*Bioorganic & Medicinal Chemistry* Vol. 1, No. 1, pp. 39-43, 1993
Printed in Great Britain

0968-0896/93 $6.00 + .00


# Kinetic Studies Of 2-(2'-Haloethyl) And 2-Ethenyl Substituted Quinazolinone Alkylating Agents. Acid-Catalyzed Dehydrohalogenation And Alkylation Involving A Quinazolinone Prototropic Tautomer

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(*Received* 5 *January* 1993)

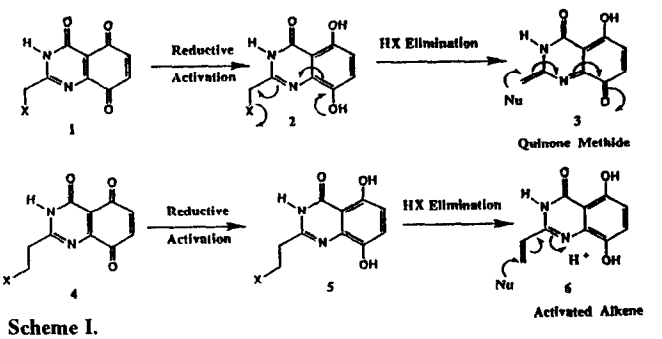
**Abstract**–The mechanism of halide elimination from 2-haloethyl-5,8-dihydroxyquinazolin-4(3*H*)-ones was studied in aqueous buffer by means of a pH-rate profile, buffer dilution studies, isotopic labeling, and kinetic isotope effects. From the results of these studies, it is apparent that a quinazolinone tautomer, arising from a prototropic shift of the C(1') proton to the N(1) position, is formed in the rate determining step of elimination. Monobasic phosphate acts as a bifunctional catalyst for the tautomerism. The halide then eliminates from the tautomer to afford the alkene derivative. Conversely, hydroxyethyl mercaptide adds to the alkene to afford the tautomer. The significance of these studies lies in the discovery of a prototropic tautomer of quinazolinone, which is reversibly formed in aqueous buffer under mild conditions, and in the discovery of alkylation chemistry useful in the design of quinazolinone-based enzyme inhibitors.

## Introduction

The quinazolinone ring system has been used in the design of inhibitors of xanthine oxidase,[1] purine nucleoside phosphorylase,[2,3] thymidylate synthase,[4] and tubulin polymerization.[5] In addition, this ring system can be found in antimalarial agents.[6] Efforts in this laboratory have therefore focused on the design of new quinazoline-based alkylating agents which could bring about enzyme-active-site-directed irreversible inhibition. Our early work dealt with the design of a quinone methide forming system which irreversibly inhibits both xanthine oxidase and purine nucleoside phosphorylase.[2] As illustrated in Scheme I, reductive activation of the quinazolinone quinone derivative **1** leads to formation of an alkylating quinone methide species **3**.[1] Reductive activation occurs by electron transfer from reduced xanthine oxidase[1] and by quinone reductive addition in the purine phosphorylase active site.[2] Recently, we have investigated the reductive activation and purine nucleoside phosphorylase inhibitory activity of the haloethyl quinazolinone system **4**.[3] As illustrated in Scheme I, reduction of **4** to **5** results in halide elimination to afford alkene **6** which can trap nucleophiles. In the present report, we provide results of detailed kinetic studies of halide elimination from **5**. Our results indicate that a quinazolinone prototropic tautomer is involved in the elimination process as well as in nucleophilic addition to alkene **6**.

## Kinetic Studies

In order to gain insights into haloethyl group reductive activation, we carried out a kinetic study of halide elimination from **5** (X=Br, **5a** and X=Cl, **5b**) in anaerobic aqueous buffers held at 30±0.2°C. A preliminary report of our findings appeared in a previous publication.[3]




**Scheme I.**

*pH-Rate profile for the conversion of* ***5a*** *to* ***6****.*

This was studied in anaerobic aqueous buffers over the pH range of 6–11.5. Product studies at pH 7.4, 8.5 and 9.5 revealed that **6** was the sole product. The rate of conversion of **5a** to **6** was followed at 368 nm with a UV-visible spectrophotometer. Absorbance vs time plots were first order in character at all pH values.

Buffer catalysis was noted over the pH range of 6–8. Rate constants pertaining to catalysis by lyate species (i.e., $H^+$, $H_2O$, $HO^-$) were obtained by measuring rate constants over a 10-fold dilution in buffer and then extrapolating to zero buffer. The type of buffer catalysis (general base vs general acid) was determined from the plot shown in Figure 1

wherein $k_{obsd}$ is the measured rate constant, $k_{lyate}$ is the rate constant obtained by extrapolating to zero buffer concentration, $B_T$ is the total buffer concentration ($[phosphate]_T$ = [phosphate dibasic] + [phosphate monobasic]), $K_a$ is the dissociation constant for monobasic phosphate ($pK_a$ = 7.10),[7] and $a_H$ is the proton activity ($10^{-pH}$) of the experiment. The slope of this plot provides the second order rate constant for general acid (monobasic phosphate) catalysis, 7.8 x $10^{-4}$ $M^{-1}s^{-1}$. The zero intercept of this plot indicates that there is no apparent general base (dibasic phosphate) catalysis, however.[8]

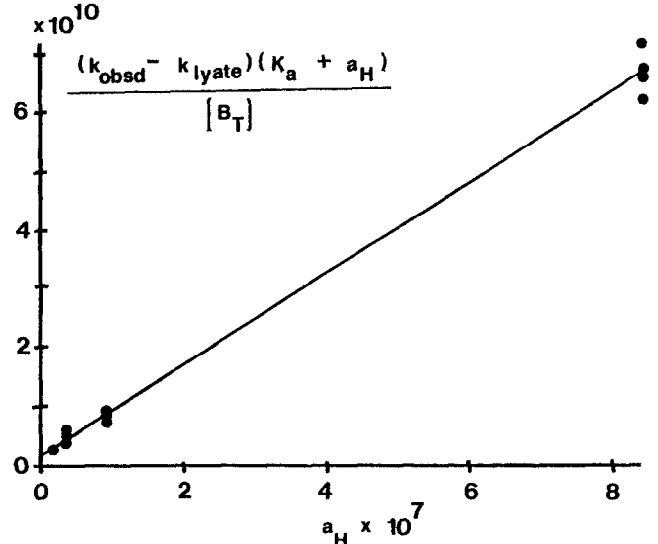



**Figure 1** Buffer dilution results obtained for the hydrolysis of **5a** in anaerobic phosphate buffer over the range of pH 6–8 with $\mu$ = 1.0 (KCl) aqueous buffers at 30.0±0.2°C.

Shown in Figure 2 are $k_{lyate}$ values, plotted as the log, vs pH for the formation of **6** from **5a**. In the pH range studied, **5a** exists in the neutral, monoanion and dianion forms. Dissociation of the 5-hydroxyl proton affords the oxygen anion with spectrophotometric $pK_a$= 8.5. Dissociation of the 8-hydroxyl proton then affords the second oxygen anion, with spectrophotometric and electrochemical $pK_a$ of 10.4.[1] Previous studies indicated that acid dissociation occurs from the 5-hydroxyl first due to stabilization of the anion by an internal hydrogen bond.[1,9] The data in Figure 2 indicate that the three forms of **5a** are involved in product formation, Scheme II.

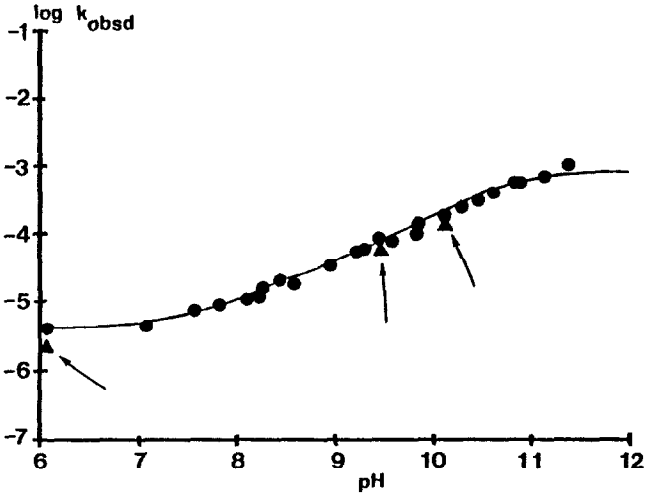



**Figure 2** pH-Rate profile for the hydrolysis of **5a** (circles) and **5b** (triangles) obtained with $\mu$ = 1.0 (KCl) aqueous buffers at 30.0±0.2°.

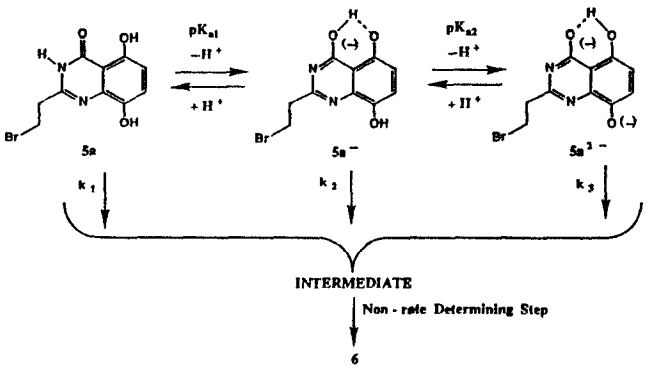
The rate law for product formation from all three forms of **5a** is found in eq. 1

$$k_{lyate} = \frac{k_1 a_H^2 + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad (1)$$

wherein the rate and equilibrium constants correspond to those shown in Scheme II. Fitting the data in Figure 2 to eq. 1, and taking the $pK_a$ values as constants, provided $k_1$= 4.07 x $10^{-6}s^{-1}$, $k_2$= 2.21 x $10^{-5}s^{-1}$ and $k_3$= 5.5 x $10^{-4}s^{-1}$ as the solution to eq. 1. This solution was used to generate the solid line in Figure 2. The inflection for the first acid dissociation at pH 8.5 is barely noticeable and a simpler rate law involving a single $pK_a$ ($k_{lyate} = (k_1 a_H + k_2 K_a)/(a_H + K_a)$) would appear to be more appropriate. The single $pK_a$ rate law was rejected because it fitted very poorly to the data and because it did not account for the contribution of both mono- and dianion species to the mechanism.

The leaving group was changed from bromide (**5a**) to chloride (**5b**) in order to assess the rate determining step in the formation of **6**. Shown in Figure 2 are the data obtained with **5b**, triangles highlighted with arrows. These data indicate that the elimination of the leaving group does not occur in the rate determining step. Indeed, studies in this research group[1,10] and elsewhere[11] have shown that there are substantial differences between rate-determining chloride and bromide elimination rates.

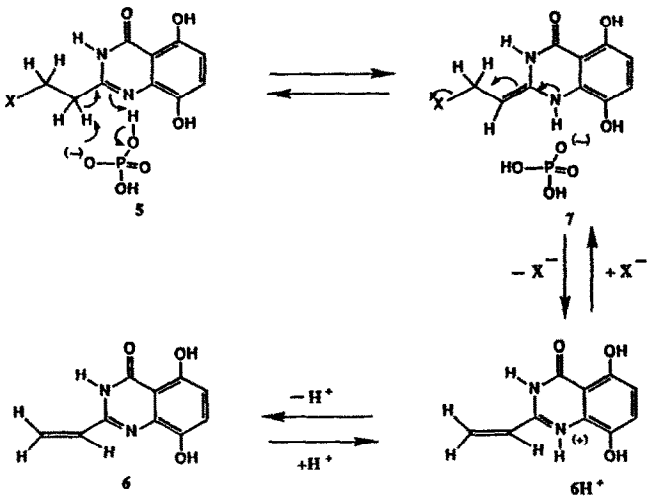
From the foregoing results it is apparent that the formation of **6** involves the following: (i) the three forms of **5a** (neutral, monoanion, and dianion) or their kinetic equivalent, (ii) general acid catalysis or specific acid/general base catalysis, and (iii) leaving group elimination in a non-rate-determining step, Scheme II.




**Scheme II.**

*Evidence of a tautomeric intermediate*

Tautomerism of the C(1')-proton to afford the enamine **7** in Scheme III is proposed based on deuterium exchange of the C(1')-protons of **5** in acid (Scheme III).[3] It is further proposed that **7** is formed in the rate determining step of the conversion of **5a** to **6**. Thus, the rate determining step is not dependent on the type of leaving group (Cl vs Br) and it possesses a KIE of 4.1 when the C(1')-position is deuterated.[3] The general acid (monobasic phosphate) catalysis observed in the conversion of **5a** to **6** is also consistent with rate determining tautomerism. As illustrated in Scheme III, monobasic phosphate can serve as a bifunctional catalyst of the prototropic shift leading to alkene **6**. Bifunctional catalysis, wherein monobasic phosphate transfers and accepts a proton in a cyclic transition state, has been proposed by others but has never been proven to occur.[12] Consistent with a bifunctional catalysis mechanism, however, imidazole buffer did not catalyze the conversion of **5a** to **6**.



**Scheme III.**

The lyate-catalyzed rates for tautomerism shown in Figure 2 are probably due to the participation of water as a general acid and/or general base. At the first plateau of the pH-rate profile of Figure 2, where neutral **5a** is reacting, the tautomerism process could involve N(1)-protonation by water as well as C(1') proton abstraction by water. As the quinazolinone ring becomes more electron rich due to monoanion and dianion formation, the N(1)-position will become more basic, which in turn will facilitate protonation of this position in the course of tautomerism. Accordingly, the rate of tautomerism increases with anion formation: neutral to monoanion, 5.2-fold increase; and monoanion to dianion, 25-fold increase (see pH-rate profile results). Prototropic tautomerism can occur in a variety of heterocyclic systems.[13] Either prototropic shifts between heteroatoms, or the shift of an alkyl proton to a heteroatom can be involved in tautomerism. The latter prototropic shifts were found to occur in methylated 1,2,4-triazine.[14] The Aldol-like condensation reactions of 2-methylquinazolines with aldehydes[15] and acetic anhydride[16] are other noteworthy examples of reactions which may go through tautomeric intermediates. In contrast to the conversion of **5** to **7**, which occurs at 30°C, the condensation reactions of 2-methylquinazolines require high temperatures (~180°C). These observations suggest that the electron rich character of the hydroquinone ring of **5** could facilitate tautomerism.

*Elimination–addition*

The facile elimination of bromide from **7** is probably due to involvement of the N(1) lone pair as shown in Scheme III. Tautomerism converts the bromoethyl group of **5a** to an allylic bromide in conjugation with the electron-rich quinazolinone ring. A similar elimination from an enamine halide has been reported in the literature.[17] Without tautomerism, alkene formation would have to proceed via a primary carbocation (E1), a carbanion on an electron rich system (ElcB), or a combination of the two (E2).[18]

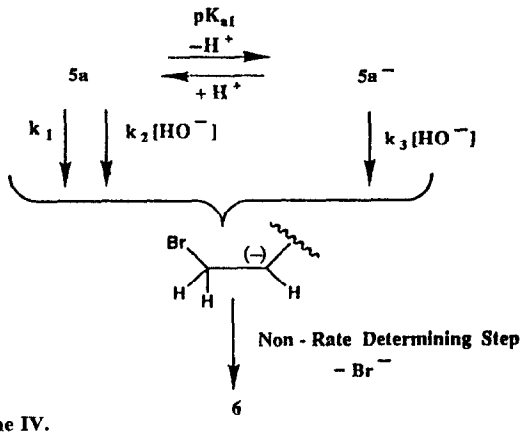
Anti-Markovnikov addition of mercaptoethanol to the alkene group of **6** occurred quantitatively in pH 7.4 buffer.[3] The rate of nucleophile trapping, according to the reverse of the dehalogenation mechanism in Scheme III, should be dependent on nucleophile concentration. Indeed, a second-order rate constant of $0.59 M^{-1}s^{-1}$ was determined for formation of the mercaptan addition product. According to the 'Principle of Microreversibility', the mechanism of addition must involve equilibrium protonation of basic N(1)-position followed by addition of mercaptide. Water may function as a general acid and protonate the N(1)-position during the addition process. This addition mechanism explains the anti-Markovnikov addition by mercaptide and bromide.[3]

*Mechanistic troubleshooting*

In this section, we will present alternative mechanisms for this conversion of **5a** to **6**. These mechanisms are kinetically equivalent to those already considered, and therefore differ only with respect to location of reacting components in the transition state.

The mechanism for tautomer formation (**5**→**7** in Scheme III) could also involve equilibrium protonation of the N(1)-position and then abstraction of the C(1')proton by dibasic phosphate. This process is an example of specific acid/general base catalysis, which is kinetically the same as the postulated general acid mechanism involving monobasic phosphate. In fact, deuterium exchange at the methyl centers of methylated pyridines occurs by a specific acid/general base mechanism.[19] At this point in time, we cannot distinguish between general acid catalysis and specific acid/general base catalysis.

An alternative elimination mechanism is the $ElcB_{irr}$ process which involves rate determining carbon anion formation followed by non-rate determining loss of the leaving group.[20] The mechanism would involve water abstraction of a proton from neutral **5a**, hydroxide abstraction of a proton from neutral **5a**, and hydroxide abstraction of a proton from anionic **5a**, Scheme IV.




**Scheme IV.**

The rate law for this mechanism is found in eq. 2

$$k_{lyate} = \frac{k_1 a_H^2 + k_2 K_w a_H + k_3 K_w K_{a1}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad (2)$$

where the rate and equilibrium constants correspond to those shown Scheme IV and $K_w$ is the autoprotolysis constant for water ($pK_w$ at 30°C in 13.83). Both eqs 1 and 2 have the same form since the mechanisms shown in Schemes II and IV are kinetically equivalent. Fitting, the data in Figure 2 to eq. 2 provided $k_1 = 4.07 \times 10^{-6}$ s$^{-1}$, $k_2 = 5.0$ s$^{-1}$, and $k_3 = 1.5$ s$^{-1}$. The E1cB$_{irr}$ mechanism was not considered as a possibility since the relative values of $k_1$, $k_2$ and $k_3$ do not reflect the unfavorable electrostatic interactions which must occur when a proton is abstracted from an electron rich substrate. Typically, unfavorable electrostatic interactions result in large decreases in rate (~30-fold).[21]

## Conclusion

The studies described herein indicated that the quinazolinone tautomer **7** readily forms in aqueous buffer at 30°C from **3**. Bifunctional catalysis by monobasic phosphate is involved in the tautomerism. Halide elimination to afford alkene **6** then occurs from the tautomer in a non-rate-determining step. Thus, the elimination process appears to be general acid (monobasic phosphate) catalyzed.

Unlike the conversion of **5** to **7**, 2-alkylquinazolines do not appear to tautomerize readily. Indeed, Aldol-like reactions, which would require tautomerism involving the 2-alkyl center, occur only at high temperatures. The facility of the conversion of **5** to **7** appears to result from the electron rich character of the hydroquinone ring, which assists the tautomerism process by increasing the basicity of the N(1)-position. In concert with protonation of the N(1)-position, the C(1')-proton departs resulting in the tautomer.

An important finding is the rapid addition of a sulfur nucleophile to alkene **6**. According to the 'Principle of Microreversibility', nucleophilic addition to **6** must occur via tautomer **7**. The stability of **7** probably facilitates the addition process. Since quinazolines are purine mimics in some enzyme systems, 2-alkenylquinazolines can be employed in the design of active-site-directed alkylating agents.

## Experimental Section

Hydroquinones were prepared according to the literature.[3] The kinetic studies were carried out in buffers prepared with doubly distilled water and adjusted to $\mu = 1.0$ with KCl. The following buffer systems were employed to hold pH: acetic acid/acetate ($pK_a = 4.55$), phosphate monobasic/phosphate dibasic ($pK_a = 6.50$), and boric acid/borate ($pK_a = 9.2$). These $pK_a$ values were obtained at 30.0±0.2°C in $\mu = 1.0$ (KCl) aqueous solutions. Measurements of pH were made with a Radiometer GK2401C combination electrode.

*Kinetic studies of hydrolysis*

The hydrolytic studies of the hydroquinones were carried out in anaerobic aqueous buffers employing Thunberg cuvettes as previously described.[22]

Both aerobic and anaerobic studies were carried out as follows: A dimethyl sulfoxide stock of the compound to be studied was prepared fresh and 50 µL of this stock was added to 2.95 mL of buffer. The absorbance vs time data were collected on a UV-vis spectrophotometer in thermostatted cells held at 30.0±0.2°C. These data were computer-fitted to single first-order rate law.

## Acknowledgement

The financial support of the National Science Foundation and a research career development award (to E. B. S.) from the National Instititutes of Health are gratefully acknowledged.

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