CRYSTAL AND MOLECULAR STRUCTURE OF THE METHYL ESTER OF URIDIN-5-OXYACETIC ACID: A MINOR CONSTITUENT OF *ESCHERICHIA COLI* tRNAs

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1. Introduction

Uridin-5-oxyacetic acid, designated as V,* is a minor constituent of *Escherichia coli* $tRNA_1^{Val}$ and $tRNA_1^{Ser}$ found at the first position of the anticodon [1,2]. The structure of V is of particular interest, because it participates directly in codon–anticodon base-pairing in the decoding process of protein biosynthesis. *E. coli* $tRNA_1^{Val}$ can recognize not only the codon GUA and GUG, but also GUU, although the binding efficiency for GUU is only 20% of those for the other two codons [3]. Similarly, *E. coli* $tRNA_1^{Ser}$, that also contains V in the first position of the anti-codon is recognized by UCU with 30% of the efficiency of recognition by UCA or UCG (2). This suggests that the ability of V to form hydrogen-bonds differs from that of U. (An infrared study [4] indicated that the association constant between adenine and uracil derivatives in $CDCl_3$ increases on substitution of CH_3^-, Br^-, or I^- at the C(5) position).

Therefore, it seemed interesting to compare the molecular structure of V, precisely determined by X-ray analysis, with those of other C(5) substituted uridine derivatives. This paper reports results of analyses of the crystal structure of a V derivative, uridin-5-oxyacetic acid methyl ester. Details of this work will be published elsewhere.

* The abbreviations used are: V: Uridin-5-oxyacetic acid, mV: uridin-5-oxyacetic acid methyl ester, FUdR: 5-Fluoro-2′-deoxy-β-uridine, ClUR: 5-Chlorouridine, BrUR: 5-Bromouridine, IUR(I): 5-Iodouridine form (I), IUR(II): 5-Iodouridine form(II), TdR=Thymidine, MeUR: 5-Methyluridine, 3′UMP: Uridine-3′-phosphate disodium salt.

2. Materials and methods

The uridin-5-oxyacetic acid used in this study was synthesized as described previously [1]. The crystals were grown by slow evaporation of methyl alcohol containing uridin-5-oxyacetic acid at 50°C. Plate-like crystals of uridin-5-oxyacetic acid methyl ester appeared in about a week. Esterification of uridin-5-oxyacetic acid presumably occurred during the crystallization procedure.

The crystal data are given in table 1. Three dimensional intensity data were obtained by a Rigaku four-

Table 1
Crystal data on uridin-5-oxyacetic acid methyl ester

Formula	$C_{12}H_{16}N_2O_9 \cdot H_2O$
Mol. wt.	332.3
a	11.159 ± 0.002 Å
b	14.461 ± 0.002 Å
c	4.821 ± 0.001 Å
β	101.15 ± 0.02°
Space group	$P2_1$
Z	2
Density	D_{cal}=1.45 g·cm^{-3}

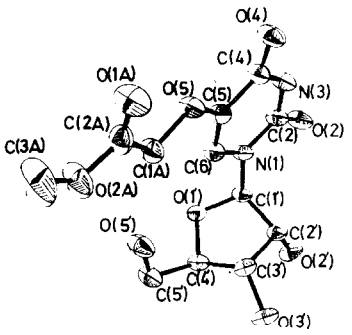

Fig. 1. Conformation of uridin-5-oxyacetic acid methyl ester. The atoms are represented by ellipsoids defined by the principal axes of thermal motion.

circle diffractometer, using Ni filtered Cu-Kα radiation. Intensity data with 2θ values up to 135° were collected by an ω-2θ scanning technique.

The structure (see fig. 1) was solved by the Patterson method and the positional and thermal parameters of atoms were refined by block-diagonal least-squares method. The final R value for 1332 reflections was 0.040. The estimated standard deviations of the distances are 0.005 – 0.008 Å and those of the bond angles are 0.3–0.4°.

3. Results

The structures of various C(5) substituted uridine derivatives have been determined by X-ray analyses. The bond lengths and angles of these derivatives are listed in tables 2 and 3 for comparison.

As seen in table 2, there are some significant differences in the bond lengths. The length of N(1)–C(2) in the V derivative is shorter than that in any other C(5) substituents while that of N(1)–C(6) is longer. Moreover, the lengths of these bonds are significantly different from those in bases of thymidine, 5-methyluridine and uridine-3′-phosphate, all of which are naturally occuring nucleic acid constituents. It is also of interest to note that the differences between the bond distances of C(2)–O(2) and C(4)–O(4) in the V derivative and in 5-bromouridine seem to be smaller than in the others, even taking the effects of hydrogen bonding in the crystals into consideration. In addition, the C(2)–O(2) distance is longer than C(4)–O(4) in the V derivative, while it is shorter than the latter in both thymidine and 5-methyluridine; the standard deviations in these C–O distance determinations are 0.005 – 0.007 Å, which are small enough to permit these conclusions. These facts indicate a slight but definite difference in the resonance structure of the base from those of other uracil derivatives, possibly representing a difference in the conjugation system along the bond C(5)–C(6)–N(1)–C(2)–O(2).

No systematic difference between the bond angles of the V derivative and other C(5) substituents is observed. However, there are small but appreciable differences in the N(1)–C(2)–N(3), C(5)–C(4)–N(3), C(6)–C(5)–C(4) and C(5)–C(6)–N(1) angles of the V derivative from the corresponding angles in thymidine, 5-methyluridine, and uridine-3′-phosphate.

Table 2
A comparison of the bond lengths (Å) in bases

Bond	mV	FUdR	ClUR	BrUR	IUR(I)	IUR(II)	T	MeU	3′UMP
N(1)–C(2)	1.366	1.394	1.375	1.40	1.37	1.39	1.385	1.377	1.383
C(2)–N(3)	1.370	1.377	1.383	1.37	1.36	1.36	1.381	1.376	1.379
N(3)–C(4)	1.381	1.373	1.375	1.39	1.44	1.37	1.378	1.384	1.381
N(1)–C(6)	1.401	1.365	1.373	1.35	1.38	1.37	1.374	1.361	1.380
C(4)–C(5)	1.437	1.433	1.437	1.43	1.44	1.49	1.453	1.437	1.440
C(5)–C(6)	1.337	1.331	1.335	1.36	1.37	1.32	1.343	1.345	1.334
C(2)–O(2)	1.227	1.198	1.215	1.23	1.23	1.23	1.206	1.196	1.219
C(4)–O(4)	1.224	1.235	1.230	1.23	1.19	1.25	1.230	1.223	1.231
N(1)–C(1′)	1.499	1.481	1.474	1.49	1.49	1.48	1.480	1.481	1.459
Standard	0.005	0.008	0.004	0.02	0.15	0.15	0.006	0.006	0.005
deviation	~0.007	~0.011	(average)	(average)	~0.3	~0.3	~0.007	~0.007	(average)
Reference		(5)	(6)	(7)	(8)	(8)	(9)	(10)	(11)

Table 3
A comparison of the bond angles (°)

Bond angle	mV	FUdR	ClUR	BrUR	IUR(I)	IUR(II)	T	MeU	3'UMP
C(6)–N(1)–C(1')	120.0	120.2	118.9	120.0	120.5	121.6	121.6	121.5	118.1
C(2)–N(1)–C(1')	116.6	117.0	118.9	117.5	115.6	116.6	116.6	116.8	119.4
C(2)–N(1)–C(6)	121.9	122.4	121.7	122.0	123.5	121.8	121.8	121.6	122.0
N(1)–C(2)–N(3)	115.2	113.9	114.5	114.0	113.7	113.7	113.7	114.1	114.0
N(1)–C(2)–O(2)	122.6	123.4	124.2	123.8	127.5	124.3	124.3	123.0	123.8
N(3)–C(2)–O(2)	122.2	122.8	121.3	121.7	118.8	122.0	122.0	122.9	122.3
C(4)–N(3)–C(2)	127.5	128.0	127.4	127.8	126.1	127.5	127.5	127.2	127.2
C(5)–C(4)–N(3)	113.5	112.6	113.7	112.8	116.7	115.8	115.8	115.4	115.0
C(5)–C(4)–O(4)	126.1	124.1	126.2	125.8	122.2	124.5	125.4	125.4	125.4
N(3)–C(4)–O(4)	120.4	123.3	120.1	121.4	121.1	119.7	119.7	119.2	119.6
C(6)–C(5)–C(4)	121.6	122.7	120.6	121.4	116.4	117.2	117.2	117.6	119.1
C(5)–C(6)–N(1)	120.3	120.3	122.0	121.3	123.3	124.1	124.1	124.2	122.6
Standard deviation	0.3–0.5	0.5–0.7	0.3 (average)	1.0 (average)	1.0–2.0	1.0–2.0	0.3–0.5	0.5 (average)	0.3 (average)
Reference		(5)	(6)	(7)	(8)	(8)	(9)	(10)	(11)

Another difference to be noted is in the planarity of the base. The ribose carbon C(1') in the V derivative is significantly displaced from the least-squares plane of the pyrimidine ring, the deviation being 0.242 Å. This deviation is the second largest among those found in the C(5) substituents so far examined. (In 5-bromouridine, it is as large as 0.255 Å [7]).

The bond length and angles in the ribose moiety are within the normal range. The glycosidic torsion is anti, $\chi = 34.3°$. The conformation of the sugar ring is C(3')-endo which is known to be preferable in pyrimidine nucleosides and nucleotides.

4. Discussion

The changes in the structure of the base residue on conversion from U to V derivatives should be attributed to oxygen substitution at the 5 position. The C(5)–O(5) distance, 1.377 Å, is shorter than the normal C–O single bond length (1.43 Å) [12], but it is very close to that often found in a C–O bond adjacent to a C=C double bond [13]. It is therefore suggested that this bond has a partial double bond character as the result of electron migration from the lone pairs of O(5) to the π-electron system of pyrimidine. Such an electron migration should cause a considerable change of the electron distribution in the pyrimidine ring, and a change in the ability for inter-base hydrogen bonding.

It is further speculated that for some reason the proton accepting power of O(2) in V is stronger than that in U or T. In fact, in a crystal of 1-ethyl-5-bromouracil O(2), instead of O(4), is involved in inter-base hydrogen bonding [14–17], and it has been shown that the structural change on conversion from U to BrUR [14–17] is quite similar to the change on conversion from U to V.

With regard to the U(codon)–V(anticodon) base-pair, the occurrence of which has previously been indicated [2,3], we propose that there is a hydrogen-bond between NH(3) of U and O=C(2) of V and between C=O (4) of U and H–N(3) of V, as shown in fig. 2. There is

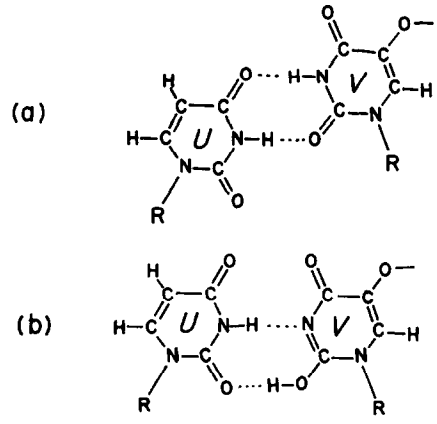

Fig. 2. Probable U–V base pairing.

no indication so far that the corresponding scheme of base pairing between U and U is involved in the possible condon–anticondon pairing [18]. It is probable that the increase in the proton accepting power of O(2) on conversion from U to V, as just suggested, is responsible for the ability of V to pair with U.

Lastly it should be pointed out, however, that there is another possibility. The observed changes in bond-lengths on going from U to V are consistent with an idea that an enol form is slightly more preferable in V than that in U. Therefore, the U–V base pair, now in question, may be what is shown in fig. 2(b), which involves the enol form of V.

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