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Pseudouridine

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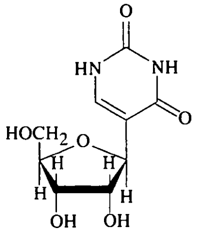
Abstract

The structure of naturally occurring pseudouridine, 5-β-D-ribofuranosyl-2,4(1*H*,3*H*)-pyrimidinedione, $C_9H_{12}N_2O_6$, was determined by direct methods. The asymmetric unit contains two independent pseudouridine molecules, with *anti* orientation of the base with respect to the ribose moiety in both molecules, but with different sugar-ring conformations. The crystal structure is characterized by an extensive hydrogen-bonding network.

Comment

Pseudouridine, (I), a uridine isomer in which the sugar–base bond is from C1′ of ribose to C5 of uracil rather than N1, was the first modified nucleoside found in RNA (Davis & Allen, 1957; Yu, Crestfield & Allen, 1959; Scanell & Allen, 1959; Cohn, 1959). Although in the past, the N1 position of uracil has been shown to transfer acyl groups into peptide linkages (Spector & Keller, 1958), only recently has a similar role been postulated for the free N1 position in pseudouridine in peptidyl transfer at the ribosome during protein biosynthesis (Lane, Ofengand & Gray, 1992, 1995).



(I)

We have recently shown that acetylation of uracil takes place exclusively at N1 (Wood *et al.*, 1995); in connection with acetylation studies of pseudouridine, we obtained crystals of pseudouridine itself and determined its structure. Although crystal structures of uridine (Green *et al.*, 1975), α-pseudouridine (Rohrer & Sundaralingam, 1970) and 4-thiopseudouridine (Barnes, Hawkinson & Wigler, 1980) have been published, this is the first report of the structure of naturally occurring β-pseudouridine.

There are two independent molecules of pseudouridine in the asymmetric unit; their molecular conformations are shown in Fig. 1. Both molecules may be described as having *anti* orientations of the base with respect to the sugar ring; the χ torsion angles (O1′—C1′—C5—C6) are 8.5 (10) and 36.9 (9)° for the *A* and *B* molecules, respectively. The two molecules differ in their sugar conformations; molecule *A* adopts a C3′-*endo* pucker ribose conformation in which C3′ is 0.59 (1) Å from the best plane through the other four ring atoms [maximum deviation of these four atoms from their plane is 0.03 (4) Å], while molecule *B* has a C2′-*endo* pucker arrangement of the ribose ring atoms [C2′ is 0.58 (1) Å from the plane of the other atoms, whose maximum deviation is also 0.03 (3) Å]. Both conformations are commonly observed in nucleoside and nucleotide structures.

Bond lengths and angles (Table 1) do not deviate significantly from expected values. A stereoscopic view

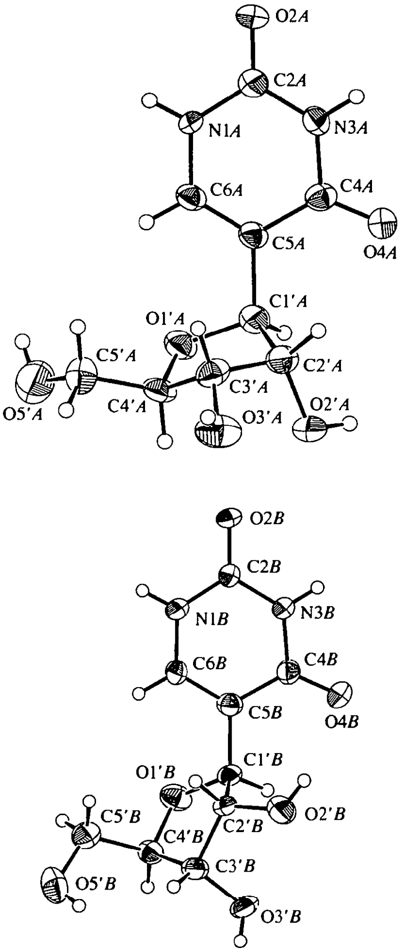


Fig. 1. *ZORTEP* (Zsolnai, 1995) view of the two independent pseudouridine molecules (*A* and *B*) showing 50% probability displacement ellipsoids. H atoms are drawn as small circles of arbitrary radii.

of the crystal packing (Burnett & Johnson, 1996) is presented in Fig. 2. An elaborate three-dimensional hydrogen-bond network binds the molecules in the crystal (Table 2). The pyrimidinedione moieties are held in stacked layers by four base-to-base hydrogen bonds of the N—H···O type, similar to the situation in the 4-thiopseudouridine structure (Barnes, Hawkinson & Wigler, 1980) where there are three independent molecules in the asymmetric unit. These layers are separated by sugar layers, themselves hydrogen-bonded by an intricate network involving sugar–sugar (O—H···O type) interactions, again similar to the crystal structure of 4-thiopseudouridine. The only sugar–base hydrogen bond is O5′*A*—H5′*A*···O4*A*. Of all possible hydrogen-bonded atoms, only O4*B* does not participate in the network.

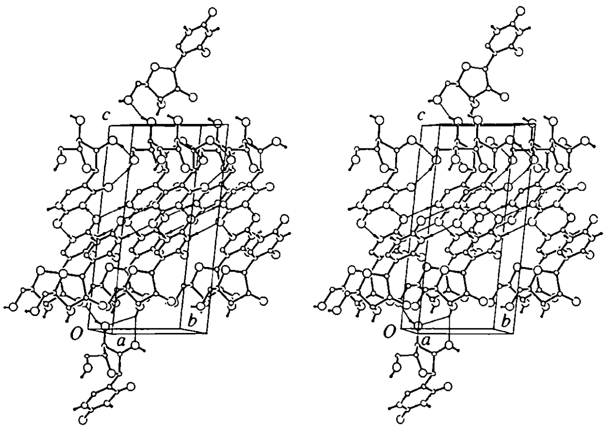


Fig. 2. *ORTEP*III (Burnett & Johnson, 1996) stereoview of the crystal structure with marked hydrogen bonds (thin lines). Diameter of the circles grows in the order: H, C, N, O.

Experimental

The title material was obtained from Sigma (isolated from wheat bran).

Crystal data

$C_9H_{12}N_2O_6$
$M_r = 244.21$
Triclinic
$P1$
$a = 5.1318\,(2)$ Å
$b = 6.9156\,(3)$ Å
$c = 14.9166\,(6)$ Å
$\alpha = 83.3087\,(14)°$
$\beta = 88.388\,(2)°$
$\gamma = 71.835\,(2)°$
$V = 499.55\,(4)$ Å^3
$Z = 2$
$D_x = 1.624$ Mg m^{-3}
D_m not measured

Mo $K\alpha$ radiation
$\lambda = 0.71013$ Å
Cell parameters from 27 reflections
$\theta = 22$–$30°$
$\mu = 0.138$ mm^{-1}
$T = 293\,(2)$ K
Plate
$0.60 \times 0.20 \times 0.02$ mm
Colourless

Data collection

Siemens *P*4 SMART CCD diffractometer
ω rotation scans
Absorption correction: none
5997 measured reflections
5997 independent reflections

2286 reflections with $I > 2\sigma(I)$
$R_{\rm int} = 0.0675$
$\theta_{\rm max} = 30.62°$
$h = -7 \rightarrow 7$
$k = -9 \rightarrow 9$
$l = -21 \rightarrow 21$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)] = 0.092$
$wR(F^2) = 0.285$
$S = 0.927$
5995 reflections
313 parameters
H atoms riding
$w = 1/[\sigma^2(F_o^2) + (0.1330P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{\rm max} = -0.001$

$\Delta\rho_{\rm max} = 0.665$ e Å^{-3}
$\Delta\rho_{\rm min} = -0.354$ e Å^{-3}
Extinction correction: none
Scattering factors from *International Tables for Crystallography* (Vol. C)
Absolute configuration: Flack (1983)
Flack parameter = 2 (2), 2948 Friedel pairs

Table 1. *Selected bond lengths (Å)*

N1*A*—C2*A*	1.357 (8)	N1*B*—C2*B*	1.339 (9)
N1*A*—C6*A*	1.380 (9)	N1*B*—C6*B*	1.392 (9)
C2*A*—O2*A*	1.231 (8)	C2*B*—O2*B*	1.240 (8)
C2*A*—N3*A*	1.361 (9)	C2*B*—N3*B*	1.378 (8)
N3*A*—C4*A*	1.397 (9)	N3*B*—C4*B*	1.400 (9)
C4*A*—O4*A*	1.236 (9)	C4*B*—O4*B*	1.217 (8)
C4*A*—C5*A*	1.422 (9)	C4*B*—C5*B*	1.448 (10)
C5*A*—C6*A*	1.347 (10)	C5*B*—C6*B*	1.341 (9)
C5*A*—C1′*A*	1.530 (10)	C5*B*—C1′*B*	1.512 (9)
C1′*A*—O1′*A*	1.437 (8)	C1′*B*—O1′*B*	1.422 (9)
C1′*A*—C2′*A*	1.531 (11)	C1′*B*—C2′*B*	1.539 (10)
O1′*A*—C4′*A*	1.422 (8)	O1′*B*—C4′*B*	1.447 (8)
C2′*A*—O2′*A*	1.429 (9)	C2′*B*—O2′*B*	1.406 (9)
C2′*A*—C3′*A*	1.521 (11)	C2′*B*—C3′*B*	1.521 (10)
C3′*A*—O3′*A*	1.389 (9)	C3′*B*—O3′*B*	1.424 (8)
C3′*A*—C4′*A*	1.526 (9)	C3′*B*—C4′*B*	1.496 (10)
C4′*A*—C5′*A*	1.504 (10)	C4′*B*—C5′*B*	1.518 (9)
C5′*A*—O5′*A*	1.420 (9)	C5′*B*—O5′*B*	1.424 (9)

Table 2. *Hydrogen-bonding geometry (Å, °)*

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N1*A*—H1*A*···O2*B*$^{\rm i}$	0.86	2.01	2.87 (2)	176
N3*A*—H3*A*···O2*B*$^{\rm ii}$	0.86	2.04	2.90 (3)	174
N1*B*—H1*B*···O2*A*$^{\rm iii}$	0.86	2.01	2.86 (3)	172
N3*B*—H3*B*···O2*A*$^{\rm iv}$	0.86	2.07	2.93 (2)	178
O2′*A*—H2′1···O5′*A*$^{\rm v}$	0.82	1.86	2.62 (3)	154
O3′*A*—H3′1···O5′*B*$^{\rm vi}$	0.82	1.93	2.70 (3)	157
O5′*A*—H5′*A*···O4*A*$^{\rm vii}$	0.82	2.03	2.80 (2)	156
O2′*B*—H2′2···O5′*B*$^{\rm v}$	0.81	2.27	3.06 (3)	165
O3′*B*—H3′2···O2′*A*$^{\rm viii}$	0.82	2.11	2.86 (3)	152
O3′*B*—H3′2···O3′*A*$^{\rm viii}$	0.82	2.38	3.01 (3)	134
O5′*B*—H5′*B*···O2′*B*$^{\rm i}$	0.81	1.96	2.75 (2)	168

Symmetry codes: (i) $x, y-1, z$; (ii) $x-1, y, z$; (iii) $1+x, y, z$; (iv) $x, 1+y, z$; (v) $x-1, 1+y, z$; (vi) $x, y, 1+z$; (vii) $1+x, y-1, z$; (viii) $x-1, y, z-1$.

The crystals were not of high quality, as evidenced by the high $R_{\rm int}$ value. Data were collected using a *P*4 Siemens diffractometer, equipped with a Siemens SMART 1K charge-coupled device area detector (using the program *SMART*; Siemens, 1996*b*) and rotating anode Mo $K\alpha$ radiation. The crystal-to-detector distance was 3.98 cm and the data collection was carried out in 512 × 512 pixel mode, utilizing 2 × 2 pixel binning. The unit-cell parameters were determined by a least-squares fit of the angular settings of 27 strong reflections; 97% of the whole sphere of data was collected. Processing was carried out using the program *SAINT* (Siemens, 1996*a*), which applied Lorentz and polarization corrections to three-dimensionally integrated diffraction spots. Except for four hydroxyl H atoms on the sugars, which were found from a difference map, all H atoms were positioned geometrically. During the refinement H atoms were 'riding' on their parent atoms. Two reflections (001 and $00\bar{1}$) were rejected due to severe extinction phenomena. The relatively high *wR* values are due to crystal quality. In the outermost 5° in θ there are approximately 1000 reflections of which about 150 are observed. Omitting these data from the refinement led to improved *R*1 (0.07) and *wR*2 (0.23), but made no significant difference to the molecular geometry (maximum difference in bond length was 0.005 Å).

Data collection: *SMART*. Cell refinement: *SMART*. Data reduction: *SAINT*. Program(s) used to solve structure: *SHELXS*86 (Sheldrick, 1990). Program(s) used to refine structure: *SHELXL*93 (Sheldrick, 1993). Molecular graphics: *ZORTEP* (Zsolnai, 1995) and *ORTEP*III (Burnett & Johnson, 1996).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: FG1303). Services for accessing these data are described at the back of the journal.

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4-[Hydroxy(phenyl)methylene]-5-oxo-3-phenyl-2-cyclohexenecarboxylic Acid

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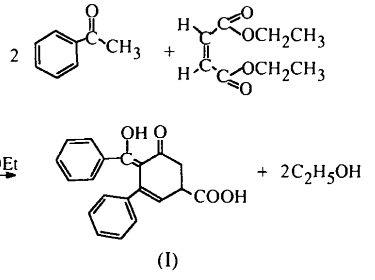
Abstract

In the title compound, $C_{20}H_{16}O_4$, the cyclohexene ring adopts a twisted-boat conformation. The molecule contains an enol sub-unit and the hydroxyl group forms an intramolecular hydrogen bond to the cyclic carbonyl group, with an O···O distance of 2.485 (3) Å and an O—H···O angle of 148 (3)°. Enantiomeric pairs of molecules form centrosymmetric dimers by mutual hydrogen bonding of carboxyl groups, with O···O distances of 2.649 (3) Å.

Comment

Many studies have been concerned with the synthesis and chemistry of cyclic ketones among which cyclic ketoacids have received special attention due to their biological activity as sexual hormones (Lazarević, Vorkapić-Furač & Mühl, 1992; Bu'Lock, Austin, Snatzke & Hruban, 1970; Bacigaluppo *et al.*, 1994; Hervouet & Guingant, 1996). In this paper, we report the structure of a new cyclohexene ketoacid, (I), prepared by base-catalysed condensation of acetophenone with diethyl maleate.



(I)

The molecule contains a chiral centre (C1), but both enantiomers are present in the crystal. The cyclohexene ring adopts a twisted-boat conformation. The dihedral angles between the least-squares best plane through