

It was expected that species such as IV would have very different uv spectra from those of V in which electrons were delocalized around C(5), the nitro group on C(5), and the carbonyl group on C(4). However, the initial absorbances obtained using a rapid reaction technique when solutions of V which were initially at pH 10, were lowered to pH 4.5 for Va, to pH 6.0 for Vb with X = NHOH, and to pH 2.0 for Vb with X = SO₃⁻, and were the same as those of the original solutions of the anionic adducts. At pH values below those stated, the elimination of X⁻ was occurring more rapidly than the mixing time in the rapid reaction spectrophotometer. These results will be discussed in more detail in a subsequent publication.

Experimental Section

Synthesis of 1,3-Dimethyl-5-nitrouracil. A mixture of 2.0 g

of 5-nitrouracil (Sigma Chemical Co.) and 30 ml of water was adjusted to pH 8 to 9 with 10% aqueous KOH. With constant stirring, 20 ml of dimethyl sulfate was added over a 30-min period, keeping the pH at 8 to 9. After adding 20 ml of ethanol, the mixture was heated to 45°, and a homogeneous solution was obtained. Stirring at room temperature for 1 hr resulted in formation of white crystals. They were collected by filtration (2.2 grams) and recrystallized from ethyl acetate, yielding 1.5 g of white plates, mp 156–158° (lit. 155¹¹ and 159–161¹²). *Anal.* Calcd for C₆H₇N₃O₄: C, 38.89; H, 3.78; N, 22.69. Found: C, 39.34; H, 3.93; N, 22.00.

Measurements of Equilibrium Constants. The apparent equilibrium constant, K_{eq}^{obsd} , was calculated as described previously.³ For all reactions studied, the absorbance at around 330 nm was used in calculation of K_{eq}^{obsd} values.

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Linear Free Energy Relationships Governing the Covalent Addition of Nucleophilic Reagents to a Nitrogen-Containing Heteroaromatic Molecule¹

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Abstract: Equilibrium and rate constants have been determined at 25° for the covalent addition of water, bisulfite ion, hydroxylamine, urea, β-mercaptoethanol, and hydrogen sulfide to the >C(4)=N⁺(3)< bond of quinazoline cation. Linear relationships were found to exist between (1) the logarithms of the equilibrium constants for this series of reactions and the γ value (*i.e.*, parameters which are a measure of the ability of the nucleophilic reagent to covalently add to aldehydes and ketones) of the nucleophilic reagent and (2) the logarithms of the second-order rate constants for addition of the nucleophilic reagents and the pK_a values of their conjugate acids. Knowledge of these relationships appears to make it possible to accurately estimate the equilibrium and rate constants for addition of other nucleophilic reagents to quinazoline cation if their γ values and basicities are known.

Many of the reactions of heteroaromatic molecules in aqueous solution are postulated to be initiated by covalent addition of water or another nucleophilic reagent across a double bond in the molecule. Some examples are the ring opening of 1-alkyl-1,2-dihydro-2-iminopyrimidines during their Dimroth rearrangement,³ the deamination of cytosine in aqueous solutions of sodium bisulfite,^{4,5} and the dehalogenation of 5-halouracils in aqueous solutions of thiols.⁶

In considering the chemical stability of heterocyclic molecules against these types of reactions, it is necessary to first of all consider their stability with respect to addition of the nucleophilic reagent and then the stability

of the adduct with respect to a subsequent reaction. In the case of covalent addition of strong nucleophilic reagents such as bisulfite ion, the reaction is frequently favorable enough that equilibrium and rate constants can be measured directly. For example, equilibrium and/or rate constants have been determined for the covalent addition of bisulfite ion to a variety of pyrimidines.^{3,4,7} However, the addition of weak nucleophilic reagents, especially water, is frequently so unfavorable that rate and equilibrium constants cannot be measured but only rough estimates could be made.⁸

The present study was undertaken to elucidate the kinetics and thermodynamics which governed the covalent addition of a series of nucleophilic reagents to a particular heteroaromatic molecule, quinazoline cation (I). The compound was known from previous studies^{9,10} to add both strong nucleophilic reagents

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(8) For example, the extent of covalent hydration of 2-hydroxypyrimidine was estimated to be in the range of 0.1 to 1%: A. R. Katritzky, M. Kingsland, and O. S. Tee, *J. Chem. Soc. B*, 1484 (1968).

(9) T. Higashino, *J. Pharm. Soc. Jap.*, 80, 245 (1960).

(10) A. Albert, W. L. F. Armarego, and E. Spinner, *J. Chem. Soc.*, 2689 (1961).

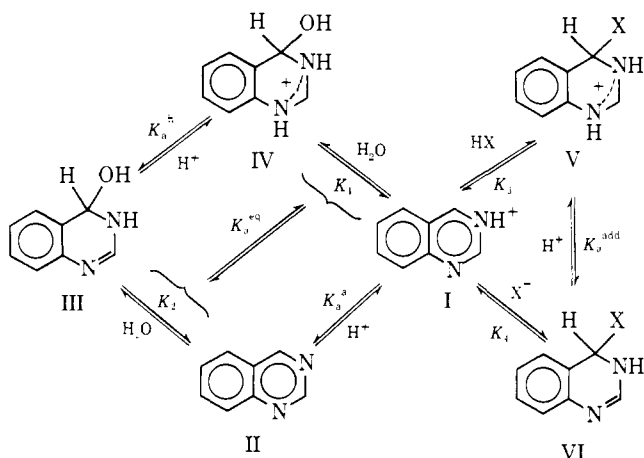
such as bisulfite ion and weak nucleophilic reagents such as water to a measurable extent. Additions in these cases occur across the $>C(4)=N^+(3)<$ bond.

It is hoped that the results of the present study will provide a quantitative means for predicting equilibrium and rate constants for the same types of reactions in other biologically, pharmaceutically, and physicochemically important systems.

Results and Discussion

It has previously been established¹¹ that quinazoline exists mainly as four different species in aqueous solutions: anhydrous quinazoline (II) and its cation (I) and the monohydrate of quinazoline (III) and its cation (IV). They are related as shown in Scheme I. In the pH

Scheme I. Multiple Equilibria Involved in an Aqueous Solution of Quinazoline Containing a Nucleophilic Reagent, HX



range 0 to 3.5, IV is the dominant species, whereas in the range above 3.5, II is the dominant species. When a nucleophilic reagent, HX, is added to an aqueous solution of quinazoline, additional equilibria are established as shown in Scheme I.

The structures of the water,¹² bisulfite,¹² and hydroxylamine¹³ adducts have been previously described and those of urea, β -mercaptoethanol, and hydrogen sulfide were believed to be similar because of similarities in the uv spectral changes that followed the addition of the nucleophilic reagents to aqueous buffered solutions of quinazoline. Spectral characteristics of aqueous solutions of quinazoline in the presence of the various reagents are given in Table I. Previous studies¹⁴ on the covalent addition to heterocyclic molecules have revealed that a variety of covalent adducts of a substrate have similar uv spectra. Supportive evidence for the structure of the β -mercaptoethanol adduct was provided by the similarity of its nmr spectrum in D_2O solutions of β -mercaptoethanol to that of the water (or D_2O) adduct.

Hydration Reaction. Equilibrium constants involved in the hydration reaction at 25°¹⁵ were calculated from experimental measurements by using

(11) D. D. Perrin, *Advan. Heterocycl. Chem.*, 4, 43 (1965), and the references therein.

(12) A. Albert, W. C. F. Armarego, and E. Spinner, *J. Chem. Soc.*, 5267 (1961).

(13) E. Ochiai, "Aromatic Amine Oxides," Elsevier, New York, N. Y., 1967, p 54.

(14) A. Albert and W. L. F. Armarego, *Advan. Heterocycl. Chem.*, 4, 1 (1965).

(15) Values of these constants at 20° have been reported by Perrin.¹¹

Table I. Ultraviolet Spectral Characteristics of Aqueous Solutions of Quinazoline in the Range of 230 to 330 nm in the Presence of Nucleophilic Reagents

| Reagent | pH | [Reagent] $\times 10^3 M$ | % fraction of adduct | λ_{max} , nm | ϵ_{max} , $10^{-3} M^{-1} cm^{-1}$ |
|--------------------------|-----|---------------------------|----------------------|----------------------|---|
| Water | 1.1 | 55,000 | 99 | 259 | 8.1 |
| | 7.5 | 55,000 | 0.1 | 270 | 2.6 |
| | | | | 305 | 2.4 |
| Sulfurous acid species | 1.1 | 1.0 | 99 | 280 | 4.0 |
| | 4.8 | 3.1 | 99 | 281 | 3.5 |
| | 7.0 | 19 | 97 | 281 | 4.3 |
| Hydroxylamine | 3.0 | 400 | 70 | 263 | 7.2 |
| | 4.5 | 20 | 38 | 265 | 4.7 |
| | 6.6 | 100 | 40 | 264 | 5.7 |
| Urea | 3.0 | 2,000 | 87 | 265 | 7.1 |
| β -Mercaptoethanol | 1.3 | 45 | 96 | 277 | 4.5 |
| | 4.1 | 60 | 91 | 276 | 3.9 |
| Hydrogen sulfide | 1.1 | 40 | 66 | 268 | 5.4 |

identical methods with those described by previous workers.^{11,16-18} The values of these constants were $K_a^a = 10^{-1.98}$, $K_a^h = 10^{-7.61}$, $K_a^{eq} = 10^{-3.72}$, and $K_1 = 1.10 M^{-1}$. The value of K_1 is included in Table II.

Table II. Equilibrium Constants for the Covalent Addition of Nucleophilic Reagents to Quinazoline Cation at 25° and Ionic Strength 0.1 M, and γ Values^c of the Reagent of the Type HX

| Nucleophilic reagent | γ^a | $K_3^b M^{-1}$ | $K_4^c M^{-1}$ |
|--------------------------------------|------------|---------------------------------|---------------------------------|
| H ₂ O | -3.58 | 1.10^d | |
| OH ⁻ | | | 1.50×10^8 ^e |
| HSO ₃ ⁻ | 4.02 | 3.25×10^8 | |
| SO ₃ ²⁻ | | | 1.13×10^8 |
| NH ₂ OH | 1.24 | 3.58×10^5 | |
| H ₂ NCONH ₂ | -1.84 | 1.21×10^2 ^f | |
| HSCH ₂ CH ₂ OH | 0.35 | 3.72×10^4 | |
| -SCH ₂ CH ₂ OH | | | 6.32×10^8 |
| H ₂ S | g | 3.08×10^3 | |

^a See ref 19. ^b The equilibrium constant for the reaction $I + HX \rightleftharpoons V$. ^c The equilibrium constant for the reaction $I + X^- \rightleftharpoons VI$; values were calculated from $K_4 = K_3 K_a^{add} / K_a$ where K_a^{add} and K_a are the ionization constants of nucleophilic adducts and the reagents HX. ^d $K_1/[H_2O]$. ^e $K_1 K_a^h / K_w$ are the ionization constant of quinazoline hydrate (IV) and H₂O. ^f Corrected by a statistical factor of 2; see ref 19. ^g Attempts to measure the equilibrium constant for H₂S addition to pyridine-4-carboxaldehyde were not successful.

The observed first-order rate constant, k_{obsd} , for the attainment of equilibrium when the pH of aqueous solutions of IV (initially at pH close to 2) is increased (essentially the dehydration reaction) or when the pH of aqueous solutions of II (initially at pH close to 8) is lowered (essentially the hydration reaction) is the sum of the pseudo-first-order rate constant, k_h , for hydration and the first-order rate constant, k_d , for dehydration. The values of k_h and k_d were calculated from k_{obsd} values using the computation method given by Perrin,^{11,16} and their dependence on pH is shown in Figure 1.

The hydration reaction can be represented as the sum of the following four simultaneous reactions: (1) spontaneous water addition to quinazoline dication

(16) J. W. Bunting and D. D. Perrin, *J. Chem. Soc. B*, 950 (1967).

(17) W. L. F. Armarego, *J. Chem. Soc. B*, 561 (1962).

(18) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962.

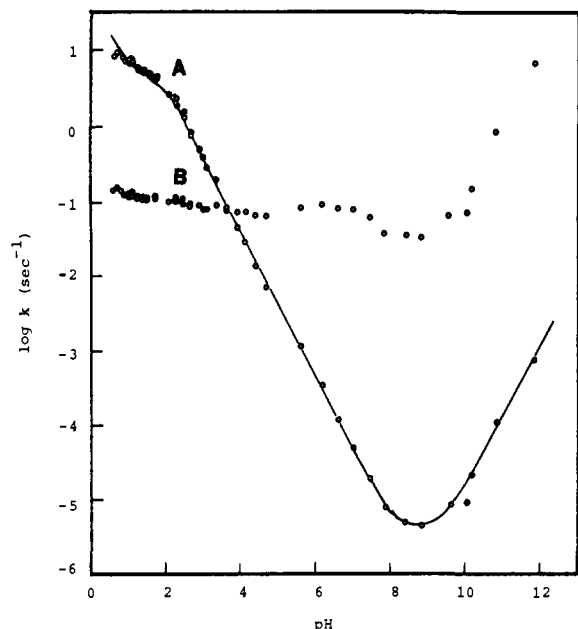


Figure 1. Dependence on pH of the hydration rate constant of quinazoline (A) and the dehydration rate constant of dehydrated quinazoline (B) at 25°. Ionic strength was maintained at 0.1 *M* except when the pH was lower than 1.0. The solid curve was calculated as described in the text.

(this is a species with a $pK_a = -5.5^{10}$ which only exists in very low concentrations in the pH range studied), (2) spontaneous water addition to I, (3) hydroxide ion addition to I, and (4) hydroxide ion addition to II. The corresponding pH-independent second-order rate constants are designated as k_1 to k_4 .

On the basis of this analysis, values of k_h are related to the microscopic rate constants k_1 to k_4 by the expression

$$k_h = \frac{k_1[\text{H}_2\text{O}][\text{H}^+]^2 + k_2K_{a1}[\text{H}_2\text{O}][\text{H}^+] + k_3K_{a1}K_w + k_4K_{a1}K_a^a[\text{OH}^-]}{[\text{H}^+]^2 + K_{a1}[\text{H}^+] + K_{a1}K_a^a} \quad (1)$$

where K_{a1} is the acid dissociation constant of anhydrous quinazoline dication,¹⁰ $K_w = [\text{H}^+][\text{OH}^-]$, and $[\text{H}_2\text{O}]$ is the activity of water which was taken to be 55.5 *M*. The solid curve in Figure 1 was drawn using the values of $k_1 = 2.13 \times 10^5$, $k_2 = 7.93 \times 10^{-2}$, $k_3 = 3.32 \times 10^6$, and $k_4 = 1.33 \times 10^{-1} \text{ sec}^{-1} \text{ M}^{-1}$ in eq 1. Of the four rate constants, the values of k_2 and k_3 are the quantities representing the nucleophilicity of H_2O and OH^- toward I and will be used in constructing a free energy relationship for rate constants.

Equilibrium Constants for Covalent Addition of Nucleophiles Other than Water. Differences in the uv absorbance of aqueous buffered solutions of quinazoline and those of the same solutions which had been equilibrated after the addition of various amounts of nucleophilic reagents were consistent with the formation of 1:1 covalent adducts of quinazoline and the nucleophilic reagents. The equilibrium constant for these reactions at a particular pH is defined as

$$K_{\text{eq}}^{\text{obsd}} = \frac{[\text{add}]_{\text{T}}[\text{Q}]_{\text{T}}}{[\text{HX}]_{\text{T}}} \quad (2)$$

where $[\text{add}]_{\text{T}}$, $[\text{Q}]_{\text{T}}$, and $[\text{HX}]_{\text{T}}$ are the equilibrium concentrations of all ionic forms of the 1:1 adduct, all

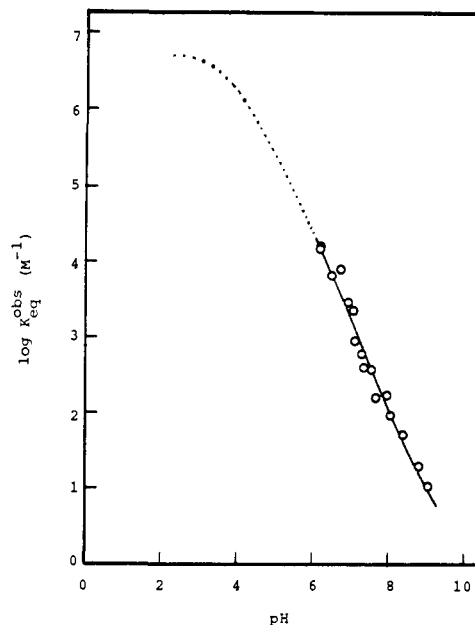


Figure 2. Dependence on pH of the apparent equilibrium constant for the formation of sulfurous acid adduct of quinazoline at 25°. Ionic strength varied from 0.1 to 0.5 *M*. The curve was calculated using eq 7 and a value of $K_3 = 3.25 \times 10^8 \text{ M}^{-1}$.

ionic forms of hydrated and anhydrous quinazoline, and all ionic forms of the nucleophilic reagent, respectively. For the purposes of the present study, it was convenient to abstract from $K_{\text{eq}}^{\text{obsd}}$ a value of K_3 , the equilibrium constant for the formation of the acid form of the adduct (V). In these reactions, the HX reagents are H_2O , HSO_3^- , $\text{HSCH}_2\text{CH}_2\text{OH}$, H_2NCONH_2 , NH_2OH , and H_2S .

Dependence on pH of $K_{\text{eq}}^{\text{obsd}}$ values is shown in Figures 2–4 for the additions of sulfurous acid species, hydroxylamine, β -mercaptoethanol, and hydrogen sulfide to quinazoline at 25° and ionic strength 0.1 *M*. In the case of urea addition, the value of $K_{\text{eq}}^{\text{obsd}}$ was determined several times at pH values around 3. The values of K_3 were calculated from these data, using

$$K_{\text{eq}}^{\text{obsd}} = \frac{K_3(1 + (K_a^{\text{add}}/[\text{H}^+]))}{(1 + (K_a/[\text{H}^+]))(K_1 + 1 + (K_a^a/[\text{H}^+]))} \quad (3)$$

for the addition of HSO_3^- , $\text{HSCH}_2\text{CH}_2\text{OH}$, and H_2S and

$$K_{\text{eq}}^{\text{obsd}} = \frac{K_3(1 + (K_a^{\text{add}}/[\text{H}^+]))}{(1 + ([\text{H}^+]/K_a))(K_1 + 1 + (K_a^a/[\text{H}^+]))} \quad (4)$$

for the addition of NH_2OH and H_2NCONH_2 . In these expressions, K_a represents the ionization constant of HSO_3^- , $\text{HSCH}_2\text{CH}_2\text{OH}$, H_2S , NH_3OH^+ , or $\text{H}_2\text{NCONH}_3^+$.

The ionization constant, K_a^{add} , of nucleophilic adducts of quinazoline had to be determined by a rapid reaction technique because, as in case of the covalent hydrate, when the pH of their solution is raised the dissociation of the adducts to quinazoline and nucleophilic moiety takes place very rapidly. Thus, the values of K_a^{add} , for the bisulfite and β -mercaptoethanol adducts, were obtained by measuring the initial absorbance of solutions obtained when the pH value of an acidic quinazoline solution containing a large excess of the reagents was increased. They were $10^{-7.4}$ and

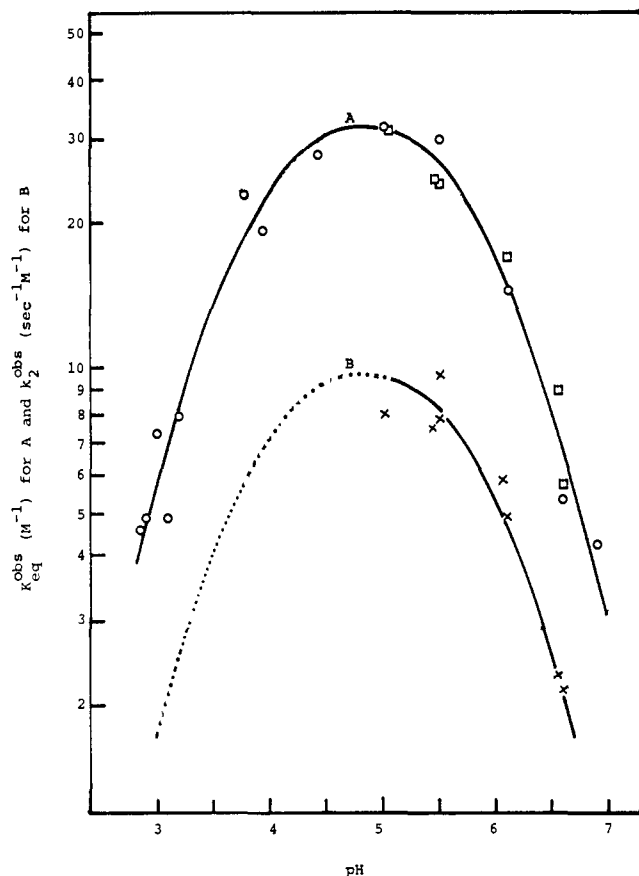


Figure 3. Dependence on pH of the apparent equilibrium constant (A) and the second-order rate constant (B) for hydroxylamine addition to quinazoline at 25°. The equilibrium constants at pH values around 3 were obtained at ionic strength as high as 1.5 *M*. The squares are the equilibrium constants obtained from kinetic experiments. The solid curves were calculated as described in the text.

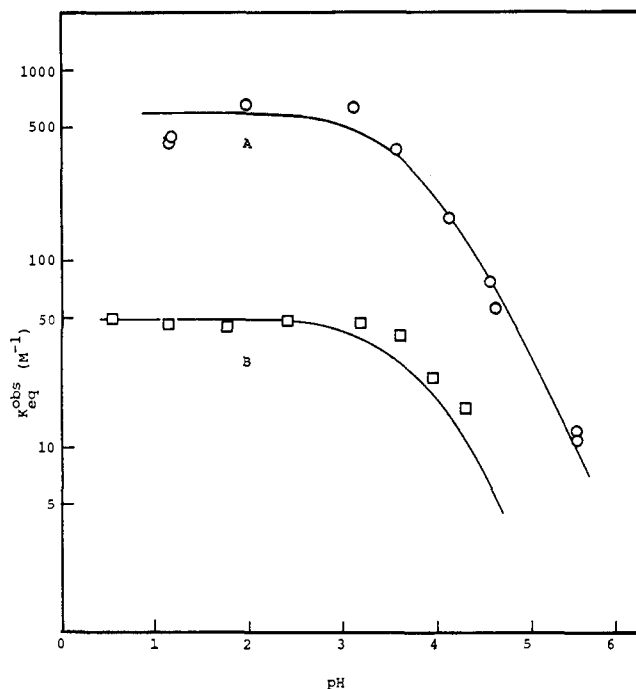


Figure 4. Dependence on pH of the apparent equilibrium constants for the formation of the β -mercaptoethanol (A) and the hydrogen sulfide (B) adducts of quinazoline at 25° and ionic strength 0.1 *M* except when pH is lower than 1.0. The solid lines were calculated as described in the text.

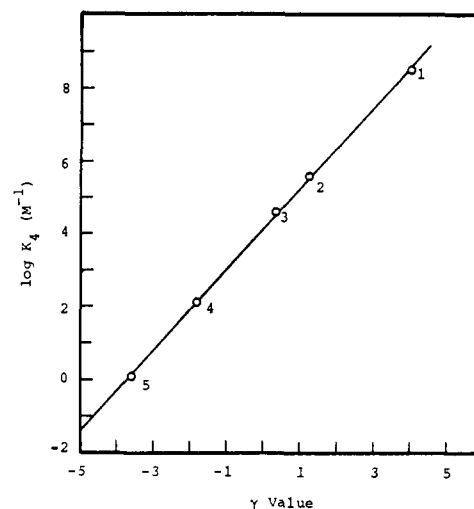


Figure 5. Log-log plot of equilibrium constants for addition of nucleophilic reagents to quinazoline cation *vs.* those for addition of the same reagents to pyridine-4-carboxaldehyde. Reagents are (1) HSO_3^- , (2) NH_2OH , (3) $\text{HSCH}_2\text{CH}_2\text{OH}$, (4) H_2NCONH_2 , and (5) H_2O .

$10^{-7.3}$, respectively. Since these and K_a^h values are close to one another, it appears to be a reasonable approximation that the ionization constants of other adducts are also of similar magnitude. Then, within most of the experimental pH range, the term $K_a^{\text{add}}/[\text{H}^+]$ in eq 3 and 4 becomes negligible and hence the values of K_3 could be calculated from experimental data using eq 3 and 4. The values of K_3 are listed in Table II and the curves on Figures 2 to 4 were calculated using these values in eq 3 or 4.

A Linear Free Energy Relationship for the Equilibrium Constants. As shown in Figure 5, linear plots were obtained when the logarithms of K_3 values ($K_3/[\text{H}_2\text{O}]$ in case of hydration) were plotted against the γ values¹⁹ of the nucleophilic reagents. Hence, knowledge of the equation of this line together with the γ value of a nucleophilic reagent makes it possible to estimate the extent to which the nucleophilic reagent would covalently add to quinazoline cation.

Values of γ were assigned to a number of nucleophilic reagents by Sander and Jencks¹⁹ and they are a measure of the tendency of the reagent to covalently add across the $>\text{C}=\text{O}$ bond pyridine-4-carboxaldehyde in aqueous solution. This parameter is defined as

$$\gamma_{\text{HX}} = \log K_{\text{HX}}/K_{\text{MeNH}_2} \quad (5)$$

where K_{HX} and K_{MeNH_2} are the equilibrium constants for addition of the nucleophilic reagent HX and methylamine, respectively, to pyridine-4-carboxaldehyde at 25°. It has subsequently been shown that the extent of addition of the same reagents to the carbonyl group of formaldehyde,¹² *p*-chlorobenzaldehyde,¹² and acetone¹³ is governed by the relationship

$$\log K_{\text{eq}} = \Delta\gamma + A \quad (6)$$

where Δ and A are constants for additions to a common substrate.

The line in Figure 5 has a slope very close to 1.0 and

(19) E. G. Sander and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 6154 (1968).

is thus very similar to the lines obtained for addition to *p*-chlorobenzaldehyde¹² and acetone.¹³

The significance of the value of I for the value of Δ in eq 6 can be seen from the following equations. By definition, the γ value of a nucleophilic reagent, HX, can be expressed as in eq 7 in terms of the standard free energies of the reactants and products involved in two reactions, $P + HX \rightleftharpoons P=HX$, and $P + MeNH_2 \rightleftharpoons P=MeNH_2$, where P is pyridine-4-carboxaldehyde and PHX and PMeNH₂ are its adducts with HX and MeNH₂, respectively

$$\gamma = \frac{(G^\circ_{HX} - G^\circ_{P=HX}) - (G^\circ_{MeNH_2} - G^\circ_{P=MeNH_2})}{2.3RT} \quad (7)$$

$$= \frac{(G^\circ_{HX} - G^\circ_{P=HX}) - C_1}{2.3RT}$$

where C_1 is a constant. Also by definition $\log K_3$ values ($K_1/[H_2O]$ in case of hydration) can be equated to standard free energy changes by the entity

$$\log K_3 = \frac{G^\circ_I + G^\circ_{HX} - G^\circ_V}{2.3RT} \quad (8)$$

Combining eq 7 and 8 leads to the expression

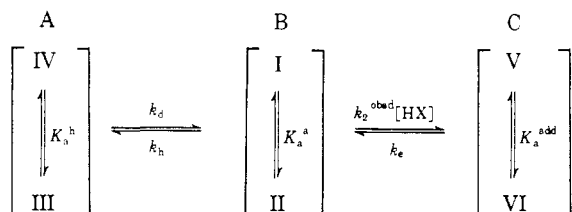
$$\log K_3 = \gamma + \frac{(G^\circ_{P=HX} - G^\circ_V) + C_2}{2.3RT} \quad (9)$$

where C_2 is a constant. Hence, a linear relationship of K_3 with the γ parameter would be observed as long as $G^\circ_{P=HX} - G^\circ_V$ is a linear function of γ . In order for a plot of $\log K_3$ ($K_1/[H_2O]$ in case of hydration) against γ to have a slope of 1 it is necessary for $G^\circ_{P=HX} - G^\circ_V$ to be 0 or to have a constant value independent of the reagent HX. The first situation is extremely unlikely but the second could arise if (1) there were no strong steric interactions between the moiety -X and other groups in the respective adducts and (2) electronic interactions between the moiety -X and the remainder of the adduct were similar for quinazoline and aldehyde adducts. It is suggested that the slope of the line in Figure 5 indicates that these conditions are applying in the system studied.

Kinetics of Addition of Nucleophiles to Quinazoline.

The kinetics of covalent addition were studied by measuring the rate of changes in uv absorbance when similarly buffered solutions of quinazoline and of the nucleophilic reagent were mixed. At pH values above 5.7 the absorbance changes exhibited first-order kinetics, but in some cases the changes which occurred at lower pH values followed more complicated rate laws.

This situation arises because the covalent addition of the nucleophilic reagent is always competing with the hydration-dehydration reactions of quinazoline. The over-all reaction scheme is



Analysis of absorbance changes for this type of two-stage reversible consecutive reaction scheme is ex-

tremely difficult. However, because of the different pH and concentration dependencies of the various reactions, it was possible to compute values of the pH-dependent second-order rate constant for each reaction of the nucleophilic reagent by carrying out experiments under conditions where the reaction scheme was simplified. This was achieved in the following ways. (1) When $k_d > k_h$, the reaction scheme reduces to $B \rightleftharpoons C$ (HX). Hence, the absorbance changes that follow addition of an excess of HX to B follow a first-order rate law with a pseudo-first-order rate constant, k_1^{obsd} . Under these conditions

$$k_1^{\text{obsd}} = k_2^{\text{obsd}}[\text{HX}]_T + k_e \quad (10)$$

and a plot of k_1^{obsd} against the total concentration of nucleophilic reagent, $[\text{HX}]_T$, yields a straight line with a slope of k_2^{obsd} and intercept of k_e . This condition applied at all pH values above 5.7 as can be seen from Figure 2. (2) When the values of all the rate constants were similar in magnitude, it was possible to simplify the system by addition of a sufficient excess of HX to make the ratio $k_2^{\text{obsd}}[\text{HX}]_T/k_e > 1$. Under these conditions, the reaction scheme reduces to $A \rightleftharpoons B \rightarrow C$ (HX_T) and absorbance changes can be interpreted in terms of rate constants by using the methods given by Perrin and Pitman.²⁰ That is, plots of $\ln(D - D_\infty)$ against time under these conditions will usually pass through a maximum or minimum and then become linear. The slope of the linear portion of the curve, y , is related to the various rate constants by the identity

$$2y = (k_h + k_d + k_2^{\text{obsd}}[\text{HX}]_T) - \{(k_h + k_d + k_2^{\text{obsd}}[\text{HX}]_T)^2 - 4k_d k_2^{\text{obsd}}[\text{HX}]_T\}^{1/2} \quad (11)$$

Hence, values of y could be used to compute k_2^{obsd} values. When $k_2^{\text{obsd}}[\text{HX}]_T \gg k_d$ and k_h , the absorbance changes again followed first-order kinetics with the rate constant k_d . This change in rate-determining step was encountered during the addition of β -mercaptoethanol to quinazoline at pH values around 4 when the total concentration of the latter was above 0.04 M.

The values of k_2^{obsd} were independent of concentrations of buffer components (succinate, phosphate, and Tris) when these were varied between 0.02 and 0.2 M. Dependence of k_2^{obsd} values on pH is shown in Figures 3, 6, and 7 for the additions of hydroxylamine, sulfurous acid species, and β -mercaptoethanol to quinazoline at 25° and ionic strength 0.1 M. The pH profile for the addition of sulfurous acid species was analyzed using

$$k_2^{\text{obsd}} = \frac{([\text{H}^+]/K_{a2})k_{2a} + k_{2b}}{(1 + (K_{a1}/[\text{H}^+]))(([\text{H}^+]^2/K_{a1}K_{a2}) + ([\text{H}^+]/K_{a2}) + 1)} \quad (12)$$

where k_{2a} and k_{2b} are the pH-independent second-order rate constants for the addition of HSO_3^- and SO_3^{2-} to I, and K_{a1} and K_{a2} are the ionization constants of H_2SO_3 and HSO_3^- , respectively.

As shown in Figure 6, the data indicate that, in addition to SO_3^{2-} , HSO_3^- is also reactive. This situation was also observed in case of the addition of sulfurous acid species to other pyrimidines.⁷ The possibility of a reaction between anhydrous quinazoline dication, the pK_a of which is about -5.5,¹⁰ and SO_3^{2-} , which is ki-

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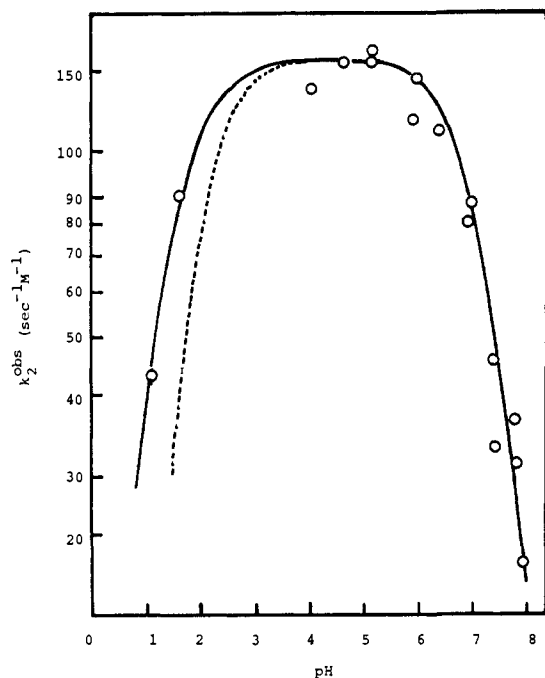


Figure 6. Dependence on pH of the second-order rate constant for the reaction of quinazoline with sulfurous acid species at 25° and ionic strength 0.1 *M*. The solid curve was calculated on the basis that both SO_3^{2-} and HSO_3^- were the nucleophilic reagents. The dotted line was calculated on the basis that only SO_3^{2-} was the nucleophilic reagent.

netically equivalent to the reaction between I and HSO_3^- , can be ruled out, because the value of the second-order rate constant for this reaction would have the magnitude of $10^{14} \text{ sec}^{-1} \text{ M}^{-1}$, which is far above the rate constant for the fastest diffusion-controlled reaction in aqueous media.

In a similar manner, k_2^{obsd} -pH profiles for the additions of hydroxylamine (Figure 3) and β -mercaptoethanol were analyzed. In the latter case, even though the data appear to fit the theoretical curve better when the thiol neutral species is also included as a reactive nucleophile in the system, it is not certain whether or not it is significant because of poor data. If so, it is believed that the neutral thiol may behave as an oxygen rather than sulfur nucleophile.

For the additions of urea and hydrogen sulfide, k_2^{obsd} values were repeatedly measured at pH values around 3 and 1, respectively. Since, under the experimental conditions, the hydration-dehydration process was occurring far more rapidly than the addition of these reagents, data were analyzed using

$$k_2^{\text{obsd}} = \frac{k_2}{(1 + ([\text{H}^+]/K_a))(1 + (K_a/[\text{H}^+] + K_1))} \quad (13)$$

where k_2 is the pH-independent second-order rate constant for the addition of H_2NCONH_2 or HS^- , and K_a is the ionization constant of $\text{H}_2\text{NCONH}_3^+$ or H_2S .

The values of k_2 are summarized in Table III. The curves shown in Figures 3, 6, and 7 were calculated using these values and appropriate rate expressions such as eq 12 or 13.

A Linear Free Energy Relationship for the Kinetics of the Addition Reaction. Figure 8 displays a plot of the logarithms of the rate constants for addition of a series of nucleophilic reagents to quinazoline cation

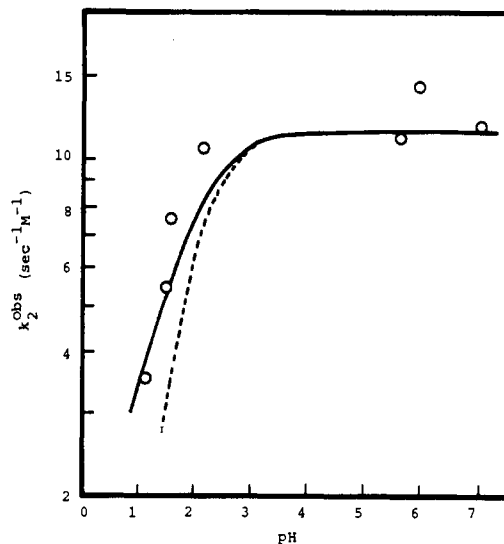


Figure 7. Dependence on pH of the second-order rate constant for the reaction between quinazoline and β -mercaptoethanol at 25° and ionic strength 0.1 *M*. The solid curve was calculated on the basis that both $\text{HSCH}_2\text{CH}_2\text{OH}$ and $-\text{SCH}_2\text{CH}_2\text{OH}$ were the nucleophilic reagents. The dotted line was calculated on the basis that only $-\text{SCH}_2\text{CH}_2\text{OH}$ was the nucleophilic reagent.

Table III. Summary of the pH-Independent Second-Order Rate Constants for the Formation of Nucleophilic Adducts of Quinazoline at 25° and Ionic Strength 0.1 *M*^a

| Reagent | p <i>K</i> _a of the conjugate acid of reagent | Rate constant, sec ⁻¹ M ⁻¹ |
|---------------------------------------|--|--|
| H ₂ O ^b | -1.74 | 2.13 × 10 ⁵ |
| H ₂ O | -1.74 | 7.93 × 10 ⁻² |
| HO ⁻ | 15.74 | 3.32 × 10 ⁶ |
| HO ^{-c} | 15.74 | 1.13 × 10 ⁻¹ |
| HSO ₃ ⁻ | 1.54 | 1.19 × 10 ² |
| SO ₃ ²⁻ | 6.94 | 1.69 × 10 ⁷ |
| NH ₂ OH | 5.95 | 1.18 × 10 ⁵ |
| H ₂ NCONH ₂ | ca. 0.2 ^d | 3.47 × 10 ^{-2 e} |
| HSCH ₂ CH ₂ OH | ca. -7 ^f | 2.5 |
| -SCH ₂ CH ₂ OH, | 9.55 | 4.55 × 10 ⁸ |
| -SH | 6.78 | 1.86 × 10 ⁷ |

^a Ionic strength was maintained with sodium chloride. ^b Addition to QH_2^{2+} species. ^c Addition to Q species. ^d Reference 18. ^e Solvent properties might be different from those in other reactions. ^f Reference 24.

against the p*K*_a values of their conjugate acids. As can be seen, the values for all the nucleophilic reagents except urea and hydroxide ion fall on a straight line with a slope (β value for the reactions) of 0.87. This result provides an empirical relationship for estimating the rate of addition of the nucleophilic reagents to quinazoline cation.

A theoretical explanation of the data is difficult because of the small number of nucleophiles of any one type (charge type, or N, S, or O nucleophiles) that were studied. However, the following points can be noted. (1) The fact that the β value is 0.87 suggests that the nucleophilic moiety becomes more positive by almost a complete unit of charge by the time it is in the transition state. (2) The various types of nucleophilic reagent (as defined above) all appear to sit on one straight line. It is especially noteworthy that the attack of both anionic and neutral nucleophilic reagents appears to be governed by the same relationship, even though the

substrate bears a formal positive charge. (3) There does not appear to be a significant " α effect"²¹ exhibited in the reactivity of sulfite ion or hydroxylamine even though addition is occurring to an unsaturated substrate²² and the reactions have a β value close to unity.²³ It may simply be coincidental that the data obtained fall on one straight line and it is believed that more information is necessary before a definitive mechanistic discussion can be conducted. The deviation shown by hydroxide ion appears to be a general observation no matter whether it behaves as a base catalyst²⁴ or as a nucleophilic reagent.²⁵

Experimental Section

Materials. Quinazoline (Aldrich Chemical Co.) was sublimed before use. Hydroxylamine hydrochloride (reagent grade) was precipitated from an ethanolic solution with ether. β -Mercaptoethanol (reagent grade) was distilled under vacuum. Hydrogen sulfide stock solutions were prepared by bubbling the gas into 0.1 *M* hydrochloric acid. All other chemicals were reagent grade or AR if available. Water was double distilled with the second distillation being from acid permanganate. Solutions containing sulfurous acid species were prepared immediately before use in water through which oxygen free nitrogen had bubbled for 1 hr. One milliliter of methanol was added to each 100 ml of sulfite solution to minimize its oxidation.²⁶ The total sulfite concentration of solutions was determined by iodometric titration²⁷ at the conclusion of both kinetic and equilibrium studies. The concentration of hydrogen sulfide solutions was determined by iodometric titration²⁷ and that of hydroxylamine solutions by potassium bromate titration.²⁷ The pH of solutions was adjusted to 2 or below with hydrochloric acid or to 11 or above with sodium hydroxide. Other pH values were maintained by mixtures of sodium hydroxide and monochloroacetic acid (2.4–3.2), succinic acid (3.6–5.8), phosphoric acid (6.4–7.3), tris(hydroxymethylaminomethane) (7.8–8.8), and carbonic acid (9.3–10.4). Whenever possible the buffer capacity of the nucleophilic reagent was used to maintain constant pH values. Ionic strengths of solutions were maintained at 0.1 *M* by the addition of sodium chloride.

Ionization Constants. The ionization constants of the nucleophilic reagents were determined potentiometrically¹⁸ at $25 \pm 0.5^\circ$ in aqueous solutions of ionic strength 0.1 *M*. The ionization constants of acids whose conjugate bases are unstable were calculated from the initial absorbance obtained when the pH's of solutions of the acids were rapidly raised. These experiments were performed on a Durrum stopped flow spectrophotometer. The initial absorbance measured as a function of pH was analyzed by conventional method.¹⁸ Analytical wavelengths adopted for the determination of K_a^a , K_a^b , and K_a of bisulfite adduct and K_a of β -mercaptoethanol adduct of quinazoline were 355, 288, 300, and 315 nm, respectively. In all cases, a quinazoline solution (1.5×10^{-4}

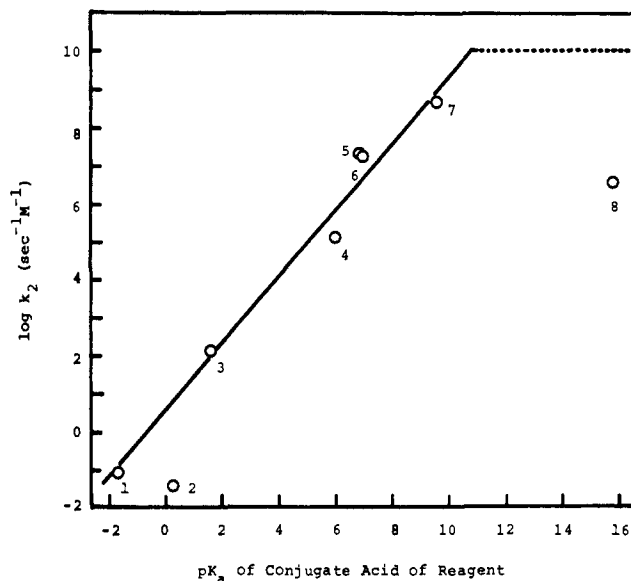


Figure 8. A linear free energy relationship of the reaction rate constant for the addition of nucleophilic reagents to quinazoline cation with the basicity of the reagents. Nucleophilic reagents are (1) H₂O, (2) H₂NCONH₂, (3) HSO₃⁻, (4) NH₂OH, (5) HS⁻, (6) SO₃²⁻, (7) SCH₂CH₂OH, and (8) ⁻OH.

M) containing an excess of nucleophilic reagent was mixed with an equal volume of buffers. Optical cell width of the stopped flow spectrophotometer was 20.0 mm.

Measurement of Equilibrium Constants. Equilibrium constants were calculated from differences in absorbance between aqueous buffered solutions of quinazoline and the same solutions which had equilibrated at $25.0 \pm 0.1^\circ$ after the addition of various concentrations of the nucleophilic reagent. Ionic strengths of all solutions were maintained at 0.1 *M* by the addition of sodium chloride. The computation of *K* values involved an iterative procedure similar to the one described by Higuchi, *et al.*²⁸

Measurement of Rate Constants. Rate constants were calculated from changes in absorbance that followed the mixing of an aqueous solution which contained the nucleophilic reagent. The initial concentration of the nucleophilic reagent in the reaction mixture was at least 25 times that of the initial quinazoline concentration. All experiments were performed at $25.0 \pm 0.1^\circ$ and ionic strength was maintained constant (0.1–0.5 *M*) by the addition of sodium chloride. The wavelengths at which absorbance changes were measured were 290 nm for addition of sulfurous acid species, 270 or 310 nm for addition of hydroxylamine, 285 nm for addition of urea, 290 or 295 nm for addition of β -mercaptoethanol, and 260 or 295 nm for addition of hydrogen sulfide. Measurements were made on either a Durrum stopped flow spectrophotometer or on a Cary (Model No. 16) spectrophotometer.

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