Carbon-13 Nmr Spectra of the tRNA "Wobble" Nucleosides 5-Carboxymethyl-, 5-Carbomethoxymethyl-, and 5-Carbamoylmethyluridine (1)

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The ^{13}C nmr chemical shifts have been measured for 5-carboxymethyluracil **2**, 5-carbomethoxymethyluracil **3**, their 2-thio derivatives **4** and **5**, respectively, as well as of the three β-D-ribonucleosides, 5-carboxymethyluridine **7**, 5-carbomethoxymethyluridine **8**, and 5-carbamoylmethyluridine **9**. In addition, the ^{13}C-^{1}H coupling constants for **2** and **7** have also been obtained.

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The β-D-ribonucleosides 5-carboxymethyluridine **7** (2), 5-carbomethoxymethyluridine **8** (3), and 5-carbamoylmethyluridine **9** (4) have been isolated from several species of tRNA, where they are found at the 5′-end of the respective anticodons. The β-D-ribonucleosides of the 2-thiouracil derivatives **4, 5** have also been found to occur naturally (5). The presence of 5-substituents in uridine bases at that anticodon position, and in some cases, replacement of the 2-keto group by a thione function, is believed to be related to their ability to undergo Watson-Crick and/or alternate "wobble" hydrogen bonding interactions (6).

We report here the ^{13}C nmr data for 5-carboxymethyluracil **2**, 5-carbomethoxymethyluracil **3**, their 2-thio derivatives **4** and **5**, respectively, as well as of the three above β-D-ribonucleosides **7, 8,** and **9**. The ^{13}C chemical shifts and ^{13}C-^{1}H coupling constants are presented in Tables I and II, respectively.

At both the uracil base **1-5** and nucleoside **7-9** level, the largest ^{13}C shift relative to uracil **1** and uridine **6** occurs as expected at C-5, the position of substitution. This shift is about 7 ppm downfield for each derivative, and is similar to that observed in ribothymidine **10** with the methyl substituent. At C-6, the result of such substitution is an upfield shift only slightly greater than 1 ppm. Similarly, at C-2 and C-4, the shifts are downfield, and less than 1 ppm in all cases. These results suggest that these three substituents have approximately the same effect as a methyl group on the electronic charge distribution of the uracil base, and this appears to be minimal at C-2 and C-4, the two possible acceptor sites for hydrogen bonding.

An alteration from the normal *anti* glycosyl conformation to a significant population of *syn* type states would be expected to alter the ^{13}C chemical shifts of the furanose carbons relative to uridine. For compounds **7-9**, all such shifts were found to be less than 0.15 ppm. On the other hand, a similar comparison of cytidine and 6-methyl-cytidine shows shifts of 1.2 to 4.9 ppm, which have been interpreted in terms of a shift from an *anti* conformation in the former to a preference for *syn* type conformers in the latter (13). Thus, the similarity of the furanose ^{13}C shifts in 5-carboxymethyluridine, 5-carbamoylmethyluridine, and 5-carbomethoxymethyluridine suggests *anti* conformations in each case. This interpretation is consistent with the measured value of $J_{C(2),H(1')} = 2$ Hz for 5-carboxymethyluridine **3** (Table II), which is similar to that reported for uracil nucleosides in an *anti* conformation (2.4 Hz), and outside the range (6.5-8.0 Hz) for those in a *syn* conformation (12). It is also in agreement with the results of our earlier X-ray crystallographic study which demonstrated that both 5-carboxymethyluridine and 5-carbamoylmethyluridine exist in an *anti* conformation in the solid state (14, 15). Thus, whatever effect the 5-substituent exerts on the function of these "wobble" bases may not be related to an alteration in the *syn/anti* equilibrium.

EXPERIMENTAL

Compounds **2-5** and **7-9** were synthesized according to procedures reported previously from this laboratory (16). Uridine (**6**) was a commercial sample (Sigma), and was used without further purification. The ^{13}C nmr spectra were obtained from a solution of 20-50 mg. of each derivative dissolved in *ca.* 1 ml. of DMSO-d_6, and were recorded on a JEOL-PFT-100 spectrometer operating in the Fourier transform mode. Chemical shifts are in ppm downfield from internal DMSO. Additional information on the exact experimental conditions used for each spectrum is contained in Tables I and II.

REFERENCES AND NOTES

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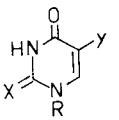
Table I

^{13}C Chemical Shifts for 5-Carboxymethyluracil and Related Compounds (a)



Compound	Substituents			δC_2	δC_4	δC_5	δC_6	δC_7	δC_8	δC_9	$\delta C_1'$	$\delta C_2'$	$\delta C_3'$	$\delta C_4'$	$\delta C_5'$
	X =	Y=	R=												
1(b)	O	-H	-H	110.95	123.76	59.78	101.63								
2	O	$-CH_2-\overset{O}{\overset{\|}{C}}-OH$	-H	111.88	124.74	67.32	100.42	-8.01	132.65						
3	O	$-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$	-H	111.78	124.59	66.64	100.61	-8.30	131.58	12.09					
4	S	$-CH_2-\overset{O}{\overset{\|}{C}}-OH$	-H	135.61	121.73	72.95	100.37	-7.91	133.02						
5	S	$-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$	-H	135.71	121.58	72.27	100.57	-8.20	131.00	12.23					
6	O	-H	-BR(d)	111.20	123.57	62.22	101.20				48.20	34.02	30.38	45.33	21.36
				(112.1)	(125.2)	(63.6)	(102.7)				(49.6)	(35.4)	(31.6)	(46.5)	(22.7)
7	O	$-CH_2-\overset{O}{\overset{\|}{C}}-OH$	-BR(d)	111.15	123.52	68.87	98.92	-7.43	132.31		48.00	33.78	30.34	45.33	21.50
8	O	$-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3$	-BR(d)	111.05	123.38	68.19	99.11	-7.77	131.29	12.13	48.00	33.78	30.29	45.28	21.45
9	O	$-CH_2-\overset{O}{\overset{\|}{C}}-NH_2$	-BR(d)	111.20	123.67	69.41	98.92	-6.26	131.77		48.20	33.83	30.23	45.19	21.40
10(e)	O	CH_3	-BR(d)	114.4	124.9	70.2	98.2	-27.4			47.8	34.3	30.7	45.5	22.0

(a) 1-10K FT accumulations. (b) Chemical shifts based upon the data of P. D. Ellis, *et al.*, (7); the assignment of C(2) and C(4) is consistent with the difference in multiplicity of these carbon signals in uracil-$^{15}N_2$-1,3 (8); corresponding assignments for the ribose carbons are based upon the work of H.H Mantsch and I. C. P. Smith (9). Assignments confirmed in some cases from the multiplicity and magnitude of ^{13}C-H coupling obtained from separate non-H-decoupled spectra (Table II). (c) Values in parenthesis from H. Sugiyama, *et al.*, (10). (d) BR = β-riboside. (e) From H. Sugiyama, *et al.*, (10).

Table II

^{13}C-^{1}H Coupling Constants for 5-Carboxymethyluridine and Related Compounds (In Hz)

Compound (a)	Base Ring								Sugar Ring (J)				
	C2		C4		C6		C7	C8					
	H6	H1′	H6	H7	H6	H7	H7	H7	C1′	C2′	C3′	C4′	C5′
2(b)	8		(c)	(c)	179	6	129	8					
Uracil (d)					180.5								
Thymine (d)					177.8								
7(e)	6	2	7	3	182	(c)	129	8	169	148	150	148	139
Uridine (f)	8.0	2.4	9.4		183.7				168.5	155.7	152.0	155.6	143.5
Thymidine (f)	7.8	2.0	9.8		181.6				169.9	134.2	149.9	149.4	143.4
5-Br-Uridine (f)	8.5	2.4	8.6		186.8				172.4	152.6	150.8	149.3	142.8
2′,3′-ip-2,5′-O-Cyclouridine (g)	7.2	6.6											

(a) For structures represented by compound numbers, see Table I. (b) 2,000 Transients; for 4 KHz sweep width, 8K data points, resolution = 1.2 Hz. (c) Value not observed. (d) From P. D. Ellis, *et al.*, (7) in DMSO-d_6 solution. (e) 13,000 Transients; for 5 KHz sweep width, 8K data points, resolution = 1.2 Hz. (f) From D. B. Davies (11); ± 0.3 Hz; in deuterium oxide solution. (g) R. U. Lemieux, *et al.*, (12).

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