Protonation, deuteration, and tritiation were performed as described by Lehn.[8]

For the X-ray structure analyses, the following programs were used: MULTAN 77,[23] X-RAY-76,[24] SHELX-75,[25] ABWI,[26] and XANADU.[27]

Table V shows important data of the crystal structure determination of the investigated complexes.

Acknowledgment. We gratefully acknowledge the financial support by the Bundesministerium für Forschung und Technologie as well as the Verband der Chemischen Industrie—Fonds der Chemischen Industrie.

(23) Main, P.; Lessinger, L.; Woolfson, M. M.; Germann, G.; Declerq, J. P. "MULTAN 77"; Universities of York, England and Louvain, Belgium, 1977.
(24) Stewart, K. M. "The X-RAY-76 System"; Computer Science Center, University of Maryland: College Park, MD, 1976; Technical Report, TR 446.
(25) Sheldrick, G. "Programs for Crystal Structure Determination"; Cambridge University Press: New York, 1975.
(26) Hoffmann, K. Dissertation, Universität Hamburg, 1976.
(27) Robert, P.; Sheldrick, G. "XANADU Program for Crystallographic Calculation"; Cambridge University Press: New York, 1975; p 975.

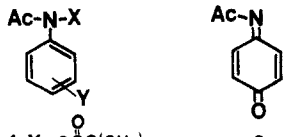
Hydrolysis of the Model Carcinogen *N*-(Pivaloyloxy)-4-methoxyacetanilide: Involvement of *N*-Acetyl-*p*-benzoquinone Imine

Michael Novak,* Maria Pelecanou, and Lee Pollack

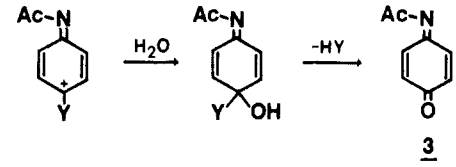
Contribution from the Department of Chemistry, Clark University, Worcester, Massachusetts 01610. *Received August 6, 1985*

Abstract: Results of kinetic, ^{1}H NMR, and HPLC studies show that *N*-(pivaloyloxy)-4-methoxyacetanilide (**1a**), a model for suspected carcinogenic metabolites of phenacetin (**10**), decomposes predominately into *N*-acetyl-*p*-benzoquinone imine (**3**) in aqueous solution. The only other product isolated from the decomposition of **1a** is 4-methoxyacetanilide (**7**), which is produced in moderate yield at pH > 6.0. The available evidence indicates that both of these materials are produced by a nitrenium ion mechanism. In aqueous solutions containing KCl at pH < 6.0, **3** decomposes in a first-order manner by an acid-catalyzed process into *p*-benzoquinone (**6**) and 3-chloro-4-hydroxyacetanilide (**5**). An intermediate in the reaction, which decomposes into **6**, has been detected. ^{1}H NMR results indicate that the intermediate is a carbinolamine (**8**). At pH > 6.0, the decomposition of **3** becomes very complicated. At high concentrations, **3** decomposes by a non-first-order path into a material which appears to be oligomeric. At sufficiently low concentrations (ca. 2.5×10^{-6} M), the decomposition of **3** returns to a first-order process. Under these conditions, the major products of the reaction are **6** and acetaminophen (**4**). The measured standard reduction potential for **3** of 0.978 ± 0.001 V indicates that it is a much stronger oxidizing agent than either *p*-benzoquinone or *p*-benzoquinone monoimine. However, at this time it is not possible to determine the identity of the species responsible for the reduction of **3** into **4** in aqueous solution.

As part of a study of the chemistry of model compounds for the proximate carcinogenic metabolites of aromatic amides,[1] we have been investigating the aqueous solution chemistry of a series of ring-substituted *N*-(pivaloyloxy)- and *N*-(sulfonatooxy)acetanilides (**1** and **2**).[2] These compounds decompose in aqueous

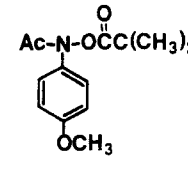


solution through the intermediacy of nitrenium ion pairs.[2] During our studies, we have obtained indirect evidence that *N*-acetyl-*p*-benzoquinone imine, **3**, may be involved in some of the decomposition pathways of these species if the ring substituent, Y, is at the 4-position and is a good leaving group.[2a,c] We have proposed that **3** may be formed by attack of H_2O at the 4-position of the nitrenium ion intermediate with subsequent loss of HY, as in eq 1.[2a] However, no direct evidence for such a process was obtained.



(1)

We can now report direct evidence based on kinetic studies and, ^{1}H NMR and HPLC experiments, that one member of this series of compounds, *N*-(pivaloyloxy)-4-methoxyacetanilide, **1a**, decomposes in aqueous solution between pH 3.0 and 8.0 predominately (>80%) through the intermediacy of **3**. As part of this



1a

study, we have investigated the hydrolysis and redox chemistry of **3** in this pH region. These results are also reported in this paper.

N-Acetyl-*p*-benzoquinone imine, **3**, is apparently an important hepatotoxic metabolite of the common analgesics acetaminophen[3]

(1) Miller, J. A. *Cancer Res.* **1970**, *30*, 559-576. Kriek, E. *Biochim. Biophys. Acta* **1974**, *355*, 177-203. Miller, E. C. *Cancer Res.* **1978**, *38*, 1479-1496. Miller, E. C.; Miller, J. A. *Cancer* **1981**, *47*, 2327-2345.
(2) (a) Novak, M.; Pelecanou, M.; Roy, A. K.; Andronico, A. F.; Plourde, F. M.; Olefirowicz, T. M.; Curtin, T. J. *J. Am. Chem. Soc.* **1984**, *106*, 5623-5631. (b) Novak, M.; Roy, A. K. *J. Org. Chem.* **1985**, *50*, 571-580. (c) Pelecanou, M.; Novak, M. *J. Am. Chem. Soc.* **1985**, *107*, 4499-4503. (d) Novak, M.; Roy, A. K. *J. Org. Chem.*, in press.
(3) (a) Blair, I. A.; Boobis, A. R.; Davies, D. S. *Tetrahedron Lett.* **1980**, *21*, 4947-4950. (b) Dahlin, D. C.; Nelson, S. D. *J. Med. Chem.* **1982**, *25*, 885-886. (c) Miner, D. J.; Kissinger, P. T. *Biochem. Pharmacol.* **1979**, *28*, 3285-3290. (d) Dahlin, D. C.; Miwa, G. T.; Lu, A. Y. H.; Nelson, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1327-1331. (e) Corcoran, G. B.; Mitchell, J. R.; Vaishnav, Y. N.; Horning, E. C. *Molec. Pharmacol.* **1980**, *18*, 536-542.

0002-7863/86/1508-0112$01.50/0

and phenacetin,[4] and the model compound **1a** is closely related to the material thought to be the precursor of **3** during the metabolism of phenacetin.[4,5] The results of our studies have implications for the biological activity of these materials, particularly with respect to the apparent weak carcinogenic properties of phenacetin.[6] These implications will be discussed herein.

Experimental Section

Synthesis of Starting Materials. All solvents used in synthetic procedures were reagent grade and were purified, if necessary, by commonly known procedures. Me_4Si or DSS were used as internal standards for NMR spectra.

***N*-(Pivaloyloxy)-4-methoxyacetanilide (1a).** The ester was prepared by the slow addition of pivaloyl chloride (1.1 equiv) in dry Et_2O to a solution of *N*-hydroxy-*p*-methoxyacetanilide and *N*-ethylmorpholine (1.0 equiv) in dry Et_2O. The reaction was performed under a positive pressure of N_2, and the mixture was stirred at 0 °C during the addition of pivaloyl chloride and for 3 subsequent h. The mixture was then extracted once with ice-cold 1 N $NaHCO_3$ and twice with ice-cold distilled water. After drying over Na_2SO_4, the ethereal solution was subjected to rotary evaporation to yield an oily liquid. This material, which HPLC and ^1H NMR analysis showed to be at least 90–95% pure, was obtained in ca. 55% yield: IR (neat) 2975, 1775, 1685, 1505, 1255, 1250, 1080 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 1.30 (9 H, s), 2.01 (3 H, s), 3.78 (3 H, s), 6.78–7.41 (4 H, m, para-substituted aromatic, J = 9.3 Hz). The ester was used immediately after its synthesis. If necessary, it was stored for short periods in CH_3CN at −10 °C. Attempts to further purify the ester proved unsuccessful since it decomposed on handling.

***N*-Hydroxy-*p*-methoxyacetanilide.** This material was synthesized from *p*-nitroanisole according to published procedures:[2a,7,8] mp 54–56 °C; IR (KBr) 3150, 2850, 1620, 1503, 1386, 1293, 1248, 825 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 2.04 (3 H, s), 3.80 (3 H, s), 6.82–7.40 (4 H, m, para-substituted aromatic, J = 8.7 Hz), 9.28 (1 H, s, br). Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.69. Found: C, 59.42; H, 6.05; N, 7.69.

***N*-Acetyl-*p*-benzoquinone Imine (3).** This material was synthesized according to published procedures[3a,b] and was obtained in crystalline form by sublimation:[3b] mp 74.5–75.5 °C [lit.[3b] mp 74–75 °C)]; IR (KBr) 3035, 1697, 1645, 1622, 1575, 1208, 1091, 873 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 2.29 (3 H, s), 6.63 (2 H, d, J = 10.15 Hz), 6.97 (2 H, d, J = 10.15 Hz).

Kinetic Measurements. All kinetics were performed in 5 vol % CH_3CN–H_2O solutions. Procedures for purification of solvents, preparation of solutions, and general methods for following the progress of reactions by UV absorption spectroscopy have been described.[2a,b]

Kinetic measurements were performed at 25.0 ± 0.1 °C over the pH range 3.0–8.0 in HCl solutions and in acetate and phosphate buffers. All solutions were maintained at 0.5 M ionic strength with KCl. All pH readings were taken at 25.0 ± 0.1 °C with an Orion Model 801 digital pH meter equipped with a Radiometer Model GK 2402C combination electrode.

It was found necessary to exclude O_2 from the kinetic solutions. This was accomplished by pipetting 3 mL of the kinetic solution into a thunberg cuvette and outgassing this solution with a slow stream of O_2-free N_2 presaturated with 5 vol % CH_3CN–H_2O. The N_2 was supplied through a thin Teflon tube inserted into the opening of the cuvette. After half an hour, 15 μL of the appropriate stock solution of **1a** or **3** in CH_3CN was injected into the cuvette, the Teflon tube was quickly removed, and the cuvette was sealed, shaken, and placed in the cell holder of the spectrophotometer.

Ordinarily, 0.01 M stock solutions of **1a** or **3** in CH_3CN were employed to obtain an initial concentration of ca. 5×10^{-5} M in the cuvette. Where necessary (see Results section) to achieve initial concentrations of ca. 2.5×10^{-6} M, 5.0×10^{-4} M stock solutions of **1a** and **3** were employed.

It was found that at most pH values, the decomposition kinetics of **1a** and **3** were characterized by consecutive first-order processes, although, under some conditions, simple first-order processes were observed. Absorbance vs. time data were fit by nonlinear least-squares procedures to either the standard first-order rate equation (three parameter fit) or to the rate equation for consecutive first-order processes (five parameter fit), eq 2.[9] The quality of these fits, as judged by agreement between observed and calculated A_0 and A_∞ values, and the standard deviations of the fits, were excellent.

Wavelengths used to monitor the reactions, isosbestic points for the various processes, and details of the data handling procedures are reported in the Results section.

The kinetics of the decomposition of **1a** and **3** and the formation of the various reaction products were also monitored at 25.0 ± 0.1 °C by HPLC methods previously described.[2a,b] It was also possible to monitor the appearance and decay of several intermediates in this fashion. Details of the HPLC methods are described below. Peak area vs. time data were fit to either the first-order rate equation or eq 2 as described above.

Product Analyses. Product studies were performed in solutions identical with those used in the kinetic studies. The progress of the reactions was monitored by HPLC. Typically, reactions were initiated by injection of 10 μL of a ca. 5.00×10^{-2} M stock solution of **1a** or **3** in CH_3CN into 5 mL of the reaction solution incubating in a water bath at 25.0 ± 0.1 °C to achieve a final concentration of ca. 1.0×10^{-4} M. Aliquots of 10 μL were periodically removed and subjected to HPLC analysis (μ-Bondapak C-18 reverse-phase column, 50/50 CH_3OH/H_2O eluent, UV absorbance monitored at 250 nm). HPLC peak areas were calibrated with known concentrations of authentic samples. At the end of the reactions, the yields of the products were determined by triplicate injections on the HPLC.

Different concentrations of **1a** or **3** were obtained, when necessary, by standard methods. For the low-concentration reactions, larger injections were made on the HPLC in order to obtain useful chromatograms.

The identities of the hydrolysis products of **3** (**4**, **5**, and **6**) were known to us from our previous work.[2a] The NMR and HPLC data obtained in this study were always in complete agreement with the previous assignments.

The reduction product, **4**, was isolated from the low-concentration 9.86 ± 0.04 × 10^{-6} M) hydrolysis of **3** in phosphate buffer to confirm its identity: a 200-μL aliquot of a 4.93×10^{-2} M stock solution of **3** was injected into 1 L of phosphate buffer (pH 7.81 ± 0.02). The reaction mixture was incubated at 25.0 ± 0.1 °C, and the reaction was allowed to reach completion. The water was removed by freeze-drying and the residue triturated with CH_3CN. The triturant was evaporated to dryness, and the residue was subjected to thin-layer chromatography on silica gel (CH_2Cl_2/EtOAc 4/1 eluent). The acetaminophen obtained in this manner was characterized by comparison of its physical and spectral properties to those of an authentic sample.

The identity of **3** as the major initial product of the decomposition of **1a** was established by the NMR (see below), HPLC, and kinetic studies. The final decomposition products subsequently obtained from **3** were identified as described above.

The reduction product 4-methoxyacetanilide, **7**, that is formed during the hydrolysis of **1a** in phosphate buffer was identified by direct isolation: 25.9 mg of **1a** was dissolved in 0.5 mL of CH_3CN and added to 1 L of phosphate buffer (pH 6.76 ± 0.02). The reaction mixture was incubated at 25.0 ± 0.1 °C, and the reaction was allowed to reach completion. The mixture was subsequently extracted with CH_2Cl_2 (5 × 200 mL). The extract was evaporated to dryness and the residue subjected to thin-layer chromatography on silica gel (CH_2Cl_2/EtOAc 4/1 eluent). The 4-methoxyacetanilide was characterized by comparison of its physical and spectral properties to those of authentic material obtained by methylation of acetaminophen (Aldrich).

NMR Studies. Deuterated phosphate buffer (0.05 M B_T and 0.5 M ionic strength with KCl) was prepared by dissolving the proper amount of KH_2PO_4, standardized KOH, and KCl in D_2O. The solution was freeze-dried, and D_2O was added to the residue to reconstitute the buffer. The solution was freeze-dried and reconstituted once more. The 1×10^{-3} M DCl solution was prepared by adding the proper amount of concentrated standardized DCl solution to D_2O. The pD of the solutions was obtained at 25.0 ± 0.1 °C.

The reaction mixtures were prepared by dissolving 4 mg of **1a** or **3** into 1 mL of the deuterated solution held at 5 °C. It was necessary to

(4) (a) Calder, I. C.; Creek, M. J.; Williams, P. J.; Funder, C. C.; Green, C. R.; Ham, K. N.; Tange, J. D. *J. Med. Chem.* **1973**, *16*, 499–502. (b) Calder, I. C.; Creek, M. J., Williams, P. J. *Chem.-Biol. Interact.* **1974**, 8, 87–90. (c) Hinson, J. A.; Nelson, S. D.; Mitchell, J. R. *Mol. Pharmacol.* **1977**, *13*, 625–633. (d) Mulder, G. J.; Hinson, J. A.; Gillette, J. R. *Biochem. Pharmacol.* **1978**, *27*, 1641–1649.

(5) Mulder, G. J.; Hinson, J. A.; Gillette, J. R. *Biochem. Pharmacol.* **1977**, *26*, 189–196. Vaught, J. B.; McGarvey, P. B.; Lee, M.-S.; Garner, C. D.; Wang, C. Y.; Linsmaier-Bednar, E. M.; King, C. M. *Cancer Res.* **1981**, *41*, 3424–3429.

(6) Liu, T.; Smith, G. W.; Rankin, J. T. *Can. Med. Assoc. J.* **1972**, *107*, 768–771. Calder, I. C.; Goss, D. E.; Williams, P. J.; Funder, C. C.; Green, C. R.; Ham, K. N.; Tange, J. D. *Pathology* **1976**, *8*, 1–6. Johansson, S.; Wahlquist, L. *Acta Pathol. Microbiol. Immunol. Scand., Sect. A* **1977**, *85*, 768–774. Bengtsson, U.; Angervall, L. *Lancet* **1979**, *1*, 305. Isaka, H.; Hirooki, Y.; Otusji, A.; Koike, M.; Nagai, Y.; Koura, M.; Sugiyasu, K.; Kanabayashi, T. *Gann* **1979**, *70*, 29–36.

(7) Rising, A. *Chem. Ber.* **1904**, *37*, 43–47.

(8) Baumgarten, H. E.; Staklis, A.; Miller, E. M. *J. Org. Chem.* **1965**, *30*, 1203–1206. Faddeava, V. K.; Svirskaya, P. I.; Baskakov, Yu. A. *Zh. Org. Khim* **1970**, *6*, 285–291.

(9) Wentworth, W. E. *J. Chem. Educ.* **1965**, *42*, 96–103.

Table I. Pseudo-First-Order Rate Constants for the Decomposition of **1a** at 25 °C

buffer[a]	concn, M	pH[b]	10^2k_0, s^{-1}	k', s^{-1}	k'', s^{-1}
HCl	0.001	3.09	1.30 ± 0.08	1.6 ± 0.2 × 10^{-1}	4.88 ± 0.03 × 10^{-4}
KOAc/HOAc	0.01	3.74			4.64 ± 0.05 × 10^{-4}
KOAc/HOAc	0.01	3.84	1.23 ± 0.04	2.37 ± 0.09 × 10^{-2}	
KOAc/HOAc	0.01	4.67	1.16 ± 0.02	3.53 ± 0.08 × 10^{-3}	6.47 ± 0.06 × 10^{-4}
KOAc/HOAC	0.01	5.67	1.23 ± 0.01	3.94 ± 0.04 × 10^{-4}	2.05 ± 0.25 × 10^{-3}
K_2HPO_4/KH_2PO_4	0.01	5.78	1.39 ± 0.01	3.15 ± 0.03 × 10^{-4}	
K_2HPO_4/KH_2PO_4	0.01	5.80			3.1 ± 0.6 × 10^{-3}
K_2HPO_4/KH_2PO_4	0.01	6.78	1.33 ± 0.04	5.86 ± 0.09 × 10^{-5}	
K_2HPO_4/KH_2PO_4	0.01	7.82	1.29 ± 0.04	2.85 ± 0.14 × 10^{-5}	

[a] Ionic strength = 0.50 M (KCl). [b] ±0.02 at 25 °C.

Table II. Pseudo-First-Order Rate Constants for the Decomposition of **3** at 25 °C

buffer[a]	concn, M	pH[b]	k', s^{-1}	k'', s^{-1}
HCl	0.001	3.09	1.13 ± 0.01 × 10^{-1}	4.70 ± 0.03 × 10^{-4}
KOAc/HOAc	0.01	3.86	1.99 ± 0.02 × 10^{-2}	4.71 ± 0.02 × 10^{-4}
KOAc/HOAc	0.01	4.67	3.46 ± 0.01 × 10^{-3}	6.55 ± 0.03 × 10^{-4}
KOAc/HOAc	0.05	4.67	3.34 ± 0.03 × 10^{-3}	6.46 ± 0.03 × 10^{-4}
KOAc/HOAC	0.01	5.67	3.98 ± 0.03 × 10^{-4}	2.34 ± 0.06 × 10^{-3}
K_2HPO_4/KH_2PO_4	0.01	5.78	3.34 ± 0.03 × 10^{-4}	2.93 ± 0.10 × 10^{-3}
K_2HPO_4/KH_2PO_4	0.03	6.77	5.69 ± 0.09 × 10^{-5}	
K_2HPO_4/KH_2PO_4	0.02	6.77	5.27 ± 0.07 × 10^{-5}	
K_2HPO_4/KH_2PO_4	0.01	6.78	5.72 ± 0.04 × 10^{-5}	
K_2HPO_4/KH_2PO_4	0.01	7.77	3.12 ± 0.07 × 10^{-5}	

[a] Ionic strength = 0.50 M (KCl). [b] ±0.02 at 25 °C.

predissolve **1a** in 50 μL of CD_3CN. The mixture was then placed in the NMR tube and quickly transferred to the probe of a WM-250 NMR spectrometer which was thermostated at 5 °C. FT ^1H NMR spectra were obtained by using the kinetics program written for the Aspect 2000 computer. DSS was used as an internal reference. Peaks were assigned on the basis of chemical shift comparisons with authentic samples run under identical conditions.

Spectrophotometric Analysis of the Redox Equilibrium of 3 and 4. The procedure used here is adapted from that of Corbett.[10] A series of phosphate buffers (0.10 M B_T, no KCl or CH_3CN present) were prepared covering the pH range from 7.8 to 11.5. Equal volumes of each of the buffers and of a 1.0 × 10^{-3} M solution of **4** in distilled–deionized water were mixed to generate 5.0 × 10^{-4} M solutions of **4** in 0.05 M B_T phosphate buffers. In the same manner, equal volumes of each of the buffers and of a 2.0 × 10^{-3} M solution of $K_3[Fe(CN)_6]$ in distilled–deionized water were mixed to generate 1.0 × 10^{-3} M solutions of $K_3[Fe(CN)_6]$ in 0.05 M B_T phosphate buffers. Blank solutions containing only 0.05 M B_T phosphate were also prepared. All solutions were incubated in a 25.0 ± 0.1 °C water bath. At each buffer pH, 1.5 mL of the solution of **4** and 1.5 mL of the $K_3[Fe(CN)_6]$ solution were simultaneously added to a cuvette which was quickly transferred to the cell holder of the spectrophotometer thermostated at 25.0 ± 0.1 °C. The absorbance of the mixture was monitored at 420 nm, where only $K_3[Fe(CN)_6]$ absorbs. Initially, as the redox reaction approached equilibrium, the absorbance decreased rapidly and exponentially, and after equilibrium was established, it continued to decrease linearly at a much slower rate. The absorbance curve was extrapolated to zero time (taken as the time of mixing), and that absorbance was recorded. Absorbance readings were also taken at each pH from a solution prepared by mixing the $K_3[Fe(CN)_6]$ solution and the blank phosphate buffer. Duplicate readings of both the redox reaction and the blank solution were made at each buffer pH. In this way, it was possible to determine [ferricyanide]/[ferrocyanide] at each pH. The conversion of this ratio into [**4**]/[**3**] and the subsequent calculation of $E°$ for the reduction of **3** is described in the Results section.

The pH values of the reaction solutions were determined from solutions equivalent to those used in the spectrophotometric study after sufficient time was allowed for the redox processes to reach equilibrium (ca. 15–60 s after mixing).

Results

The kinetics of the decomposition of both **1a** and **3** were followed by UV spectrophotometric methods at 25 °C in 5 vol % CH_3CN–H_2O at 0.5 M ionic strength in the pH region from 3.0 to 8.0. The reproducibility of kinetic runs could be improved by excluding O_2 from the kinetic solutions, so all solutions were routinely outgassed with N_2 prior to kinetic experiments. The kinetics of the decomposition of both **1a** and **3** were quite complicated and in both cases exhibited considerably different characteristics at pH < 6.0 than at pH > 6.0.

The situation at pH < 6.0 will be discussed first. Under these conditions, repetitive wavelength scans of the decomposition of both **1a** and **3** were complex, and it was clear that in both cases, several processes were occurring. However, in both cases, an isosbestic point was observed at 266.5 nm for part of the reaction. Absorbance vs. time data taken for **1a** at this wavelength were fit[9] well by eq 2, where A_∞ is the absorbance at infinite time, k_1 and k_2 are pseudo-first-order rate constants, and A_1 and A_2 are amplitude factors. Equation 2 is valid for consecutive first-order processes.[11] Values of the rate constants obtained from these

$$A_t = A_\infty + A_1e^{-k_1t} + A_2e^{-k_2t} \quad (2)$$

fits are reported in Table I as k_0 and k'. One of these rate constants, k_0, is independent of pH, while the other, k', depends strongly on pH.

Absorbance vs. time data taken at 266.5 nm for **3** were fit[9] well by the standard first-order rate equation. The values of these rate constants are reported in Table II as k'. Note that k' values reported for both **1a** and **3** are, within experimental error, equivalent. Figure 1 shows representative plots of absorbance vs. time for both **1a** and **3** at 266.5 nm in 0.01 M acetate buffer at pH 4.67.

Absorbance vs. time data were also collected in 245 nm, where considerable absorbance changes associated with the process having the isosbestic point at 266.5 nm occurred. Data collected for **3** could be fit quite well by eq 2 to give two rate constants. One of these was equivalent to k' measured at 266.5 nm. For example, in 0.01 M acetate buffer at pH 4.67, k' measured at 266.5 nm was 3.46 ± 0.03 × 10^{-3} s^{-1}, while the two rate constants calculated from a fit of the data taken at 245 nm to eq 2 were 3.37 ± 0.03 × 10^{-3} and 6.55 ± 0.03 × 10^{-4} s^{-1}. The new rate constant observed at 245 nm is reported in Table II as k''.

Data taken at 245 nm for **1a**, could be laboriously fit to an equation containing three exponential terms;[12] however, it was more convenient to fit data taken after 10 half-lives of the k_0

(10) (a) Corbett, J. F. *J. Chem. Soc. B* **1969**, 207–212. (b) Corbett, J. F. *J. Chem. Soc. B* **1969**, 213–216.

(11) Moore, J. W.; Pearson, R. G. "Kinetics and Mechanism", 3rd ed.; Wiley: New York, 1981; pp 290–296.

(12) Such a fit requires seven parameters (A_∞, three rate constants, and three amplitude factors) and will converge only if the initial guesses for these parameters are chosen very carefully. This fit was attempted in some cases, and it always gave rate constants comparable to those obtained by the other methods. An example of such a fit is shown in Figure 1C.

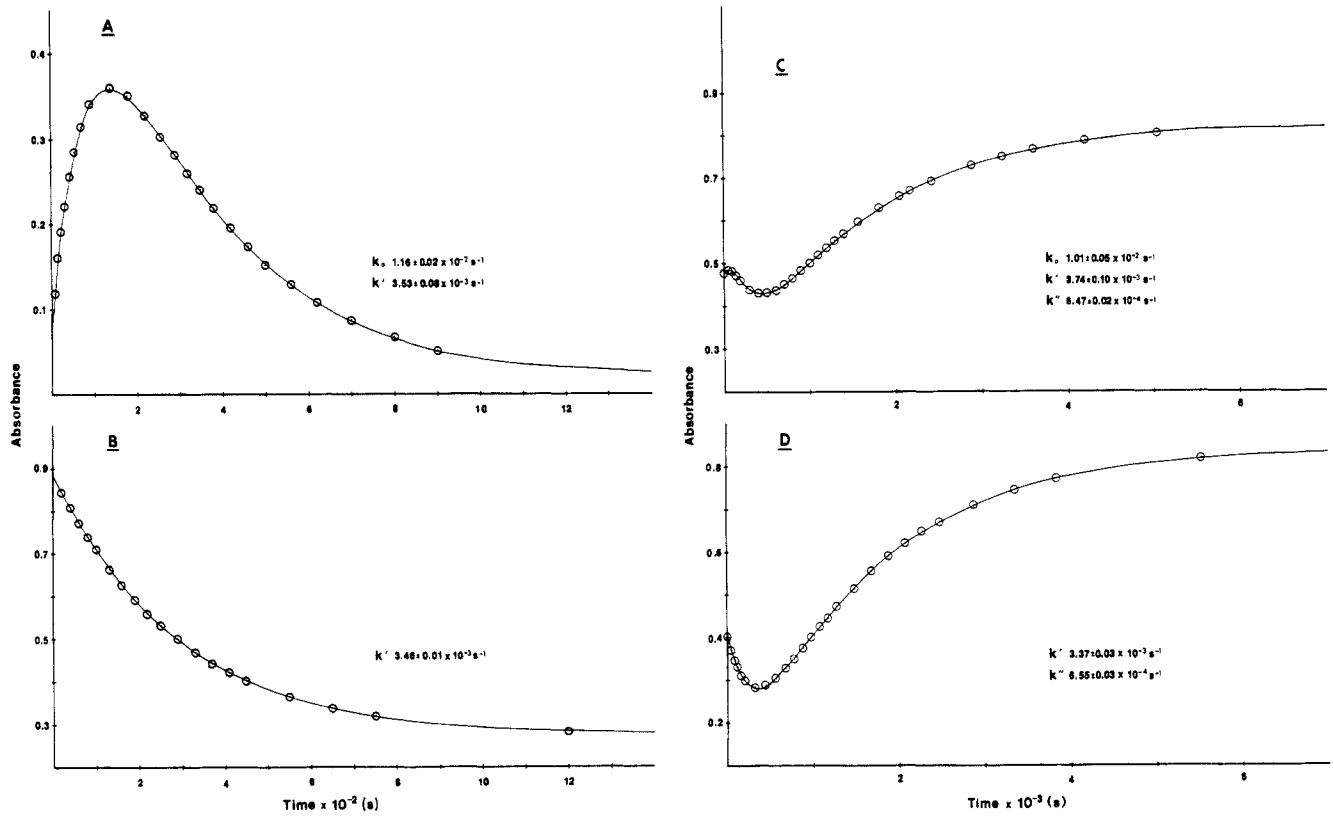


Figure 1. Plots of absorbance vs. time recorded during the decomposition of **1a** and **3** in 0.01 M acetate buffer at pH 4.67. Rate constants are those obtained from least-squares fits. A. Decomposition of **1a** monitored at 266.5 nm. Theoretical line obtained from a fit to eq 2. B. Decomposition of **3** monitored at 266.5 nm. Theoretical line obtained from a fit to the first-order rate equation. C. Decomposition of **1a** monitored at 245 nm. Theoretical line obtained from a fit to an equation containing three exponential terms (ref 12). D. Decomposition of **3** monitored at 245 nm. Theoretical line obtained from a fit to eq 2.

Table III. Rate Parameters Derived for the Decomposition of **1a** and **3** at 25 °C[a]

compound	k_0, s^{-1}	k_c', s^{-1} [b]	k_H', M^{-1} s^{-1} [b]	k_c'', s^{-1} [c]	k_{OH}'', M^{-1} s^{-1} [c]
1a	$1.28 \pm 0.08 \times 10^{-2}$	$2.77 \pm 0.13 \times 10^{-5}$	137 ± 15	$4.74 \pm 0.11 \times 10^{-4}$	$3.6 \pm 0.6 \times 10^{5}$
3		$2.67 \pm 0.12 \times 10^{-5}$	105 ± 7	$4.49 \pm 0.07 \times 10^{-4}$	$4.1 \pm 0.3 \times 10^{5}$

[a] All parameters are reported with their standard deviations. [b] Parameters derived from weighted nonliner least-squares fit of observed k' values to eq 3. The values of the exponent, x, were 0.98 ± 0.01 for **1a** and 0.96 ± 0.01 for **3**. [c] Parameters derived from a weighted nonlinear least-squares fit of observed k'' values to eq 4.

process[13] (600 s) to eq 2. One of the rate constants obtained in this fashion was equivalent to k' obtained at 266.5 nm,[13] while the other was equivalent to k'' obtained from the decompostion of **3** at 245 nm. For example, k' measured for **1a** in 0.01 M acetate buffer, pH 4.67, at 266.5 nm was $3.53 \pm 0.08 \times 10^{-3}$ s^{-1}. The two rate constants obtained at 245 nm were $3.81 \pm 0.25 \times 10^{-3}$ s^{-1} and $6.47 \pm 0.06 \times 10^{-4}$ s^{-1}. The correspondence of these numbers with those obtained for the decomposition of **3** is obvious. Values of k'' obtained at 245 nm for the decomposition of **1a** are reported in Table I. It can be seen from both Tables I and II that k'' increases in magnitude as the pH increases. Figure 1 also shows representative plots of absorbance vs. time for both **1a** and **3** at 245 nm in 0.01 M acetate buffer at pH 4.67.

At pH > 6.0, the characteristics of the decomposition of **1a** and **3** change significantly in a number of ways. First, the process described by the rate constant k'' can no longer be observed. Secondly, the kinetic behavior becomes dependent on the initial concentration of **1a** and **3** at pH > 6.0. A non-first-order process competes with the previously observed first-order processes. The kinetic behavior of the decomposition of **1a** and **3** returns to clean first-order or consecutive first-order if initial concentrations of **1a** and **3** are kept low at ca. 2.5×10^{-6} M. The kinetic data obtained at pH < 6.0 were determined primarily at initial concentrations of ca. 5.0×10^{-5} M. Finally, at pH > 6.0, the decomposition of one of the reaction products, *p*-benzoquinone, **6**, becomes sufficiently rapid that this reaction interferes with the observation of the decomposition of **1a** and **3**. However, the decomposition of **6** exhibits an isosbestic point at 262.1 nm so that the reactions of interest can be examined at this wavelength.[14]

Absorbance vs. time data taken at 262.1 nm for **1a** are fit well by eq 2. One of the two rate constants obtained in this manner is equivalent to k_0 determined at pH < 6.0; the other appears to be consistent with the pH dependence of k'. Both of these constants are reported for buffers with pH > 6.0 in Table I. Data obtained at this wavelength for **3** can be fit well to the first-order rate equation. The rate constants so obtained are reported as k' in Table II. Again, k' values measured for both **1a** and **3** are equivalent within experimental error.

The pH dependence of k' can be expressed by eq 3, where k_c' is a pH-independent first-order rate constant, and k_H' is a second-order rate constant ($x \approx 1.0$) for a hydronium-ion-dependent process.[15] The data collected in Table II show that k' is inde-

$$k' = k_c' + k_H'(a_{H^+})^x \qquad (3)$$

(13) At the lowest pH examined (3.09), $k' > k_0$ so that the data after ten half-lives of the process governed by k' were fit to eq 2. The rate constants obtained from this fit were then k_0 and k''.

(14) Corbett encountered the same problem and used the same solution in his study of the hydrolysis of *p*-benzoquinone monoimine. He reported an isosbestic at 261 nm at 30 °C (ref 10b).

(15) Data were fit by a weighted nonlinear least-squares method to eq 3. Three variable parameters, k_c', k_H', and x were used. The value of x differed only slightly from 1.0 (Table III).

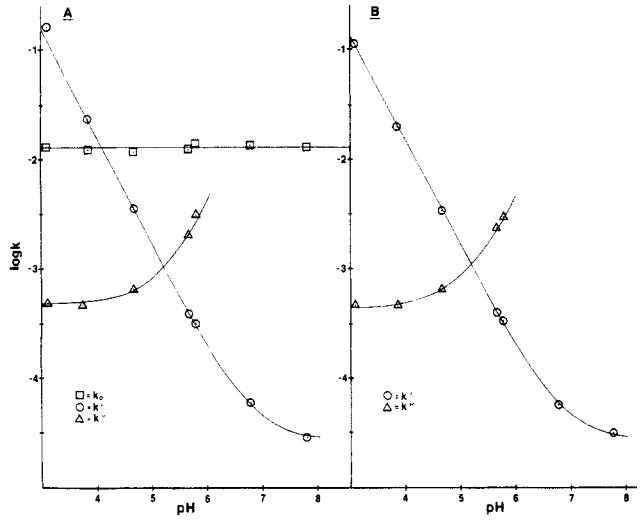


Figure 2. Plots of the logarithms of k_o, k', and k'' as a function of pH for **1a** and **3**. Theoretical lines were obtained from least-squares fits as described in the text. A. Data for **1a**. B. Data for **3**.

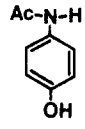
pendent of buffer concentration in both acetate and phosphate buffers. The pH dependence of k'' is best expressed by eq 4, where k_c'' is a pH-independent first-order rate constant and k_{OH}'' is a second-order rate constant for a hydroxide-ion-dependent process.[16]

$$k'' = k_c'' + k_{OH}''(a_{OH^-}) \quad (4)$$

The data in Table II show that k'' is independent of buffer concentration. Values of the derived rate constants for both **1a** and **3**, as well as the average value of k_o for **1a**, are collected in Table III. Within experimental error, the derived rate constants for both compounds are equivalent. Figure 2 shows the quality of the fit of the kinetic data for **1a** and **3** to eq 3 and 4.

Activation parameters were determined for k_o from rate data taken in 0.01 M acetate buffers, pH 5.74, in the temperature range 25.0–50.0 °C. The calculated values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were 15.8 ± 0.5 kcal/mol and −14.2 ± 1.5 eu, respectively.

Table IV summarizes the results of product studies performed on the decomposition of **3** in aqueous solution over the pH range 3.0–8.0. As we had previously reported,[2a] acetaminophen, **4**, 3-chloro-4-hydroxyacetanilide, **5**, and *p*-benzoquinone, **6**, are produced as a result of the decomposition of **3**. At pH < 6.0,



4

the yields of reaction products are independent of the initial concentration of **3** and the predominant reaction products are **5** and **6**. At pH > 6.0, **4** became a major product, while the yield of **5** became vanishingly small. In addition, the yields of both **4** and **6** were observed to depend on the initial concentration of **3**. At low concentrations (2.5×10^{-6} M), at which the decomposition of **3** was a first-order process kinetically, **4** and **6** could account for most, if not all, of the reactant. The yields reported for **6** at the highest pH examined must be considered lower limits because **6** decomposes at a significant rate at this pH. The studies at low concentrations were further complicated by the fact that **4** and **6** were produced in amounts near their UV detection limits. Nevertheless, at higher initial concentrations of **3**, the yields of both of these materials decreased, and at initial concentrations of **3** > 1.0×10^{-4} M a new product, a brown amorphous precipitate, could be observed. This material has not been completely characterized, but it appears to be oligomeric in nature.

(16) Data were fit by a weighted nonlinear least-squares method to eq 4. Two variable parameters, k_c'' and k_{OH}'', were used. It was assumed that pOH = 14.00 − pH.

Table IV. Yields of Products Obtained from the Hydrolysis of **3** at 25 °C under Various pH Conditions

buffer[a]		initial concn of **3**, M	% yields[b] **4**	**5**	**6**
HCl	3.1	1.0×10^{-4}		33 ± 2	65 ± 2
KOAc/HOAc	3.8	1.0×10^{-4}		29 ± 2	66 ± 3
KOAc/HOAc	5.7	1.0×10^{-4}	10 ± 1	17 ± 2	65 ± 3
K_2HPO_4/KH_2PO_4	6.8	5.0×10^{-4}	24 ± 2	trace[c]	12 ± 1
K_2HPO_4/KH_2PO_4	6.8	1.0×10^{-4}	28 ± 3	trace[c]	26 ± 2
K_2HPO_4/KH_2PO_4	6.8	5.0×10^{-5}	28 ± 4	trace[c]	31 ± 2
K_2HPO_4/KH_2PO_4	6.8	1.0×10^{-5}	25 ± 2	trace[c]	40 ± 3
K_2HPO_4/KH_2PO_4	6.8	2.5×10^{-6}	55 ± 12	trace[c]	55 ± 8
K_2HPO_4/KH_2PO_4	7.8	5.0×10^{-4}	26 ± 3		4 ± 2[d]
K_2HPO_4/KH_2PO_4	7.8	1.0×10^{-4}	36 ± 5		11 ± 2[d]
K_2HPO_4/KH_2PO_4	7.8	5.0×10^{-5}	39 ± 1		11 ± 1[d]
K_2HPO_4/KH_2PO_4	7.8	1.0×10^{-5}	50 ± 4		17 ± 5[d]
K_2HPO_4/KH_2PO_4	7.8	2.5×10^{-6}	53 ± 4		19 ± 3[d]

[a] Concentration of all buffers was 0.01 M. Ionic strength was maintained in all solutions at 0.50 M with KCl. [b] Yields were determined by HPLC methods and are reported with respect to **3** initially present. [c] Less than 0.5%. [d] These must be regarded as lower limits since significant decomposition of benzoquinone occurs at this pH.

Table V. Yields of Products Obtained from the Hydrolysis of **1a** at 25 °C under Various pH Conditions

buffer[a]	pH	% yields[b] **4**	**5**	**6**	**7**
KOAc/HOAc	3.8		16 ± 2	56 ± 2	
K_2HPO_4/KH_2PO_4	5.8	10 ± 1	9 ± 2	41 ± 2	4 ± 1
K_2HPO_4/KH_2PO_4	6.8	27 ± 1	trace[c]	14 ± 1	13 ± 3
K_2HPO_4/KH_2PO_4	7.8	34 ± 2		5 ± 2[d]	15 ± 3

[a] Concentration of all buffers was 0.01 M. Ionic strength was maintained at 0.50 M with KCl. Initial concentration of **1a** was ca. 1.0×10^{-4} M. [b] Yields were determined by HPLC methods and are reported with respect to **1a** initially estimated to be present. [c] Less than 0.5%. [d] This is a lower limit since significant decomposition of benzoquinone occurs at this pH.

Quantitative product studies of the reactions of **1a** were complicated by the fact that this material decomposes quite rapidly, primarily by intramolecular rearrangement,[2] when stored neat or in nonaqueous solvents. It was not possible to prepare an analytically pure sample of **1a**; so the product yields reported in Table V should be considered as lower limits. The kinetic studies showed that **3** is an intermediate during the decomposition of **1a**. The magnitude of the absorbance changes observed during the kinetic experiments indicated that 80–100% of **1a** decomposed via a path involving **3**. The product studies reported in Table V confirm that at all pH values examined, the decomposition products of **3** account for the major portion of the product yield. In fact, only one material not associated with the decomposition of **3**, 4-methoxyacetanilide, **7**, was detected among the reaction products. This material accounts for a significant part of the yield in the more basic buffers, but is not observed in the acidic buffers. Acetanilides have been detected previously among the reaction products of the *N*-(sulfonatooxy)acetanilides and *N*-(pivaloyloxy)acetanilides, but they are usually formed only in the presence of reducing agents such as KI.[2] The *N*-(sulfonatooxy)- and *N*-(pivaloyloxy)acetanilides commonly yield a number of products associated with nitrenium ion intermediates when they undergo decomposition under the conditions of this study. Among these are ring-chlorinated and ring-hydroxylated species, as well as products of intramolecular rearrangement.[2] None of these is produced as a result of decomposition of **1a** in an aqueous environment.[17]

The reactions of **1a** and **3** were monitored as a function of time by HPLC and ^1H NMR techniques in an effort to detect reactive intermediates. These studies were performed under conditions

(17) The products of intramolecular rearrangement, 2-(pivaloyloxy)-4-methoxyacetanilide and 3-(pivaloyloxy)-4-methoxyacetanilide, were already present as minor impurities. However, our results indicate that the amounts of these materials do not increase when **1a** is incubated in aqueous media.

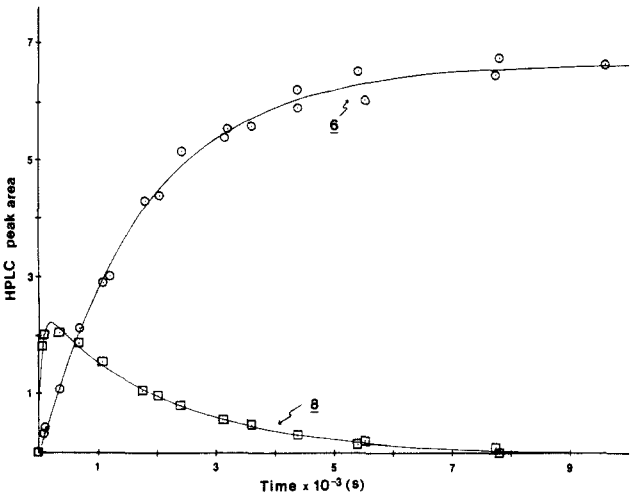


Figure 3. Plots of HPLC peak area (arbitrary units) vs. time for **6** and **8** during the decomposition of **3** in 0.01 M acetate buffer at pH 3.88. Theoretical lines were obtained from a fit of the data to eq 2. Rate constants are given in the text.

similar to those employed in the UV kinetic studies except for higher initial concentrations of **1a** and **3** and, in the case of the NMR studies, at lower temperature (ca. 5 °C).

In 0.01 M acetate buffer at pH 3.88, HPLC analysis (μ-Bondapak C-18 column; 50/50 MeOH/H_2O eluent; 1 mL/min) showed that **3** (retention time (rt) = 4.3 min) disappeared at a very rapid rate ($t_{1/2}$ < 1 min). One of the products, **5**, (rt = 4.5 min) was produced at apparently the same rate. However, **6** (rt = 4.1 min) grew in much more slowly, and its appearance was coupled to the appearance and decay of an intermediate, **8** (rt = 3.1 min). Peak area vs. time data for both **6** and **8** were fit well by eq 2. The two rate constants obtained for **6** were 1.4 ± 1.4 × 10^{-2} s^{-1} and 5.5 ± 0.4 × 10^{-4} s^{-1}. Those obtained for **8** were 2.1 ± 0.3 × 10^{-2} and 4.6 ± 0.2 × 10^{-4} s^{-1}. Figure 3 shows a plot of peak area vs. time for both **6** and **8** at this pH. These rate constants compare favorably with k' and k'' measured spectrophotometrically at pH 3.86, 1.99 ± 0.02 × 10^{-2} and 4.71 ± 0.02 × 10^{-4} s^{-1}, respectively. These results indicate that at this pH, **3** decomposes at a rate governed by k' to yield **5** and **8**, and **8** subsequently decomposes to **6** at a rate governed by k''.

At pH 3.09 in HCl, **3** disappears even more rapidly. In fact, it cannot be observed as early as 2 min after initiation of the reaction. Since the half-life for the process associated with k' at this pH is only 6.1 s, this result is consistent with the disappearance of **3** via a process governed by k'. Under these conditions, the peak area vs. time data for both **6** and **8** can be adequately fit by the standard first-order rate equation. Since k'/k'' = 240 at this pH, this is a reasonable result. The rate constants obtained were 4.5 ± 0.5 × 10^{-4} s^{-1} for the appearance of **6** and 4.9 ± 1.2 × 10^{-4} s^{-1} for the disappearance of **8**. These values agree well with the spectrophotometric value of k'' of 4.70 ± 0.03 × 10^{-4} s^{-1} measured at this pH.

In DCl solution at pD 3.17, the ^{1}H NMR spectrum of **3** (below) cannot be observed even at early reaction times. Instead, a spectrum which is apparently that of **8** can be clearly observed: ^{1}H NMR (D_2O, 250 MHz) δ 1.98 (3 H, s), 6.26 (2 H, d, J = 10.18 Hz), 7.10 (2 H, d, J = 10.18 Hz). This material decomposes cleanly into **6** (δ 6.86) and acetamide (δ 1.99) at a slow rate.

In 0.01 M acetate buffer at pH 3.74, HPLC analysis shows that **1a** very rapidly decomposes into **3** which subsequently decomposes as indicated above. Under these conditions, the only products of the decomposition of **1a** which can be observed are those already identified as products of the decomposition of **3**. In DCl at pD 3.17, ^{1}H NMR spectra show the same behavior already described for **3**. The only difference is that methanol (δ 3.34), which is produced as **1a** decomposes into **3**, can be observed.

HPLC analysis shows that in the more basic buffers, **3** disappears more slowly. For example, in 0.01 M acetate buffer at pH 5.67, **3** disappears with a half-life of approximately 30 min. This half-life is consistent with the magnitude of k' measured spectrophotometrically under these conditions. The intermediate, **8**, can still be observed, although it is present at much lower concentration than at lower pH. This is to be expected since k' is smaller and k'' larger at this pH than at lower pH. In 0.01 M phosphate buffer at pH 7.83, **3** decomposes to yield a mixture of **4** and **6**. At the concentrations employed in this study (1.0 × 10^{-4} M), the overall kinetics are not first-order. This is consistent with the spectrophotometric results. At this pH, the intermediate, **8**, cannot be observed. The combined yields of **4** and **6** are far from quantitative (~40–50%), but no other products can be observed by HPLC.

In 0.05 M deuterated phosphate buffer at pD 6.81, the ^{1}H NMR spectrum of **3** can be observed clearly at early reaction times: ^{1}H NMR (D_2O, 250 MHz) δ 2.38 (3 H, s), 6.73 (2 H, d, J = 10.1 Hz), 7.13 (2 H, d, J = 10.1 Hz). Under these conditions, the observed products are **4** and small amounts of acetamide. The ^{1}H NMR signal for **6** is obscured by the aromatic signals of **4**, but presumably it is present since the byproduct of its formation, acetamide, can be detected. At the concentrations of **3** employed in this study (ca. 10 mM), the combined yields of **4** and acetamide are no more than about 30%. A considerable amount of a brownish precipitate is formed in the NMR tube as the reaction progresses. Under these conditions, it is not possible to detect the NMR signals of the intermediate, **8**.

Since the rate of decomposition of **1a** into **3** is rapid ($t_{1/2} \approx 60$ s) and pH-independent (see k_o, Table I), the HPLC chromatograms obtained for the decomposition of **1a** strongly resemble those obtained for **3** at all pH values. In 0.01 M phosphate buffer at pH 7.83, the only significant difference is that in the case of **1a**, the reduction product, **7**, is rapidly formed simultaneously with **3** as **1a** decomposes.

^{1}H NMR analysis shows that in 0.05 M deuterated phosphate buffer at pD 6.81, **1a** rapidly decomposes into **3** and **7**. Subsequently, **3** decomposes as indicated above.

It was not clear how **3** is reduced to **4**. There is no obvious reducing agent present, and the hydrolysis of the closely related compound, *p*-benzoquinone monoimine, apparently proceeds under very similar conditions to yield only *p*-benzoquinone. No *p*-aminophenol was detected.[10b] Each of the buffer components (except H_2O) was removed or replaced in turn in an attempt to find a possible reducing agent. However, the yield of **4** obtained from 1.0 × 10^{-4} M **3** did not vary significantly in any of the following solutions: 0.01 M phosphate buffer, μ = 0.50 M (KCl), pH 6.78; 0.01 M phosphate buffer, μ = 0.50 M (KNO_3), pH 6.74; 0.01 M phosphate buffer, no added salts, pH 6.76; and 0.01 M succinate buffer, μ = 0.50 M (KCl), pH 6.68. The yield of **4** was also insensitive to the removal of the cosolvent, CH_3CN, from the buffers.

The standard reduction potential, $E°$, for the conversion of **3** into **4** was determined by a method previously used by Corbett[10a] to measure the redox equilibrium between *p*-benzoquinone monoimine and *p*-aminophenol. If the redox equilibrium between **3** and **4** is described by eq 5, then this reaction may be in equi-

$$3 + 2H^+ + 2e^- \rightleftharpoons 4 \qquad (5)$$

librium with the ferricyanide/ferrocyanide redox pair as in eq 6. This reaction can be readily monitored since all other species are transparent at the visible λ_{max} of ferricyanide at 420 nm.

$$4 + 2[Fe(CN)_6]^{3-} \rightleftharpoons 3 + 2[Fe(CN)_6]^{4-} + 2H^+ \qquad (6)$$

Equation 5 is assumed by analogy to the *p*-benzoquinone monoimine/*p*-aminophenol system.[10a] Its validity was supported by the observation that the electrochemical generation of **3** from **4** is a two-electron process.[3c,18] Our results (below) confirm the validity of eq 5.

A rapid decrease in absorbance at 420 nm occurs when equal volumes of 5.0 × 10^{-4} M **4** and 1.0 × 10^{-3} M ferricyanide in 0.05 M phosphate buffers in the pH range 7.8–11.5 are mixed. This

(18) Miner, D. J.; Rice, J. R.; Riggin, R. M.; Kissinger, P. T. *Anal. Chem.* **1981**, *53*, 2258–2263.

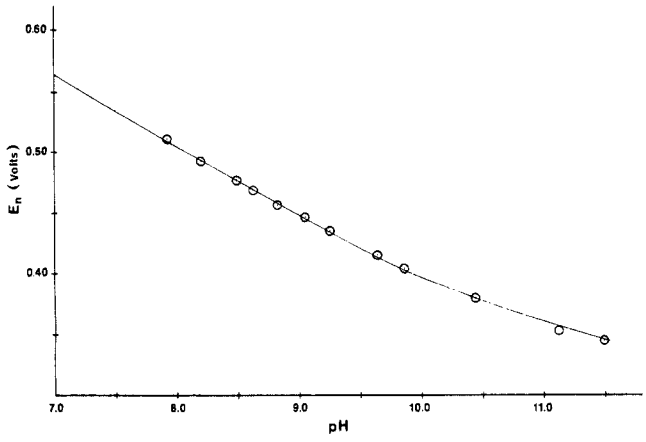


Figure 4. Plot of reduction potential of **3**, E_n, as a function of pH. Theoretical line obtained from a least-squares fit of the data to eq 8.

exponential decay of the absorbance is complete in about 15 s (pH 11.5) to 60 s (pH 7.8). Thereafter, the absorbance decreases slowly in a linear fashion. This latter phenomenon is most likely due to the disruption of the redox equilibrium by the decomposition of **3** in the aqueous media. Extrapolation of the linear portion of the absorbance curve to zero time yields the absorbance at equilibrium which can be converted into the equilibrium ratio of [ferricyanide]/[ferrocyanide]. Corbett has shown that under the conditions of these experiments, E_n, the standard reduction potential for **3** at a given pH, is given by eq 7,[10a] where E_n' is the standard reduction potential of ferricyanide at the same pH. At

$$E_n = E_n' + (3RT/2F) \ln ([\text{ferricyanide}]/[\text{ferrocyanide}]) \quad (7)$$

pH > 4, E_n' has a constant value of 0.42 V.[19] It can be readily shown that $E°$ for eq 5 is related to E_n through eq 8, where K_a is the ionization constant of **4**.[20]

$$E_n = E° + (RT/F) \ln [H^+] - (RT/2F) \ln ([H^+]/(K_a + [H^+])) \quad (8)$$

E_n vs. pH data are plotted in Figure 4. These data were fit to eq 8 by a least-squares method in which $E°$ and K_a were used as variable parameters.[9] The best fit values of $E°$ and pK_a were 0.978 ± 0.001 V and 9.91 ± 0.05. The calculated pK_a of **4** is in good agreement with a value of 9.82 determined by spectrophotometric titration under similar conditions.[21] As expected, $E°$ for **3** is considerably larger than that determined for *p*-benzoquinone monoimine (0.728 V).[22]

Discussion

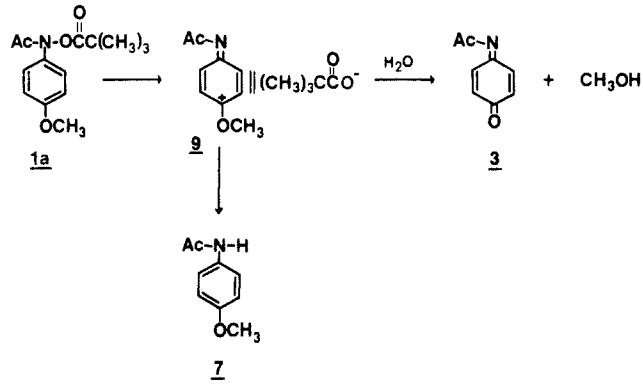
Three separate lines of evidence show that in aqueous solution in the pH range 3.0–8.0, *N*-(pivaloyloxy)-4-methoxyacetanilide, **1a**, decomposes predominately into *N*-acetyl-*p*-benzoquinone imine, **3**. First, the UV kinetics results establish that **1a** decomposes into a material which has identical kinetic properties with those observed for **3**. Tables I and II show that the rate constants k' and k'' obtained from either **1a** or **3** are identical, within experimental error, both in magnitude and dependence. Moreover, comparison of the magnitude of absorbance changes observed during the kinetics experiments indicates that 80–100% of **1a** decomposes via a path involving **3** at all pH values examined. Second, HPLC experiments confirm that in phosphate buffer at pH 7.83, **1a** rapidly decomposes into a material with retention time equivalent to that of authentic **3**. This material subsequently decomposes in a manner identical with that of **3**. Under these conditions, the only reaction product not derived from **3** is 4-methoxyacetanilide, **7**, which is produced simultaneously with **3** as **1a** decomposes. However, **7** accounts for only about 15% of the **1a** originally present; **3** and its reaction products account for the rest of the product yield. In acetate buffer at pH 3.74, small amounts of **3** can be observed at early reaction times by HPLC as **1a** decomposes. However, the intermediate, **8**, derived from **3** (see below) builds up to substantial levels and decay to *p*-benzoquinone, **6**, in a manner identical with that observed for authentic **3**. Tables IV and V show that at pH < 6.0, the only products observed to form during the decomposition of **1a** are those derived from **3**. Finally, ^1H NMR data show that in deuterated phosphate buffer at pD 6.81, **1a** decomposes into a material which has an NMR spectrum identical with that of authentic **3**. In DCl solution at pD 3.17, **1a** yields a material with a ^1H NMR spectrum identical with that of the intermediate **8** derived from authentic **3**.

We have shown previously that the *N*-(sulfonatooxy)acetanilides[2a–c] and *N*-(pivaloyloxy)-*p*-methylacetanilide[2d] decompose in aqueous solution via the intermediacy of nitrenium ion pairs.[23] Gassman and Granrud have shown that the closely related methanesulfonic acid esters of the *N*-hydroxyacetanilides also decompose under various conditions by mechanisms involving nitrenium ion pairs.[24] The available evidence indicates that the decomposition of **1a** also proceeds via a nitrenium ion mechanism as shown in Scheme I. The rate constant k_o, which describes the decomposition of **1a**, is insensitive to changes in pH and buffer composition (Table I). This characteristic has also been noted in the reactions of the *N*-(sulfonatooxy)acetanilides[2a,b] and *N*-(pivaloyloxy)-*p*-methylacetanilide[2d] in aqueous solution. The magnitudes of $\Delta S^‡$ for the decomposition of the *N*-(sulfonatooxy)acetanilides under these conditions are typically between −13 and −22 eu.[2a] $\Delta S^‡$ determined for **1a** was within this range at −14.2 ± 1.5 eu. Extrapolation of rate data taken at lower temperatures to 70 °C gave a value of k_o for **1a** of 5.1 ± 0.3 × 10^{-1} s^{-1}. Correlation of this rate constant and the corresponding rate constants determined at 70 °C for *N*-(pivaloyloxy)-*p*-methylacetanilide[2d] and three other *N*-(pivaloyloxy)acetanilides (**1**; Y = H, *p*-Cl, and *p*-Br)[25] with Brown's σ^+ parameter gave a ρ of −5.6 ± 0.8 (r = 0.97). This value is similar to the ρ of −4.4 ± 0.9 determined for the *N*-(sulfonatooxy)acetanilides from data taken at 40 °C[2a] and most definitely indicates that a significant amount of positive charge has developed in the aromatic ring in the rate-determining step of the reaction. Finally, reduction of the nitrenium ions derived from ester derivatives of the *N*-hydroxyacetanilides into the corresponding acetanilides is a characteristic reaction of these compounds which is also observed for **1a** at pH > 6.0. Although these reductions occur more efficiently in the presence of a good reducing agent such as KI,[2c] it has been shown that *N*-(pivaloyloxy)-*p*-methylacetanilide also undergoes this reaction in phosphate buffers in the absence of added reducing agents.[2d] Gassman has previously shown that the solvolysis of the *N*-*tert*-butyl-*N*-chloroanilines in MeOH occurs with some reduction to the corresponding anilines.[26] These reactions also most certainly involve nitrenium ion intermediates.[26] It is possible that the reductions which occur in the absence of obvious reducing agents proceed through the intermediacy of a triplet nitrenium ion,[27] but we have no evidence which would require this conclusion.

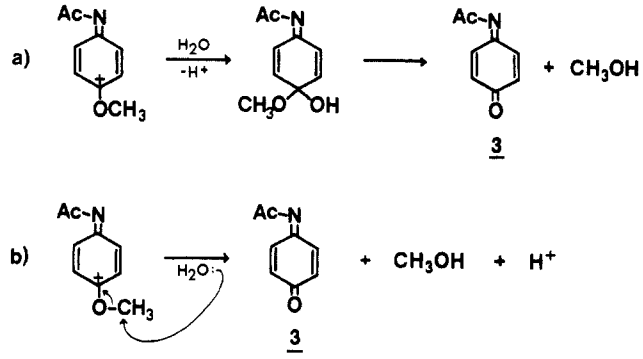
All previously examined sulfuric or pivalic acid esters of the *N*-hydroxyacetanilides have yielded significant amounts of rearranged products, predominately 2-(sulfonatooxy)- or 2-(pivaloyloxy)acetanilides, which are thought to have arisen from the collapse of tight ion pair intermediates.[2,24] The solvent-separated nitrenium ion pairs derived from these species are also susceptible

(19) Clark, W. M. "Determination of Hydrogen Ions", 2nd ed.; Williams and Wilkins: Baltimore, 1922; p 387.
(20) In deriving eq 8, it was assumed that only one ionization equilibrium (that for **4**) was important in the pH range of the study. Protonation of **3** and **4** will occur only in very strong acid solutions (ref 31 and 32).
(21) Legler, G.; Sinott, M. L.; Withers, S. G. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1376–1383.
(22) Fieser, L. F. *J. Am. Chem. Soc.* **1930**, *52*, 4915–4940.
(23) Gassmann, P. G. *Acc. Chem. Res.* **1971**, *3*, 26–33.
(24) Gassman, P. G.; Granrud, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 1498–1499. Gassman, P. G.; Granrud, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 2448–2449.
(25) Novak, M.; Andronico, A. F., unpublished results.
(26) Gassman, P. G.; Campbell, G. A. *J. Am. Chem. Soc.* **1972**, *94*, 3891–3896. Gassman, P. G.; Campbell, G. A.; Frederick, R. C. *J. Am. Chem. Soc.* **1972**, *94*, 3884–3891.
(27) Gassman, P. G.; Cryberg, R. L. *J. Am. Chem. Soc.* **1969**, *91*, 5176–5178.

Scheme I



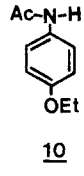
Scheme II



to attack by external nucleophiles to yield 2- or 4-substituted acetanilides.[2,24] Surprisingly, within the limits of our ability to detect them, none of these products were found in the present case.

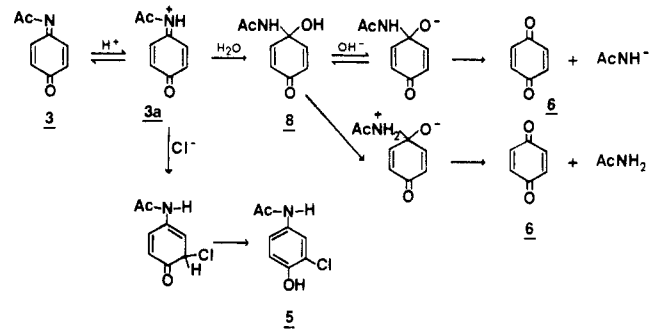
It has been previously noted that the yield of the rearranged products decreases as the electron-donating ability of the substituent increases for both the *N*-(sulfonatooxy)acetanilides[2b] and *N*-*tert*-butyl-*N*-chloroanilines.[26] These observations are consistent with the idea that the lifetime of the tight ion pair (in contrast to the solvent-separated ion pair) decreases with the increasing stability of the nitrenium ion. Since no rearranged products are obtained from the decomposition of **1a** in aqueous solution, it appears that the lifetime of the tight ion pair is too short to allow internal return but that this species rapidly and irreversibly decomposes into the solvent-separated ion pair (**9**).[28] The decomposition of the solvent-separated ion pair into **3** must be a very efficient process since none of the ring-substituted products, which are commonly observed to arise from nucleophilic attack in the other cases, can be detected. Scheme II presents two alternative mechanisms for the formation of **3** from the nitrenium ion. The *N*-(sulfonatooxy)acetanilides which also apparently produce **3**, the *p*-Cl and *p*-Br compounds,[2a] must do so by a mechanism similar to that of path a. However, in the present case, the two mechanisms cannot be distinguished by the available data. Experiments which can distinguish these possibilities are under way.

The best available evidence indicates that phenacetin, **10**, is a weak carcinogen.[6] Since phenacetin is known to be metabolized to *N*-hydroxyphenacetin in several species,[29] it has been suggested,

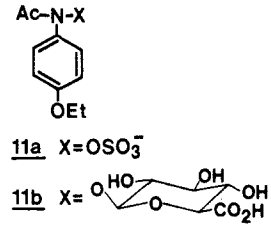


10

Scheme III



by analogy to the metabolism of polycyclic amides,[1] that the sulfate or glucuronide derivatives of *N*-hydroxyphenacetin, **11a** or **11b**, are possible carcinogenic metabolites.[4d,5] The similarity of **1a** to these species is obvious. It has been suggested that **11a** and **b** and *N*-hydroxyphenacetin decompose in aqueous solution into *N*-acetyl-*p*-benzoquinone imine, **3**, but only indirect evidence sup-



porting this claim has been offered.[4] This study provides the first unequivocal evidence that **3** is generated during the decomposition of such species. Although **3** is apparently highly toxic,[3,4] it cannot be the ultimate carcinogen derived from phenacetin since it has been recently demonstrated that **3** is also formed during the metabolism of acetaminophen, **4**, which is not carcinogenic.[3d,30] If species such as **11** are responsible for the carcinogenic properties of phenacetin, then it is likely that the nitrenium ions derived from them are the ultimate carcinogenic electrophiles. The rather weak carcinogenicity of phenacetin may then be directly linked to the demonstrated tendency of such ions to decompose into **3** rather than react with cellular nucleophiles.

Although **3** has been thought to be a toxic metabolite of acetaminophen and phenacetin for over a decade, a systematic study of its aqueous solution chemistry has not been reported.[3,4] For that reason, we have investigated the chemistry of **3** in the pH range 3.0–8.0.

At pH <6.0, the predominant reactions of **3** under the conditions of our experiments are acid-catalyzed hydrolysis to form *p*-benzoquinone, **6**, and acid-catalyzed nucleophilic attack of Cl^- to form 3-chloro-4-hydroxyacetanilide, **5**. The overall kinetics of the decomposition of **3** in this pH range are biphasic and can be characterized by two pseudo-first-order processes with rate constants k' and k'' (Table II). HPLC experiments show that k' is associated with the decomposition of **3** into **5** and an intermediate species **8**. HPLC experiments again showed that **8** subsequently decomposes into **6** with a rate governed by k''. This is most clearly seen in Figure 3 which shows plots of HPLC peak area vs. time for **6** and **8** at pH 3.88.

Figure 2B shows that at pH < 6.0, the initial decomposition of **3** is subject to acid catalysis (k_H'), while the decomposition of **8** has both pH-independent and base-catalyzed paths (k_c'' and k_{OH}''). Rate data in Table II show that buffer concentrations have no effect on k' or k'', so that both of the pH-dependent processes are examples of specific catalysis.

The mechanism of Scheme III is consistent with all the results obtained at pH < 6.0. Both **5** and **8** are formed by an acid-catalyzed process which most likely involves the N-protonated conjugate acid of **3**, **3a**. A similar intermediate was invoked to

(28) The available data do not allow us to distinguish between solvent-separated ion pairs and entirely dissociated ions. In view of the substantial amount of internal return observed in other systems (ref 2, 24, and 26), it is likely that either type of intermediate is produced in a stepwise fashion from tight ion pairs.

(29) McLean, S.; Davies, N. W.; Watson, H.; Favretto, W. A.; Bignall, J. C. *Drug. Metab. Dispos.* **1981**, *9*, 255–260 and references therein.

(30) Ham, K. N.; Calder, I. C. In "Advances in Inflammation Research"; Rainsford, K. D., Velo, G. P., Eds.; Raven Press: New York, 1984; Vol. 6, pp 139–148.

explain the acid-catalyzed hydrolysis of *p*-benzoquinone monoimine.[10b] Since the pK_a of that species is ca. 3.7, it was possible to obtain kinetic evidence for its existence.[10b] However, the pK_a of the conjugate acid of acetaminophen is ca. −1.0,[31] and the pK_a of **3a** must be more negative than that;[32] so over the pH range of this study, **3a** will not be present in detectable concentrations, and its presence cannot be demonstrated from kinetic data. The observation of specific acid catalysis requires that the protonation of **3** be a rapid and reversible process.

Attack of Cl^- at C-3 of **3a** will lead to the keto form of **5** which would rapidly isomerize to **5**. Although in principle H_2O could also attack at this site, we have found no evidence that this occurs. It has been previously shown by others that **3** undergoes attack by various nucleophiles at C-3.[3,4b]

Attack of H_2O at C-1 of **3a** would yield the carbinolamine which we have identified as **8**. The ^1H NMR spectrum of **8** obtained in DCl solution is consistent with this structure. Specifically, the upfield shift of the acyl methyl resonance from δ 2.30 in **3** to δ 1.98 in **8** is consistent with the change in hybridization of N from sp^2 to sp^3. The carbinolamine structure also readily explains the decomposition of **8** into the observed products, **6** and acetamide. The specific base-catalyzed decomposition of **8** is envisioned to proceed via deprotonation of the hydroxyl group to yield an anionic species which can expel the acetamide anion. The uncatalyzed decomposition of **8** may proceed through a zwitterionic species as indicated in Scheme III.

We have obtained no evidence which requires that the conversion of **3** into **8** is a reversible process. If it is, the equilibrium constant for the formation of **8** is large. At pH < 4.0, the decomposition of **8** is slow. HPLC results show that under these conditions, **3** disappears rapidly and none of it can be detected for the greater part of the reaction as **8** disappears. It would have been possible to detect **3** if a mobile equilibrium with $K_{eq} < 25$ existed.

The conversion of **8** into **6** is the process which gave nonreproducible kinetic data in the presence of O_2. Control experiments showed that under the conditions of our study, **6** underwent an O_2-dependent reaction which did not give reproducible absorbance vs. time data. We did not characterize this process further. In the absence of O_2, **6** was stable, except for the base-catalyzed hydrolysis at pH > 6.0.[10b] If solutions were routinely outgassed with N_2 prior to experiments, the kinetic data for the conversion of **8** into **6** could be fit very well to the standard first-order rate equation.

At pH > 6.0, the kinetic study was complicated by a competing non-first-order process. At sufficiently low concentrations of **3** (ca. 2.5×10^{-6} M), its decomposition returned to a slow ($t_{1/2}$ > 1 h) first-order process. Other workers have performed kinetic studies of the decomposition of **3** at essentially neutral pH and high initial concentrations (>1.0×10^{-4} M).[3b,c] They reported relatively rapid decomposition of **3** and both half-order[3b,c] and second-order[3b] kinetics. We have not investigated the kinetics in detail under these conditions, but the apparent product of the reaction at high initial concentrations of **3** is an amorphous brown precipitate which we have not yet fully characterized.

Since the rate of decomposition of **3** is increased by the presence of acetaminophen, **4**, under these conditions, it has been suggested that **3** and **4** undergo a comproportionation reaction to form a semiquinone imine radical which then undergoes further reactions.[3b] However, Corbett has shown that *p*-benzoquinone monoimine reacts with *p*-aminophenol to form dimeric and trimeric products by simple electrophilic attack of the quinone imine on the electron-rich *p*-aminophenol.[33] This quinone imine undergoes condensation reactions with a number of other electron-rich aromatics by similar mechanisms.[34]

It appears that **4**, which is a product of the first-order decomposition of **3** at pH > 6.0 (below), plays a role in the rapid disappearance of **3** in the experiments done at high initial concentrations. At the present time, it is not possible to conclude whether this occurs by a radical process or by electrophilic attack of **3** on **4**. However, our experiments indicate that this process is insensitive to the presence of O_2. This observation would seem to favor the latter possibility.

At low initial concentrations of **3**, the overall decomposition reaction is first-order at pH > 6.0, and the process governed by the rate constant k'' cannot be detected. At pH 6.78, the calculated value of k'', based on data obtained at lower pH, is 2.5×10^{-2} s^{-1}. The observed value of k' in 0.01 M phosphate buffer at this pH is $5.86 \pm 0.09 \times 10^{-5}$ s^{-1}. Since $k''/k' \approx 440$ at this pH, it would, indeed, be difficult to detect the faster process. HPLC and ^1H NMR results confirm that **8** cannot be detected under these conditions. Since **6** is still a product of the reaction at this pH (Table IV), **8** must be formed, but it must also decompose at a very rapid rate.

At pH > 6.0, both a pH-independent (k_c') and pH-dependent (k_H') term contribute significantly to k' (Figure 2B and Table III). Table IV shows that in this pH range, the products of the reaction also change significantly. Acetaminophen, **4**, becomes a major product of the first-order decomposition of **3**. If the pH-dependent hydrolysis of **3** behaves as it does at lower pH, the predominant product of the pH-independent reaction must be **4**. At pH 7.8, the pH-independent reaction accounts for 89 ± 5% of the decomposition of **3**, while **4** accounts for 53 ± 4% of the reaction products. If the pH-dependent reaction does not produce **4**, 60 ± 6% of the product of the pH-independent process is **4**. Significant amounts of the only other reaction product observed at this pH, **6**, must also be produced by the pH-independent route.[35] This product is likely produced by uncatalyzed attack of H_2O on **3** to form **8**, which undergoes rapid base-catalyzed decomposition. Corbett has shown that the hydrolysis of *p*-benzoquinone monoimine also involves a pH-independent process which becomes important at pH > 6.0.[10b] However, the only product detected from this reaction was **6**.

We examined the redox equilibrium between **3** and **4** by a method developed by Corbett to study the *p*-benzoquinone imine/*p*-aminophenol system.[10a] Figure 4 shows that E_n, the standard reduction potential of **3** at a given pH, is, in fact, pH-dependent. The data in Figure 4 were fit very nicely by eq 8, which was derived by assuming that the electrochemical reduction of **3** is described by eq 5.[20] The standard reduction potential, $E°$, for **3** of 0.978 ± 0.001 V shows that **3** is a considerably stronger oxidizing agent than either *p*-benzoquinone, **6** ($E°$ = 0.700 V),[36] or *p*-benzoquinone monoimine ($E°$ = 0.728 V).[22]

We have not yet been able to identify the agent which reduces **3** to **4**. The phosphate buffer, KCl, and CH_3CN have been eliminated as possibilities by appropriate control experiments. The magnitude of $E°$ for **3** is not large enough for reduction by H_2O to be thermodynamically feasible. Since the product study results were quite reproducible, it is unlikely that an impurity is responsible for the reaction. We cannot rule out the possibility that some unidentified product of the decomposition of **3** is responsible for the reduction.

We are continuing our studies of **3** with emphasis on its redox chemistry and the nature of the process observed at high concentrations of **3** at pH > 6.0.

Acknowledgment. The HPLC used in this study was purchased with funds obtained from the Cotrell Research Grant Program of the Research Corp. This work was supported by a grant from the American Cancer Society (BC-348).

(31) Giffney, C. J.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 2* **1975**, 706–712.

(32) For example, the pK_a of the N-protonated conjugate acid of *p*-aminophenol is 5.65, while that of the conjugate acid of *p*-benzoquinone monoimine is 3.70 (ref 10).

(33) Brown, K. C.; Corbett, J. F. *J. Chem. Soc., Perkin Trans. 2* **1979**, 308–311.

(34) Corbett, J. F. *J. Chem. Soc. B* **1969**, 823–826. Corbett, J. F. *J. Chem. Soc. B* **1970**, 1502–1509. Corbett, J. F. *J. Chem. Soc., Perkin Trans. 2* **1972**, 999–1005. Brown, K. C.; Corbett, J. F.; Labinson, R. *J. Chem. Soc., Perkin Trans. 2* **1978**, 1292–1296.

(35) Since **6** decomposes rather rapidly at this pH, the yields reported in Table IV are lower limits.

(36) Bates, R. G. "Electrometric pH Determinations"; Wiley: New York, 1954; p 175.