

$[(C_6H_5)_4P]^+$ un nombre assez important de contacts relativement courts (Tableau 4), qui ont sans doute quelque relation avec les écarts aux symétries idéales des cations et des anions. Entre ces couches, les contacts interioniques sont normaux.

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The Molecular and Crystal Structure of the Fluorescent Base Yt (1H-4,6-Dimethylimidazo[1,2-a]purine-9-one) in tRNA

BY G. NYGJERD, J. MCALISTER AND M. SUNDARALINGAM*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

AND S. MATSUURA

Department of General Education, Nagoya University, Nagoya, Japan

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The fluorescent base Yt of *Torulopsis utilis* tRNA^{Phe}, adjacent to the 3' end of the tRNA anticodon, has been determined by single-crystal X-ray diffraction techniques. The structure suggested by chemical and spectroscopic studies is confirmed. The molecule is slightly buckled with the two imidazole rings bent to the same side of the pyrimidine ring. In the crystal the molecules stack on top of each other forming infinite columns along the *a* axis. There is one intermolecular hydrogen bond between the imidazole ring nitrogens N(7)–H···N(9). The crystals are monoclinic with space group $P2_1/c$. The unit-cell constants are $a = 8.831$ (3), $b = 9.563$ (2), $c = 10.667$ (7) Å and $\beta = 95.40$ (1)°. The structure was solved by direct methods and refined to $R = 0.057$ for 1031 reflections. Standard deviations in bond lengths and angles involving nonhydrogen atoms average 0.006 Å and 0.3°.

Introduction

The 3' side of the anticodon of a number of phenylalanine tRNA's incorporates a modified base, Y, that is characterized by an intense fluorescence. For a review of the molecular structures of the modified bases, nucleosides and nucleotides known as of 1971 see Sundaralingam (1972). The base Y has been observed in

slightly different forms: Y in baker's yeast tRNA^{Phe} (Fig. 1) (Nakanishi, Furutachi, Funamizu, Grunberger & Weinstein, 1970); Yw in wheat germ tRNA^{Phe} (Dudock, Katz, Taylor & Holley, 1969); and Yt in *Torulopsis utilis* tRNA^{Phe} (Fig. 2) (Kasai, Goto, Take-mura, Goto & Matsuura, 1971). The Y bases have attracted great interest because of their biochemical importance and unique position in the anticodon loop. Those tRNA's that contain a Y base and whose sequences have been determined (Dudock *et al.*, 1969;

* To whom correspondence should be addressed.

RajBhandary, Chang, Stuart, Faulkner, Hoskinson & Khorana, 1967; Keith, Picaud, Weissenbach, Ebel, Petrissant & Dirheimer, 1973) all have the same base sequence in the anticodon loop, with GAA as the sequence of bases of the anticodon triplet. Nakanishi *et al.* (1970) first suggested the structure shown in Fig. 1 for the base Y from baker's yeast tRNA. Kasai *et al.* (1971) later suggested the structure in Fig. 2 for the base Yt from *Torulopsis* tRNA. The present investigation was carried out in order to determine unequivocally the chemical structure and to provide accurate geometrical parameters for this novel base.

Experimental

Crystals of the Yt base were grown in aqueous ethanol solutions by one of us (S.M.). Preliminary photographs show systematically absent reflections for $l=2n+1$ ($h0l$) and $k=2n+1$ ($0k0$). This indicates that the crystals of the base Yt have the monoclinic space group $P2_1/c$. Accurate unit-cell dimensions were determined by the least-squares fit of the Bragg angles (θ) of 12 reflections with $2\theta > 40^\circ$ as measured on a Picker diffractometer. These dimensions are $a=8.831$ (3), $b=9.563$ (2), $c=10.667$ (7) Å and $\beta=95.40$ (1)° ($\lambda=1.5418$ Å). $D_{\text{calc}}=1.386$ g cm⁻³ for 4 molecules per unit cell. Suitable crystals were not available for density measurement. 1245 independent reflections were collected using the θ - 2θ scan technique and nickel-filtered Cu $K\alpha$ radiation. Scan speed was 2° min⁻¹. Of the 1245 reflections that were measured 868 were considered observed by the criterion that F_o should be greater than $3\sigma(F_o)$. The crystal used was very small, with dimensions 0.15 × 0.15 × 0.05 mm. Corrections for absorption and extinction were therefore not applied.

Determination and refinement of the structure

The structure was solved by direct methods using the program *MULTAN* developed by Main, Germain & Woolfson (1970). Out of eight generated solutions, the

one with FOM=1.13 and $R_E=31.8$ was outstanding. The 190 phased normalized structure factors with $|E| > 1.3$ yielded an E map that revealed all the heavy atoms in the structure.

Two cycles of full-matrix least-squares refinement with isotropic temperature factors followed by two cycles of refinement with anisotropic temperature factors for the atoms gave an R value of 0.12, where $R = \sum ||F_o| - |F_c|| / \sum |F_o|$. A difference electron-density map calculated at this point with all heavy atoms included in the structure-factor calculation revealed all the hydrogen atoms. Two more cycles of anisotropic refine-

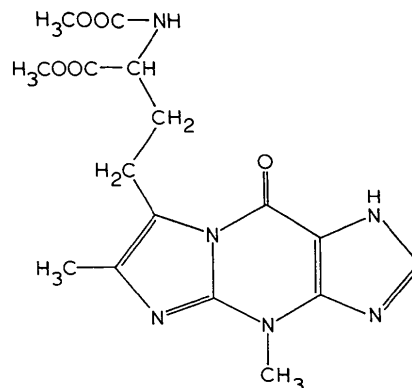


Fig. 1. Proposed structure for the base Y (baker's yeast tRNA) (Nakanishi *et al.*, 1970). The base nucleus is the same in Yw and Yt.

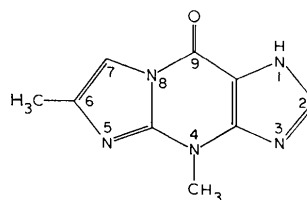


Fig. 2. Proposed structure for the base Yt (*Torulopsis utilis* tRNA) (Kasai *et al.*, 1971).

Table 1. Positional and thermal parameters of the heavy atoms multiplied by 10⁴

Standard deviations are listed in parentheses. Anisotropic thermal parameters are of the form $\exp[-(\beta_{11}h^2 + \dots + 2\beta_{12}hk + \dots)]$. Numbering of atoms as in Fig. 3.

	x/a	y/b	z/c	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	2629 (4)	6212 (3)	-449 (3)	96 (5)	67 (4)	53 (3)	-8 (4)	22 (3)	3 (3)
C(2)	2923 (5)	4789 (4)	-458 (4)	83 (6)	60 (5)	65 (4)	5 (4)	17 (4)	-1 (3)
N(3)	2348 (4)	3945 (3)	425 (3)	101 (5)	51 (4)	68 (3)	3 (3)	30 (3)	3 (3)
C(3)	2620 (8)	2433 (5)	424 (6)	153 (10)	54 (5)	102 (6)	13 (6)	41 (6)	-4 (4)
C(4)	1470 (5)	4582 (4)	1248 (4)	74 (6)	60 (5)	55 (4)	-6 (4)	13 (4)	0 (3)
C(5)	1193 (5)	5999 (4)	1227 (4)	88 (6)	58 (5)	51 (4)	-6 (4)	20 (4)	-3 (3)
C(6)	1727 (5)	6946 (4)	348 (4)	102 (6)	63 (5)	58 (4)	-4 (5)	13 (4)	1 (3)
O(6)	1496 (4)	8205 (3)	218 (3)	173 (6)	56 (4)	78 (3)	15 (3)	42 (3)	8 (2)
N(7)	259 (4)	6219 (4)	2186 (3)	117 (6)	64 (4)	54 (3)	-3 (4)	30 (3)	-4 (3)
C(8)	59 (5)	4976 (4)	2709 (4)	113 (7)	64 (5)	68 (4)	-8 (5)	29 (5)	-1 (4)
N(9)	772 (4)	3927 (3)	2175 (3)	105 (5)	57 (4)	70 (4)	-8 (4)	31 (4)	3 (3)
C(10)	3347 (5)	6757 (5)	-1463 (4)	116 (7)	73 (6)	67 (4)	-3 (5)	31 (4)	9 (4)
C(11)	4006 (5)	5663 (5)	-1988 (4)	90 (6)	94 (6)	68 (4)	-13 (5)	23 (4)	-2 (4)
N(12)	3748 (4)	4411 (4)	-1365 (3)	95 (5)	69 (4)	78 (4)	-2 (4)	32 (4)	-7 (3)
C(13)	4894 (7)	5672 (7)	-3121 (6)	142 (9)	105 (8)	91 (6)	-3 (4)	54 (6)	6 (5)

Table 3. Deviations (\AA) of the heavy atoms from least-squares planes in the molecule

Atoms included in calculation of the plane are marked with an asterisk.

Equations of the least-squares planes

Coefficients are direction cosines relative to the axes a, b, c^* . $X, Y,$ and Z are in \AA measured along these axes.

$$\begin{aligned} A: & 0.780X + 0.147Y + 0.608Z = 2.430 \\ B: & 0.760X + 0.156Y + 0.630Z = 2.415 \\ C: & 0.752X + 0.150Y + 0.642Z = 2.395 \\ D: & 0.762X + 0.153Y + 0.629Z = 2.388 \\ E: & 0.763X + 0.144Y + 0.630Z = 2.360 \end{aligned}$$

	Plane A	Plane B	Plane C	Plane D	Plane E
N(1)	-0.002*	-0.012*	0.028	-0.030*	-0.002*
C(2)	0.001*	0.008*	0.043	-0.015*	0.002*
N(3)	0.015	-0.003*	0.011	-0.024*	-0.018
C(3)	0.041	0.040	0.048	0.015	
C(4)	0.064	0.004*	0.003*	-0.010*	-0.002*
C(5)	0.067	-0.008*	-0.004*	-0.019*	0.002*
C(6)	0.064	0.012*	0.038	0.001*	0.032
O(6)	0.120	0.057	0.089	0.049	
N(7)	0.136	0.018	0.004*	0.014*	
C(8)	0.152	0.028	-0.002*	0.022*	
N(9)	0.111	0.022	-0.001*	0.010*	
C(10)	0.002*	0.024	0.087	0.004*	
C(11)	-0.001*	0.055	0.124	0.029*	
N(12)	0.000*	0.046	0.098	0.019*	
C(13)	0.029	0.128	0.220	0.097	
r.m.s. Δ	0.001	0.009	0.003	0.018	0.002
σ (r.m.s. Δ)	0.004	0.004	0.004	0.004	0.004

deviations are 0.006 \AA and 0.3° . The only double bond in the pyrimidine ring is the C(4)–C(5) bond. The only other known purine structure with C(4)–C(5) as the only double bond in the pyrimidine ring is isocaffeine (1,3,9-trimethyl-2,6-deoxypurine, Rasmussen & Sletten, 1973). None of the bond lengths in the pyrimidine ring deviates from the corresponding ones in isocaffeine by more than 2σ , even though all their correspond-

ing bond angles are significantly different. The bond angles about N(1) and C(2) are distorted from the values expected for guanine derivatives because of the presence of the second imidazole ring. The C(8)–N(9) and the N(7)–C(8) bonds are unexpectedly similar in length in the Yt base whereas they are considerably different in isocaffeine. In the latter structure, in which atom N(9) is methylated, the C(8)–N(9) and N(7)–C(8) bond distances are 1.376 and 1.299 \AA , respectively. Both the difference electron-density maps and the least-squares refinement of hydrogen atom H(7) indicate that this atom is bonded to atom N(7). The electron density found in the difference map at the position of H(7) is the same as that of H(8) and H(10). There was no significant peak in the difference map within bonding distance of atom N(9). The difference electron-density map through the best plane of the base in which all hydrogen and nonhydrogen atoms were included in the structure factor-calculation is shown in Fig. 5.

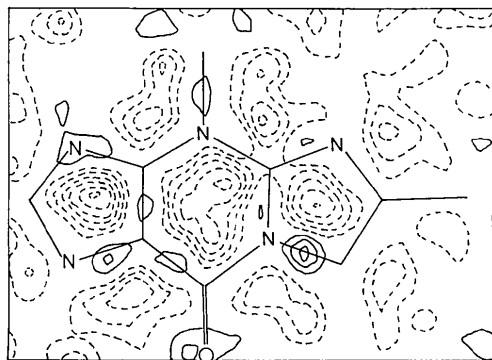


Fig. 5. Difference electron density map through the least-squares plane of the base. All hydrogen and nonhydrogen atoms were included in the structure-factor calculation. Both positive (solid lines) and negative (broken lines) contours are at intervals of 0.05 e \AA^{-3} , starting at 0.00 e \AA^{-3} .

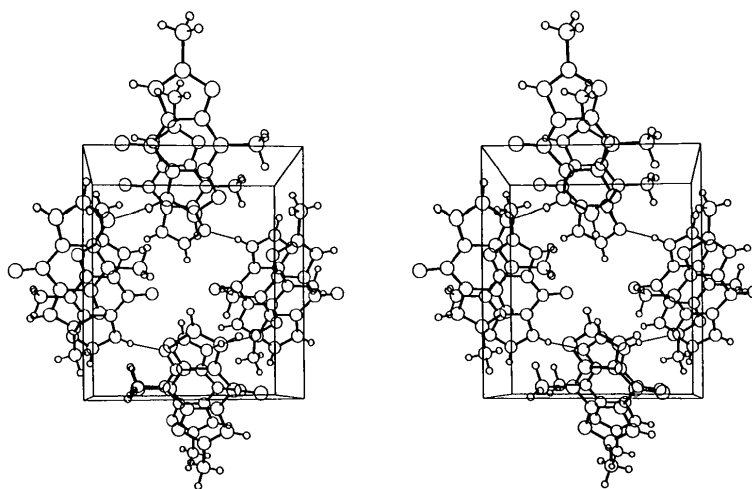


Fig. 6. A stereoscopic plot of the molecular packing as viewed along the a^* axis.

The significantly larger valence angle at N(7) compared with N(9) further demonstrates that protonation is at N(7) (Prusiner & Sundaralingam, 1975). The bond distances and angles involving the hydrogen atoms are in the usual range and are not tabulated.

Both methyl groups have the same conformation relative to the molecular plane. The torsion angles C(4)–N(3)–C(3)–H(32) and N(12)–C(11)–C(13)–H(132) are both 80°.

Molecular packing and hydrogen bonding

A stereoscopic drawing of the molecular packing is shown in Fig. 6. The molecules stack on top of each other in infinite columns along the *a* axis with alternate interplanar distances between adjacent molecules of 3.29 and 3.46 Å. The C(13) methyl group is involved in 'stacking' interactions with every second base. The molecule exhibits one normal intermolecular hydrogen bond between N(7)–H(7) and N(9). The two molecules involved in this interaction lie approximately in the same plane but in adjacent stacks. This hydrogen-bond distance is 2.85 Å and the angle N(7)–H(7)···N(9) is 169°. There is also a weak C–H···O interaction between C(8)–H(8) and O(6). The C(8)···O(6) distance is 3.20 Å, the H(8)···O(6) distance is 2.30 Å and the angle C(8)–H(8)···O(6) is 147°.

The computer programs used in this analysis were the full-matrix least-squares program of Busing, Martin & Levy (1962) modified by Rao (1969) of the University of Wisconsin, Madison, the Rao (1968) Fourier synthesis program, and a number of structure interpretation programs of Rao (1968, 1974). All calculations were carried out on the University Univac 1110 computer.

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