

A Linear Free Energy Relationship Governing the Covalent Addition of Anionic Reagents to 1,3-Dimethyl-5-nitrouracil¹

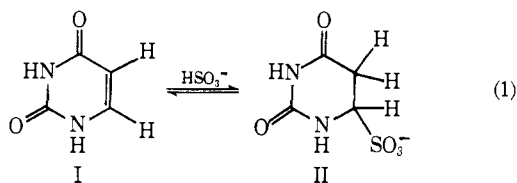
Ian H. Pitman,* Moo J. Cho, and Gerald S. Rork

Contribution from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66044. Received September 20, 1973

Abstract: Equilibrium constants have been calculated for the covalent addition to 1,3-dimethyl-5-nitrouracil at 25° of hydroxide and sulfite ions and the anions of β -mercaptoethanol and hydroxylamine. The logarithms of the product of equilibrium constants and the ionization constant of the conjugate acid of the anion were linearly related to the γ value of the conjugate acid of the anion.

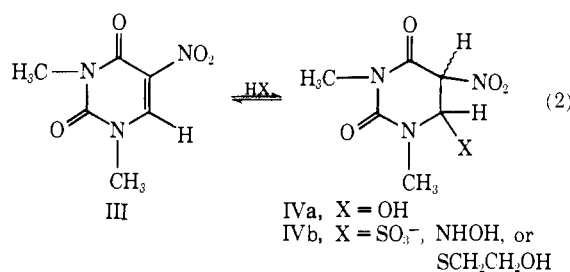
It has been established² that the logarithms of the equilibrium constants for the covalent addition of nucleophilic reagents, ranging from the weak nucleophile, water, to the strong nucleophile, bisulfite ion, across the $>C(4)=N^+(3)<$ bond of quinazoline cation are linearly related to the γ value³ of the nucleophilic reagent. This latter parameter is a measure of the ability of a reagent to covalently add across the carbonyl group of aldehydes and ketones. Knowledge of this linear free energy relationship facilitates the making of precise estimates of the equilibrium constants for covalent addition to quinazoline of any nucleophilic reagent whose γ value is known.²

The present study was undertaken to ascertain whether a similar linear free energy relationship governed the addition of nucleophilic reagents across a $>C=C<$ bond in heteroaromatic molecules such as uracil (I). Uracil is known to covalently add a variety of nucleophilic reagents across the $>C(5)=C(6)<$ double bond. For example, the covalent addition of bisulfite ion to yield 5,6-dihydrouracil-6-sulfonate (II)



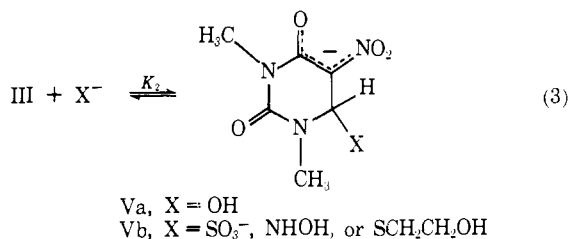
has recently been the subject of several studies.^{4,5} It was anticipated that the nitro group at C(5) would facilitate the rate of the addition of nucleophilic reagent, HX , to 1,3-dimethyl-5-nitrouracil (III). Another advantage that III was expected to have over I was that it possesses no ionizable protons and would thus exist as a neutral molecule throughout the pH range 1 to 10, where experiments would be performed. The plan was to study the covalent addition to III of water, bisulfite

ion, hydroxylamine, and β -mercaptoethanol. However, adducts such as IVa were not detected to any



significant extent in the pH range 2 to 10 during a few days. This could arise because of their very high acidity or because the kinetics of their formation were unfavorable, compared to other possible reactions. This problem is currently being studied in our laboratory.

The reaction that could be observed and which was consequently studied was the covalent addition of sulfite ion, hydroxide ion, and the anions of β -mercaptoethanol and hydroxylamine (*i.e.*, X^- species) to III to yield the anionic adduct, V.



Results and Discussion

Addition Reactions. The uv spectra of aqueous solutions of III with pH values between 1.0 and 7.5 were identical (λ_{max} 308 nm; ϵ_{max} 1.0×10^4 $cm^{-1} M^{-1}$) and similar to the spectra obtained in acetonitrile (λ_{max} 307 nm; ϵ_{max} 9.1×10^3 $cm^{-1} M^{-1}$) and in methanol (λ_{max} 303 nm; ϵ_{max} 9.0×10^3 $cm^{-1} M^{-1}$). This finding suggests that 1,3-dimethyl-5-nitrouracil exists as the anhydrous neutral species, III, within this pH range. However, a bathochromic shift with an increase in molar absorptivity took place when the pH of solutions containing III was increased beyond 7.5, even though the compound does not contain ionizable protons. An isoabsorptive point was observed at 302 nm (see Table

(1) This work was supported in part by a National Institute of Health Grant (No. 5 ROI-GM-18348-03) and was facilitated by the award to I. H. Pitman of a Public Health Service Career Development Award (No. IK4-GM-70, 100-01).

(2) M. J. Cho and I. H. Pitman, *J. Amer. Chem. Soc.*, **96**, 1843 (1974).

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

(4) H. Hayatsu, Y. Wataya, and K. Kai, *J. Amer. Chem. Soc.*, **92**, 724 (1970).

(5) R. Shapiro, R. E. Servis, and M. Welcher, *J. Amer. Chem. Soc.*, **92**, 422 (1970).

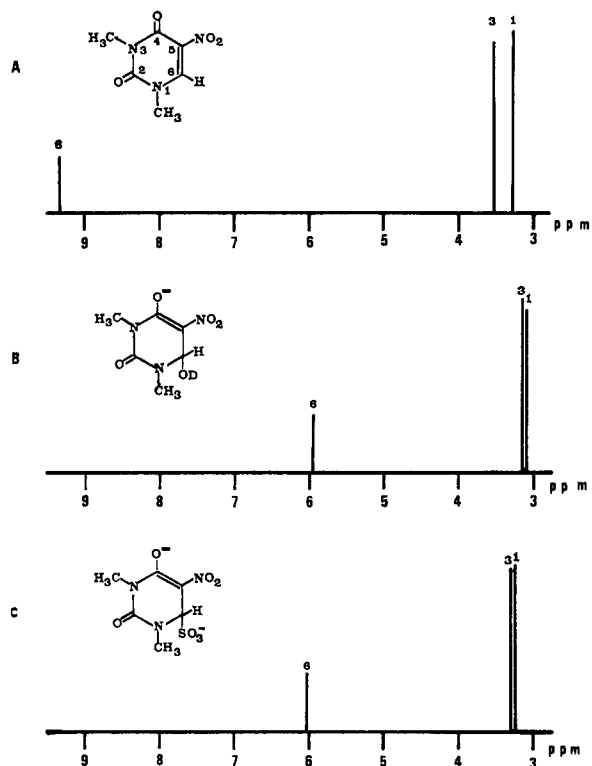


Figure 1. Nmr spectra of 1,3-dimethyl-5-nitrouracil in D_2O (A), in D_2O containing NaOD (B), and in D_2O containing Na_2SO_3 and $NaHSO_3$ (C). The pD of sample C was 6.5.

Table I. Ultraviolet Spectral Characteristics of Aqueous Solutions of 1,3-Dimethyl-5-nitrouracil in the Range of 230 to 350 nm in the Presence of Nucleophilic Reagent

Reagent	pH	[Re-agent] $\times 10^3$ M	% fraction of adduct	λ_{max} , nm	ϵ_{max} , 10^{-4} $cm^{-1} M^{-1}$	λ_{iso} , nm
Water	2.0		0.1	308	1.04	
	11.5 ^a		99.9	325	1.83	302
Sulfurous acid species	4.95	6.0	74	326	1.25	312
Hydroxylamine	6.50	200	65	321	1.37	305
β -Mercaptoethanol	7.00	200	74	320	0.94	317

^a Freshly prepared.

I). Since a hypsochromic shift would be expected if IVa is formed,⁶ this observation suggests that HO^- , rather than H_2O , adds to the substrate to form the anionic hydrate, Va. This postulate was also supported by the fact that the lowest pH value at which the uv spectrum of III starts to change was as high as 7.5. The pK_a of IVa is expected to be lower than 7.5, since that of nitroacetone,⁷ for example, is 5.1.

Nmr data suggest that the major species in aqueous solutions at pH below 7.5 is III (with essentially no detectable amount of covalent hydrate with the structure IVa) and that at pH above 9.5 the predominant species is the adduct, Va. The nmr spectra of solutions III in D_2O at pD 4 and 11 are displayed in Figure 1. The

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

(7) R. G. Pearson and R. L. Dillon, *J. Amer. Chem. Soc.*, **75**, 2439 (1953).

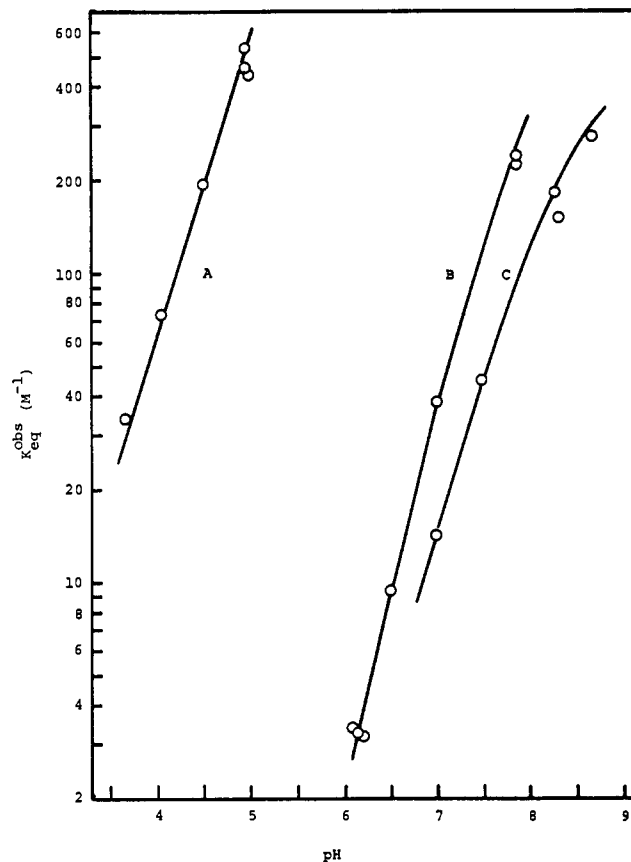
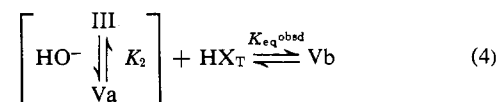


Figure 2. Dependence on pH of the apparent equilibrium constant for the additions of bisulfite ion (A), hydroxylamine (B), and β -mercaptoethanol (C) to 1,3-dimethyl-5-nitrouracil at 25° and ionic strength 1.0 M. The solid lines were calculated as described in the text.

ring proton on C(6) of III occurs as a singlet at 9.32 ppm, which undergoes an upfield shift to 5.90 ppm at pD 11. As shown in Figure 1c, a similar chemical shift was observed at pD about 6.5 in the presence of sulfurous acid species.

Addition of other nucleophilic reagents to equilibrated buffered aqueous solutions of III also produced uv spectral changes similar to those produced by hydroxide ion. The uv spectral characteristics of the products of these reactions are shown in Table I. Because of the similarities of all these nmr and uv spectra to those of Va, it is believed that they are caused by the substances Vb where $X = SO_3^-$, $NHOH$, or SCH_2CH_2OH . Hence, the equilibrium reactions are believed to be



where the observed equilibrium constant, K_{eq}^{obsd} , is defined as

$$K_{eq}^{obsd} = [\text{Vb}] / ([\text{III}] + [\text{Va}])[\text{HX}]_T \quad (5)$$

where $[\text{HX}]_T$ indicated the total concentration of the nucleophilic reagent.

Plots of K_{eq}^{obsd} as a function of pH are shown in Figure 2. A pH-dependent equilibrium constant, K_2 , is defined as

$$K_2 = [\text{Vb}] / [\text{III}][\text{X}^-] \quad (6)$$

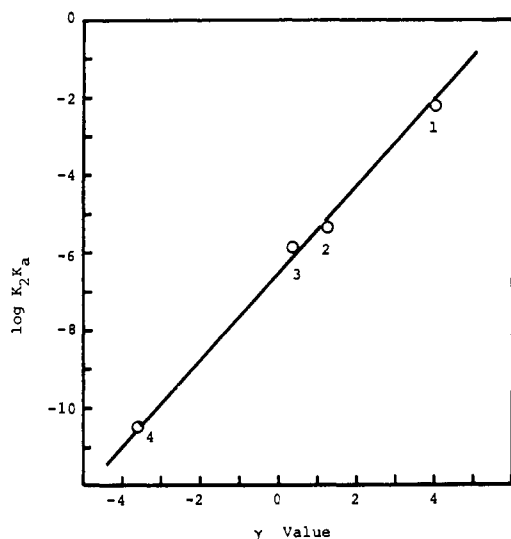


Figure 3. Plot of the logarithmic value of the product of the equilibrium constant, K_2 , for the formation of an anionic nucleophilic adduct of 1,3-dimethyl-5-nitrouracil and the ionization constant, K_a , of the nucleophilic reagent vs. the γ parameter which is a measure of the ability of a reagent to add across the carbonyl group of aldehydes or ketones. Reagents are (1) HSO_3^- , (2) NH_2OH , (3) $\text{HSCH}_2\text{CH}_2\text{OH}$, and (4) H_2O .

Therefore, the dependence on pH of $K_{\text{eq}}^{\text{obsd}}$ values for the additions of sulfurous acid species and β -mercaptoethanol can be expressed as

$$K_{\text{eq}}^{\text{obsd}} = \frac{K_2}{(1 + (K_1K_w/[\text{H}^+]))(1 + ([\text{H}^+]/K_a))} \quad (7)$$

where K_a is the ionization constant of HSO_3^- or $\text{HS-CH}_2\text{CH}_2\text{OH}$, and the pH-independent equilibrium constant, K_1 , is defined as

$$K_1 = [\text{Va}]/[\text{III}][\text{OH}^-] \quad (8)$$

The value of K_1 was calculated from the change in absorbance at a given wavelength as a function of pH of aqueous solutions containing III and is listed in Table II. Since eq 7 contains only one unknown, K_2 , its

Table II. The Value of K_2 , the Equilibrium Constant for the Formation of an Anionic Adduct of 1,3-Dimethyl-5-nitrouracil, and the Product of K_2 and the Ionization Constant of the Nucleophilic Reagent, K_a , at 25° and Ionic Strength 1.0 M

Nucleophilic reagent	γ^a	$K_2, M^{-1}{}^b$	$K_2K_a^c$
Water	-3.58	1.78×10^5	3.21×10^{-11}
β -Mercaptoethanol	0.35	4.48×10^3	1.38×10^{-6}
Hydroxylamine	1.24		4.27×10^{-6}
Sulfurous acid species ^d	4.02	1.85×10^4	5.85×10^{-3}

^a Reference 1. ^b K_1 when the reagent is water. ^c $K_1K_w/[\text{H}_2\text{O}]$ when the reagent is water. ^d Bisulfite ion.

values can be calculated from experimental data. Substituting the average value of K_2 into eq 7, the solid curves on Figure 2 were calculated. In the case of hydroxylamine addition, eq 7 becomes

$$K_{\text{eq}}^{\text{obsd}} = \frac{K_2}{(1 + (K_1K_w/[\text{H}^+]))(([\text{H}^+]^2/K_{a1}K_a) + ([\text{H}^+]/K_a) + 1)} \quad (9)$$

where K_{a1} is the ionization constant of NH_3OH^+ and K_a is that of NH_2OH , a value of which is not available. However, it is most likely that $K_a \gg [\text{H}^+]$ for the experimental pH range, and, hence, eq 9 becomes

$$K_{\text{eq}}^{\text{obsd}} = \frac{K_2K_a}{(1 + (K_1K_w/[\text{H}^+]))([\text{H}^+]^2/K_{a1}) + [\text{H}^+]} \quad (10)$$

The value of K_2K_a is the only unknown in eq 10 and, hence, could be calculated from experimental data. The significance of this quantity is discussed in the subsequent section. The solid line on Figure 2 was calculated by substituting the average value of K_2K_a into eq 10.

A Linear Free Energy Relationship for the Equilibrium Constants. A linear free energy relationship for two series of reactions should be based on an equivalent stoichiometry in both series of reactions. Since the addition reactions of nucleophilic reagents to quinazoline cation² and to carbonyl compounds^{3,8} have a stoichiometry of substrate + HX, the reaction series of the present discussion should be represented as $\text{III} + \text{HX} \rightleftharpoons \text{Vb} + \text{H}^+$. The overall change in the standard free energy, ΔG° , associated with this reaction is then

$$\Delta G^\circ = -2.3RT \log K_2K_a \quad (11)$$

The values of K_2K_a are listed in Table II. A plot of the latter quantities against the γ value⁴ of HX is shown in Figure 3. A straight line of slope 1.10 best covers the data. A knowledge of the equation of this line, together with that of the ionization constant and γ value of a reagent HX, would enable precise estimates to be made of the extent of addition of the reagent X^- to III.

The fact that the plot in Figure 3 is linear with a slope close to 1.0 is interpreted⁹ to indicate that the differences in standard free energy between adducts of both pyridine-4-carboxaldehyde and III with a particular nucleophilic reagent are essentially independent of the nature of the substituent X^- . (These adducts are designated as PHX and V, respectively.) The substituent X^- is attached to a tetrahedral carbon atom in both adducts, PHX and V, so it is not unreasonable to assume that its electronic contribution to the standard free energy of both types of adducts would be similar. The carbon atom adjacent to that at which X^- is attached in V is most likely to have an sp^2 configuration by virtue of the distribution of the electronic charge between the nitro group on C(5) and the carbonyl group on C(4).¹⁰ Hence, it is unlikely that the substituent X^- interacts sterically to a significant extent with the nitro group on C(5) in V. The fact that the value of the term, $G^\circ_{\text{PHX}} - G^\circ_{\text{V}}$, is essentially independent of the nature of the substituent X^- suggests that X^- does not interact sterically to any great extent with other groups in the PHX adduct either.

A very different situation could, however, apply in neutral adducts such as IV, if they were formed. If the adducts were formed by trans addition of HX across the $>\text{C}(6)=\text{C}(5)<$ bond of III, certainly the $-\text{X}$ and $-\text{NO}_2$ groups (which would be cis to each other) would be expected to interact sterically. However, the effect of steric interactions of this type could not be examined because neutral adducts such as IV were not detected in the systems studied.

(8) J. Hine and R. W. Redding, *J. Org. Chem.*, **35**, 2769 (1970).

(9) See ref 2 for explanation of this point.

(10) B. M. Wepster, *Progr. Stereochem.*, **2**, 99 (1958).

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.

(11) D. J. Brown, *J. Chem. Soc.*, 211 (1955).

(12) M. Prystas and J. Gut, *Collect. Czech. Chem. Commun.*, 28, 2501 (1963).

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

Moo J. Cho and Ian H. Pitman*²

Contribution from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66044.

Received September 20, 1973

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

(1) This work was supported in part by a National Institute of Health Grant (No. 5 ROI GM 18348) and was facilitated by the award to I. H. P. of a Public Health Service Career Development Award (No. IK4-GM-70).

(2) Author to whom all correspondence should be addressed.

(3) D. D. Perrin and I. H. Pitman, *J. Chem. Soc.*, 7071 (1965).

(4) R. Shapiro, R. E. Servis, and M. Welcher, *J. Amer. Chem. Soc.*, 92, 422 (1970).

(5) H. Hayatsu, Y. Wataya, and K. Kai, *J. Amer. Chem. Soc.*, 92, 724 (1970).

(6) F. A. Sedor and E. G. Sander, *Biochem. Biophys. Res. Commun.*, 50, 328 (1973).

(7) I. H. Pitman and M. A. Ziser, *J. Pharm. Sci.*, 59, 1295 (1970).

(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).