30 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to afford a solid, which was flash chromatographed (1.5 × 10 cm, 5% Et_2O/hexanes) to give, after concentration and high-vacuum drying, 55 mg of **2g** (88%), which proved identical (^1H NMR, TLC) with that obtained by reduction of epivitamin D_2 with Cp_2TiCl_2–"aluminum hydride".

Reduction of Benzoyloxy Tosylate 2k to the Ether 4. A solution of lithium triethylborohydride in THF (850 μL, 0.85 mmol, 1 M) was added via syringe to a solution of **2k** (80 mg, 0.12 mmol) at 0 °C under argon. The resulting mixture was stirred at room temperature for 2 h and then at reflux for 4 h. Quenching and workup as above gave, after flash chromatography (1.5 × 20 cm, 7% Et_2O/hexanes), 47 mg of **4** (100%): ^1H NMR δ 6.17 and 5.76 (2 H, AB q, J = 11.3 Hz, H-6, H-7), 5.19 (2 H, m, H-22, H-23), 3.97–3.90 (3 H, m, 2H-19, H-3β); UV (Et_2O) λ_{max} 246, 254, 264 nm; MS, m/e 396 (M^+, 26), 271 (24), 149 (20), 147 (16), 145 (13), 137 (32), 133 (40), 107 (100); HRMS calcd for $C_{28}H_{44}O$ 396.3392, found 396.3360.

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Registry No. **2a**, 50-14-6; **2b**, 65377-86-8; **2c**, 807-27-2; **2d**, 104846-62-0; **2e**, 116559-84-3; **2f**, 116559-85-4; **2g**, 116559-86-5; **2h**, 116559-87-6; **2i**, 116467-93-7; **2j**, 116559-88-7; **2k**, 116561-02-5; **2l**, 116559-89-8; **2m**, 116561-03-6; **3a**, 51744-66-2; **3b**, 65377-91-5; **3c**, 67-96-9; **3d**, 115540-26-6; **4**, 116559-90-1.

Studies of Extended Quinone Methides. Design of Reductive Alkylating Agents Based on the Quinazoline Ring System

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This report discusses the quinone methide reactivity, electrochemistry, and xanthine oxidase alkylation properties of a quinazoline-based reductive alkylating agent. The design of this alkylating agent involved functionalizing the quinazoline ring as a quinone with a leaving group placed so as to afford a quinone methide species upon reduction. pH–rate profiles, nucleophile-trapping studies, and product studies indicate the presence of a steady-state quinone methide species. The quinone methide species reacts by either trapping nucleophiles or ketonizing to a quinone. It is concluded that the fate of this and similar quinone methides can be predicted from the redox potential of the quinone resulting from quinone methide ketonization. If a low potential quinone is the ketonization product, ketonization is thermodynamically favored over nucleophile trapping. The opposite is true if a high redox potential quinone (such as the quinazoline-based system) results from ketonization. Finally, the reductive alkylation of the xanthine oxidase active site is demonstrated with the title systems.

The low reduction potentials exhibited by some tumor cells[2] has generated an interest in reductive alkylating agents as selective antitumor agents.[3,4] Reductive alkylating agents are quinones functionalized with a leaving group so as to permit quinone methide formation upon reduction. The quinone methide species can trap nucleophiles important to cellular function as well as ketonize to a quinone derivative. Indeed, many naturally occurring quinone antitumor agents such as mitomycin[5] and the anthracyclines[6] act as reductive alkylating agents.

Efforts in this laboratory have been direct toward the design of reductive alkylating agents based on heterocyclic ring systems. Thus the benzimidazole[7] and imidazo[4,5-*g*]quinazoline[8,9] ring systems have been functionalized as such. The goals of these efforts have been to gain insights into the structure–reactivity relationship for quinone methide fate (nucleophile trapping vs ketonization) and to design enzyme-directed reductive alkylating agents. Most, if not all, naturally occurring reductive alkylating agents are directed toward DNA rather than enzymes. However, the imidazo[4,5-*g*]quinazoline agents alkylate the active site of xanthine oxidase and thus represent the first enzyme-directed reductive alkylation system.[9]

The present report discusses the quinone methide reactivity, electrochemistry, and xanthine oxidase alkylation

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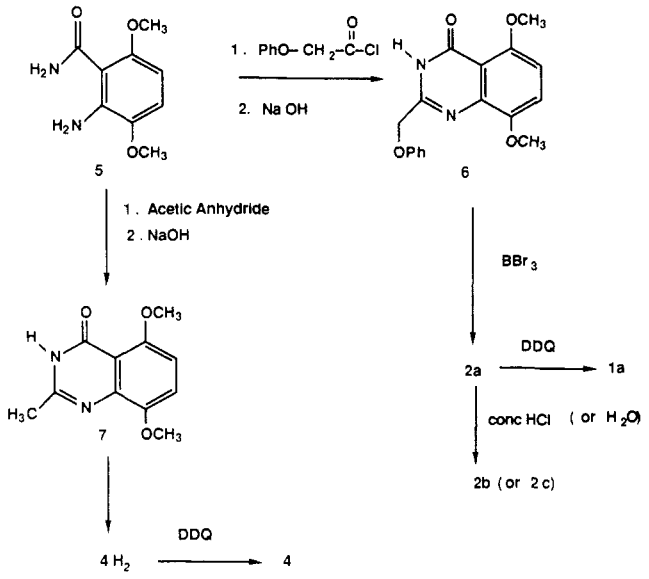
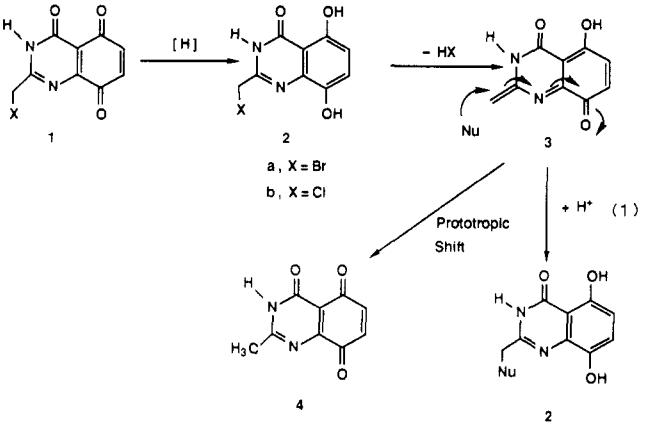
Scheme I



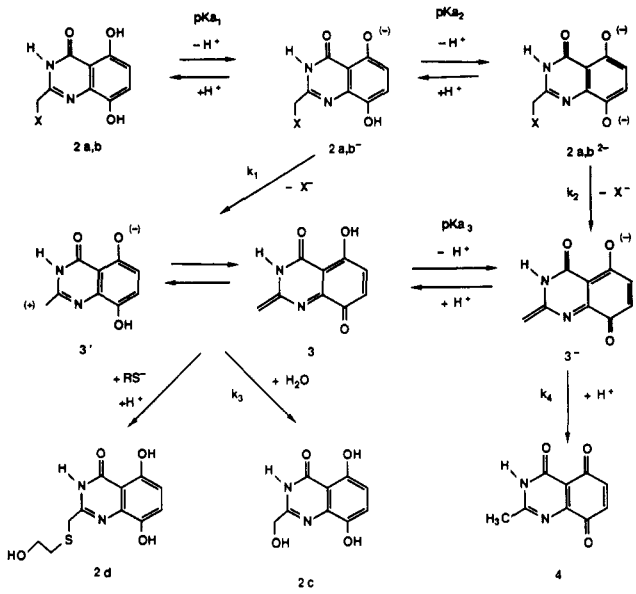
properties of a quinazoline-based reductive alkylation system (eq. 1). Kinetic evidence is provided for the formation of the quinone methide intermediate **3**. Electrochemical studies of **4** provide a rationale for the tendency of **3** to act as a nucleophile trap. It is concluded from this and other studies that quinone methides in general prefer to trap nucleophiles if a high-potential quinone results from ketonization. Finally, the active-site-directed reductive alkylation of xanthine oxidase is demonstrated with the alkylation systems in eq 1.



Results and Discussion

Quinone Methide Formation and Fate. As in previous studies,[7,9] the presence of a steady-state quinone methide intermediate was determined by employing pH–rate profiles, trapping studies with added 2-hydroxyethyl mercaptide, and product studies. All of these studies were carried out with the purified hydroquinones **2a,b**, at 5×10^{-5} M in anaerobic aqueous buffers, over the pH range of 6–12 (μ = 1.0, KCl) at 30.0 ± 0.2 °C. Found in Schemes I and II, respectively, are an outline of the synthetic methods employed to prepare the title systems and the proposed hydrolysis mechanism of **2a,b**. In the rate-determining step, halide (Br, Cl) is eliminated from the hydroquinone anion and dianion species to afford quinone methide species 3_T (3_T = 3′ + 3 + 3^-). Quinone methide ketonization to **4** then competes with nucleophile trapping in non-rate-determining steps. Details of the studies that led to the mechanism in Scheme II are provided below.

Scheme II



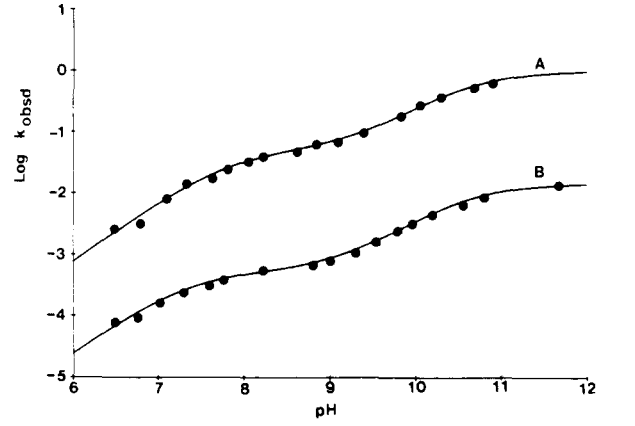


Figure 1. Plots of k_{obsd} vs pH for the first-order hydrolysis of **2a** (plot A) and **2b** (plot B) in anaerobic buffer (μ = 1.0, KCl) at 30.0 ± 0.2 °C.

Since the hydrolytic behavior of **2a,b** is similar, except for the rates of halide elimination, some aspects of **2a,b** hydrolysis are discussed together. The course of hydrolysis was followed spectrophotometrically at either 344 or 410 nm. Absorbance vs time (s) plots obtained at either wavelength are first-order in character over the concentration of **2a,b** equal to $(1–5) \times 10^{-5}$ M. The products arising from the first-order process are the hydroxy hydroquinone **2c** and quinone **4**. Preparative hydrolysis of **2a** in aqueous dimethyl sulfoxide afforded **2c** (see the Experimental Section). The UV–visible spectra obtained after **2a,b** hydrolysis in aqueous buffer (pH >6) distinctly show the presence of **2c** (λ_{max} = 344 nm) and **4** (λ_{max} = 410 nm). The extinction coefficients of both compounds were employed to obtain yields: 57 ± 7% **2c** and 43 ± 6% **4**, which are independent of the reaction's pH. In reactions where concentrations of **2a,b** were $\geq 5 \times 10^{-5}$ M, a second absorbance change followed the initial first-order process. The second absorbance change is dependent on the initial concentration of **2a,b** and probably results from oxidation of **2c** by **4** when both are present in high concentrations (see ref 10 for an example of this reaction in the benzimidazole system).

(10) Hydroquinone–quinone reactions have also been observed during hydrolysis of the benzimidazole reductive alkylator, ref 7.

Table I. First-Order Rate Constants and pK_a Values Obtained from Figure 1[a]

	2a	2b
k_1	0.049 s^{-1}	5.1×10^{-4} s^{-1}
k_2	0.96 s^{-1}	1.25×10^{-2} s^{-1}
pK_{a_1}	7.8	7.3
pK_{a_2}	10.60	10.50

[a] Measured in anaerobic aqueous buffer (μ = 1.0, KCl) a 30.0 ± 0.2 °C.

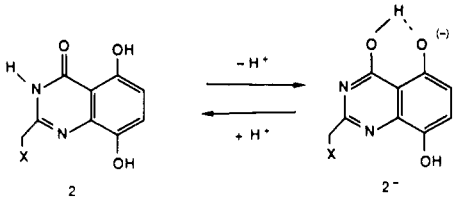
Values of k_{obsd} for the first-order hydrolysis of **2a** (plot A) and **2b** (plot B) are plotted against pH in Figure 1. The presence of two plateaus in both plots indicate product formation occurs from both a monoanion (the first plateau) and a dianion (the second plateau). A mechanism that would account for these observations is shown in Scheme II. The pH–rate law for the disappearance of **2a,b** according to this mechanism is provided in eq 2 where k_1,

$$k_{obsd} = \frac{k_1 K_{a_1}}{a_H + K_{a_1}} + \frac{k_2 K_{a_2}}{a_H + K_{a_2}} \quad (2)$$

k_2, K_{a_1} and K_{a_2} are constants in Scheme II and a_H is the proton activity determined with a pH meter. Computer fitting of eq 2 to the k_{obsd} vs pH data for **2a,b** hydrolysis afforded the solid curves shown in plots A and B of Figure 1. The parameters obtained from these fits are provided in Table I.

Kinetic pK_a values, obtained from pH–rate data, could either represent the actual pK_a values of the reactant or composites of rate and equilibrium constants.[11] The spectrophotometric pK_a value for the acid dissociation **2b** $\rightleftarrows$ **2b**$^-$ + H$^+$ is 7.6 ± 0.2,[12] which is nearly identical with the kinetically obtained value of pK_{a_1} for **2b**. Thus, the values of pK_{a_1} and pK_{a_2} in Table I represent acid dissociations from **2b**. Consistent with the similar electron-withdrawing inductive effects of chloro and bromo substituents, the pK_a values of **2a** in Table I are essentially the same as those of **2b**.

The constants pK_{a_1} and pK_{a_2} likely correspond to successive acid dissociations from the 5- and 8-hydroxyl groups of **2a,b**. The reactants **2a,b** possess three protons capable of dissociation in aqueous buffer: N(3)H and the protons of the 5- and 8-hydroxyl groups. Initial acid dissociation of the N(3) proton would not occur at low pH; studies of acid dissociation from substituted quinazolin-4(3*H*)-ones indicate electron-rich derivatives possess pK_a values > 10.[13] Initial acid dissociation from the 5-hydroxyl, on the other hand, affords an anion stabilized by internal hydrogen bonding (eq 3). Previous studies in



(3)

this laboratory have demonstrated the presence of internal hydrogen bonding in species related to **2**$^-$ as well as the low pK_a (~8) for 5-hydroxyl dissociation.[14]

(11) Bruice, T. C.; Schmir, G. L. *J. Am. Chem. Soc.* **1959**, *81*, 4552.
(12) Measured by following absorbance changes at 360 nm with pH (μ = 1.0, KCl), at 30.0 ± 0.2 °C.
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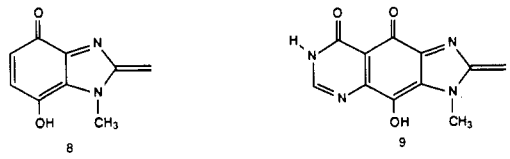
The results discussed so far indicate **2a,b** hydrolysis involves a rate-determining reaction of the hydroquinone monoanion and dianion species. The approximately 100-fold difference in rate constants between chloro (**2b**) and bromo (**2a**) derivatives (Figure 1, plots B and A, respectively) indicate halide elimination from the monoanion and dianion is rate determining.

Formation of quinone **4** and the hydroxy-substituted hydroquinone species **2a** during hydrolysis requires the presence of a quinone methide intermediate. Such an intermediate would be capable of ketonization[6f-h,7,9] and nucleophile trapping[7,9] resulting in **4** and **2c**, respectively. To confirm the presence of a steady-state quinone methide intermediate, kinetic studies of **2a** hydrolysis were carried out in pH 7 buffer containing 2-mercaptoethanol (RSH) with [**2a**] = 3×10^{-5} M [RSH] = 3×10^{-4} to 3×10^{-3} M. The rate constants for **2a** hydrolysis are independent of [RSH] and are essentially the same as that measured for **2a** hydrolysis in pH 7 buffer. Spectral studies of completed reactions, containing 2-mercaptoethanol, revealed the presence of hydroquinone species (**2d**) and the absence of the ketonization product **4**. Identical results were obtained with the benzimidazole[7] and imidazo[4,5-*g*]quinazoline[9] based quinone methides. From these findings it is apparent that **2a**$^-$ eliminates bromide in a rate-determining step and the resulting intermediate traps the mercaptide nucleophile (RS$^-$) in a fast step.

The mechanism of **2a,b** hydrolysis is now summarized below. Leaving-group elimination from **2**$^-$ affords zwitterion **3**′, which may then tautomerize to quinone methide **3**. Either **3**′ or **3** can act as nucleophile traps resulting in **2c,d** formation. Leaving-group elimination from **2**$^{2-}$ to afford the quinone methide **3**$^-$ is associated with large rate constants. The unfavorable electrostatic influence of the two anions of **2**$^{2-}$ no doubt facilitates leaving group elimination. Protonation of **3**$^-$ at the exocyclic methylene affords the ketonized product **4**. The constant ratio of [**2c**]/[**4**] ≃ 1 observed at all reaction pH values is explained by the mechanism in Scheme II. This mechanism shows **2c** and **4** formation occurring by parallel pH–rate profiles. The pH independence of the product ratio is readily apparent by expressing the ratio in terms of constants and reactants—the proton activity terms cancel out (eq 4).

$$1 \sim \frac{[2c]}{[4]} = \frac{[H_2O]k_3}{K_{a_3}k_4} \quad (4)$$

Comparisons are made in the following paragraphs between the present quinone methide system and the previously studied benzimidazole[7] and imidazo[4,5-*g*]-quinazoline[9] quinone methides (**8** and **9**, respectively, in eq 5). In contrast to **8** and **9**, the present quinone methide



(5)

system does not trap the chloride employed to hold ionic strength (see ref 7 and 9 for descriptions of chloride trapping studies). The Reactivity–Selectivity Principle[15] is invoked to explain this observation. Many reactions adhere to this principle including the trapping of carbocations by nucleophiles. Highly reactive carbocations, for

(15) Pross, A. *Adv. Phys. Org. Chem.* **1977**, *14*, 69.

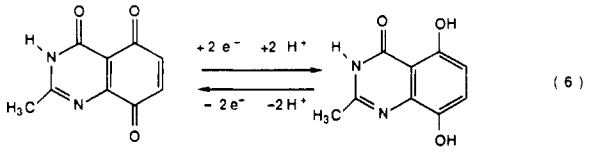
example, are unselective and do not distinguish between good nucleophiles and the water employed as solvent.[16] Relatively stable carbocations, on the other hand, can select the most nucleophilic species in solution.

According to the above principle, the relative stabilities of the quinone methides is in the order 8 > 9 > 3. Quinone methide 8 is somewhat stable since it selects chloride over the poorer nucleophile water[17] while quinone methide 9 traps both water and chloride. The present quinone methide system may be somewhat unstable since it traps the most abundant nucleophile water. However, all three quinone methides will trap the excellent nucleophile 2-hydroxyethyl mercaptide[18] in aqueous solutions.

Trapping of 8 and 9 by weak nucleophiles (water and chloride) is a reversible process whereas ketonization to the corresponding quinones is irreversible. Thus, the reaction of 8 and 9 in aqueous buffer ultimately provides quantitative yields of the corresponding quinones. In contrast, the present quinone methide system traps water irreversibly (the product **2c** is stable in aqueous buffers at pH values > 6). The observations noted above can be explained by considering the two-electron reduction potentials of the quinones arising from quinone methide ketonization. The quinazoline quinone 4 possesses a high reduction potential (vide infra, Electrochemistry), indicating the hydroquinone form of 4 ($4H_2$) as well as the derivative **2c** are more stable than 4. Thus, **2c** represents the thermodynamically favored product of quinone methide hydrolysis in aqueous buffer. The benzimidazole and imidazo[4,5-*g*]quinazoline quinones, on the other hand, possess much lower reduction potentials than 4.[8] As a consequence, the trapping of 8 and 9 by weak nucleophiles tends to be reversible, and the ketonized products are thermodynamically favored.

The relationship between quinone redox potential and quinone methide reactivity described above is further substantiated by the chemistry of the reductive alkylating agent daunomycin. The quinone methide derived from daunomycin effectively traps electrophiles[6f,g] (protons and benzaldehyde) to afford low-potential quinones. Nucleophilic trapping of the quinone methide on the other hand, affords highly unstable hydroquinones.[6h-j]

Electrochemistry. Electrochemical studies of the two-electron couple $4/4H_2$ (eq 6) provided insights into the relative stability of nucleophile trapping and ketonization products arising from quinone methide 3. The high



4 $4H_2$ (6)

potentials of this couple (E_0 = 805 mV, NHE) indicate stabilization of the hydroquinone form. Thus, nucleophile trapping of quinone methide 3 to afford hydroquinones is thermodynamically favored over ketonization. The electrochemical studies also contributed to an ongoing electrochemical survey of heterocyclic quinones. With a knowledge of the effect of fused heterocycles on quinone potentials, reductive alkylating agents can be designed possessing specific redox potentials and quinone methide reactivity.

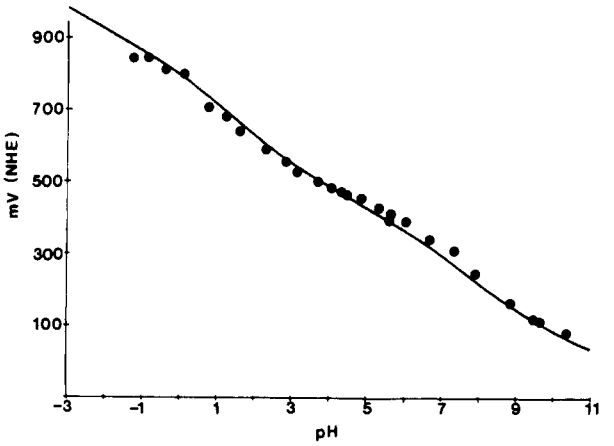


Figure 2. E_m vs pH data for the two-electron couple $4/4H_2$ measured at 25–26 °C in anaerobic buffer (μ = 1.0, $NaClO_4$). The solid curve was generated by employing the Nernst equation.

Table II. Quinone and Hydroquinone pK_a Values Determined for $4/4H_2$ in μ = 1.0 (KCl) Buffer at 30 °C

entry	acid dissociation	acid species	pK_a
1	$4H^+ \rightleftarrows 4 + H^+$	N(1)-protonated quinone	−0.05 ± 0.02
2	$4 \rightleftarrows 4^- + H^+$	N(3)-H of quinone	6.79 ± 0.05
3	$4H_3^+ \rightleftarrows 4H_2 + H^+$	N(1)-protonated hydroquinone	2.80 ± 0.08
4	$4H_2 \rightleftarrows 4H^- + H^+$	5-OH of hydroquinone	8.33 ± 0.05
5	$4H^- \rightleftarrows 4^{2-} + H^+$	8-OH of hydroquinone	~11
6	$4^{2-} \rightleftarrows 4^{3-} + H^+$	N(3)-H of hydroquinone	>14

Electrochemical redox potentials for $4/4H_2$ were determined, as a function of pH, by employing conventional cyclic voltammetry with a glassy carbon electrode (see the Experimental Section). The measurements were carried out in anaerobic μ = 1.0 ($NaClO_4$) aqueous buffer over the pH range −1.3 to 10.3 at 25–26 °C. Base-catalyzed hydrolysis of 4 above pH 6 is slow enough to permit potential measurements at basic pH values. The cyclic voltammograms are quasi-reversible[19] in nature and highly symmetric ($\alpha \sim 0.5$).

Found in Figure 2 is a plot of the two-electron potentials (E_m) for $4/4H_2$ vs pH. The solid line in this figure was computer generated from the Nernst equation[20] employing the E_m vs pH data and the pK_a values of 4 and $4H_2$ found in Table II. Fitting these data to the Nernst equation requires the hydroquinone species to have two more acid dissociations than the quinone species. Acid dissociations from the protonated and neutral quinone were measured spectrophotometrically, entries 1 and 2 in Table II. Only two of the four acid dissociations from the hydroquinone could be measured spectrophotometrically, entries 3 and 4 in Table II. The acid dissociations to afford the hydroquinone dianion (entry 5) and trianion (entry 6) are estimated values.[21]

Previous studies[14] of the influence of the fused quinazolin-4(3*H*)-one ring system on benzoquinone redox potentials revealed that this ring exerts two opposing effects:

(16) (a) Swain, C. G.; Scott, C. B.; Lohmann, K. H. *J. Am. Chem. Soc.* **1953**, *75*, 136. (b) Sneen, R. A.; Carter, J. V.; Kay, P. S. *J. Am. Chem. Soc.* **1966**, *88*, 2594. (c) Raber, D. J.; Harris, J. M.; Hall, R. E.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1971**, *93*, 4821.

(17) Chloride is a better nucleophile than water: Wells, P. R. *Chem. Rev.* **1963**, *63*, 171 (Table XXXI, p 212).

(18) 2-Hydroxyethyl mercaptide is an excellent electrophilic center trap: Ritchie, C. D.; Gandler, J. *J. Am. Chem. Soc.* **1979**, *101*, 7318.

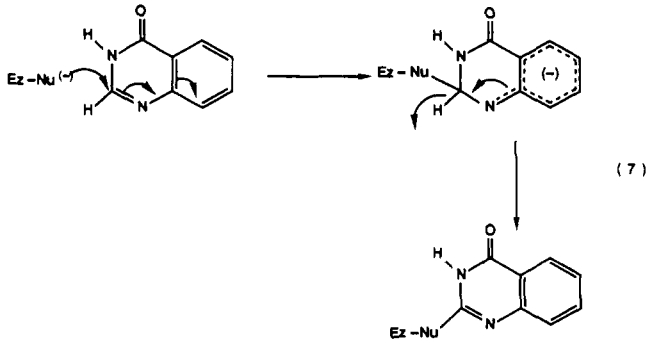
(19) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; pp 227–231.

(20) (a) Clark, W. M. *Oxidation Reduction Potentials of Organic Systems*; Williams and Wilkins: Baltimore, 1960; p 118. (b) Eberlein, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 6685.

(21) The pK_a in entry 5 of Table II is based on the pK_a values for hydroquinone dianion formation found in Table I. An exact value for the pK_a entry 6 is not necessary since it falls outside the pH range studied.

stabilization of the quinone by electron release and stabilization of the hydroquinone by internal hydrogen bonding (i.e. 2^- in eq 3). Indeed, benzimidazole and imidazo[4,5-*g*]quinazoline based quinones possess similar E_m values over a wide pH range (E_0 = 601 and 595 mV, respectively) due to the balancing of these opposing effects.[8,14] The Nernst fit in Figure 2 provides, at a glance, the redox potentials of the various ionic and neutral forms of the couple $4/4H_2$. The inflections in the curve correspond to the quinone and hydroquinone pK_a values shown in Table II. The opposing effects of the quinazolin-4-(3*H*)-one ring on redox potentials is evident from the E_7 of $4/4H_2$, 300 mV, which is nearly the same as the E_7 of benzoquinone, 287 mV.[22] At pH 7, 4 is present as the anion (4^-) and should possess a much lower redox potential than benzoquinone.[23] However, the internal hydrogen bonding of the hydroquinone $4H_2$ offsets this effect by raising the redox potential. The presence of high-potential protonated forms of $4/4H_2$ at low pH values results in E_m values much higher than those of benzoquinone (E_0 for $4/4H_2$ = 805 mV as opposed to E_0 for benzoquinone = 699 mV[24]).

Xanthine Oxidase Inactivation. Previous work in this laboratory demonstrated that the purine-like imidazo-[4,5-*g*]quinazoline reductive alkylating agents could enter and alkylate the active site of this enzyme.[9] We have also shown that the enzyme oxidizes quinazoline substrates by nucleophile transfer to the substrate and hydride (or its equivalent) transfer to the active-site molybdenum center (eq 7).[13] Hydrolysis of the resulting adduct then affords the 2-oxo quinazoline. The above findings suggested that



(7)

the quinazoline reductive alkylating agent described here would also inactivate the enzyme, perhaps by quinone methide trapping of the nucleophile involved in catalysis.

Inactivation of the enzyme by the reduced reductive alkylating agents **2a,b** is illustrated in Figure 3. The control is a plot of V_{max} for enzymatic oxidation of xanthine versus the concentration of enzyme. After anaerobic incubation of the enzyme with **2a,b**, V_{max} vs [XO] plots possess positive *x* intercepts as well as much lower velocities than the control. These observations indicate that hydroquinones **2a,b** are irreversibly inactivating the enzyme (the plots would pass through the origin if inactivation is reversible).[25] A probable mechanism of inactivation is formation of the quinone methide species from **2a,b** followed by trapping of the nucleophile involved in catalysis. In fact, the rate of enzyme inactivation parallels the rate of quinone methide formation from **2b** (see the Experimental Section).

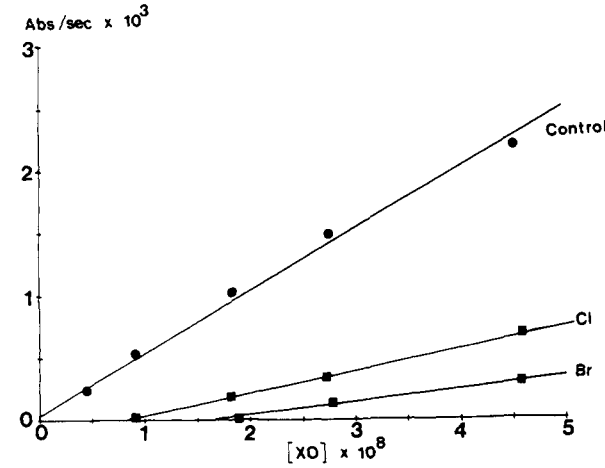
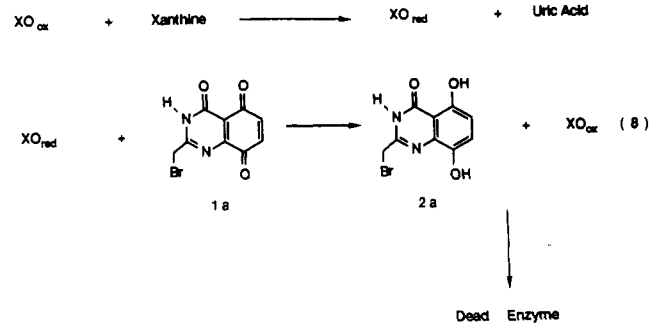


Figure 3. Plots of velocities of uric acid formation (at 290 nm) vs [XO] obtained in 0.05 M pH 7.40 phosphate buffer (μ = 0.1, KCl) containing 22 μM EDTA at 30 °C: Control, native enzyme only; Cl, after treatment with 68.5 μM **2b** for 3 h under anaerobic conditions: Br, after treatment with 68.5 μM **2a** for 1 h under anaerobic conditions.

It has been shown that reduced xanthine oxidase will transfer electrons to imidazo[4,5-*g*]quinazoline based quinones.[9] Thus, the reduced enzyme can activate imidazo[4,5-*g*]quinazoline reductive alkylators, thereby causing its irreversible inactivation. As discussed below in conjunction with eq 8, the quinazoline reductive alkylating agents do likewise.



Incubation of 51 μM **1a** with 0.01 μM xanthine oxidase under aerobic conditions results in no loss of xanthine–oxygen reductase activity. The same experiment, carried out in the presence of 51 μM xanthine under anaerobic conditions, results in complete loss of activity. Under anaerobic conditions, the reduced enzyme (XO red), formed upon oxidation of xanthine to uric acid, transfers electrons to **1a** rather than the normal electron-acceptor oxygen. The resulting hydroquinone **2a** then inactivates the enzyme as discussed above.

Conclusions

This study demonstrates the feasibility of designing reductive alkylating agents based on the quinazoline ring system (e.g. **1a,b**). Mechanistic details are provided for quinone methide (**3**) formation from the reduced reductive alkylators **2a,b**. Electrochemical studies of the two-electron couple $4/4H_2$ provided insights into fate of quinone methide **3** (nucleophile trapping vs ketonization). Finally, the reductive alkylation of xanthine oxidase is demonstrated with the title alkylators.

Quinone methide formation from **2a,b** occurs by halide elimination from the hydroquinone monoanion and dianion. Nucleophile-trapping studies and the formation of a ketonization product **4** indicate the presence of a steady-state quinone methide species.

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(23) Electron-rich quinones possess low redox potentials: Becker, H.-D. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: New York, 1979; Chapter 7.
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(25) Segel, I. H. *Enzyme Kinetics*; Wiley: New York, 1975; p 128.

It is concluded that the fate of quinone methides in general can be predicted from the redox potential of the quinone resulting from quinone methide ketonization. If a low-potential quinone is the ketonization product, quinone methide ketonization is thermodynamically favored over nucleophile trapping. The opposite is true if a high redox potential quinone such as **4** results from quinone methide ketonization.

The ability of quinazolines to enter the xanthine oxidase active site and the presence of an active-site nucleophile near the alkylating center of **2a,b** likely account for enzyme inactivation by these hydroquinones. The quinone **1a** acts as an oxidizing suicide substrate: **1a** oxidizes reduced enzyme and the resulting hydroquinone (**2a**) inactivates the enzyme. It is concluded that xanthine oxidase may likewise activate other quinazoline reductive alkylating agents targeted toward cellular structures important to the cancer cell. The design of other quinazoline reductive alkylating agents is currently under study in this laboratory.

Experimental Section

Elemental analyses were performed by Desert, Analytics, Tucson, AZ. All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some of these compounds still contained water of crystallization that was determined from the elemental analyses found. No elemental analysis was obtained for quinone **4**; spectral data support the assigned structure, and the hydroquinone form ($4H_2$) was fully characterized. Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. All TLC's were run with Merck silica gel 60 (F_{254}) plates employing a variety of solvents. IR spectra were taken as KBr pellets or thin films, employing a Nicolet MX-1 FT IR spectrophotometer; the strongest IR absorbances are reported. ^{1}H NMR spectra were obtained with a Bruker WH-90 spectrometer. The ^{1}H NMR spectra of **2c** was obtained with a Bruker AM-400 narrow-bore spectrometer employing very dry dimethyl-d_6 sulfoxide as solvent. It was thus possible to make chemical shift assignments for all the protons of **2c** (vide infra).

pK_a constants were determined by spectrophotometric titration in μ = 1.0 (KCl) aqueous solvent at 30 ± 0.2 °C with a Perkin-Elmer 559 or a Lambda-3 spectrometer. Measurements were usually carried out under aerobic conditions; acid dissociations from hydroquinones in strong base were measured under an argon atmosphere with Thunberg cuvettes. Details of the methodology employed are found in a previous publication.[26]

Kinetic Studies of Hydrolysis. The hydrolytic studies of **2a,b** were carried out in aqueous buffer at 30.0 ± 0.2 °C under an argon atmosphere with Thunberg cuvettes. A dimethyl sulfoxide stock of **2a,b** was placed in the top port, and the aqueous buffer was placed in the bottom port. After a stream of purified argon was passed into each port for 30 min, the cuvette was sealed and equilibrated at 30 °C in a thermostated cell holder for 20 min. The ports were then mixed, and absorbance vs time data were obtained with a Perkin-Elmer 559 or a Lambda-3 UV–vis spectrophotometer. These data were computer fit to a first-order rate law.

Electrochemistry. Determination of E_m values was carried out with a BAS 27 voltamograph. The working electrode material was glassy carbon, and the reference electrode was Ag/AgCl, which was calibrated with a Calomel electrode. Measurements were carried out in μ = 1.0 ($NaClO_4$) aqueous buffer at 25–26 °C under an atmosphere of argon by employing scan speeds of 100 mV s^{-1}. The midpoint potential E_m was determined from the average of the anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) potentials.

Nernst Fit. For the redox couple $4/4H_2$, 26 E_m determinations were made over the pH range studied. For each E_m value, an E_0 value was calculated from the Nernst equation,[20] containing the acid dissociation constants in Table II and the proton activity determined with a pH meter. The average of all E_0 determinations (805 mV) was substituted into the Nernst equation, with which the solid curve in Figure 2 was generated.

Enzyme Inhibition Studies. Inhibition studies were carried out in 0.05 M, pH 7.40 phosphate buffer (μ = 0.1, KCl) containing 22 μM ethylenediaminetetraacetic acid (EDTA). The approximate molarity of enzyme was determined from an absorbance measurement at 450 nm and the ϵ_{450} value of 37 800 M^{-1} cm^{-1} per enzyme-bound FAD.[27] The xanthine oxidase employed, grade III (Sigma), is chromatographically pure and is reported to contain 10 units/mL. Stock solutions of the **2a,b** and quinone **1a** were prepared in dimethyl sulfoxide. The hydroquinones decompose slowly in this solvent, and stock solutions were always prepared fresh for an inhibition study. Anaerobic incubation of **2a,b** and **1a** with xanthine oxidase were carried out in Thunberg cuvettes degassed with argon (loc. cit., Kinetic Studies of Hydrolysis). After an incubation, the mixture was aerated, and uric acid formation followed at 290 nm in the presence of 6 μM xanthine. Comparisons of enzyme activities with controls permitted assessments to be made concerning the irreversible nature of inhibition (see Figure 3). The loss of enzyme activity in the presence of **2b** is first-order in nature; the k_{obsd} value is approximately the same as that for quinone methide formation from **2b**. The data shown in Figure 3 were obtained after the inactivation process was complete (time needed for complete hydrolysis of **2a,b**).

Synthesis and physical properties of new compounds are provided below.

2-(Phenoxymethyl)-5,8-dimethoxyquinazolin-4(3*H*)-one (6). To 1 g (5.1 mmol) of **5**[28] was added 3 mL of phenoxyacetyl chloride, and the resulting slurry was stirred for 1 h at room temperature. The completed reaction was combined with 5 mL of ethanol; crystallization of 2-(phenoxyacetamido)-3,6-dimethoxybenzamide occurred upon addition of water (10 mL) to this solution. The product was filtered, washed with water and benzene, and then dried: 1.4 g (83%) yield; mp 198–200 °C; IR (KBr) 3388, 1697, 1648, 1500, 1486, 1435, 1263, 1247, 1060, 758 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.20 (1 H, s, acetamide NH), 7.41–6.94 (7 H, complex multiplets, aromatic), 4.58 (2 H, s, methylene), 3.74 and 3.71 (6 H, 2 s, methoxys). Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.58; H, 5.30; N, 8.46.

The compound obtained above was converted to **6** by the following procedure. To a solution of 2-(phenyoxyacetamido)-3,6-dimethoxybenzamide (1 g, 3.03 mmol) in 50 mL of ethanol was added 150 mL of 20% NaOH. The resulting solution was refluxed for 24 h and then cooled to room temperature. Neutralization of the reaction mixture with concentrated HCl resulted in the crystallization of **6**. Recrystallization was carried out by dissolving the product in a large volume of chloroform, evaporating the solution to a small volume, and then adding hexane: 0.83 g (88%) yield; mp 242–245 °C; TLC ($CHCl_3$, ethanol [95:5]) R_f = 0.5; IR (KBr) 1680, 1596, 1484, 1267, 1247, 1220, 1175, 1155, 1091, 1078 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.41–6.89 (7 H, complex multiplets, aromatic), 4.93 (2 H, s, acetamido methylene), 3.81 and 3.80 (6 H, 2 s, 5,8-dimethoxy); mass spectrum (EI), m/z 312 (P^+). Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.05; H, 5.00; N, 8.94.

2-(Bromomethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (2a). Combination of 1 g (3.30 mmol) of **6** in 35 mL of dry benzene was followed by addition of 1 mL of BBr_3. The reaction mixture was heated at reflux for 90 min, cooled, and then evaporated to dryness. The residue was recrystallized twice from aqueous ethanol: 586 mg (68%) yield; mp 211–212 °C; TLC (chloroform, methanol [9:1]) R_f = 0.60; IR (KBr) 3471, 3232, 1690, 1680, 1586, 1470, 1222, 805, 641 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.16 and 6.75 (2 H, AB system, J = 8.8 Hz, 6-H and 7-H), 4.40 (2 H, s, bromomethyl); mass spectrum (EI), m/z 270 (P^+, ^{79}Br), 272 (P^+, ^{81}Br), 191 (P^+ – Br). Anal. Calcd for $C_9H_7BrN_2O_3$: C, 39.88; H, 2.90; N, 10.33. Found: C, 40.22; H, 2.72; N, 9.68.

2-(Chloromethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (2b). A mixture consisting of 103 mg (0.38 mmol) of **2a** in 5 mL of concentrated HCl was stirred at room temperature for 22 h. The

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solid was filtered off and dried: 80 mg of the hydrate (87%) yield; TLC (chloroform, methanol [9:1]) R_f = 0.56; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.17 and 6.75 (2 H, AB system, J = 9 Hz, 6-H and 7-H), 4.55 (2 H, s, chloromethyl); mass spectrum, m/z 226 (P^+), 191 (P^+ – Cl). Anal. Calcd for $C_9H_7ClN_2O_3 \cdot 0.75H_2O$: C, 45.01; H, 3.56; N, 11.66. Found: C, 44.83; H, 2.82; N, 11.21. The hydrogen percentage obtained experimentally deviates widely from the theoretical value. The ^{1}H NMR and mass spectra indicate that the material is pure and the assigned structure correct, however.

2-(Hydroxymethyl)-5,8-dihydroxyquinazolin-4(3*H*)-one (2c). To a mixture consisting of dimethyl sulfoxide (10 mL) and water (2 mL) was added 33 mg (0.12 mmol) of **2b**. The reaction mixture was degassed at room temperature for 30 min with N_2 and then heated at 55 °C under an N_2 atmosphere for 10 h. The completed reaction was extracted with ethyl acetate to remove **2c**. Evaporation of the extracts to a residue in vacuo was followed by addition of 1 mL of ethanol. Crystallization of **2c** occurred upon addition of 5 mL of water: 16 mg (64%) yield; TLC (butanol, acetic acid, water [5:2:3]) Rf = 0.64; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 12.35 (1 H, br s, 5-OH), 10.98 (1 H, br s, 8-OH), 8.96 (1 H, br s, N(3)-H), 7.13 and 6.69 (2 H, AB system, J = 9 Hz, aromatic), 5.42 (1 H, br s, OH of 2-hydroxymethyl), 4.42 (2 H, s, methylene of 2-hydroxymethyl); mass spectrum (EI), m/z 208 (P^+).

2-(Bromomethyl)quinazoline-4,5,8(3*H*)-trione (1a). To an ice bath chilled mixture of 50 mg (0.184 mmol) **2a** in 1 mL of dry methanol was added 70 mg (0.308 mmol) of DDQ. The reaction mixture was stirred for 30 min with continued chilling. Crystallized **1a** was filtered off and washed with diethyl ether. Recrystallization of **1a** was carried out by dissolution in hot acetone followed by addition of hexane: 15.8 mg of the hydrate (32%) yield; mp 177–181 °C dec; TLC (chloroform, methanol [9:1]) R_f = 0.09; IR (KBr) 3182, 3156, 3070, 1722, 1701, 1677, 1569, 1546, 1465, 1104 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.00 and 6.87 (2 H, AB system, J = 10.4 Hz, aromatic), 4.42 (2 H, s, bromomethyl); mass spectrum (EI), m/z 268 (P^+, ^{79}Br) 270 (P^+, ^{81}Br). Anal. Calcd for $C_9H_5BrN_2O_3 \cdot 0.5H_2O$: C, 39.16; H, 1.46; N, 10.16. Found: C, 40.34; H, 1.89; N, 9.92.

2-Methyl-5,8-dimethoxyquinazolin-4(3*H*)-one (7). A solution of 325 mg (1.65 mmol) of **5**[28] in 15 mL of acetic anhydride was stirred at room temperature for 3 h. Acetylated **5** formed as a white precipitate, which was filtered and dried under vacuum: 310 mg (79%) yield; mp 195–196 °C. Anal. Calcd for $C_{11}H_{14}N_2O_4 \cdot 0.25H_2O$: C, 54.42; H, 6.64; N, 11.53. Found: C, 54.30; H, 6.11; N, 11.59.

The acetylated derivative (90 mg, 0.378 mmol) was combined with 20% aqueous NaOH (20 mL) and refluxed for 4 h. The reaction mixture was then diluted with 50 mL of water and adjusted to pH 6 with acetic acid. The cyclized product **7** crystallized from solution upon chilling: 70 mg (84%) yield; mp 264–265 °C; TLC (chloroform, methanol [9:1]) R_f = 0.26; IR (KBr) 3317, 2990, 2903, 1689, 1635, 1581, 1489, 1329, 1268, 1178, 1094, 815 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.23 and 6.85 (2 H, AB system, J = 9 Hz, aromatic), 3.80 and 3.77 (6 H, 2 s, 5,8-dimethoxy) 2.29 (3 H, s, 2-methyl). Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.92; H, 5.62; N, 12.53. Found: C, 60.01; H, 5.42; N, 12.72.

2-Methyl-5,8-dihydroxyquinazolin-4(3*H*)-one ($4H_2$). A solution of **7** (13 mg, 0.059 mmol) in 1 mL of 48% HBr was heated at 150 °C for 3 h. The reaction mixture was then cooled to room temperature, resulting in crystallization of $4H_2$ as the hydrobromide salt: 9.5 mg (86%) yield; mp 277–280 °C dec; TLC (chloroform, methanol [9:1]) R_f = 0.45; IR (KBr) 3360, 3025, 1661, 1500, 1362, 1204 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 10.89 (1 H, br s, amide proton), 7.32 and 6.87 (2 H, AB system, J = 8.8 Hz, aromatic), 2.58 (3 H, s, 2-methyl); mass spectrum (EI), m/z 192 (P^+). Anal. Calcd for $C_9H_8N_2O_3 \cdot HBr$: C, 39.58; H, 3.32; N, 10.26. Found: C, 39.36; H, 3.30; N, 10.13.

2-Methylquinazoline-4,5,8(3*H*)-trione (4). To an ice bath chilled mixture of $4H_2 \cdot HBr$ (70 mg, 0.256 mmol) in 1 mL of methanol was added 88 mg (0.387 mmol) of DDQ. The reaction was stirred at ice-bath temperature for 30 min and then diluted with ~10 mL of ethyl acetate. Crystallized **4** was filtered off and washed with ethyl acetate: 35.8 mg (74%) yield; mp 165–168 °C dec; TLC (chloroform, methanol [9:1]) R_f = 0.19; IR (KBr) 1700, 1683, 1577, 1548, 1481, 1107 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 6.97 and 6.84 (2 H, AB system, J = 10.4 Hz, aromatic), 2.42 (3 H, s, 2-methyl); mass spectrum (EI), m/z 190 (P^+).

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Registry No. **1a**, 117526-26-8; **2a**, 117498-05-2; **2b**, 117498-06-3; **2c**, 117498-07-4; **4**, 117498-11-0; **4H$_2$**, 117498-10-9; **5**, 98991-68-5; **5** (acetyl deriv.), 117498-08-5; **6**, 117498-04-1; **7**, 117498-09-6; 2-(phenoxyacetamido)-3,6-dimethoxybenzamide, 117498-03-0; xanthine oxidase, 9002-17-9.

Notes

Effect of Conjugation on the Rates of the Acid-Catalyzed Hydrolyses of Acetals

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The hydrolyses of acetals and ketals have been extensively investigated.[1] Our recent studies[2] have focused on the effect of acetal structure on the importance of general acid catalysis (*i.e.*, on the relationship between structure and the Brønsted α). For general acid catalysis to be observable for a diethyl acetal/ketal, the structure must be such as to render the acetal/ketal quite reactive. One of the structural features that accomplishes this is conjugation of the reaction center with an aromatic group not containing an electron-withdrawing group. For example, the diethyl acetal of benzaldehyde is 2 orders of magnitude more reactive than the diethyl acetal of acetaldehyde, and general acid catalysis is barely discernable for the former[2a] and has never been observed for the latter.[1]

The question that has generated all this attention is: Does the lack of observing general acid catalysis imply a different mechanism of hydrolysis or does is simply reflect experimental limitations put in place by Brønsted α values approaching unity? The importance of providing a definitive answer to this matter has been diminished by the

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