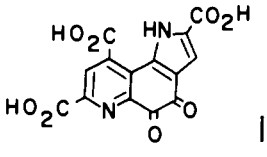
Synthesis of 7,9-Didecarboxymethoxatin (4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid) and Comparison of Its Chemical Properties with Those of Methoxatin and Analogous *o*-Quinones. Model Studies Directed Toward the Action of PQQ Requiring Bacterial Oxidoreductases and Mammalian Plasma Amine Oxidase

Paul R. Sleath, J. Barry Noar, Gert A. Eberlein, and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. *Received November 5, 1984*

Abstract: The synthesis of 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic acid (7,9-didecarboxymethoxatin, 7_{ox}) and its ester (6_{ox}) are described and their acid–base properties, electrochemistry, and chemical properties are compared to the cofactor Methoxatin, as well as to those of other *o*-quinones. The two-electron redox potentials of 6_{ox} and 7_{ox} are shown to be ca. 110 mV less than those of the penanthroline-5,6-quinones but to be comparable to those of methoxatin at all pH values. Replacement of the pyridine ring of 7_{ox} by a benzene ring reduces its oxidation potential by 100 mV. 7_{ox} forms a C-5 adduct with acetone and a cyclic bis(carbinolamine) adduct with urea. The rate constants for formation and dissociation of the urea adducts of methoxatin, 6_{ox}, and the most electrophilic phenanthrolinequinone (3_{ox}) are compared. The *o*-quinone moiety of 7_{ox} undergoes hydration with $pK_{app} = 10.15$, and under vigorous conditions, base-catalyzed ring contraction of the *o*-quinone ring occurs to yield the pyrrolopyrindinone hydrate (**15**). 7_{ox} apparently reacts with ammonia, in an analogous manner to methoxatin, and is capable of the oxidation of primary amines (ethylamine, glycine, benzylamine, and glycinamide) while a secondary amine (morpholine) and a tertiary amine (*N*,*N*-dimethylbenzylamine) are not oxidized. Oxidation of primary amines by 7_{ox} (anaerobic) converts the latter to the corresponding quinol (7_{red}) with some formation of the aminophenol (**17**). These products arise via two competing covalent addition base-catalyzed elimination mechanisms through intermediate carbinolamine and imine, respectively. The oxidation of benzylamine by 7_{ox} is catalyzed by general acid, and the absence of an isotope effect (k_H/k_D) in the oxidation of α,α-dideuteriobenzylamine establishes that intermediate carbinolamine and imine formation are rate-determining. Under "aerobic autorecycling" conditions, the oxidation of primary amines by 6_{ox} and 7_{ox} leads to conversion of the quinones into redox inactive oxazole derivatives. Hydrazine readily reduces 7_{ox} to the quinol 7_{red} at low pH, but under basic conditions, the hydrazone of 7_{ox} is obtained. The latter is not an intermediate in the reduction. The chemistry and electrochemistry of methoxatin and 7,9-didecarboxymethoxatin are quantitatively very much alike, showing that 7_{ox} serves as a good model for chemical investigations.

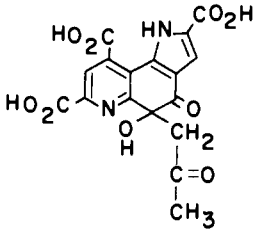
In addition to the nicotinamide- and flavin-dependent alcohol dehydrogenases, a third class of dehydrogenases exists containing the novel *o*-quinone cofactor 1_{ox}.[1-3] By analogy with the names



1_{ox}

"flavoproteins" and "hemoproteins", this class of enzymes has been designated "quinoproteins"[4a] and the trivial name "methoxatin" has been applied to the pyrroloquinolinequinone cofactor (PQQ).[5] Methoxatin can be isolated from the methanol dehydrogenases of a variety of methylotrophic bacteria[1,2,6] as well as from bacterial glucose dehydrogenases.[4b] Also, 1_{ox} serves as a covalently bound coenzyme for bovine serum amine oxidase.[7] This finding suggests the possibility that methoxatin may be required as a cofactor for other mammalian enzymes and that it may also be a dietary requirement (vitamin).

Interpretation of ESR and ENDOR spectra obtained from the methanol dehydrogenase of *Hyphomicrobium X* led to the conclusion that the prosthetic group of this enzyme is "a quinone with two nitrogen atoms".[8,9] Subsequently, a chemical structure was proposed for the cofactor based on an X-ray diffraction study of its C-5 acetone adduct **2**.[5] Development of procedures for the



2

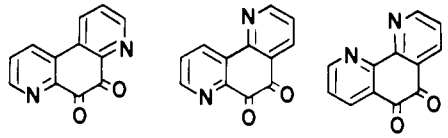
isolation and purification of methoxatin allowed its structure to be confirmed by spectroscopic techniques.[10] Methoxatin has been synthesized via four different routes,[11-14] and the synthetic cofactor (or its reduced derivative) has been reconstituted with the apoenzyme of glucose dehydrogenase from *Acinetobacter calcoaceticus* to yield enzyme with full activity.[15]

(1) Ohta, S.; Fujita, T.; Taobari, J. *J. Biochem.* **1981**, *90*, 205.
(2) Duine, J. A.; Frank, J. *Biochem. J.* **1980**, *187*, 213.
(3) Ameyama, M.; Matsushita, K.; Ohno, Y.; Shinagawa, E.; Adachi, O. *FEBS Letts.* **1981**, *130*, 179.
(4) (a) Duine, J. A.; Frank, J.; Verwiel, P. E. J. *Eur. J. Biochem.* **1980**, *108*, 187. (b) Conlin, M.; Forrest, H. S.; Bruice, T. C., unpublished results.
(5) Salisbury, S. A.; Forrest, H. S.; Cruse, W. B. T.; Kennard, O. *Nature (London)* **1979**, *280*, 843.
(6) Duine, J. A.; Frank, J. *Biochem. J.* **1980**, *187*, 221.
(7) Lobenstein-Verbeek, C. L.; Jongejan, J. A.; Frank, J.; Duine, J. A. *FEBS* **1984**, *170*, 305.
(8) de Beer, R.; van Ormondt, D.; van Ast, M. A.; Banen, R. *J. Chem. Phys.* **1979**, *70*, 4491.
(9) Westerling, J.; Frank, J.; Duine, J. A. *Biochem. Biophys. Res. Commun.* **1979**, *87*, 719.
(10) Duine, J. A.; Frank, J. J.; Verwiel, P. E. J. *Eur. J. Biochem.* **1981**, *118*, 395.
(11) Corey, E. J.; Tramontano, A. *J. Am. Chem. Soc.* **1981**, *103*, 5599.
(12) Gainor, J. A.; Weinreb, S. M. *J. Org. Chem.* **1981**, *46*, 4317.
(13) Hendrickson, J. B.; de Vries, J. G. *J. Org. Chem.* **1982**, *47*, 1150.
(14) MacKenzie, A. R.; Moody, C. J.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1983**, 1372.
(15) Kilty, C. G.; Maruyama, K.; Forrest, H. S. *Arch. Biochem. Biophys.* **1982**, *218*, 623.

0002-7863/85/1507-3328$01.50/0

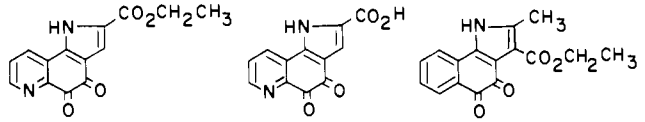
The spectral properties of methoxatin ($\mathbf{1}_{ox}$), its semiquinone ($\mathbf{1}_{sem}$), quinol ($\mathbf{1}_{red}$), and dihydroquinol have been described[4a,10] together with some of their chemistry,[16,17] and a mechanism for alcohol oxidation by PQQ containing enzymes has been proposed, which takes into account the requirement of ammonia as an activator by many PQQ dehydrogenases.[18]

The availability of only small quantities of methoxatin (even from the multistep synthetic routes) and the complex functionality of the molecule have hampered mechanistic studies of oxidations performed by this cofactor. Reconstitution studies have shown, however, that the glucose dehydrogenase from *Acinetobacter calcoaceticus* is active when reconstituted with derivatives of 1,7- and 4,7-phenanthroline-5,6-quinone although these *o*-quinones bind poorly to the enzyme.[4a,b] With this in mind, the reactions and properties of three isomeric phenanthroline-5,6-quinones ($\mathbf{3}_{ox}$, $\mathbf{4}_{ox}$, and $\mathbf{5}_{ox}$) have been studied.[17,19] We describe herein the



$\mathbf{3}_{ox}$ $\mathbf{4}_{ox}$ $\mathbf{5}_{ox}$

synthesis of 7,9-didecarboxymethoxatin ($\mathbf{7}_{ox}$), its corresponding ethyl ester ($\mathbf{6}_{ox}$), and the electrochemical and chemical properties of these compounds as amine oxidants and electrophiles. An objective of the present study has been to assess the similarities of $\mathbf{7}_{ox}$ and methoxatin and to compare them to the phenanthroline-*o*-quinones and $\mathbf{8}_{ox}$. The quinoquinone $\mathbf{7}_{ox}$ is shown to possess



$\mathbf{6}_{ox}$ $\mathbf{7}_{ox}$ $\mathbf{8}_{ox}$

electrochemical and dynamic properties which are virtually identical with those of methoxatin. These observations are deemed to be highly useful since $\mathbf{7}_{ox}$ can be easily prepared in gram quantities whereas methoxatin must still be considered as a rare and not too stable chemical.

Experimental Section

Melting points were obtained in open capillary tubes on a Mel-temp apparatus and are uncorrected. Ultraviolet and visible absorption spectra were recorded on Cary Models 118C and 15 or Perkin-Elmer Lambda 3 spectrophotometers. ^1H and ^{13}C NMR spectra were recorded on a Varian CFT-20 (90 MHz) or Nicolet NT-300 (300 MHz) spectrometer. Chemical shifts are reported in δ units downfield of tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded as Nujol mulls on a Perkin-Elmer 137 spectrophotometer. Thin-layer cyclic voltammetry was performed by using a modified PAR Model 174 polarographic analyzer. Measurements of pH were performed by using either a doubly standardized Radiometer 26 or Beckman 4500 digital pH meter. Mass spectra were obtained on a V.G. Micromass ZAB-2F mass spectrometer operating in either the electron impact mode at 70 eV, the negative ion mode at 100 eV, or the positive chemical ion mode at 200–450 eV. Elemental analyses were determined by Galbraith Laboratories Inc., Knoxville, TN. Thin-layer chromatography was performed by using 0.2-mm silica gel 60 F_{254} plates (Merck) or reverse-phase KC-18 plates (Whatman).

Synthetic Procedures. 5-Amino-8-hydroxyquinoline dihydrochloride (9). A suspension of 5-nitro-8-hydroxyquinoline (Aldrich) (9.5 g, 0.05 mol), 10% Pd on charcoal (0.5 g) in H_2O (100 mL), and concentrated HCl (10 mL) was hydrogenated at 3 atm for 1.5 h. The catalyst was filtered off and the filtrate evaporated down to yield **9** (11.26 g, 97%) as an orange–yellow solid which was washed with ethanol and ether before being air-dried: mp 241–243 °C.

5-[1-Azo-2-oxo-3-methyl-3-(methoxycarbonyl)propyl]-8-hydroxyquinoline (10). Sodium nitrite (3.31 g, 0.048 mol) was added to a stirred solution of **9** (11.19 g, 0.048 mol) in ice/water (190 mL). The resulting dark red diazonium salt was stirred for a further 15 min at 0 °C before being added to a stirred ice-cold solution of methyl α-methylacetoacetate (7.54 g, 0.058 mol) and KOH (3.25 g, 0.058 mol) in MeOH (190 mL). The mixture was stirred for 1.5 h at 0 °C and then stored at 4 °C for 15 h. The solvent was removed under reduced pressure before water (240 mL) and ether (750 mL) were added to the resulting solid. The contents were stirred rapidly for 10 min and filtered. The ether layer was separated, washed with H_2O (3 times), dried ($MgSO_4$), and concentrated in vacuo to leave a dark red oil which crystallized on scratching. Recrystallization from ether/hexane and treatment with charcoal gave **10** (2.3 g, 16%) as a yellow solid: mp 111–113 °C; TLC (silica) R_f = 0.6 ($CHCl_3$/MeOH, 4:1); IR 1715, 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.74 (3 H, s, CH_3), 2.40 (3 H, s, $COCH_3$), 3.80 (3 H, s, CO_2CH_3), 7.12 (1 H, d, H_4), 7.47 (1 H, dd, H_3), 7.69 (br, OH), 7.77 (1 H, d, H_2), 8.67–9.00 (2 H, m, $H_{6,7}$).

Methyl Ester of 5-Hydroxy-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid (11). A suspension of **10** (1.81 g, 6.0 mmol) in a saturated solution of HCl in MeOH (50 mL) was stirred for 15 h. The solvent was removed, and the residue was dissolved in H_2O (300 mL) before being neutralized with saturated $NaHCO_3$ solution. The precipitated product was filtered off, washed with water, stirred in acetone, and refiltered before being dried to give **11** (0.856 g, 59%) as a light grey solid: mp 288–296 °C; IR 1680, 3300 cm^{-1}; MS, *m/e* 242 (M^+); ^1H NMR (Me_2SO-d_6) δ 3.90 (3 H, s, CO_2CH_3), 7.20 (1 H, s, H_3), 7.24 (1 H, s, H_4), 7.66 (1 H, dd, H_8), 8.86 (1 H, dd, H_9), 8.97 (br, OH), 9.11 (1 H, dd, H_7), 11.16 (br, NH).

Methyl Ester of 4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-Carboxylic Acid. A suspension of **11** (0.83 g, 3.43 mmoles) in acetonitrile (40 mL) at 0 °C was added to an ice-cold solution of ammonium ceric nitrate (10.37 g, 18.9 mmol) in water (10 mL). The mixture was sonicated for 15 min before the solvent was removed under reduced pressure. The residue was dissolved in H_2O (500 mL), and on addition of a little $CHCl_3$ and scratching, the orange quinone precipitated. The product was filtered off, washed with H_2O and acetone, and dried to yield the methyl ester of 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic acid (0.55 g, 63%): mp >300 °C (dec >250 °C); TLC (silica) R_f = 0.6 ($CHCl_3$/MeOH, 5:1), IR 1645, 1710 cm^{-1}; MS, *m/e* 256 (M^+); exact mass calcd for $C_{13}H_8N_2O_4$ 256.0484, found 256.0482; ^1H NMR (Me_2SO-d_6) δ 3.86 (3 H, s, CH_3), 7.17 (1 H, s, H_3), 7.66 (1 H, dd, H_8), 8.62–8.73 (2 H, m, $H_{7,9}$), 13.27 (br, NH). Anal. Calcd for $C_{13}H_8N_2O_4$: C, 60.94; H, 3.13; N, 10.94. Found: C, 60.82; H, 3.30; N, 10.81.

Ethyl Ester of 4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid ($\mathbf{6}_{ox}$). When ethyl α-methylacetoacetate (Aldrich) was used, a 20% yield of ethyl ester (based on 5-nitro-8-hydroxyquinoline) was obtained as compared to a 6% overall yield of the methyl ester provided above. The ethyl ester is designated as $\mathbf{6}_{ox}$ and was used in all studies in this manuscript. The ethyl ester was characterized in the same manner as the methyl ester (^1H NMR, MS, elemental analysis, UV–vis spectra).

4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid ($\mathbf{7}_{ox}$). A mixture of $\mathbf{6}_{ox}$ (270 mg, 1 mmol) and 0.5 M K_2CO_3 solution (25 mL) was stirred at 85 °C for 24 h. The solution was allowed to cool before being acidified to pH 4 with concentrated HCl. The resulting precipitate was filtered off, washed with water and ethanol, and dried to yield $\mathbf{7}_{ox}$ (182 mg, 75%) as an ocher solid: mp >300 °C; TLC (reverse phase) R_f = 0.85 (H_2O/EtOH 7:3); IR 1655, 3250 cm^{-1}; MS, *m/e* 242 (M^+); exact mass calcd for $C_{12}H_6N_2O_4$ 242.0323, found 242.0300; ^1H NMR (Me_2SO-d_6) δ 7.11 (1 H, s, H_3), 7.69 (1 H, dd, H_8), 8.62–8.73 (2 H, m, $H_{7,9}$); ^{13}C NMR (Me_2SO-d_6) δ 113.61 (C-3), 121.81, 126.94, 127.83, 129.20, 130.91, 137.59, 145.92, 149.22 (nine aromatic carbons), 161.44 (CO_2H); 173.38, 179.74 (two carbonyl carbons). Anal. Calcd for $C_{12}H_6N_2O_4 \cdot {}^1/_4\ H_2O$: C, 58.42; H, 2.64; N, 11.36. Found: C, 58.69; H, 2.80; N, 11.25.

2-Carboxy-1*H*-pyrrolo[2,3-*f*]-4*H*-1-pyrindin-4-one Hydrate (15). The filtrate from the hydrolysis of $\mathbf{6}_{ox}$ (see immediately preceeding paragraph) was allowed to stand for several days to yield **15** (31 mg) as golden needles: mp 287–288 °C dec; IR 1670, 3400, 3800 cm^{-1}; UV (water) λ_{max} 266 nm; ^1H NMR (Me_2SO-d_6) δ 7.06 (1 H, d, H_3), 7.60 (1 H, dd, H_7), 7.85 (1 H, d, H_8), 8.65 (1 H, d, H_6), 12.43 (4 H, br, exchangeable); MS, (positive chem ion) *m/e* 233 (M + 1); exact mass calcd for $C_{11}H_8N_2O_4$ 232.0480, found 232.0502.

Ethyl Ester of 4,5-Dihydro-4,5-dioxo-2-methyl-1*H*-pyrrolo[2,3-*f*]-naphthalene-3-carboxylic Acid ($\mathbf{8}_{ox}$). This compound was prepared ac-

(16) Dekker, R. H.; Duine, J. A.; Frank, J.; Verwiel, P. E. J.; Westerling, J. *Eur. J. Biochem.* **1982**, *125*, 69.
(17) Eckert, T. S.; Bruice, T. C.; Gainor, J. A.; Weinreb, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2533.
(18) Forrest, H. S.; Salisbury, S. A.; Kilty, C. G. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 248.
(19) Eckert, T. S.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 4431.

cording to the method of Teuber and Thaler[20] and was recrystallized from pyridine/H_2O before use: mp 305–307 °C dec (lit. 305 °C, dec); MS, m/e 283 (M^+); ^1H NMR (Me_2SO-d_6) δ 1.29 (3 H, t, CH_2CH_3), 2.42 (3 H, s, CH_3), 4.22 (2 H, q, CH_2CH_3), 7.38–7.91 (4 H, m, aromatic).

Electrochemical Reduction of 7_{ox} To Yield 4,5-Dihydroxy-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid (7_{red}). A 2.5×10^{-2} M solution of 7_{ox} in 1 M phosphate buffer (pH 7) was reduced under a nitrogen atmosphere at a potential of 525 mV (vs. NHE) for 64 h by using platinum working and auxiliary electrodes and a silver/silver chloride reference electrode. The electrochemical cell was then transferred into a glovebox and the pH of the solution was adjusted to ca. pH 2 with 1 M HCl. The resulting precipitate was filtered off, washed with water, and sucked dry: UV (0.1 M phosphate buffer, pH 7) λ_{max} 238 nm (ϵ 16 100 M^{-1} cm^{-1}), 286 (ϵ 34 100), 313 (ϵ 19 900), ^{13}C NMR (Me_2SO-d_6) δ 106.12 (C-3), 112.34, 117.66, 118.42, 126.53, 127.10, 129.79, 130.26, 136.65, 139.93, 146.35 (11 aromatic carbons), 162.31 (CO_2H).

Reduction of 4,5-Dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic Acid (7_{ox}) with Sodium Dithionite. A suspension of 7_{ox} (121 mg, 0.5 mmol) in water (5 mL) was treated with a solution of sodium dithionite (410 mg, 2 mmol) in water (4 mL) in a nitrogen-filled glovebox. The mixture was stirred at room temperature for 2.5 h after which time the dark brown solid was filtered off, washed with water, and dried under vacuum to yield 7_{red} (70 mg, 57%): mp >300 °C; UV (1 M phosphate buffer, pH 7) λ_{max} 238 nm (ϵ 16 100 M^{-1} cm^{-1}), 286 (ϵ 34 100), 313 (ϵ 19 900). Anal. Calcd for $C_{12}H_8N_2O_4 \cdot {}^7/_8H_2O$: C, 55.44; H, 3.76; N, 10.48. Found: C, 55.55; H, 3.75; N, 10.64.

Reduction of the ethyl ester of 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic acid (6_{ox}) with sodium dithionite was carried out in the same manner as the dithionite reduction of 7_{ox} (94%): mp >300 °C; IR no *o*-quinone carbonyl absorption; UV (H_2O) λ_{max} 285 nm; ^1H NMR (Me_2SO-d_6) δ 1.33 (3 H, t, CH_2CH_3), 4.33 (2 H, q, CH_2CH_3), 7.32 (1 H, s, H_3), 7.42 (1 H, m, H_8), 8.54 (1 H, br, OH), 8.77 (1 H, d, H_{7or9}), 9.03 (1 H, d, H_{7or9}), 9.66 (1 H, br, OH), 12.90 (1 H, s, NH).

Preparation of Quinone Adducts. Acetone Adduct Formation. Acetone (40 mL), neutral alumina (100 mg), and 6_{ox} (100 mg, 0.37 mmol) were stirred at room temperature until a color change from orange to white was observed (ca. 2 h). The solution was filtered and the solid washed with acetone (2 × 25 mL). The combined filtrate was reduced down to give a cream-colored solid which was recrystallized from acetone to yield white crystals (85 mg, 70%): mp 232–234 °C dec; UV (CH_3CN) λ_{max} 241 nm (ϵ 21 870 M^{-1} cm^{-1}), 279 (ϵ 13 740), 307 (ϵ 19 320); MS, m/e 328 (M^+, 2%), 327 ($M^+ - 1$, 11%), 271 (100%); ^1H NMR (Me_2SO-d_6) δ 1.33 (3 H, t, ethyl-CH_3), 1.98 (3 H, s, acetonyl-CH_3), 3.73 (2 H, dd, acetonyl-CH_2, restricted rotation), 4.38 (2 H, q, ethyl-CH_2), 6.05 (1 H, s, OH), 7.12 (1 H, s, H_3), 7.43 (1 H, dd, H_8), 8.62, 8.49 (2 H, dd, $H_{7,9}$), 13.10 (1 H, br, NH). Anal. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53. Found: C, 62.22; H, 4.99; N, 8.42.

Urea Adduct Formation. 6_{ox} (135 mg, 0.5 mmol) and urea (6.0 g, 0.1 mol) were refluxed in ethanol (20 mL) on a steam bath for 5 min before the mixture was cooled, filtered from excess urea, and evaporated under reduced pressure. The residue was dissolved in water (20 mL), and unreacted starting material was filtered off after 15 h. The filtrate was allowed to stand for 3 days during which time a white precipitate formed which was filtered off, washed with EtOH, and dried to yield the urea adduct of 6_{ox} (20 mg, 12%): mp > 156 °C dec; IR 1680 cm^{-1}; TLC (silica) R_f = 0.55 (EtOH); UV (H_2O) λ_{max} 312 nm (ϵ 26 000 M^{-1} cm^{-1}); MS, m/e 270 (M^+ – urea); ^1H NMR (Me_2SO-d_6) δ 1.31 (3 H, t, CH_3), 4.28 (2 H, q, CH_2), 6.03 (1 H, s, exchangeable), 6.15 (1 H, s, exchangeable), 7.03 (1 H, s, exchangeable), 7.09 (1 H, s, exchangeable), 7.44 (1 H, dd, H_8), 7.54 (1 H, s, H_3), 8.41 (2 H, m, $H_{7,9}$). Anal. Calcd for $C_{15}H_{14}N_4O_5 \cdot {}^1/_4H_2O$: C, 53.81; H, 4.33; N, 16.74. Found: C, 53.84; H, 4.53; N, 16.68.

3_{ox} (100 mg, 0.48 mmol), ethanol (100%, 40 mL), and urea (2 g) were refluxed for 2 h at 95 °C during which time a color change from orange to white was observed. After cooling, the white solid was filtered off, washed with water (3 × 20 mL), and air-dried: UV (H_2O) λ_{max} 262, 290 nm; ^1H NMR (Me_2SO-d_6) δ 6.36 (2 H, s, exchangeable, OH or NH), 7.32 (2 H, s, exchangeable, OH or NH), 7.56 (2 H, dd, $H_{2,9}$), 8.50 (2 H, d, $H_{3,8}$), 8.64 (2 H, d, $H_{1,10}$). Anal. Calcd for $C_{13}H_{10}N_4O_3$: C, 57.77; H, 3.72; N, 20.73. Found: C, 57.80; H, 3.95; N, 20.71.

Hydrazone Formation. 6_{ox} (135 mg, 0.5 mmol) was suspended in water (20 mL) before hydrazine hydrate (118 mg, 2.0 mmol) was added and the mixture heated on a steam bath for 90 min. The contents were cooled, and the yellow/green suspension was filtered off, washed with EtOH, and recrystallized from DMF/H_2O to yield 39 mg (27%) of the hydrazone as a pink solid: mp 233–238 °C dec; TLC (silica) R_f = 0.6 (EtOH); IR 1720, 2130, 3200 cm^{-1}; UV (EtOH) λ_{max} 254, 285, 329 nm; MS, m/e 254 ($M^+ - N_2H_2$). Anal. Calcd for $C_{14}H_{12}N_4O_3$: C, 59.15; H, 4.23; N, 19.72. Found: C, 59.33; H, 4.11; N, 19.80.

Products from the Reaction of Amines with Quinones. Anaerobic Reactions with Glycinamide, Benzylamine, and Ethylamine with 7_{ox}. All procedures were carried out in a nitrogen-filled glovebox. As a general method, 7_{ox} (121 mg, 0.5 mmol) was treated with a 10–150-fold excess of an aqueous solution of the amine hydrochloride in water (10–20 mL), and the pH was adjusted to 8–9 with 1 M KOH. The resulting solutions were allowed to stir at room temperature and were monitored by running the UV spectra of diluted aliquots in 0.1 M phosphate buffer at pH 7. When no further spectral changes were observed (6–72 h), the pH of the solutions was adjusted to 4 with concentrated HCL. The resulting precipitates were filtered off, washed with water and ethanol, and dried under vacuum. UV spectra (0.1 M phosphate buffer, pH 7) were similar to 7_{red} (7_{red}, λ_{max} 238, 286, 313 (s) nm; product with glycinamide, λ_{max} 241, 286, 312 (s) nm; product with benzylamine, λ_{max} 287,314 (s) nm; product with ethylamine, λ_{max} 286, 315 (s) nm). The products were analyzed for percentage ammonia released on aerobic reoxidation of any aminophenol present by a modification of the Nessler technique[21] and the results were as follows: reaction with glycinamide (13%), benzylamine (20%), and ethylamine (6%). The isolated products are described as primarily 7_{red} plus the aminophenol (and in the case of glycinamide and ethylamine contamination by these amines). Product from benzylamine. Anal. Calcd (on the basis of 15% **17**, 85% 7_{red}): C, 59.0; H, 3.3; N, 12.3. Found: C, 58.8; H, 3.8; N, 12.0. Product from glycinamide. Anal. Calcd (on the basis of 19% **17**, 6% glycinamide hydrochloride, 75% 7_{red}): C, 53.0; H, 3.9; N, 12.8; Cl, 2.0. Found: C, 53.0 H, 3.7; N, 13.2; Cl, 2.2. Product from ethylamine. Anal. Calcd (on the basis of 9% **17**, 6% ethylamine hydrochloride, 85% 7_{red}): C, 57.4; H. 3.8; N, 12.6. Found: C, 57.4; H, 4.0; N, 12.7.

Aerobic Reactions of Glycinamide, Benzylamine, and Ethylamine with 7_{ox}. 7_{ox} (121 mg, 0.5 mmol) was treated with a 10–400-fold excess of an aqueous solution of the amine hydrochloride in water (50 mL) and the pH adjusted to 7 with 10 M KOH, ensuring dissolution of 7_{ox} by sonication. The solutions were allowed to stir at room temperature and were monitored by running the UV spectra of diluted aliquots. When no further spectral change was observed (1–6 days), the pH of the solutions was adjusted to 4 with concentrated HCl. The resulting precipitates were filtered off, washed with water and ethanol, and dried under vacuum to yield the oxazolo derivatives **20a**, **20b**, and **20c** (65–78%). The λ_{max} values of the products were similar to 7_{ox} but the absorbances were broader. A much higher absorbance around 400 nm was also observed. Mass spectra (positive chemical ionization) showed, in all three products, M + 1 peaks that were low by 44 amu (loss of CO_2). This was due to thermal decarboxylation of the carboxylic acid at the high source temperature required (>400 °C). **20a**: exact mass calcd for $C_{13}H_9N_4O_2$ 253.0723, found 253.0726. **20b**: exact mass calcd for $C_{18}H_{12}N_3O$ 286.0979, found 286.0963. **20c**: exact mass calcd for $C_{13}H_{10}N_3O$ 224.0823, found 224.0829.

Aerobic reaction of 6_{ox} with glycinamide was carried out under the conditions described in the preceding paragraph to yield the ethyl ester of **20a** (105 mg, 65%): UV (water) λ_{max} 269, 370 (s) nm; MS (positive chemical ionization), m/e 325 (M + 1); exact mass calcd for $C_{16}H_{13}N_4O_4$ 325.0933, found 325.0945.

Reaction of glycine and 6_{ox} under aerobic conditions was performed as follows. Glycine hydrochloride (3.72 g, 33.3 mmol) in water (4 mL) was adjusted to pH 9.5 with 10 M KOH. 6_{ox} (135 mg, 0.5 mmol) was added, and the mixture was stirred at room temperature for 24 h. The resulting brown suspension was adjusted to pH 4 with concentrated HCl, filtered off, washed with water, and dried. Recrystallization from ethanol (with charcoaling) and then from DMF yielded **21**: mp >300 °C; IR 1630, 1700 cm^{-1}; UV (water) λ_{max} 241, 269, 377 nm; exact mass calcd for $C_{15}H_{11}N_3O_3$ 281.0797, found 281.0804; ^1H NMR (Me_2SO-d_6) δ 1.36 (3 H, t, CH_2CH_3), 4.36 (2 H, q, CH_2CH_3), 7.30 (1 H, s, H_3), 7.34 (1 H, m, H_9), 8.36 (1 H, d, H_{8or10}), 8.74 (1 H, s, H_4), 8.80 (1 H, s, H_{8or10}). Anal. Calcd for $C_{15}H_{11}N_3O_3 \cdot {}^1/_4H_2O$: C, 63.05; H, 4.20; N, 14.71. Found: C, 62.95; H, 4.60; N, 14.62.

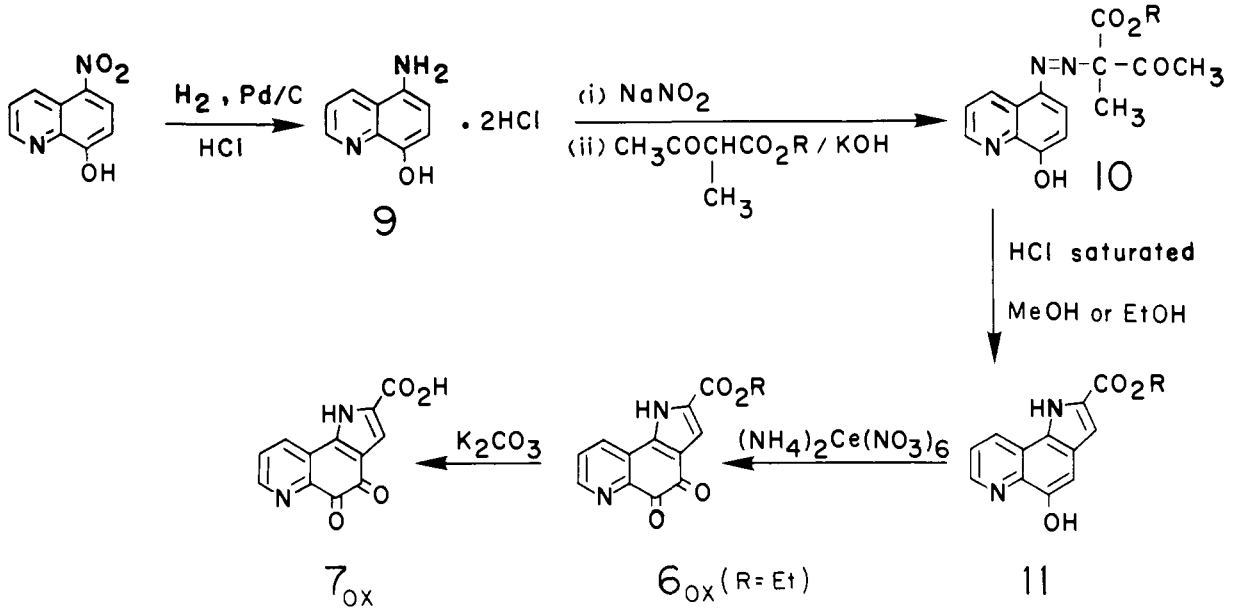
Kinetics and Properties of *o*-Quinones. Acid–Base Titrations. The acid–base dissociation constants for 6_{ox} and 7_{ox} were determined at 30 ± 0.2 °C by spectrophotometric titration of the compounds in a 25-mL titration cell with a path length of 3.387 ± 0.004 cm. Solutions from 7.11×10^{-6} to 6.87×10^{-6} M in dione were titrated with 1 M HCl (Baker Dilut-it). Ionic strength was adjusted to 1.0 by the addition of KCl. The acid–base dissociation constants for 6_{red} were determined in an identical manner except that the titration was performed under N_2 with a quinol concentration of 1.48×10^{-5} M.

Base-Catalyzed Exchange of ^{18}O into 6_{ox}. 6_{ox} (5 mg) was suspended in H_2 ^{18}O (200 μL, 97.17% ^{18}O) before 0.1 M KOH (50 μL) was added.

(20) Teuber, H.-J.; Thaler, G. *Chem. Ber.* **1959**, 92, 666.

(21) Mayer, S. W.; Kelly, F. H.; Morton, M. E. *Anal. Chem.* **1955**, *27*, 837.

Scheme I



The orange solid immediately dissolved. After 10 s, 0.1 M HCl (50 μL) was added and the orange quinone precipitated out of solution. The solid was filtered off, washed with water, and dried before being analyzed by mass spectroscopy.

Electrochemistry. The reduction potentials of **6** and **7** were determined by use of the thin-layer cyclic voltammetry technique of Hubbard.[22,23] The number of electrons transferred in a reversible process was determined as described previously.[24] Operations were carried out by using N_2-purged solutions under an atmosphere of N_2 at 26.3–27.0 °C. A scan rate of 2 mV s^{-1} was employed toward negative potential over a 750-mV scan range at a sensitivity of 50 μA. The reference electrode employed was Ag/AgCl/1 M NaCl (reference potential 225 mV vs. NHE at 25 °C). All potentials are given vs. NHE. Stock solutions of **6** and **7** were prepared in Me_2SO at concentrations of 1.40×10^{-2} and 1.39×10^{-2} M, respectively. Buffer solutions were prepared as indicated below with 0.1 mL of stock solution being added to the buffer prior to a run: H_0 −0.22, 3 mL of 1 M perchloric acid; pH 1.23, 2.7 mL of 1 M $NaClO_4$, 0.3 mL of 0.5 M sodium sulfate buffer; pH 4.51, 2.925 mL of 1 M $NaClO_4$, 0.075 mL of 2 M sodium acetate buffer; pH 5.91, 2.6 mL of 1 M $NaClO_4$, 0.3 mL of 0.5 M sodium phosphate buffer; pH 9.48, 2.7 mL of 1 M $NaClO_4$, 0.3 mL of 0.5 M sodium carbonate buffer; pH 12.53, 2.7 mL of 1 M $NaClO_4$, 0.3 mL of 1 M sodium hydroxide.

The oxidation potential of quinone **8** was determined in a similar manner except that 0.4 mL of a 7×10^{-4} M solution of the quinone in Me_2SO was added to each buffer.

Determination of the Equilibrium Constant for the Formation of Semiquinone from 6_{ox} and 6_{red}. Solutions of 6_{ox} (2.10×10^{-3} M) and 6_{red} (4.49×10^{-3} M) in trifluoroacetic acid were placed in separate compartments of two tandem cuvettes (path lengths 0.438/0.439 and 0.438/0.437 cm). One cuvette was placed in the reference compartment of a Cary 118C spectrophotometer whilst the other was placed in the sample compartment. After mixing the two solutions in the cuvette in the sample compartment, the spectrum was recorded from 700–300 nm.

The ESR spectrum of the semiquinone was recorded on a Bruker ER200D-SRC spectrometer connected to a Nicolet signal averager. The quantitative measurement of the spin number was taken relative to a ruby reference (Cr^{3+} in Al_2O_3). The *g* factor was determined relative to a DPPH standard ($g_0 = 2.0036 \pm 0.0003$), and the sweep width and sweep time were 300 G and 10 s, respectively. The sweep number was 120 times, $J_0 = 9.48$ GHz, $H_0 = 33750$ G. The sample contained oxidized and reduced quinone (4.49×10^{-3} M and 2.10×10^{-3} M, respectively) in trifluoroacetic acid, the mixture being prepared anaerobically.

Oxidation of Amines by 7_{ox}. The hydrochloride salts of ethylamine, morpholine, benzylamine, and *N,N*-dimethylbenzylamine were prepared by dropwise addition of the amine to an excess of anhydrous ethanolic HCl. The EtOH was then removed under reduced pressure, and the HCl salt was recrystallized twice from EtOH/ether before use. Glycinamide hydrochloride was recrystallized from $EtOH/H_2O$ before use.

α,α-Dideuteriobenzylamine was prepared by reduction of benzamide (7.26 g, 0.06 mol) with lithium aluminum deuteride (5.0 g, 0.133 mol) in dry THF/ether (150:300 mL) under a nitrogen atmosphere. After destruction of excess reductant with water, the solution was filtered and reduced down before the residue was dissolved in acetone. Addition of ethanolic HCl yielded white crystals of α,α-dideuteriobenzylamine hydrochloride; yield 6.1 g (70%). Product was >98.5% dideuterated by ^1H NMR and MS.

Solutions were prepared with total amine concentrations of 8.58×10^{-3} M with 50% of the amine hydrochloride being neutralized using 1 M KOH to give the pH = pK_a of the amine ±0.05 for each buffer. The concentration of 7_{ox} used was 4.29×10^{-5} M. The reactions were performed under an atmosphere of argon in Thunberg cuvettes at 30.0 ± 0.2 °C and were followed by repetitive scanning on a spectrophotometer between 400 and 200 nm over a period of 12 h. Subsequently, reaction kinetics were followed at 285 and 420 nm for the oxidation of glycinamide and at 287 nm for the oxidation of benzylamine.

Determination of the Equilibrium Constants for the Reaction of 1_{ox}, 3_{ox} and 6_{ox} with Urea. Six solutions of urea (recrystallized from EtOH) with initial concentrations from 1 to 0.1 M were prepared by using 0.1 M phosphate buffer at pH 7. Aqueous solutions of the diones were added to the urea solutions to give final dione concentrations as follows: 1_{ox}, 2.83×10^{-3} M; 3_{ox}, 4.35×10^{-3} M; 6_{ox}, 1.2×10^{-5} M. Adduct formation was followed spectrophotometrically with time at 340, 260, and 276 nm, respectively, at 30 ± 0.2 °C until equilibrium had been obtained. The reactions were conducted aerobically after it had been shown (for each of the diones) that the reactions were not affected by air. First-order rate constants were then calculated for each concentration of urea, and a least-squares program was used to calculate the equilibrium constants for adduct formation.

Reduction of 7_{ox} by Hydrazine at pH 1.0. A mixture of hydrazine monohydrochloride (recrystallized from $EtOH/H_2O$) (3.33×10^{-3} M) and 7_{ox} (4.29×10^{-5} M) in 0.1 M HCl under N_2 was monitored spectrophotometrically from 400 to 200 nm, repetitively. The pseudo-first-order rate constant for the formation of 7_{red} was calculated from the absorbance change at 290 nm.

Results and Discussion

The Synthesis of 7_{ox} (Scheme I) is much more simplistic than is the synthesis of methoxatin. This is due primarily to the initiation of the synthetic sequence with the readily available 5-nitro-8-hydroxyquinoline rather than by assembling the quinoline ring in the course of synthesis. The synthetic procedures leading from **9** to 7_{ox} have been used by Corey and Tramontano[11] in their synthesis of methoxatin. Minor alterations include the use of methanolic or ethanolic HCl in preference to formic acid in the conversion of **10** to **11** and the direct hydrolysis of 6_{ox} to 7_{ox} without the need for protection of the quinone moiety by conversion to a ketal.

(22) Hubbard, A. T. "CRC Critical Reviews in Analytical Chemistry"; Meites, L., Campbell, B. H., Eds.; The Analytical Rubber Co.: Cleveland, 1973; Vol. 3, Issue 2, pp 210–243.
(23) Hubbard, A. T. *J. Electroanal. Chem.* **1969**, *22*, 165.
(24) Eberlein, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 6679.

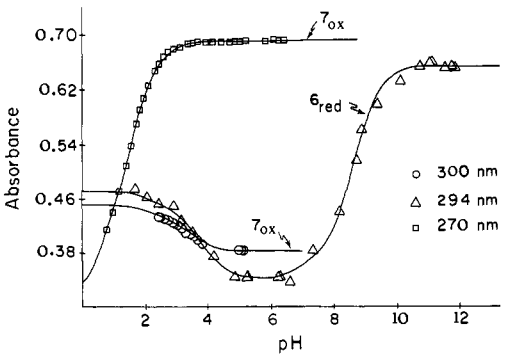


Figure 1. Spectrophotometric pK_a determinations (30 °C, N_2, μ = 1.0 with KCl, path length 3.387 cm) for 7_{ox} at 270 nm (pyridine N), 7_{ox} at 300 nm (CO_2H), and 6_{red} at 294 nm (pyridine N and OH).

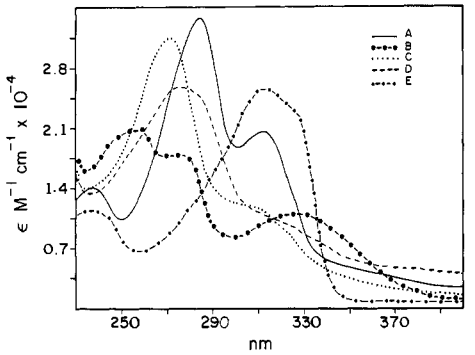
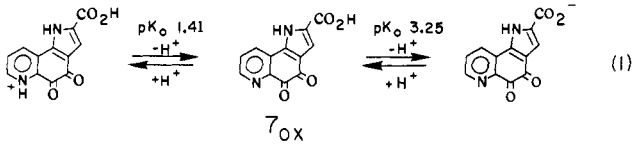


Figure 2. UV spectra of (A) 7_{red} at pH 7 (N_2, 0.1 M phosphate buffer), (B) 7_{ox} at H_0 = −0.22 (1 M perchloric acid) (C) 7_{ox} at pH 5.91 (0.1 M phosphate buffer), (D) 7_{ox} at pH 12.53 (1 M NaOH), and (E) urea adduct of 6_{ox} (H_2O).

The pK_a Values of 6_{ox} and 7_{ox} are required in order to carry out both electrochemical and kinetic studies. These pK_a values were determined by monitoring the absorbances at fixed wavelength as a function of pH. For 6_{ox}, change in pH from 5.3 to 0.8 was accompanied by a decrease in A_{275} with isosbestic points at 316 and 258 nm. The decrease in absorbance at 275 nm followed a least-squares titration curve with pK_a = 1.40 ± 0.05 for protonation of the pyridine nitrogen. The dissociation constants of 7_{ox} were determined by monitoring the increase in A_{270} from pH 0 to 6.0 and the decrease in A_{300} from pH 2.0 to 5.0. The increase in absorbance at 270 nm and the decrease at 300 nm were fitted to least-squares titration curves which yielded pK_a values of 1.41 ± 0.01 and 3.25 ± 0.1 (eq 1) (Figure 1). The larger error



(1)

7_{ox}

in the latter pK_a value stems from the small change in the electronic absorbance spectrum of 7_{ox} caused by dissociation of the carboxyl group. The similarity of the first ionization constants for 6_{ox} and 7_{ox} establishes that the two pK_a values of 7_{ox} are microscopic.

The quinol 7_{red} was prepared by the electrochemical reduction of 7_{ox} (2.5×10^{-2} M in 1 M phosphate buffer at pH 7) at its $E^{\circ\prime}$ in water (525 mV vs. NHE, vide infra) using a platinum working electrode. Once the theoretical count of 48.25 C had been reached, the solution was brought to ca. pH 2 and the precipitated quinol was collected by filtration, washed with copious volumes of cold water, and dried. Electrochemical reduction and work up were carried out under an N_2 atmosphere. The UV/vis spectrum of 7_{red} at pH 7 is shown in Figure 2. 7_{red} is rapidly reoxidized to 7_{ox} by air, when in solution at pH 7.

The quinol 6_{red} was Prepared by reduction of 6_{ox} with sodium dithionite. The pK_a values for 6_{red} were determined under nitrogen to prevent rapid reoxidation of the quinol anion by air. Monitoring

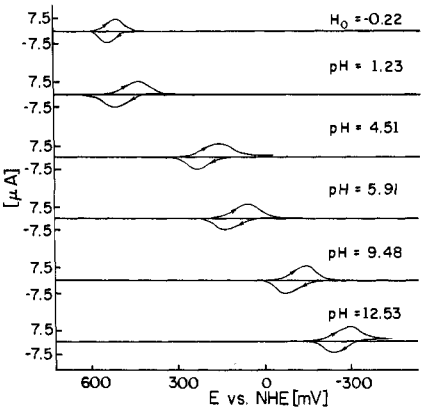
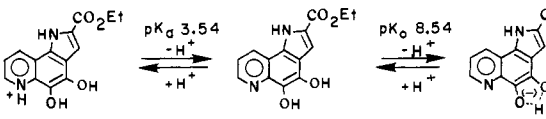


Figure 3. pH dependence of thin-layer cyclic voltammograms for **7** (~4.5×10^{-4} M) at a scan speed of 2 mV s^{-1} (solvent 97% H_2O, 3% Me_2SO). Direction of scan is from positive to negative.

Table I. Mean Values of Anodic and Cathodic Peak Separation (ΔE), Calculated Number of Electrons Transferred in Those Peak Potentials, and the Standard Redox Potentials for **6**, **7**, and **8** as Determined by TLCV

	ΔE, mV	no. of elect transferred	E°, mV	$E^{\circ\prime}$, mV
6	91.5 ± 61.5	2.2 ± 0.5	550	70
7	68.5 ± 35.5	2.1 ± 0.5	550	70
8	50.0 ± 32.0	2.4 ± 0.5	440	10

the increase in A_{294} from pH 6.0–11.5 and the decrease in A_{294} from pH 1.6–5.0 yielded pK_a values of 3.54 ± 0.09 and 8.54 ± 0.04 (eq 2) (Figure 1). The acid–base dissociation constant for



(2)

6_{red}

the pyridine ring of 6_{red} clearly reflects the greater electron density on the pyridine nitrogen in the reduced species (pK_a = 3.54) when compared to the oxidized species (pK_a = 1.40). The pK_a for the hydroxyl function of 6_{red} is ca. one unit less than that of catechol (pK_a = 9.48)[25] and indicates that the combined electronic effect of the annelated pyridine and indole ring is approximately equivalent to a 4-bromo substituent on catechol (pK_a 4-bromocatechol = 8.70).[26]

Electrochemical redox potentials of 7 have been determined, as a function of pH, by employing thin-layer cyclic voltammetry (TLCV) with a platinum billet electrode (vs. NHE, 26 ± 1 °C). The solvent employed was 97% H_2O–3% Me_2SO (v/v). In separate experiments, it was shown that the Me_2SO content does not influence the value of determined potentials when compared to H_2O. Figure 3 shows the pH dependence of the thin-layer cyclic voltammograms for **7** and reveals a single transfer wave for oxidation and reduction between pH −0.22 and 12.5. Table I shows the mean values for the separation of cathodic and anodic peak potentials for **6**, **7**, and **8** together with the number of electrons transferred for each peak potential. The values given are averaged from the six scans at different pHs for each *o*-quinone. It is apparent that each potential is associated with the transfer of $2e^-$ and that the reactions are essentially thermodynamically and electrochemically reversible. Repeated scans on the same sample yielded identical potentials and electron counts.

Figure 4A is a plot showing the pH dependence of the $2e^-$ transfer potentials for **7**. The equations employed to generate the

(25) Jencks, W. P.; Regenstein, J. "Handbook of Biochemistry"; Sober, H. A., Ed.; CRC Press: Cleveland, 1973; p J-187.
(26) Serjeant, E. P.; Dempsey, B. "Ionisation Constants of Organic Acids in Aqueous Solution"; Pergamon: Oxford, England, 1979.

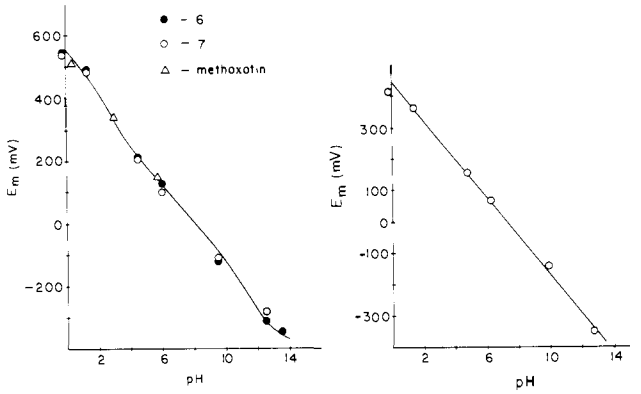
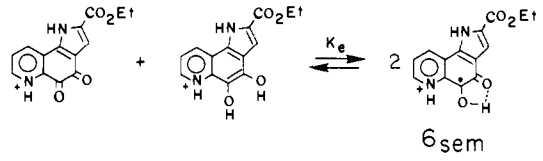


Figure 4. Nernst–Clark plots showing the pH dependence of $2e^-$ transfer potentials for (A, left) 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]-quinoline-2-carboxylic acid (**7**), its ethyl ester (**6**), and methoxatin, and (B, right) ethyl ester of 4,5-dihydro-4,5-dioxo-2-methyl-1*H*-pyrrolo[2,3-*f*]naphthalene-3-carboxylic acid (**8**).

line of Figure 4A are of general form provided by Clark[27] but appended to be useful for acid dissociation constants in the H_0 range.[24] For this purpose, the pK_a values of eq 1 were employed for $\mathbf{7}_{ox}$, while the pK_a values of eq 2 were used for $\mathbf{7}_{red}$. The plot does not deviate greatly from a slope of 60 mV/pH because of the near cancellation of the influence of the pK_a values of $\mathbf{7}_{ox}$ and $\mathbf{7}_{red}$. Included in Figure 4A are potentials determined for **6** (at the same pHs as those determined for **7**) and for methoxatin at pH 0.30, 2.98, and 5.60.[17] It can be seen that the points for **6** and methoxatin lie well within the error range of the plot for **7**. Figure 4B shows the pH dependence of the $2e^-$ transfer potentials for **8** which yield a linear plot of slope 60 mV/pH.

Knowledge of the redox potentials of methoxatin and of **6**, **7**, and **8** allows the following conclusions to be drawn: Replacement of the benzene ring of **8** by a pyridine ring to give a pyrroloquinolinequinone increases the redox potential by ca. 100 mV. The potential remains essentially unaltered when carboxyl groups are appended to **7** to yield methoxatin. Thus, methoxatin, 7,9-didecarboxymethoxatin ($\mathbf{7}_{ox}$) and its ethyl ester ($\mathbf{6}_{ox}$) are of equal thermodynamic capacity to carry out $2e^-$ oxidations in aqueous solution. Compounds $\mathbf{6}_{ox}$ and $\mathbf{7}_{ox}$ are interchanged, for convenience, in the study of the reactions which are to follow.

Disproportionation of $\mathbf{6}_{ox}$ and $\mathbf{6}_{red}$ to provide $\mathbf{6}_{sem}$ (eq 3) was examined by employing trifluoroacetic acid as the solvent. The ESR spectrum of the radical, obtained from a solution containing



(3)

$\mathbf{6}_{ox}$ (2.10×10^{-3} M) and $\mathbf{6}_{red}$ (4.49×10^{-3} M), is highly unsymmetrical resembling that of the enzyme bound semiquinone of methoxatin.[9] From the spectrum (Figure 5), it can be deduced that there are at least three different radical species present in solution with resonances at $P_1 = 2.0152$ G, $P_2 = 2.0051$ G, and $P_3 = 1.9899$ G. The total concentration of radical was found to be $(6.5 \pm 0.2) \times 10^{-6}$ M, allowing the equilibrium constant, K_e, for the formation of the radical species to be calculated as 4.48×10^{-6} with the assumption of eq 4. The total radical concentration also allows the calculation of an apparent extinction coefficient for semiquinone species of 4500 M^{-1} cm^{-1} at 444 nm in trifluoroacetic acid. Using the relationship $\Delta G° = -RT \ln K$, the change in standard free energy for formation of the radical

$$K_e = \frac{[\mathbf{6}_{sem}]^2}{[\mathbf{6}_{ox}][\mathbf{6}_{red}]} \quad (4)$$

(27) Clark, W. M. "Oxidation Reduction Potentials of Organic Systems"; Crieger, R. E., Ed.; Huntington: New York, 1972; p 129.

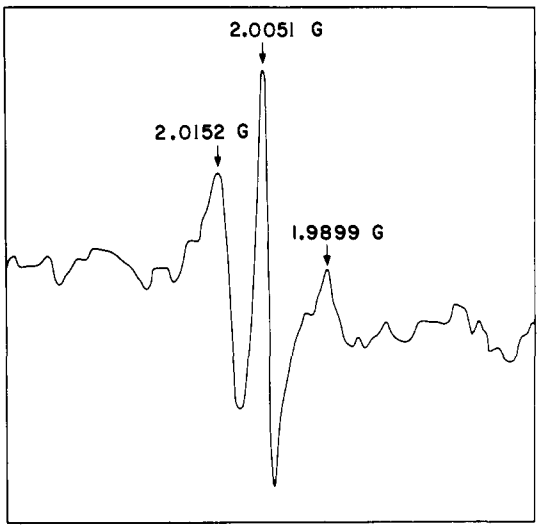
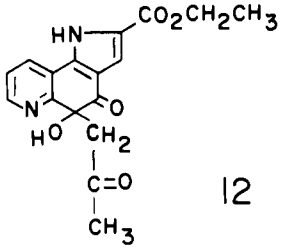


Figure 5. ESR spectrum of $\mathbf{6}_{sem}$ obtained by mixing $\mathbf{6}_{ox}$ (2.13×10^{-3} M) and $\mathbf{6}_{red}$ (4.49×10^{-3} M) in trifluoroacetic acid under anaerobic conditions. *g* values are given with reference to a DPPH standard.

via comproportionation is calculated as 31 kJ mol^{-1}.

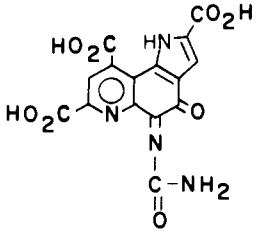
Carbonyl Adduct Formation. $\mathbf{6}_{ox}$ forms a stable white crystalline adduct with acetone, in an analogous reaction to that of methoxatin[5] and other *o*-quinones,[28] in which C-1 of acetone adds to one of the quinone carbonyls to yield the hydroxy diketone **12**.



12

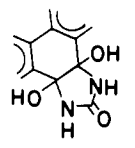
The position of nucleophilic addition has been shown to be at C(5) in the case of methoxatin and is thus assumed to be at C(5) in the conversion of $\mathbf{6}_{ox}$ to **12**. The ^1H NMR spectrum of **12** shows a pair of doublets at δ 3.89 and 3.56 which is assigned to the CH_2 group of the acetonyl chain. This splitting is attributed to hindered rotation of the acetonyl group which can be easily demonstrated by using models. Raising the sample temperature to 80 °C resulted in partial coalescence of this splitting pattern.

Duine et al. report that methoxatin forms an adduct with urea and have assigned the 5-imino structure **13**.[16] In our hands, the 4,7-phenanthroline-5,6-quinone ($\mathbf{3}_{ox}$), as well as the ester of the



13

didecarboxymethoxatin $\mathbf{6}_{ox}$ and methoxatin itself, reacts with urea. When dissolved in Me_2SO, the adducts prove to be cyclic bis(carbinolamines) **14**. This can be shown from their ^1H NMR



14

(28) Magnusson, R. *Acta Chem. Scand.* **1960**, *14*, 1643.

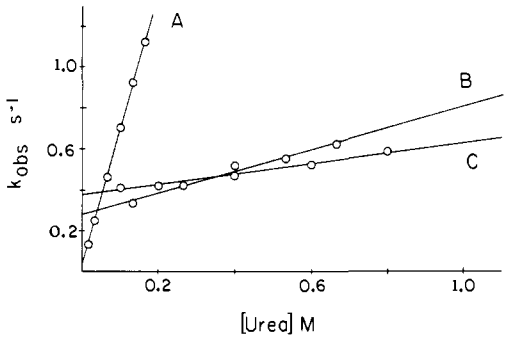


Figure 6. Pseudo-first-order rate constants (k_{obsd}, s^{-1}) vs. urea concentration (M) for the formation of dicarbinolamine adducts (pH 7, 30 °C) with (A) 4,7-phenanthrolinequinone ($\mathbf{3}_{ox}$), (B) 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2-carboxylic acid ethyl ester ($\mathbf{6}_{ox}$), and (C) methoxatin ($\mathbf{1}_{ox}$).

Table II. Values of k_1, k_{-1}, and K for the Formation of Dicarbinolamine Adducts from *o*-Quinones and Urea

o-quinone	k_1, s^{-1}	k_{-1}, s^{-1}	K, M^{-1}
$\mathbf{1}_{ox}$	2.53×10^{-4}	3.77×10^{-4}	0.67 ± 0.06
$\mathbf{3}_{ox}$	6.64×10^{-3}	2.57×10^{-5}	258.92 ± 83.74
$\mathbf{6}_{ox}$	5.28×10^{-4}	2.79×10^{-4}	1.89 ± 0.21

spectra in dry Me_2SO-d_6. The ^1H NMR spectrum of the adduct of $\mathbf{3}_{ox}$ shows two singlets at δ 7.31 and 6.35, each equivalent to two protons, which vanish when D_2O is added. The presence of four exchangeable singlets between δ 6.03 and 6.09, each equivalent to one proton, in the spectrum of the adduct of $\mathbf{6}_{ox}$ and urea clearly shows that the adduct has the bis(carbinolamine) structure. The UV spectrum of the urea adduct of $\mathbf{6}_{ox}$ is shown in Figure 2.

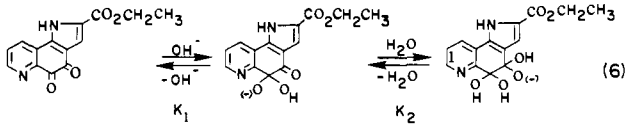
The kinetics of formation of the urea adducts of methoxatin, $\mathbf{3}_{ox}$ and $\mathbf{6}_{ox}$, were studied at pH 7 (30 °C) under the pseudo-first-order conditions of excess urea. Formation of urea adducts was accompanied by an increase in A_{340} for $\mathbf{1}_{ox}$, A_{260} for $\mathbf{3}_{ox}$, and A_{276} for $\mathbf{6}_{ox}$. These increases in absorbance followed the first-order rate law. Plotting the pseudo-first-order rate constants (k_{obsd} (s^{-1})) against the concentration of urea (0.8–0.02 M) yielded linear plots with slopes of k_1 and intercepts of k_{-1} (eq 5) as shown in Figure 6. Values for k_1, k_{-1}, and K ($=k_1/k_{-1}$) are given in Table II.

Quinone $\mathbf{8}_{ox}$ did not react with urea under identical conditions with those reported above, indicating that $\mathbf{8}_{ox}$ is less susceptible to nucleophilic addition than the other quinones investigated. The

$$\text{quinone} + \text{urea} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{bis(carbinolamine) adduct} \qquad (5)$$

order of the equilibrium constants for urea adduct formation parallels the redox potentials of the quinones ($\mathbf{3}_{ox} > \mathbf{6}_{ox}$, $\mathbf{1}_{ox} > \mathbf{8}_{ox}$). The similarity of the constants k_1 and k_{-1} for the reactions of methoxatin and $\mathbf{6}_{ox}$ with urea suggest that adduct formation in any covalent oxidation mechanism should be as favorable with the model $\mathbf{6}_{ox}$ (and, therefore, $\mathbf{7}_{ox}$) as with methoxatin.

Pseudo-base formation is seen by adjusting the pH of an aqueous solution of the didecarboxymethoxatin $\mathbf{7}_{ox}$ from pH 5.2 to 11.0 which causes a shift in λ_{max} from 272 nm (ϵ 31 900 M^{-1} cm^{-1}) to 275 nm (ϵ 26 300 M^{-1} cm^{-1}). Readjustment of the pH to 4.5 brings about the reappearance of the initial spectrum establishing that the spectral change is rapid and reversible. Even after 12 h at pH 11.0 (30 °C), the reaction was fully reversible. The pH dependence of the rapid covalent hydration is described by eq 6. The UV spectrum of $\mathbf{7}_{ox}$ showed a decrease in absorbance



(6)

at 270 nm on increase of pH from 7 to 11, and a plot of A_{270} vs.

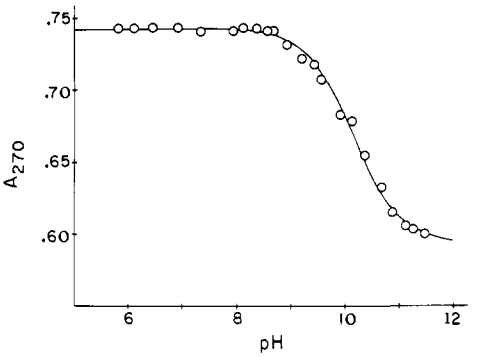


Figure 7. Spectroscopic determination of pK_{app} for pseudo-base formation with $\mathbf{7}_{ox}$ at 270 nm (30 °C, N_2, μ = 1.0 with KCl, path length 3.387 cm).

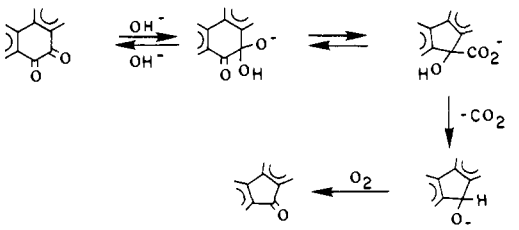
pH yielded a least-squares titration curve with a pK_{app} for pseudo-base formation of 10.15 ± 0.03 (Figure 7). Equation 7

$$\Delta A = \frac{a_H}{K_w K_1(1 + K_2) + a_H} \qquad (7)$$

describes the titration curve for pseudo-base formation and thus $K_1(1 + K_2)$ can be calculated as 4.82×10^3 M^{-1} for $\mathbf{7}_{ox}$ compared to the value of 5.50×10^3 M^{-1} for $\mathbf{3}_{ox}$.[19] The UV spectra of $\mathbf{7}_{ox}$ at $H_0 = -0.22$, pH 5.91, and pH 12.53 are shown in Figure 2.

Pseudo-base formation was confirmed via an ^{18}O exchange study in which a small quantity of $\mathbf{6}_{ox}$ was suspended in H_2 ^{18}O (97.17% ^{18}O). Addition of base to the suspended quinone caused it to go into solution where upon an equivalent amount of acid was added to reprecipitate the quinone. The reprecipitated quinone was analyzed by mass spectrometry and showed a large peak at m/e 274 indicative of two ^{18}O atoms in the molecule. Thus, both quinone carbonyl groups are able to undergo rapid exchange of ^{18}O in the presence of base as shown in eq 6.

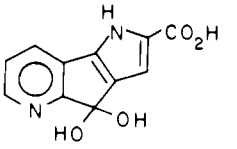
Contraction of the quinone ring of phenanthrolinequinones has been observed at high pH to yield diazofluoren-9-one derivatives (eq 8).[19] Ring contraction with $\mathbf{7}_{ox}$ in base is a much less facile



(8)

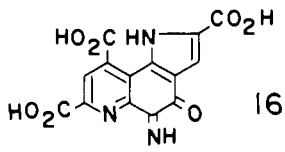
reaction than in the case of the phenanthrolinequinones. However, when a solution of $\mathbf{6}_{ox}$ in water at pH 11.0 is heated on a steam bath for 20 h, the pyrrolopyrindinone hydrate (**15**) is formed.

The Reaction of $\mathbf{7}_{ox}$ with ammonia was investigated at pH 8.25, 9.0, and 9.5. At pH 8.25, the spectrum of $\mathbf{7}_{ox}$ was identical in ammonia/ammonium (1 M) and phosphate (0.1 M) buffers. At



15

pH 9.0 and 9.5, however, an increase in A_{307} and a decrease in A_{270} were noted in the spectrum of $\mathbf{7}_{ox}$ in ammonia/ammonium buffer (1 M) when compared to that in pyrophosphate buffer (0.1 M) at the same pH. The latter observation is similar to that of Dekker et al. who report that the spectrum of methoxatin in pH 9 ammonia buffer is different from that in pH 9 pyrophosphate buffer.[16] They attribute this difference to the addition of ammonia to the C-5 position of methoxatin to generate imine **16**. It should be noted that the reported spectral differences obtained on addition of NH_3, for both methoxatin and $\mathbf{7}_{ox}$, are rather small when



16

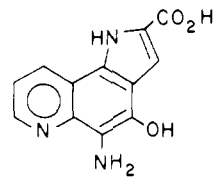
compared to the changes observed for hydration (Figure 2) or for C-5 adduct formation. Thus, it is concluded that the difference in spectra could be due to (a) formation of an imine as suggested by Dekker et al., (b) carbinolamine formation, or (c) a specific salt effect arising from comparison of spectra in different buffers, although both the ammonia and pyrophosphate buffers were of equal ionic strength (μ = 1.0).

The reaction of 7_{ox} with a variety of amines has been studied under both anaerobic and aerobic conditions. An investigation of amine oxidation is particularly timely with the recent finding that methoxatin is the cofactor of plasma amine oxidase.[7] Dekker et al.[16] report that methoxatin does not oxidize amino acids. In view of their claim (results not published) that methoxatin oxidizes primary amines, the lack of any reactivity of methoxatin with amino acids is difficult to rationalize. Indeed, the rate of oxidation of glycine by the phenanthrolinequinones has been shown to be much greater than the rate of oxidation of primary, secondary, and tertiary amines by these quinones.[19] Thus, the ability of 7_{ox} and its ethyl ester (6_{ox}) to oxidize glycine and glycinamide has been investigated.

No reaction was observed by repetitive scanning (from 400 to 200 nm over a period of 24 h) (H_2O, μ = 1.0, 30 °C, argon atmosphere) of reaction solutions containing morpholine or *N*,*N*-dimethylbenzylamine at pH = amine pK_a with total amine buffer concentrations equal to 8.58×10^{-3} M and $[7_{ox}] = 4.29 \times 10^{-5}$ M. When ethylamine, benzylamine, glycine, and glycinamide were employed under identical conditions, a slow reaction was observed for ethylamine (3 days) with a λ_{max} shift from 272 to 285 nm and isosbestic points at 241 and 274 nm. The reactions were more rapid with benzylamine (λ_{max} 272 → 286 nm), glycine (λ_{max} 272 → 284 nm), and glycinamide (λ_{max} 271 → 286 nm). The final spectra closely resembled that of 7_{red}, and the spectrum of 7_{ox} was regenerated in each case upon allowing air into the cuvette. In the case of the benzylamine reaction, the λ_{maxima} and extinction coefficients at 286 and 314 nm were identical with those of 7_{red}. From these experiments, it is concluded that the tertiary amine (*N*,*N*-dimethylbenzylamine) and secondary amine (morpholine) are not oxidized by 7_{ox} under anaerobic conditions while the primary amines (ethylamine, benzylamine, glycine, and glycinamide) are oxidized.

On a preparative scale, 7_{ox} was treated with a large excess of an aqueous solution of the appropriate amine hydrochloride at pH 8–9. When a reaction had come to completion, the pH was adjusted to 4 and resulting precipitate collected, washed with water and ethanol, and dried under vacuum. (Crude yields calculated as 7_{red}: 90%, glycinamide; 41%, ethylamine; and 18%, benzylamine). The spectra of the reaction mixtures (prior to precipitate formation) and the precipitates were superimposable when normalized. Thus, the composition of the precipitates reflect the composition of the reaction solutions. Aminophenol content was determined by reoxidation of the product with O_2 to yield 7_{ox} plus NH_3 and assay of the latter by Nesslerization. When combined with microanaiytical data (Experimental), the precipitated crude reaction products may be concluded to consist primarily of 7_{red} together with some aminophenol **17**. The yields of **17** in the precipitated products were as follows: 19%, glycinamide; 9%, ethylamine; and 15%, benzylamine.

The kinetics for reaction of benzylamine with 7_{ox} were studied (30 °C, μ = 1.0 with KCl) under the pseudo-first-order conditions of total benzylamine concentration (8.75×10^{-2} to 8.75×10^{-3} M) greatly exceeding $[7_{ox}] = 4.29 \times 10^{-5}$ M. Kinetic studies with benzylamine buffers at pHs higher than 9.73 are complex due to competing hydration of the quinone moiety by HO^-. At pH values of 9.73, 9.28, and 8.79, the increase in A_{287} yielded first-order kinetics and plots of $[BzNH_2]_{total}$ vs. k_{obsd} (s^{-1}) yield upwardly curving lines indicative of a greater than first-order dependence upon benzylamine species concentration. That the reaction is both second and first order in amine is shown by the linearity of plots of $k_{obsd}/[BzNH_2]$ vs. $[BzNH_2]$ (Figure 8) and by their positive intercepts. Within the pH range investigated, the kinetics of the reaction of benzylamine with 7_{ox} have been shown to follow the rate law of eq 9 which exhibits terms for



17

$$k_{obsd} = k_1[BzNH_2] + k_2[BzNH_2][BzNH_3^+] \quad (9a)$$

$$\frac{k_{obsd}}{[BzNH_2]} = k_1 + \left(\frac{k_2 a_H}{K_a}\right)[BzNH_2] \quad (9b)$$

spontaneous (or H_2O catalysis) and for $BzNH_3^+$ general-acid catalysis. Thus, the plots provide k_2a_H/k_a as slopes and k_1 as intercept = $(5.3 \pm 0.1) \times 10^{-2}$ M^{-1} s^{-1}. A plot of the slopes vs. a_H yields a straight line (Figure 8 inset) with k_2/K_a as the slope from which k_2 is calculated as (0.7 ± 0.2) M^{-2} s^{-1}.

For comparison, the oxidation of benzylamine (8.75×10^{-3} M) by 4,7-phenanthroline-5,6-quinone (3_{ox}) (6.95×10^{-5} M) was investigated (H_2O, μ = 1.0, 30 °C, under argon) by repetitive scanning from 400 to 200 nm. The final spectrum obtained was identical with that of the aminophenol of 3_{ox}.[19] Reactions were then followed at 365 nm and found to follow the first-order rate law when using total amine concentrations from 8.75×10^{-2} to 8.75×10^{-3} M at pHs 9.73, 9.28, and 8.79. Plots of the pseudo-first-order rate constants (k_{obsd} (s^{-1})) vs. $[BzNH_2]_{tot}$ are linear (Figure 9) with the reaction being faster at higher pH. The reaction of 3_{ox} with $BzNH_2$ thus follows rate eq 10. When a pK_a

$$k_{obsd} = k_1[BzNH_2] = k_1\left(\frac{K_a}{K_a + a_H}\right)[BzNH_2]_{tot} \quad (10)$$

value of 9.34 is used for benzylamine, k_1 can be calculated as 0.34 $\pm$ 0.03 s^{-1} from the six values of k_{obsd} determined at each of three pH values (Figure 9).

To gain further information about the mechanism of benzylamine oxidation by 3_{ox} and 7_{ox}, the kinetic isotope effects (k_H/k_D) for the reactions were measured by using benzylamine-d_2. Two identical buffers were prepared containing deuterated and undeuterated benzylamine (8.75×10^{-2} M), and the rate of benzylamine oxidation by 7_{ox} (4.29×10^{-5} M) (pH 9.61, argon atmosphere, 30 °C) was followed by the increase in A_{287}. The k_{obsd} values obtained were used to calculate the isotope effect (k_H/k_D) for the reaction, and this was found to be 1.2 $\pm$ 0.3 from three determinations. When the same procedure was used, the isotope effect for the oxidation of benzylamine by 3_{ox} was calculated as 2.0 $\pm$ 0.3 from three determinations. The absence of an isotope effect in the oxidation of benzylamine by 7_{ox} shows that C–H bond breaking is not occurring in the rate-determining step of the reaction. It is thus concluded that carbinolamine and imine formation are the rate-determining steps in the oxidation process. The fact that the oxidation of benzylamine by 7_{ox} is general-acid-catalyzed is in agreement with the formation of carbinolamine and/or imine intermediates in the rate-determining step since both carbinolamine and imine formation are known to be general-acid-catalyzed.[29] The lack of general-acid catalysis for reaction of benzylamine with 3_{ox} may reflect the greater electrophilicity of this quinone. The observation of a small isotope effect in the oxidation of benzylamine by 3_{ox} shows that C–H bond breaking is at least partially rate-determining. Since adduct formation has

(29) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; Chapter 10.

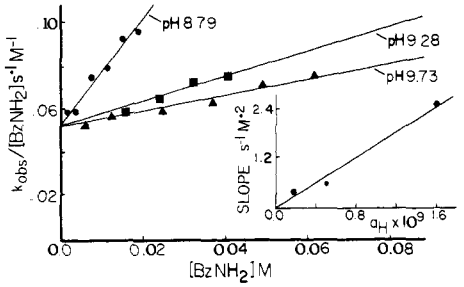


Figure 8. Plot of the pseudo-first-order rate constants $k_{obsd}/[BzNH_2]$ vs. $[BzNH_2]$ for the reduction of 7_{ox} by benzylamine at pH 9.73, 9.28, and 8.79. Inset: Plot of slopes vs. hydrogen activation coefficient (a_H).

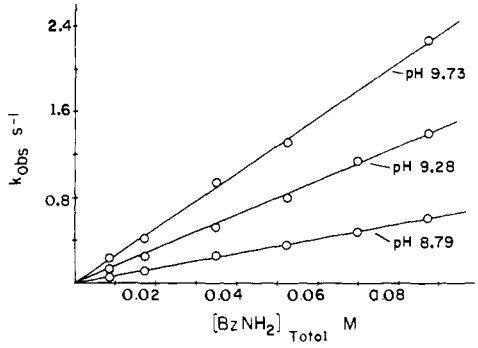


Figure 9. Plot of the pseudo-first-order rate constants (k_{obsd}, s^{-1}) vs. $[BzNH_2]_{tot}$ for the reduction of 3_{ox} by benzylamine at pH 9.73, 9.28, and 8.79.

been shown to be more favorable with 3_{ox} than with 7_{ox}, it is reasonable that carbinolamine and/or imine formation should not be as rate-determining with 3_{ox} as with 7_{ox}.

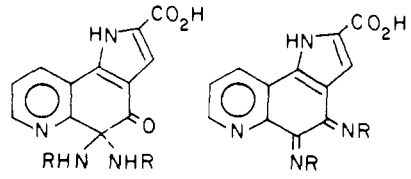
The time course for reaction of 7_{ox} with glycinamide under the pseduo-first-order conditions of [glycinamide] = 8.75×10^{-2} M to 8.75×10^{-3} M $\gg$ $[7_{ox}] = 4.13 \times 10^{-5}$ M (H_2O, 30 °C, μ = 1 with KCl, argon atmosphere) has been followed by repetitive scanning of the spectrum between 200 and 600 nm at pH values between 7.3 and 9.3. With glycinamide concentrations of 8.75×10^{-3} M, reasonable pseudo-first-order kinetics were observed. The pseudo-first-order rate constants for product formation with [glycinamide] = 8.75×10^{-3} M were found to be as follows: pH 7.3, $(5.06 \pm 0.05) \times 10^{-5}$ s^{-1} (four half-lives); pH 8.3, $(1.92 \pm 0.02) \times 10^{-4}$ s^{-1} (nine half-lives); pH 9.3, $(2.70 \pm 0.02) \times 10^{-4}$ s^{-1} (five half-lives). From these k_{obsd} values there may be calculated the second-order rate constant ($v = k_2$[glycinamide][7_{ox}]) $k_2 = (3.8 \pm 0.4) \times 10^{-2}$ M^{-1} s^{-1}. Much above [glycinamide] = 8.73×10^{-3} M, the increase in absorbance at 285 nm exhibits an initial lag phase followed by an exponential change. The lag phase becomes more pronounced with increase in [glycinamide]. At 420 nm, an increase followed by a decrease in absorbance was observed. The appearance and disappearance of an absorbing species at 420 nm is reasonably accounted for by the formation of 7_{sem} species which arise by $1e^-$ transfer from product 7_{red} to reactant 7_{ox}. This view is supported by the observation that mixing equimolar (8.17×10^{-4} M) samples of 7_{ox} and 7_{red} in bicarbonate buffer under an argon atmosphere generates a species with λ_{max} = ca. 400 nm. The multiphasic absorbance at 285 nm may reflect this comproportionation as well as the overlapping of absorbance by intermediate carbinolamine and imine adducts of 7_{ox} and glycinamide (eq 11). The reactions of eq 11 are not sufficient,

$$7_{ox} + H_2N\text{–}CH_2CONH_2 \rightleftharpoons \text{carbinolamine} \rightleftharpoons \text{imine} \rightleftharpoons 7_{red} + HOCCONH_2 \quad (11)$$

$$7_{ox} + 7_{red} \rightleftharpoons 2\ 7_{sem}$$

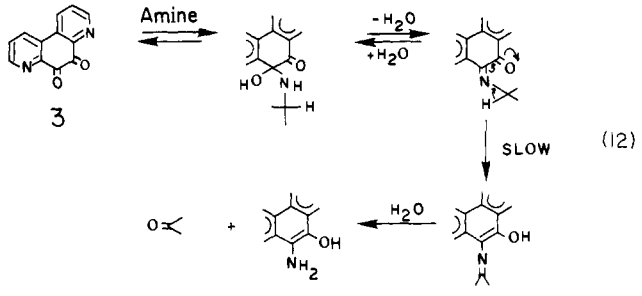
however, to explain the observation that the lag phase observed at 285 nm becomes more pronounced with increase in [glycinamide]. This may be attributed to the reversible reaction of a second molecule of glycinamide with carbinolamine or imine intermediates to provide species such as **18** or **19**.

It is worth noting that in a previous study of amine oxidation by the isomeric phenanthrolinequinones, it was found that the ability to oxidize primary, secondary, and tertiary amines, under



18 19

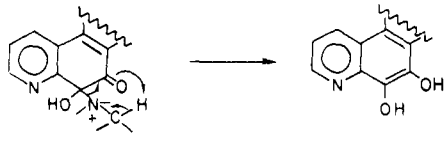
an inert atmosphere, is dependent upon the structures of the quinones rather than upon their redox potentials with the order in oxidation rates being $3_{ox} > 4_{ox} > 5_{ox}$. Thus the rate of amine oxidation by phenanthrolinequinones increases as the pyridine nitrogens are moved peri to the quinone carbonyls. The mechanisms of these oxidations were shown to involve the formation of covalent intermediates (eq 12) by isolation of the aminoquinol



(12)

products. When comparing 3_{ox} and 7_{ox} (or methoxatin), the second pyridine ring of the former should subject its quinone moiety to a greater electron deficiency than does the indole ring of 7_{ox}. This is apparent in their redox potentials and equilibrium constants for urea addition.

It is interesting to note that 7_{ox} reacts most readily with primary amines (as does plasma amine oxidase) whereas 3_{ox} reacts most readily with glycine, tertiary amines (*N*,*N*-dimethylbenzylamine), and secondary amines (morpholine). The didecarboxymethoxatin (7_{ox}) is reduced primarily to its quinol (7_{red}) with formation of some aminophenol (**17**). In comparison, 3_{ox} yields quinol only with tertiary amines and aminophenol products with secondary and primary amines. Thus, the mechanism of eq 13 comes into play with 3_{ox} when there is no other alternative, and the preferred product arises via the reaction of eq 12.[19] In the case of 7_{ox}, the



(13)

preference in mechanism is reversed. A rationale for these observations is not immediately evident. For 3_{ox} prototropy is at least partially rate-determining while for 7_{ox} carbinolamine formation is rate-determining.

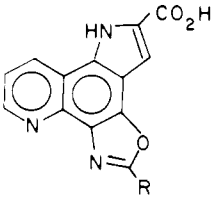
Ohshiro et al.[30] have repported that methoxatin is capable of the oxidation of amines when coupled to the reduction of O_2—so-called "aerobic autorecycling catalysis". We find that 7_{ox} is a poor aerobic autorecycling catalyst due to its conversion, in the presence of amine and O_2, to redox inactive derivatives. Thus, on admittance of air, at completion of the reduction of 7_{ox}, by glycinamide, benzylamine, or ethylamine, the generated spectra of 7_{ox} possessed to broadened λ_{max} suggestive of the formation of another product in addition to 7_{ox}. In order to investigate this phenomenon and isolate the unknown product(s), the reactions of 7_{ox} with glycinamide, benzylamine, and ethylamine were performed on a preparative scale *aerobically*. In each case, 7_{ox}

(30) Ohshiro, Y.; Itoh, S.; Kurokawa, K.; Kato, J. *Tetrahedron Lett.* **1983**, *24*, 3465–3468.

Table III. Pseudo-First-Order Rate Constants for the Reduction of Five *o*-Quinones by Hydrazine (3.33×10^{-3} M) at pH 1.0

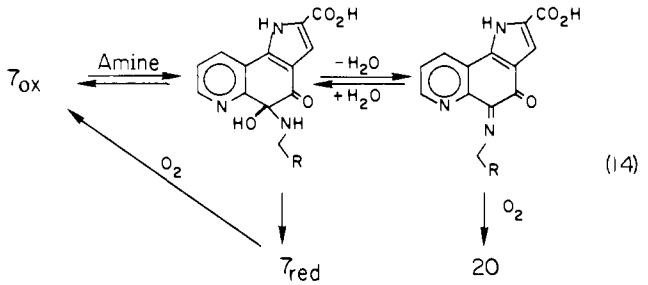
quinone	k_{obsd}, s^{-1}	quinone	k_{obsd}, s^{-1}
methoxatin	6.33×10^{-5}	$\mathbf{5}_{ox}$	1.13×10^{-5}
$\mathbf{3}_{ox}$	2.72×10^{-5}	$\mathbf{7}_{ox}$	6.55×10^{-5}
$\mathbf{4}_{ox}$	2.13×10^{-4}		

was treated with a large excess of an aqueous solution of the appropriate amine/amine hydrochloride at pH 7. When a reaction came to completion, the pH was adjusted to 4 and the resulting precipitates filtered off, washed with water and ethanol, and dried under vacuum. UV spectra of the products showed a λ_{max} in the same region as $\mathbf{7}_{ox}$ but with a broader peak. There was also observed a much higher absorbance around 400 nm. The products were identified by mass spectrometry (positive chemical ionization) and high-resolution mass spectrometry as oxazoles **20a**, **b**, and **c**). Thus, in air there are two competing reactions: formation



20a, R = $CONH_2$ (from glycinamide)
20b, R = Ph (from benzylamine)
20c, R = CH_3 (from ethylamine)

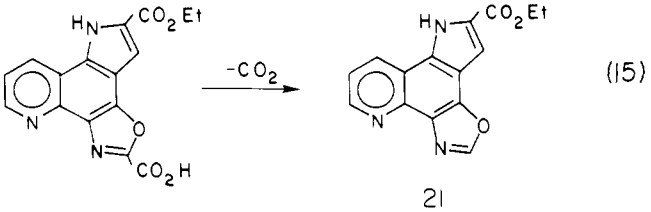
of $\mathbf{7}_{red}$ which is oxidized back to $\mathbf{7}_{ox}$, and ring closure and oxidation (irreversible) to the oxazole (eq 14). In the characterization of



(14)

compounds **20 a**, **b**, and **c**, mass spectral analysis resulted in thermal decarboxylation due to the high source temperature used. In order to observe the parent M + 1 peak, $\mathbf{6}_{ox}$ was reacted with glycinamide in the same manner as $\mathbf{7}_{ox}$ to yield the ethyl ester of **20a**: exact mass calcd for $C_{16}H_{13}N_4O_4$ 325.0933, found 325.0945.

A preparative scale experiment employing excess glycine and $\mathbf{7}_{ox}$ (pH 9.5, aerobic conditions) yielded the oxazole **21**, which has been characterized by high-resolution mass spectrometry, elemental analysis, and ^1H NMR. A plausible mechanism for the formation of **21** is provided in eq 15. It should be noted that Duine



(15)

et al.[16] reported that methoxatin reacts with amino acids to give unknown compounds.

The pseduo-first-order rate constant for the reduction of $\mathbf{7}_{ox}$ with hydrazine (3.33×10^{-3} M, 0.1 M HCl, 30 °C, under argon) is compared to the rate constants for hydrazine reduction of methoxatin and the phenanthroline-5,6-quinones[19] in Table III. The reduction of $\mathbf{7}_{ox}$ was followed by monitoring the increase in A_{290} which is accompanied by isosbestic points at 350, 319, 273, and 231 nm, indicating that no intermediates are accumulated in the course of the reaction. The rate constants determined for methoxatin and the didecarboxymethoxatin ($\mathbf{7}_{ox}$) are similar, whilst phenanthrolinequinones $\mathbf{3}_{ox}$ and $\mathbf{4}_{ox}$ are reduced more readily. The reduction of quinone $\mathbf{8}_{ox}$ was not investigated since it has neglible solubility in 0.1 M HCl due to its lack of a pyridine function.

Attempts to reduce $\mathbf{7}_{ox}$ with hydrazine at pH 8.3 (anaerobic) yielded complex kinetics, and the final spectrum was not that of $^7_{red}$. Preparatively, $\mathbf{6}_{ox}$ and hydrazine hydrate were reacted together at pH 10 under aerobic conditions and the 5-hydrazone of $\mathbf{6}_{ox}$ was isolated (Experimental Section). The UV spectrum of this product was similar to that obtained from the kinetic experiments with $\mathbf{7}_{ox}$, indicating that at higher pH, hydrazone formation competes with quinone reduction.

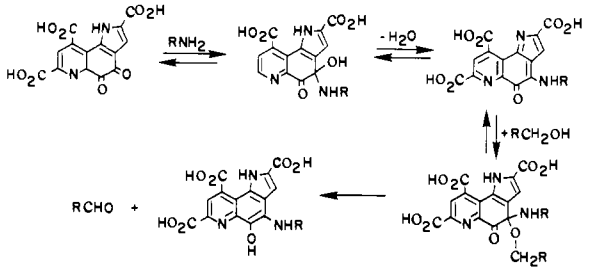
The reduction of protonated $\mathbf{7}_{ox}$ by hydrazine cannot occur via imine formation since the hydrazone is stable and does not reduce in the presence of excess hydrazine. If a covalent mechanism is involved, the redox reaction would be required to occur via a carbinolamine as shown in eq 13. (Alternatively, the reduction of hydrazine may be radical in nature since stepwise one-electron oxidations of hydrazines are well documented.[31]) It has been observed previously,[32] in the hydrazine reduction of an "*o*-quinone" grouping of 6-amino-5-oxo-[3*H*,5*H*]-uracil, that the hydrazone was not an intermediate in the redox reaction.

Conclusions

In the comparison of the reactions of methoxatin, its close analogue 7,9-didecarboxymethoxatin ($\mathbf{7}_{ox}$) (and ethyl ester $\mathbf{6}_{ox}$), and the phenanthroline-5,6-quinones (in particular $\mathbf{3}_{ox}$), expected similarities and marked differences arise. It has been observed previously that the rates of reactions of the phenanthroline-5,6-quinones differ ($\mathbf{3}_{ox}$ being the most reactive oxidant) though their redox potentials are essentially identical.[19] In the present study, we find that $\mathbf{6}_{ox}$, $\mathbf{7}_{ox}$, and methoxatin are electrochemically indistinguishable in aqueous solution. The values of $E°$ for these compounds are ~110 mV less positive than the $E°$ values for the phenanthroline-5,6-quinones. Consequently, $\mathbf{7}_{ox}$ is unable to perform as wide a range of oxidations as $\mathbf{3}_{ox}$.

Pseudo-base formation has been shown to occur with the phenanthrolinequinones[19] and with $\mathbf{7}_{ox}$ by addition of HO^- on a quinone carbonyl moiety followed by hydration of the second carbonyl group (eq 6), the order in ease of hydration being $\mathbf{3}_{ox} > \mathbf{7}_{ox} > \mathbf{4}_{ox} > \mathbf{5}_{ox}$. In contrast to the phenanthroline-5,6-quinones, $\mathbf{7}_{ox}$ undergoes base-catalyzed ring contraction of the quinone moiety only with difficulty (eq 8). Urea also condenses with the quinones to generate cyclic dicarbinolamine adducts (**14**) with the ease of adduct formation being $\mathbf{3}_{ox} \gg \mathbf{6}_{ox} >$ methoxatin (Table II).

Methoxatin has been reported to react with ammonia to yield an imine adduct.[16] We show that $\mathbf{7}_{ox}$ appears to react with ammonia in a similar manner to methoxatin although we feel unable to conclude that imine formation is definitely occurring due to the rather small spectral change observed. The possibility of a reaction with ammonia is pertinent to the mechanism of methoxatin-mediated alcohol oxidation offered by Forrest et al.[18] to account for the need of ammonia as an activator by many alcohol dehydrogenases (eq 16). This mechanism involves the formation



(16)

of an iminoquinone species which would be expected to have a higher oxidation potential than the corresponding quinone (cf.

(31) Patai, S. "The Chemistry of the Hydrazo, Azo and Azoxy Groups"; Wiley: New York, 1975.
(32) Wessiak, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 4809.

$E_{1/2}$ for 2-aminophenol = 0.48 V vs. SCE at pH 7.4[33] and $E_{1/2}$ for catechol = 0.18 V vs. SCE at pH 7.0[34]).

The model 7_{ox} is able to oxidize primary amines (ethylamine and benzylamine) and amino acids and their derivatives (glycine and glycinamide) but does not oxidize a secondary (morpholine) or a tertiary amine (*N,N*-dimethylbenzylamine). This is surprising on the basis of amine oxidation potentials[19] which predict that *N,N*-dimethylbenzylamine would be much easier to oxidize than ethylamine. Also, *N,N*-dimethylbenzylamine and morpholine are oxidized by 3_{ox}. The finding that 7_{ox} reacts preferentially with primary amines and that some aminophenol **17** is formed supports these oxidations to occur via covalent addition–elimination mechanisms (eq 12 and 13). The absence of a kinetic isotope effect in the oxidation of benzylamine and benzylamine-d_2 by 7_{ox} shows that the rate-determining processes in the reaction must be carbinolamine and imine formation. Under the conditions of "aerobic autorecycling" oxidation of primary amines, the didecarboxymethoxatin (7_{ox}) and its ethyl ester (6_{ox}) are converted to redox inactive oxazoles (**20**) (eq 14).

Acknowledgment. This work was supported by a grant from the National Institutes of Health.

Registry No. 1_{ox}, 72909-34-3; 3_{ox}, 84-12-8; 3_{ox} (urea adduct), 95912-06-4; **3** (aminophenol), 95912-15-5; 4_{ox}, 82701-91-5; 5_{ox}, 27318-90-7; 6_{ox}, 95911-95-8; 6_{ox} (methyl ester), 95912-14-4; 6_{ox} (hydrazone), 95911-98-1; 6_{ox} (urea adduct), 95912-05-3; 6_{red}, 95911-96-9; 6_{sem}, 95911-97-0; 7_{ox}, 95911-99-2; 7_{red}, 95912-00-8; 8_{ox}, 95912-01-9; **9**·2HCl, 21302-43-2; **9** (diazonium chloride), 95912-13-3; **10** (R = Me), 95912-02-0; **11** (R = Me), 95912-03-1; **12**, 95912-04-2; **15**, 95912-07-5; **17**, 95912-08-6; **20a**, 95912-09-7; **20a** (ethyl ester), 95935-32-3; **20b**, 95912-10-0; **20c**, 95912-11-1; **21**, 95912-12-2; $CH_3COCH(CH_3)CO_2Me$, 17094-21-2; $CH_3COCH(CH_3)CO_2Et$, 609-14-3; $(CH_3)_2CO$, 67-64-1; $(H_2N)_2CO$, 57-13-6; $H_2NCH_2CONH_2$·HCl, 1668-10-6; $PhCH_2NH_2$·HCl, 3287-99-8; $EtNH_2$·HCl, 557-66-4; $H_2NCH_2CO_2H$·HCl, 6000-43-7; $PhCONH_2$, 55-21-0; $PhCD_2NH_2$, 15185-02-1; 5-nitro-8-hydroxyquinoline, 4008-48-4.

(33) Bezuglyi, V. D.; Beills, Yu I. J. *Anal. Chem. USSR (Engl. Transl.)* **1965**, *20*, 1060.
(34) Pungor, E.; Szepesuary, E. *Anal. Chim. Acta* **1968**, *43*, 298.

Communications to the Editor

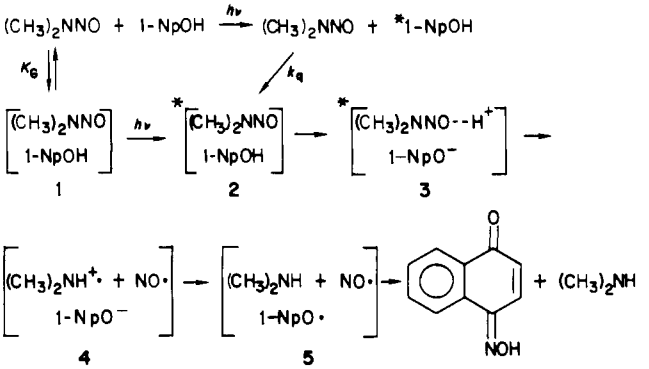
Photonitrosation Promoted by Enhanced Acidity of Singlet-State Phenols

Yuan L. Chow* and Zheng-Zhi Wu

Department of Chemistry, Simon Fraser University
Burnaby, British Columbia, Canada V5A 1S6

Received October 22, 1984

We wish to demonstrate that singlet-excited-state naphthols[1,2] and anthrols can induce decomposition of *N*-nitrosodimethylamine (NND) by dual proton and energy-transfer processes occurring from an exciplex within the lifetimes of these phenol–phenolate couples. Although intramolecular proton transfer of excited-state phenols has been demonstrated,[3] the claim that the enhanced acidity of singlet-state 2-naphthol nitrosation[4] has been disputed on the grounds of the short excited-state lifetimes.[5] Due to highly dipolar nature, nitrosamines are known to associate extensively with aromatic π-electron clouds[6] as well as Lewis acids.[7] Upon excitation, an acid complex of NND rapidly dissociates[8-10] ($>10^9$ s^{-1}) to the aminium radical and nitric oxide.[11,12] This paper describes the utilization of the enhanced acidity and excitation energy of singlet state phenols to cause the NND photodissociation as shown in **1** → **2** → **3** → **4**.



Photolysis of 1-naphthol (1-NpOH, 25 mM) and NND (25 mM) in a variety of neutral solvents, e.g., THF, dioxane, methanol, acetonitrile, or toluene, through a Pyrex filter, gave 1,4-naphthoquinone monooxime and dimethylamine, the former in limiting quantum yields Φ_{ox} of 0.14–0.02. The chemical yields of quinone monooximes from various phenols under similar reaction conditions are listed in Table I together with the pertinent data. With appropriate filter systems or monochromatic light, irradiation of NND at 360–380 nm under these conditions did not cause the reaction; irradiation of 1-NpOH at 290–310 nm caused the photonitrosation. In the presence of HCl, irradiation of a similar solution induced the decomposition of NND[9,10] but no nitrosation of 1-NpOH. That the reaction is initiated from singlet state 1-NpOH is indicated by the quenching of 1-NpOH fluorescence by NND (vide infra) and by the failure of xanthone triplet (E_T = 74 kcal/mol)[18] to sensitize the photoreaction. The participation of enhanced acidity in the singlet excited state of 1-NpOH was shown by the following evidence. First, irradiation

(1) (a) Weller, A. *Prog. React. Kinet.* **1961**, *1*, 189. (b) Ireland, J. F.; Wyatt, P. A. H. *Adv. Phys. Org. Chem.* **1976**, *12*, 131.
(2) Förster, T. *Z. Electrochem.* **1950**, *54*, 42; **1950**, *54*, 531.
(3) Isaks, M.; Yates, K.; Kalanderopoulos, P. *J. Am. Chem. Soc.* **1984**, *106*, 2730.
(4) Saeva, F. D.; Olin, G. R. *J. Am. Chem. Soc.* **1975**, *97*, 5631.
(5) Chandross, E. A. *J. Am. Chem. Soc.* **1976**, *98*, 1053.
(6) (a) Chow, Y. L.; Fesser, M. M. *Chem. Commun.* **1967**, 239. (b) Williams, D. H.; Wilson, D. A. *J. Chem. Soc. B* **1966**, 144.
(7) Layne, W. S.; Jaffe, H. H.; Zimmer, H. *J. Am. Chem. Soc.* **1963**, *85*, 435, 1816.
(8) It has not been possible to detect excited states of *N*-nitrosopiperidine associated with an acid by subnanosecond excitation, which implies the photodissociation process is faster than 10^9 s^{-1}.
(9) Chow, Y. L. *Acc. Chem. Res.* **1973**, *6*, 354.
(10) Cessna, A. J.; Sugamori, S. E.; Yip, R. W.; Lau, M. P.; Snyder, R. S.; Chow, Y. L. *J. Am. Chem. Soc.* **1977**, *99*, 4044.
(11) The photodissociation of NND in solution does not occur under neutral conditions.[9] In the gas phase NND is photolyzed at 363.5 nm to $(CH_3)_2N\cdot$ and NO· with Φ = 1, which recombine extremely rapidly leaving no net reaction; the rate constant of the singlet-excited-state decomposition is estimated to be $>8.5 \times 10^9$ s^{-1}.[12]
(12) Geiger, G.; Huber, J. R. *Helv. Chim. Acta* **1981**, *64*, 989. Geiger, G.; Stafast, H.; Brühlmann, Y.; Huber, J. R. *Chem. Phys. Lett.* **1981**, *79*, 521. Müller, R. P.; Murata, S.; Huber, J. R. *Chem. Phys.* **1982**, *66*, 237.
(13) McClure, D. S. *J. Chem. Phys.* **1949**, *17*, 905.
(14) Harris, C. M.; Selinger, B. K. *J. Phys. Chem.* **1980**, *84*, 1366.
(15) Weller, A. *Z. Phys. Chem. (Munich)* **1958**, *15*, 438.
(16) Yamamoto, S. A.; Kikuchi, K.; Kokubun, H. *J. Photochem.* **1976**, *5*, 469.
(17) Young, J. F.; Schulman, S. G. *Talanta* **1973**, *20*, 399.
(18) Scaiano, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 7747.

0002-7863/85/1507-3338$01.50/0