These phenomena are illustrated in Fig. 9, which contains photomicrographs of these forms after 5 min.

The overall rate of crystal growth for Form I of SK&F 30097 was derived according to the method of Edmundson and Lees (11). A plot of percentage number cumulative frequency oversize against time for diameters from 14 to 25 μ is shown in Fig. 10. This size represents the faster growing end of the distribution obtained from Fig. 7. Horizontal lines corresponding to various percentage cumulative counts were made. The intercepts of these lines at the 1, 2, and 3% levels for the various time intervals give the equivalent diameter, which is then plotted against time. Figure 11 shows the rate plot for a typical crystal growth study. The slope of the lines represents the rate of growth of the crystals expressed as an increase in diameter per unit time. Table I contains values showing the relative rates of crystal growth of Form I in the presence and absence of various protective colloids.

The crystal growth in the presence of methylcellulose was approximately 300 times slower than in the presence of the other colloids. Photomicrographs showing the change in particle size with time in the presence of methylcellulose are shown in Fig. 12. There was no apparent increase in crystal size after 3 days; however, after 8 days, there was some evidence of growth. The fact that the Coulter counter measurements indicated a growth rate of 3×10^{-4} μ/min. indicates the extreme sensitivity of this instrument to change in crystal size with time.

SUMMARY

1. The presence of two polymorphic forms and a hydrate of SK&F 30097 has been confirmed by X-ray diffraction, IR spectroscopy, and differential scanning calorimetry.

2. Form II, which was less stable thermodynamically, had a more rapid dissolution rate than Form I in 50% ethanol solution; however, in artificial gastric fluid, there was no apparent difference.

3. Forms I and II readily formed the hydrate in aqueous suspension; protective colloids were shown to affect the rate of hydrate formation.

4. Coulter counter measurements were used to follow the change in particle-size distribution for Form I in electrolyte solution. The relative rates of crystal growth were determined in the presence of various protective colloids. Methylcellulose retarded the rate of crystal growth of Form I significantly.

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Thermodynamics and Kinetics of Covalent Addition of Bisulfite Ion to Pyrimidinium Ions

IAN H. PITMAN* and MARK A. ZISER

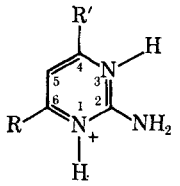
Abstract □ Equilibrium and rate constants have been calculated for the reversible covalent addition of bisulfite ion to 2-aminopyrimidinium ion and to its 1-methyl and 4-methyl derivatives. 2-Amino-4,6-dimethylpyrimidinium ion did not appear to add bisulfite ion under the experimental conditions. The 1:1 covalent adducts had very low solubility in aqueous buffers around pH 4. This was consistent with their being zwitterions. Rate-determining steps in adduct formation appeared to involve attack of both bisulfite ion and sulfite ion on the pyrimidinium cation. In the reverse reactions, the zwitterionic adducts appeared to decompose by both nonbase-catalyzed and specific base-catalyzed reactions.

Keyphrases □ Bisulfite ion, kinetics, thermodynamics—covalent addition to pyrimidinium ions □ Covalent addition—bisulfite ion to pyrimidinium ions □ 2-Amino-1,6-dihydropyrimidinium-6-sulfonate—synthesis □ 2-Amino-1,6-dihydro-4-methylpyrimidinium-6-sulfonate—synthesis □ UV spectrophotometry—identification

Many of the known reactions of sodium bisulfite with organic and inorganic molecules have been studied quantitatively, and a considerable amount of thermodynamic and kinetic data is available for use when considering the inclusion of sodium bisulfite as an antioxidant in drug formulations (1). However, very little quantitative data are available on the reversible covalent additions of bisulfite ion to nitrogen-containing heteroaromatics such as pyridines (2), pyrimidines (3, 4), pteridines (5–8), and quinazolines (9, 10), although many drugs belong to these classes of compounds. The occurrence of the addition reaction in a drug formulation would reduce the effective concentration of the bisulfite ion, and the covalent adduct may have different chemical reactivity to that of the augend.

The present study was undertaken to determine the effects of temperature and pH on the kinetics and thermodynamics of addition of bisulfite ion to 2-aminopyrimidinium ion (I; R = R′ = H), 2-amino-1-methylpyrimidinium ion (II), 2-amino-4-methylpyrimidinium ion (I; R = H, R′ = Me), and 2-amino-4,6-dimethylpyrimidinium ion (I; R = R′ = Me). Studies on addi-

tions to molecules which are widely used as drugs are continuing in the authors' laboratories.



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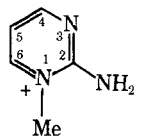

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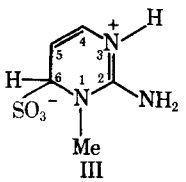

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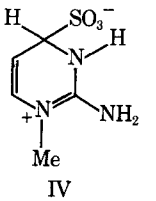
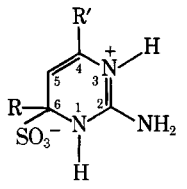


IV

RESULTS AND DISCUSSION

The Addition Reaction—Compound II has previously been shown (4) to react with bisulfite ion reversibly to yield, in dilute aqueous solution at 25°, an approximately 0.8:1 mixture of the covalent adducts III and IV. These adducts appear to have similar UV spectra (4), and the spectrum of their equilibrium mixture has the characteristics shown in Table I. Because addition of sodium bisulfite (0.2 *M*) to dilute aqueous solutions of (I; R = R′ = H) and (I; R = H, R′ = Me) produced solutions with similar UV spectra to that of a mixture of III and IV (Table I), it was deduced that covalent addition of bisulfite ion was occurring in these cases also. The addition of sodium bisulfite (1.0 *M*) to saturated aqueous solutions of the pyrimidinium ions (I; R = R′ = H) (II), and (I; R = H, R′ = Me) resulted in the formation of white crystalline products which had the elemental analysis of 1:1 adducts of the respective pyrimidinium ion and bisulfite ion. The reversibility of all of the addition reactions was indicated by the fact that dilution of solutions of the adducts in aqueous sodium bisulfite with water led to reformation of the original pyrimidine. Addition of sodium bisulfite (0.2 *M*) to dilute aqueous solutions of (I; R = R′ = Me) did not lead to any significant changes in its UV spectrum (when the absorbance of the bisulfite ion was taken into account), and it was assumed that covalent addition of bisulfite ion was not occurring to this compound under these conditions.



V

The 1:1 adduct of Compound (I; R = R′ = H) would have the same structure whether addition occurred at C_4 or C_6 and was thus believed to be 2-amino-1,6-dihydropyrimidinium-6-sulfonate (V; R = R′ = H). The strong blocking effects of methyl groups at C_4 and C_6 in Compound (I; R = R′ = Me) to addition at those sites strongly suggest that addition to (I; R = H, R′ = Me) would occur predominantly at C_6 to yield 2-amino-1,6-dihydro-4-methyl-pyrimidinium-6-sulfonate (V; R = H, R′ = Me). Similar blocking effects of *C*-alkyl groups to nucleophilic additions at that site have been observed (11) for nucleophilic additions to other heteroaromatic molecules. More exact structural determinations by NMR spectroscopy of the adducts (V; R = R′ = H) and (V; R = H, R′ = Me) were difficult because of their low solubility in aqueous sodium bisulfite (<1 g./100 ml.). This low solubility is consistent with their being zwitterionic in aqueous buffers between pH 3 and 5.

Because of the acidic or basic nature of the reactants and products in the addition reactions, the reaction mixtures always contained varying concentrations of pyrimidinium ion PyH^+, neutral pyrimidine Py, sulfurous acid H_2SO_3, bisulfite ion HSO_3^-, sulfite ion $SO_3^=$, cation H_2Add^+, zwitterion $HAdd^\pm$, and anion Add^- of the covalent adduct. At any particular pH, the overall addition reaction was

$$Py_T + S_T \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Add_T \qquad \text{(Eq. 1)}$$

where Py_T, S_T, and Add_T were the total pyrimidine, sulfurous acid, and adduct species, respectively, present at any time. The acid-dissociation constants of the various acidic species were measured under experimental conditions and are listed in Table II.

Equilibrium Constants for Addition—Apparent equilibrium constants, $K_{app.}$ values, were calculated from differences in UV absorbance at fixed wavelengths between aqueous buffered solutions of the pyrimidines and similar solutions which had equilibrated after the addition of different amounts of sodium bisulfite. As shown in Fig. 1, the $K_{app.}$ values for addition to the very weakly acidic compound (II) were independent of pH in the pH region investigated, but the values for the more strongly acidic compounds, (I; R = R′ = H) and (I; R = H, R′ = Me), decreased with increasing pH. No evidence could be found for adduct formation by any of the compounds at pH values above 8.5 or below 0.5.

The $K_{app.}$ values were the combined equilibrium constants at each pH for the reactions represented by Eq. 1 and are defined as

$$K_{app.} = \frac{[Add_T]}{[Py_T][S_T]} \qquad \text{(Eq. 2)}$$

If the true addition reactions were

$$PyH^+ + HSO_3^- \overset{K}{\rightleftharpoons} HAdd^\pm \qquad \text{(Eq. 3)}$$

then, in the pH range where $[HSO_3^-] \gg [H_2SO_3]$ or $[SO_3^=]$ and $[HAdd^\pm] \gg [H_2Add^+]$ or $[Add^-]$, values of $K_{app.}$ and K would be related by the identity

$$K_{app.} = \frac{K[H^+]}{(K_{a_p} + [H^+])} \qquad \text{(Eq. 4)}$$

where K_{a_p} is the acid-dissociation constant of the pyrimidinium ion.

Because plots of $K_{app.}$ $([H^+] + K_{a_p})$ against $[H^+]$ were linear between pH 3.3 and 5 for addition to each of the pyrimidinium ions studied, it is believed that these addition reactions are adequately described by Eq. 3 and that the zwitterionic forms of the adducts are the most stable forms in this pH region. Values of the

Table I—UV Spectral Characteristics of 1:1 Bisulfite Adducts

Augend	$\lambda_{max.}$ (log ϵ)
I; R = R′ = H′	255 (3.38)[a]
II	258 (3.36)[a]
I; R = H, R′ = Me	254 (3.17)

[a] These values were corrected for absorbance due to free pyrimidine species at equilibrium and are considered to be more accurate than those in *Reference 3*.

Table II—Acid-Dissociation Constants, at Ionic Strength 10^{-1} *M*, of Pyrimidinium Ions and Sulfurous Acid

Compound	Temperature	pKa (± spread)
I; R = R′ = H	15.5°	3.85 (0.02)
	25.3°	3.77 (0.03)
	36.6°	3.70 (0.03)
II	20.0°	10.75[a] (0.1)
I; R = H, R′ = Me	25.0°	4.41 (0.04)
HSO_3^-	15.1°	6.85 (0.04)
	24.0°	6.94 (0.05)
	37.0°	6.92 (0.03)

[a] At I = 0.01, D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, **1955**, 4035.

Table III—Thermodynamic Data for Addition of Bisulfite Ion to Pyrimidinium Ions

Augend	Temperature	K,[a] M^{-1}	K_6,[b] M^{-1}	K_4,[b] M^{-1}	$\Delta H°$, kcal. M^{-1}	$\Delta S°$, cal. M^{-1}
I; R=R′=H	12.45°	4110				
	25.00°	1800			−11.3	−23.1
	36.00°	905				
II	12.45°	1098				
	25.00°	501	222	278	−9.70	−20.1
	36.00°	282				
	36.50°	273				
I; R=H, R′=Me	25.00°	49.0				
I; R=R′=Me	25.00°	<0.1				

[a] K = [HAdd±]/([PyH⁺][HSO_3^-]). [b] K_6 and K_4 are defined in the text.

pH-independent equilibrium constant K were calculated from the slopes of the graphs and are listed in Table III, together with standard enthalpy and entropy changes for addition.

The K values for addition to Compounds (I; R=R′=H) and (I; R=H, R′=Me) are each related to the differences in free energy between a particular adduct and the reactants, because only one covalent adduct is formed in each reaction. On the other hand, an approximately 0.80:1 ratio of III and IV results (4) from addition to II at 25°. Thus, the K value for the addition reaction is really the sum of two microequilibrium constants, $K_6 \simeq K/2.25$ and $K_4 \simeq K/1.8$, where K_6 and K_4 relate to addition at C_6 to yield III and at C_4 to yield IV, respectively. The values of K_6 and K_4 have to be compared with K values for the other compounds in discussion of differences in free energy between a particular adduct and its pyrimidinium ion augend.

It can be seen from Table III that substitution of methyl groups for protons anywhere in the pyrimidinium ring decreases the extent to which covalent addition occurs. The very marked reduction in the value of K produced by methyl groups at the potential sites of addition [comparing additions to (I; R=R′=Me) and (I; R=R′=H)] is probably due to both steric and electronic (+I) destabilization of the adduct and electronic stabilization (+I) of the original pyrimidinium ion. The electronic effects are expected to be the major ones when methyl substitution is at sites distant from the site of addition, *i.e.*, at C_4 and N_1 of the 2-aminopyrimidinium ion. A possible explanation for the lower K value obtained for addition to the compound with the *C*-methyl substituent (I; R=H, R′=Me) than to that with the *N*-methyl substituent (II) would be that the +I effect of the *N*-methyl substituent was partially shielded from the ring by the positive charge on the nitrogen atom.

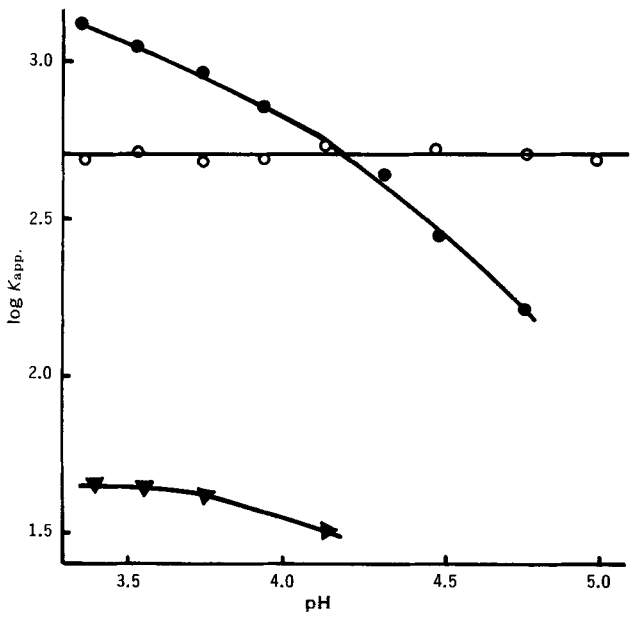


Figure 1—*Plot against pH of log* $K_{app.}$ *for covalent addition of bisulfite ion to:* (●) *(I; R=R′=H),* (○) *(II), and* (▲) *(I; R=H, R′=Me).*

Covalent addition of bisulfite ion to pyrimidinium ions involves saturation of charged imino group >C=$\overset{+}{N}$< and can be compared with the well-known addition to aldehydic or ketonic carbonyl groups >C=O. These reactions have many similarities; they occur readily at room temperature, they are reversible, and they are both inhibited by alkyl substitution at the site of addition. However, as seen by comparison of the thermodynamic data in Table IV with that in Table III, the standard enthalpy change is more favorable and entropy change less favorable for addition to the carbonyl compounds. These differences are most likely due largely to the different "charge types" of the reactions, and they reflect the changes in solvation of reactants and products which accompany the reactions.

Kinetics of Formation and Decomposition of Covalent Adducts (V; R=R′=H) and (V; R=H, R′=Me)—The kinetics of the reversible formation of (V; R=R′=H) and (V; R=H, R′=Me) were studied to determine the effect of total sulfurous acid species concentrations, pH, and buffer-ion concentrations on the covalent addition of bisulfite ion to pyrimidinium ions. These systems were chosen for study because they each involved only one covalent adduct and proceeded cleanly and rapidly at room temperature.

First-order rate constants, $k_{obs.}$ values, for the consumption of the pyrimidinium ion plus the pyrimidine were calculated from the changes in UV absorbance at 302 mμ, which followed mixing of aqueous buffered (succinic acid and borax) solutions of the (I; R=R′=H) or (I; R=H, R′=Me) (6.26×10^{-4} *M*) with equal volumes of solutions containing sodium bisulfite at an initial concentration that was at least 10 times that of the pyrimidine. Values of $k_{obs.}$ increased with increasing pH in the pH range 3.3–4.8 when the initial bisulfite-ion concentration was kept constant, and they increased linearly with increasing initial sodium bisulfite concentrations at constant pH. Examples of this behavior for addition to (I; R=R′=H) are shown as the circles in Fig. 2. Values of the $k_{obs.}$ did not change more than ±2% when the total buffer concentration was varied between 10^{-1} *M* and 10^{-3} *M* at pH 4.20. The ionic strength of all reaction mixtures was maintained at 0.1 *M* with sodium chloride.

When only one covalent adduct is formed in the addition reaction, the rate of consumption of total pyrimidine species according to Eq. 1 would be

$$\text{rate} = k_f[\text{Py}_T][\text{S}_T] - k_b[\text{A}_T] \qquad \text{(Eq. 5)}$$

Table IV—Thermodynamic Data for Addition of Bisulfite Ion to Carbonyl Compounds

Compound	Temperature	K,[a] M^{-1}	$\Delta H°$	$\Delta S°$
CH_3CH_2CHO[b]	20°	12,000		
$CH_3—\overset{\overset{O}{\|}}{C}—CH_3$[b]	20°	290		
C_6H_5CHO[c]	20°	5,000	−17.7	−43

[a] K = [adduct⁻]/([carbonyl compound][HSO_3^-]). [b] M. A. Gubareva, *J. Gen. Chem., USSR*, **17**, 2529(1947). [c] J. A. Sousa and J. D. Margerum, *J. Amer. Chem. Soc.*, **82**, 3013(1960).

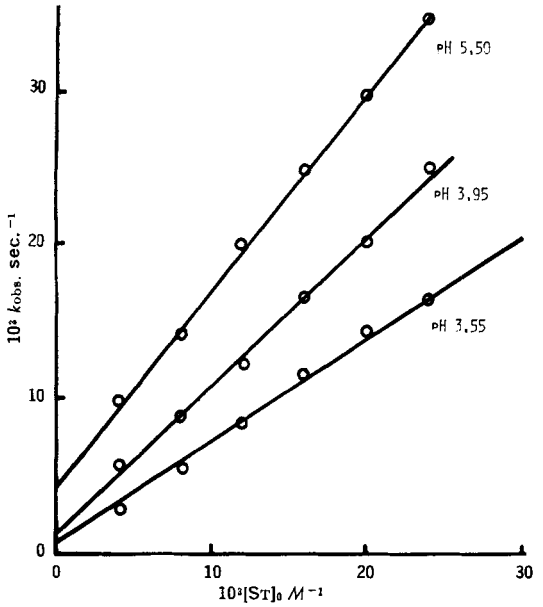


Figure 2—*Plot against* $[S_T]_0$ *of* $k_{obs.}$ *for covalent addition to 2-aminopyrimidinium ion at 25°. Open circles are experimental points and lines were calculated from values of pH-independent rate constants as described in text.*

and the observed first-order rate constant for approach to equilibrium in the presence of a considerable excess of total sulfurous acid species could be related to k_f and k_b values by the identity:

$$k_{obs.} = k_f[S_T]_0 + k_b \qquad \text{(Eq. 6)}$$

where $[S_T]_0$ was the initial sodium bisulfite concentration. Values of k_f were thus calculated from the slope of linear plots of $k_{obs.}$ against $[S_T]_0$ and are listed in Table V.

Although it should be possible to calculate k_b values from the Y-axis intercepts of these plots, this was only done in a few cases because of the extreme sensitivity of these values to small variations in the slopes of the lines. The k_b values were usually calculated from the k_f value and the appropriate $K_{app.}$ value by assuming the principle of microscopic reversibility and using the identity

$$k_b = k_f/K_{app.} \qquad \text{(Eq. 7)}$$

Justification for this assumption came from the fact that at high pH values, the Y-axis intercepts of plots of $k_{obs.}v[S_T]_0$ were relatively large and the graphically determined and calculated k_b values did not vary more than ±3%.

Table V—Rate Constants for Reversible Addition of Bisulfite Ion to Pyrimidinium Ions

Augend	Temperature	pH	$10\,k_f$ M^{-1} sec.$^{-1}$	$10^4\,k_b$ sec.$^{-1}$
I; R = R′ = H	12.00°	3.58	3.50	1.23
		3.98	5.60	4.22
		4.54	7.65	9.99
	25.00°	3.36	5.40	4.16
		3.55	6.90	6.13
		3.76	8.78	9.61
		3.95	9.63	13.4
		4.13	10.8	19.6
		4.32	11.0	27.6
		4.50	12.7	44.7
		4.77	13.5	81.8
	37.00°	3.52	9.85	19.3
		3.93	14.7	47.0
		3.96	15.8	52.8
		4.45	19.8	156.0
I; R = H, R′ = Me	25.00°	3.40	0.703	14.7
		3.56	0.880	21.8
		3.76	1.137	30.2
		4.15	2.24	61.1

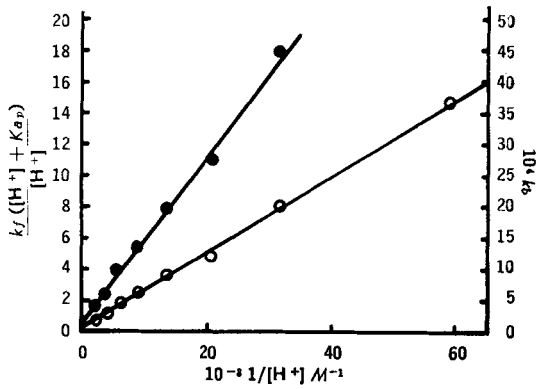


Figure 3—*Plots against* $1/[H^+]$ *of* (○) k_f $([H^+] + K_{a_p})/[H^+]$ and (●) k_b *for addition of bisulfite ion to (I; R = R′ = H) at 25°.*

The pH dependence of k_f and k_b values is consistent with a hypothesis that two independent and competitive reaction paths are followed in the equilibrium reactions. In the forward reaction, both bisulfite ion and sulfite ion react with the pyrimidinium ion; in the reverse reactions, the neutral (zwitterionic) adduct decomposes in both nonbase-catalyzed and specific base-catalyzed reactions. This hypothesis requires that the rate of consumption of pyrimidinium ion would be

$$\text{rate} = k_1[PyH^+][HSO_3^-] + k_2[PyH^+][SO_3^-] - k_{-1}[HAdd^\pm] - k_{-2}[HAdd^\pm][OH^-] \qquad \text{(Eq. 8)}$$

In terms of the total pyrimidine concentration $[Py_T]$ and total sulfurous acid concentrations $[S_T]$, and assuming that the adduct exists predominantly as a zwitterion in the pH region studied, this rate law becomes:[1]

$$\text{rate} = \frac{k_1[H^+]^2 + k_2[H^+]K_{a_{S,2}}}{(K_{a_p} + [H^+])(K_{a_{S,2}} + [H^+])}[Py_T][S_T] - (k_{-1} + k_{-2}[OH^-])[A_T] \qquad \text{(Eq. 9)}$$

Comparison of rate laws 5 and 9 shows that if this hypothesis is correct, plots of $\{k_f(K_{a_p} + [H^+])\}/[H^+]$ against $1/[H^+]$ and of k_b against $1/[H^+]$ should be linear between pH 3 and 5.5 with intercepts on the Y-axis greater than 0.

Plots of this type were obtained for addition to (I; R = R′ = H) and (I; R = H, R′ = Me). An example of results for addition to (I; R = R′ = H) at 25° is shown in Fig. 3. Least-squares treatments of the 8 data points for the forward reaction shown in Fig. 3 showed that they fell on a line with a slope of $2.46 \pm 0.03 \times 10^{-4}$ and with an intercept on the Y-axis (at $1/[H^+] = 0$) of 0.19 ± 0.08. These results indicated that attack of sulfite ion on the pyrimidinium ion ($k_2 = 2103\ M^{-1}$ sec.$^{-1}$) was a much more favorable reaction than attack of bisulfite ion on the pyrimidinium ion ($k_1 \simeq 0.19\ M^{-1}$ sec.$^{-1}$). Similarly, for the decomposition of the covalent adduct (V; R = R′ = H), the 8 data points shown in Fig. 3 lay on a line with slope $1.376 \pm 0.017 \times 10^{-7}$ and an intercept on the Y-axis of $1.03 \pm 0.46 \times 10^{-4}$. These results indicated that the second-order rate constant for the specific base-catalyzed decomposition of the adduct was $1.367 \times 10^7\ M^{-1}$ sec.$^{-1}$, while the first-order rate constant for the nonbase-catalyzed reaction was 10.31×10^{-5} sec.$^{-1}$. The consistency of this data with the proposed mechanisms was demonstrated by the close correspondence between the experimentally determined values of $k_{obs.}$, which are shown as circles in Fig. 2, and the solid lines which were calculated from values of k_1, k_{-1}, k_2, and k_{-2}.

Because of the large standard errors associated with k_1 and k_{-1} values, they could not be used to make meaningful calculations on the enthalpy and entropy of the reactions between bisulfite ion

[1] K_{a_p} and $K_{a_{S,2}}$ are the acid dissociation constants of the pyrimidinium ion and bisulfite ion, respectively. The concentration of H_2SO_3 has been neglected in this equation because it is very much less than $[HSO_3^-]$ between pH 3 and 5.

Table VI—Kinetic Data for Formation of Bisulfite Adducts by Attack of Sulfite Ion on the Pyrimidinium Ion (k_2) and the Base-Catalyzed Decomposition of the Adduct (k_{-2})

Augend	Temperature	k_2, M^{-1} sec.$^{-1}$	ΔH_2^*, kcal. M^{-1}	ΔS_2^*, cal. M^{-1}	$10^{-6}k$, M^{-1} sec.$^{-1}$	ΔH_{-2}^*, kcal. M^{-1}	ΔS_{-2}^*, cal. M^{-1}
I; R = R′ = H	12.0°	754			7.7		
	25.0°	2103	−11.0	−6.2	13.7	−7.7	−2.1
	37.0°	3935			23.2		
I; R = H, R′ = Me	25.0°	170			39.9		

Table VII—Kinetic Data for Formation and Decomposition of Bisulfite Adducts of II at 25°

pH	$10(k_{f_4} + k_{f_6})$, M^{-1} sec.$^{-1}$	$10^4\left(\frac{k_{b_4}K^* + k_{b_6}}{1 + K^*}\right)$ sec.$^{-1}$
3.36	5.00	9.98
3.53	7.60	15.2
3.74	11.4	22.7
3.93	17.6	35.1

and (I; R = R′ = H) or the nonbase-catalyzed decomposition of the adduct. However, the errors associated with k_2 and k_{-2} values were much less, and they were used in Arrhenius plots to calculate enthalpies and entropies of reaction between sulfite ion and (I; R = R′ = H) and for the specific base-catalyzed decomposition of the adduct (V; R = R′ = H). These values are listed in Table VI.

Whereas both bisulfite ion and sulfite ion appeared to be attacking nucleophiles in the addition reaction, it has been reported that the rate-determining step in additions to aldehydes and ketones (12) only involves the attack of sulfite ion on the neutral carbonyl compound. These differences probably arise because of the greater electrophilicity of the charged pyrimidinium ion compared to the uncharged carbonyl compound. This is exemplified by the fact that the second-order rate constant for attack of sulfite ion on neutral benzaldehyde is 240 M^{-1} at 13°, whereas it is 754 M^{-1} for attack on 2-aminopyrimidinium ion at 12°.

Kinetics of Reversible Addition of Bisulfite Ion to II—The changes in UV absorbance which followed mixing of aqueous buffered solutions of II (5.2×10^{-4} M) with solutions of sodium bisulfite ($4.0–20.0 \times 10^{-3}$ M) appeared to be similar to those observed for additions to (I; R = R′ = H) and (I; R = H, R′ = Me). Thus, plots against time of log $(D - D_\infty)$ values which had been calculated from measurements at a wavelength where II was the main absorbing species (303 mμ) were linear through at least two half-lives. Apparent first-order rate constants, $k_{obs.}$ values, were calculated from these types of plots.

The precise interpretation of the meaning of $k_{obs.}$ values for this reaction was complicated by the fact that addition to II yields the isomeric products III and IV, and their relative concentrations (≃1.3:1) at the time when most of the pyrimidine has been consumed is different from that (≃0.8:1) at thermodynamic equilibrium (4). In the extreme, the kinetics for approach to equilibrium of such a system would not be first order. However, because approximate first-order kinetics were observed, it was assumed that the initial reactions between II and bisulfite and sulfite ions led to a "pseudoequilibrium" system and that the subsequent isomerization of the products took place much more slowly.

On this basis, when $[S_T]_0 \gg [Py_T]_0$, the apparent first-order rate constant for approach to the "pseudoequilibrium" condition would be given by Eq. 6:

$$k_{obs.} = (k_{f_4} + k_{f_6})[S_T]_0 + \frac{k_{b_4}K^* + k_{b_6}}{1 + K^*} \qquad \text{(Eq. 10)}$$

where k_{f_4} and k_{f_6} are rate constants for addition at C_4 and C_6, respectively; k_{b_4} and k_{b_6} are the rate constants for decomposition of isomers III and IV, respectively; and $K^* = [III]/[IV]$ at "psuedo-equilibrium." This treatment was consistent with the experimental results, and plots of $k_{obs.}$ against $[S_T]_0$ were linear at several pH values and temperatures. Values of $(k_{f_4} + k_{f_6})$ and $(k_{b_4}K^* + k_{b_6})/(1 + K^*)$ were calculated from such plots and are listed in Table VII.

The pH dependence of $(k_{f_4} + k_{f_6})$ and $(k_{b_4}K^* + k_{b_6})/(1 + K^*)$ values appeared to be similar to that for addition to (I; R = R′ = H), and (I; R = H, R′ = Me) because plots of both $(k_{f_4} + k_{f_6})$ and $(k_{b_4}K^* + k_{b_6})/(1 + K^*)$ against $1/[H^+]$ at constant temperature were linear with intercepts on the Y-axis (when $1/[H^+] = 0$) greater than 0. Thus, it appears that the additions of bisulfite ion to II and decomposition of both III and IV occur by similar mechanisms to those described for addition to (I; R = R′ = H) and (I; R = H, R′ = Me).

The fact that [III]/[IV] ≃1.3 when most of II had been consumed, together with the observation that the pyrimidinium ion was apparently consumed in a first-order reaction, strongly suggests that addition to the carbon atom in II, which is adjacent to the alkylated nitrogen atom, occurs more rapidly than addition to the carbon atom adjacent to the nonalkylated nitrogen atom.

EXPERIMENTAL

Materials and Apparatus—2-Aminopyrimidine and 2-amino-4-methylpyrimidine (K & K Laboratories Inc.) were crystallized from petroleum ether and sublimed before use. 1,2-Dihydro-2-imino-1-methylpyrimidine hydrochloride was prepared as described by Brown and Harper (13), m.p. 277–278°. Sodium bisulfite solutions were prepared immediately before use by dissolving sodium pyrosulfite (Mallinckrodt A.R.) in freshly boiled and cooled distilled water through which oxygen-free nitrogen had been bubbled for 1 hr. One milliliter of methanol was added per 100 ml. of sodium bisulfite solution to stabilize it against oxidation (1). Final bisulfite concentrations were determined by iodometric titration. All solutions were made with freshly boiled and cooled distilled water through which oxygen-free nitrogen had been bubbled for 1 hr.

Spectrophotometric measurements were made on Cary 14 and 16 UV spectrophotometers. Temperatures were controlled to within 0.01° with circulating water from a Lo-temptral 154 water bath (Precision Scientific Co.). The pH values were measured using an Orion model 801/digital pH meter.

Isolation of Bisulfite Adducts—The 1:1 bisulfite adducts of the pyrimidinium ions (I; R = R′ = H) and (I; R = H, R′ = Me) were obtained as white microcrystalline powders following addition of sodium bisulfite (1M) to saturated aqueous solutions of the pyrimidines at 25°. After washing the powders with a little water and drying over calcium chloride at atmospheric pressure, the adducts had the following elemental analysis.

Anal.—Calcd. for 2-amino-1,6-dihydropyrimidinium-6-sulfonate (V; R = R′ = H): C, 27.12; H, 3.98; S, 18.10. Found: C, 27.15; H, 4.12; S, 18.33. Calcd. for 2-amino-1,6-dihydro-4-methylpyrimidinium-6-sulfonate (V; R = H, R′ = Me): C, 31.40; H, 4.73; N, 21.98; S, 16.77. Found: C, 31.18; H, 4.82; N, 21.70; S, 16.88.

Equilibrium Constants—The pKa values were measured by potentiometric titration using the method described by Albert and Serjeant (14). The ionic strength was maintained at 10^{-1} M with sodium chloride. Equilibrium constants for addition of bisulfite ion to the pyrimidinium ions were calculated from the equilibrium concentrations of pyrimidinium ion, bisulfite ion, and covalent adduct which existed following mixing of equal volumes of solutions of the pyrimidine in borax–succinic acid buffers and sodium bisulfite solutions. The ionic strength of all reaction mixtures was maintained at 10^{-1} M with sodium chloride. The equilibrium concentrations were calculated from spectrophotometric measurements at wavelengths where the pyrimidine or pyrimidinium ion was the main absorbing species, and corrections were made for the absorbance of the other species.

Kinetics—Rate constants were calculated from spectrophotometric measurements of concentration changes. Standard kinetic procedures were followed. The ionic strength of all reaction mixtures was maintained at 10^{-1} *M* with sodium chloride.

CONCLUSIONS

1. Covalent addition of bisulfite ion occurs to Position C_4 or C_6 of 2-aminopyrimidinium ion derivatives so long as these positions are not blocked by alkyl substituents and the pH of the system is such that the reactants exist to an appreciable extent as the monoanion (HSO_3^-) and the cation (pyrimidinium ion).
2. Alkyl substituents at any position in the pyrimidinium ring reduce the extent and rate of addition.
3. The covalent adducts are zwitterions, between pH 3 and 5, and have very low water solubility.
4. The mechanism of addition appears to involve attack of both the bisulfite ion and sulfite ion on the pyrimidinium ion. The reverse reaction involves both a nonbase-catalyzed and a base-catalyzed decomposition of the neutral adduct.

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Kinetics of the Hydrolysis of Pilocarpine in Aqueous Solution

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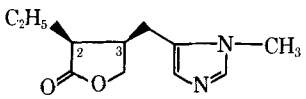
Abstract □ The kinetics of the hydrolysis of pilocarpine in aqueous solution were investigated utilizing pH-stat titrimetry and polarimetry. A cyclic mechanism was proposed for the hydrolysis, which is catalyzed by both hydrogen ion and hydroxide ion. The appropriate rate constants, equilibrium constants, and the energy of activation for the hydroxide-ion catalyzed hydrolysis were calculated. The hydrolysis in high alkaline pH was found to be accompanied by some epimerization. An optimum condition for the preparation of pilocarpine ophthalmic solution was also suggested.

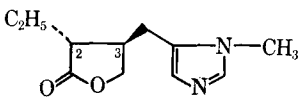
Keyphrases □ Pilocarpine in aqueous solution—hydrolysis □ Kinetics—pilocarpine hydrolysis □ Hydrolysis, pilocarpine—hydrogen-ion catalyzed □ TLC—separation □ UV spectrophotometry—identity □ Titration, pH-stat—pilocarpine degradation determination □ Polarimetry—pilocarpine cyclization

Pilocarpine is used topically as a miotic in the treatment of glaucoma. The isomer, isopilocarpine, although qualitatively similar in its pharmacological effects, is almost completely inactive as a miotic (1). However, little clinical data are available concerning isopilocarpine. Pilocarpine solutions ranging from 0.5 to 10% have been used, although there seems to be no advantage in concentrations above 4%. Pilocarpine is less irritating than physostigmine salicylate and can be employed for long periods without producing undesirable side effects (2).

Although pilocarpine was isolated early in 1875, its structure and that of the isomer were elucidated by Jowett (3, 4) in 1900; total synthesis was achieved by several workers in different ways during the 1930's (5–7). In 1966, the absolute configurations of these compounds were established as (2S:3R) (I) and (2R:3R) (II) for pilocarpine and isopilocarpine, respectively (8).



pilocarpine (2S:3R)
I



isopilocarpine (2R:3R)
II

Pilocarpine possesses a γ-lactone with two asymmetric centers. In the dry state and at high temperature, the two isomers, pilocarpine and isopilocarpine, interconvert, isopilocarpine predominating at equilibrium (8). This property has been used for the preparation of isopilocarpine hydrochloride from pilocarpine hydrochloride (9). It has been shown recently that, in the presence of alkali, isomerization may proceed *via* the enol intermediate (10).

In aqueous solution, pilocarpine presents at least two possible pathways of degradation, including hy-