Carbon-13 Nuclear Magnetic Resonance Studies of Bisulfite–Pyrimidine Addition Reactions: Stereoselective Formation and Reactions of the 5-Halouridines

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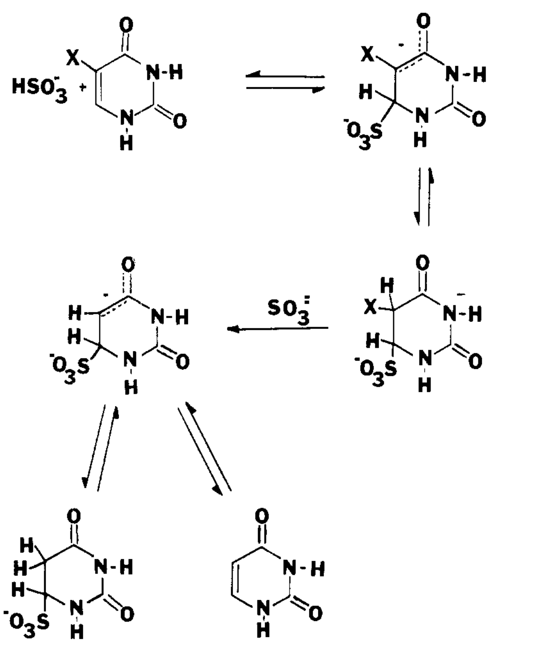
The interaction of bisulfite with 5-halouridines is investigated utilizing ^{13}C NMR. Reaction mixtures (equimolar) of sodium bisulfite and the halouridine at equilibrium are studied, and individual equilibrium constants are calculated for each diastereomeric product. The pH of the reaction mixture is then raised, causing dehalogenation of the 5-halouridine. By comparative analysis of peak position and intensity patterns, the addition of the elements of bisulfite to 5-fluorouridine is found to be a trans process. 5-Chlorouridine adds bisulfite in both a cis and a trans manner, resulting in four diastereomeric adducts. 5-Bromouridine and 5-iodouridine add bisulfite cis exclusively. The data also suggest that only those bisulfite–5-halouridine adducts in which the halogen is oriented trans to the sulfonic acid moiety can dehalogenate under the influence of sulfite dianion. Further, it is shown that uridine is not an intermediate in the dehalogenation (i.e., the bisulfite adduct of the halouridine dehalogenates directly to the bisulfite adduct of uridine).

The nucleophilic attack of bisulfite anion on the uracil ring system has been much studied as a model for the initiating step of the methylation of 2′-deoxyuridine by the enzyme thymidylate synthetase.[1-13] Both the enzyme[14] and bisulfite have also been shown to catalyze the dehalogenation of 5-halouracils and 5-halouridines (except for the 5-fluoro compounds).

Rork and Pitman[7] have presented a mechanism (Scheme I) for the dehalogenation of the halouracils. In this mechanism the initiating step is nucleophilic attack at carbon 6 of the pyrimidine, resulting in 5-halo-5,6-dihydrouracil-6-sulfonate. Sulfite dianion then attacks the halogen in an S_N2 manner, resulting in the formation of a 5,6-dihydrouracil anion and the halosulfonic acid whose subsequent protonation results in the formation of the uracil–bisulfite adduct which equilibrates with uracil. Also in this mechanism is a pathway from the 5-halo-5,6-dihydrouracil-6-sulfonate to uracil which then equilibrates with the bisulfite adducts. Hayatsu et al.[16] have suggested that the Rork mechanism may be valid for the dehalogenation of halouridines, but not for halouracils as originally proposed.

Scheme I



Investigations into the stereochemistry of the addition of bisulfite to uracil[4] and uridine[2] have revealed that the reaction occurs as a trans process. The work of Sander et al.[15] indicates that bisulfite attacks 5-fluorouracil in a manner that is stereochemically similar to that observed in the case of uracil (i.e., a trans process). Rork and Pitman have suggested that in the case of bisulfite addition to 5-chlorouracil, the addition occurs by both a cis process and a trans process.[7] According to their mechanism, bisulfite addition at carbon 6 results in an enolate anion which, in the case of chlorouracil, is sufficiently stable to allow proton addition from both above and below the plane. Uridine has been shown to add bisulfite in a manner resulting in the formation of a pair of diastereomeric adducts. This occurs because the newly formed asymmetric center (carbon 6) is a mixture of two configurations. In the case of the halouridine–bisulfite adducts, there could potentially be four diastereomers formed (Scheme II).

Such complex systems as the equilibrium established between 5-chlorouridine and the four corresponding diastereomeric bisulfite adducts can be evaluated (not only can the presence of all four products be detected, but an estimate of the relative concentration of the individual components can be made, permitting determination of the corresponding equilibrium constants). This paper describes the use of ^{13}C NMR to elucidate the stereochemical details of the mechanism of bisulfite-catalyzed dehalogenation of the 5-halouri-

Scheme II

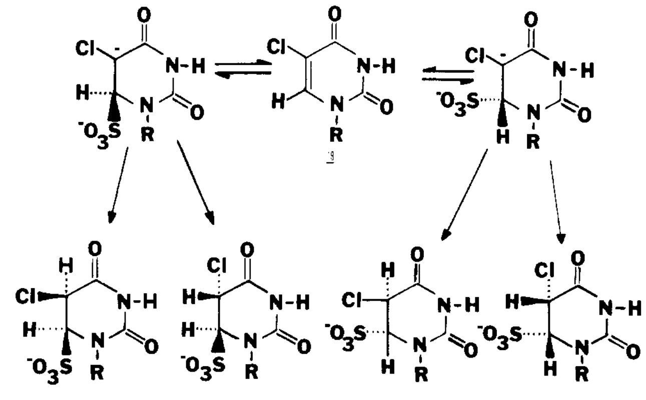


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Table I. Chemical Shift Assignments (ppm) for the Carbon Signals in the ^{13}C NMR Spectra of the 5-Halouridines and Their Bisulfite Adducts

compd[a]	C_2	C_4	C_5	C_6	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{5'}$
5-Cl-Urd	151.82	162.38	110.03	139.82	91.29	70.58	75.27	85.72	61.99
5-Br-Urd	151.64	162.37	97.25	141.96	90.78	69.89	75.18	85.04	61.20
5-F-Urd	152.81	165.11 (J_{CF} = 20.9 Hz)	142.35 (J_{CF} = 236.6 Hz)	125.56 (J_{CF} = 35.3 Hz)	90.28	70.38	74.74	85.00	61.78
5-I-Urd	152.61	163.84	69.11	147.54	91.30	70.58	75.35	85.63	61.99
5-Cl-Urd-A	152.71	167.94	53.70	68.55	90.87	71.15	75.26	83.96	61.59
5-Cl-Urd-B	151.85	168.64	53.70	67.34	92.54	69.71	72.93	84.34	62.28
5-Cl-Urd-C	152.12		50.16		91.27	69.30	74.10	84.75	62.00
5-Cl-Urd-D	153.11		50.16		92.04	72.65		83.69	
5-Br-Urd-A	153.20	168.32	42.67	69.81	90.95	72.74	74.46	84.64	62.30
5-Br-Urd-B	153.60	168.32	42.67	69.13	92.97	71.16	75.17	84.14	61.42
5-F-Urd-A	155.16	169.58 (J_{CF} = 20.8 Hz)	83.36 (J_{CF} = 194.5 Hz)	67.30 (J_{CF} = 37.6 Hz)	91.23	70.98	75.02	84.51	62.22
5-F-Urd-B	153.34	169.58 (J_{CF} = 20.8 Hz)	83.36 (J_{CF} = 194.5 Hz)	66.38 (J_{CF} = 36.5 Hz)	93.78	69.83	72.81	83.91	61.76

[a] Registry No.: 5-Cl-Urd, 2880-89-9; 5-Br-Urd, 957-75-5; 5-F-Urd, 316-46-1; 5-I-Urd, 1024-99-3.

Table II. The Individual Equilibrium Constants for the Formation of 5-Halouridine–Bisulfite Adducts

compd	$[HSO_3^-]_T$	pH	$[HSO_3^-]_e$	$K_{eq\,A}$	$K_{eq\,B}$	$K_{eq\,C}$	$K_{eq\,D}$
5-F-Urd	1.0	6.90	0.21	4.13	3.38		
5-Cl-Urd	2.5	5.58	1.04	0.81	0.75	0.31	0.25
5-Br-Urd	2.5	5.62		0.66	0.53		

dines. The data indicate that the bisulfite-catalyzed dehalogenation of the 5-halouridines follows a mechanism very similar to that proposed by Rork and Pitman.[7] This is in agreement with Hayatsu et al.[16] However, there is no evidence supporting a mechanism which includes uridine as an intermediate. The evidence supports an argument that the ability of the adduct to dehalogenate is dependent on its configuration. Specifically, the bisulfite adducts in which the halide is cis to the sulfonic acid (resulting from bisulfite addition across the $C_5{=}C_6$ bond in a trans fashion) do not dehalogenate. Those species which add bisulfite in a cis manner (those in which the halogen is oriented trans to the sulfonic acid moiety) undergo dehalogenation in a pH-dependent reaction.

Experimental Section

5-Fluorouridine and 5-chlorouridine were obtained from Calbiochem, San Diego, Calif. 5-Bromouridine and 5-iodouridine were obtained from Aldrich Chemical Co., Milwaukee, Wis. These products were found to be spectroscopically pure by ^{13}C NMR and were used as received.

Preparation of Samples and Acquisition of ^{13}C NMR Spectra. (1) Preparation of Samples of the 5-Halouridines. The spectra of all of the 5-halouridines were acquired from a 1.0 mL aqueous sample containing 1.0 mmol of the compound (the singular exception being 5-iodouridine, which was not sufficiently soluble for molar concentration). The lock signal was provided by a capillary tube containing D_2O.

(2) Preparation of Equilibrium Systems Containing the 5-Halouridine and Sodium Bisulfite. Sodium bisulfite (104 mg, 1.0 mmol) was added to the aqueous sample of the 5-halouridine. After 24 h, the ^{13}C NMR spectrum was measured and the pH of the sample determined (generally, the pH was found to be between 5.2 and 5.3). Preliminary studies of samples allowed to equilibrate for longer periods indicate that the equilibrium is established in less than 24 h. In the case of 5-iodouridine, the amount of sodium bisulfite added to the sample was equimolar with the 5-iodouridine.

(3) Preparation of Samples under Conditions for Dehalogenation. The pH of the equilibrium system of the 5-halouridine in aqueous bisulfite solution was adjusted to 6.9, and the sample was allowed to equilibrate for 24 h before the spectrum was measured.

(4) Measurement of Nuclear Magnetic Resonance Spectra. Carbon-13 nuclear magnetic resonance spectra were measured on 0.5–1.0 mL solutions in 8 mm sample tubes with a Varian Associates (Walnut Creek, Calif.) CFT-20 spectrometer. The following were standard operating conditions: pulse width = 7 μs; acquisition time for a 4K FID = 0.512 s; total cycle time = 0.512 s (no delay); sweep width = 4000 Hz (ppm); accumulated transients = 7000–100 000. Probe temperatures were measured with a Leeds Northrup potentiometer and thermocouple. Ambient probe temperature was 31 °C.

Results and Discussion

The spectral assignments of the bisulfite–5-halouridine equilibrium systems at low pH (5.0–5.2) are reported in Table I, and the corresponding equilibrium constants, calculated from ^{13}C NMR intensity data,[17] are reported in Table II. In each instance, the system has been allowed to equilibrate; thus, the diastereomer present in the greatest quantity is the most stable species. Carbon 1′ is used to obtain comparative intensities because the signals are well separated in all of the systems studied. Of special interest is the 5-chlorouridine system, in which all four possible diastereomers are present. In the case of 5-fluoro-, 5-bromo-, and 5-iodouridines, the spectral evidence indicates that only two diastereomeric bisulfite adducts are formed. Addition of bisulfite to 5-fluorouracil has been shown to occur as a trans process (yielding a product with the halogen oriented cis to the sulfonic acid moiety[8]). Thus, by analogy, the addition of bisulfite to 5-fluorouridine is assumed to be a trans process. The spectral pattern observed for carbon 1′ of the 5-fluorouridine–bisulfite equilibrium system is characterized by the signal from the starting material at 90.28 ppm, the major diastereomer signal downfield at 91.23 ppm, and the minor diastereomer signal further downfield (93.78 ppm) (Table I). When the pH of the 5-fluorouridine–bisulfite sample is raised, there is no sign of dehalogenation. In fact, efforts to force the dehalogenation (high temperature and more concentrated bisulfite solutions

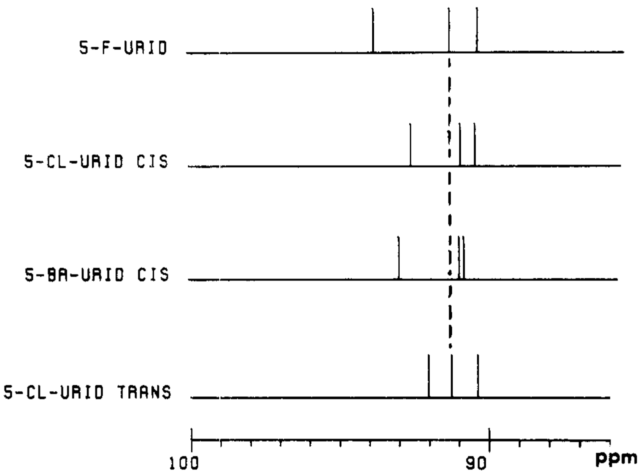

Figure 1. The relative location of the signals from carbon 1′ for the systems assigned to be cis and trans. One signal from the minor part of 5-chlorouridine–bisulfite adducts aligns with the signal from one of the diastereomers of the 5-fluorouridine–bisulfite adduct, a system known to add bisulfite in a trans manner.

of various pH values) produced no evidence of dehalogenated products.

The carbon-13 NMR spectrum of the 5-chlorouridine–bisulfite system observed at pH 5.2 is found to be a composite of the spectra of five individual chemical species: 5-chlorouridine and four diastereomeric bisulfite adducts (Scheme II). Evaluation of the signal pattern for carbon 1′ reveals one intense signal (91.29 ppm) from the starting material and two pairs of signals arising from diastereomeric bisulfite adducts of 5-chlorouridine. The adduct signals can be arranged into two pairs based on their intensity. The more intense pair of signals represents the more populous species, which in an equilibrium system would be the most stable pair of diastereomeric bisulfite adducts. The less intense signals represent the less stable pair. It is reasonable to assume that one pair of signals would represent those two diastereomers with the bisulfite moiety cis to the sulfonic acid moiety while the other pair of signals represents the two diastereomers with the halogen oriented trans to the sulfonic acid moiety. It is likely that there is less steric hindrance in the molecules in which the chlorine is oriented trans to the sulfonic acid. Thus, the major pair of signals (90.87 and 92.54 ppm) has been tentatively assigned as representing the two diastereomeric molecules with the halogen oriented trans to the sulfonic acid moiety. This orientation results when the bisulfite addition across the $C_5{=}C_6$ bond proceeds as a cis addition process. Similarly, the minor pair of signals has been tentatively assigned to the pair of diastereomers in which the chlorine is oriented cis to the sulfonic acid group. This orientation would be the product of trans addition across the $C_5{=}C_6$ bond. In support of the above assignment, it is noted that the signal from carbon 1′ of one of the diastereomeric bisulfite adducts of 5-fluorouridine (a system shown to add bisulfite in a trans fashion) is very close to that of one of the minor diastereomers of the 5-chlorouridine system (Figure 1).

If the 5-chlorouridine–bisulfite equilibrium system is established at pH 6.9, the resulting spectrum shows evidence of dehalogenation. This evidence manifests itself in two ways: (a) there is no spectral evidence for the signals assigned to the major diastereomeric adducts, and (b) there are new signals present in the spectrum which are coincidental with those observed for the bisulfite adducts of uridine.[17] Clearly, the system has undergone a regioselective dehalogenation. The more stable pair of diastereomeric bisulfite adducts (tentatively assigned as having the halogen trans to the sulfonic acid moiety) has been selectively dehalogenated.

This observation is consistent with the above assignment in that the trans orientation is sterically more conducive to dehalogenation via S_N2 attack on the halogen by a sulfite dianion. The nucleophilic attack on the halogen would be coulombically inhibited by the sulfonic acid anion when the halogen and the sulfonic acid moiety are oriented cis, but not when they are oriented trans. Therefore, the favored reaction should be the dehalogenation of that pair of bisulfite adducts of 5-chlorouridine in which the halogen is situated trans. This dehalogenation would result in the formation of bisulfite adducts of uridine.

Bisulfite adds to 5-bromouridine in a manner similar to that in which it adds to 5-chlorouridine, except that there is spectral evidence for the formation of only two diastereomeric bisulfite adducts. It is important that the signals observed for carbon 1′ (Table II) reside in almost exactly the same positions (90.95 and 92.97 ppm) as those in which the signals from carbon 1′ of the major pair of 5-chlorouridine–bisulfite adducts are observed (90.87 and 92.54 ppm) (Figure 1). Thus, if the stereochemical assignment tentatively presented for the 5-chlorouridine bisulfite adducts is correct, then the stereochemistry of the 5-bromouridine adducts must be assigned as trans. The addition of bisulfite to 5-bromouridine would therefore have to proceed as a cis process. This is certainly reasonable considering the size of the bromine atom relative to chlorine or fluorine. When the equilibrium system is prepared at pH 6.9 and the spectrum is accumulated 24 h later, there is spectral evidence indicating that the sample has been extensively dehalogenated. This evidence is similar to that seen in the case of 5-chlorouridine, in that the signals assigned as bisulfite adducts of 5-bromouridine with the halogen oriented trans to the sulfonic acid moiety are no longer present. There are, however, signals present which are coincident with those previously assigned as bisulfite adducts of uridine.

Aqueous solutions of 5-iodouridine and sodium bisulfite at various pH values are unstable, dehalogenating too rapidly to allow the accumulation of spectral evidence of bisulfite adducts of 5-iodouridine. In all cases, the only species found in the solution were attributable to 5-iodouridine and the two diastereomeric bisulfite adducts of uridine.

The present study indicates that bisulfite addition to the various 5-halouridines can be summarized as follows. (a) Bisulfite adds to 5-fluorouridine, forming two diastereomers previously shown to add the sulfonic acid moiety in a trans process. The resultant adducts do not dehalogenate under the experimental conditions. (b) Bisulfite adds to 5-chlorouridine by both a cis and a trans process, and at equilibrium (pH 5.2) the pair of diastereomers produced by cis addition predominates. At pH 6.9 the pair of adducts resulting from cis addition dehalogenates (the adducts resulting from trans addition are stable to dehalogenation). (c) Bisulfite adds exclusively cis to 5-bromouridine, and at pH 6.9 the 5-bromouridine–bisulfite adducts dehalogenate. (d) Bisulfite adds to 5-iodouridine, and the adduct (orientation unknown) is unstable and rapidly dehalogenates, yielding an equilibrium system consisting of uridine and its bisulfite adducts.

When dehalogenation occurs, not only do the signals corresponding to the halouridine–bisulfite adducts disappear, but signals corresponding to the uridine–bisulfite adducts appear. These signals are also observed when the adducts are formed directly by addition of bisulfite to uridine.[17] Significantly, while the peaks for the uridine–bisulfite adducts formed by dehalogenation of the 5-halouridine–bisulfite adducts occur in the same position as those observed upon direct bisulfite addition to uridine, *their intensities are different*.

The intensity pattern can be summarized as follows (Figure 2). (a) In the case of uridine and 5-fluorouridine, the pattern of peak intensity is that the more intense signal (assigned to the more stable diastereomer) is upfield from the signal as-

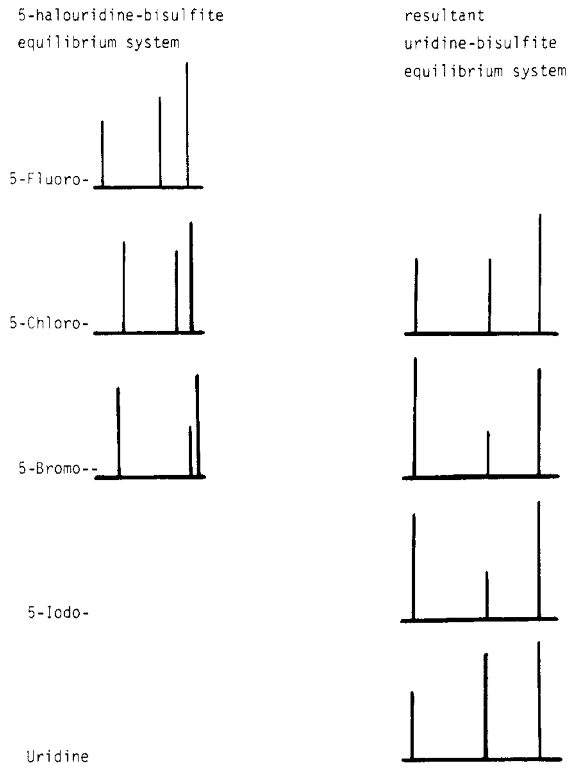

Figure 2. The intensity patterns for the uridine–bisulfite adducts, the 5-halouridine–bisulfite adducts, and the uridine–bisulfite adducts produced by sulfite-catalyzed dehalogenation of the 5-halouridine.

signed to the less stable diastereomer. (b) In the case of both pairs of diastereomers from 5-chlorouridine, the pattern is the same except that the difference in intensity is greatly reduced (i.e., the difference in stability is relatively small). (c) In the case of 5-bromouridine, the intensity pattern is the reverse of that observed for uridine and 5-fluorouridine. Thus, substitution at carbon 5 of the parent molecule (uridine) affects the stereoselectivity of the nucleophilic attack at carbon 6. Replacing the proton with a fluorine has little effect, chlorine tends to equalize the diastereomers, and the very large bromine directs addition in a manner which results in reversal of the intensity pattern.

In the case of chloro- and bromouridines, the concentration ratio of the resultant uridine–bisulfite adducts is found to match that observed for the parent halouridine rather than that observed when bisulfite is added directly to uridine (i.e., the stereochemistry about carbon 6 after dehalogenation is identical with that observed before dehalogenation). Therefore, the dehalogenation must proceed from the bisulfite adduct of the halouridine to the bisulfite adduct of uridine without the intermediacy of uridine. Similarly, this would eliminate mechanisms in which the bisulfite moiety is removed and readded.

Since 5-iodouridine shows the same product intensity ratio, this suggests that 5-iodouridine adds bisulfite in the same manner as 5-bromouridine, and in the subsequent dehalogenation the sulfonic acid moiety remains attached to carbon 6.

Bisulfite addition has been shown to occur in two stereochemically different ways. Early work has shown that uracil and 5-fluorouracil add bisulfite in a trans fashion. By analogy, it is inferred that uridine and 5-fluorouridine also add bisulfite trans. 5-Chlorouridine adds bisulfite both cis and trans, thus generating four diastereomers. 5-Bromouridine and 5-iodouridine add bisulfite exclusively cis. Evidence has been presented supporting the hypothesis that only the diastereomers with the halide and the sulfonic acid trans can dehalogenate at neutral pH. Those in which the sulfonic acid and the halide are cis are resistant to 5-dehalogenation. Intensity patterns support the hypothesis that the dehalogenation proceeds from the halouridine adducts directly to the uridine bisulfite adducts without the intermediacy of uridine.

Registry No.—5-Cl-Urd (isomer 1), 67598-41-8; 5-Cl-Urd (isomer 2), 67650-72-0; 5-Cl-Urd (isomer 3), 67650-73-1; 5-Cl-Urd (isomer 4), 67650-74-2; 5-Br-Urd (isomer 1), 67598-42-9; 5-Br-Urd (isomer 2), 67650-75-3; 5-F-Urd (isomer 1), 67598-43-0; 5-F-Urd (isomer 2), 67650-76-4; sodium bisulfite, 7631-90-5.

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