

Uridine-5-oxycetic Acid Methyl Ester Monohydrate

BY KOSUKE MORIKAWA, KAZUO TORII, YOICHI IITAKA AND MASAMICHI TSUBOI

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Tokyo 113, Japan

(Received 31 October 1974; accepted 14 November 1974)

The title compound, $C_{12}H_{16}O_9N_2 \cdot H_2O$, F.W. 332.3, crystallizes with $a = 11.159$ (2), $b = 14.461$ (2), $c = 4.821$ (1) Å, $\beta = 101.15$ (2)°, $U = 763.2$ Å³, $D_x = 1.45$ g cm⁻³ and space group $P2_1$, $Z = 2$. The crystal structure was refined to an R value of 0.04 for 1332 observed reflexions including 13 hydrogen atoms out of 18. The conformation of the sugar moiety is C(3')-endo with $\chi = 34.3^\circ$ (*anti*), $\varphi_{OO} = -58.7$ and $\varphi_{OC} = 58.8^\circ$ (*gg*). The bases are stacked along the c axis but are tilted by $46^\circ 42'$ relative to the c axis. No direct hydrogen bonds are found between the bases.

Experimental

Crystals were grown by slow evaporation of a methanol solution of uridine-5-oxycetic acid at 50°C. Plate-like crystals of the methyl ester appeared in about a week. The lattice constants and the intensity data were measured on a Rigaku four-circle diffractometer using Ni-filtered Cu $K\alpha$ radiation. Intensities of reflexions with 2θ values up to 135° were collected by the θ - 2θ scan method with a scan rate of 2θ 2° min⁻¹. Background was measured at each end of the scan for 10 s. The size of the crystal was about 0.1 × 0.25 × 0.7 mm. The intensities were corrected for Lorentz and polarization factors and were then converted to normalized structure factors. The total number of independent observed reflexions above the 2σ level was 1332 out of 1356 theoretically possible reflexions.

Attempts at determination of the crystal structure by direct methods gave no conclusive result. The Patterson map, on the other hand, gave the orientation of a hexagonal pyrimidine nucleus along with two substituent atoms at the *para* positions lying in the plane. The structure factors for the $h0l$ reflexions were therefore calculated by assuming the disposition of eight atoms which may form a 1,4-disubstituted pyrimidine base. A two-dimensional R map was then calculated by translating these atoms together along the a and c axes. The most probable x and z coordinates of an arbitrarily chosen origin fixed to the atomic group could be found on the R map. The y coordinate was chosen arbitrarily. More precise values of x and z were determined by searching the lowest R values near the position obtained above, but in this case the structure factors of all hkl reflexions were taken into account. The electron density map synthesized by using the phase angles calculated on the basis of the eight atoms showed a further 13 atoms along with a similar number of spurious peaks. The whole structure was revealed by successive use of Fourier and difference Fourier syntheses.

Refinement of the structure was carried out by the block-diagonal least-squares method (program by

Okaya & Ashida, 1967). 13 Hydrogen atoms out of 18 could be located on the difference electron density map calculated at the stage when $R = 0.07$. Subsequent least-squares refinement in which anisotropic thermal motions were assumed for non-hydrogen atoms and isotropic for hydrogen atoms gave $R = 0.04$. Unit weight was applied for each reflexion in this calculation. The atomic scattering factors for C, N and O were those cited in *International Tables for X-ray Crystallography* (1962) as SX-6, 7 and 8 respectively, and for H those given by Stewart, Davidson & Simpson (1965). The final atomic coordinates are listed in Table 1.*

Discussion

Uridine-5-oxycetic acid is a minor constituent occurring at the first position of the anticodon of *E. coli* RNA₁^{val} (Murao, Saneyoshi, Harada & Nishimura, 1970). This molecule takes part in a codon-anticodon base pairing in the decoding process of protein biosynthesis.

The bond lengths and angles are listed in Table 2 together with their estimated standard deviations. Among the lengths of the bonds involved in the uracil base, it was noticed that N(1)-C(6) and C(2)-O(2) are longer and N(1)-C(2) is shorter than usual, and a comparison of these values with those found in various C(5)-substituted pyrimidine derivatives as well as a discussion about the effects of introducing an oxycetate group at the C(5) position have already been presented in a previous paper (Morikawa, Torii, Iitaka, Tsuboi & Nishimura, 1974).

The conformation of the molecule can be seen in Fig. 1. The planarity of the base is illustrated in Table 3. It is to be noted that although the displacements of

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30787 (7pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 1. *Atomic parameters*(a) Heavy atoms. Values are $\times 10^4$.Anisotropic temperature factors are of the form: $T = \exp \{ -(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl) \}$.

	<i>x</i>	<i>y</i>	<i>z</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(2)	662 (4)	2343 (0)	2104 (10)	58 (4)	20 (2)	319 (24)	-3 (3)	0 (8)	-4 (6)
N(3)	893 (4)	1675 (3)	4155 (9)	61 (4)	26 (2)	320 (20)	-12 (2)	-20 (7)	21 (5)
C(4)	1892 (4)	1613 (4)	6344 (10)	52 (4)	27 (2)	280 (22)	-4 (3)	6 (8)	10 (6)
C(5)	2775 (4)	2334 (4)	6313 (10)	42 (4)	25 (2)	277 (21)	-2 (3)	-15 (7)	1 (6)
C(6)	2604 (4)	2997 (3)	4345 (10)	44 (4)	24 (2)	251 (21)	0 (3)	-13 (7)	2 (6)
N(1)	1546 (3)	3003 (3)	2235 (8)	48 (3)	17 (2)	289 (18)	-0 (2)	-13 (6)	5 (5)
O(2)	-278 (3)	2350 (3)	298 (8)	65 (3)	29 (2)	399 (18)	-10 (2)	-58 (6)	14 (5)
O(4)	1950 (3)	1000 (3)	8116 (8)	66 (3)	37 (2)	424 (19)	-12 (2)	-23 (6)	59 (5)
O(5)	3771 (3)	2265 (3)	8493 (7)	52 (3)	30 (2)	338 (17)	-4 (2)	-40 (5)	26 (5)
C(1A)	4643 (4)	2993 (4)	8622 (11)	50 (4)	35 (3)	345 (24)	-5 (3)	-7 (8)	14 (7)
C(2A)	5610 (4)	2829 (4)	11247 (11)	45 (4)	46 (3)	357 (26)	-3 (3)	2 (8)	-20 (8)
O(1A)	5594 (4)	2242 (3)	12942 (8)	91 (4)	53 (2)	417 (20)	-14 (3)	-58 (7)	45 (6)
O(2A)	6474 (4)	3466 (3)	11333 (9)	62 (3)	68 (3)	560 (23)	-24 (3)	-41 (7)	29 (7)
C(3A)	7463 (6)	3472 (7)	13869 (16)	75 (6)	108 (6)	663 (42)	-33 (6)	-107 (13)	13 (15)
C(1')	1238 (4)	3825 (3)	339 (9)	52 (4)	21 (2)	240 (20)	1 (3)	-2 (7)	12 (6)
C(2')	561 (4)	4548 (3)	1719 (10)	50 (4)	23 (2)	285 (22)	6 (3)	8 (7)	14 (6)
C(3')	1624 (4)	5177 (3)	3107 (9)	60 (4)	23 (2)	237 (20)	7 (3)	5 (7)	-6 (6)
C(4')	2436 (4)	5166 (3)	952 (10)	59 (4)	19 (2)	249 (20)	-1 (3)	3 (7)	-2 (6)
C(5')	3770 (5)	5378 (4)	1997 (11)	68 (5)	30 (3)	400 (25)	-12 (3)	8 (9)	9 (7)
O(1')	2320 (3)	4224 (2)	-170 (6)	55 (3)	21 (2)	246 (14)	-1 (2)	23 (5)	-6 (4)
O(2')	-228 (3)	5022 (3)	-520 (7)	62 (3)	32 (2)	358 (18)	13 (2)	-30 (6)	2 (5)
O(3')	1252 (3)	6066 (2)	3821 (7)	81 (3)	22 (2)	325 (16)	12 (2)	-1 (6)	-16 (5)
O(5')	4267 (3)	4726 (3)	4118 (9)	64 (3)	38 (2)	536 (22)	-14 (2)	-47 (7)	35 (6)
O(W)	3894 (4)	731 (3)	12832 (9)	90 (4)	53 (3)	500 (22)	10 (3)	-43 (8)	42 (6)

Table 1 (cont.)

(b) Hydrogen atoms. Positional parameters are $\times 10^3$.

	<i>x</i> ($\times 10^3$)	<i>y</i> ($\times 10^3$)	<i>z</i> ($\times 10^3$)	<i>B</i> (\AA^2)
H(N3)	25 (5)	134 (5)	454 (13)	5.1 (1.6)
H(C6)	325 (5)	344 (4)	395 (12)	4.6 (1.4)
H(C1A)	517 (4)	296 (3)	695 (9)	2.0 (1.0)
H'(C1A)	419 (5)	362 (4)	895 (11)	4.6 (1.4)
H(C1')	72 (4)	354 (4)	-157 (10)	2.6 (1.1)
H(C2')	2 (4)	430 (4)	303 (10)	2.5 (1.1)
H(C3')	218 (5)	493 (4)	512 (10)	2.7 (1.1)
H(C4')	218 (5)	560 (4)	-55 (10)	3.3 (1.2)
H(C5')	369 (5)	606 (4)	262 (11)	4.5 (1.4)
H'(C5')	427 (5)	534 (4)	40 (11)	4.2 (1.3)
H(O2')	-79 (5)	532 (4)	41 (11)	4.3 (1.4)
H(O3')	110 (5)	638 (4)	254 (11)	3.8 (1.3)
H(O5')	474 (6)	483 (5)	490 (15)	6.2 (1.8)

O(2), O(4) and O(5) from the plane of the pyrimidine ring are not so significant, C(1') deviates 0.242 Å from the plane on the same side as C(2'). This displacement is the largest among the C(5)-substituted uracil bases so far determined and is comparable with that found in 5-bromouridine (0.255 Å: Iball, Morgan & Wilson, 1966).

The oxyacetate group takes a *trans* planar conformation (the torsional angles are listed in Table 4), and extends from C(5) almost on the same plane of the base. The deviations of the atoms from the base plane are shown in Table 3.

The torsional angles in the ribose moiety are also listed in Table 4. The χ angle, 34.3°, lies in the *anti* region. The $\tau_0 \sim \tau_4$ values indicate that the conforma-

Table 2. *Bond lengths* (Å) *and angles* (°)

N(1)—C(2)	1.366 (5)	N(3)—C(2)—O(2)	122.3 (0.4)
C(2)—N(3)	1.370 (5)	C(4)—N(3)—C(2)	127.5 (0.4)
N(3)—C(4)	1.381 (6)	C(5)—C(4)—N(3)	113.5 (0.4)
N(1)—C(6)	1.401 (5)	C(5)—C(4)—O(4)	126.1 (0.5)
C(4)—C(5)	1.437 (7)	N(3)—C(4)—O(4)	120.4 (0.5)
C(5)—C(6)	1.337 (7)	C(4)—C(5)—C(6)	121.6 (0.4)
C(2)—O(2)	1.227 (5)	C(4)—C(5)—O(5)	113.1 (0.4)
C(4)—O(4)	1.224 (6)	C(6)—C(5)—O(5)	125.3 (0.4)
C(5)—O(5)	1.377 (5)	C(5)—C(6)—N(1)	120.3 (0.4)
N(1)—C(1')	1.499 (6)	C(5)—O(5)—C(1A)	115.0 (0.4)
O(5)—C(1A)	1.428 (6)	O(5)—C(1A)—C(2A)	106.9 (0.4)
C(1A)—C(2A)	1.514 (7)	C(1A)—C(2A)—O(2A)	108.2 (0.5)
C(2A)—O(1A)	1.181 (7)	O(1A)—C(2A)—C(1A)	126.0 (0.5)
C(2A)—O(2A)	1.329 (7)	O(1A)—C(2A)—O(2A)	125.8 (0.5)
O(2A)—C(3A)	1.480 (8)	C(2A)—O(2A)—C(3A)	116.8 (0.5)
O(1')—C(1')	1.402 (6)	O(1')—C(1')—N(1)	109.2 (0.4)
C(1')—C(2')	1.516 (7)	O(1')—C(1')—C(2')	108.2 (0.4)
C(2')—C(3')	1.540 (6)	N(1)—C(1')—C(2')	110.8 (0.4)
C(3')—C(4')	1.504 (7)	C(1')—C(2')—C(3')	101.0 (0.4)
C(4')—O(1')	1.463 (6)	C(1')—C(2')—O(2')	106.6 (0.4)
C(2')—O(2')	1.429 (6)	C(3')—C(2')—O(2')	110.9 (0.4)
C(3')—O(3')	1.414 (6)	C(2')—C(3')—C(4')	102.1 (0.4)
C(4')—C(5')	1.508 (7)	C(2')—C(3')—O(3')	113.8 (0.4)
C(5')—O(5')	1.422 (7)	C(4')—C(3')—O(3')	115.0 (0.4)
C(6)—N(1)—C(1')	120.6 (0.4)	C(3')—C(4')—O(1')	104.0 (0.4)
C(2)—N(1)—C(1')	116.6 (0.4)	C(3')—C(4')—C(5')	117.0 (0.4)
C(2)—N(1)—C(6)	121.9 (0.4)	O(1')—C(4')—C(5')	108.9 (0.4)
N(1)—C(2)—N(3)	115.2 (0.4)	C(4')—O(1')—C(1')	109.6 (0.3)
N(1)—C(2)—O(2)	122.6 (0.4)	C(4')—C(5')—O(5')	109.2 (0.4)

tion of the sugar ring is C(3')-endo, the displacement of the C(3') atom from the least-squares plane formed by C(1'), C(2'), C(4') and O(1') being 0.589 Å on the same side as C(5'). This plane makes an angle of 82.9° with the pyrimidine base plane, which is significantly

Table 3. *Deviations of atoms from the least-squares plane of the base*

Equation of the least-squares plane is $-0.4659X + 0.5592Y + 0.6858Z = 2.1495$, where $X \parallel a^*$, $Y \parallel b$ and $Z \parallel c$, measured in Å.

Deviations			
N(1)	0.001 Å	C(1')*	0.242 Å
C(2)	0.005	C(2')*	1.728
N(3)	-0.009	O(1')*	-0.316
C(4)	0.007	C(1A)*	0.066
C(5)	-0.001	C(2A)*	0.164
C(6)	-0.002	O(1A)*	0.261
O(2)*	0.033	O(2A)*	0.140
O(4)*	0.059	C(3A)*	0.332
O(5)*	0.008	O(W)*	0.121

* Not included in the least-squares calculation.

Table 4. *Torsional angles (°) and the conformation of the ribose moiety*

Oxyacetate group

C(4)—C(5)—O(5)—C(1A)	-177.1
C(5)—O(5)—C(1A)—C(2A)	176.2
O(5)—C(1A)—C(2A)—O(2A)	176.4
C(1A)—C(2A)—O(2A)—C(3A)	175.4

Ribose group

χ [C(6)—N(1)—C(1')—O(1')]	34.3 (<i>anti</i>)
τ_0 [C(4')—O(1')—C(1')—C(2')]	3.2
τ_1 [O(1')—C(1')—C(2')—C(3')]	-25.4
τ_2 [C(1')—C(2')—C(3')—C(4')]	37.0
τ_3 [C(2')—C(3')—C(4')—O(1')]	-36.1
τ_4 [C(3')—C(4')—O(1')—C(1')]	21.2

Puckering: C(3')—*endo*

φ_{000} [O(1')—C(4')—C(5')—O(5')]	-58.7
φ_{00c} [C(3')—C(4')—C(5')—O(5')]	58.8
Conformation about C(4')—C(5'): <i>gauche-gauche</i>	

large when compared with the usual pyrimidine nucleosides and nucleotides with the *anti* conformation.

The projections of the crystal structure along the *c* and *a* axes are shown in Figs. 1 and 2. As seen in Fig. 2, the base planes are tilted and stacked along [001]. The normal to the base plane makes an angle of $46^\circ 42'$ with the *c* axis, and the perpendicular distance between successive base planes is 3.306 Å. There are many short contacts of atoms between these bases (Table 5). Note also that most of the close approaches of atoms occur at contacts between the molecules translated along the *c* axis.

Contrary to expectation, there is no hydrogen bond between the uracil bases. The atoms N(3) and O(4), which are known to form hydrogen bonds of Watson-Crick or Hoogsteen types, and O(2), which also often participates in hydrogen bonds between bases,* form hydrogen bonds to the ribose hydroxyl groups, O(2') and O(3'), and to the water oxygen atom. The interatomic distances calculated for the presumed hydrogen bonds are also listed in Table 5. As shown in Figs. 1 and 2, hydrophilic groups are arranged roughly in layers parallel to (010), and the hydrogen bonds are formed within the layer connecting the molecules related by the screw dyad axes.

The authors wish to express their sincere thanks to Dr S. Nishimura of the National Cancer Centre Research Institute in Tokyo for much valuable advice and discussion.

* Such as those found in reversed Watson-Crick or reversed Hoogsteen type base pairs.

Table 5. *Intermolecular atomic distances (Å) less than 3.5 Å*

The molecules are at the positions:

i	x, y, z	iv	$x, y, -1+z$
ii	$-x, -\frac{1}{2}+y, -z$	v	$-x, \frac{1}{2}+y, -z$
iii	$-x, -\frac{1}{2}+y, 1-z$	vi	$1-x, -\frac{1}{2}+y, 2-z$

From atom at i	to atom	at	Distance	From atom at i	to atom	at	Distance
C(2)	C(4)	iv	3.491 (7)	O(2)	O(3')	ii	2.782 (5)
C(2)	O(4)	iv	3.256 (6)	O(2)*	H(O3')	ii	2.05 (5)
C(2)	O(2')	ii	3.412 (5)	N(3)	O(2')	ii	2.972 (6)
O(2)	O(4)	iv	3.480 (5)	H(N3)	O(2')	ii	2.72 (6)
O(2)	O(2')	ii	3.412 (5)	N(3)	O(3')	iii	2.892 (6)
N(3)	O(4)	iv	3.485 (6)	H(N3)*	O(3')	iii	2.02 (7)
C(4)	O(W)	iv	3.309 (7)	O(4)	O(2')	iii	2.810 (5)
C(5)	O(W)	iv	3.250 (7)	O(4)*	H(O2')	iii	1.87 (6)
C(6)	O(5)	iv	3.492 (6)	O(W)*	O(4)	i	2.850 (5)
O(1')	C(5)	iv	3.307 (6)	O(W)*	O(1A)	i	2.887 (6)
O(1')	O(5)	iv	3.386 (5)	O(W)	O(5')	vi	2.705 (5)
O(1')	C(6)	iv	3.250 (6)	O(W)*	H(O5')	vi	2.14 (7)
O(1')	C(3')	iv	3.468 (5)	O(W)	O(5)	i	3.034 (6)
O(1')	C(1A)	iv	3.288 (6)				
C(1A)	O(1A)	iv	3.306 (7)				
O(W)	C(5')	vi	3.279 (6)				

* Hydrogen bond suggested.

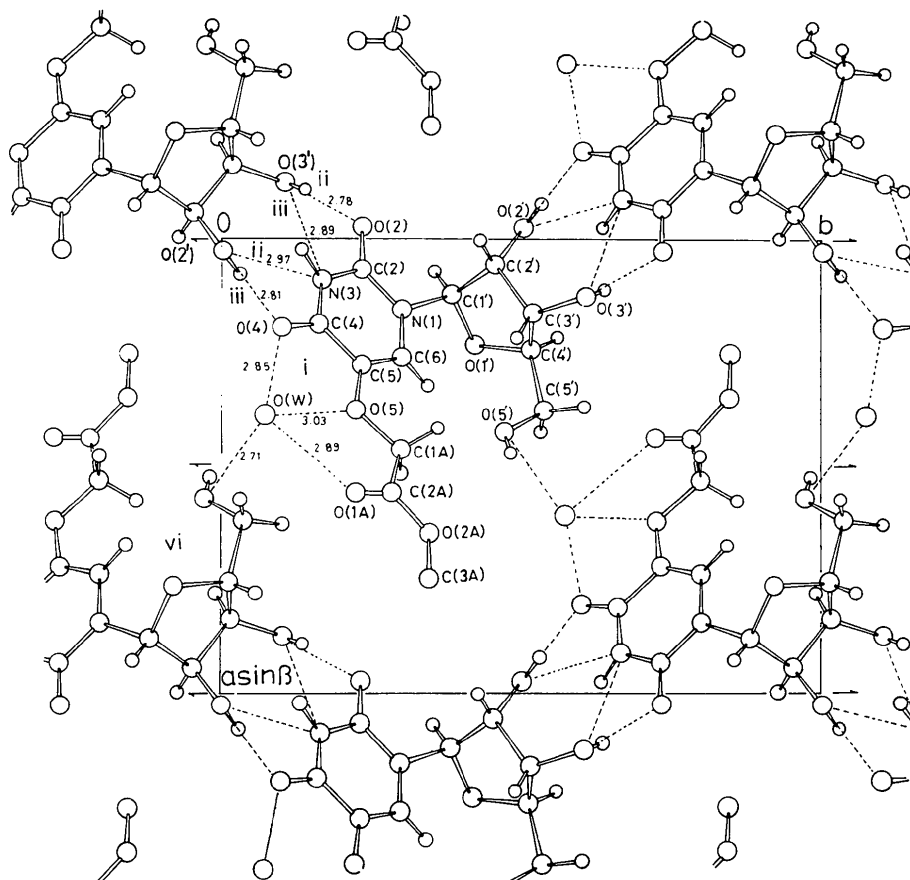


Fig. 1. Projection of the crystal structure along the c axis. Symmetry operations are shown in Table 5.

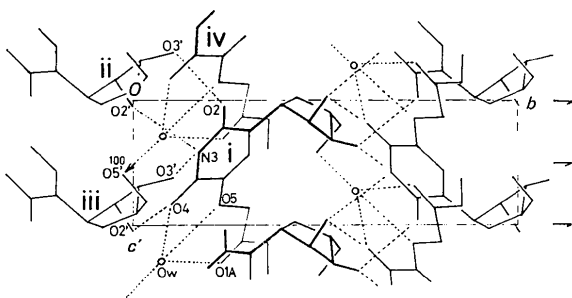


Fig. 2. The crystal structure viewed along the a axis showing the stacking of the molecules along $[001]$.

References

- IBALL, J., MORGAN, C. H. & WILSON, H. R. (1966). *Proc. Roy. Soc. A* **295**, 320–333.
- International Tables for X-ray Crystallography* (1962). Vol. III. Birmingham: Kynoch Press.
- MORIKAWA, K., TORII, K., IITAKA, Y., TSUBOI, M. & NISHIMURA, S. (1974). *F.E.B.S. Lett.* **48**, 279–282.
- MURAO, K., SANEYOSHI, M., HARADA, F. & NISHIMURA, S. (1970). *Biochem. Biophys. Res. Commun.* **38**, 657–661.
- OKAYA, Y. & ASHIDA, T. (1967). *HBL5 4. The Universal Crystallographic Computing System* (I), p. 65. Tokyo: The Crystallographic Society of Japan.
- STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.