2NH-dependent term may be due to ester aminolysis (eq 2), which generates the hydroxylamine **6**.[7] Indeed, at pH 11.2 (μ = 1.0 M, T = 25 °C) in the absence of Et_2NH, **6** is isolated in only 3 ± 1% yield.

The presence of **9a** shows that the nucleophilic displacement of eq 1 can compete with S_N1 solvolysis of **2a** under aqueous conditions. The decomposition of the more reactive ester **2b**[2b] in an Et_2NH buffer identical with that described previously generates **9b** in 1.0 ± 0.1% yield. This compound is similar in reactivity to the suspected carcinogenic metabolites of polycyclic aromatic amines.[2b] The substituent effects noted in these product studies indicate that if ρ^+ is −6.0 for the S_N1 solvolysis of **2** in an aqueous solution,[8] then ρ^+ is ca. −3 for the S_N2 substitution of **2** by Et_2NH. This relatively low sensitivity to the aromatic substituent is in accord with expectations.[2b]

These results demonstrate that nucleophilic attack on the nitrogen of ester derivatives of *N*-arylhydroxylamines can compete with S_N1 solvolysis in aqueous solutions, but the solvolysis will predominate at low to moderate concentrations of the nucleophile (≤1 M). The results with OH^- show that acyl transfer (eq 2) can also occur efficiently. We are continuing to examine the nature of the bimolecular nucleophilic displacement reactions of **2** in an effort to understand the factors that determine the site of nucleophilic attack.

Experimental Section

The syntheses of **2a** and **2b** have been described.[2b,4] All water used in the kinetic and product studies was distilled, deionized, and then distilled again in an all-glass apparatus. The purification of CH_3CN has been described.[9] All reactions were run in glassware or plasticware that had been soaked in an EDTA solution (pH ≈ 12) and rinsed with deionized water. All aqueous solutions contained 5% CH_3CN by volume, and ionic strength was maintained at 0.5 M with KCl; pH was maintained with phosphate, borate, or carbonate buffers or KOH. $(Et)_2NH$ was distilled from CaH under a N_2 atmosphere prior to use.

Kinetics. The appropriate solution (3 mL) was transferred to a thunberg cuvette and outgassed with a rapid stream of N_2 for ca. 30 min before it was equilibrated at 40 °C in the thermostated cell holder of a Cary 2290 UV–vis spectrophotometer. Reactions were initiated by injection of 15 μL of a ca. 0.015 M stock solution of **2a** in CH_3CN to obtain an initial concentration of ca. 7.5×10^{-5} M. Progress of the reaction was monitored at 233 and 260 nm. The absorbance vs time data were fit to the appropriate rate equation by nonlinear least-squares methods. The pH of solutions was measured at 40 °C after the kinetic run. An apparent pK_w of 13.52 ± 0.02 was obtained for the solvent system at 40 °C by measurement of pH at known concentrations of OH^- in the range 0.01–0.50 M.

Product Studies. These studies were run at the same concentrations as the kinetic runs on a 25-mL scale. The buffer was outgassed with N_2 for 3–4 h before the reaction was initiated. After ca. 10 half-lives, the products were quantified by HPLC (μ-Bondapak-C_{18} column, 6/4 MeOH/H_2O, 1.0 mL/min, 250 nm, 20-μL injections). Comparisons were made to authentic compounds in all cases by HPLC and GC/MS. It was necessary to quench the KOH solutions with appropriate amounts of 1 M KH_2PO_4 to avoid oxidation of the hydroxylamine **6** during quantification.

^{18}O Experiment. The addition of 0.5 mL of 45% ^{18}O-enriched H_2O (determined by MS analysis of a sample of lauric acid generated by hydrolysis of lauroyl chloride in $[^{18}O]H_2O$) to 1.0 mL of a 0.75 M KOH solution generated a 0.5 M KOH solution with an ^{18}O enrichment of ca. 15%. After outgassing of the solution and incubation at 40 °C for an appropriate time, the mixture was brought to 7.5×10^{-4} M in **2a** by injection of a ca. 1 M stock solution of **2a** in CH_3CN. After completion, the reaction was quenched by addition of 1 M KH_2PO_4 and the reaction products were extracted into CH_2Cl_2 (3 × 5 mL), dried briefly over Na_2SO_4, concentrated, and analyzed by GC/MS on a Hewlett-Packard 5890 gas chromatograph equipped with a 5971A mass-selective detector. The column used was a 25 m × 0.1 mm fused silica column with a 0.1 μ-bonded methyl silicone stationary phase. The reaction was run in duplicate and compared to duplicate runs in ordinary H_2O.

Et_2NH Reactions. These reactions were run under conditions similar to the other product studies except that Et_2NH was used as the buffer, ionic strength was maintained at 1 M, and reactions were done at 25 °C. The hydrazine **9a** was compared to an authentic sample prepared in an earlier study.[10] An authentic sample of **9b** was prepared by decomposition of **2b** in neat Et_2NH. After 24 h, the Et_2NH was removed by rotary evaporation, and the residue was taken up into CH_2Cl_2. This solution was extracted with 5% $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness. The yellow oil was then purified by chromatography on silica gel (CH_2Cl_2 eluent): IR (neat) 3286, 3015, 2972, 1614, 1514, 1251 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.07 (6 H, t, J = 7.0 Hz), 2.24 (3 H, s), 2.75 (4 H, q, J = 7.0 Hz), 4.20, (1 H, s, broad) 6.78 (d, J = 8.4 Hz), 6.99 (d, J = 8.4 Hz); GC/MS m/e 178 (M^+), 163, 149, 135, 106, 91; high-resolution MS m/e 178.1492, $C_{11}H_{18}N_2$ requires m/e 178.1471.

The yield of **9a** was obtained by HPLC as described previously. Quantification of the yield of **9b** was performed by GC/MS on the same column used for the ^{18}O analysis. The authentic samples of **9a** and **9b** were used to calibrate peak areas in a standard fashion.

Acknowledgment. This work was supported by a grant from the American Cancer Society (CN-23H).

Supplementary Material Available: Table of rate constants vs pH for **2a** (1 page). Ordering information is given on any current masthead page.

(6) Hydrolysis in phosphate and acetate buffers at pH < 7 provided k_0 under these conditions, $k_{OH^-}[OH^-]$ was obtained from measurements in KOH solution at pH 11.2, and k_{Et_2NH} was obtained from the slope of k_{obs} vs $[Et_2NH]$ at pH 11.2 in the Et_2NH concentration range 0.0–1.0 M.

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Photochemistry of Large-Ring 2-Phenylcycloalkanones in Various Environments. Intramolecular Para Coupling Products of Acyl Benzyl Biradicals

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The photochemistry of five- and six-membered cycloalkanones has played an important role in mechanistic organic chemistry and in our knowledge of biradicals.[1,2] The photolysis of 2-phenylcyclopentanone and -cyclo-

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