Scheme I

$$PhMgX + O_2 \longrightarrow PhO_2MgX \longrightarrow Ph\cdot + MgO_2X$$

$$Ph\cdot + MgX_2 \longrightarrow PhMgX + X\cdot$$

$$X\cdot + PhMgX \longrightarrow XPhMgX\cdot \overset{R}{\longrightarrow} R\cdot + XPhMgX$$

$$XPhMgX + O_2 \longrightarrow XPhO_2MgX$$

$$XPhO_2MgX + PhMgX \longrightarrow XPh\text{-}Ph^* + 2MgOX \quad (I)$$

$$XPh\text{-}Ph^* \longrightarrow XPh\text{-}Ph + h\nu$$
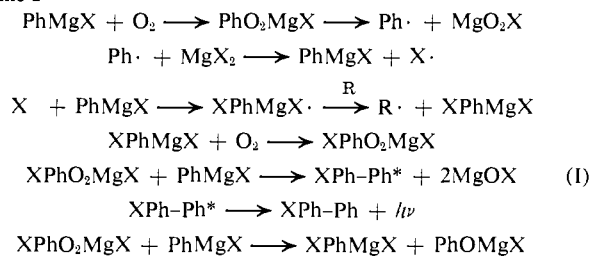
$$XPhO_2MgX + PhMgX \longrightarrow XPhMgX + PhOMgX$$
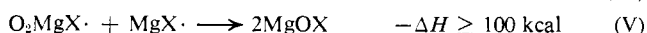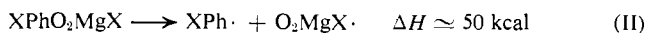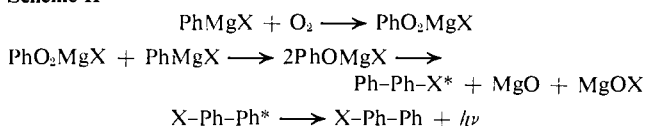
This gives an overall exothermicity of at least 100 kcal of which 90 kcal is necessary for exciting the brominated biphenyl.

$$XPhO_2MgX \longrightarrow XPh\cdot + O_2MgX\cdot \qquad \Delta H \simeq 50 \text{ kcal} \qquad (II)$$

$$PhMgX \longrightarrow Ph\cdot + MgX\cdot \qquad \Delta H \simeq 70\text{-}90 \text{ kcal} \qquad (III)$$

$$XPh\cdot + Ph\cdot \longrightarrow XPh\text{-}Ph \qquad -\Delta H \simeq 140 \text{ kcal} \qquad (IV)$$

$$O_2MgX\cdot + MgX\cdot \longrightarrow 2MgOX \qquad -\Delta H \geq 100 \text{ kcal} \qquad (V)$$

Alternatively, we could assume that the brominated biphenyls are formed through a different reaction path than the unsubstituted biphenyl. Such a reaction scheme is outlined in Scheme II. The energetics of this

Scheme II

$$PhMgX + O_2 \longrightarrow PhO_2MgX$$

$$PhO_2MgX + PhMgX \longrightarrow 2PhOMgX \longrightarrow$$
$$Ph\text{-}Ph\text{-}X^* + MgO + MgOX$$

$$X\text{-}Ph\text{-}Ph^* \longrightarrow X\text{-}Ph\text{-}Ph + h\nu$$

second alternative are basically the same as that of the first, but it is to be noted that the second alternative is not explicitly dependent on radical formation. In an effort to obtain information regarding the role of radicals in the chemiluminescence reaction we attempted to add radical quenchers to the Grignard solution before oxygenation. However, all the radical quenchers that

we are aware of are unsatisfactory due either to insolubility or reaction with the Grignard reagent. A study of the magnetic field effect of the intensity of the chemiluminescence would be helpful in elucidating the role, if any, of radicals in the chemiluminescence.

Summary

The oxygen induced chemiluminescence of aryl Grignard reagents is a well-known though much neglected phenomenon. In this investigation the emitting species in the oxygenation of phenylmagnesium bromide and p-chlorophenylmagnesium bromide have been identified as the radical pathway isomeric distribution of brominated biphenyls. This conclusion was reached by comparing the chemiluminescence spectra, the fluorescence spectra of the oxygenation products, and the fluorescence spectra of brominated biphenyls formed through the photolysis of the Grignard precursor, as shown in Figures 1–3. An epr study showed that free radicals are formed during the oxygenation of phenylmagnesium bromide, but the full role of these radicals in the chemiluminescence could not be ascertained. To account for the chemiluminescence we proposed two reaction schemes, one of which depends explicitly on radical intermediates, and the other involves only nonradical species. Since the quantum yield of the chemiluminescence is quite low, at most 10^{-2}, these suggested mechanisms do not contradict the mechanism for the main reaction products proposed by Garst, et al.[11]

Elimination of Bisulfite Ion from a Series of Uracil–Bisulfite Adducts. Evidence for a Two-Step Mechanism[1]

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Abstract: Kinetic studies have been carried out on the elimination of bisulfite ion from the 5,6-dihydro-6-sulfonates of uracil, 1-methyluracil, 3-methyluracil, 1,3-dimethyluracil, and from the 5,5-dideuterio, cis-5-deuterio, and trans-5-deuterio analogs of 5,6-dihydrouracil-6-sulfonate. In the absence of significant concentrations of general acids, the reactions in aqueous solution were catalyzed by hydroxide ion and general bases and the 5,5-dideuterio and trans-5-deuterio derivatives were desulfonated less rapidly than their protio analogs. When larger concentrations of general acids were included in the reaction solution, the rates of desulfonation became less sensitive to the concentration of general bases and exchange of the trans deuterium atoms of the 5,5-dideuterio and trans-5-deuterio derivatives with protons from the solvent occurred more rapidly than desulfonation. These observations strongly suggest that elimination is occurring via a multi-step rather than a one-step mechanism as has previously been suggested.

Many reactions of uracil derivatives and related pyrimidines are now recognized to be initiated

by covalent addition of a nucleophilic reagent across the $>C\text{-}6=C\text{-}5<$ bond of the pyrimidine molecule. Some

examples of such reactions are the bisulfite ion catalyzed deamination of cytosine,[2] the bisulfite ion dehalogenation of 5-halouracils,[3] and the bisulfite ion catalyzed exchange of the C-5 proton of uracil for a deuteron in deuterium oxide solutions of sodium bisulfite and amines.[4] It is believed that the initial step in all of these reactions is the covalent addition of bisulfite ion to the pyrimidine molecule. A typical reaction is illustrated in Scheme I. This is the addition of bisulfite
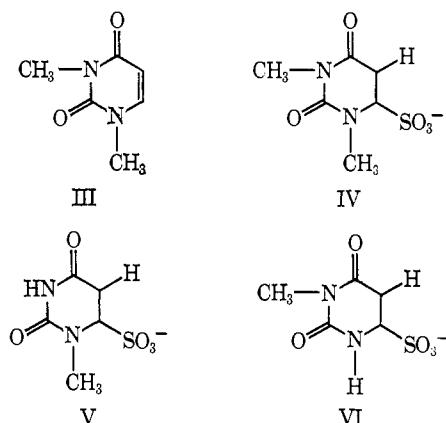
Scheme I
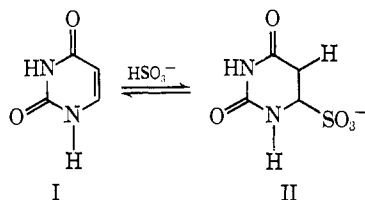
ion (HSO_3^-) to uracil (I) to yield 5,6-dihydrouracil-6-sulfonate (II).

It has previously been suggested by Sander, et al.,[5] that the reversible addition of HSO_3^- to 1,3-dimethyluracil (III) to yield 5,6-dihydro-1,3-dimethyluracil-6-sulfonate (IV) occurs by a concerted mechanism. This suggestion was based on the stereoselectivity of the reactions, the kinetic deuterium isotope effect on the desulfonation reaction ($k_H/k_D = 4.1$), and on an interpretation of some specific and general base catalysis of the desulfonation reaction.[6]

This manuscript describes the results of a kinetic study on the elimination of HSO_3^- (or DSO_3^-) from II, IV, 5,6-dihydro-1-methyluracil-6-sulfonate (V), 5,6-dihydro-3-methyluracil-6-sulfonate (VI), and from the

5,5-dideuterio and the cis-5-deuterio and the trans-5-deuterio analogs of II (VII, VIII, and IX, respectively). It will be argued that the results of these studies are more consistent with a postulate that the elimination reaction occurs by a multi-step rather than a one-step mechanism. It will also be argued, by analogy, that

addition of bisulfite ion to uracils occurs by a multi-step mechanism.

Although the three previously mentioned examples of reactions which are initiated by the covalent addition of a nucleophilic reagent to a pyrimidine molecule all involved the addition of bisulfite ion, they are believed to be representative of a much wider class of reaction. Hence, it has been postulated that hydrogen-deuterium exchange at C-5 of uridine is initiated by the addition of either water,[7] glutathione,[8] or other thiols[9] across the $>C-6=C-5<$ bond of uridine. Addition of enzymes across this bond has also been implicated[10] in a variety of biologically important processes. Consequently, it is anticipated that an understanding of the mechanism of addition of bisulfite ion to uracil and its derivatives will help to account for the reactivity of uracil nucleosides and nucleotides in aqueous environments which contain nucleophilic species.

Results

Stoichiometry of the Elimination Reactions. The methods of synthesis of the 5,6-dihydro-6-sulfonates of uracils which were used in this study are described in the Experimental Section. The product of addition of bisulfite ion to I has previously been characterized[2] as II. The molar absorptivities at 260 nm and nmr spectral characteristics of I and II are listed in Table I.

Table I. Uv[a] and Nmr[b] Spectral Data for Uracils and Their Bisulfite Adducts

Compd	Log ϵ^c at 260 nm	Chemical shift δ, ppm—— C-5-H	C-6-H
I	3.92	5.8 (doublet)[d]	7.7 (doublet)[d] $J_{5-6} = 6{-}7$ Hz
II	<1.0	3.1 (multiplet)	4.6 (multiplet)
III	3.91		
1-Methyluracil	3.96		
3-Methyluracil	3.86[e]		
IV	<1.0		
V	<1.0		
VI	<1.0		
VII	<1.0		4.6 (singlet)
VIII	<1.0	3.1 (broad doublet)	4.6 (doublet) $J_{5-6} = 8$ Hz
IX	<1.0	3.1 (broad singlet)	4.6 (singlet) $J_{5-6} < 2{-}3$ Hz

[a] In 0.1 N HCl. [b] In D_2O. Except for that of I, all spectra were obtained on solutions of the potassium salts of the compounds to which sufficient 40% DCl had been added to shift the HOD peak to 6.0 ppm. Internal reference was 3-(trimethylsilyl)propanesulfonic acid sodium salt. [c] ϵ is the molar absorptivity in M^{-1} cm^{-1}. [d] Measured on an NaOD solution in D_2O. [e] Lit. value: H. A. Sober, Ed., "Handbook of Biochemistry," 2nd ed, Chemical Rubber Co., Cleveland, Ohio, 1970, pp 6–18.

The ultraviolet and nmr spectral changes that accompanied the reactions of III, 1-methyluracil, and 3-methyluracil with bisulfite ions in water strongly suggested that the products of these reactions were IV, V, and VI. Spectral characteristics of these adducts and their parent uracils are included in Table I.

It has previously been reported[2,11] that both the

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(5) R. W. Erickson and E. G. Sander, J. Amer. Chem. Soc., 94, 2086 (1972).
(6) When this manuscript was being prepared we received a personal communication from Dr. E. G. Sander informing us that he had obtained evidence that the reversible addition of HSO_3^- to 5-fluorouracil occurred via a two-step mechanism. These results will be communicated in Bioorganic Chemistry.

(7) D. V. Santi and C. F. Brewer, J. Amer. Chem. Soc., 90, 6236 (1968).
(8) T. I. Kalman, Biochemistry, 10, 2567 (1971).
(9) S. R. Heller, Biochem. Biophys. Res. Commun., 32, 998 (1968).
(10) D. V. Santi and C. F. Brewer, Biochemistry, 12, 2416 (1973).
(11) H. Hayatsu, Y. Wataya, K. Kai, and S. Iida, Biochemistry, 9, 2858 (1970).
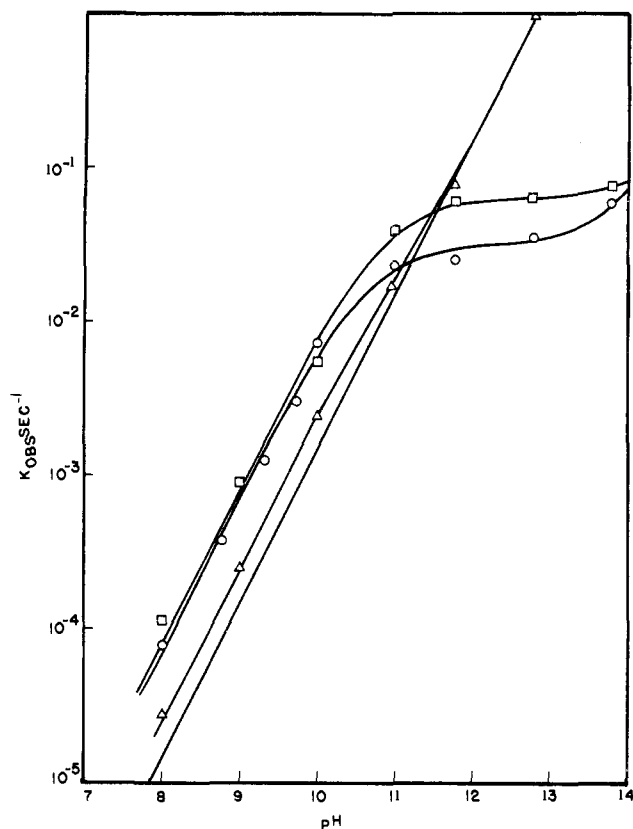
Figure 1. pH–rate profiles for the desulfonation of II (○), V (□), and VI (△), in lightly buffered aqueous solutions at 25° and $I = 1.0\ M$. The lines were calculated by using eq 3 and the data in Table II. The solid line without points is for IV, based on the data of Sander. *et al.*,[5] as listed in Table II.

addition of bisulfite ion to I in aqueous solutions and the elimination of bisulfite ion from II in alkaline solutions are highly stereoselective reactions. Although no assignments have been made of the absolute configurations of these covalent adducts, it has been assumed that the proton which is added to C-5 is trans to the sulfonate group on C-6. In the present study, two geometrical isomers of the C-5 monodeuterio derivative of II were synthesized, and the kinetics of their desulfonation were studied. For convenience, these isomers were named the *cis*- and *trans*-5-deuterio-5,6-dihydrouracil-6-sulfonates. The trans isomer (IX) was synthesized by adding DSO_3^- to I in deuterium oxide solution, while the cis isomer (VIII) was synthesized by adding HSO_3^- to 5-deuteriouracil in water. The 5,5-dideuterio-5,6-dihydrouracil-6-sulfonate was synthesized by reacting DSO_3^- with 5-iodouracil in D_2O.[3]

Elimination of bisulfite ion from all the adducts studied resulted in the formation of compounds which had identical ultraviolet spectra with the original uracils. Nmr spectral observations indicated that the major product of desulfonation of *cis*-5-deuterio- and 5,5-dideuterio-5,6-dihydrouracil-6-sulfonates was 5-deuteriouracil, whereas uracil was the major product formed by *trans*-5-deuterio-5,6-dihydrouracil-6-sulfonate.

Kinetics of Elimination in Aqueous Solutions. Measurements of changes in ultraviolet absorbance which accompanied the desulfonation of II, IV, V, and VI in aqueous solutions of potassium hydroxide or in solutions which contained low concentrations of buffers ($<10^{-2}\ M$) indicated that no significant buildup of an

intermediate occurred, and that the elimination reaction was first order in the total adduct concentration, $adduct_T$. Hence, plots of $\log(A - A_\infty)$ against time, at any wavelength where the adduct and the parent uracil had different molar absorptivities, were linear for at least 5 half-lives. The logarithms of the pseudo-first-order rate constants for these reactions are plotted against pH in Figure 1. It will be shown later that the rates of elimination were sensitive to concentrations of general acids and general bases. Hence, because the values of k_{obsd} in Figure 1 were not corrected for the concentrations of buffer species in the solutions, their absolute values will be in error. However, in view of the fact that concentrations of buffer species were low in all these experiments, the k_{obsd} values in Figure 1 are not expected to differ by more than $\pm 20\%$ from the true hydroxide ion catalyzed rate constants.

Compounds II, V, and VI each contain an acidic (imino) proton and are expected to ionize appreciably (to dianions) within the pH range where measurements were made. Hence, the rate law for desulfonation under pseudo-first-order conditions would be

$$rate = k_{obsd}([adduct^-] + [adduct^{2-}]) \qquad (1)$$

where [adduct$^-$] and [adduct^{2-}] are the concentrations of the adduct (with one negative charge on the sulfonate group) and of its anion (formed by ionization of an imino proton), respectively.

The data in Figure 1 are consistent with a postulate that, in the absence of significant concentrations of general acids and bases, the rate-determining steps in the desulfonation reactions involve a reaction between hydroxide ion and both the adduct and its anion. The rate law for this reaction scheme would be

$$rate = k_{OH}[adduct^-][OH^-] + k_{OH}'[adduct^{2-}][OH^-] \qquad (2)$$

Hence, k_{obsd} values should be related to k_{OH} and k_{OH}' values by the identity

$$k_{obsd} = \frac{k_{OH}K_w + k_{OH}'[OH^-]K_a^A}{K_a^A + [H^+]} \qquad (3)$$

In this identity, K_a^A is the acid dissociation constant of the adduct and K_w ($= 10^{-14}\ M^2$) is the autodissociation constant of water. The values of K_a^A, k_{OH}, and k_{OH}' which are listed in Table II were obtained by a process of

Table II. Parameters Used to Calculate[a] the Solid Lines in Figure 1

Compd	k_{OH}, $M^{-1}\ sec^{-1}$	k_{OH}', $M^{-1}\ sec^{-1}$	pK_a^A
II	70	0.04	10.65
IV[b]	14.7		
V	80	0.02	10.90
VI	25.0	13.0	11.00

[a] Calculations were made by using eq 2. [b] Buffer-independent rate constants reported in ref 5 based on hydroxide ion activity.

curve fitting. The solid lines in Figure 1 were calculated by inserting the values of the parameters in Table II into the identity (eq 3).

The kinetics of elimination of HSO_3^- or DSO_3^- from the deuterio derivatives of II in 0.01 M KOH also obeyed a first-order rate law and plots of $\log(A - A_\infty)$ against time were linear for at least 5 half-lives. The
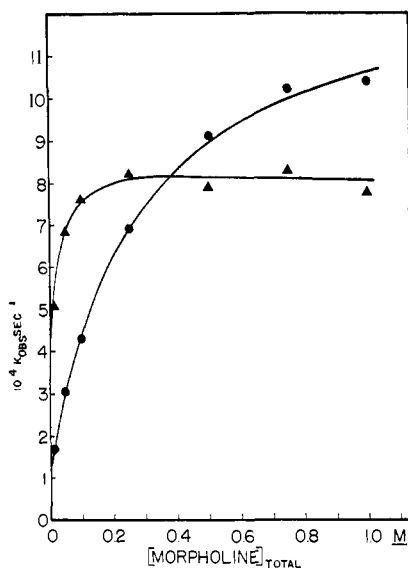
Figure 2. Plots against the total concentration of morpholine buffer of the k_{obsd} values for desulfonation of II (▲) and IV (●) at 25°. The pH was 8.77 and $I = 1.0$ M. The lines were calculated as described in the text.
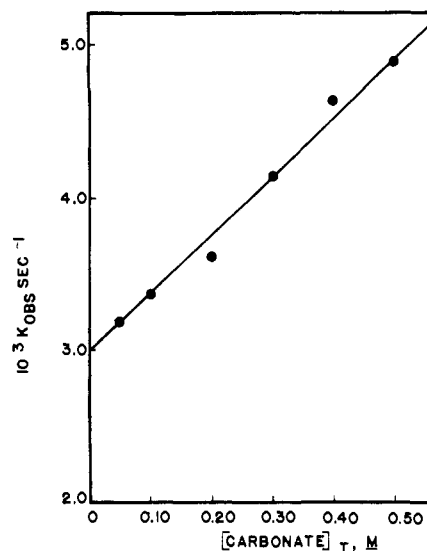

Figure 3. A plot against the total concentration of carbonate buffer of the k_{obsd} values for desulfonation of II at 25°. The pH was 9.73 and $I = 1.0$ M.

Table III. Desulfonation Rate Constants in 0.01 M KOH at $I = 1.0$ M^a and 25°

Compd	$10^3 k_{obsd}$, sec^{-1}	Compd	$10^3 k_{obsd}$, sec^{-1}
II	27.6	VIII	25.4
VII	5.9	IX	6.6

^{a} Adjusted with KCl.

k_{obsd} values for these reactions are listed in Table III. Thus, in 0.01 M KOH (i.e., at a pH where the imino protons of the adducts would be largely ionized) the ratio of $k_{obsd}{}^{II}/k_{obsd}{}^{IX} = 4.2$, $k_{obsd}{}^{II}/k_{obsd}{}^{VIII} = 1.1$, and the ratio $k_{obsd}{}^{II}/k_{obsd}{}^{VII} = 4.7$. Because the elimination from II and VIII involves removal of a proton, whereas elimination from VII and IX involves removal of a deuteron, the first of these ratios is believed to result from a primary kinetic isotope effect, and the second ratio is believed to result from a secondary kinetic isotope effect, and the third ratio is believed to result from a mixture of primary and secondary isotope effects. The similarities of the values of the ratios $k_{obsd}{}^{II}/k_{obsd}{}^{VII}$ and $k_{obsd}{}^{II}/k_{obsd}{}^{IX}$ strongly suggest that no exchange of deuterium atoms for protons occurs at C-5 of the adduct during the elimination of DSO_3^- from VII and IX under these conditions. In other words, it appears that removal of the hydrogen isotope from C-5 of the adduct is rapidly followed by elimination of $SO_3{}^{2-}$.

Kinetics of Elimination from II and IV in Strongly

Buffered Solutions. The rate laws for desulfonation of II and IV in solutions which contained relatively high concentrations of general acids and general bases were also first order in the total adduct concentration. Although k_{obsd} values under these conditions were sensitive to the concentrations of general acids and general bases, this sensitivity changed with variations in both the total concentration of general acids and general bases and in the ratio of their concentrations. This behavior is illustrated in Figure 2 where k_{obsd} values for desulfonation of II or IV are plotted against total concentrations of morpholine plus morpholinium ion in the solutions. Similar results were obtained for the desulfonation of IV in piperidine and benzylmethylamine solutions. The results for these experiments are in Table IV. As shown in Figure 3, the k_{obsd} values for desulfonation of II in a carbonate-bicarbonate buffer at pH 9.73 (the pK_a of HCO_3^-) were linearly related to the total buffer concentration up to a value of 0.5 M for the latter parameter. The data in these figures and table were calculated from experiments in which the pH was kept constant but in which the concentration of buffer species was varied. All the experiments reported in these figures and tables were carried out at pH values where imino protons of the adducts would not be ionized to a greater extent than 10% (based on the fact that the pK_a values of the adducts were within the range 10.5–11.0). Hence, based on the fact that the diionized forms of the adducts do not eliminate bisulfite any more rapidly than the monoionized forms

Table IV. Rate Constants for Desulfonation Reactions in Aqueous Buffers at 25° and $I = 1.0$ M

Compd	Buffer	pH	C_2,^{a} M^{-1} sec^{-1}	C_3,^{a} M^{-1}	k_{BH^+}/k_E, M^{-1}	k_B,^{b} M^{-1} sec^{-1}
IV	Morpholine	8.77^c	0.00485	3.68	7.4	0.0097
IV	Benzylmethylamine	9.85^c	0.083	7.61	15.2	0.166
IV	Piperidine	11.33^c	1.00	4.12	8.2	2.00
II	Morpholine	8.77^c	0.0331	38.8	78.0	0.064
II	Morpholine	9.72	0.0554	8.66	87.0	0.062
II	Carbonate	9.73^c	0.0038	<0.1	<0.1	0.008

^{a} The constants defined in the empirical identity in eq 9. ^{b} The rate constants defined in the rate law in eq 10. ^{c} The pH of a 50% neutralized solution of the buffer.
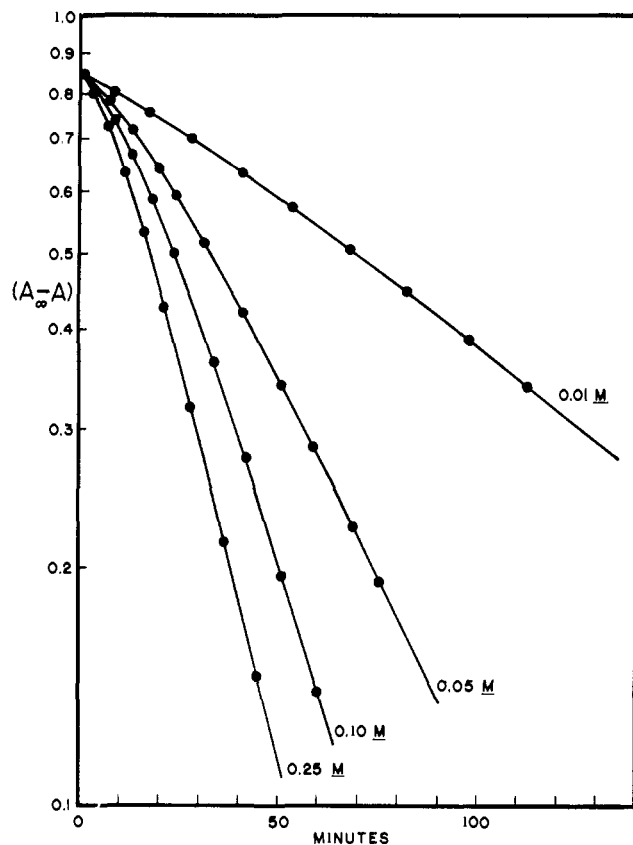
Figure 4. Semilogarithmic plots against time of absorbance changes that occurred during the desulfonation of IX in aqueous morpholine buffers at 25°. The pH was 8.77 and $I = 1.0\ M$. The lines were calculated as described in the text. The experimental points and the lines were normalized to facilitate the making of visual comparisons.

Figure 5. Semilogarithmic plots against time of the absorbance changes that occurred during the desulfonation of II in a 1 M ethanolamine buffer at pH 8.89 (A), and in a 2 M potassium bicarbonate buffer at pH 8.77 (B).

(see Table II), the measured rates of elimination in the pH range 9.5–10 will be largely those for reactions of the monoionized adducts, adduct⁻. Consequently, the rate law at a particular pH value and buffer concentration would be

$$\text{rate} = k_{\text{obsd}}[\text{adduct}^-] \tag{4}$$

It could be argued that the curves in Figure 2 arise because general acids or general bases form some type of complex with the reactant and that these complexes are less reactive than the uncomplexed molecules with respect to elimination. However, this explanation is not believed to be valid because the addition of N,N-dimethylmorpholinium ion or dioxane (molecules which would be expected to have similar complexing properties to morpholinium ion and morpholine but which are not general acids or general bases) had very little effect on the rate of desulfonation of II or IV when compared to the effect of similar concentrations of morpholinium ion and morpholine. The results of these experiments are displayed in Table V.

Elimination from Deuterio Adducts in Strongly Buffered Aqueous Solutions. The deuterio adducts VII, VIII, and IX also eliminated HSO₃⁻ or DSO₃⁻ in solutions which contained general acids. However, plots of log $(A - A_\infty)$ against time for these reactions were not linear. Some typical results are displayed in Figures 4,[12] 5, and 6. Similar results were obtained

(12) The data points in this figure were normalized to facilitate comparisons of the curvature of the lines that were obtained under the different experimental conditions.
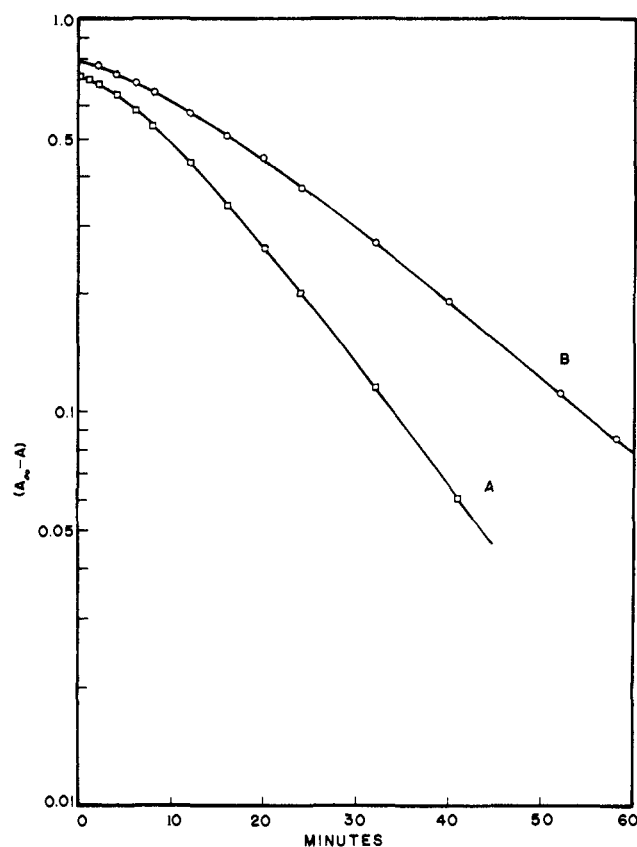
Table V. Pseudo-First Order Rate Constants for the Desulfonation of II in 0.05 M Morpholine Buffers[a] Which Contained Dioxane or N,N-Dimethylmorpholinium Iodide

[Dioxane], M	[N,N-Dimethyl-morpholinium iodide], M	pH	$10^3 k_{\text{obsd}}$, sec⁻¹
0		8.77	0.68
0.10		8.77	0.65
0.25		8.77	0.60
0.50		8.77	0.56
	0.00	9.72	4.25
	0.04	9.72	3.79
	0.24	9.72	3.34
	0.40	9.72	2.88

[a] All solutions had an ionic strength of 1.0 M (adjusted with KCl) and were at 25°.

during the desulfonation of VIII in 0.8 M Tris buffer at pH 8.75, in a 0.9 M triethylamine buffer at pH 8.77, and in a 1.0 M ethanolamine buffer at pH 6.4. Curved plots of this type were also obtained during the desulfonation in aqueous buffers of the adducts that were obtained by adding DSO₃⁻ to 1-methyl-3-methyl-, 1,3-dimethyl-, and 5-fluorouracil. Two slightly different types of behavior were exhibited during these reactions depending on whether the adduct had a trans or a cis deuterium atom. In the former case, the plots were curved initially but rapidly became linear. In the case of compounds with a cis deuterium atom the plots were slightly curved throughout the life of the reaction.

As will be discussed later, it is believed that the curvature arises because the deuterio adducts are both elim-

inating HSO$_3^-$ or DSO$_3^-$ and exchanging their C-5 deuterons for protons from the solvent in parallel reactions. The rapid approach to linearity that is exhibited by the curves for the trans deuterio compounds is thus thought to occur because the trans atom is rapidly exchanged. Exchange of the cis atom is believed to be a much slower process.

Discussion

The concerted mechanism which was previously proposed to account for the desulfonation of IV is illustrated in Scheme II. In this scheme, B represents a

Scheme II

$$SO_3^{2-} + BH^+ \text{ and } H_2O$$
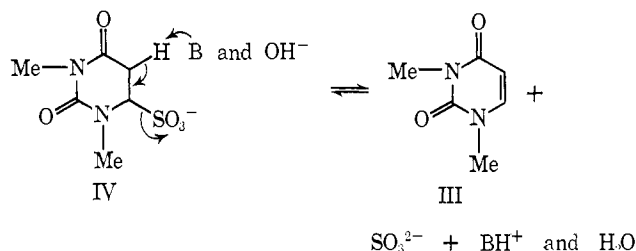
general base and BH$^+$ is its conjugate acid. The rate law for desulfonation of IV by this route under conditions where the reaction goes essentially to completion would thus be

$$\text{rate} = (k_B[B] + k_{OH}[OH^-])[\text{adduct}^-] \quad (5)$$

and the observed pseudo-first-order rate constant for desulfonation, k_{obsd}, would be related to [OH$^-$] and [B] by the identity

$$k_{obsd} = k_B[B] + k_{OH}[OH^-] \quad (6)$$

If the reaction solution contained one general base other than water and OH$^-$, and its conjugate acid, k_{obsd} values would be related to the term ([BH$^+$] + [B]), i.e., [B]$_T$, by the following identity in which K_a^B is the acid dissociation constant of BH$^+$. Hence, if the E2

$$k_{obsd} = k_{OH}[OH^-] + \frac{k_B K_a^B[B]_T}{K_a^B + [H^+]} \quad (7)$$

mechanism which is shown in Scheme II was operating, it would be expected that k_{obsd} values would be linearly related to [OH$^-$] when [B]$_T K_a^B/(K_a^B + [H^+])$ was small or constant and linearly related to [B]$_T$ when the pH was constant and [B]$_T$ varied. The results in Figure 1 are consistent with the first of these requirements. That is, log k_{obsd} values for desulfonation of IV or of II, V, and VI, and of the diionized forms of the latter three compounds were linearly related to pH when [B]$_T$ was small. However, the results in Figure 2 show clearly that k_{obsd} values were not linearly related to [B]$_T$ at all constant pH values.

The results in Figure 2 were more consistent with a rate law with the form

$$\text{rate} = \frac{C_1[OH^-] + C_2[B]_T}{1 + C_3[B]_T} [\text{adduct}^-] \quad (8)$$

In this rate law, C_1, C_2, and C_3 are constants when the pH is kept constant. Because the results in these experiments were obtained at pH values where the imino proton of II was not appreciably ionized, only the reactions of the adduct$^-$ species are included in the rate
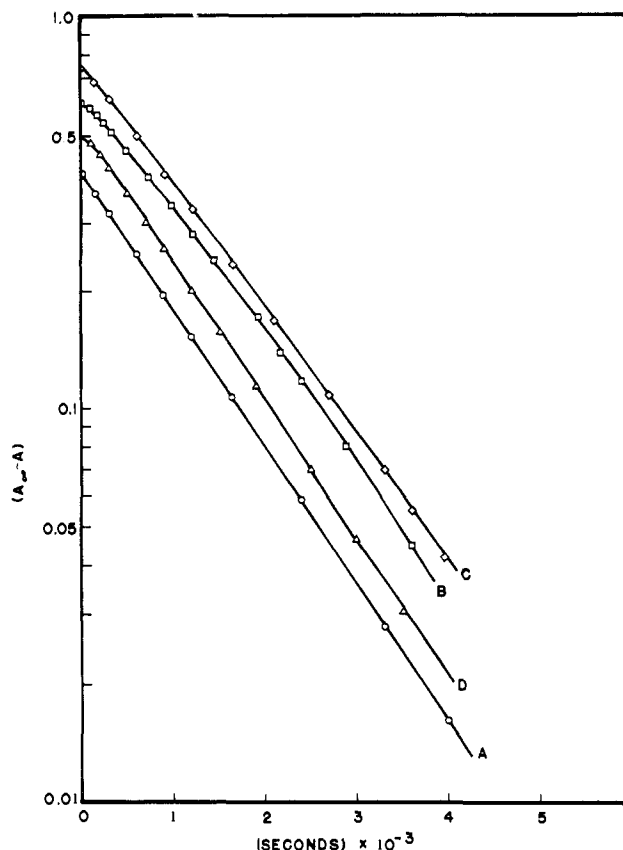
Figure 6. Semilogarithmic plots against time of the absorbance changes that occurred during the desulfonation of II (A), VII (B), VIII (C), and IX (D), in 1.0 M morpholine buffer at pH 8.77.

law (8). According to this rate law, k_{obsd} values would be related to [B]$_T$ by the identity

$$k_{obsd} = \frac{C_1[OH^-] + C_2[B]_T}{1 + C_3[B]_T} \quad (9)$$

By making the assumption that C_1 is the rate constant for the hydroxide ion catalyzed desulfonation of adduct$^-$ (i.e., the k_{OH} value from Table II), the values of C_2 and C_3 in Table IV could be calculated by curve fitting data such as that in Figure 2. The solid lines in this figure were, in turn, calculated by using the appropriate values of C_1, C_2, C_3, and [B]$_T$.

There are at least two reaction schemes which appear to be consistent with the rate law in eq 8. The first of these would be one in which either a molecule of general acid or of general base in the reaction mixture formed a relatively unreactive complex (with respect to desulfonation) with a molecule of the adduct. This possibility is, however, thought to be unlikely because dioxane (which is structurally related to morpholine) and N,N-dimethylmorpholinium ion (which is structurally related to morpholinium ion) have only a slight depressing effect on the rates of desulfonation of IV in morpholine buffers. The results of these experiments are displayed in Table V. The stability constants for complexation between IV and dioxane and N,N-dimethylmorpholinium ion are expected to be similar in magnitude to those for complexation of IV with morpholine and morpholinium ion, respectively. Hence, the fact that neither of the former substances had a marked effect on the rate of desulfonation of IV sug-
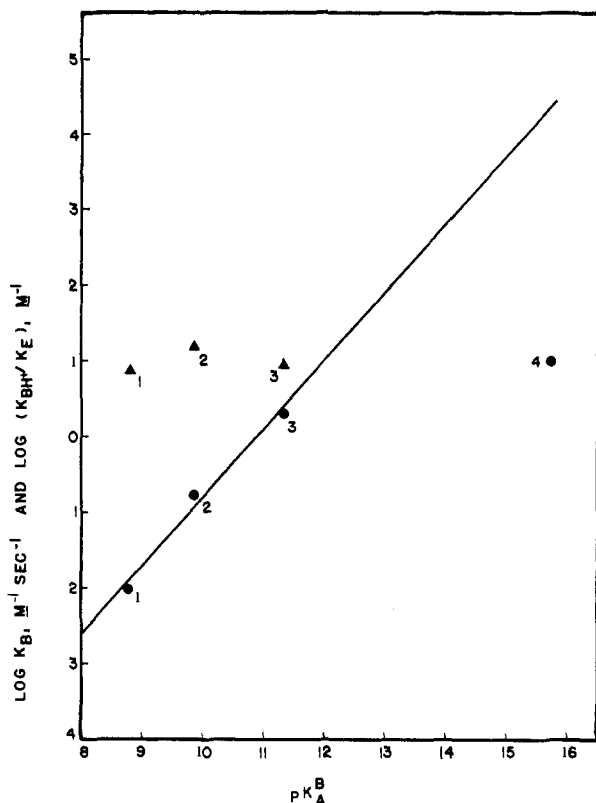
Figure 7. Plots against pK_a^B values of the logarithms of k_B (●) and k_{BH^+}/k_E (▲) values for the desulfonation of IV in aqueous buffers at 25°. The ionic strength was 1 M. The numbers refer to different buffers—1 is morpholine, 2 is benzylmethylamine, 3 is piperidine, and 4 is hydroxide ion.

gests that complexation of the reactant with buffer species did not lead to the behavior observed in Figure 2.

An alternative explanation of the results is a two-step reaction sequence such as the one shown in Scheme III. If the assumption is made that the concentration of

Scheme III
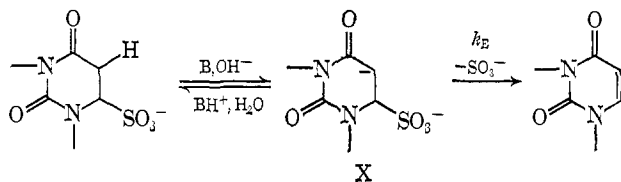
the carbanion intermediate in this sequence remains in low steady state concentrations, the k_{obsd} values generated by these reactions would be related to $[B]_T$ and $[OH^-]$ by the identity

$$k_{obsd} = \frac{k_{OH}[OH^-] + k_B \left(\dfrac{K_a^B}{K_a^B + [H^+]} \right)[B]_T}{1 + \dfrac{k_{H_2O}[H_2O]}{k_E} + \dfrac{k_{BH^+}}{k_E} \left(\dfrac{[H^+]}{K_a^B + [H^+]} \right)[B]_T} \quad (10)$$

This identity has the same form as the empirical eq 9 if $k_{H_2O}[H_2O]/k_E < 1$. If it is assumed that this is the case, the values of C_2 and C_3 in Table IV are $k_B[K_a^B/(K_a^B + [H^+])]$ and $k_{BH^+}[[H^+]/(K_a^B + [H^+])]$ respectively, and, at pH values where $K_a^B = [H^+]$ (i.e., at the pK_a of BH^+), $2C_2 = k_B$, and $2C_3 = k_{BH^+}/k_E$. Values of these rate

constants were calculated from C_2 and C_3 values and are listed in Table IV.

Figure 7 displays a plot of log k_B and log k_{BH^+}/k_E for reactions of IV against the pK_a^B values. These data suggest that the β value for removal of the proton from C-5 of IV by amines is approximately 0.9. This value is very close to the previously calculated β value (0.87) for this reaction.[5] If the reaction sequence shown in Scheme III did accurately represent the desulfonation of IV, it would be expected that the α value for protonation of the carbanion to yield the adduct (the reverse reaction to proton removal) would be small. Consequently, it is surprising that the value of the term $k_{H_2O}[H_2O]/k_E$ is apparently so much smaller than that of $k_{BH^+}[BH^+]/k_E$, because a small α value for this step would suggest that there should not be much difference in the rate at which water and other general acids would protonate the carbanion. Furthermore, k_E values should be independent of the pK_a values of BH^+, and, hence, it would be expected that values of log k_{BH^+}/k_E would be linearly related to pK_a^B values for structurally related acids. As shown in Figure 7, however, this was not the case.

It is also noteworthy that the k_{OH} value falls so far below the line in Figure 7 for the amines[13] and that the value of k_B for carbonate in its reaction with II (pK_a for bicarbonate $= 9.75$) is so much smaller than the k_B value for the less basic morpholine ($pK_a = 8.77$) in the same reaction. These facts, together with the low values of k_{BH^+}/k_E for HCO_3^- in the desulfonation of II, strongly suggest that the structure and charge on both general acids and general bases are important factors in determining the effect that they have on the desulfonation. These points will be discussed further later.

The results in Tables II and IV for the rates of proton removal from C-5 of the adducts indicate that an increase in electron density at N-3 decreases the rate whereas an increase in electron density at N-1 has little effect. Thus, proton removal is slowest from adducts which carry a negative charge on N-3 (cf. k_{OH} and k_{OH}' values in Table II) and it is slower for compounds that carry a methyl substituent on N-3 than for those that carry a proton (cf. k_{OH} and k_B values in Tables II and IV). The presence or absence of a proton on N-1 apparently has little effect on the rate constants for proton removal from C-5 (cf. k_{OH} and k_{OH}' values for VI in Table II).

The hydrogen–deuterium exchange reactions that accompanied desulfonation of VII and IX in aqueous buffers could be accommodated by adding one step to the reaction sequence in Scheme III. This extended sequence is displayed in Scheme IV. According to this sequence, OH^- and B react with the deuterio adduct in a way that results in the removal of a proton or deuterium atom and formation of a carbanion. This latter species can then either add a proton at C-5 to re-form the adduct or eliminate SO_3^{2-} to form the parent uracil. The stereochemistry of these reactions will be discussed later.
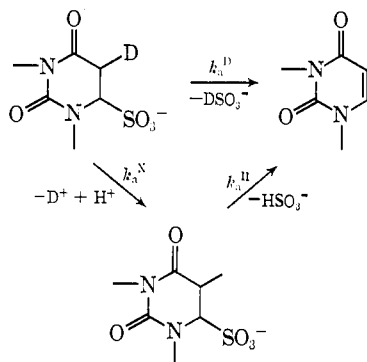
To simplify the handling of the kinetic laws for reactions proceeding by the steps in Scheme IV, it is convenient to also represent the reactions by the sequence of events in Scheme V. In Scheme V it is visualized that the deuterio adduct participates in two

(13) A similar observation was made in ref 4.

Scheme IV
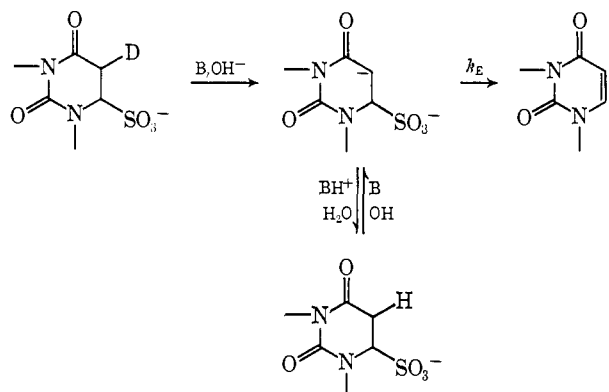
Scheme V

parallel reactions which are pseudo first order when the pH is kept constant. One of these is the elimination of DSO_3^- with an apparent first-order rate constant k_a^D, and the other results in the exchange of a proton for a deuteron at C-5 and has an apparent first-order rate constant, k_a^X. The protio adduct which is formed as a result of the exchange reaction eliminates HSO_3^- with an apparent first-order rate constant, k_a^H. The rate laws for the sequences in Schemes IV and V will be identical if it is assumed that the concentration of the common intermediate for elimination of DSO_3^- and HSO_3^- and for the exchange reaction remains in low steady state concentrations. Values of k_a^D, k_a^H, and k_a^X are related to the microscopic rate constants in Scheme IV by the identities in eq 11–13. As men-

$$k_a^D = \frac{k_{OH}^D[OH^-] + k_B^D\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_{H_2O}[H_2O]}{k_E} + \frac{k_{BH^+}}{k_E}\left(\frac{[H^+]}{K_a^B + [H^+]}\right)[B]_T} \quad (11)$$

$$k_a^H = \frac{k_{OH}^H[OH^-] + k_B^H\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_{H_2O}[H_2O]}{k_E} + \frac{k_{BH^+}}{k_E}\left(\frac{[H^+]}{K_a^B + [H^+]}\right)[B]_T} \quad (12)$$

$$k_a^X = \frac{k_{OH}^D[OH^-] + k_B^D\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_E}{k_{H_2O}[H_2O] + k_{BH^+}\left(\frac{[H^+]}{K_a^B + [H^+]}\right)[B]_T}} \quad (13)$$

tioned earlier, the equation relating k_{obsd} for elimination of HSO_3^- from the protio adducts to $[B]_T$ fits the experimental data when the conclusion was drawn that k_{H_2O} $[H_2O] \ll k_E$ and $\ll k_{BH^+}[BH^+]$ for finite values of $[BH^+]$.

If this conclusion was correct for elimination from the protio adducts, then it must also apply in the elimination and exchange reactions of deuterio adducts. Hence, the values of k_a^H, k_a^D, and k_a^X identities 11, 12, and 13 are expected to be related to $[B]_T$ and $[BH^+]$ by the simpler eq 14–16. These identities appear to give

$$k_a^D = \frac{k_{OH}^D[OH^-] + k_B^D\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_{BH^+}}{k_E}\left(\frac{[H^+]}{K_a^B + [H^+]}\right)[B]_T} \quad (14)$$

$$k_a^H = \frac{k_{OH}^H[OH^-] + k_B^H\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_{BH^+}}{k_E}\left(\frac{[H^+]}{K_a^B + [H^+]}\right)[B]_T} \quad (15)$$

$$k_a^X = \frac{k_{OH}^D[OH^-] + k_B^D\left(\frac{K_a^B}{K_a^B + [H^+]}\right)[B]_T}{1 + \frac{k_E}{k_{BH^+}}\left(\frac{K_a^B + [H^+]}{[H^+][B]_T}\right)} \quad (16)$$

both a qualitative and a quantitative description of what is occurring during the desulfonation of VII, VIII, and IX in aqueous solutions. Hence, when $[B]_T \rightarrow 0$, $k_a^X \rightarrow 0$, and the rate constant for desulfonation should be k_a^D and would equal $k_{OH}^D[OH^-]$. In alkaline solutions which contained no bases other than OH^- and H_2O plots of log $(A - A_\infty)$ against time for the reactions of VII were linear and these plots were used to calculate the values of k_{OH}^D which are listed in Table III. It was also observed that plots of log $(A - A_\infty)$ against time were not linear when the significant amounts of general acids were present. Under these conditions, exchange was competing with elimination of DSO_3^-. The nonlinear plots which were obtained in these experiments (Figures 4–6) could be analyzed to obtain values of the constant k_a^X at different values of $[B]_T$. This was done for the reactions of IX in morpholine buffers (data in Figure 4) by fitting the data from these experiments to eq 17.[14] This equation was derived by integrating the

log $(A_\infty - A)$ = constant +

$$\log \frac{k_a^X e^{-k_a^H t} + (k_a^D - k_a^H)e^{-(k_a^X + k_a^D)t}}{k_a^X + k_a^D - k_a^H} \quad (17)$$

rate laws for disappearance of the deuterio and protio adducts in Scheme V and by fitting the values of the concentrations so obtained into eq 17 which equates the change in total absorbance at any wavelength to the concentrations and absorptivities of reactants and products. It was assumed that the molar absorptivities of both the deuterio and protio adducts would be the same. In the curve fitting procedure, known or estimated values of k_a^H and k_a^D were plugged into eq 17, and values of the constant k_a^X were varied until the calculated curves fit the experimental points. The value of k_a^H that was used was the observed rate constant for desulfonation of II in the same buffer that desulfonation of IX was studied. The value of k_a^D was estimated by making the assumption that the ratios k_{OH}^H/k_{OH}^D and k_B^H/k_B^D were equal. If this assumption is correct, the ratio of k_a^H/k_a^D would be equal to k_{OH}^H/k_{OH}^D. In this way, the values of k_a^D were estimated to be 1.00, 1.60,

(14) A similar equation was developed to handle reactions of this type in R. A. More O'Ferrall and S. Slae, *J. Chem. Soc. B*, 260 (1970).

1.83, and 1.98 $\times$ 10^{-4} sec^{-1} at [B]$_T$ values of 0.01, 0.05, 0.10, and 0.25 M, respectively. The values of $k_a{}^x$ which were required to calculate the line in Figure 4 were 6.16, 30.0, 75.0, and 167 $\times$ 10^{-5} sec^{-1} at [B]$_T$ values of 0.01, 0.05, 0.10, and 0.25 M, respectively.

By substituting known or estimated values of $k_{OH}{}^D$, $k_B{}^D$, [OH$^-$], and [B]$_T$ into eq 16, it was possible to solve it for a value of the constant k_{BH^+}/k_E. The value so obtained, 87 M^{-1}, was very close to the value of the same constant, 78 M^{-1} (in Table IV), which was, in turn, calculated from the data in Table IV. This fact strongly supports the postulate that a sequence of steps such as that in Scheme IV does describe the exchange and desulfonation reactions, and that they all proceed through a common intermediate.[15]

It has been previously noted that both addition of HSO$_3{}^-$ to uracils and elimination of HSO$_3{}^-$ from uracil-bisulfite adducts are largely stereoselective reactions. Hence, it seems reasonable to conclude that either addition of a proton (or deuteron) trans to the sulfonate group is faster than cis addition or *vice versa*. Furthermore, if trans addition is faster than cis addition, it follows that removal of the trans proton (or deuteron) from the adduct by a base would be a faster reaction than removal of a cis proton or deuteron. The present experimental results support these conclusions, at least qualitatively. Hence, the isomer of the monodeuterated adduct of II which we have designated as the trans isomer appears to rapidly exchange its deuteron with a proton in aqueous buffers. Consequently, although plots of log ($A - A_\infty$) against time for these reactions are curved initially, they become linear fairly quickly. It is believed that the curve becomes linear because the exchange reaction has been completed. These results are in Figure 6.

On the other hand, the plots for desulfonation of the cis deuterio isomer have very slight curvature throughout the course of the reaction. If no exchange of the deuteron for a proton occurred during desulfonation of this compound, the plot of log ($A - A_\infty$) against time would be linear. The fact that very slight curvature persists throughout the course of desulfonation suggests that a very slow exchange is occurring.

These results thus strongly suggest that the elimination of HSO$_3{}^-$ or DSO$_3{}^-$ from the adducts that were studied occurs *via* at least a two-step mechanism. Consequently, it appears very likely that addition of HSO$_3{}^-$ to uracils would occur in at least a two-step mechanism.

However, the results in this paper do suggest that the detailed mechanism of the elimination and addition reactions may be more complex than would be inferred from the simplified reaction sequences in Schemes III–V. For example, the failure to experimentally observe protonation of C-5 of the carbanionic intermediate by water suggests that the α value for this reaction must be very large. However, the opposite conclusion would be drawn about the magnitude of the α value for this re-

action from the fact that the β value for the reverse of reaction is large. Another line of reasoning also leads to the expectation that the α value for addition of a proton to C-5 of the carbanion should be small. Hence, the ketonic adducts such as II and IV are expected to be extremely weak carbon acids (acetone is reported[16] to have a pK_a of approximately 20, and the adducts with N(H)C(O)NH and CHSO$_3{}^-$ substituents in place of a CH$_3$ and H, respectively, are not likely to be much more acidic), and their anions are expected to be strong bases. Consequently, it would be expected that they would rapidly extract protons from any more acidic species and that these reactions would have low α values. Support for this proposal comes from the fact that addition of a proton (H$^+$) to the strongly basic enolate ion of acetone is virtually a diffusion controlled reaction,[16] whereas the rates of addition to less basic carbanions such as the anions of acetylacetone[17] or acetoacetic ester are slower by a factor of approximately 10^3.

One possible explanation of the kinetics of protonation at C-5 of the carbanions in the reactions that were studied comes from the observation of Kreevoy and Oh[18] that in protonation reactions of carbanions "considerably more than a simple encounter (between the anion and the proton donor)[19] is required to generate a structure in which proton transfer will take place. The work required to form the reaction complex is W^r. Operationally, it is that part of $\Delta F^*{}_{HA}$ ($\Delta F^*{}_{HA}$ is the free energy of activation of a reaction catalyzed by HA)[19] which is insensitive to changes in pK_a." This observation was, in turn, an elaboration of Marcus' theory[20] of proton transfer.

In the context of the present study, the reaction mechanism can be visualized as follows. The most stable form of the carbanionic intermediate (which would be generated by SO$_3{}^{2-}$ addition to the uracil or by proton removal from C-5 of the adduct) is likely to have a structure such as XI in which the negative charge is largely localized on the oxygen atom. This structure, in turn, is expected to be stabilized by hydrogen bond formation with solvent molecules. To generate a structure in which proton addition to C-5 could occur, it can be visualized that work would have to be done to generate a complex with a structure such as XII. This

XI   XII
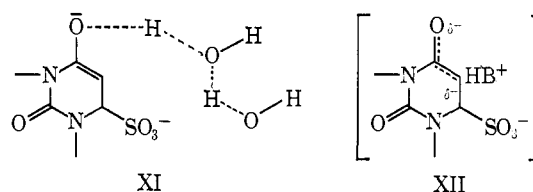
work would involve diffusion together of the reactants and their desolvation and orientation. The inability of water to act as an effective proton donor could arise from the fact that the water molecules in the vicinity of the reaction center are highly structured as a result of hydrogen bonding between water molecules and the oxygen atom of the anion. A considerable amount of work would be required to break up this structure and

(15) A referee raised the point that a carbanion could be formed in a blind-alley reaction rather than along the reaction coordinate of the desulfonation reaction. This situation is analogous to one which was discussed by A. Williams and K. T. Douglas (*J. Chem. Soc., Perkin Trans. 2*, 318 (1973)). However, a combination of the evidence which was obtained from both buffer catalysis experiments and kinetic and solvent isotope effects data strongly suggests that the major pathway leading to the desulfonations in aqueous buffers does include a carbanion intermediate along the reaction coordinate.

(16) R. P. Bell in "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959.
(17) M. Eigen, *Angew. Chem. Int. Ed. Engl.*, 3, 1 (1964).
(18) M. M. Kreevoy and S. Oh, *J. Amer. Chem. Soc.*, 95, 4805 (1973).
(19) The words in parentheses have been added for clarification.
(20) R. A. Marcus, *J. Phys. Chem.*, 72, 891 (1968).

to free water molecules which could act as proton donors. However, other general acids, especially those with positive charges, would be less affected by the water structure than would water molecules, and the work required for them to enter into a structure such as XII could be much less. The experimental results in Table IV do suggest that positively charged acids such as protonated amines were more effective proton donors than the negatively charged bicarbonate ion, although more data would be necessary to clarify this point. As well as being sensitive to the charge on the acid, the work required to form a structure such as XII would also be expected to be sensitive to the structure of the acid. Again, from the limited amount of data generated by this study, it appears that the structure of the conjugate acids of the three amines, morpholine, piperidine, and benzylmethylamine, has as much effect as their pK_a in determining their effectiveness as proton donors.

Similar factors seem to influence the catalytic efficiency of bases in the elimination reactions. Hence, the low catalytic constants for hydroxide ion relative to the neutral amines for proton removal from IV, or for carbonate ion relative to morpholine for proton removal from II, suggest that negatively charged bases must do more work than neutral bases to become orientated in such a way that they can remove a proton from C-5 of the negatively charged adduct.

Experimental Section

Materials. All distilled water was redistilled in a Pyrex apparatus and bubbled with N_2 before use. All inorganic materials were reagent grade and were used without purification. Uracil was obtained from Sigma Chemical Co., and 1-methyl-, 3-methyl-, and 1,3-dimethyluracil were obtained from Cyclo Chemical Co. All were used without purification. Tris(hydroxymethyl)aminomethane was obtained from Aldrich Chemical Co., Inc. and was recrystallized from EtOH–H_2O. Morpholine, piperidine, and all other liquid amines were dried over anhydrous $CaSO_4$ and distilled.

1-Methyl, 3-methyl, and 1,3-dimethyl derivatives of 5,6-dihydrouracil-6-sulfonate were prepared as 0.10 M solutions in bisulfite buffers (1.0 M, pH or pD 5) as described by Sander, et al.[5] These solutions served as stock solutions of these compounds in the kinetic study of their desulfonation reactions.

5,6-Dihydrouracil-6-sulfonate (II) precipitated as a monohydrate potassium salt after stirring 0.56 g (0.005 mol) of uracil in 10 ml of aqueous 2 M K_2SO_3 (previously adjusted to pH 6–7 with concentrated HCl) at room temperature for several hours. The crystals were collected by filtration and recrystallized from H_2O. Anal. Calcd for $KC_4N_2H_7O_6S$: N, 11.23; C, 19.26; H, 2.80. Found: N, 10.86; C, 19.52; H, 2.57. A 0.10 M stock solution of this compound in H_2O was prepared for the kinetic study of its desulfonation reaction.

Potassium 5,5-dideuterio-5,6-dihydrouracil-6-sulfonate deuterate (VII) was isolated from the reaction of 5-iodouracil in a pD 6 bisulfite buffer in D_2O; 1.0 g of 5-iodouracil (Sigma Chemical Co.) was suspended in 10 ml of 2 M K_2SO_3 in D_2O, adjusted to pD 6 with 40% DCl in D_2O, and the mixture was warmed to 80–90° over a 10-min period, until a homogeneous solution was obtained. On cooling to room temperature, white crystals formed and were col-

lected by filtration and recrystallized from D_2O. (This reaction, in H_2O, has previously been shown to yield 5,6-dihydrouracil-6-sulfonate.[3]) A 0.10 M stock solution of this compound was made up in D_2O for the kinetic study of the desulfonation reaction.

5-Deuteriouracil was prepared by treating 1.0 g of VII with 10 ml of 1 M NaOD solution in D_2O for 15 min and then acidifying the solution with 40% DCl in D_2O to pD 1–2 to precipitate the 5-deuteriouracil, which was collected by filtration and recrystallized from D_2O.

The two geometrical isomers of 5-deuterio-5,6-dihydrouracil-6-sulfonate (VIII and IX) were prepared by addition of DSO_3^- to uracil and HSO_3^- to 5-deuteriouracil; 0.56 g of uracil was added to 10 ml of 2 M K_2SO_3 in D_2O, previously adjusted to pD 6–7, and allowed to stir several hours at room temperature. Fine crystals of the monodeuterate of IX were collected. (No recrystallization was attempted, to avoid any isotopic exchange at C-5 during heating.) Prepared in this manner, it is thought that this isomer has the deuterium on C-5 oriented trans to the sulfonate on C-6.[2] Similarly, reacting 5-deuteriouracil with HSO_3^- in H_2O led to the other geometrical isomer, presumably with the deuterium on C-5 oriented cis to the sulfonate on C-6 (VIII). Nmr spectral data of these compounds in D_2O, obtained on a Varian T-60 instrument, are listed in Table II and confirm that the two compounds are geometrical isomers, but offer no basis for absolute assignment. A 0.1 M solution of the trans isomer was made up in D_2O, and a 0.1 M solution of the cis isomer was made up in H_2O as stock solutions for the kinetic study of their desulfonation reactions.

N,N-Dimethylmorpholinium iodide was prepared by the methylation of N-methylmorpholine with methyl iodide in diethyl ether. The salt was recrystallized from ethyl acetate, mp 246–248° (lit.[21] 246°).

Kinetics and Methods. Desulfonation rates of all 5,6-dihydrouracil-6-sulfonate derivatives were measured by monitoring the appearance of the particular uracil derivative's absorbance in the 260–290-nm region after diluting a 0.1 M solution of the sulfonate derivative into 3 ml or more of various buffer solutions. All buffer solutions were maintained at ionic strength 1.0 M by suitable concentrations of KCl, and all kinetic runs were carried out at 25.0 ± 0.1°. Typically, 5–10 μl of a 0.1 M solution of the sulfonate derivative was added to a cuvette, previously filled with buffer and equilibrated at 25° in the cell compartment of a Cary 14, 15, or 16 spectrophotometer, and the cuvette was quickly capped and inverted several times to ensure mixing. The resultant increase in optical density was followed with time. At low buffer concentrations, 5- or 10-cm uv cells, containing 15 or 30 ml of buffer, were used to increase the effective buffer capacity of the solution. After the reaction, the pH of the solution was checked using either a Corning Model 12 or Radiometer Model 26 pH meter equipped with Corning electrodes No. 476002 (calomel) and No. 476022 (glass). The observed first-order rate constants for desulfonation were obtained from semilogarithmic plots of $(A_\infty - A)$ vs. time, plotted through 3–5 half-lives. For desulfonation runs with C-5 deuterium labeled derivatives which were typically non-first order, rate constants were obtained as described in Results.

The pK_a's of the buffers were determined from the measured pH's at ionic strength 1.0 M and the percentage of conjugate acid and base forms in the buffer as analytically prepared, using the Henderson–Hasselbach equation.

(21) "Dictionary of Organic Compounds," Vol. 4, 4th ed, Oxford University Press, London, 1965, p 2354.