Sigmatropic Reactions of the Aziridinyl Semiquinone Species. Why Aziridinyl Benzoquinones Are Metabolically More Stable than Aziridinyl Indoloquinones†

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ABSTRACT: Described herein is the chemistry of aziridinyl semiquinone species, which are formed upon one-electron metabolic reduction of aziridinyl quinone antitumor agents. The semiquinone species undergo a type of electrocyclic reaction known as a 1,5-sigmatropic shift of hydrogen. This reaction converts the aziridinyl group to both ethylamino and amino groups resulting in a loss of cytotoxicity. Since the radical anion conjugate base does not undergo ring opening as fast as the semiquinone, it was possible to determine the semiquinone pK_a values by plotting the percent sigmatropic products versus pH. Aziridinyl quinones based on benzoquinones, such as DZQ and AZQ, possess semiquinone pK_a values below neutrality. In contrast, an indole-based aziridinyl quinone possesses a semiquinone pK_a value of 9.3. Single electron reduction of DZQ and AZQ by NADPH: cytochrome P-450 reductase at physiological pH therefore affords the radical anion without any sigmatropic rearrangement products. In contrast, the same reduction of an aziridinyl indoloquinone affords the semiquinone which is rapidly converted to sigmatropic rearrangement products. These findings suggest that aziridinyl quinone antitumor agents based on indoles will be rapidly inactivated by one electron-reductive metabolism. A noteworthy example is the indoloquinone agent EO9, which is rapidly metabolized in vivo. In contrast, benzoquinone-based aziridinyl quinone antitumor agents such as AZQ, DZQ, and the new benzoquinone analogue RH1 do not suffer from this problem.

The aziridinyl quinone-based antitumor agents are functionalized to be activated as alkylating agents by two-electron reduction. The reductive activation process merely involves an increase in the pK_a of the protonated aziridine nitrogen upon reduction to the hydroquinone (*1*), so that this reactive species can exist at physiological pH and alkylate DNA N(7) nucleophiles (*2*−*7*). In the absence of reduction, the protonated aziridinyl quinone alkylating species exists only in strong acid (*8*). Besides two electron reduction, the aziridinyl quinones can also be activated by one electron reductive activation to afford DNA alkylation products, as well as oxygen radical species upon reaction with oxygen (*9*−*12*, *13*). Examples of aziridinyl quinone antitumor agents include the simple benzoquinones DZQ and AZQ (*14*) as well as the more complex EO9 (*15*−*17*) and PBI systems, Chart 1.

The antitumor agent EO9 (*18*, *19*) suffers from a short plasma half-life due to facile opening of the aziridinyl ring. In contrast, DZQ is a clinically useful antitumor agent and does not appear to have this problem. Recently, the substituted aziridinyl benzoquinone RH1 was documented to be metabolically stable compared to EO9 (*20*). We therefore began a study of ring opening reactions of aziridinyl quinones activated by one- and two-electron reduction. The usual two-electron reduction products of aziridinyl quinones illustrated in Chart 2 include the following: nucleophile trapping, tautomerization followed by aziridine elimination, and the 1,5-sigmatropic shift reaction. The sigmatropic shift reaction has only been documented for some of the PBIs (*21*). This process is essentially a redox reaction between the aziridine and the hydroquinone rings to afford the noncytotoxic derivatives. Tautomerization of electron-rich aziridinyl hydroquinones serves to stabilize overly electron-rich systems, leading to elimination of the aziridine ring in some cases. Finally, the nucleophilic trapping reaction is the process responsible for cytotoxicity when DNA nucleophiles are the target. However, the trapping of water is an inactivation process of reduced aziridinyl quinones and is, in fact, one of the metabolites of EO9 (*22*).

At the outset of this study, we postulated that the sigmatropic shift reaction upon one-electron activation could also be an important inactivation path. Herein we report that the one-electron reduction of aziridinyl quinones, either catalytically or by NADPH:cytochrome P-450 reductase, can result in a facile sigmatropic shift reaction of the semiquinone intermediate. This process is dependent on the semiquinone pK_a such that electron-rich systems (higher pK_a) undergo the reaction at physiological pH and electron-deficient systems (lower pK_a) do not. The sigmatropic process may therefore be involved in the metabolic inactivation of some aziridinyl quinones. Since the radical anion conjugate slowly undergoes this rearrangement, it was possible to determine semiquinone pK_a values by measuring rearrangement products as a function of pH. Indole-based aziridinyl quinones (e.g., EO9)

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Chart 1

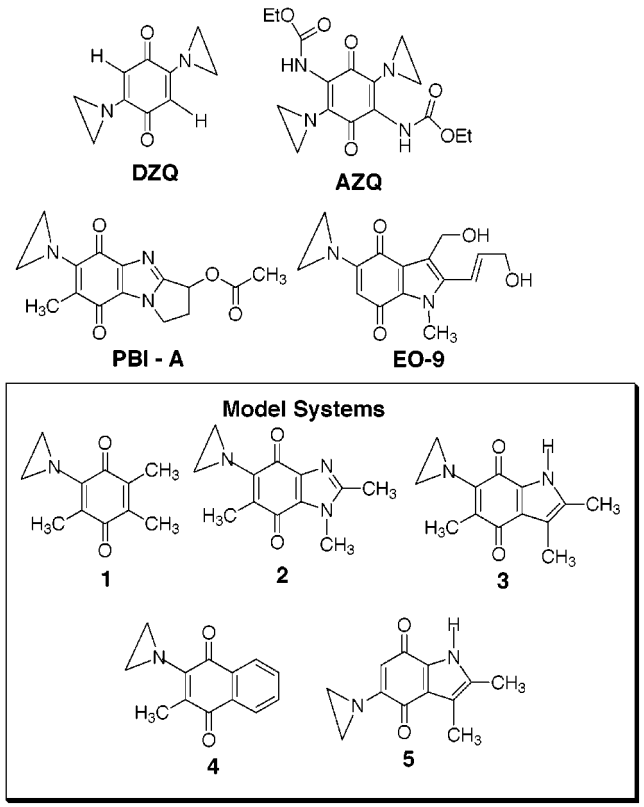
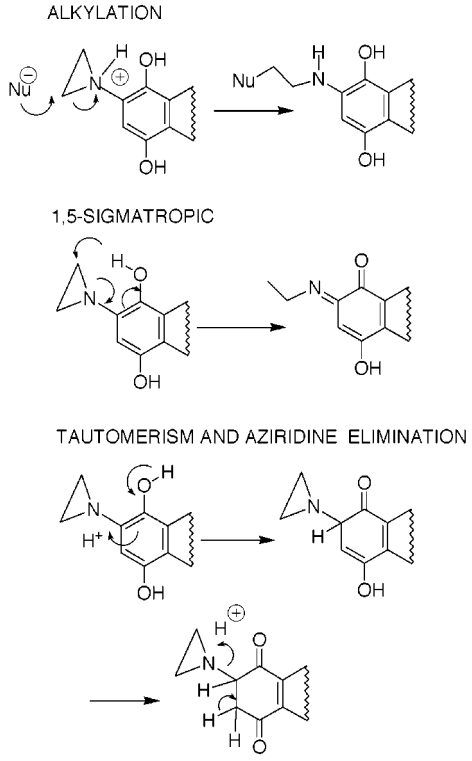


Chart 2



possess a high semiquinone pK_a, due to the presence of the indole nitrogen lone pair, resulting in significant amounts of semiquinone at physiological pH. In contrast, the DZQ semiquinone possesses a pK_a below neutrality and largely exists as the radical anion at physiological pH. We have thus concluded that indole-base aziridinyl quinones are more susceptible to cytochrome-mediated ring opening than benzoquinone-based systems due to this difference in semiquinone pK_a value.

MATERIALS AND METHODS

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some compounds contained fractional amounts of water of crystallization that was determined from the elemental analyses found. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, GA. All of the bulk sigmatropic shift products (**1**−**5a**,**b**) were characterized by elemental analysis, but the AZQ hydroquinone hydrolysis products in Chart 8 were only characterized by ^{1}H NMR and mass spectrometry due to the low yields. Uncorrected melting points and decomposition points were determined with a Mel-Temp apparatus. All TLCs were run with silica gel plates with fluorescent indicator, employing a variety of solvents. IR spectra were taken as KBr pellets or thin films; the strongest IR absorbances are reported. ^{1}H NMR spectra were obtained on a 300 MHz spectrometer, and chemical shifts are reported relative to TMS. UV−vis spectroscopic/kinetic studies were carried out on an OLIS-modified Cary 14. Computer fits of rate laws were carried out with a Scientist mathematical fitting program. Preparative enzymatic reactions were carried out a Thunberg flask, which resembles a Thunberg cuvette, except the bottom port is a 50 mL flask. Hydroquinone derivatives were prepared and handled in a Vacuum Atmospheres nitrogen glovebox.

DT-diaphorase was purified from Hooded rat liver. NADH, NADPH, and cytochrome P-450 reductase were purchased from Sigma, stored under −20 °C, and used within 2 weeks.

Catalytic Reduction of Aziridinyl Quinones in Methanol with H_2. To 20 mg of the aziridinyl quinone (**1**−**5**,DZQ, AZQ) in 15 mL of MeOH was added 3 mg of 5% Pd on carbon and the solution was degassed with argon for 5 min, followed by H_2 bubbling until color disappeared. The solution was then bubbled with argon for another 5 min and opened to the air. The catalyst was filtered off using Celite and the filtrate was concentrated to a residue. The products were purified by flash chromatography using CH_2Cl_2 as the eluent.

Catalytic Reduction of Aziridinyl Quinones in pH 7.96 Buffer with H_2. To a solution of 20 mL of 0.05 M pH 7.96 phosphate buffer was added 3 mg of 5% Pd on carbon and a solution of 15 mg of aziridinyl quinone (**1**−**5**, DZQ, AZQ) in 2 mL of DMSO. The solution was degassed with argon for 5 min, followed by H_2 until the color disappeared. The solution was then bubbled with argon for another 5 min and opened to the air, extracted six times with 10 mL portions of CH_2Cl_2. The extract was washed twice with 20 mL of water, dried over Na_2SO_4, and concentrated to a residue. The products were separated by flash chromatography using CH_2Cl_2 as eluent.

Reduction of DZQ in 0.05 M pH 5.99 Acetate Buffer. To a solution of 40 mL of pH 5.99 0.05 M acetate buffer was added 35 mg of 5% Pd on carbon and a solution of 50 mg of DZQ in 6 mL of DMSO. The solution was degassed with argon for 5 min, followed by H_2 until the color disappeared. The solution was then bubbled with argon for another 5 min and opened to the air, extracted three times with 60 mL portions of CH_2Cl_2. The extract was washed twice with 20

mL of water, dried over Na_2SO_4, and concentrated to a residue. The products were separated by flash chromatography using CH_2Cl_2 as eluent and identified by NMR, Chart 4. Yields were obtained spectrophotometrically from the extinction coefficents of the products.

Catalytic Reduction of DZQ in d_1-Methanol with H_2. The same procedure for the catalytic reduction of DZQ in methanol was employed except d_1-methanol was used as the solvent. The DZQa isolated from reactions in methanol and d_1-methanol were compared by MS and NMR. DZQa from d_1-methanol was a mixture nondeuterated, ($C_{10}H_{14}N_2O_2$), *m*/*z* 194, and monodeuterated ($C_{10}H_{13}D_1N_2O_2$), *m*/*z* 195. The intensity of *m*/*z* 195 is about one-third that of *m*/*z* 194 in the d_1-methanol product, while the intensity of *m*/*z* 195 is about one-fifth that of *m*/*z* 194 in the methanol product. Either parent ion afforded *m*/*z* 179 due to loss of CH_3 or CH_2D and the d_1-methanol product showed a molecular fragment with *m*/*z* 16, corresponding to CH_2D. The NMR spectrum nondeuterated DZQa has one triplet for both methyls of the two aminoethyl groups due to symmetry. In contrast, the deuterated DZQa has two closely spaced triplets for these methyls indicating that not all the methyls are the same due to deuterium substitution in some of these methyls.

2,5-Diaziridinyl-1,4-benzohydroquinone ($DZQH_2$). To 35.6 mg (0.187 mmol) of DZQ in 10 mL of methanol was added 22 mg of $NaBH_4$. The solution was stirred at RT for 20 min and centrifuged for 10 min. The liquor was decanted and the precipitate was washed with cold methanol twice: 29 mg (80%). ^{1}H NMR (d_6-DMSO) δ 8.23 (2H, bs, hydroquinone protons), 6.19 (2H, s, 3,6 -protons), 2.48 (8H, s, 2,5-diaziridinyl protons); MS (EI mode) *m*/*z* 192 (M^+), 177, 164, 149, 136, 122.

2-Aziridinyl-3, 5, 6-trimethyl-1,4-benzohydroquinone ($1H_2$). To 31 mg of **1** in 10 mL of methanol was added 15 mg of $NaBH_4$ under strict anaerobic conditions. The solution was stirred at room temperature for 2 h and centrifuged for 10 min. The liquor was decanted and the precipitate was washed with cold MeOH twice: 13 mg (42%). ^{1}H NMR (d_6-DMSO) δ 7.33 and 7.18 (2H, 2s, 1,4-hydroxy protons), 2.48 (4H, s, 2-aziridinyl), 2.08, 1.99 and 1.97 (9H, 3s, 3,5,6-trimethyls).

*The hydroquinones of **2**, **3**, **4**, and **5*** could not be isolated because they did not readily crystallize from methanolic solutions. Studies with these hydroquinones were carried out in methanolic stock solutions. Aeration of these stock solutions afforded the corresponding quinones in pure form.

Deuterium Exchange during $DZQH_2$ Hydrolysis. The pD 7.22 deuterium buffer was prepared as follows: K_2HPO_4 435.7 mg and KH_2PO_4 340.4 mg were dissolved in 25 mL of D_2O. To the pD 7.22 buffer were added 3 mg of 5% Pd on carbon and a solution of 15 mg of DZQ in 2 mL of DMSO. The reaction was reduced with H_2 and then incubated under anaerobic conditions for 18 h. The products were isolated by preparative thin-layer chromatography: DZQ, 3.6 mg; DZQa, 0.43 mg; and nucleophile trapping product, 3.2 mg. Mass spectra of these products revealed that DZQ did not incorporate deuterium but the DZQa incorporated deuterium at the 3,6-positions at nearly 100%. DZQa: MS (*m*/*z*) $C_{10}H_{12}N_2D_2O_2$, 196 (M^+), 181 ($M^+ - CH_3$), 167, 153, 139, 127, 111, 96, 83, 69. The ^{1}H NMR spectrum of DZQa revealed that the peak at δ 5.93 is decreased to only 2%.

*DT-Diaphorase−NADH Reduction of **1**, **3**, and DZQ.* To a solution of 20 mL of 50 mM pH 7.4 Tris-HCl buffer in the lower port of a Thunberg flask, was added 3.5 mg of quinone in 0.5 mL of DMSO solution. To the top port of the Thunberg flask was added 0.2 mL of DT-diaphorase from Hooded rat liver and 30 mg of NADH in 3 mL of buffer. Both solutions were degassed for 5 min with argon and the ports mixed. The reaction was incubated at 30 °C for 2 h, during which time the quinone color disappeared. The solution was then opened to the air and stirred for 20 min, extracted six times with 10 mL portions of CH_2Cl_2. The extract was washed twice with 20 mL portions of water, dried over Na_2SO_4 and concentrated for flash chromatography using CH_2Cl_2 as the eluent. In all cases, only starting quinone was isolated as the final product.

*Hydrolysis of Two-Electron Reduced **1** in 0.2 M pH 6.60 Phosphate Buffer.* To a solution of 6 mg of **1** in 2 mL of DMSO was added 2 mL of anaerobic methanol containing 1−2 mg of $NaBH_4$ under nitrogen. After the reaction mixture turned colorless, it was combined with a solution of 50 mL of 0.2 M pH .60 phosphate buffer and then incubated at room temperature for 4 h under nitrogen. The solution was then stirred aerobically for 24 h to reoxidize the hydroquinone species and then extracted three times with 40 mL portions of CH_2Cl_2. The extracts were washed twice with 40 mL portions of water, dried over Na_2SO_4, and concentrated to a residue. The extracted products were purified by flash chromatography on silica gel using CH_2Cl_2 as eluent: 2-(2-hydroxyethylamino)-3,5,6-trimethyl-1,4-benzoquinone (21.4% yield) and 2-hydroxy-3,5,6-trimethyl-1,4-benzoquinone (5.6% yield). The aqueous solution was concentrated and purified on Baker Phenyl reverse phase using water as the eluent: 2-(2-hydroxyethylamino-*O*-phosphate)-3,5,6-trimethyl-1,4-benzoquinone **1c** (33.6%yield).

*Measurement of Semiquinone pK_a of AZQ, DZQ, **1**, and **5**.* To 30 mg of the quinone in 2 mL of DMSO was added 25 mg of 5% Pd on carbon, and it was mixed with 18 mL of buffer resulting in the final buffer concentration of 50 mM. The solution was degassed with argon for 5 min followed by bubbling H_2 for 5 min. Then the solution was degassed again with argon for 5 min and then poured into 50 mL of saturated $NaHCO_3$ aqueous solution. The solution was extracted three times with 40 mL portions of CH_2Cl_2. The extract was washed two times with 50 mL portions of H_2O and dried over Na_2SO_4. The residue was either isolated with chromatography and the percent yield obtained by weighing the sigmatropic product, Figure 1. Alternatively, the percent yield of sigmatropic rearrangement product was obtained from the UV−vis spectrum of the product, Figure 2.

*Cytochrome P-450 Reductase/NADPH Mediated Reduction of AZQ, DZQ, and **5**.* To the lower port of the Thunberg flask was added 20 mL of argon-degassed 50 mM buffer, either pH 7.40 tris or pH 6.4 acetate, 3.5 mg of quinone in 0.5 mL of DMSO solution, and 25 mg of NADPH in 3 mL of the reaction buffer. To the top port of the Thunberg flask was added 50 μg of cytochrome P-450 reductase in 1 mL of the reaction buffer and both ports were degassed for 5 min with argon. The pre-degassing of the 20 mL of buffer served to decrease the final degassing time. Also, the Teflon tube used to degas the top port was not inserted into the enzyme solution to avoid denaturing. The flask was sealed after the degassing was complete and the ports mixed. Incubation times were determined from the time required for the quinone

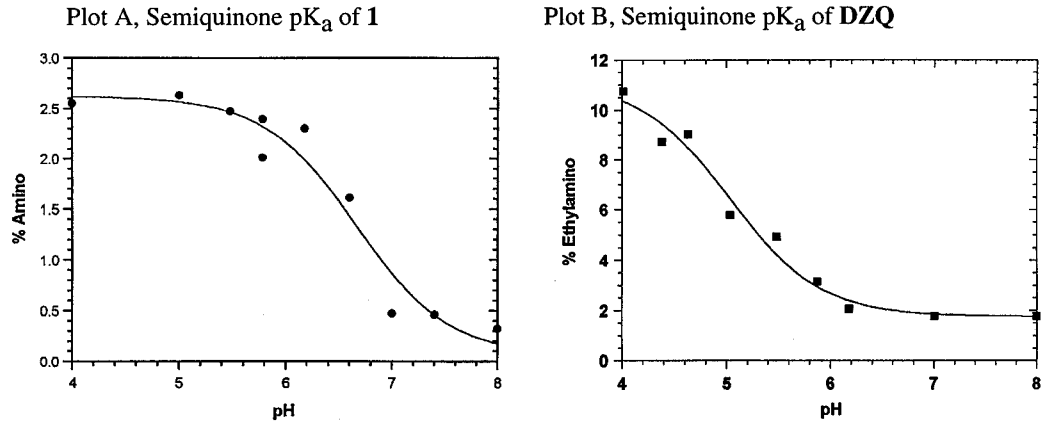


FIGURE 1: Percent sigmatropic shift products, either ethylamino or amino quinone products, versus the pH. The percent yields were determined at each pH by isolation and weighing of the product. The solid lines were computer- generated from eq 1.

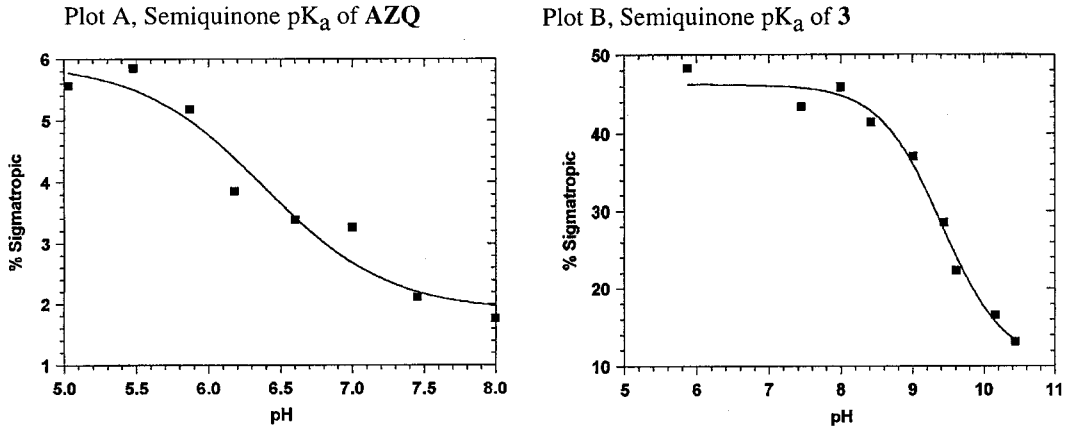


FIGURE 2: Percent sigmatropic shift products, either ethylamino or amino quinone products, versus the pH. The percent yields were determined at each pH from extinction coefficient and absorbance data. The solid lines were computer-generated from eq 1.

color to disappear, usually 3 h. The solution was then opened to the air and stirred for 20 min followed by extraction six times with 10 mL portions of CH_2Cl_2. The extract was washed twice with 20 mL portions water, dried over Na_2SO_4, and separated by flash chromatography using CH_2Cl_2 as the eluent. For DZQ and AZQ, reductions in pH 7.4 reactions afforded only the starting quinones, while **5** afforded sigmatropic shift and nucleophile-trapping products, **5a**−**c**. DZQ afforded starting material again at pH 6.4, but the AZQ reaction at this pH afforded the sigmatropic rearrangement product 2-aziridinyl-3,6-diethoxycarbamyl-5-(ethylamino)-1,4-benzoquinone(AZQa′) along with starting material in a ratio 1:3.

*2-Ethylamino-3,5,6-trimethyl-1,4-benzoquinone (**1a**).* Mp 117−118 °C; TLC ($CHCl_3$/MeOH, 95:5). R_f = 0.75; IR (KBr pellet) 3448, 3240, 2966, 2926, 2361, 1647, 1597, 1510, 1467, 1386, 1261, 1124, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.35 (1H, bs, amino proton), 3.49 (2H,q, J = 6.9 Hz, methylene of ethyl), 2.067, 2.027 and 1.952 (9H, 3s, 3,5,6 methyls), 1.227 (3H, t, J = 6.9 Hz, methyl of ethyl); MS (EI mode) m/z 193 (M^+), 178 ($M^+ - CH_3$), 163, 150, 138, 122, 108. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.39; H, 7.90; N, 7.18.

*2-Amino-3,5,6-trimethyl-1,4-benzoquinone (**1b**).* Mp 155−156 °C; TLC ($CHCl_3$/MeOH, 95:5) R_f = 0.55; IR (KBr pellet) 3477, 3375, 3227, 2920, 2362, 1655, 1595, 1489, 1390, 1300, 1269, 1097, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.68 (2H, bs, amino proton), 2.023, 1.974, 1.838 (9H, 3s, 3,5,6 methyls), MS (EI mode) m/z 165 (M^+), 150 ($M^+ - CH_3$), 137,123. Anal. Calcd for $C_9H_{11}NO_2$: C, 65.43; H, 6.71; N, 8.48. Found: C, 65.34, H, 6.74, N, 8.44.

*2-(2-Hydroxyethylamino-O-phosphate)-3,5,6-trimethyl-1,4-benzoquinone (**1c**).* TLC (H_2O:*n*-BuOH:acetic acid, 5:3:2). R_f = 0.25; ^{1}H NMR (D_3O) δ 3.75 (2H, q, J = 5.4 Hz, *O*-methylene), 3.56 (2H, t, J = 5.4 Hz, *N*-methylene), 1.83, 1.81, and 1.77 (9H, 3s, 3,5,6-methyls); ^{31}P NMR (D_2O) δ 4.51; From GHELCO(D_2O) spectra, protons at 3.75 were coupled to phosphorus at δ 4.51 confirming the phosphate nucleophile trapping product.

*5-Ethylamino-1,2,6-trimethylbenzimidazole-4,7-dione (**2a**).* Mp 152−155 °C; TLC ($CHCl_3$/MeOH, 9:1) R_f = 0.54; IR (KBr pellet) 3464, 2960, 2926, 2868, 2360, 2353, 1674, 1626, 1514, 1475, 1305, 1130, 1057, 603 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.76 (1H, bs, 5-amino protons), 3.89 (3H, s, 1-methyl), 3.58 (2H, q, J = 7.2 Hz, methylene of 5-amino-ethyl), 2.44 and 2.08 (6H, 2s, 2,6-methyls), 1.26 (3H, t, J = 7.2 Hz, methyl of 5-aminoethyl); MS (EI mode) m/z 233 (M^+), 218 ($M^+ - CH_3$), 204, 192,177. Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.78; H, 6.48; N, 18.16. Found: C, 61.67; H, 6.63; N, 18.24.

*5-Amino-1,2,6-trimethylbenzimidazole-4,7-dione (**2b**).* Mp 213-215 °C; TLC ($CHCl_3$/MeOH, 9:1) R_f = 0.35; IR (KBr pellet) 3551, 3454, 3416, 3317, 3240, 2926, 2856, 2651, 2346, 1662, 1597, 1529, 1471, 1438, 1384, 1319, 1091, 1030, 925, 746, 642, 603 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.85 (2H, bs, 5-amino protons), 3.89 (3H, s, 1-methyl), 2.46 and 1.86 (6H,

2s, 2,6-methyls); MS (EI mode) *m/z* 205 (M^+), 190 ($M^+ - CH_3$), 176, 161, 148, 121. Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.52; H, 5.40; N, 20.48. Found: C, 58.76; H, 5.47; N, 20.32.

*6-Ethylamino-2,3,5-trimethylindole-4,7-dione (**3a**).* Mp 185−187 °C; TLC ($CHCl_3$/MeOH, 98:2) $R_f = 0.74$; IR (KBr pellet) 3549, 3481, 3416, 3236, 2984, 2926, 2361, 2343, 1637, 1601, 1510, 1475, 1383, 1284, 1151, 1049, 771, 607, 480 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.28 (H, bs, indole proton), 5.37 (1H, bs, 6-amino protons), 3.51 (2H, q, $J = 6.9$ Hz, methylene of 6-aminoethyl), 2.25, 2.24, and 2.08 (9H, 3s, 2,3,5-methyls), 1.24 (3H, t, $J = 6.9$ Hz, methyl of 6-aminoethyl); MS (EI mode) *m/z* 232 (M^+), 217 ($M^+ - CH_3$), 203, 189, 175,148. Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.2; H, 6.94; N, 12.06. Found: C, 67.34; H, 6.87; N, 12.12.

*6-Amino-2,3,5-trimethylindole-4,7-dione (**3b**).* Mp 235−238 °C; TLC ($CHCl_3$/MeOH, 98:2) $R_f = 0.56$; IR (KBr pellet) 3568, 3475, 3416, 3367, 3223, 2920, 2854, 2364, 2345, 1656, 1618, 1595, 1574, 1491, 1390, 1302, 1276, 1172, 1099, 754, 626 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.80 (H, bs, indole proton), 4.66 (2H, bs, 6-amino protons), 2.24, 2.24 and 1.85 (9H, 3s, 2,3,5-methyls); MS (EI mode) *m/z* 204 (M^+), 189 ($M^+ - CH_3$), 177, 161, 147, 122. Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 6.91; N, 13.71. Found: C, 64.34; H, 7.04; N, 13.90.

*2-Ethylamino-3-methyl-1,4-naphthoquinone (**4a**).* Mp 119−121 °C; TLC ($CHCl_3$) $R_f = 0.8$; ^1H NMR ($CDCl_3$) δ 8.08, 7.99 (2H, 2m, 5,8 protons), 7.66, 7.58 (2H, 2m, 6,7 protons), 5.70 (1H, bs, 2-amino protons), 3.60 (2H,q, $J = 6.3$ Hz, methylene of 2-aminoethyl), 2.24 (3H, s, 3-methyl), 1.29 (3H, t, $J = 6.3$ Hz, methyl of ethyl); MS (EI mode) *m/z* 215 (M^+), 200 ($M^+ - CH_3$), 186, 172. Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.73; H, 6.05; N, 6.37.

*2-Amino-3-methyl-1,4-naphthoquinone (**4b**).* Mp 165−167 °C; TLC ($CHCl_3$) $R_f = 0.65$; ^1H NMR ($CDCl_3$) δ 8.08, 7.99 (2H, 2m, 5,8 protons), 7.66, 7.58 (2H, 2m, 6,7 protons), 4.96 (2H, bs, 2-amino protons), 2.02 (3H, s, 3-methyl); MS (EI mode) *m/z* 187 (M^+), 172 ($M^+ - CH_3$), 171 ($M^+ - NH_2$). Anal. Calcd for $C_{11}H_9NO_2$: C, 70.57; H, 4.84; N, 7.48. Found: C, 70.72; H, 4.78; N, 7.54.

*5-Ethylamino-2,3-dimethylindole-4,7-dione (**5a**).* Mp 200−202 °C; TLC ($CHCl_3$/acetone, 90:10) $R_f = 0.42$; IR (KBr pellet) 3448, 3317, 3146, 3051, 2972, 2924, 2854, 1662, 1606, 1570, 1485, 1392. 1249. 1105. 1058, 827 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.12 (H, bs, indole proton), 5.91 (1H, bs, 5-amino protons), 5.11 (1H, s, 6-proton), 3.16 (2H, q, $J = 6.6$ Hz, methylene of 5-aminoethyl), 2.20 and 2.19 (6H, 2s, 2,3 methyls), 1.29 (3H, t, $J = 6.6$ Hz, methyl of 5-aminoethyl); MS (EI mode) *m/z* 218 (M^+), 202 ($M^+ - CH_3$), 189, 175, 163 and 148. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.89; H, 6.57; N, 12.78.

*5-Amino-2, 3-dimethylindole-4,7-dione (**5b**).* Mp 234−236 °C; TLC ($CHCl_3$/acetone, 90:10) $R_f = 0.30$; IR (KBr pellet) 3381, 3277, 3207, 2920, 1660, 1591, 1568, 1498, 1477, 1384, 1253, 1201, 1132, 1051, 964, 825 cm^{-1}; ^1H NMR ($CDCl_3$ and d_6-DMSO) δ 11.12 (1H, bs, indole proton), 5.54 (2H, bs, 5-amino protons), 5.22 (1H, s, 6-proton), 2.05 and 2.02 (6H, 2s, 2,3 methyls); MS (EI mode) *m/z* 190 (M^+), 175 ($M^+ - CH_3$), 163, 148, 120, 109. Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 65.12; H, 5.42; N, 14.59.

*5-Hydroxyethylamino-2,3-dimethyl-4,7-indoloquinone (**5c**).* Mp 256−258 °C; TLC ($CHCl_3$/MeOH, 90:10) $R_f = 0.45$; IR (KBr pellet) 3443, 3306, 3281, 2928, 2872, 1658, 1601, 1566, 1475, 1392, 1244, 1190, 1138, 1057, 966 cm^{-1}; ^1H NMR ($CDCl_3$ and d_6-DMSO) δ 11.03 (1H, bs, indole proton), 6.36 (1H, bs, 5-amino), 4.93 (1H, s, 6-proton), 4.24 (1H, t, $J = 6.0$ Hz, hydroxy), 3.65 (2H, m, methylene of hydroxymethyl), 3.07 (2H, m, methylene of aminomethyl), 2.04 and 2.02 (6H, 2s, 2,3-methyls); MS (EI mode) *m/z* 234 (M^+), 216 ($M^+ - H_2O$), 203, 175, 161, 143, 111. Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.43; H, 6.09; N, 11.89.

2,5-Di(ethylamino)-1,4-benzoquinone (DZQa). Mp 188−189 °C; TLC ($CHCl_3$/MeOH, 95:5) $R_f = 0.66$; IR (KBr pellet) 3448, 3279, 2972, 2928, 1639, 1560, 1483, 1458, 1365, 1267, 1057, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.56 (2H, bs, amino protons), 5.29 (2H, s, 3,6 protons), 3.19 (4H, q, $J = 6$ Hz, methylenes of ethyls), 1.286 (6H, t, $J = 6$ Hz, methyls of ethyl); MS (EI mode) *m/z* 194 (M^+), 179 ($M^+ - CH_3$), 165, 151, 137, 123, 109. Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.83; H, 7.20; N, 14.42. Found: C, 61.95, H, 7.10, N, 14.56.

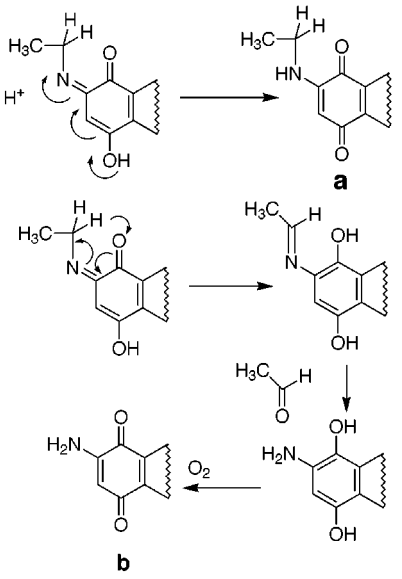
2-Amino-5-(ethylamino)-1,4-benzoquinone (DZQb). Mp 170−172 °C; TLC ($CHCl_3$/MeOH, 95:5) $R_f = 0.45$; IR (KBr pellet) 3448, 3273, 2980, 2926, 2853, 2326, 1655, 1568, 1543, 1498, 1458, 1354, 1292, 1055, 815, 669, 603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.38 (1H, bs, 5-amino protons), 5.536 and 5.307 (2H, 2s, 3,6 protons), 5.50 (2H, bs, 2-amino protons), 3.20 (2H, q, $J = 6$ Hz, methylene of 5-aminoethyl), 1.30 (3H, t, $J = 6$ Hz, methyl of ethyl); MS (EI mode) *m/z* 166 (M^+), 151 ($M^+ - CH_3$), 137, 123, 111, 96. Anal. Calcd for $C_8H_{10}N_2O_2$: C, 57.81; H, 6.07; N, 16.86. Found: C, 58.02; H, 6.12; N, 17.01.

2,5-Di(ethylamino)-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQa). Mp 223−225 °C; TLC ($CHCl_3$/MeOH, 90:10) $R_f = 0.45$; IR (KBr pellet) 3448, 3271, 2982, 2928, 2854, 2368, 2345, 1701, 1585, 1502, 1448, 1348, 1255, 1091, 1028, 771, 669, 603 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.74 and 5.65 (4H, 2bs, amino protons), 4.16 (4H, q, $J = 6.3$ Hz, 3,6-methylenes of ethyls), 3.58 (4H,q, $J = 6$ Hz, 2,5-methylenes of ethyls), 1.27 (12H, m, methyls of ethyl); MS (EI mode) *m/z* 368 (M^+), 353 ($M^+ - CH_3$), 339, 323, 307, 292, 276, 260, 249, 235. Anal. Calcd for $C_{16}H_{24}N_4O_6$: C, 52.16; H, 6.57; N, 15.21. Found: C, 52.11; H, 6.72; N, 15.32.

2-Aziridinyl-5-(ethylamino)-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQa′). TLC ($CHCl_3$/MeOH, 90:10) $R_f = 0.52$; ^1H NMR ($CDCl_3$) δ 6.36 and 6.12 (3H,bs, amino protons), 4.16 (4H, m, 3,6 methylenes of ethyls), 3.66 (2H,q, $J = 6$ Hz, 5-methylene of aminoethyl), 2.38 (4H, s, 2-aziridinyl), 1.27 (9H, m, methyls of ethyl); MS (EI mode) *m/z* 366 (M^+), 351 ($M^+ - CH_3$), 337, 321.

2-Amino-5-(ethylamino)-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQb). Mp 203−205 °C; TLC ($CHCl_3$/MeOH, 90:10) $R_f = 0.40$; IR (KBr pellet) 3483, 3414, 3271, 2980, 2933, 2364, 2345, 1701, 1664, 1616, 1508, 1460, 1345, 1249, 1091, 1068, 773, 669, 597, 499 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.67 and 6.42 (5H, bs, amino protons), 4.16 (4H, m, methylenes of ethoxys), 3.61 (2H, q, $J = 6$ Hz, methylene of aminoethyl), 1.27 (9H, m, methyls of ethoxys); MS (EI mode) *m/z* 340 (M^+), 325 ($M^+ - CH_3$), 312, 294, 266, 253, 248, 220, 207, 193, 166. Anal. Calcd for $C_{14}H_{20}N_4O_6$: C, 49.40; H, 5.92; N, 16.46. Found: C, 49.21; H, 5.97; N, 16.23.

Chart 3



2-Amino-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQc). ^{1}H NMR ($CDCl_3$) δ 7.53 and 7.23 (2H, 2s, amide NH), 6.99 (1H, s, 5-H), 5.54 (2H, s, amino), 4.2 (4H, 2q, methylenes of ethoxy groups), 1.3 (6H, 2t, methyls of ethoxy groups); MS (EI mode) *m*/*z* 297 (M^+).

2-(2′-Acetoxyethylamino)-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQd). ^{1}H NMR ($CDCl_3$) δ 7.46 and 7.06 (2H, 2s, amide NH), 6.43 (1H, s, 5-H), 5.72 (1H, t, amino), 4.2 (6H, m, methylenes attached to oxygen), 3.6 (2H, q, methylene attached to nitrogen), 2.09 (3H, s, acetate methyl), 1.3 (6H, 2t, methyls of ethoxy groups); MS (EI mode) *m*/*z* 383 (M^+).

2,5-Di(2′-acetoxyethylamino)-3,6-di(ethoxycarbamyl)-1,4-benzoquinone (AZQe). ^{1}H NMR ($CDCl_3$) δ 6.94 (2H, 2t, amino NH), 5.75 (2H, bs, amide NH), 4.2 (8H, m, methylenes attached to oxygen), 3.84 (4H, q, methylenes attached to nitrogen), 2.08 (3H, s, acetate methyl), 1.3 (6H, t, methyls of ethoxy groups); MS (EI mode) *m*/*z* 484 (M^+).

RESULTS AND DISCUSSION

One-Electron Reduction Products of Aziridinyl Quinones. One-electron reduction of a quinone in a biological system is usually associated with the generation of cytotoxic oxygen radicals by redox cycling (*23*). In this section we show that the addition of hydrogen atom to an aziridinyl quinone can result in rapid aziridine ring opening by a sigmatropic process, depending on the pH and solvent.

The presence of sigmatropic rearrangements in aziridinyl hydroquinones based on the pyrrolo[1,2-*a*]benzimidazole (PBI) was previously studied in this laboratory (*21*, *24*). Once the 1,5-sigmatropic shift occurs, tautomerism affords an *N*-ethylamino quinone derivative (product a), Chart 3. Since this process occurs under anaerobic conditions, the reaction mixtures become red or blue during the incubation. Upon aerobic workup, the red or blue color becomes more intense due to oxidation to afford an amino quinone derivative (product b), Chart 3. The formation of product b involves a second 1,5-sigmatropic shift followed by solvolysis of the imine intermediate. The rates of these rearrangements were found to be independent of solvent and therefore ascribed to an electrocyclic (sigmatropic) process (*21*).

Chart 4

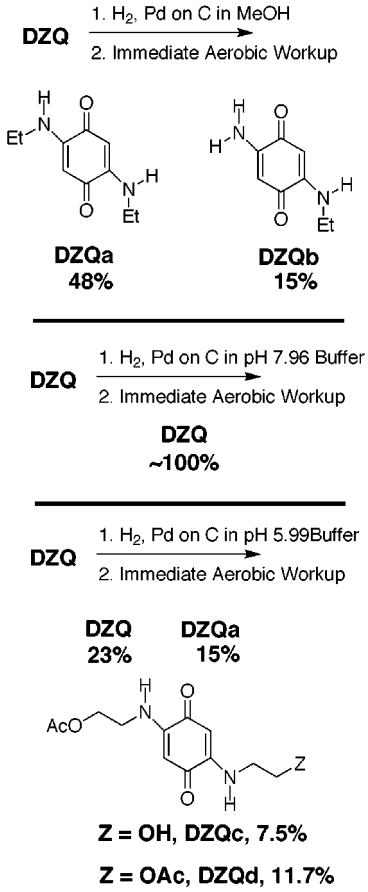


Table 1: Percent Yield of Products Obtained upon Catalytic Reduction in Methanol and Immediate Aerobic Workup

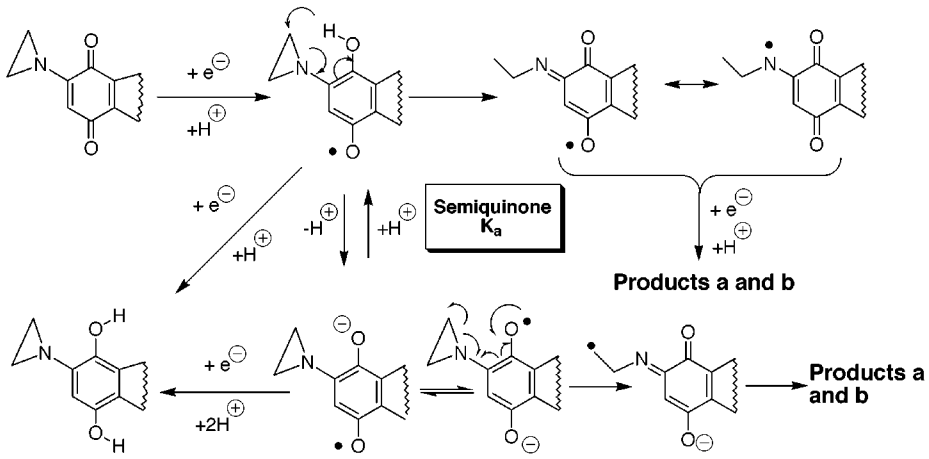
	AZQ	DZQ	1	2	3	4	5
yield a	46	48	56	46	61	56	65
yield b	32	15	22	17	24	19	12

To prepare the aziridinyl quinone one-electron reduction products in bulk, catalytic hydrogenation was carried out in anaerobic methanol at one atmosphere. Catalytic hydrogenation is known to occur by hydrogen atom transfer resulting in a "half hydrogenated state" at low hydrogen pressure (*25*) and therefore radical rearrangements have been observed in some reduction products (*26*). The sigmatropic rearrangement of the semiquinone species will occur only if the second hydrogen atom transfer is relatively slow. When DZQ was catalytically reduced at one atmosphere and immediately worked up, the sigmatropic rearrangement products DZQa and DZQb were isolated, Chart 4. Similarly, the other aziridinyl quinones in the series **1**−**5** provided the ethylamino (product a) and the amino derivative (product b) as the sole products, Table 1. This series of aziridinyl quinones and DZQ represents a range in electronic character from electron deficient (**1**) to the electron rich (**3** and **5**) (*8*).

The following results support the general mechanism shown in Chart 5. In this mechanism the DZQ semiquinone radical undergoes a sigmatropic rearrangement while the radical anion undergoes further reduction to the hydroquinone along with slow aziridinyl ring opening.

(1) The sigmatropic rearrangements were not observed when the hydroquinone form of DZQ was prepared and then

Chart 5



added to anaerobic methanol. Therefore, a reduction intermediate rather than the hydroquinone must be involved in the rearrangement.

(2) When the catalytic hydrogenation of DZQ was carried out in anaerobic deuterated methanol, mass spectral studies revealed the presence of deuterium in the methyl of the ethylamino product. This product is consistent with the sigmatropic rearrangement of the semiquinone species.

(3) The sigmatropic rearrangements were *not* observed in high yield when the catalytic hydrogenation of DZQ was carried out in basic solutions. Indeed, the catalytic reduction of DZQ in pH 7.96 phosphate buffer affords the hydroquinone with only trace amounts of the rearrangement products shown in Chart 4.

(4) The presence of aziridinyl ring opening products in basic solutions suggests that the radical anion could also rearrange.

(5) Consistent with the importance of the conjugate acid (i.e., the semiquinone) in the rearrangement, the catalytic reduction of DZQ in pH 5.99 acetate buffer afforded a sigmatropic product along with acetate nucleophile-trapping products and unreacted DZQ, Chart 4.

The radical anion ring opening reaction shown in Chart 5 has precedents in the chemical and biochemical literature. The radical-mediated opening of the aziridinyl ring is similar to that of the "azacyclopropylcarbinyl" radical intermediate occurring in the reaction of lysine 2,3-aminomutase (*27*, *28*). Ring opening of cyclopropylcarbinyl radical anions is another well-known chemical precedent (*29*−*31*). However, the present study shows that aziridinyl ring opening by semiquinone sigmatropic shift is more favored than opening via the radical anion. The explanation is that resonance stabilization of the radical species occurs both before and after the ring opening by sigmatropic rearrangement. In contrast, cyclopropylcarbinyl radicals stabilized by resonance rearrange slowly because of the loss of resonance accompanying primary radical formation (*30*, *31*).

Since the semiquinone species undergoes the sigmatropic rearrangement with facility, the ring opening products could indirectly provide the semiquinone pK_a value. The measurement of semiquinone pK_a values has been carried out by pulse radiolysis. The tetramethyl benzoquinone semiquinone has a pK_a of 5.1 (*32*) while the more electron-rich indole based semiquinone has a pK_a of 6.8 (*33*). Inspection of the data in Table 2 reveals how the electronic character of the aziridinyl quinone influences the rearrangement. At pH 7.96 different aziridinyl quinones will afford different amounts of rearrangement products, presumably due to differences in the semiquinone pK_a. Consistent with a semiquinone pK_a below neutrality, both **1** and DZQ reductions afford the corresponding hydroquinones, which crystallize from solution. In contrast, the more electron-rich AZQ shows some rearrangement products while the heterocyclic systems **2** and **3** afforded larger amounts of rearrangement products.

Table 2: Percent Yield of Products Obtained upon Catalytic Reduction in pH 7.96 Phosphate Buffer and Immediate Aerobic Workup

yield %	AZQ	DZQ	1	2	3
SM	52	major	major	24	10
a	16	trace	trace	22	44
b	11	not found	not found	15	trace

Shown in Figure 1 are the titration curves for **1** and DZQ determined from percent rearrangement vs pH plots. In these reactions the percent rearrangement was determined by isolation. The solid lines in the plots of Figure 1 were obtained by computer fitting to eq 1:

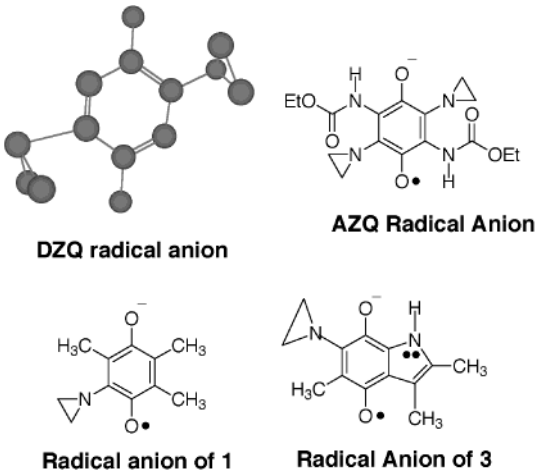
$$\%\ \text{measured} = [a_H\ (\%\ \text{yield from semiquinone}) + K_a\ (\%\ \text{yield from radical anion})]/(a_H - K_a) \quad (1)$$

where a_H is the proton activity, K_a is the acid dissociation constant of the semiquinone, and "% measured" is the yield of sigmatropic rearrangement products determined from isolation, either the "a" or "b" products in Chart 3. The "% yield from semiquinone", "% yield from radical anion", and K_a were calculated from the computer fit. The semiquinone pK_a values obtained from these fits for **1** and DZQ were 6.6 and 5.0, respectively.

Spectrophotometric yields of the sigmatropic products were determined using the absorbance measurements and extinction coefficients. The percent yield versus pH data for AZQ and **5** were fit to eq 1 providing semiquinone pK_a values of 6.3 and 9.3 respectively, Figure 2. The spectrophotometric method is a rapid and accurate means to determine semiquinone pK_a values compared to the isolation and weighing method.

The calculated yield for rearrangement from the radical anion was always very small while that from the semiquinone

Chart 6

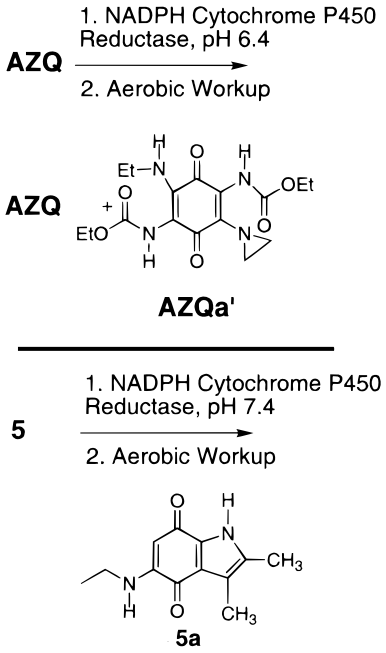


varied between 5 and 50%. The variability in yield from the semiquinone is attributed to the rate of the rearrangement of the semiquinone compared to its further reduction to the hydroquinone. The electron-rich indole semiquinone affords higher sigmatropic rearrangement products than the benzo semiquinone system. The balance of products in these reactions was hydroquinone, which was isolated as the quinone starting material upon aerobic workup.

The semiquinone pK_a values, measured using either isolation or spectrophotometric means, fall in the range of known values for semiquinones (*32*, *33*). Significantly, these pK_a values do not match those previously observed for *N*-protonated aziridinyl hydroquinones, which possess pK_a values >8 for the protonated aziridinyl nitrogen and even higher values for hydroxyl acid dissociation (*1*). Variations in the values for **1**, **5**, DZQ, and AZQ are in accord with substituent effects on the radical anion species, Chart 6. The DZQ radical anion has the aziridinyl rings twisted out of the plane of the benzene ring resulting in inductive electron withdrawal by the nitrogen rather than electron release. Consequently, the DZQ radical anion will be stabilized and the pK_a of the semiquinone will be relatively low (5.0). The addition of electron releasing methyl or carbamyl groups will destabilize the anion by electron release and the semiquinone pK_a will increase to near neutrality, as in **1** and AZQ, respectively. Finally, the indole radical anion of **5** will be even more destabilized due to the presence of the nitrogen lone pair in the aromatic system resulting in a semiquinone pK_a significantly above neutrality. The indole nitrogen lone pair of the semiquinone of **5** also facilitates the rearrangement resulting in yields of up to 50%.

The implication of the findings cited above is that rapid sigmatropic ring opening of aziridinyl quinones will occur upon metabolic one-electron reduction if the pK_a is near or above physiological pH, particularly in electron-rich systems. To illustrate the metabolic importance of semiquinone pK_a values, DZQ and AZQ were reduced by NADPH:cytochrome P-450 reductase in anaerobic pH 7.4 and 6.4 buffers. When DZQ was reduced in either buffer, the ultimate reduction product was the hydroquinone, which was isolated as the quinone upon aerobic workup. In contrast, AZQ reduction by NADPH:cytochrome P-450 reductase at pH 6.4 afforded a sigmatropic shift product AZQ a′ while the pH 7.4 reaction did not, Chart 7. These results are consistent with an AZQ

Chart 7



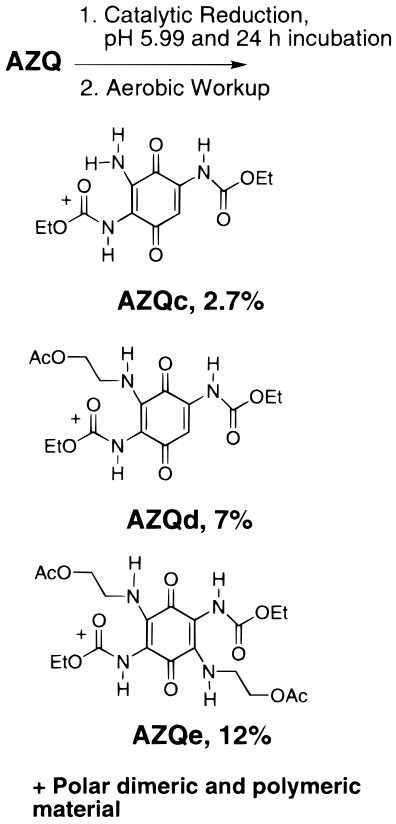
semiquinone pK_a value greater than that of DZQ, 6.3 versus 5.0.

In contrast to DZQ, indole or benzimidazole-based aziridinyl quinones should be converted to inactive ring-opened products in high yield upon one electron reduction by the cytrochrome P-450 system. With semiquinone pK_a values of ∼9, the sigmatropic ring opening process will readily occur in these systems at physiological pH. The cytochrome P-450 reduction of the indole-based aziridinyl quinone **5** at pH 7.4, in fact, afforded a high yield of the sigmatropic shift products in addition to water-trapping product. These findings may explain the short plasma half-life of the indole-based aziridinyl quinone EO9. With only 20% of the EO9 metabolites having been accounted for, the presence of sigmatropic metabolites of EO9 have yet been verified.

Two-Electron Reduction Products of Aziridinyl Quinones. The preparation of aziridinyl hydroquinones were carried out by catalytic reduction at pH ∼9, DT-diaphorase reduction, or borohydride reduction. These hydroquinones were isolated under strict anaerobic conditions and characterized by ^{1}H NMR. In contrast to the semiquinone species, the hydroquinones slowly react over a period of hours or days, depending on the pH, to afford nucleophilic trapping products or in some cases tautomerization products. A noteworthy example is reduced **5**, which remains unchanged for hours at pH values where the semiquinone species rapidly rearranges. In this section, the chemistry of the aziridinyl hydroquinone species is assessed with product and kinetic studies. The goal is to confirm that the sigmatropic rearrangement products arise mainly from the semiquinone species and not from the hydroquinone species.

A rare reaction of aziridinyl hydroquinones is tautomeric equilibrium between hydroquinone and ketone forms, Chart 2. This tautomerization serves to relieve the excessively electron rich character of some aziridinyl hydroquinones. The presence of this equilibrium was assessed by the presence of deuterium incorporation at the 3,6-positions when the hydroquinone was incubated in deuterated buffer. An indirect

Chart 8



method is the determination of the presence of deaziridination, which could arise from the elimination of aziridine from the tautomer. Reduction of DZQ in D_2O did not result in deuterium exchange at the 3,6-positions, but the ring opened analogue DZQa readily exchanged deuterium upon reduction in D_2O, see Materials and Methods. When reduced AZQ was incubated in acetate buffer, deaziridinated products AZQc and AZQd were observed along with nucleophile trapping products, Chart 8. The tautomerization of quinones occurs only in electron rich systems such as DZQa and to a degree in AZQ. Such hydroquinone tautomerizations are more common in reduced naphthoquinones(*34*) and anthraquinones (*35*, *36*).

The products shown in Chart 8 show that reduced AZQ traps acetate nucleophiles as well as deaziridinates via tautomers. However, the material balance is poor for this reaction due to intermolecular reactions; the presence of a dimer of unknown structure was determined by mass spectrometry.

In contrast, reduced **1** cleanly affords the water and phosphate (**1c**) trapping without any evidence of sigmatropic shifts, tautomerization, or polymerization, Chart 9. The mechanism of nucleophile trapping involves equilibrium protonation of the aziridinyl nitrogen followed by the reaction of the protonated species with a nucleophile. The rate law for this process is found in eq 2

$$k_{obsd} = a_H k/(a_H - K_a) \qquad (2)$$

where a_H is the proton activity, K_a is the acid dissociation constant of the protonated hydroquinone, and k is the rate constant for nucleophilic attack on the protonated aziridinyl hydroquinone. The k_{obsd} versus pH data are found in Figure

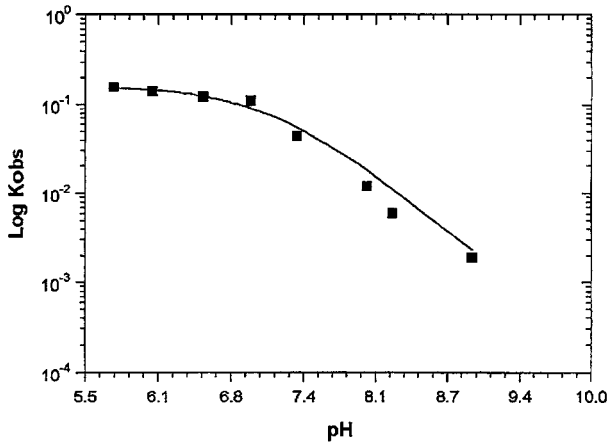
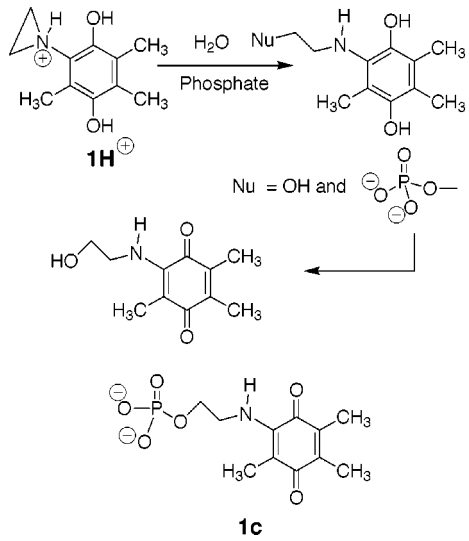


FIGURE 3: Log k_{odsd} versus pH plot for the hydrolysis of reduced **1** under strict anaerobic conditions. The solid line was computer generated from eq 2.

Chart 9



3 for the reaction of reduced **1** in anaerobic buffers. Computer fitting of these data to eq 2 provided the solution, $k = 0.15$ M^{-1} s^{-1} and $pK_a = 7.10$. This solution was used to generate the solid line in Figure 3. The chemistry of the hydroquinone of **1** is distinctly different than the semiquinone species. (i) The products are different with the hydroquinone undergoing nucleophile trapping reactions whereas the semiquinone rapidly undergoes sigmatropic rearrangements. (ii) The pK_a values of the protonated aziridinyl hydroquinone (7.10) is different from that of the semiquinone (6.6). (iii) The semiquinone species reacts rapidly (reactions complete in minutes if not seconds) compared to the hydroquinone (reactions complete in hours or days).

CONCLUSIONS

The above findings provide evidence that sigmatropic rearrangements occur in the semiquinone species of structurally diverse systems. Previously, we observed these rearrangements only in a PBI aziridinyl hydroquinone and its *N*-protonated form (*21*). The high energy semiquinone species readily undergoes this reaction because of the loss of ring strain without loss of radical delocalization. In contrast, the hydroquinone species slowly undergo nucleophile trapping reactions and tautomerization.

The sigmatropic rearrangement is pH dependent because the semiquinone rather than the radical anion conjugate base is the reacting species. This feature permitted the measurement of aziridinyl semiquinone pK_a values for the first time. While the benzoquinone-based systems DZQ and AZQ possess semiquinone pK_a values below neutrality, the indole-based analogue **5** possesses a pK_a value of 9.3. The nitrogen lone pair in the indole aromatic system results in the high semiquinone pK_a. Consequently, single-electron reduction of the indoloquinone at physiological pH (mostly semiquinone is present) provides sigmatropic rearrangement products in high yield. On the other hand, single electron reduction of the benzoquinones at physiological pH (mostly radical anion present) provides insignificant amounts of sigmatropic rearrangement products. To make metabolic connections, single-electron reductions of aziridinyl quinones were carried out with NADPH:cytochrome P-450 reductase. The indoloquinone **5** was converted to sigmatropic rearrangement products at physiological pH while the benzoquinones were merely converted to their corresponding hydroquinones. In contrast, DT-diaphorase converted all of the quinone systems to the corresponding hydroquinones at physiological pH without any sigmatropic rearrangements.

Ongoing work in this laboratory has shown that structural modification in series **5** can change the semiquinone pK_a. Substitution of the 2-position of **5** with an ethoxycarbonyl group results in a semiquinone pK_a decrease to 8.6 along with an increase in in vivo activity.

The above findings suggest that aziridinyl quinone antitumor agents based on indoles or benzimidazoles will be rapidly inactivated by one-electron reductive metabolism. A noteworthy example is the agent indoloquinone EO9, which is rapidly metabolized in vivo. In contrast, benzoquinone-based aziridinyl quinone antitumor agents do not suffer from this problem (*20*). A missing part of this study is the documentation of sigmatropic metabolites arising in vivo from aziridinyl indoloquinone antitumor agents. In the case of EO9, 20% of the metabolites are formed by water trapping and the rest are unidentified (*37*).

The obvious solution to the one-electron inactivation of aziridinyl indoloquinones is to lower the pK_a of the semiquinone species to below neutrality. A recent article from this laboratory revealed that the in vivo activity of an aziridinyl indoloquinone was improved greatly by *N*-acetylation (*38*), which served to lower the semiquinone pK_a by reducing the influence of the nitrogen lone pair.

Our findings seem to be at odds with role of NADPH: cytochrome P-450 reductase in the bioactivation of EO9 since semiquinone formation would lead to noncytotoxic products. A recent report clearly indicates that cytochrome reduction activates this aziridinyl quinone as a cytotoxic species (*39*). Preliminary studies from this laboratory indicate that when an indole or benzimidazole aziridinyl semiquinone is generated in the presence of DNA, alkylation processes effectively compete with the sigmatropic rearrangement. Indeed the aziridinyl semiquinone is ~10-fold better DNA alkylating agent than the aziridinyl hydroquinone. Perhaps semiquinone mediated alkylation of DNA, as well as oxygen radicals generated from the reaction of aziridinyl semiquinone with oxygen (*39*), contribute to cytotoxicity.

REFERENCES

1. Driebergen, R. J., Hartigh, J. D., Holthuis, J. J. M., Hulshoff, A., Van Oort, W. J., Postma Kelder, S. J., Verboom, W., Reinhoudt, D. N., Bos, M., and Van Der Linden, W. E. (1990) *Anal. Chim. Acta 233*, 251−268.
2. Hargreaves, R. H. J., Mayalarp, S. P., Butler, J., McAdam, S. R., OHare, C. C., and Hartley, J. A. (1997) *J. Med. Chem. 40*, 357−361.
3. Hartley, J. A., Berardini, M., Ponti, M., Gibson, N. W., Thompson, A. S., Thurston, A. S., Hoey, B. M., and Butler, J. (1991) *Biochemistry 30*, 11719−11724.
4. Lee, C. S., Hartley, J. A., Berardini, M. D., Butler, J., Siegel, D., Ross, D., and Gibson, N. W. (1992) *Biochemistry 31*, 3019−3025.
5. Lee, C. S., Pfeifer, G. P., and Gibson, N. W. (1994) *Cancer Res. 54*, 1622−1626.
6. Mayalarp, S. P., Hargreaves, R. H. J., Butler, J., O'Hare, C. C., and Hartley, J. A. (1996) *J. Med. Chem. 39*, 531−537.
7. Alley, S. C., Brameld, K. A., and Hopkins, P. B. (1994) *J. Am. Chem. Soc. 116*, 2734−2741.
8. Skibo, E. B., and Xing, C. (1998) *Biochemistry 37*, 15199−15213.
9. Giulivi, C., and Cadenas, E. (1998) *Chem.-Biol. Interact. 113*, 191−204.
10. Butler, J., Spanswick, V. J., and Cummings, J. (1996) *Free Radical Res. 25*, 141−148.
11. Goin, J., Giulivi, C., Butler, J., and Cadenas, E. (1995) *Free Radical Biol. Med. 18*, 525−536.
12. Lusthof, K. J., Demol, N. J., Janssen, L. H. M., Egberink, R. J. M., Verboom, W., and Reinhoudt, D. N. (1990) *Chem.-Biol. Interact. 76*, 193−209.
13. Li, B. B., Gutierrez, P. L., Amstad, P., and Blough, N. V. (1999) *Chem. Res. Toxicol. 12*, 1042−1049.
14. Reynolds, R. C. (1995) in *Cancer Chemotherapeutic Agents* (Foye, W. O., Ed.) pp 187−197, American Chemical Society, Washington, DC.
15. Hendriks, H. R., Pizao, P. E., Berger, D. P., Kooistra, K. L., Bibby, M. C., Boven, E., Dreef-van der Meulen, H. C., Henrar, H. H., Fiebig, H. H., Double, J. A., Hornstra, H. W., Pinedo, H. M., Workman, P., and Schwartsmann, G. (1993) *Eur. J. Cancer 29A*, 8997−906.
16. Maliepaard, M., Wolfs, A., Groot, S. E., de Mol, N. J., and Janssen, L. H. M. (1995) *Br. J. Cancer 71*, 836−839.
17. Jaffar, M., Naylor, M. A., Robertson, N., and Stratford, I. J. (1998) *Anti-Cancer Drug Des. 13*, 593−609.
18. Schellens, J. H. M., Planting, A. S. T., Vanacker, B. A. C., Loos, W. J., Deboerdennert, M., Vanderburg, M. E. L., Koier, I., Krediet, R. T., Stoter, G., and Verweij, J. (1994) *J. Natl. Cancer Inst. 86*, 906−912.
19. Aamdal, S., Lund, B., Koier, I., Houten, M., Wanders, J., and Verweij, J. (2000) *Cancer Chemother. Pharmacol. 45*, 85−88.
20. Loadman, P. M., Phillips, R. M., Lim, L. E., and Bibby, M. C. (2000) *Biochem. Pharmacol. 59*, 831−837.
21. Zhou, R., and Skibo, E. B. (1996) *J. Med. Chem. 39*, 4321−4331.
22. Schellens, J. H. M., Loos, W., Beijnen, J. H., Stoter, G., and Verweij, J. (1993) *J. Chromatogr.-Biomed. Appl. 615*, 309−315.
23. Butler, J. (1998) in *DNA and Free Radicals: Techniques, Mechanisms & Applications* (Aruoma, O. I., and Halliwell, B., Eds.) pp 131−159, Oica International, Micoud.
24. Skibo, E. B., Islam, I., Schulz, W. G., Zhou, R., Bess, L., and Boruah, R. (1996) *Synlett* 297−309.
25. Horiuti, I., and Polanyi, M. (1934) *Trans. Faraday Soc. 30*, 1165−1172.
26. Siegel, S., Dunkel, M., Smith, G. V., Halpern, W., and Cozort, J. (1966) *J. Org. Chem. 31*, 2802−2806.
27. Wu, W. M., Lieder, K. W., Reed, G. H., and Frey, P. A. (1995) *Biochemistry 34*, 10532−10537.
28. Ballinger, M. D., Frey, P. A., and Reed, G. H. (1992) *Biochemistry 31*, 10782−10789.
29. Tanko, J. M., and Phillips, J. P. (1999) *J. Am. Chem. Soc. 121*, 6078−6079.

30. Tanko, J. M., Drumright, R. E., Suleman, N. K., and Brammer, L. E. (1994) *J. Am. Chem. Soc. 116*, 1785−1791.
31. Tanko, J. M., and Drumright, R. E. (1992) *J. Am. Chem. Soc.* 114, 1844−1854.
32. Willson, R. I. (1971) *Chem. Commun.* 1249−1250.
33. Al-Kazwini, A. T., O'Neill, P., Adams, G. E., Cundall, R. B., Janquet, B., Lang, G., and Junino, A. (1990) *J. Phys. Chem. 94*, 6666−6670.
34. Papageorgiou, V. P., Assimopoulou, A. N., Couladouros, E. A., Hepworth, D., and Nicolaou, K. C. (1999) *Angew. Chem., Int. Ed. 38*, 271−300.
35. Bird, D. M., Gaudiano, G., and Koch, T. H. (1991) *J. Am. Chem. Soc. 113*, 308−315.
36. Brand, D. J., and Fisher, J. (1986) *J. Am. Chem. Soc. 108*, 3088−3096.
37. Workman, P., Binger, M., and Kooistra, K. L. (1992) *Int. J. Radiat. Oncol. Biol. Phys. 22*, 713−716.
38. Xing, C. G., Wu, P., Skibo, E. B., and Dorr, R. T. (2000) *J. Med. Chem. 43*, 457−466.
39. Saunders, M. P., Jaffar, M., Patterson, A. V., Nolan, J., Naylor, M. A., Phillips, R. M., Harris, A. L., and Stratford, I. J. (2000) *Biochem. Pharmacol. 59*, 993−996.

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