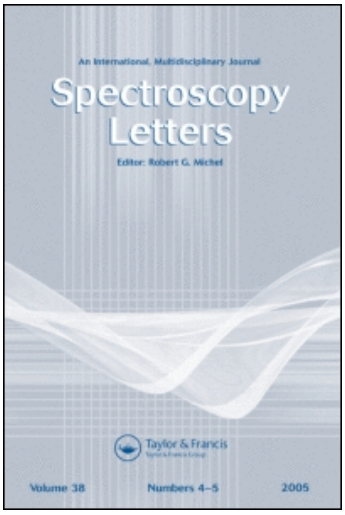

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Carbon-13 Chemical Shift Assignments for the Bisulfite Adducts of Uracil, 5-Deuterouracil, 5-Fluorouracil and 5-Chlorouracil

J. W. Triplett[a]; G. A. Digenis[a]; W. J. Layton[b]; S. L. Smith[b]
[a] College of Pharmacy University of Kentucky, Lexington, Kentucky [b] Department of Chemistry, University of Kentucky, Lexington, Kentucky

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CARBON-13 CHEMICAL SHIFT ASSIGNMENTS FOR THE BISULFITE ADDUCTS OF URACIL, 5-DEUTEROURACIL, 5-FLUOROURACIL AND 5-CHLOROURACIL

Key words: uracil, bisulfite, 5-deuterouracil, 5-fluorouracil, 5-chlorouracil, carbon-13 NMR

J. W. Triplett*, G. A. Digenis*,
W. J. Layton+, and S. L. Smith+

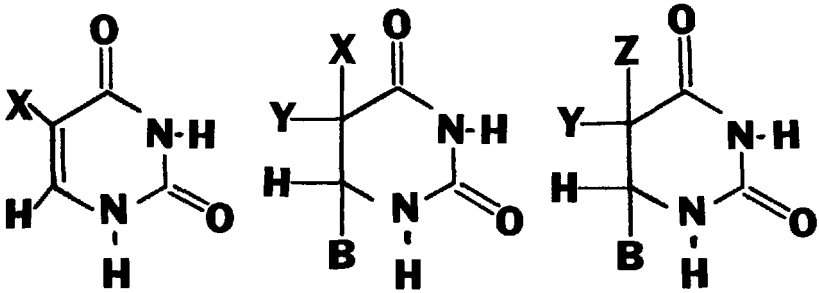
*College of Pharmacy and +Department of Chemistry, University of Kentucky, Lexington Kentucky 40506

ABSTRACT

Chemical shift assignments are made for the carbon atoms of the uracil-bisulfite adduct by use of specific labeling with deuterium and carbon-13. The signals of the analogous adducts from 5-chlorouracil and the pharmacologically important 5-fluorouracil are assigned.

Nucleophilic attack at C_6 is postulated as a key step in enzymatic reactions involving substitution at C_5 of biologically important pyrimidines, notably in the conversion of 2'-deoxyuridine-5'-monophosphate (5'-dUMP) to thymidine-5'monophosphate

by thymidylate synthetase; the reaction which is blocked by anti-tumor agents such as 5-fluorouracil and 5-fluro-2'-deoxyuridine (1). Reaction of bisulfite with uracil and its derivatives as shown in the first half of Scheme I is assumed to provide a valid model for these more complicated biological processes. Extensive study has shown these adducts[2-6] to be intermediates in the exchange[7] and dehalogenation[8-15] reactions of substituted uracils as shown in the second half of Scheme I. Further, the fact that bisulfite is the aqueous form of the common pollutant sulfur dioxide gives these reactions intrensic importance. For example, bisulfite has been shown to catalyse the deamination of cytosine to uracil[4] and to inhibit ribosomal activity suggesting a possible mutagenic role for bisulfite[16]. Our interest



X = H or D ; Y = H or D ; Z = H or D ; B = HSO_3^-

Scheme I

in using ^{13}C NMR to study the structure of complex intermediates and products from biological processes and to study the reaction of bisulfite with nucleic acids required unambiguous assignment of the ^{13}C chemical shifts of the simple bisulfite adducts of uracil and halouracils which we report here.

Uracil (U), 5-fluorouracil (5-FU), and 5-chlorouracil (5-ClU) were obtained from Aldrich Chemical Company and Calbiochem and used without further pruification. 5-deutereouracil (5-dU) was prepared by the method of Rork and Pitman[6]. Uracil enriched to 5% ^{13}C at position 6 was synthesised by methods reported elsewhere[17]. The spectra of these materials in d_6-DMSO (they are practically insoluble in water) are reported in Table 1. The chemical shift assignments are consistent with those reported previously by a variety of investigators[17] and are confirmed here by the expected couplings or intensity changes.

Reaction of uracil with bisulfite in water led to 5,6-dihydrouracil-6-sulfonate (U/HSO_3^-) whose ^{13}C NMR spectrum (Table 1) showed the disappearance of the signals at 100 and 141 ppm and the appearance of two new signals in the aliphatic region at 30.8 and 62.9 ppm. Off resonance decoupled or coupled spectra showed the upfield signal as a triplet and the downfield signal as a doublet establishing that the sulfonate group was attached to the carbon giving the downfield signal. When the adduct was formed from 5-deuterouracil and HSO_3^-, or from

Table 1

Carbon-13 Chemical Shifts[a]

Compound	Solvent	C-2	C-4	C-5	C-6
Uracil	DMSO	151.3	164.1	100.8	141.8
5-dU	DMSO	151.5	164.6	100.2(t) (J_{CD}=20.4Hz)	142.1
6-^{13}C-U	DMSO	151.7	164.7	100.5	142.4 (more intense)
5-FU	DMSO	149.6	157.4(d) (J_{CF}=25.4Hz)	139.3(d) (J_{FC}=226.7 Hz)	125.7(d) (J_{CF}=31.3Hz)
5-ClU	DMSO	150.2	159.6	105.6	139.2
U/HSO_3^-	H_2O	*[b]	*	30.8	63.0
5-dU/HSO_3^-	H_2O	*	*	30.8(t) (J_{CD}=21Hz)	63.8
6-^{13}C-U/HSO_3^-	H_2O	*	*	30.9	63.0 (more intense)
5-FU/HSO_3^-	H_2O	161.4	174.5(d) (J_{CF}=19.8 Hz)	91.5(d) (J_{CF}=187.6 Hz)	68.4(d) (J_{CF}=17.7Hz)
5-ClU/HSO_3^-	H_2O	*	*	50.0	70.3

a. Chemical shifts referenced to DMSO solvent or Dioxane in water as _____ and _____ respectively relative to TMS.
b. Signal too weak to detect.

uracil and DSO_3^- / D_2O, the same spectrum was observed with the signal at 30.8 ppm split into a triplet (J_{CD}= 21 Hz) confirming its assignment to C_5 of the adduct. The adduct formed from uracil enriched with ^{13}C at carbon 6 shows the expected in-

crease in intensity for the signal at 63 ppm confirming its assignment to C_6 of the adduct.

The adducts of 5-ClU and 5-FU with bisulfite give analogous spectra reported in Table 1. The shifts for C_6 (70.3 ppm) and C_5 (49.97 ppm) in 5-ClU/HSO_3^- can be assigned by analogy with the uracil adduct shifts after allowing for the expected effect of the chlorine. It has been suggested that bisulfite addition at carbon-6 of 5-ClU results in the formation of an anionic species at carbon-5, stable enough to allow proton addition both cis and trans giving two isomers[19]. In this investigation only one bisulfite adduct of 5-ClU was observed. The large one-bond C_5-F coupling (187.6 Hz) and the smaller C_6-F coupling (17.7 Hz) permit the unambiguous assignment of C_5 (91.5 ppm) and C_6 (63.8 ppm) in the adduct from the antitumor agent 5-FU.

The fact that C-5 of uracil (ca. 100 ppm) and C-5 of its adduct (ca. 30 ppm) occur in regions of the ^{13}C NMR spectrum which, for most biological samples of interest, do not contain other signals makes them particularity useful for the detection of the pyrimidine-bisulfite reaction in complex systems.

Spectra were measured with a Varian CFT-20 spectrometer. Typically, a 4K spectrum covering 200 ppm was accumulated using a pulse width of 7 microseconds (35° flip), no delay, an aquisition time of 0.512 seconds and broad band proton decoupling. Spectra from DMSO solutions required accumulation of a

few thousand scans. Spectra of the insoluable adducts in H_2O required 100,000 to 500,000 scans. Probe temperature was 30°C. The pH of all aqueous samples was 6.5-7.1.

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