(13) A. W. Johnson and R. T. Amel, *Tetrahedron Lett.*, 819 (1966).
(14) K. W. Ratts and A. N. Yao, *J. Org. Chem.*, **31**, 1185 (1966).
(15) A. J. Speziale, C. C. Tung, K. W. Ratts, and A. N. Yao, *J. Am. Chem. Soc.*, **87**, 3460 (1965).
(16) A. W. Johnson, "Ylid Chemistry", Academic Press, New York, N.Y., 1966, pp 310–344.
(17) G. B. Payne, *J. Org. Chem.*, **33**, 3517 (1968).
(18) K. W. Ratts, *Mech. React. Sulfur Compd.*, **2**, 167 (1968).
(19) W. Ando, *Int. J. Sulfur Chem., Part B*, **7**, 189 (1972).
(20) K. Kim and H. J. Shine, *Tetrahedron Lett.*, 4413 (1974).
(21) M. T. Cox, T. T. Howarth, A. H. Jackson, and G. W. Kenner, *J. Am. Chem. Soc.*, **91**, 1232 (1969).
(22) H. Meerwein et al., *Chem. Ber.*, **89**, 2060 (1956); *J. Prakt. Chem.*, **147**, 251 (1937); **154**, 83 (1939).
(23) S. Winstein, B. Appel, R. Baker, and A. Diaz, "Organic Reaction Mechanisms", *Chem. Soc., Spec. Publ.*, **No. 19**, 109 (1965).
(24) H. Weiner and R. A. Sneen, *J. Am. Chem. Soc.*, **87**, 287 (1965).
(25) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, Elmsford, N.Y., 1969, p 291. We thank Dr. Sternhell for helpful discussions.
(26) K. Kim and H. J. Shine, *J. Org. Chem.*, **39**, 2537 (1974).
(27) Y. Murata and H. J. Shine, *J. Org. Chem.*, **34**, 3368 (1969).
(28) A. I. Vogel, "A Textbook of Practical Organic Chemistry", Longmans, Green and Co., New York, N.Y., 1954, p 483.
(29) I. Rabcewicz-Zubkowski and H. Kaflinska, *Rocz. Chem.*, **10**, 555 (1930); *Chem. Abstr.*, **25**, 505 (1931).
(30) J. H. Boyer and D. Straw, *J. Am. Chem. Soc.*, **74**, 4506 (1952).
(31) E. D. Sych, *Ukr. Khim. Zh.*, **22**, 80 (1956); *Chem. Abstr.*, **50**, 16751*i* (1956).
(32) I. Rabcewicz-Zubkowski, *Rocz. Chem.*, **9**, 538 (1929); *Chem. Abstr.*, **24**, 106 (1930).
(33) M. L. Tamayo and F. P. Guerrero, *An. Fis. Quim*, **40**, 390 (1944); *Chem. Abstr.*, **44**, 1470f (1950).
(34) H. Rheinboldt and M. Perrier, *Quimica*, 110 (1951); *Chem. Abstr.*, **46**, 7553*h* (1952); *J. Am. Chem. Soc.*, **69**, 3148 (1947).
(35) J. H. Boyer and D. Straw, *J. Am. Chem. Soc.*, **75**, 1642 (1953).
(36) To be described in detail at another time.

Different Reactivities of 5-Bromo-2′-deoxyuridine and 5-Bromouracil in the Bisulfite-Mediated Debromination

Hikoya Hayatsu,* Toshiyuki Chikuma, and Kazuo Negishi

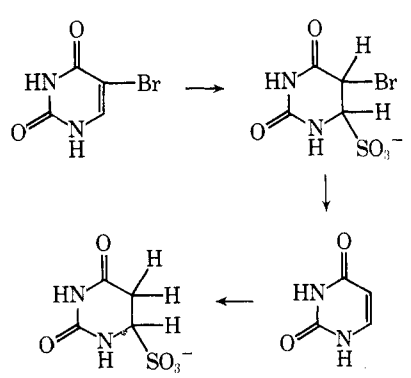
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo, Japan 113

Received June 2, 1975

Sodium bisulfite mediated debromination of 5-bromo-2′-deoxyuridine, 1-methyl-5-bromouracil, and 5-bromouracil was studied. Spectroscopic determination of the velocity at pH 7.0 and 17° showed that 5-bromo-2′-deoxyuridine undergoes debromination two orders of magnitude more slowly than 5-bromouracil. The debromination of 1-methyl-5-bromouracil in this system was also slow, only several times faster than that of 5-bromo-2′-deoxyuridine. The optimum pH for the debromination of both 5-bromo-2′-deoxyuridine and 5-bromouracil was about 7. In the debromination of 5-bromo-2′-deoxyuridine, the existence of the intermediate 5,6-dihydro-5-bromo-2′-deoxyuridine 6-sulfonate was proved by NMR and by the reversal to 5-bromo-2′-deoxyuridine upon dilution of the reaction mixture. The formation of the intermediate from 5-bromo-2′-deoxyuridine was a rapid process, whereas the subsequent debromination was a slow process which was the rate-limiting step of the overall reaction. The facile debromination of 5,6-dihydro-5-bromouracil 6-sulfonate, in contrast to its N^1-substituted derivatives, was explained in terms of participation of an intermediate formed by elimination of HSO_3^- from the N^1–C^6 linkage of this dihydro compound.

Recent research in several laboratories has shown that sulfur nucleophiles, such as bisulfite and cysteine, bring about dehalogenation of 5-halogenouracil derivatives under mild conditions in aqueous solution.[1-4] Sander and coworkers[1a,b] reported that the bisulfite-mediated decomposition of 5-bromouracil proceeds as illustrated in Scheme I,

Scheme I



which involves addition of bisulfite across the 5,6 double bond of the pyrimidine ring followed by elimination of bromonium and sulfite ions to give uracil. The uracil in turn produces 5,6-dihydrouracil 6-sulfonate upon reaction with bisulfite.

The formation of the intermediate, 5,6-dihydro-5-bromouracil 6-sulfonate, was assumed by the analogy to the well-established 5,6-dihydrouracil 6-sulfonate formation from uracil and bisulfite.[5,6] This assumption was supported by the fact that in the case of the reaction between 5-fluorouracil and bisulfite, the formation of 5,6-dihydro-5-fluorouracil 6-sulfonate was demonstrated both by NMR studies and by reversal to 5-fluorouracil.[1a] However, since the bisulfite adduct of 5-bromouracil cannot be observed as a discrete species, it was not possible to determine whether the rate-determining step of the bisulfite-promoted debromination was the addition of bisulfite to 5-bromouracil or the subsequent dehalogenation.

Although Fourrey[2] reported that 5-bromouridine can also be converted to 5,6-dihydrouridine 6-sulfonate by treatment with sodium bisulfite, the study was not performed under kinetically controlled conditions. When we compared reactivities of 5-bromouracil, 1-methyl-5-bromouracil, and 5-bromo-2′-deoxyuridine toward bisulfite under defined conditions, a great difference was observed between these substrates; the N^1-substituted substrates react much more slowly than 5-bromouracil, and the intermediate 5,6-dihydro-5-bromo-2′-deoxyuridine 6-sulfonate can be detected as a discrete species. This paper reports the results of these studies, which show that in the bisulfite-promoted debromination of 5-bromo-2′-deoxyuridine the rate-determining step is the debromination reaction

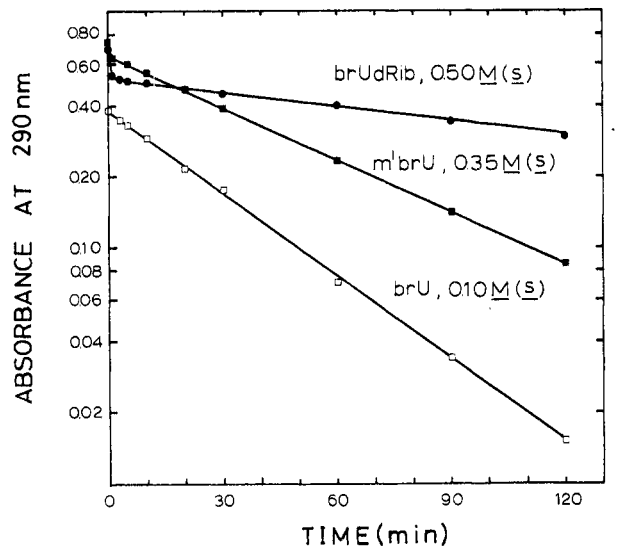


Figure 1. Comparison of absorbance changes of 5-bromouracil, 1-methyl-5-bromouracil, and 5-bromo-2′-deoxyuridine in the reaction with sodium bisulfite. Concentration of the bromouracil derivatives at time zero was 1.0×10^{-3} *M*. Incubations were at pH 7.0 and 17°. (*s*) represents total bisulfite buffer concentration.

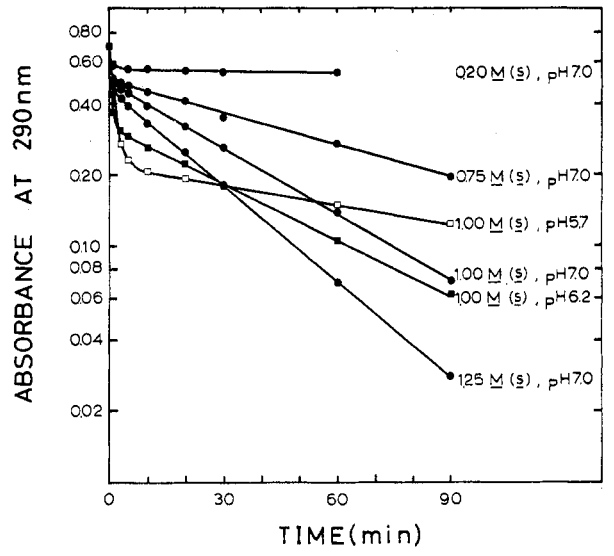


Figure 2. Reaction of 5-bromo-2′-deoxyuridine (1.0×10^{-3} *M*) with sodium bisulfite as functions of the bisulfite buffer concentration (*s*) and the pH. Reaction temperature was 17°.

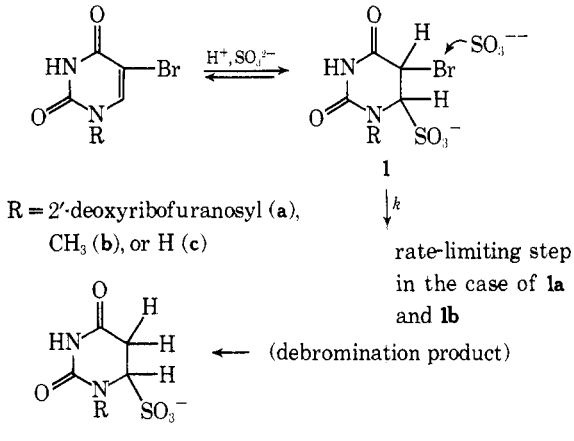
but not the initial addition of bisulfite to the pyrimidine ring.

Results

The reactions were carried out with 1.0×10^{-3} *M* brU (see ref 7), m¹brU or brUdRib, in sodium bisulfite buffer [5×10^{-2} to 1.25 *M* (see ref 8)] at 17°, and the progress of the reactions was followed spectrophotometrically. The change in ultraviolet spectra of brU that occurred on treatment with 0.10 *M* bisulfite at pH 7.0 was similar to that previously reported:[1a] a rapid decrease of the absorbance at the 280–290-nm region and transient appearance of a 260-nm peak, which indicated uracil formation, were observed, followed by a final establishment of an end absorption. On the other hand, in the reaction of brUdRib only a very slow spectroscopic change was detected under identical conditions. At higher bisulfite concentrations, spectral changes of brUdRib were more evident, but in such conditions intermediate formation of 2′-deoxyuridine was difficult to detect since any deoxyuridine formed would have been rapidly converted to 5,6-dihydro-2′-deoxyuridine 6-sulfonate (see below). Thus, the spectra of a solution of brUdRib in 1.0 *M* sodium bisulfite, pH 7.0, did not give any detectable 260-nm peak during the course of the reaction and became finally an end absorption. When the reaction mixture was treated with sodium hydroxide and then analyzed by paper chromatography (solvent, 1-butanol–acetic acid–water, 2:1:1 v/v), 2′-deoxyuridine was recovered as a sole uv-absorbing product. The identification of 2′-deoxyuridine was made by comparing the R_f value and the uv spectra in neutral and alkaline media with those of an authentic specimen.

The progress of the reactions was determined by the decrease in absorbance of the derivatives at 290 nm where uracil, 1-methyluracil, or 2′-deoxyuridine, if they were formed, do not exhibit any absorbance. Typical examples are shown in Figures 1 and 2, in which A_{290} is plotted on a semilogarithmic scale against time of reaction. Both in the brUdRib– and the m¹brU–bisulfite reactions, a rapid, initial drop and a subsequent, slow and linear decrease of the absorbance were observed. In contrast, the brU reaction did not show such an initial drop and instead gave a straight line, consistent with the previous observation of other workers.[1a] This linear decrease should represent the decrease of the starting material, brU. As can be seen from the figures, the extent of the initial drop was a function of both the bisulfite concentration and the pH of the solution. Thus, the drop was larger at higher bisulfite concentrations and at more acidic conditions. The drop was obviously due to the equilibrium between brUdRib (or m¹brU) and 5,6-dihydro-5-bromo-2′-deoxyuridine 6-sulfonate (**1a**) (Scheme II). It is known that bisulfite adds reversibly to the 5,6 double bond of uracil, thymine, and cytosine forming 5,6-dihydropyrimidine 6-sulfonates, and that the latter compounds are stable in acid.[5,6] The formation of the bisulfite adduct **1a** was demonstrated by the following experiments. A solution of 1.0×10^{-3} *M* brUdRib in 1 *M* sodium bisulfite, pH 5.7, was allowed to stand at 17° for 10 min. The A_{290} value of this solution measured in a cuvette of 1 mm light path was approximately 30% of the value for 1.0×10^{-3} *M* brUdRib in water. When the solution was diluted 100 times with 0.1 *M* sodium phosphate buffer of pM 5.8, and the A_{290} was measured in a 10-mm light-path cuvette, a gradual increase of the absorbance was observed. On standing for 90 min, the spectral curve of the solution became identical with that of 1.0×10^{-5} *M* brUdRib (both at pH 5.8 and pH 13), indicating quantitative regeneration of brUdRib from the adduct **1a**. Furthermore, the ^1H NMR spectrum of a 10-min incubated solution of brUdRib in 1 *M* sodium bisulfite (pD 5.7) in D_2O gave two singlets at δ 5.23

Scheme II



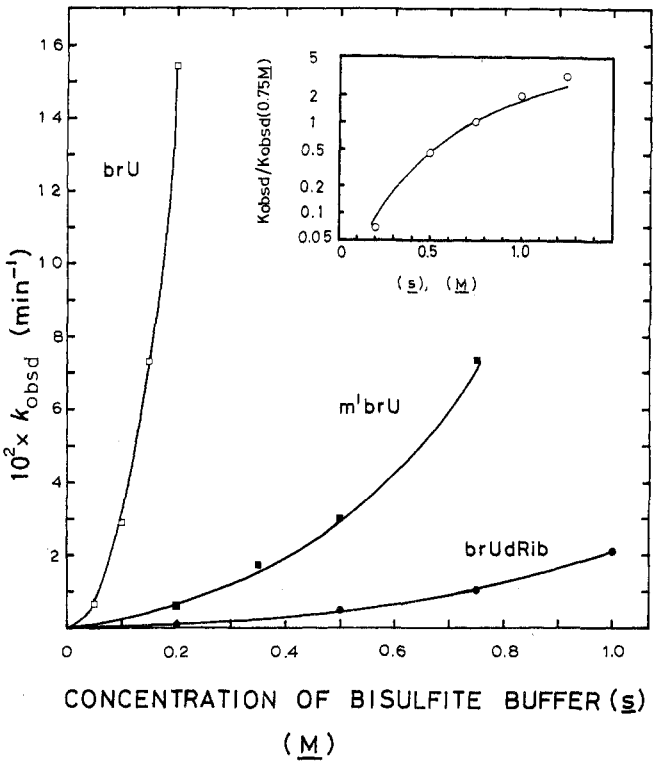


Figure 3. Pseudo-first-order rate constants of the reaction between 5-bromouracil derivatives and sodium bisulfite as a function of the bisulfite buffer concentration. In the inset, the relative rates for the brUdRib–bisulfite reaction are shown in semilogarithmic scale against concentration of bisulfite buffer. The curve in the inset was drawn by the calculation described in the text and the points represent the experimentally observed values.

and 5.35 ppm, assignable[5,6] to the protons at position 6 of two epimers of the adduct **1a**. When the sum of the areas of these two singlets was compared with that of the 6-H signal (8.23 ppm) of brUdRib in this solution, it was found that the ratio of the former to the latter was 7:3. This value is coincident with that (69:31) obtained by the uv measurement (Figure 2).

It can therefore be concluded that the initial rapid decrease of the A_{290} value in the brUdRib and m¹brU reactions represents the accumulation of the bisulfite adduct (1). Subsequent slow, linear decrease must be a reflection of further decomposition of the adduct (1) into debrominated product(s). Furthermore, it is clear that this debromination is the rate-determining step of the overall reaction for brUdRib.

By extrapolating the linear portions to time zero, the A_{290} values at the equilibrium were determined and the values of [**1a**]/[brUdRib][total bisulfite] were found to be 0.51 M^{-1} with 0.5 M bisulfite, 0.47 M^{-1} with 0.75 M bisulfite, 0.44 M^{-1} with 1.0 M bisulfite, and 0.45 M^{-1} with 1.25 M bisulfite. The equilibrium constant for buUdRib + sodium bisulfite $\rightleftarrows$ **1a** was thus estimated at 0.47 ± 0.02 M^{-1} (pH 7.0, 17°).

In the brU–bisulfite reaction, in which no initial drop of A_{290} was noted, there are two possibilities concerning the rate-determining step. First the addition of bisulfite to the 5,6 double bond of brU is the rate-determining step in the overall reaction sequence, and the subsequent debromination of the adduct **1c** is faster than the first step. In this case, the linear decrease in A_{290} should represent the velocity of addition of bisulfite across the 5,6 double bond of brU [assuming that the reverse reaction (elimination) is much slower than the forward reaction]. Second, the amount of the intermediate **1c** is undetectably small and the rate-determining step is the debromination rather than the formation of **1c**. These two alternatives cannot be distinguished by the present data.

Figure 3 summarizes the apparent pseudo-first-order rate constants obtained from the linear portions of the curves such as those in Figures 1 and 2, and shows them as a function of the bisulfite concentration. A strikingly great difference in the reactivity of brU and brUdRib is obvious from this figure. At one bisulfite concentration the rates for these three substrates were compared. Thus, the k_{obsd} values in 0.20 M sodium bisulfite, pH 7.0, were 0.154, 0.0059, and 0.00072 min^{-1} for brU, m¹brU, and brUdRib. It can therefore be estimated that the debromination of brU is two orders of magnitude faster than that of brUdRib. It should be noted that the rates for brU may represent merely the velocity of the adduct **1c** formation and, if so, the rate of the subsequent debromination step must be larger than the observed rate. m¹BrU was more reactive than brUdRib but the difference between brU and m¹brU was much larger than that between m¹brU and brUdRib.

We examined the possibility that the pH profile of the reaction might be greatly different among the substrates and the phenomenon we were observing was an extreme case. That this was not so was shown by the fact that both the brUdRib– and the brU–bisulfite reactions are optimal at pH about 7. Thus, the k_{obsd} (min^{-1}) values follow: with brUdRib (in 1.0 M bisulfite), 0.0061 at pH 5.7, 0.018 at pH 6.2, 0.021 at pH 7.0, and 0.003 at pH 7.9; with brU (in 0.10 M bisulfite), 0.0077 at pH 5.7, 0.029 at pH 7.0, and 0.0088 at pH 8.0.

The results in Figure 3 also indicate that the rate for either of the three substrates is a function of more than first order of the total bisulfite–buffer concentration. A similar relationship was previously observed for the brU–bisulfite system.[1b]

In the reaction of brUdRib (and m¹brU) with bisulfite, the expression

$$k_{obsd} = kK(s)^2/[1 + K(s)] \quad (1)$$

can be derived, where k represents the rate constant for the debromination, K the equilibrium constant for brUdRib + HSO_3^- (total buffer) $\rightleftarrows$ **1a**, and (s) total bisulfite buffer concentration. Expression 1 indicates that the experimentally determined k_{obsd} values at various bisulfite concentrations should be related by [k_{obsd} at buffer concn (s)]/[k_{obsd} at buffer concn (s')] = $(s)^2[1 + K(s')]/(s')^2[1 + K(s)]$. Employing the K value 0.47 M^{-1} experimentally determined as already described, theoretical relative rates were plotted against (s) and compared with those observed. As shown in the inset of Figure 3, the experimental values were reasonably coincident with the theoretical ones. It was therefore concluded that the reaction scheme illustrated in Scheme II is basically correct.

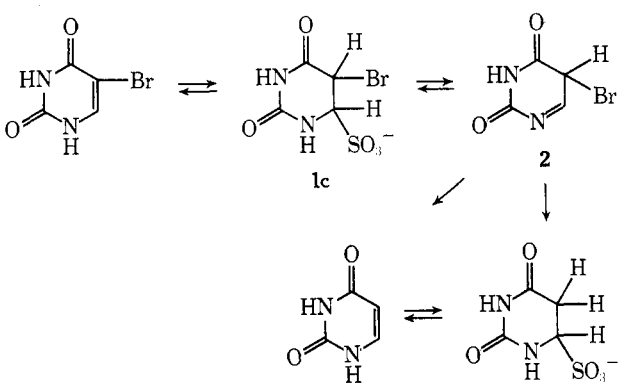
By use of expression 1 and the k_{obsd} values, the rate constant k can be calculated. From the k_{obsd} values found for the 0.5, 0.75, 1.0, and 1.25 M bisulfite reactions, the k value was estimated to be 0.061 ± 0.008 l. mol^{-1} min^{-1}.

We measured velocity of bisulfite addition to 2′-deoxyuridine under conditions identical with those employed in the brUdRib–bisulfite reaction and found that it is greater than the velocity of the decomposition of the adduct **1a**. The apparent pseudo-first-order rate constants found were 0.0533 min^{-1}/1.0 M sodium bisulfite, and 0.0147 min^{-1}/0.50 M sodium bisulfite at pH 7.0 and 17°, the initial deoxyuridine concentration being 0.010 M. This finding indicates that if ever deoxyuridine is produced from the adduct **1a** it will escape detection.

Discussion

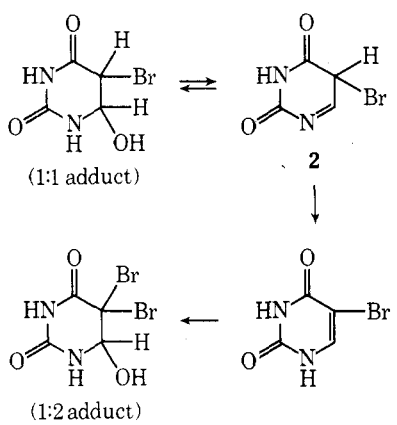
The results presented above showed that the breakdown of the adduct **1c** is very much faster compared with the N^1-substituted adducts **1a** and **1b**. Although the different reactivities of these substrates might be attributable to steric hindrance of the N^1 substituents, a more likely explanation for the very large reactivity difference is provided by postulating the intermediate **2** in the brU–bisulfite reaction (Scheme III). Sulfite will reductively subtract the bromine

Scheme III



atom[1b] of **2** to give uracil and 5,6-dihydrouracil 6-sulfonate. It is known that the 5,6-dihydrouracil 6-sulfonate is produced not necessarily via uracil.[4] Support for the possibility of the existence of intermediate **2** is found in the recent finding that titration of uracil with bromine results in the formation of an uracil–bromo (1:2) adduct, whereas titration of 1-methyluracil gives a 1:1 adduct.[10] In explanation, the intermediate **2** was postulated, whose formation is the

Scheme IV



crucial step for the generation of the 1:2 adduct (Scheme IV). In contrast to the sulfite-mediated debromination, in which a bromonium ion rather than a proton is subtracted at position 5 of **2**, bromine deprotonates **2** to give brU. The workers[10] postulated that this occurs via the formation of an *N*-bromo derivative of **2**.

In the brUdRib- and m^1brU–bisulfite reactions, the formation of intermediate **2** would be blocked or extremely difficult because it requires quaternization of the nitrogen at position 1. Direct reduction of the adduct **1** by sulfite anion will also be a slow reaction owing to electronic repulsion by the sulfonate group at position 6. Therefore, the debromination of brUdRib and m^1brU will proceed much more slowly than that of brU, by taking either the direct route or the indirect route to the final product.

Besides bisulfite, cysteine debrominates both brU and brUdRib under mild conditions. In the case of cysteine, however, no great difference is existent between the reactivities of brU[1c] and brUdRib.[3] An explanation for this is the following. In the cysteine-mediated debromination of brUdRib, the initial addition of the nucleophile across the 5,6 double bond of the pyrimidine was supposed to be the rate-determining step. In this regard, the bisulfite-mediated debromination of brUdRib is different from the cysteine reaction, because its rate-determining step is not the initial addition of the nucleophile but the subsequent debromination.

It is interesting that the reactivity at the position β to the glycosidic linkage is so much different between a nucleoside and the corresponding base. The present finding indicates that reactions at position 5 of pyrimidine nucleosides and bases should always be carefully compared.

Experimental Section

General. BrUdRib and brU were products of Sigma Chemical Co. and were used without further purification. m^1BrU was prepared according to the literature.[11] Proton magnetic resonance spectra (100 MHz) were measured on a Jeol NM-4H-100 spectrometer.

Kinetic Measurements. All reactions were performed in deionized, distilled water. Sodium bisulfite buffers were always freshly prepared before use. The pH was fixed by mixing appropriate amounts of $NaHSO_3$ and Na_2SO_3. The reactions were run under nitrogen atmosphere at room temperature which was maintained at 17 ± 0.5°. Progress of the reaction was monitored by determining A_{290} in a cuvette of 1-mm light path against a reference in which brU derivative was omitted from the reaction mixture, using a Beckman Acta CIII spectrophotometer. The pH of the reaction mixture was measured both at zero time and after the incubation was over. The value generally did not exhibit any change, except in the case where 0.050 *M* bisulfite buffer was used, and the pH was 7.0 at zero time and 6.85 after 180-min incubation. The zero time A_{290} values employed were those obtained with solutions omitting the bisulfite buffer from the reaction mixture (reference, H_2O).

Acknowledgments. We thank Dr. Y. Kawazoe of National Cancer Center Research Institute and Mr. M. Shiragami of this laboratory for valuable discussions.

Registry No.—5-Bromo-2′-deoxyuridine, 59-14-3; 1-methyl-5-bromouracil, 6327-97-5; 5-bromouracil, 51-20-7.

References and Notes

(1) (a) E. G. Sander and C. L. Deyrup, *Arch. Biochem. Biophys.*, **150**, 600–605 (1972); (b) F. A. Sedor and E. G. Sander, *ibid.*, **161**, 632–637 (1974); (c) F. A. Sedor and E. G. Sander, *Biochem. Biophys. Res. Commun.*, **50**, 328–336 (1973); (d) F. A. Sedor, D. G. Jacobson, and E. G. Sander, *Bioorg. Chem.*, **3**, 154–162 (1974).
(2) J. L. Fourrey, *Bull. Soc. Chim. Fr.*, 4580–4581 (1972).
(3) Y. Wataya, K. Negishi, and H. Hayatsu, *Biochemistry*, **12**, 3992–3998 (1973).
(4) I. A. Pitman and G. S. Rork, *J. Pharm. Pharmacol.*, **26**, Suppl., 69P–70P (1974).
(5) R. Shapiro, R. E. Servis, and M. Welcher, *J. Am. Chem. Soc.*, **92**, 422–424 (1970).
(6) (a) H. Hayatsu, Y. Wataya, and K. Kai, *J. Am. Chem. Soc.*, **92**, 724–726 (1970); (b) H. Hayatsu, Y. Wataya, K. Kai, and S. Iida, *Biochemistry*, **9**, 2858–2865 (1970).
(7) Abbreviations used are: brU, 5-bromouracil; m^1brU, 1-methyl-5-bromouracil; and brUdRib, 5-bromo-2′-deoxyuridine.
(8) The concentrations represent total buffer concentrations.
(9) F. A. Sedor, D. G. Jacobson, and E. G. Sander, *Bioorg. Chem.*, **3**, 221–228 (1974).
(10) S. Banerjee and O. S. Tee, *J. Chem. Soc., Chem. Commun.*, 535–536 (1974).
(11) T. B. Johnson and I. Matsuo, *J. Am. Chem. Soc.*, **41**, 782–789 (1919).