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X-ray Study of 1,5,*N*(4),*N*(4)-Tetramethylcytosine – an Overcrowded Molecule with Planar Structure

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1,5,*N*(4),*N*(4)-Tetramethylcytosine ($C_8H_{13}N_3O$) is monoclinic, space group $P2_1/c$, with $a = 15{\cdot}362$, $b = 10{\cdot}240$, $c = 12{\cdot}452$ Å, $\beta = 114{\cdot}98°$, $Z = 8$. The structure was solved by direct methods and refined to an R of 8·0% for 1984 diffractometer data. Average standard deviations are 0·006 Å and 0·4°. The dimethylamino group is not rotated, to avoid steric hindrance with the *ortho* methyl group at C(5), but is coplanar with the pyrimidine ring. The sp^2 configuration of N(4) is retained and steric strain is relieved by spreading of the angles at C(4), C(5) and N(4).

Introduction

Recently, two papers on the 'barrier to rotation and conformation of the $-NR_2$ group in cytosine and its derivatives' have been published (Geller & Lesyng, 1975; Kulakowska, Geller, Lesyng, Bolewska & Wierzchowski, 1975). Among other cytosine derivatives, 1,5,*N*(4),*N*(4)-tetramethylcytosine (TMC, Fig. 1) was investigated with CNDO/2 and INDO calculations and experimental dipole moments were evaluated. One of the authors' suggestions was that the dimethylamino group should be rotated by about 30° relative to the pyrimidine plane. Further, the configuration of the amino N atom should be sp^3 rather than sp^2. These results prompted us to carry out an X-ray analysis to establish the geometrical details of TMC.

Experimental

A prismatic single crystal 0·35 × 0·5 × 0·15 mm was sealed in a capillary because of its hygroscopic properties and used for all subsequent X-ray measurements. Crystallographic data are given in Table 1. X-ray intensities were collected in the $2\theta/\omega$ scan mode on an automated Stoe diffractometer equipped with a Cu tube (Ni filter, Cu $K\alpha = 1{\cdot}54182$ Å) and corrected for geometrical factors but not for absorption. Three check reflexions monitored every 100 measurements showed no significant change in intensity.

Wilson's (1942) method was used to obtain overall temperature ($B = 3{\cdot}6$ Å^2) and scale factors and normalized structure amplitudes were evaluated (Karle & Hauptman, 1956). The structure was solved with *MULTAN* (Main, Germain & Woolfson, 1970) on the

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basis of all E's $> 1{\cdot}5$. An E map obtained from the most consistent set revealed the non-hydrogen atoms of the two molecules in the asymmetric unit. Isotropic and anisotropic full-matrix least-squares refinement (Busing, Martin & Levy, 1962) and a difference synthesis provided the positions of all the H atoms except those attached to methyl group C(8). As a consequence of the rather high thermal motion of the dimethylamino groups (Fig. 2), the attached H atoms were not located as easily as those bonded to the aromatic and other methyl groups. In the refinement cycles, data with $F_{hkl} < 3\sigma_{hkl}$ were treated as unobserved, with σ_{hkl} the standard deviation according to counting statistics (Stout & Jensen, 1968). The refinement converged at $R = \Sigma(|F_o - F_c|)/\Sigma F_o = 8{\cdot}0\%$ for all 1984 measured data, with average parameter changes in the last cycle less than $\frac{1}{3}\sigma$.

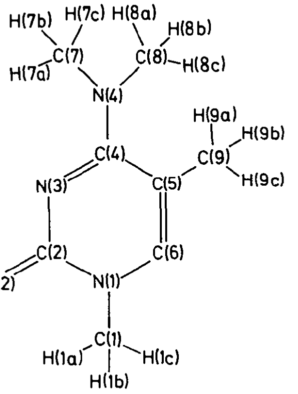

Fig. 1. Chemical structure and numbering scheme for the 1,5,*N*(4),*N*(4)-tetramethylcytosine molecule.

Table 1. *Crystal data*

Chemical formula	$C_8H_{13}N_3O$
Crystal system	Monoclinic
Space group	$P2_1/c$
Cell dimensions	$a = 15{\cdot}362\,(3)$ Å
	$b = 10{\cdot}240\,(2)$
	$c = 12{\cdot}452\,(2)$
	$\beta = 114{\cdot}98°$
Density ($Z = 8$)	$1{\cdot}25$ g cm^{-3}

Discussion

In Tables 2 to 5 are presented final atomic parameters, bond distances and angles, deviations of atoms from least-squares planes through the pyrimidine rings and some relevant dihedral angles.* In Figs 2 to 4 the molecules and their packing within the unit cell are shown.

The bond lengths (Table 3) agree well with average values cited for cytidine by Voet & Rich (1970). Only C(4)–N(4), 1·350 Å, appears to be lengthened beyond the 3σ limit ($3\sigma = 0{\cdot}018$ Å), a peculiarity of this structure which will be interpreted below. The C–N single-bond distances for the methyl groups correspond to the standard value of 1·465 Å (Pauling, 1960), the C(5)–C(9) distances, 1·520 and 1·528 Å, are also close to the standard C–C distance of 1·533 Å (Pauling, 1960).

Least-squares planes calculated for the six pyrimidine ring atoms indicate that both molecules are essentially planar, with the dimethylamino C atoms and C(9) deviating by not more than 0·18 Å. Expressed in terms of torsion angles (Table 5), the dimethylamino groups are rotated from the heterocycle plane by less than 6·6° [N(3)–C(4)–N(4)–C(8) in molecule 1] or the angles between the least-squares planes through the pyrimidine heterocycles and the dimethylamino groups [C(4), N(4), C(7), C(8)] are 4·1° for molecule 1 and 3·9° for molecule 2.

The planarity of the TMC molecule must be explained by the partial double-bond character of

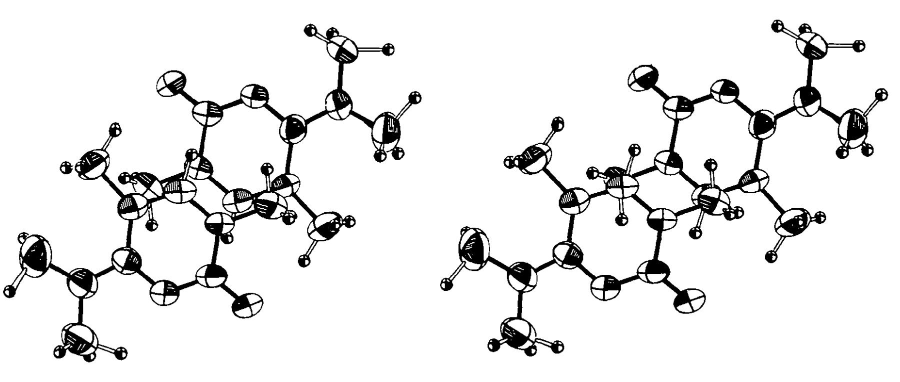

Fig. 2. Stereo view of the two 1,5,*N*(4),*N*(4)-tetramethylcytosine molecules in the asymmetric unit. Thermal ellipsoids are represented at the 50% probability level.

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* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32023 (12 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. *Atomic coordinates of* 1,5,*N*(4),*N*(4)-*tetramethylcytosine in fractions of the crystallographic axes* ($\times 10^4$) *and thermal parameters* ($\times 10^4$) *in the form:* $T = exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	765 (4)	3909 (5)	1368 (4)	136 (5)	129 (6)	122 (5)	6 (4)	78 (4)	−25 (4)
N(1)	959 (2)	2595 (3)	1078 (3)	91 (3)	112 (5)	82 (3)	4 (3)	52 (2)	−7 (3)
C(2)	936 (3)	2349 (5)	−25 (4)	82 (3)	128 (6)	82 (4)	2 (2)	51 (3)	−4 (4)
O(2)	776 (3)	3263 (3)	−737 (3)	167 (3)	124 (4)	117 (3)	31 (3)	96 (3)	37 (3)
N(3)	1106 (2)	1121 (3)	−279 (3)	74 (2)	112 (5)	79 (3)	6 (3)	46 (2)	−1 (3)
C(4)	1261 (3)	135 (4)	−1120 (4)	57 (3)	105 (5)	88 (4)	0 (3)	35 (3)	5 (4)
C(5)	1289 (3)	371 (4)	1651 (3)	67 (3)	125 (6)	74 (4)	2 (3)	38 (3)	17 (4)
C(6)	1118 (3)	1596 (5)	1872 (3)	62 (3)	133 (6)	73 (4)	−4 (3)	34 (3)	7 (4)
N(4)	1401 (2)	−1040 (3)	90 (3)	79 (3)	103 (5)	111 (4)	8 (3)	47 (3)	1 (3)
C(7)	1326 (4)	−1162 (5)	−1120 (4)	120 (4)	136 (7)	98 (4)	6 (4)	57 (4)	−23 (4)
C(8)	1681 (4)	−2244 (5)	794 (5)	117 (5)	115 (7)	174 (7)	16 (4)	75 (5)	17 (5)
C(9)	1388 (4)	−645 (5)	2585 (4)	119 (4)	172 (8)	106 (5)	20 (4)	59 (4)	46 (5)
C(1′)	5877 (4)	1081 (5)	4465 (4)	151 (5)	149 (7)	101 (5)	−24 (5)	69 (4)	−23 (5)
N(1′)	6003 (2)	2418 (3)	4912 (3)	94 (3)	118 (5)	71 (3)	−3 (3)	48 (2)	−2 (3)
C(2′)	6033 (3)	2662 (5)	6030 (4)	83 (3)	142 (7)	75 (4)	5 (4)	47 (3)	3 (4)
O(2′)	5910 (2)	1727 (3)	6584 (3)	146 (3)	135 (4)	92 (3)	−2 (3)	77 (3)	18 (3)
N(3′)	6179 (2)	3897 (4)	6438 (3)	76 (3)	117 (5)	79 (3)	1 (3)	44 (2)	−1 (3)
C(4′)	6264 (3)	4880 (4)	5790 (4)	56 (3)	120 (6)	89 (4)	4 (3)	36 (3)	−8 (4)
C(5′)	6183 (3)	4680 (5)	4605 (3)	57 (3)	130 (6)	86 (4)	4 (3)	34 (3)	11 (4)
C(6′)	6065 (3)	3447 (5)	4238 (3)	63 (3)	150 (6)	73 (4)	1 (3)	34 (3)	4 (4)
N(4′)	6437 (3)	6051 (4)	6336 (3)	89 (3)	128 (5)	122 (4)	−7 (3)	61 (3)	−16 (4)
C(7′)	6517 (4)	6164 (5)	7546 (5)	156 (5)	177 (8)	152 (6)	−45 (5)	99 (5)	−54 (5)
C(8′)	6614 (4)	7287 (5)	5850 (5)	137 (5)	135 (7)	153 (6)	−36 (5)	24 (5)	−15 (6)
C(9′)	6218 (3)	5691 (5)	3722 (4)	99 (3)	156 (5)	124 (5)	17 (3)	67 (5)	44 (6)
H(1*A*)	1273 (0)	4549 (0)	1440 (0)	80 (0)	148 (0)	122 (0)	0 (0)	42 (0)	0 (0)
H(1*B*)	886 (0)	3830 (0)	2250 (0)	80 (0)	148 (0)	122 (0)	0 (0)	42 (0)	0 (0)
H(1*C*)	−52 (0)	4152 (0)	750 (0)	80 (0)	148 (0)	122 (0)	0 (0)	42 (0)	0 (0)
H(6)	1148 (0)	1897 (0)	2680 (0)	59 (0)	109 (0)	90 (0)	0 (0)	31 (0)	0 (0)
H(7*A*)	739 (0)	−951 (0)	−1768 (0)	81 (0)	149 (0)	123 (0)	0 (0)	42 (0)	0 (0)
H(7*B*)	1824 (0)	−318 (0)	−1279 (0)	81 (0)	149 (0)	123 (0)	0 (0)	42 (0)	0 (0)
H(7*C*)	1150 (0)	−2370 (0)	−1197 (0)	81 (0)	149 (0)	123 (0)	0 (0)	42 (0)	0 (0)
H(8*A*)	1156 (0)	−2589 (0)	939 (0)	98 (0)	181 (0)	149 (0)	0 (0)	51 (0)	0 (0)
H(8*B*)	1770 (0)	−3004 (0)	159 (0)	98 (0)	181 (0)	149 (0)	0 (0)	51 (0)	0 (0)
H(8*C*)	2469 (0)	−2032 (0)	1671 (0)	98 (0)	181 (0)	149 (0)	0 (0)	51 (0)	0 (0)
H(9*A*)	827 (0)	−1320 (0)	2197 (0)	86 (0)	159 (0)	131 (0)	0 (0)	45 (0)	0 (0)
H(9*B*)	1400 (0)	−78 (0)	3315 (0)	86 (0)	159 (0)	131 (0)	0 (0)	45 (0)	0 (0)
H(9*C*)	2033 (0)	−843 (0)	2899 (0)	86 (0)	159 (0)	131 (0)	0 (0)	45 (0)	0 (0)
H(1′*A*)	5848 (0)	1154 (0)	3678 (0)	82 (0)	151 (0)	124 (0)	0 (0)	43 (0)	0 (0)
H(1′*B*)	6320 (0)	640 (0)	4978 (0)	82 (0)	151 (0)	124 (0)	0 (0)	43 (0)	0 (0)
H(1′*C*)	5108 (0)	514 (0)	4303 (0)	82 (0)	151 (0)	124 (0)	0 (0)	43 (0)	0 (0)
H(6′)	5992 (0)	3119 (0)	3435 (0)	56 (0)	103 (0)	85 (0)	0 (0)	29 (0)	0 (0)
H(7′*A*)	6024 (0)	5865 (0)	7628 (0)	97 (0)	180 (0)	148 (0)	0 (0)	51 (0)	0 (0)
H(7′*B*)	6893 (0)	5113 (0)	8043 (0)	97 (0)	180 (0)	148 (0)	0 (0)	51 (0)	0 (0)
H(7′*C*)	7219 (0)	6745 (0)	8168 (0)	97 (0)	180 (0)	148 (0)	0 (0)	51 (0)	0 (0)
H(8′*A*)	5845 (0)	7510 (0)	5084 (0)	108 (0)	201 (0)	165 (0)	0 (0)	57 (0)	0 (0)
H(8′*B*)	6836 (0)	7996 (0)	6700 (0)	108 (0)	201 (0)	165 (0)	0 (0)	57 (0)	0 (0)
H(9′*A*)	6115 (0)	5105 (0)	2976 (0)	69 (0)	128 (0)	105 (0)	0 (0)	36 (0)	0 (0)
H(9′*B*)	5685 (0)	6373 (0)	3508 (0)	69 (0)	128 (0)	105 (0)	0 (0)	36 (0)	0 (0)
H(9′*C*)	6884 (0)	6070 (0)	4124 (0)	69 (0)	128 (0)	105 (0)	0 (0)	36 (0)	0 (0)

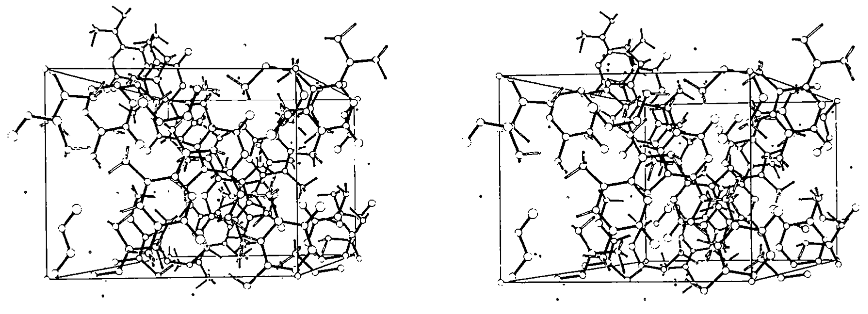

Fig. 3. Stereo view showing packing of 1,5,*N*(4),*N*(4)-tetramethylcytosine molecules in the unit cell viewed down the a^* axis.

C(4)–N(4). In unsubstituted cytidine, this bond is shorter by 0·141 Å than a standard C–N single bond of 1·465 Å but in TMC it is only shortened by 0·115 Å, presumably an effect of the steric hindrance between the methyl groups. From the empirical formula $1/d^2 = 0{\cdot}316 + 0{\cdot}144p$ (Staab, 1960) the double-bond character *p* of a C–N bond is related to the bond distance *d*. For cytidine and TMC the respective values for *p* are 1·77 and 1·61, *i.e.* both molecules display considerable double-bond character for the exocyclic C–N bond. The distortion of the exocyclic angles at

Table 3. *Bond distances* (Å) *and angles* (°)

Averaged standard deviation in lengths is 0·006 Å and in angles 0·4°. Positions of hydrogen atoms given in Table 2 were located from a difference map. C–H distances are between 0·84 and 1·26 Å, angles involving hydrogen atoms between 83 and 126°.

	Molecule 1	Molecule 2	Cytidine (Voet & Rich, 1970)
C(1)–N(1)	1·456	1·460	1·468
N(1)–C(2)	1·382	1·396	1·392
N(1)–C(6)	1·371	1·375	1·360
C(2)–O(2)	1·240	1·240	1·237
C(2)–N(3)	1·349	1·346	1·358
N(3)–C(4)	1·338	1·331	1·339
C(4)–C(5)	1·455	1·442	1·433
C(4)–N(4)	1·351	1·348	1·324
C(5)–C(6)	1·334	1·329	1·357
C(5)–C(9)	1·520	1·528	
N(4)–C(7)	1·467	1·464	
N(4)–C(8)	1·468	1·477	
C(1)–N(1)–C(2)	119·5	119·4	118·2
C(1)–N(1)–C(6)	120·2	121·2	121·5
C(2)–N(1)–C(6)	120·2	119·3	121·2
N(1)–C(2)–O(2)	119·1	117·9	118·9
N(1)–C(2)–N(3)	118·5	118·3	118·6
O(2)–C(2)–N(3)	122·4	123·7	122·5
C(2)–N(3)–C(4)	122·1	121·7	120·5
N(3)–C(4)–C(5)	120·4	121·7	121·5
N(3)–C(4)–N(4)	114·9	114·6	118·3
C(5)–C(4)–N(4)	124·7	123·8	120·1
C(4)–C(5)–C(6)	115·9	115·3	117·0
C(4)–C(5)–C(9)	127·0	128·8	
C(6)–C(5)–C(9)	116·8	115·9	
N(1)–C(6)–C(5)	122·9	123·5	121·2
C(4)–N(4)–C(7)	119·6	120·2	
C(4)–N(4)–C(8)	125·6	125·9	
C(7)–N(4)–C(8)	114·7	113·8	

Table 4. *Least-squares planes*

The equations are of the form $lx + my + nz + p = 0$ where x,y,z are along **a**,**b**,**c**. Atoms which define the planes are marked with an asterisk.

	Displacement (Å) Molecule 1	Molecule 2
C(1)	−0·028	−0·042
N(1)*	0·007	−0·019
C(2)*	−0·007	0·020
O(2)	0·016	0·092
N(3)*	0·009	−0·001
C(4)*	−0·009	−0·017
C(5)*	0·009	0·017
C(6)*	−0·008	0·001
N(4)	−0·017	−0·079
C(7)	−0·074	−0·123
C(8)	0·102	−0·183
C(9)	−0·103	0·065

Direction cosines of the equation	*l*	*m*	*n*	*p*
Molecule 1	0·9089	0·1876	0·3724	−1·7676
Molecule 2	0·9130	−0·1309	0·3863	−4·3860

Table 5. *Some torsion angles* (°) *describing the conformation of* 1,5,*N*(4),*N*(4)-*tetramethylcytosine*

These four-atom angles *A–B–C–D* are defined as zero if, when looking from *B* to *C*, bonds *A–B* and *C–D* are *cis*-planar. The angle is counted positive if the far bond *C–D* is rotated clockwise with respect to the near bond *A–B*.

A–B–C–D	Molecule 1	Molecule 2
C(1)–N(1)–C(2)–O(2)	2·0	−3·0
C(1)–N(1)–C(6)–C(5)	179·3	−180·0
N(1)–C(6)–C(5)–C(9)	−176·2	−179·0
O(2)–C(2)–N(3)–C(4)	−178·8	−177·0
N(4)–C(4)–C(5)–C(6)	−179·0	176·4
N(3)–C(4)–N(4)–C(7)	−3·1	0·02
N(3)–C(4)–N(4)–C(8)	173·4	176·5

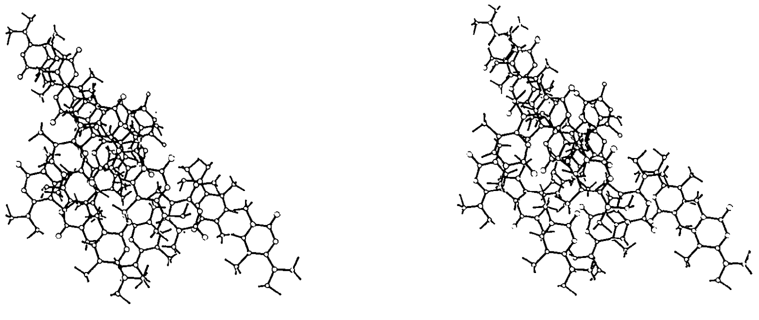

Fig. 4. Stereo view showing overlapping of the molecules.

N(4), C(4) and C(5) (Fig. 5) obviously requires less energy than the rotation of the dimethylamino group from coplanarity with the heterocycle, *i.e.* the sp^2 character of N(4) is preserved.

Space-filling models indicate that a planar TMC molecule should be strained considerably. The strain is not relieved by rotation of the dimethylamino group about C(4)—N(4) as anticipated by the quantum chemical calculations but rather by distortion of the bond angles involving N(4), C(4), C(5). Table 3 and Fig. 5 show that the endocyclic angles within the cytosine ring and the exocyclic angles at N(1) and C(2) are comparable to those averaged for cytidine. However, the angular deviations of the other exocyclic angles (Fig. 5) are far beyond the 3σ ($=1\cdot2°$) limit and can clearly be interpreted as arising from repulsion between the methyl groups C(8) and C(9).

It can be postulated that the planarity of the TMC molecule is not a consequence of crystal packing forces but an intrinsic property of the molecules because in this crystal structure no hydrogen bonds can form and intermolecular interactions are restricted to van der Waals forces. Mutual overlap between adjacent molecules occurs with pyrimidine rings and dimethylamino groups but not with the carbonyl groups as in 1,3-dimethyluracil (Banerjee, Dattagupta, Saenger & Rabczenko, 1977), another nucleobase derivative unable to form hydrogen bonds.

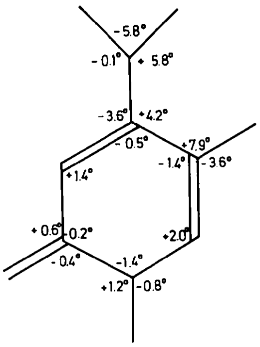

Fig. 5. Differences in angles between 1,5,*N*(4),*N*(4)-tetramethylcytosine and the standards: around N(4) = 120° (sp^2), around C(5) = 120° (sp^2), around C(4) and other angles as in cytidine.

The layered packing of the TMC molecules within the crystal structure is illustrated in the stereo views (Figs. 3 and 4). It is evident from Fig. 2 that the two molecules constituting the asymmetric unit are related by a pseudosymmetry: Table 2 shows that a pseudomirror occurs approximately at $z = 0\cdot3$.

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