34.7, 33.8, 32.3, 25.8, 24.8, 22.5, 13.6.

Acknowledgment. We are grateful for support of this research by the National Institutes of Health (GM-31750) and by the Robert A. Welch Foundation (F-626).

Registry No. **1a**, 65253-04-5; **1b**, 80595-59-1; **1c**, 104197-95-7; **1d**, 84312-20-9; **2a**, 2216-51-5; **2d**, 26315-61-7; **3a**, 75419-02-2; **3b**, 104197-96-8; **3c**, 104197-82-2; **3d**, 104264-93-9; (2*S*)-**3e**, 104197-83-3; (2*R*)-**3e**, 104264-95-1; **4a**, 104197-76-4; **4b**, 104197-97-9; **4c**, 104198-04-1; **4d**, 104198-12-1; **4e**, 104197-84-4; **5a**, 104197-77-5; **5b**, 104197-98-0; **5c**, 104198-05-2; **5d**, 104198-13-2; (2*S*)-**5e**, 104197-85-5; (2*R*)-**5e**, 104264-96-2; **6a**, 5947-19-3; **6b**, 104197-99-1; **6c**, 104198-06-3; **6d**, 104213-46-9; **6e** (isomer 1), 104197-86-6; **6e** (isomer 2), 104264-97-3; **7a**, 104197-78-6; **7b**, 104198-00-7; **7c**, 104198-07-4; **7d**, 104198-14-3; **7e** (isomer 1), 104197-87-7; **7e** (isomer 2), 104264-98-4; **8a**, 104197-79-7; **8b**, 104198-01-8; **8c**, 104198-08-5; **8d**, 104213-47-0; (2*S*)-**8e**, 104197-88-8; (2*R*)-**8e**, 104264-99-5; **9a**, 104197-80-0; **9d**, 104198-15-4; (2*S*)-**9e**, 104197-89-9; (2*R*)-**9e**, 104265-00-1; **10a**, 2035-93-0; **10b**, 104198-02-9; **10c**, 104198-10-9; **10d**, 99457-86-0; **10e** (isomer 1), 104197-90-2; **10e** (isomer 2), 104265-01-2; **11a**, 104197-81-1; **11b**, 104198-03-0; **11c**, 104198-11-0; **11d**, 104198-16-5; **11e** (isomer 1), 104197-91-3; **11e** (isomer 2), 104265-02-3; **12a**, 2362-61-0; **12b**, 104197-93-5; **12c**, 104197-94-6; **12d**, 98779-11-4; **12e** (isomer 1), 104264-94-0; **12e** (isomer 2), 104265-03-4; **13a**, 6531-86-8; **13d**, 104198-17-6; **13e** (isomer 1), 104197-92-4; **13e** (isomer 2), 104265-04-5; **14**, 5682-83-7; **15**, 30614-39-2; **16** (isomer 1), 104198-20-1; **16** (isomer 2), 104198-21-2; **17**, 104265-05-6; $SnCl_4$, 7646-78-8; (1*R*,2*S*,5*R*)-2-*tert*-butyl-5-methylcyclohexanone, 56782-80-0; methyllithium, 917-54-4; (*R*)-(+)-pulegone, 89-82-7; 1-bromoacetic acid, 79-08-3; 1-hexene, 592-41-6; glyoxylic acid, 298-12-4; bromobenzene, 108-86-1; cyclohexene oxide, 286-20-4; para-fluorophenyl bromide, 460-00-4; meta-bromoanisole, 2398-37-0; benzylmagnesium chloride, 6921-34-2; cyclohexanone, 108-94-1; benzaldehyde, 100-52-7; phenylmagnesium chloride, 100-59-4; *cis*-2-diphenylmethylcyclohexanol, 104198-18-7; *cis*-2-diphenylmethylcyclohexyl bromoacetate, 104198-19-8; (1*R*,2*S*,5*R*)-2-(1-methyl-1-cyclohexylethyl)-5-methylcyclohexanol acrylate, 104198-09-6; acryloyl chloride, 814-68-6; 2-cyclohexenone, 930-68-7; *trans*-1-phenyl-1,3-butadiene, 16939-57-4; (±)-(4aα,8aβ)-decahydro-8β-phenyl-1α-naphthalenol, 104265-06-7; cyclohexyl bromide, 108-85-0; *trans*-2-cyclohexylcyclohexyl acrylate, 104198-22-3.

Supplementary Material Available: Experimental details for the preparation of and spectral data for **4a–e**, **6a–e**, **7a–e**, and **8a–e**; **5a–c** and **11a–c**; **9a,d,e**; **10b–e**; **13d,e**; and **14–17** (21 pages). Ordering information is given on any current masthead page.

Synthesis, Electrochemistry, and Xanthine Oxidase Substrate Reactivity of Imidazo[4,5-*g*]quinazoline-4,9-diones. Studies Directed toward the Design of Purine-like Reductive Alkylators

Chang-Hee Lee, James H. Gilchrist, and Edward B. Skibo*

Department of Chemistry, Arizona State University, Tempe, Arizona 85287

Received August 22, 1986

The synthesis of imidazo[4,5-*g*]quinazoline-4,9-diones related to hypoxanthine and xanthine, **1** and **2**, respectively, was carried out in conjunction with the design of quinone-like purine mimics. These derivatives may exhibit purine-like binding to enzymes as well as quinone-mediated reactions such as reductive alkylation. Potential reductive alkylators are represented by compounds possessing a leaving group in the 2α-position: 2-(methoxymethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (**1d**); the 2-(bromomethyl) derivative of **1d** (**1e**); and 2-(methoxymethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,6,8,9(3*H*,5*H*,7*H*)-tetrone (**2b**). Reduction of these systems, perhaps in low-potential tumor cells, should activate the leaving group and thereby facilitate the alkylation of purine-utilizing enzymes. Elaboration of the 4,9-dione (benzoquinone) moiety of **1** was carried out by either oxidation of 4-aminoimidazo[4,5-*g*]quinazoline derivatives with Fremy's radical or oxidation of 4,9-unsubstituted derivatives with nitrogen dioxide. The xanthine derivatives were prepared from **1** by xanthine oxidase mediated oxidation. A study of the enzymatic oxidation of **1** → **2** (pH 7.40) indicated that the associated catalytic parameters are comparable to the natural substrates, even though the hypoxanthine derivatives **1** exist largely in the anionic form and the natural substrates do not. Thus, the title quinones are purine mimics, at least in the case of xanthine oxidase oxidation. Comparative electrochemical studies of 2,3-dimethylimidazo-[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (**1b**) and 1,2-dimethylbenzimidazole-4,7-dione (**14**) provided insights into the influence of the fused pyrimidine ring on the quinone redox potential. The neutral fused pyrimidine ring has no effect on the potential whereas the anionic form (pK_a of **1b** is 6.15) lowers the potential. The expected low potentials for the title quinones at or above neutrality are desirable in terms of reductive alkylation; reduction will only occur in a low-potential environment. The electrochemical studies also revealed that a high-potential diprotonated quinone species (**1b**·H_2^{2+}) is present in strong-acid solutions. In hydrobromic acid solutions **1b**·H_2^{2+} readily oxidizes bromide to bromine, presumably by two-electron transfer from a bromo adduct. The conclusion of the study is that the design of reductive alkylators directed toward the active sites of purine-utilizing enzymes is feasible; preliminary studies suggest that this is indeed the case.

Although many imidazo[4,5-*g*]quinazolines have been reported in the literature,[1] there are no reports of derivatives bearing 4,9-dione substitution. The work of Leonard and co-workers[1b,2] has shown that analogues of this ring system are purine mimics in many enzymatic reactions. Their findings led to our interest in imidazo[4,5-*g*]-quinazoline-4,9-diones; such derivatives may exhibit purine-like binding to enzymes as well as some of the chemical properties of quinones. Thus, quinone-mediated reactions such as oxygen radical generation,[3] carbonyl addition,[4] and reductive alkylation[5] may be exploited for the

(1) (a) Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975**, *40*, 356. (b) Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. *J. Am. Chem. Soc.* **1976**, *98*, 3987. (c) Keyser, G. E.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 3529. (d) Leonard, N. J. *Heterocycles* **1979**, *12*, 129. (e) Alkhader, M. A.; Perera, R. C.; Sinha, R. P.; Smalley, R. K. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1056. (f) Schneller, S. W.; Christ, W. J. *J. Org. Chem.* **1981**, *46*, 1699. (g) For a review see: Preston, P. N.; Tennant, G. In *Benzimidazoles and Congeneric Tricyclic Compounds*, Part 1; Preston, P. N., Ed.; Wiley: New York, 1981; Chapter 5, pp 601–636.

(2) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128.

(3) Begleiter, A. *Cancer Res.* **1983**, *43*, 481.

(4) (a) Finley, K. T. In *The Chemistry of the Quinonoid Compounds*, Part II; Patai, S., Ed.; Wiley Interscience: New York, 1974; pp 950–963. (b) Horspool, W. M. *Q. Rev., Chem. Soc.* **1969**, *23*, 204.

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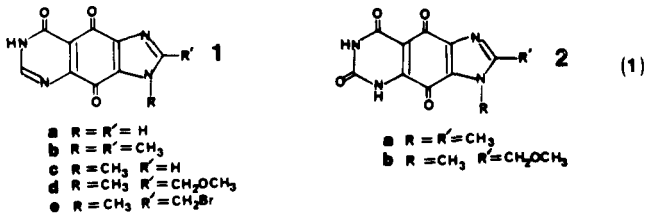
design of purine antimetabolites.

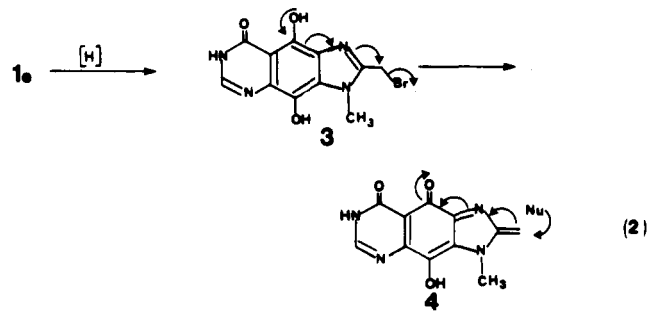
Reported here are the synthesis, electrochemistry, and xanthine oxidase substrate activity of imidazo[4,5-g]-quinazoline-4,9-diones related to hypoxanthine and xanthine, 1 and 2, respectively, in eq 1. The hypoxanthine



(1)

analogues (1) are excellent substrates for xanthine oxidase; catalytic parameters are comparable to those of the natural substrates. Indeed, the enzyme-catalyzed oxidation constitutes a convenient route to the xanthine analogues 2.

The substrate activity of the 4,9-dione derivatives suggests the feasibility of designing reductive alkylators directed toward the active sites of purine-utilizing enzymes. Potential reductive alkylating systems are represented by compounds **1d**, **1e**, and **2b** in eq 1. As with many naturally occurring[5] and synthetic[6] quinone antitumor agents, these systems may be activated as alkylating agents upon reduction. For example, reduction of **1e** to the hydroquinone **3** followed by 1,6 elimination of HBr will afford the reactive extended quinone methide **4** (eq 2).



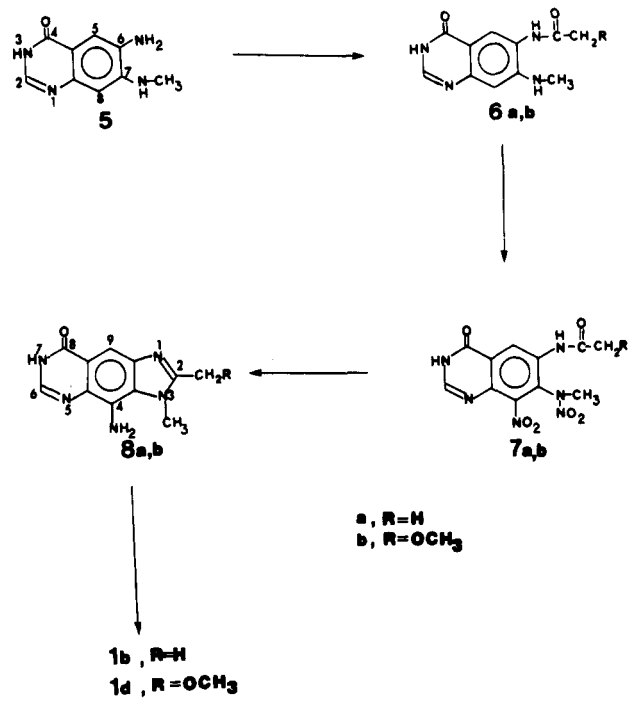
(2)

The extended quinone methide derived from the benzimidazole analogue of **3** has been documented and shown to be an effective nucleophile trap.[7] Thus, the potential reductive alkylators in eq 1 may alkylate some purine-utilizing enzymes upon enzymatic reduction in low reduction potential tumor cells.[8] The electrochemical studies described herein provided insights into the influence of the fused pyrimidine ring on the quinone reduction potential. The title quinones exhibit a high redox potential in acidic solutions and readily oxidize hydrobromic acid to bromine. The results of electrochemical and kinetic studies are used to postulate a mechanism for oxidation.

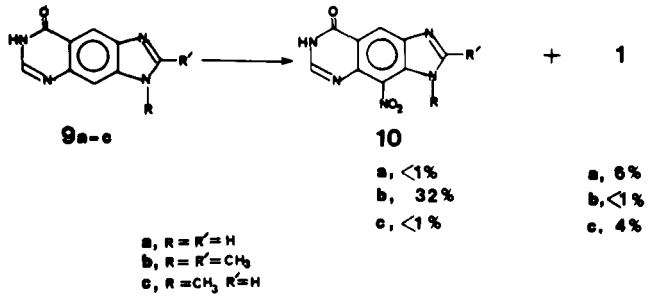
Results and Discussion

Synthesis. In a previous report we described the attempted preparation of the imidazo[4,5-g]quinazoline-4,9-dione system by imidazole ring annelation to a substituted quinazoline-5,8-dione;[9] an unusual carbonyl addition reaction afforded another product. As is described below, elaboration of the target compounds **1a–d** was carried out by Fremy radical oxidation[10] of 4-aminoimidazo[4,5-g]quinazoline derivatives (Scheme I) or by nitrogen dioxide oxidation of 4,9-unsubstituted derivatives (Scheme II). Neither approach afforded the xanthine derivatives **2**; these were prepared from the hypoxanthine derivatives by enzymatic oxidation (vide infra). Both synthetic approaches provide 4,9-dione derivatives with unambiguous $N(3)$-methyl placement. The methyl group serves both to prevent elimination of the leaving group from the 2α-position utilizing the $N(3)$ anion[11] and to act as a spectral model of the $N(3)$-ribosyl derivatives currently under study. Oxidations employing Fremy's radical are best suited for the preparation of 2-substituted derivatives. On the other hand, nitrogen dioxide oxidations are best suited for the preparation of 2-unsubstituted derivatives.

Scheme I



Scheme II



The reaction sequence found in Scheme I relies on the relative electron densities of positions in the quinazoline ring:[12] 8 > 6 > 5 > 7. Because the 6-amino group of **5** is located at an electron-rich center of the quinazoline ring,

(5) (a) Moore, H. W. *Science (Washington, D.C.)* **1977**, *197*, 527. (b) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.

(6) (a) Lin, A. J.; Cosby, L. A.; Shansky, C. W.; Sortorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1247. (b) Lin, A. J.; Pardini, R. S.; Cosby, L. A.; Lillis, B. J.; Shansky, C. W.; Sartorelli, A. J. *J. Med. Chem.* **1973**, *16*, 1268. (c) Lin, A. J.; Lillis, B. J.; Sartorelli, A. C. *J. Med. Chem.* **1975**, *18*, 917. (d) Lin, A. J.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1974**, *17*, 558. (e) Lin, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1976**, *19*, 1336. (f) Lin, T.-S.; Teicher, B. A.; Sartorelli, A. C. *J. Med. Chem.* **1980**, *23*, 1237.

(7) Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 522.

(8) (a) Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, A. C. *Biochem. Pharm.* **1980**, *29*, 1. (b) Lin, A. J.; Sartorelli, A. C. *Biochem. Pharm.* **1976**, *25*, 206.

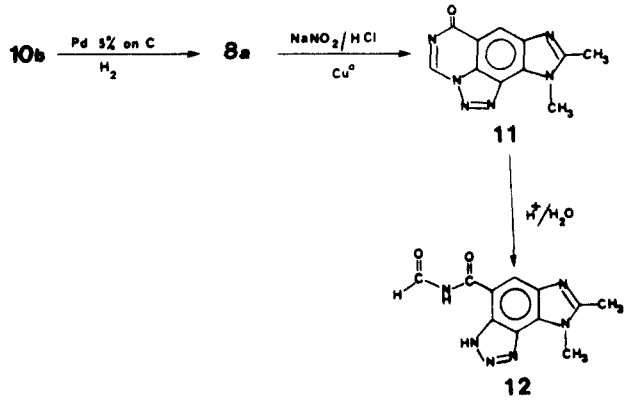
(9) Skibo, E. B. *J. Org. Chem.* **1985**, *50*, 4861.

(10) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

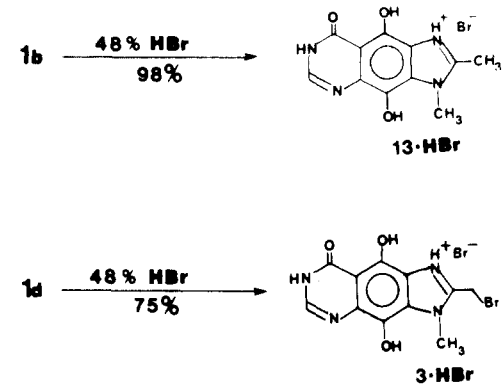
(11) Elimination utilizing the N(3) anion will afford a fused diazafulvene species: Holan, G.; Samuel, E. L. *J. Chem. Soc. C* **1967**, 25.

(12) Dewar, M. J. S.; Maitlis, P. M. *J. Chem. Soc.* **1957**, 2521.

Scheme III



Scheme IV



it should be readily acetylated. The 7-methylamino group, on the other hand, is at an electron-deficient center and should be less susceptible to acetylation. Thus, the monoacetylated derivatives **6a,b** could be obtained by treatment of **5** with acetic anhydride and methoxyacetyl chloride, respectively, under mild conditions. Evidence of only 6-acetamide formation was obtained from the ^1H NMR spectrum of **6a** (Me_2SO-d_6): the 7-methylamino substituent is split into a doublet, which collapses to a singlet upon addition of D_2O (see the Experimental Section). The nitration of **6a,b** under mild conditions afforded the dinitrated products **7a,b**. On the basis of the electron-rich nature of the 8-position, this is the more reasonable site for ring nitration. The cyclization study, described below in conjunction with Scheme III, showed that this is indeed the case. Evidence of N-nitration in **7a,b** was obtained from elemental analyses and the absence of a doublet for the 7-methylamino substituent in the ^1H NMR spectra (see the Experimental Section). We suspect that N-nitration occurs after ring nitration since nitroanilines are readily N-nitrated.[13] Catalytic reduction of **7a,b** and in situ base-catalyzed cyclization afforded **8a,b**, which were oxidized to **1b** and **1d**, respectively, with Fremy's radical.

The key feature of the approach discussed above is the isolation of **6a,b** without cyclization to the imidazo[4,5-*g*]quinazolines. This feature permits nitration under conditions that leave both the acetamido and methoxyacetamido groups intact. Nitration of imidazo[4,5-*g*]quinazolines, on the other hand, requires rather harsh conditions, which likely oxidize the 2-substituent (vide infra).

Attempted monoformylation of **5**, employing the mixed anhydride of formic and acetic acids in formic acid solvent at room temperature, provided the cyclized product **9a** and starting material. Apparently the monoformylated derivative rapidly cyclizes, even at room temperature. Thus, the approach outlined in Scheme I cannot be applied to 2-unsubstituted imidazo[4,5-*g*]quinazolines. However, treatment of the imidazo[4,5-*g*]quinazolines **9a–c** with a 90% nitric acid–acetic acid–sulfuric acid (3.6:1:1) mixture afforded very poor isolated yields of the respective quinones **1a–c** and 4-nitro derivatives **10a–c** (Scheme II). Cyclization of **6b** to the imidazo[4,5-*g*]quinazoline followed by the above treatment resulted in extensive decomposition.

The balance of mass at the conclusion of the reactions in Scheme II is largely starting material. Poor yields aside, this approach is the only route elucidated thus far to the 2-unsubstituted derivatives. The presence of oxidation and nitration products is highly dependent on the heating sequence and the ratio of acids employed. Reaction mixtures were stirred at room temperature for 2 days, then heated at 50–60 °C for 1 day, and finally refluxed for 12 h. If the reaction was simply refluxed for several days, only starting material was recovered. Even removal of acetic acid, or its replacement with acetic anhydride, with observance of the heating sequence, afforded only starting material. The long incubation times likely pertain to the slow buildup of the odd-electron species nitrogen dioxide, which would initiate oxidation by hydrogen atom abstraction.[14] Immediate heating will remove these gases as they are formed, resulting in the absence of product formation. Consistent with this view of the oxidation process, addition of $NaNO_2$[15] to a reaction containing **9c** gave **1c** (6% crude yield) and no **10c** after only 2 h of reflux.

The transformations depicted in Scheme III indicate that nitration occurs at the 4-position of imidazo[4,5-*g*]quinazolines. Reduction of **10b** gave an amine whose ^1H NMR spectrum is identical with that of **8a** (Scheme I). Evidence of 4-nitration was obtained by treatment of **8a** with $NaNO_2$ in the presence of Cu powder and HCl. If nitration had occurred at the 9-position, this treatment should provide the 9-chloro derivative by substitution of the intermediate diazonium salt.[16] On the other hand, the 4-diazonium salt could cyclize to **11** before chloride substitution occurs. The only product obtained from the reaction was **12**, which is a hydrolysis product of **11**.

It has been shown that hydrochloric and hydrobromic acids undergo reductive addition to some benzoquinone derivatives.[17] Hot hydrochloric acid had no effect on **1b** and is actually used as a recrystallization solvent. Treatment with hot 48% HBr, on the other hand, resulted in the evolution of bromine concomitant with crystallization of **13**·HBr in nearly quantitative yield (Scheme IV). A related reaction was observed by Moore and co-workers during hydrohalide-mediated dealkylations of substituted benzoquinones.[18] Mechanistic aspects of this reaction will be discussed under Electrochemistry. The HBr-mediated reduction discussed above provided a single-step route from **1d** to the potential activated alkylator **3** found in eq 2. Treatment of **1d** in refluxing 48% HBr for 2.5 h resulted in both reduction and replacement of methoxy with bromo;

(13) Weaver, W. M. In *The Chemistry of the Nitro and Nitroso Groups*, Part 2; Feurer, H., Ed.; Interscience: New York, 1970; Chapter 2, p 35.

(14) (a) Ogata, Y.; Sawaki, Y.; Matsunaga, F.; Tezuka, H. *Tetrahedron* **1966**, *22*, 2655. (b) Ogata, Y.; Sawaki, Y. *J. Am. Chem. Soc.* **1966**, *88*, 5832. (c) Ogata, Y.; Tezuka, H.; Sawaki, Y. *Tetrahedron* **1969**, *23*, 1007.

(15) The nitrous acid formed in the reaction is converted to nitrogen dioxide; see ref 14.

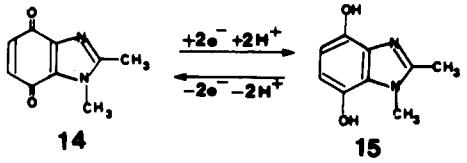
(16) Talik, T.; Talik, Z.; Ban-Oganowska, H. *Synthesis* **1974**, 293.

(17) Reference 4, pp 929–933.

(18) Moore, H. W.; Mauere, D. L.; Pearce, D. S.; Lee, M. S. *J. Org. Chem.* **1972**, *37*, 1984.

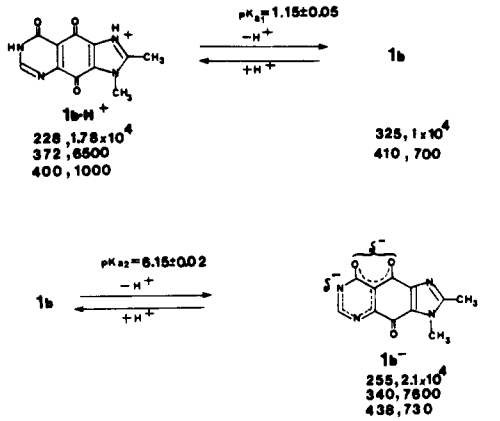
the hydrobromide salt (3·HBr) crystallized from the reaction mixture on cooling (Scheme IV). Preparation of the quinone species **1e** was carried out by treatment of 3·HBr with dichlorodicyanobenzoquinone (DDQ).

Electrochemistry. Comparative electrochemical studies of the two-electron couples **1b**/13 and 14/15 provided insights into the mechanism of hydrobromic acid oxidation and the influence of the fused pyrimidine ring on the redox potential (eq 3).



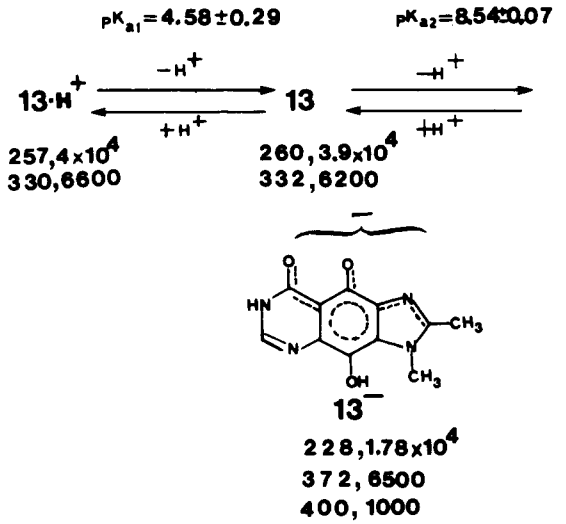
(3)

Fits of E_m vs. pH data to the Nernst equation required a knowledge of the pK_a values for the oxidized and reduced species of both couples. These were determined spectrophotometrically in aqueous solvent (μ = 1.0, KCl) at 30 ± 0.2 °C. Found in eq 4 are the pK_a values and associated UV/vis spectral data (λ_{max}, nm, ϵ) for acid dissociation from **1b**·H⁺ and **1b**.



(4)

The acidity of the N(7)-proton of **1b** is a result of anion delocalization into the quinone ring. Similar acid dissociation constants have been measured for quinazoline-2,4,5,8(1H,3*H*)-tetrones.[9] The acid dissociation constant (pK_a) for the N(3)-protonated species 14·H⁺ was determined to be 2.08 ± 0.2. Found in eq 5 are the pK_a values and associated UV/vis spectral data (λ_{max}, nm, ϵ) for acid dissociation from 13·H⁺ and 13 measured under strict anaerobic conditions.



(5)

The large error in pK_{a_1} arises from the small absorbance changes accompanying dissociation of the N(1)-proton from 13·H⁺. The first acid dissociation from 13 could involve either the 4-hydroxyl, 9-hydroxyl, or N(7)-protons. The N(7)-proton of **9b** possesses an acid dissociation constant (pK_a) of 10.33. The electron-releasing resonance effect of the 4,9-dihydroxy substituents of 13 should actually increase the pK_a value for this dissociation rather than lower it to 8.54. Ionization of the 9-hydroxyl before the 4-hydroxyl is considered since the former will result in an anion able to delocalize into the fused imidazole and pyrimidine rings. In the case of **15**, it was shown that anion delocalization into the fused imidazole ring occurs upon acid dissociation from the 4-hydroxyl group.[7] The pK_a values have been reported for N(3)-proton dissociation from 15·H⁺ (6.47) and for 4-hydroxyl proton dissociation from 15 (9.75).[7]

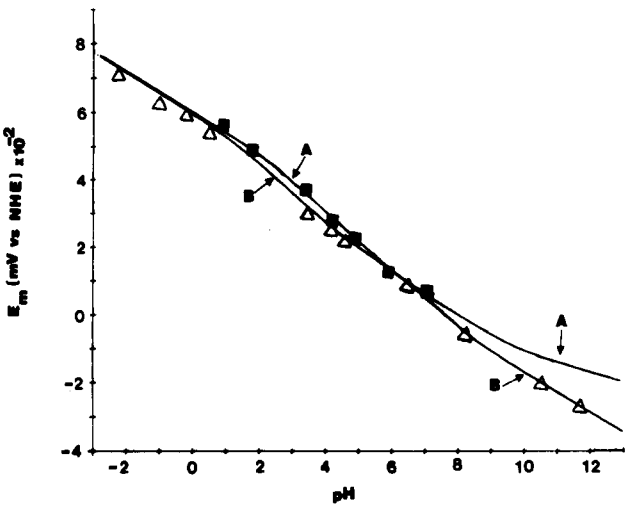
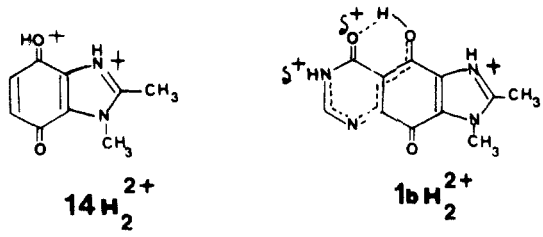


Figure 1. E_m vs. pH data for the two-electron couples 14/15 (■) and **1b**/13 (△) measured at 25–26 °C in anaerobic μ = 1.0 ($NaClO_4$) buffer. Curves A and B were computer generated for the respective couples employing the Nernst Equation.

Electrochemical redox potentials for 14/15 were determined, as a function of pH, by employing thin-layer cyclic voltammetry with a platinum electrode (vs. NHE) (see the Experimental Section). The measurements were carried out in anaerobic μ = 1.0 ($NaClO_4$) aqueous buffer over the pH range 0.95–7.30 at 25–26 °C. Base-catalyzed hydrolysis of 14 precluded studies at more basic pH values. The cyclic voltammograms are quasi-reversible,[19] with anodic and cathodic peaks separated by 200–250 mV at a scan speed of 2 mV s^{-1}. The number of electrons transferred was determined to be 2.0 ± 0.1 over the entire pH range for both the anodic and cathodic waves (see the Experimental Section). Found in Figure 1 (■) is a plot of the two-electron potentials (E_m) for 14/15 vs. pH. The solid line (plot A) was computer generated from the Nernst equation,[20] employing the acid dissociation constants for 14/15 presented above as well as pK_a > 13 for acid dissociation from the 7-hydroxyl of 15⁻ and pK_a < 0 for acid dissociation from the protonated carbonyl of 14·H_2^{2+} (eq 6). The value of E_0 in the Nernst equation (the E_m value when a_H = 1.0) was calculated as 601 mV. If acid dissociations from 14·H_2^{2+} and 15⁻ are not considered, the computer-generated curve deviates from high- and low-pH data by >100 mV.[21] The formation of 14·H_2^{2+} at high

(19) A reversible two-electron couple will exhibit anodic and cathodic peak separations of about 29 mV regardless of the scan speed. The quasi-reversible process is characterized by anodic and cathodic peaks whose separation is dependent on the scan speed: Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; pp 227–231.

(20) Clark, W. M. *Oxidation Reduction Potentials of Organic Systems*; Williams and Wilkins: Baltimore, 1960; p 118. For the derivation of pH- and H_0-dependent Nernst equations, also ref 34.



(6)

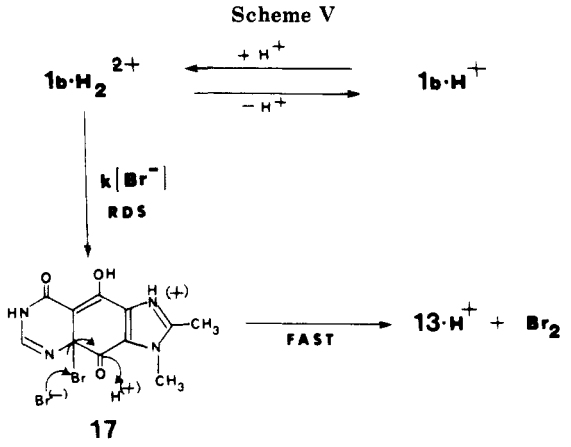
acidity is not unreasonable; O-protonated quinones have been documented in strong-acid solutions.[22] Furthermore, the analogous species **1b**·H_2^{2+} (eq 6) is implicated as the oxidant of bromide on the basis of kinetic studies (vide infra).

Studies of the couple **1b/13** employing thin-layer cyclic voltammetry with a platinum or gold thin-layer electrode indicated the presence of an irreversible system. When starting with **1b**, a single cathodic wave was observed followed by what appears to be two closely spaced anodic waves. However, conventional cyclic voltammetry with a carbon paste electrode over the pH range −2.3 to +11.7 afforded voltammograms that are reversible in nature. The voltammograms exhibit single anodic and cathodic peaks separated by 20–60 mV regardless of the scan speed. Found in Figure 1 (△) is a plot of potentials (E_m) for **1b/13** vs. pH. The solid line (plot B) was computer generated from the Nernst equation, employing the pK_a values found in eq 4 and 5 as well as pK_a < 0 for acid dissociation from the protonated carbonyl of **1b**·H_2^2 and pK_a > 13 for successive dissociation of the 4-hydroxyl and N(7)-protons from **13**$^-$. The E_0 value was calculated at 595 mV. As is seen with **14/15**, consideration of pK_a values outside the pH range studied is required in order to fit these data to the Nernst equation.

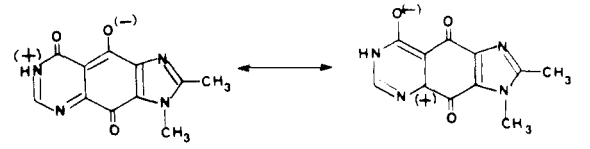
The similarities in E_m vs. pH data for both couples at low pH suggest that the diprotonated species **14**·H_2^{2+} and **1b**·H_2^{2+} possess analogous structures. Thus, **1b**·H_2^{2+} is not considered to be an N(1),N(5)-diprotonated species. Protonation of the 9-carbonyl is considered on the basis of hydrogen-bonding interaction and charge delocalization (eq 6), both of which may stabilize the additional positive charge of **1b**·H_2^{2+}. Since conventional cyclic voltametry was employed for the study of **1b/13**, an assessment could not be made of the number of electrons transferred. The fit of E_m vs. pH data to the Nernst equation (Figure 1, plot B) employing the pK_a values for **13** suggests that the cathodic waves represent two-electron transfer to afford the hydroquinone species.

Inspection of the plots in Figure 1 reveals that the E_m values for **14/15** and **1b/13** are quite similar below pH 6. Apparently, the neutral pyrimidine ring of **1b**, pK_a for N(7)-proton dissociation is 6.15, has little net electronic effect on the central quinone ring. A net electron-releasing or -withdrawing effect would have lowered or raised the quinone redox potential, respectively.[23] Theoretical studies of the quinazoline system indicate that the fused pyrimidine ring does influence electron densities in the benzene ring, however.[12] Furthermore, the electronic effect of the pyrimidine ring influences the products obtained in the synthetic sequence shown in Scheme I (loc. cit.).

Scheme V



Thus, it is unreasonable to conclude that the pyrimidine ring of **1b** does not influence electron density in the central quinone ring at all. A possible explanation for the absence of a net electronic effect is electron release to the 9-carbonyl of **1b** balanced by electron withdrawal as shown with the resonance structures in eq 7.



(7)

Above pH 6.0, E_m values for **1b/13** are much lower than extrapolated E_m values for **14/15**. This is likely due to ionization of **1b** resulting in an electron-rich quinone (**1b**$^-$ in eq 4) and the absence of anion formation by **14** in this pH range.

The conclusions of the electrochemical studies described above are as follows: (i) High-potential diprotonated quinone species (**1b**·H_2^{2+} and **14**·H_2^{2+} in eq 6) exist in acidic solutions. (ii) The pyrimidine ring of **1b** has no net effect on the quinone redox potential below pH 6 but decreases the potential much above this pH value. The first conclusion provided mechanistic insights into the **1b**-mediated bromide oxidation discussed below. The second conclusion indicates that imidazo[4,5-*g*]quinazoline reductive alkylators will possess a low potential in a biological system, $E_m \sim -100$ mV (NHE) at pH 7.4. Significantly, low-potential reductive alkylators have been shown to be the most effective antitumor agents,[8] probably because of selective activation in low-potential environments.

The **1b**-mediated oxidation of HBr was studied over the H_0 range of −1.05 to −3.61[24] in aqueous hydrobromic acid at 30.0 ± 0.2 °C. Product studies of these reactions indicated that **13**·HBr is formed in near quantitative (>90%) yields. The formation of bromine was assessed with cyclohexene trapping. A completed reaction mixture at H_0 = −3.61 was extracted with CCl_4; drying of the extracts ($NaHCO_3$) was followed by addition of cyclohexene. Workup and purification afforded *trans*-1,2-dibromocyclohexane (>90%), identified as such by ^{1}H NMR and IR. The oxidation of HBr by **1b** (5×10^{-5} M) was followed spectrophotometrically at 330 nm. Absorbance vs. time (*s*) plots were computer fit to a simple first-order rate law. These data obey the rate law, $k_{obsd} = k_H a_H$, where $k_H = 7.1 \times 10^{-7}$ M^{-1} s^{-1} and a_H is the proton activity determined from the H_0 values. Although the electrochemical study indicates that **1b** and **14** are equally good oxidants at high acidity, the treatment of **14** with refluxing 48% HBr (H_0

(21) Approximate pK_a values for acid dissociation from **14**·H_2^{2+} and **15**$^-$, well outside the pH range studied, were utilized to generate the theoretical curve in plot A of Figure 1. Thus, the curve exhibits a negative slope at both high- and low-pH values. Exact pK_a values are necessary only if they fall within the pH range studied.

(22) (a) Biedermann, G. *Acta Chem. Scand.* **1956**, *10*, 1340. (b) Handa, T. *Bull. Chem. Soc. Jpn.* **1955**, *28*, 483. (c) Beauchamp, A.; Benoit, R. L. *Can. J. Chem.* **1966**, *44*, 1607.

(23) Brown, E. R.; Finley, K. T.; Reeves, R. L. *J. Org. Chem.* **1971**, *36*, 2849.

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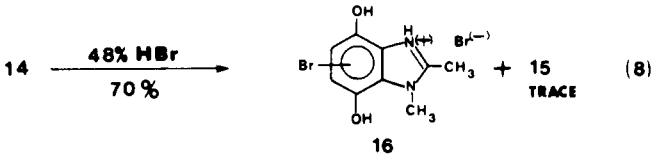
Table I

reaction	K_m, M^{-1}	V_{max},[b] $\mu M\ min^{-1}$
1b → **2a**	1.75×10^{-5}	28.2
1d → **2b**	6.4×10^{-6}	16.2
9b → 6-oxo-**9b**[a]	4.8×10^{-6}	18.0
hypoxanthine → xanthine → uric acid	5.0×10^{-6}	11.0
	2.0×10^{-6}	7.3

[a] 2,3-Dimethylimidazo[4,5-g]quinazoline-6,8(3H,5H,7H)-dione. [b] All V_{max} values were obtained under the stated conditions with 0.017 μM enzyme. V_{max} ($\mu M\ min^{-1}$) was calculated as follows: maximum OD $min^{-1}/\Delta\epsilon$. Oxidations were monitored at the following wavelengths and resulted in the following $\Delta\epsilon$ values [λ_{max}, nm ($\Delta\epsilon$)]: **1b**, 290 (1860); **1d**, 332 (4100); **9b**, 250 (2.5×10^4).

~ 3.6) for 2 min provided the brominated hydroquinone **16**[25] and only a trace amount of **15** (eq 8).



(8)

The kinetic study described above supports some aspects of the mechanism in Scheme V. In the H_0 region **1b** is nearly all N(1)-protonated (**1b**·H^+, $pK_a = 1.15$); the presence of acid catalysis thus requires that equilibrium protonation of **1b**·H^+ be followed by the rate-determining step (RDS). The oxidant must therefore be the high-potential species **1b**·H_2^{2+} described in the electrochemical study. In Scheme V we propose that two-electron transfer to the quinone occurs via adduct **17**. This assessment is based on the apparent intermediacy of a similar adduct during the conversion of **14** to **16** (eq 8). It should be pointed out, however, that mechanisms have not been rigorously excluded that involve single-electron or direct two-electron transfer to **1b**·H_2^{2+} from bromide.

Xanthine Oxidase Substrate Reactivity. The xanthine oxidase mediated oxidation of **1b**, **1d**, and **9b** was studied in aerobic 0.05 M pH 7.40 phosphate buffer (μ = 0.1, KCl) containing 22 μM EDTA and 0.017 μM purified buttermilk xanthine oxidase at 30.0 ± 0.2 °C. UV/visible spectra of completed reactions (substrate concentration 5×10^{-5} M) indicate that the oxidations are nearly quantitative; the oxidation products are shown in Table I. Preparative studies of the oxidations **1b** → **2a** (30% yield) and **1d** → **2b** (70% yield) are described in the Experimental Section. The parameters for **1b** and **1d** oxidation obtained from Lineweaver–Burk plots[26] are summarized in Table I. Also found in Table I are parameters for **9b**, hypoxanthine, and xanthine oxidation. A feature common to all of the above substrates is inhibition of the enzyme at high substrate concentrations (>1×10^{-4} M).[16,27] This is likely due to the formation of an inactive enzyme–substrate–substrate complex at low substrate dilutions.[28] Thus, the preparative oxidation of **1b** affords a poor yield of **2a** whereas this oxidation is quantitative at 5×10^{-5} M.

Leonard and co-workers[1b,2] have noted that xanthine oxidase can accept imidazo[4,5-g]quinazolines and other dimensionally altered purine analogues as substrates. Indeed, the enzyme has been shown to accept a diversity of compounds as substrates.[29] The data in Table I indicate that the enzyme tolerates both dimensionally altered and anionic purine substrates. At pH 7.40 both **1b** and **1d** exist predominately (>90%) as the anionic species **1b**$^-$ and **1d**$^-$; pK_a values are 6.15 and 6.17, respectively. On the other hand, **9b** ($pK_a = 10.33$) and the natural purine substrates exist largely as their neutral forms at this pH value. The facility with which **1b** and **1d** are oxidized is surprising in view of the proposed enzymatic oxidation mechanism.[30] Enzymatic oxidation is thought to involve nucleophilic attack on the substrate in concert with hydride transfer from the substrate. The anionic substrate should, of course, preclude nucleophilic attack. It is possible, however, that only the neutral forms of **1b** and **1d** (<10% of the total species at pH 7.4) are being oxidized, in which case the actual K_m and V_{max} values are $<1 \times 10^{-6}\ M^{-1}$ and >150 $\mu M\ min^{-1}$, respectively. The forms of **1b** and **1d** undergoing oxidation could have a bearing on the enzymatic oxidation mechanism, and this possibility is currently under study.

The conclusion of the enzymatic oxidation study is that systems **1** and **2** (eq 1) could act as purine mimics in spite of the electronic change brought about by the benzoquinone ring. The facility of enzymatic oxidation also provides a convenient route to the xanthine derivatives **2**. It is thus concluded that **1d**, **1e**, and **2e** could function as purine active site directed reductive alkylators (loc. cit., introduction). Preliminary results with xanthine oxidase indicate that **1e** is a substrate whereas the reduced form **3** (eq 2) inactivates the enzyme at high concentrations. These results will be discussed in a future publication.

Experimental Section

Elemental analyses were performed by MicAnal Laboratories, Tucson, AZ. All analytical pure compounds were dried over KOH pellets under high vacuum for 24 h. Some of these contained water or crystallization that was calculated from the elemental analyses found. Experimental nitrogen percentages for **7a**, **7b**, and **10a** deviated from theoretical values by >0.4%. Experimental carbon and hydrogen percentages as well as spectral data (MS, ^1H NMR) support the assigned structures, however. Uncorrected melting points were determined with a Mel-Temp apparatus. All TLCs were run with Merck silica gel 60 (F_{254}) plates employing butanol–acetic acid–water (5:2:3) as the solvent. IR spectra were taken as KBr pellets, employing a Nicolet MX-1 FT IR spectrophotometer; the strongest IR absorbances are reported. ^1H NMR spectra were obtained with a Bruker WH-90 spectrometer. ^{13}C NMR spectra were obtained with a Bruker AM-400 narrow-bore spectrometer operating at 100 MHz. All chemical shifts (δ) are reported relative to tetramethylsilane. UV/visible spectra and kinetic measurements were obtained with Perkin-Elmer 559 and Lambda-3 spectrometers. Mass measurements were carried out in the electron-impact mode with a Varian MAT 200 spectrometer. Measurements of pH were made with a Radiometer PHM84 pH meter equipped with a Radiometer GK2401C combination electrode.

pK_a constants were determined by spectrophotometric titration in μ = 1.0 (KCl) aqueous solvent at 30 ± 0.2 °C. Measurements were usually carried out under aerobic conditions; acid dissociations from hydroquinones were measured under an argon atmosphere, however. Details of the methodology employed are found in a previous publication.[31]

Electrochemistry. Determination of E_m values was carried out with a modified Princeton Applied Research Model 174 po-

(25) Cooling the reaction mixture afforded crude **16**; recrystallization was carried out from ethyl acetate/ethanol: NMR (Me_2SO-d_6) δ 7.02 and 6.99 (1 H, 2 s, Ar, mixture of 5- and 6-bromo isomers), 4.03 (3 H, s, N(1)-methyl), 2.73 (3 H, s, 2-methyl). Anal. Calcd for $C_9H_9BrN_2O_2 \cdot HBr \cdot 0.5H_2O$: C, 31.13; H, 3.17; N, 8.06. Found: C, 31.02; H, 3.30; N, 7.73. The ^1H NMR (Me_2SO-d_6) of crude **16** also shows a peak at δ 6.72 which is the chemical shift associated with the aromatic protons of **15**·HBr.

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(27) Inhibition of xanthine oxidase at high substrate concentrations has been documented. Massey, V. In *Iron-Sulfur Proteins*; Lovenberg, W., Ed.; Academic: New York, 1973; Vol. 1, Chapter 10, pp 316, 325–526.

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(29) Krenitsky, T. A.; Neil, S. M.; Elion, G. B.; Hitchings, G. H. *Arch. Biochem. Biophys.* **1972**, *150*, 585. See also: Reference 27, pp 318–321.

(30) (a) Olson, J. S.; Ballou, D. P.; Palmer, G.; Massey, V. *J. Biol. Chem.* **1974**, *249*, 4363. (b) Bray, R. C.; Gutteridge, S.; Stotter, D. A.; Tanner, S. J. *Biochem. J.* **1979**, *177*, 357.

(31) Skibo, E. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 3304.

larographic analyzer as previously described.[31] The working electrode material was a carbon paste consisting of 3 g of 325-mesh carbon graphite and 2 mL of paraffin oil[31,32] or a platinum thin-layer electrode.[33] Measurements were carried out in μ = 1.0 ($NaClO_4$) aqueous buffers at 25–26 °C under an atmosphere of N_2. The anodic and cathodic waves are highly symmetric ($\alpha \sim 0.5$) and the respective potentials, $E_{p,a}$ and $E_{p,c}$, could be determined within ±3 mV. The midpoint potential E_m was determined from the average of $E_{p,a}$ and $E_{p,c}$. The number of electrons transferred was determined by use of thin-layer cyclic voltammetry as previously described.[34]

Xanthine oxidase substrate activity was studied in 0.05 M pH 7.40 phosphate buffer (μ = 0.1, KCl) containing 22 μM EDTA at 30 ± 0.2 °C. Double-distilled water was employed in the preparation of all solutions. The concentration of xanthine oxidase (Sigma grade III) employed was 0.017 μM. Enzyme concentration was determined from an absorbance measurement at 450 nm.[35]

Synthetic Procedures. Preparations of **9a**,[1a] **14**, and **15**[7] were carried out as previously described. The preparation of **9b** was carried out by treatment of **5** with acetic anhydride followed by acid-catalyzed ring closure; the physical properties are identical with those previously reported.[1e] The preparations of as yet unreported compounds are described below.

6-Amino-7-(methylamino)quinazolin-4(3*H*)-one (5). A stirred mixture of 10 g (0.05 mol) of 6-nitro-7-chloroquinazolin-4(3*H*)-one[36] in 100 mL of 40% aqueous methylamine was heated at 110–120 °C for 15 h. The reaction mixture was then cooled to room temperature and the crude 6-nitro-7-(methylamino)-quinazolin-4(3*H*)-one collected by filtration and washed several times with cold water. Yield upon drying was 5.8 g (53%).

Reduction of the crude nitro derivative, 7 g (0.03 mol) in 150 mL of 1 N KOH containing 0.7 g 5% Pd on charcoal, was carried out for 15 h under 50 psi H_2. After reduction was completed, the catalyst was removed by filtration through Celite and the filtrate adjusted to pH 6 with acetic acid. Crude **5**, suitable for carrying to the next step, crystallized from the filtrate; yield 5 g (53%). Recrystallization was carried out from hot water: mp 289–290 °C dec; R_f 0.57; ^{1}H NMR (Me_2SO-d_6) δ 7.72, 7.18, and 6.45 (3 H, 3 s, 2-H, 5-H, 8-H, respectively[37]), 5.68 (1 H, q, J = 3.6 Hz, NH of methylamino), 2.82 (3 H, d, J = 3.6 Hz, methylamino); IR (KBr) 3430, 3369, 3149, 1665, 1609, 1509, 1485, 1300, 1284 cm^{-1}. Anal. Calcd for $C_9H_{10}N_4O$·0.1H_2O: C, 56.29; H, 5.30; N, 29.18. Found: C, 56.19; H, 5.22; N, 29.15.

6-Acetamido-7-(methylamino)quinazolin-4(3*H*)-one (6a). A solution consisting of 3 g (0.016 mol) of **5**, 10 mL of acetic anhydride, and 20 mL of acetic acid was stirred for 4 h at room temperature. After 20 mL of water was added to the reaction mixture, the pH was adjusted to 6 with 20% aqueous NaOH, resulting in crystallization of **6a**. Recrystallization was carried out from 95% aqueous ethanol: yield 3 g (90%); dec pt >350 °C; R_f 0.18; ^{1}H NMR (Me_2SO-d_6) δ 11.72 and 9.17 (2 H, two br s, N(3)-H and NH of acetamide), 7.91, 7.87, and 6.58 (3 H, 3 s, 2-H, 5-H, 8-H, respectively[37]), 6.09 (1 H, q, J = 5 Hz, NH of methylamino), 2.80 (3 H, d, J = 5 Hz, methylamino), 2.07 (3 H, s, acetamido methyl); IR (KBr) 3266, 1675, 1653, 1612, 1570, 1525, 1521, 1514, 1496, 1478 cm^{-1}; MS (EI) m/z 232.2 (M^+). Anal. Calcd for $C_{11}H_{12}N_4O_2$·0.2H_2O: C, 56.02; H, 5.30; N, 23.76. Found: C, 56.38; H, 5.07; N, 23.58.

6-(Methoxyacetamido)-7-(methylamino)quinazolin-4-(3*H*)-one Hydrochloride (6b·HCl). Methoxyacetyl chloride (1.5 g, 0.014 mol) was added dropwise at room temperature to a stirred solution of **5** (2.4 g, 0.012 mol) in a mixture consisting of 3 mL of dry pyridine and 30 mL of dry benzene. The reaction mixture was then stirred for 12 h at room temperature. Evaporation of the reaction mixture in vacuo afforded a solid residue that was suspended in 60 mL of water and stirred for 30 min. The resulting white solid was collected by filtration and recrystallized from 80% aqueous ethanol: yield 2.0 g (64%); mp 175–177 °C; R_f 0.61; ^{1}H NMR nMe_2SO-d_6) δ 8.67, 7.91, and 6.68 (3 H, 3 s, 2-H, 5-H, and 8-H, respectively[37]), 4.09 (2 H, s, methylene), 3.39 (3 H, s, methylamino), 2.81 (3 H, s, methoxy); IR (KBr) 3249, 3199, 3102, 3015, 1721, 1688, 1667, 1617, 1577, 1538 cm^{-1}; MS (EI) m/z 262 (M^+). Anal. Calcd for $C_{12}H_{14}N_4O_3$·HCl·0.3H_2O: C, 47.39; H, 5.17; N, 18.42. Found: C, 47.40; H, 5.13; N, 18.19.

6-Acetamido-7-(*N*-nitro-*N*-methylamino)-8-nitroquinazolin-4(3*H*)-one (7a). **6a** (2 g, 8.6 mmol) was added to an ice-cooled solution consisting of 5 mL of glacial acetic acid, 5 mL of concentrated sulfuric acid, and 15 mL of 90% nitric acid. The reaction was allowed to warm to room temperature and stirred for 1.5 h. Addition of the reaction mixture to 100 g of cracked ice afforded **7a** as a yellow precipitate. Recrystallization and activated charcoal decolorization were carried out with boiling water: yield 1.4 g (60%); mp 234–235 °C; R_f 0.61; ^{1}H NMR (Me_2SO-d_6) δ 12.76 (1 H, br d, N(3)-H), 10.13 (1 H, s, acetamide NH), 9.07 (1 H, s, 5-H), 8.19 (1 H, d, J = 3.6 Hz, 2-H), 3.56 (3 H, s, *N*-methyl), 2.46 (3 H, s, acetamido methyl); ^{13}C NMR (Me_2SO-d_6) δ 169.6, 158.5, 147.8, 143.4, 136.5, 134.0, 128.2, 125.0, 121.3, 39.0, 23.8 (no assignments made); IR (KBr) 3215, 3135, 1712, 1670, 1602, 1552, 1454, 1373, 1282, 1250 cm^{-1}; MS (EI) m/z 277 ($M^+ + H - NO_2$). Anal. Calcd for $C_{11}H_{10}N_6O_6$: C, 41.00; H, 3.12; N, 26.07. Found: C, 40.92; H, 3.04; N, 25.36.

6-(Methoxyacetamido)-7-(*N*-nitro-*N*-methylamino)-8-nitroquinazolin-4(3*H*)-one (7b) was prepared from **6b**·HCl by the method employed for the preparation of **7a**. Recrystallization and activated charcoal decolorization in hot 90% aqueous ethanol gave analytically pure **7b** as a colorless solid: yield 75%; dec pt >375 °C; R_f 0.32; ^{1}H NMR (Me_2SO-d_6) δ 8.93 (1 H, s, 2-H), 8.22 (1 H, s, 5-H), 4.14 (2 H, s, methylene), 3.56 (3 H, s, methylamino), 3.38 (3 H, s, methoxy), chemical shifts associated with the acetamido and N(3)-H protons not observed; ^{13}C NMR (Me_2SO-d_6) δ 169.4, 158.5, 148.1, 143.5, 137.2, 133.2, 127.4, 125.0, 122.5, 71.2, 58.5, 39.7 (no assignments made); IR (KBr) 3235, 3130, 1710, 1685, 1603, 1509, 1282 cm^{-1}; MS (EI) m/z 307 ($M^+ + H - NO_2$). Anal. Calcd for $C_{12}H_{12}N_6O_7$: C, 40.92; H, 3.43; N, 23.86. Found: C, 41.17; H, 3.39; N, 23.30.

4-Amino-2,3-dimethylimidazo[4,5-*g*]quinazolin-8-(3*H*,7*H*)-one (8a·HCl). A solution of 1.0 g (3.8 mmol) of **7a** in 150 mL of 1% methanolic NaOH containing 400 mg of 5% Pd on charcoal was reduced under 50 psi H_2 for 2 h. The catalyst was removed by filtration through Celite and the filtrate acidified by addition of 4 mL of concentrated HCl. Chilling in an ice bath afforded **8a**·HCl as an analytically pure colorless solid: yield 0.7 g (80%); mp 326–327 °C; R_f 0.58; ^{1}H NMR (Me_2SO-d_6) δ 8.01 (1 H, s, 6-H), 7.55 (1 H, s, 9-H), 4.12 (3 H, s, N(3)-methyl), 2.72 (3 H, s, 2-methyl), coupling between 6-H and N(7)-H not observed (see **8b**·HCl); IR (KBr) 3446, 1700, 1632, 1605, 1591, cm^{-1}; MS (EI) m/z 229 (M^+ of free base). Anal. Calcd for $C_{11}H_{11}N_5O$·HCl·0.9H_2O: C, 46.86; H, 4.93; N, 24.84. Found: C, 46.70; H, 4.37; N, 24.86. The experimental hydrogen percentage deviates from the theoretical value by 0.56%; mass spectral and ^{1}H NMR data support the assigned structure.

4-Amino-2-(methoxymethyl)-3-methylimidazo[4,5-*g*]-quinazolin-4(3*H*,7*H*)-one (8b·HCl) was prepared from **7b** by the method employed for the preparation of **8a**·HCl. Evaporation of the filtrate from the reduction afforded crude **8b**·HCl, which was then recrystallized by dissolution in ~1 mL of concentrated HCl followed by addition of 100 mL of 100% ethanol: yield 78%; mp 262–264 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 11.89 (1 H, br d, N(7)-H), 7.92 (1 H, d, J = 4 Hz, 6-H), 7.65 (1 H, s, 9-H), 4.70 (2 H, s, methylene), 4.12 (3 H, s, *N*(3)-methyl), 3.35 (3 H, s, methoxy); ^{13}C NMR (Me_2SO-d_6) δ 160.9, 152.9, 141.6, 133.1, 131.9, 131.6, 124.3, 120.6, 99.0, 64.5, 58.9, 33.4 (no assignments made); IR (KBr) 3435, 3341, 3049, 1697, 1630, 1608, 1125 cm^{-1}; MS (EI) m/z 259 (M^+ of free base). Anal. Calcd for $C_{12}H_{13}N_5O_2$·HCl·1.2H_2O: C, 45.41; H, 5.20; N, 22.06. Found: C, 45.20; H, 4.80; N, 21.79.

Oxidation of 8a and 8b with Fremy's Radical. A solution of potassium nitrosodisulfonate[10] (1 g, 4 mmol) in 40 mL of water containing 1 g of monobasic potassium phosphate was added dropwise with stirring to a solution of **8a**·HCl or **8b**·HCl (0.78 mmol) in 20 mL of 50% aqueous acetone. The reaction mixture

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was then stirred for 5 h during which time the oxidation product crystallized from the reaction mixture. The description of the purification and physical properties of the respective oxidation products—**1b** and **1d**—follow.

2,3-Dimethylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione(1b): This oxidation product crystallized from the reaction mixture in an analytically pure form (79%). Recrystallization from hot 4 N HCl afforded yellow needles: dec pt >390 °C; R_f 0.15; ^{1}H NMR (Me_2SO-d_6) δ 8.53 (1 H, s, 6-H), 3.88 (3 H, s, *N*(3)-methyl), 2.47 (3 H, s, 2-methyl); ^{13}C NMR (trifluoroacetic acid-d_1 with two drops of D_2O) δ 173.7, 173.2, 160.7, 157.3, 155.9, 155.5, 133.6, 130.8, 119.1, 35.4, 11.8; IR (KBr) 3410, 2825, 2675, 1708, 1678, 1483 cm^{-1}; MS (EI) *m/z* 244.1 (M^+). Anal. Calcd for $C_{11}H_8N_4O_3 \cdot 0.1H_2O$: C, 53.70; H, 3.35; N, 22.76. Found: C, 53.30; H, 3.25; N, 22.77.

2-(Methoxymethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1d). Recrystallization was carried out by dissolution in a minimum amount of 4 N HCl followed by dilution to 20× the volume with 100% ethanol: yield 55%; mp 295–297 °C dec; R_f 0.19; ^{1}H NMR (trifluoroacetic acid-d_1) δ 9.04 (1 H, s, 6-H), 5.03 (2 H, s, methylene), 4.29 (3 H, s, *N*(3)-methyl), 3.70 (3 H, s, methoxy); IR (KBr) 3410, 1697, 1682 cm^{-1}; MS (EI) *m/z* 274 (M^+). Anal. Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.44; H, 3.61; N, 20.52.

pK_a for N(7)-proton dissociation is 6.17 ± 0.02.

3-Methylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (9c). A solution of **5** (0.41 g, 2.15 mmol) in a mixture consisting of 12 mL of 88% formic acid and 3.2 mL of acetic anhydride was heated at reflux for 1 day. Dilution of the completed reaction with 10 mL of water was followed by evaporation in vacuo to a dry solid. The solid was dissolved in dilute HCl and crystallized by buffering to pH 5 with acetate. Analytically pure **9c** was obtained by recrystallization using the above procedure: yield 0.27 g (63%); dec pt >340 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.45, 8.38, 8.03, and 7.84 (4 H, 4 s, 2-H, 4-H, 6-H, 9-H, no assignments made), 3.92 (3 H, s, *N*(3)-methyl); IR (KBr) 3089, 3053, 2863, 2818, 2654, 1682, 1617, 1457, 1331, 1266, 925 cm^{-1}. Anal. Calcd for $C_{10}H_8N_4O \cdot 0.8H_2O$: C, 55.97; H, 4.51; N, 26.11. Found: C, 55.68; H, 4.31; N, 26.06.

Oxidation and Nitration of 9a–c. A solution of 1.5 g of the imidazo[4,5-*g*]quinazoline in a mixture consisting of 10 mL of 90% nitric acid, 3 mL of concentrated H_2SO_4, and 3 mL of acetic acid was stirred at room temperature for 2 days then heated at 50–60 °C for 1 day, and finally heated at reflux for 12 h. The completed reaction was poured over ~50 g of ice, and the precipitated solids were collected by filtration. The solid consisted of quinone, the 4-nitro derivative, and trace amounts of starting material whereas the liquor contained starting material and trace amounts of the 4-nitro derivative. In the case of **10c**, crystallization from the liquor occurred upon prolonged chilling in a refrigerator. Separation of the 4-nitro derivative and quinone was carried out by ion-exchange chromatography with a 700-mL Dowex 1-X2 100–200-mesh chloride column. The solid obtained above was dissolved in 500 mL of dilute buffer held at pH 7.0 and placed on the column. After the column was washed with 1.5 L of distilled water to remove salts, the 4-nitro derivative and trace amounts of starting material were removed by elution with 0.1 N HCl. The elutant was concentrated to a residue that was dissolved in hot 4 N HCl; adjusting the pH to 3.0 with acetate afforded the analytically pure 4-nitro derivative as a light yellow solid. Upon elution with 0.1 N HCl, the quinone had crystallized on the column; its removal was facilitated by elution with ~15% HCl. Concentration of the eluant to ~20 mL resulted in crystallization of the quinone as a bright yellow solid. Recrystallization from hot acetic acid–concentrated HCl (80:20) afforded an analytically pure sample. In what follows are spectral and analytical data for compounds **1a**,**c** and **10a–c**.

Imidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1a): dec pt >300 °C; R_f 0.37; ^{1}H NMR (Me_2SO-d_6) δ 8.55 and 8.23 (2 H, 2 s, 2-H and 6-H, respectively); ^{13}C NMR (Me_2SO-d_6) δ 175.4, 174.7, 153.8, 154.8, 153.7, 142.3, 136.8, 136.0, 117.1 (no assignments made); IR (KBr) 3386, 3074, 3024, 2859, 1700, 1530, 1514, 1458, 1398, 1307 cm^{-1}; MS (EI) *m/z* 216 (M^+), 188 (M^+ – CO). Anal. Calcd for $C_9H_7N_4O_3 \cdot 0.3H_2O$: C, 48.79; H, 2.09; N, 25.29. Found: C, 48.48; H, 1.81; N, 25.28.

pK_a for N(7)-H acid dissociation is 5.99 ± 0.04. UV/vis [λ_{max}, nm (ϵ)]: 232 (1.62 × 10^4), 321 (8100), 400 (924); (**1a**$^-$) 220 (1.50 × 10^4), 252 (1.83 × 10^4), 330 (5900), 430 (1350).

3-Methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1c): dec pt >350 °C; R_f 0.22; ^{1}H NMR (Me_2SO-d_6) δ 8.55 and 8.23 (2 H, 2 s, 2-H and 6-H, respectively), 3.95 (3 H, s, *N*(3)-methyl); IR (KBr) 3085, 3025, 2827, 2669, 2634, 1710, 1691, 1539, 1527, 1307, 1246 cm^{-1}; MS (EI) *m/z* 230 (M^+), 202 (M^+ – CO). Anal. Calcd for $C_{10}H_6N_4O_3 \cdot 0.5H_2O$: C, 50.21; H, 2.95; N, 23.42. Found: C, 50.01; H, 2.49; N, 23.02.

pK_a for N(7)-H acid dissociation is 6.09 ± 0.02. UV/vis [λ_{max}, nm (ϵ)]: (**1c**) 237 (1.72 × 10^4), 322 (9100), 410 (720); (**1c**$^-$) 222 (1.50 × 10^4), 254 (1.83 × 10^4), 330 (6400), 43 (1100).

4-Nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (10a): dec pt >310 °C; R_f 0.58; ^{1}H NMR (Me_2SO-d_6) δ 8.70, 8.52, and 8.16 (3 H, 3 s, 2-H, 6-H, 9-H, no assignments made); IR (KBr) 3064, 2929, 1685, 1634, 1593, 1534, 1401, 1335, 1270 cm^{-1}; MS (EI) *m/z* 231 (M^+), 201 (M^+ – NO), 185 (M^+ – NO_2). Anal. Calcd for $C_9H_5N_5O_3 \cdot 0.3H_2O$: C, 45.69; H, 2.39; N, 29.60. Found: C, 46.05; H, 2.03; N, 29.00.

4-Nitro-2,3-dimethylimidazo[4,5-*g*]quinazolin-8-(3*H*,7*H*)-one (10b): dec pt >338 °C; R_f 0.6; ^{1}H NMR (trifluoroacetic acid-d_1) δ 8.96 and 8.43 (2 H, 2 s, 6-H and 9-H, no assignments made), 4.03 (3 H, s, *N*(3)-methyl), 3.08 (3 H, s, 2-methyl); IR (KBr) 3360, 3215, 3130, 1691, 1613, 1530 cm^{-1}; MS (EI) *m/z* 259.1 (M^+). Anal. Calcd for $C_{11}H_9N_5O_3 \cdot 1.75H_2O$: C, 45.44; H, 3.12; N, 24.09. Found: C, 45.33; H, 2.89; N, 23.75.

4-Nitro-3-methylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (10c): dec pt >300 °C; R_f 0.64; ^{1}H NMR (Me_2SO-d_6) δ 8.64, 8.56, and 8.18 (3 H, 3 s, 2-H, 6-H, 9-H, no assignments made); IR (KBr) 3205, 3091, 2929, 2659, 1697, 1616, 1534, 1378, 1262, 877 cm^{-1}; MS (EI) *m/z* 245 (M^+), 215 (M^+ – NO). Anal. Calcd for $C_{10}H_7N_5O_3 \cdot 0.25H_2O$: C, 48.10; H, 3.03; N, 28.05. Found: C, 48.14; H, 2.77; N, 27.70.

7,8-Dimethyl-4-(*N*-formylcarbamyl)-3,8-dihydroimidazo-[4,5-*e*][1,2,3]benzotriazole (12). A mixture consisting of 50 mg (0.2 mmol) of **8a**, 3 mL of concentrated HCl, 2 mL of water and 2 mg of powdered copper was cooled to ~0 °C. A solution of 1.2 g of sodium nitrite in 4 mL of water was added to this mixture with stirring and continued cooling at ~0 °C. After addition was completed, the reaction was stirred for 10 min at ~0 °C and then diluted with 50 mL of water. The crystallized product was filtered and washed with water. Recrystallization was carried out in 50% aqueous DMF: yield 35 mg (67%); mp 303 °C; R_f 0.5; ^{1}H NMR (Me_2SO-d_6) δ 16.11 (1 H, br s, 3-H), 11.94 (1 H, br d, J = 8 Hz, amide proton), 9.37 (1 H, d, J = 8 Hz, formyl proton), 8.65 (1 H, s, 5-H), 4.24 (3 H, s, *N*(8)-methyl), 2.62 (3 H, s, 7-methyl); IR (KBr) 3560, 3288, 1732, 1651, 1519, 1329, 1316, 1240, 1214, 1173, 990 cm^{-1}; MS (EI) *m/z* 258 (M^+). Anal. Calcd for $C_{11}H_{10}N_6O_2 \cdot 0.2H_2O$: C, 50.46; H, 4.00; N, 32.10. Found: C, 50.53; H, 3.72; N, 31.93.

2-(Bromomethyl)-3-methyl-4,9-dihydroxyimidazo[4,5-*g*]-quinazolin-8(3*H*,7*H*)-one Hydrobromide (3·HBr). A mixture of 30 mg (0.1 mmol) of **1d** and 1.5 mL of 48% HBr was heated at 100 °C for 2.5 h. After the reaction mixture was cooled in a refrigerator overnight, **3**·HBr crystallized as a yellow analytically pure solid: 31 mg (75%); dec pt >279 °C; R_f 0.18; ^{1}H NMR (Me_2SO-d_6) δ 8.11 (1 H, s, 6-H), 4.95 (2 H, s, bromomethyl), 4.18 (3 H, s, *N*(3)-methyl); IR (KBr) 3380, 3194, 3185, 1680, 1628, 1584, 1337, 1024 cm^{-1}; MS (EI) *m/z* 324.3 (M^+). Anal. Calcd for $C_{11}H_9BrN_4O_3 \cdot HBr \cdot 0.4H_2O$: C, 31.97; H, 2.63; N, 13.56. Found: C, 32.11; H, 2.82; N, 13.35.

2-(Bromomethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1e). A mixture of 20 mg (0.05 mmol) of **3**·HBr and 20 mg of DDQ in methanol was heated at 60 °C for 20 min. The crystallized solids were removed by filtration and washed with methanol. Recrystallization from 5% aqueous Me_2SO afforded analytically pure **1e**: 12 mg (82%); dec pt >315 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.55 (1 H, s, 6-H), 4.92 (2 H, s, bromomethyl), 3.97 (3 H, s, *N*(3)-methyl); IR (KBr) 3430, 1707, 1683, 1475, 1315, 1251; MS (EI) *m/z* 322 (M^+), 324 (M^+ + 2).

2,3-Dimethyl-4,9-dihydroxyimidazo[4,5-*g*]quinazolin-8-(3*H*,7*H*)-one Hydrobromide (13·HBr). A mixture of 100 mg (0.36 mmol) of **1b** in 5 mL of 48% HBr was heated at reflux for 2 min. After the reaction mixture was cooled to room temperature, **13**·HBr crystallized as an analytically pure solid: yield 125 mg (98%); dec pt >325 °C; R_f 0.13; ^{1}H NMR (Me_2SO-d_6) δ 11.9 (1 H, s, N(7)-H), 10.5 (2 H, s, hydroxyl), 8.08 (1 H, s, 6-H), 4.12 (3 H, s, *N*(3)-methyl), 2.75 (3 H, s, 2-methyl); ^{13}C NMR (Me_2SO-d_6)

δ 165.8, 154.3, 141.9, 138.8, 132.0, 131.2, 126.2, 118.2, 103.9, 33.5, 11.8 (no assignments made); IR (KBr) 3421, 3358, 1682, 1644, 1606, 1499, 1235, 1293, 1022 cm^{-1}; MS (EI) m/z 246 (M^+). Anal. Calcd for $C_{11}H_{10}N_4O_3 \cdot HBr \cdot H_2O$: C, 38.28; H, 3.80; N, 16.23. Found: C, 38.04; H, 3.44; N, 16.07.

Xanthine Oxidase Mediated Oxidation of Imidazo[4,5-*g*]quinazolinetriones 1b and 1d. The imidazo[4,5-*g*]-quinazolinetrione (0.13 mmol) was either dispersed or dissolved in 5 mL of 0.05 M pH 7.4 phosphate buffer (μ = 0.1, KCl) containing 22 μM EDTA. Addition of 6.3 units of Sigma grade IV xanthine oxidase was followed by stirring for 3 h at room temperature. During this time the reaction mixture became dark amber concomitant with crystallization of the product as its potassium salt. The completed reaction mixture was diluted to 100 mL with distilled water, resulting in a homogeneous amber solution. This solution was placed on a 25-mL Dowex 1-X2 50–100-mesh ion-exchange resin column that was then washed with 500 mL of distilled water to remove salts and the enzyme. The product was removed by elution with 0.1 N HCl; evaporation of eluants to ~5 mL resulted in crystallization of the product in an analytically pure form. In what follows are spectral and analytical data for the respective oxidation products **2a** and **2b**.

2,3-Dimethylimidazo[4,5-*g*]quinazoline-4,6,8,9-(3*H*,5*H*,7*H*)-tetrone (2a): dec pt >350 °C; ^{1}H NMR (trifluoroacetic acid-d_1 with two drops of D_2O) δ 4.25 (3 H, s, *N*-(3)-methyl), 2.94 (3 H, s, 2-methyl); IR (KBr) 3541, 3488, 1726, 1700, 1516, 1405 cm^{-1}. Anal. Calcd for $C_{11}H_8N_4O_4$: C, 50.77; H, 3.10; N, 21.53. Found: C, 50.62; H, 2.98; N, 21.49.

pK_a for N(5)-H acid dissociation is 5.82 ± 0.04. UV/vis [λ_{max}, nm (ϵ)]: (**2a**) 245 (1.35 × 10^4), 312 (1.12 × 10^4); (**2a**$^-$) 232 (1.8 × 10^4), 262 (1.0 × 10^4), 325 (1.06 × 10^4), 450 (1000).

2-(Methoxymethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,6,8,9(3*H*,5*H*,7*H*)-tetrone (2b): dec pt >300 °C; R_f 0.36; ^{1}H NMR (Me_2SO-d_6) δ 4.64 (2 H, s, methylene), 3.92 (3 H, s, *N*-(3)-methyl), 3.32 (3 H, s, methoxy); IR (KBr) 3566, 3465, 3008, 2777, 1730, 1699, 1686, 1634, 1584, 1531, 1521, 1499, 1413 cm^{-1}; MS (EI) m/z 290 (M^+), 260 ($M^+ - CH_2O$). Anal. Calcd for $C_{12}H_{10}N_4O_5 \cdot 1H_2O$: C, 47.45; H, 3.81; N, 18.43. Found: C, 47.65; H, 3.53; N, 18.08.

Acknowledgment. The research was supported by a Faculty Grant-in-Aid from Arizona State University, a Cottrell Research Grant from the Research Corp., a grant from the donors of the Petroleum Research Foundation, administered by the American Chemical Society, and an award from the National Cancer Institute, Grant DHHS (PHS #1 R01 CA 36876-01). The Bruker AM-400 was purchased and supported by an instrumentation grant from the National Science Foundation (CHE-8409644).

Photochemical Reinvestigation of a 5-Phenyl-2-pyrazoline and Its Product Azocyclopropanes

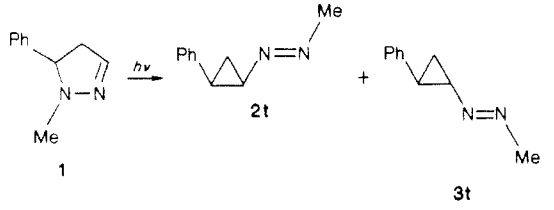
Paul S. Engel* and Gregory A. Bodager

Department of Chemistry and Rice Quantum Institute, Rice University, Houston, Texas 77251

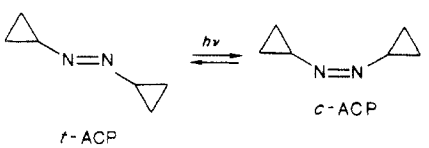
Received June 19, 1986

Irradiation of 1-methyl-5-phenyl-2-pyrazoline (**1**) affords the previously reported azocyclopropanes **2t** and **3t**; however, the major product of this reaction is β-(methylamino)-β-phenylpropionitrile (**4**). Although the azo linkage in **2t** and **3t** has the trans configuration, irradiation of either isomer causes azo trans → cis isomerization, slower interconversion of the ring isomers, reversion to pyrazoline **1**, and cleavage to styrene. Thermolysis of **2t** proceeds twice as fast as that of **3t** to interconvert the ring isomers and ultimately to afford exclusively **1**. The rapid thermolysis rate of **2t** and **3t** relative to a model phenylvinylcyclopropane is interpreted in terms of an unusually high facile formation of the α-azo (hydrazonyl) radical.

In 1968, Rosenkranz and Schmid[1] reported that UV irradiation of 1-methyl-5-phenyl-2-pyrazoline (**1**) produced isomers **2t** and **3t**. These product azoalkanes, which were



the first azocyclopropanes in the literature, caught our attention because of their unexpected photochemical behavior relative to azocyclopropane (*t*-ACP).[2] Whereas irradiation of *t*-ACP gave exclusive trans–cis isomerization of the azo group (*t*-ACP ⇄ *c*-ACP), Rosenkranz and Schmid said nothing about the configuration of the N=N linkage in **2t** and **3t**; in fact, they originally depicted the MeN=N group as semilinear. Irradiation of **2t** and **3t** was said to interconvert these ring isomers without causing reversion to **1**.[1] We found this result unusual because cis–trans ring isomerization most likely involves a 1,3-biradical[3,4] that should sometimes reclose to a pyrazoline. In fact, **2t** and **3t** are interconverted by thermolysis but they also lead to **1**.[1]



Other aspects of the system **1**–**3** appeared worthy of further exploration. For example, the total yield of **2t** and **3t** from **1** was only 35%, leading us to wonder whether any other photoproducts were formed. The use of benzene as solvent seemed unusual because it would absorb the UV light intended for **1**. Moreover, the broad band UV irradiation employed in the original work would not only mask any wavelength effects but could also cause secondary reactions of the UV-absorbing primary photoproducts. Finally, we were interested in the thermolysis rates of **2t** and **3t** because they should exhibit the large rate enhancement previously seen with *t*-ACP.[2]

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