cis-Thymidine 3',5'-Cyclic N,N-Dimethylphosphoramidate Acetone Solvate, a Cyclic Nucleotide with an Axial Dimethylamino Substituent on Phosphorus

BY WESLEY G. BENTRUDGE,* ALAN E. SOPCHIK AND WILLIAM N. SETZER
Department of Chemistry, The University of Utah, Salt Lake City, Utah 84112, USA
AND ROBERT B. BATES AND RICHARD B. ORTEGA
Department of Chemistry, The University of Arizona, Tucson, Arizona 85721, USA

(Received 7 June 1985; accepted 15 October 1985)

Abstract. C19H18N3O6P.C3H6O, Mr = 389.34, orthorhombic, P212121, a = 12.004 (9), b = 13.702 (9), c = 23.557 (7) Å, V = 3875 (4) Å3, Z = 8, Dm = 1.33 g cm⁻³, λ(Mo Kα) = 0.71073 Å, μ(Mo Kα) = 1.81 cm⁻¹, F(000) = 1648, T = 298 K, R = 0.091 for 3306 unique data, 2531 of which were considered observed. There are two independent hydrogen-bonded (base-paired) nucleotide molecules and two acetone molecules in the asymmetric unit. The dioxaphosphorinane ring of each cyclic nucleotide adopts a distorted chair conformation with the pyramidal dimethylamino substituent axial. The pyrimidine ring exists in an anti conformation.

Introduction. There is considerable interest in the understanding of the effects of heteroatom substitution on the conformational properties of saturated six-membered rings. We have been concerned with the effects of various substituents on P on the solid-state conformations of 1,3,2-dioxaphosphorinanes (Day, Bentrude, Yee, Setzer, Deiters & Holmes, 1984; Warren, Caughlan, Hargis, Yee & Bentrude, 1978; Haque, Caughlan, Hargis & Bentrude, 1970) and 1,3,2-oxazaphosphorinanes (Holmes, Day, Setzer, Sopchik & Bentrude, 1984; Bentrude, Day, Quin, Setzer, Sopchik & Holmes, 1984; Newton, Pantaleo, Bentrude & Chandrasekaran 1982; Bajwa, Bentrude, Pantaleo, Newton & Hargis, 1979). Recently we have extended our studies to include the conformational analysis of neutral derivatives of nucleoside 3',5'-cyclic monophosphates using NMR (Nelson, Sopchik & Bentrude, 1983; Sopchik, Bajwa, Nelson & Bentrude, 1981; Sopchik & Bentrude, 1980; Bajwa & Bentrude, 1978, 1980) and X-ray techniques (Newton, Pantaleo, Bajwa & Bentrude, 1982).

3',5'-Cyclic nucleotides play an important role in cell metabolism; adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) are important bioregulator molecules. There has been growing interest in the synthesis and biological activity of cyclic nucleotides in which the P centers are derivatized as triesters or phosphoramidates (Bajwa & Bentrude, 1978, and references therein). These derivatives may potentially act as cyclic nucleotide mimics, as antagonists of cyclic nucleotide action, or as storage forms of the natural diester nucleotides themselves (Nargeot, Nerbonne, Engels & Lester, 1983, and references therein).

This paper reports the crystal structure of cis-thymidine 3',5'-cyclic N,N-dimethylphosphoramidate, (1), a cyclic nucleotide with an axially disposed dimethylamino group on the P atom of the dioxaphosphorinane ring.

---

* To whom correspondence should be addressed.

0108-2701/86/050584-04$01.50 © 1986 International Union of Crystallography
Preparation of thymidine 3',5'-cyclic N,N-dimethylphosphoramidate. Dimethyl chloramine (0.5 mL) (Bock & Kompa, 1966) was added to a cooled (195 K) stirred solution of methyl thymidine 3',5'-cyclic phosphate (1-0 g, 3.31 mmol) (Bajwa & Bentrude, 1978) in anhydrous dichloromethane (20 mL). The reaction mixture was allowed to warm to room temperature and stirred for 3 h. All volatile materials were removed from the reaction mixture in vacuo and the residue chromatographed by MLC on a 15 × 1000 mm column of silica gel (Merck, 230-400 mesh), eluting with CHCl3/CH3OH (97:3), to give 500 mg of pure trans-phosphoramidate: 31P NMR (CDCl3) δ 8-63 (Baschang & Kvita, 1973) and 200 mg of pure cis-phosphoramidate: 31P NMR (CDCl3) δ 7-22 (Sopchik & Bentrude, 1980). Crystals of the cis-phosphoramidate, suitable for X-ray crystallography, were obtained by slow evaporation of the compound in acetone.

Structure determination. Syntex P1 diffractometer; cell parameters refined by least-squares method from the setting angles of 15 reflections in the 2θ range 5-7-21.7°; intensities collected with Mo Kα radiation (λ = 0.71073 Å), 4-0 ≤ 2θ ≤ 50-0°, 0-2θ scan; no absorption or extinction correction; data reduced to Fo E 5.7-21.7°; largest peak in final difference map = 0.5 e Å⁻³. Range of hkIl: h 0-14, k 0-16, l 0-28. (A/σ)max = 0.9. Three standard reflections (600, 060, 0,0,12) monitored every 97 reflections; no discernible decomposition of the crystal. Major programs used during structure determination were FORDAP (Fourier summation program by A. Zalkin) and NUCLS [structure factor calculations and least-squares refinement by J. A. Ibers, itself a modification of ORFLES (Busing, Martin & Levy, 1962)].

Discussion. The final atomic parameters with their standard deviations are listed in Table 1. * There are two independent hydrogen-bonded (base-paired) nucleotide molecules and two acetone molecules in the asymmetric unit.

<table>
<thead>
<tr>
<th>First molecule</th>
<th>Second molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uiso or Ueq</td>
<td>x</td>
</tr>
<tr>
<td>p</td>
<td>0.3609 (3)</td>
</tr>
<tr>
<td>O4'</td>
<td>0.6660 (5)</td>
</tr>
<tr>
<td>O3'</td>
<td>0.3763 (6)</td>
</tr>
<tr>
<td>O2'</td>
<td>0.4673 (8)</td>
</tr>
<tr>
<td>N'</td>
<td>0.3716 (10)</td>
</tr>
<tr>
<td>O'</td>
<td>0.2629 (8)</td>
</tr>
<tr>
<td>C8(3)</td>
<td>0.4077 (16)</td>
</tr>
<tr>
<td>C9(3)</td>
<td>0.2941 (14)</td>
</tr>
<tr>
<td>C10(3)</td>
<td>0.5653 (8)</td>
</tr>
<tr>
<td>C5(3)</td>
<td>0.5578 (10)</td>
</tr>
<tr>
<td>O(4)</td>
<td>0.9416 (6)</td>
</tr>
<tr>
<td>C7</td>
<td>0.2941 (14)</td>
</tr>
<tr>
<td>N(3)</td>
<td>0.3716 (10)</td>
</tr>
<tr>
<td>O(5)</td>
<td>0.4673 (8)</td>
</tr>
<tr>
<td>C6(4)</td>
<td>0.3716 (10)</td>
</tr>
<tr>
<td>O(A)</td>
<td>0.5334 (23)</td>
</tr>
</tbody>
</table>

* Lists of structure factors, anisotropic thermal parameters, H-atom coordinates and bond lengths, bond angles, and torsion angles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42541 (17 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.
The two independent nucleotide molecules are hydrogen bonded to each other by way of mutual N(3)-H(3)...O(4) hydrogen bonds (Fig. 1). The N(3)...O(4) intermolecular distances are 2.86 (1) and 2.84 (1) Å. The acetone molecules both appear to have attractive dipole-dipole interactions with nucleotide 4-carbonyl groups: in the first molecule, the C(4) of the carbonyl group is 3.33 (2) Å from an acetone O whereas, in the second molecule, the nucleotide carbonyl oxygen, O(4), is 3.51 (2) Å from the C of the other acetone.

The conformation adopted by the thymine base in compound (1) is anti. The glycosyl torsion angles, \( \chi[O(4')-C(1')-N(1)-C(2')] \), for the two independent molecules are 252.8 and 233.1°.* The anti conformation is found in crystalline thymidine (Young, Tollin & Wilson, 1969), thymidylyl thymidylate (Camerman, Fawcett & Camerman, 1976), and 6-azathymidine (Banerjee & Saenger, 1978). Uridine 3',5'-cyclic monophosphate (Coulter, 1969) and trans-5-isopropyl-2'-deoxyuridine 3',5'-cyclic N-benzylphosphoramidate (Béres, Bentrude, Kálman, Párkányi, Balzarini & De Clercq, 1985) have syn conformations about the glycosidic bond.

The geometrical parameters (bond lengths and bond angles) in the ribose ring and the base are comparable to those found in thymidine itself (Young, Tollin & Wilson, 1969) with the exception of the C(3')...C(4') bond length. In thymidine this bond length is 1.53 Å, whereas in (1) it is 1.45 (1) and 1.47 (1) Å. The 1,3,2-dioxaphosphorinane ring in (1) shows bond lengths and bond angles similar to those of other neutral cyclic nucleotide derivatives (Béres, Sândor, Kálman, Koritsánszky & Ötvös, 1984; Cotton, Gillen, Gohil, Hazen, Kirchner, Nagyvary, Rouse, Stanisłowski, Stevens & Tucker, 1975).

The ribose rings of (1) adopt severely twisted \( 4T^3 \), C(4')-exo/C(3')-endo, conformations (Sundaralingam & Abola, 1972) as evidenced by the relatively small O(4')...C(1')...C(2')...C(3') torsion angles, \( \nu_1 \), of 1.0 and 2.1°, and the large C(4')...O(4')...C(1')...C(2') torsion angles, \( \nu_0 \), of -27.2 and -29.3°. The 1,3,2-dioxaphosphorinane ring of (1) is in a distorted chair conformation, although in solution the twist conformation is populated to the extent of 64–75%. The P end of the six-membered ring is flattened as is generally observed in 1,3,2-dioxaphosphorinanes (Warrent, Caughlan, Hargis, Yee & Bentrude, 1978). The P-O(3')-C(3') angles are 114.5 (6) and 114.7 (6)° while the P-O(5')-C(5') angles are opened to 123.0 (7) and 123.4 (7)°. These bond angles are similar to those found in other cyclic nucleotides (Newton, Pantaleo, Bajwa & Bentrude, 1977, and references therein).

* The atom labeling and definitions of \( \chi \), \( \nu_0 \) and \( \nu_1 \) are according to Eur. J. Biochem. (1983), 131, 9–15.
References


Structure of Benzamidine Hydrochloride Monohydrate

BY V. G. THAILAMBAL AND VASANTHA PATTABHI*
Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Madras – 600 025, India

and T. N. GURU ROW

Physical Chemistry Division, National Chemical Laboratories, Pune – 411 008, India

(Received 2 September 1985; accepted 3 December 1985)

Abstract. C_6H_6N_2Cl^-·H_2O, M_r = 174-5, orthorhombic, P_2_1_2_1_2_1, a = 7-328 (2), b = 8-500 (2), c = 14-573 (3) Å, V = 907-7 (4) Å^3, Z = 4, D_m (flotation) = 1-279, D_x = 1-277 g cm^-3, Mo Ka, λ = 0-7107 Å, μ = 3-78 cm^{-1}, F(000) = 368, T = 293 K, final R = 0-051 for 707 observed reflections with I > 2σ(I). The imine group is protonated. Chlorine is involved in five hydrogen bonds, three of N···H···Cl and two of O(W)···H···Cl type. The terminal C–C\_N\_N\_N plane makes an angle of 36-6° (8) with the benzene-ring plane, which prevents conjugation between the two unsaturated systems.

* Contribution No. 676.

0108-2701/86/050587-03$01.50 © 1986 International Union of Crystallography