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Structure and conformation of the cyclic phosphate of Ganciclovir, a broad-spectrum antiviral agent

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Abstract

The title compound, the cyclic phosphate of the antiviral acyclonucleoside Ganciclovir (2′-NDG, DHPG), is itself a potent broad-spectrum antiviral agent, but with a different mechanism of action. The cyclic phosphate, 9-[[[(2-hydroxy-1,3,2-dioxophosphorinan-5-yl)oxy]methyl]-P-oxide]guanine (2′-nor-cGMP, DHPG-cMP), crystallizes in the monoclinic space group $P2_1/n$ with unit cell dimensions a = 6.612(1) Å, b = 11.562(4) Å, c = 19.231(5) Å and β = 91.786(2)° at −165°C. The N7 of the guanine base is protonated, so that the molecule is in a zwitterionic form, with two water molecules in the asymmetric unit. The principal conformational features of DHPG-cMP in the crystal are as follows: the acyclic chain is partially folded; the six-membered cyclic phosphate ring is in a chair form with C3′, O3′, C5′ and O5′ in a plane; P and C4′ are displaced in diametrically opposite directions from this plane; the O4′ is in the axial orientation with respect to this ring; and the aglycon is in the *high syn* conformation about the glycosidic bond. The conformation of the cyclic phosphate ring in aqueous medium, determined by means of ^{1}H-NMR spectroscopy, is similar to that in the crystalline form. The conformational features of DHPG-cMP were compared with those of the parent DHPG and other related compounds and, in particular, with those of the second messenger 3′:5′-cGMP, of which it is a close structural analogue. Previously reported substrate/inhibitor properties of these compounds in several enzyme systems are examined in relation to the possible mechanism of antiviral activity of DHPG-cMP as a second messenger analogue of cGMP.

Key words: Acyclonucleotide; Cyclic phosphate; Molecular structure; Molecular conformation; X-Ray diffraction; NMR; Antiviral activity

1. Introduction

The potent antiherpes activity of the clinically approved Acyclovir (ACV, 9-(2-hydroxyethoxymethyl) guanine, Fig. 1) stimulated the synthesis of a multitude of acyclonucleosides of purine and pyrimidine analogues. A number of these, including methylphosphonate derivatives, exhibit promising antiviral, and in some instances, antitumor activities; while many are excellent inhibitors of enzymes which play key roles in chemotherapy [1], and references cited.

A closely related analogue of Acyclovir, Ganciclovir (2′-NDG, DHPG, 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine, see Fig. 1) exhibits broad-spectrum activity against a variety of DNA viruses [2]. Both ACV and DHPG, like most acyclonucleosides, require intracellular phosphorylation, usually by viral thymidine kinase, in infected cells for expression of antiviral activity. It was then found that the cyclic phosphate of Ganciclovir (DHPG-cMP, see Fig. 1) displays potent broad-spectrum antiviral activity [3]. In particular, DHPG-cMP proved to be up to 20-fold more effective, with a selectivity index 10-fold higher, than its parental

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Abbreviations: Acyclovir, ACV, 9-(2-hydroxyethoxymethyl)guanine; Ganciclovir, 2′-NDG, DHPG, 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine; DHPG-cMP, DHPG-3′:5′-cyclic phosphate, 9-[[[(2-hydroxy-1,3,2-dioxophosphorinan-5-yl)oxy]methyl]-P-oxide]guanine; DHPAde, 9-[(1,3-dihydroxy-2-propoxy)methyl]adenine; DHPAde-cMP, DHPAde-3′:5′-cyclic phosphate, 9-[[[(2-hydroxy-1,3,2-dioxophosphorinan-5-yl]oxy]methyl]-P-oxide]adenine; TSP, 3-trimethylsilyl-(2,2,3,3-$^2H_4$ propionate) sodium salt.

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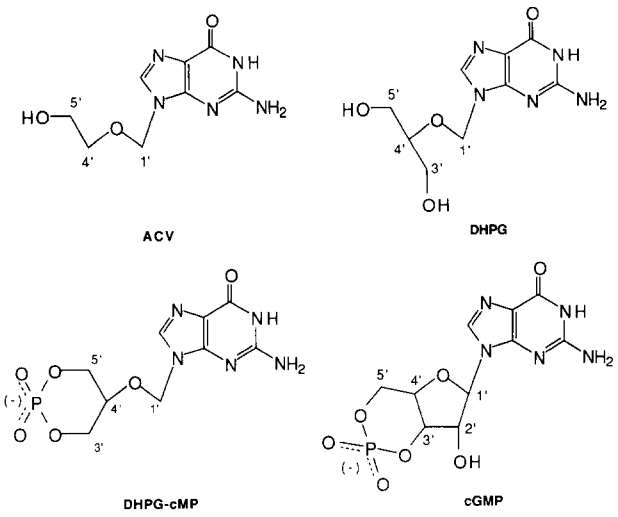

Fig. 1. Structures of ACV, DHPG, DHPG-cMP and cGMP. Note that the carbons in the acyclic chains are numbered like the corresponding carbons of the pentose ring in cGMP.

drug DHPG against a cytomegalovirus infection in guinea pigs [4]. This was, in fact, the first nucleotide analogue, and the first cyclic phosphate of an acyclonucleoside, with potent antiviral activity. It is also not dependent on prior 'activation' by intracellular phosphorylation by viral thymidine kinase, and is active vs. viruses which do not code for a thymidine kinase [5].

However, the mechanism of action of DHPG-cMP, which is taken up intact by cells [6], has not been elucidated. Although its cyclic phosphate ring is slowly hydrolyzed in intact cells, the level of librated DHPG-monophosphate is inadequate to account for its antiviral activity [6]. We have elsewhere postulated that its activity may be related to the fact that it is a close structural analogue of the second messenger cGMP (Fig. 1), and have drawn attention to numerous reports on the modulation of viral replication by cAMP and cGMP [1], further underlined by more recent similar findings [7,8].

If DHPG-cMP is indeed active as such, and not dependent on its intracellular metabolism, it is clearly of interest to examine its conformation, which should be 'recognized' by some constituent(s) of infected cells. The conformations of several acyclonucleosides, and their relevance to recognition by phosphorylating and nucleolytic enzymes, have been widely investigated [2,9,10]. Particulary striking are two recent reports describing the phosphorylation of DHPG in cells infected with human cytomegalovirus by a viral-coded protein which is not a thymidine kinase, but possesses the sequence characteristics of the catalytic domain of a protein kinase [11,12], leading to the inference that the conformation of DHPG may mimic some region of a protein kinase substrate.

We describe here the structure and conformation of the cyclic phosphate of DHPG, both in the solid state and in solution, and a comparison with other related molecules, including cGMP.

2. Materials and methods

The title compound, DHPG-cMP, was synthesized as previously described [13]. Slow diffusion of acetone into an aqueous solution of the free acid at room temperature led to formation of suitable crystals, in the form of platelets, with two molecules of water per molecule DHPG-cMP, and with the N7 of the base protonated. A crystal with dimensions $0.40 \times 0.28 \times 0.13$ mm was mounted and the cell dimensions determined from 19 reflections with 2θ angles in the range 20–35°. The low-temperature (−165°C) crystal data are as follows: $PO_6N_5C_9H_{12} \cdot 2\,H_2O$, mol wt. = 353.22, monoclinic spacegroup $P2_1/n$ with unit cell dimensions $a = 6.612(1)$ Å, $b = 11.562(4)$ Å, $c = 19.231(5)$ Å, $\beta = 91.786(2)°$, $V = 1469.5$ Å^3, $\rho_c = 1.597$ Mg · m^{-3}, $Z = 4$, $F(000) = 736.77$, $\mu = 0.20$ mm^{-1}, λ (MoK$_\alpha$) = 0.70932 Å, $2\theta(\max) = 49.8°$. No absorption correction was considered necessary due to the low μ value.

The intensity data, collected at −165°C on a Rigaku diffractometer, using the $\theta/2\theta$ scan mode, consists of 2060 reflections with intensities greater than 2.5σ and were considered observed. The intensities of two reflections, 002 and 011, were cut off due to the backstop being too broad and were thus excluded. The structure was solved on the NRCVAX system [14] employing the symbolic addition method and refined with full-matrix least squares to: $R = 0.0401$ ($R' = 0.0506$) for the observed reflections, $R = 0.0548$ ($R' = 0.0517$) for all reflections, with 39 atoms, 273 parameters (including the secondary extinction coefficient) and 2058 observed out of 2575 reflections up to $2\theta_{max}$ of 49.8°. Weights based on counting-statistics were used: $w = 1/(\sigma^2 F_0 + k^2)$ with a k value of 0.0002. No shift was larger than 0.01σ after the last cycle of refinement. The deepest hole in the last difference Fourier map was −0.32 e/Å^3 and the highest peak 0.31 e/Å^3.

^{1}H-NMR spectra of DHPG-cMP in the neutral form (Na^+ salt), at a frequency of 500.13 MHz, were recorded on a Bruker 500 AM instrument at a concentration of 0.02 M in ^2H_2O at 30°C, with TSP as internal standard.

3. Results and discussion

Solid-state conformation of DHPG-CMP

The title molecule, crystallized from acid medium, is in the zwitterionic form, with the guanine ring protonated at N7 and the phosphate group negatively

charged. The final atomic coordinates and thermal parameters, listed in Tables 1a and 1b, respectively, were employed to calculate the bond lengths and bond angles (Table 2). These are in reasonably good agreement with those in other related structures. The bond lengths C1′-O4′ (1.396(3) Å) and C4′-O4′ (1.445(3) Å), agree with those of other acyclonucleosides, in contrast to the equal values found in cGMP, 1.430 Å and 1.425 Å, respectively [15].

The three-dimensional structure and conformation is described by the torsion angles (Tables 3a, b) and the stereoscopic view in Fig. 2. Since the space group is a centrosymmetric one, the crystals contain a racemic mixture, with both enantiomers present in equal amounts; hence the torsion angles for the other enantiomer have signs opposite to those listed.

Table 1
(a) Final atomic parameters x, y, z and biso

	x	y	z	Biso *
N 1	0.7826 (4)	0.43162 (21)	0.05036 (12)	1.44 (9)
C 2	0.7283 (4)	0.51427 (23)	0.09769 (14)	1.43 (10)
N 2	0.7364 (4)	0.48365 (24)	0.16442 (13)	1.96 (11)
N 3	0.6671 (3)	0.61956 (19)	0.08000 (11)	1.41 (9)
C 4	0.6730 (4)	0.63608 (23)	0.01063 (13)	1.21 (10)
C 5	0.7297 (4)	0.56020 (24)	−0.04024 (14)	1.44 (11)
C 6	0.7887 (4)	0.44571 (23)	−0.02218 (14)	1.35 (10)
O 6	0.8398 (3)	0.36566 (16)	−0.05956 (10)	1.74 (7)
N 7	0.7110 (3)	0.61812 (20)	−0.10366 (12)	1.61 (9)
C 8	0.6454 (4)	0.72302 (25)	−0.09158 (14)	1.65 (11)
N 9	0.6191 (3)	0.73854 (19)	−0.02249 (11)	1.46 (9)
C 1′	0.5401 (5)	0.8428 (3)	0.01074 (16)	1.81 (12)
O 4′	0.6938 (3)	0.92083 (16)	0.02902 (10)	1.76 (8)
C 4′	0.8069 (5)	0.8935 (3)	0.09240 (15)	2.01 (12)
C 3′	0.6907 (6)	0.9312 (3)	0.15446 (17)	2.78 (15)
O 3′	0.6679 (3)	1.05674 (18)	0.15493 (10)	2.27 (8)
P	0.87482 (12)	1.12819 (6)	0.15716 (4)	1.58 (3)
O 1′	0.8251 (4)	1.24958 (18)	0.14119 (10)	2.72 (10)
O 2′	0.9889 (3)	1.10138 (17)	0.22368 (10)	2.21 (9)
O 5′	0.9898 (3)	1.07714 (17)	0.09220 (10)	2.01 (8)
C 5′	1.0094 (5)	0.9524 (3)	0.08881 (17)	2.10 (12)
W 1	0.7672 (4)	0.56019 (23)	−0.23279 (11)	2.49 (10)
W 2	0.2174 (4)	0.24494 (21)	0.31191 (14)	3.54 (11)
H 1	0.808 (5)	0.366 (3)	0.0660 (18)	3.6 (9)
H 2A	0.770 (5)	0.407 (3)	0.1737 (18)	3.7 (8)
H 2B	0.693 (5)	0.535 (3)	0.1941 (18)	2.7 (8)
H 7	0.744 (6)	0.589 (3)	−0.1503 (19)	4.1 (9)
H 8	0.622 (4)	0.7829 (24)	−0.1249 (14)	1.2 (6)
H 1′A	0.465 (4)	0.8201 (24)	0.0493 (15)	1.5 (6)
H 1′B	0.453 (4)	0.881 (3)	−0.0259 (15)	1.9 (6)
H 4′	0.826 (4)	0.814 (3)	0.0932 (15)	2.1 (7)
H 3′A	0.558 (6)	0.900 (3)	0.1550 (18)	3.8 (9)
H 3′B	0.760 (5)	0.908 (3)	0.1946 (19)	3.3 (8)
H 5′A	1.075 (5)	0.936 (3)	0.0466 (16)	2.0 (6)
H 5′B	1.091 (5)	0.9282 (25)	0.1271 (16)	1.8 (6)
H W1A	0.683 (6)	0.509 (3)	−0.2494 (19)	3.4 (9)
H W1B	0.755 (7)	0.625 (4)	−0.254 (3)	6.4 (13)
H W2A	0.143 (7)	0.201 (4)	0.2838 (23)	6.0 (12)
H W2B	0.265 (9)	0.186 (5)	0.342 (3)	8.9 (16)

The e.s.d's refer to the last digit printed.
* Biso is the mean of the principal axes of the thermal ellipsoid.

Base. As can be seen from Plane 1 of Table 4, the guanine residue is fairly flat, with the endocyclic atoms, C2 and C6, deviating slightly from this plane (0.021 Å and −0.017 Å). All the exocyclic atoms, *viz*. N2, O6, and C1′ are displaced from this plane to a certain extent, but the largest deviation, −0.079 for C1′, is much less than in cGMP, where it is 0.184 Å [15].

The orientation of the base relative to the sugar moiety is given by the glycosidic torsion angle χ (O4′-C1′-N9-C4) of 93.1°, hence in the range characterized as *high syn* (+ac, [16]).

Side chain. The geometrical arrangement of the acyclic chain, i.e., the part comprised of the atoms C1′, O4′, C4′, and C3′, is close to that of a sugar ring in the C4′*endo* envelope (^{4}E) conformation, but with the C2′ atom missing. This can be seen by calculating a plane through the three atoms C1′, C3′, and C4′ (Plane 3 in Table 4). The two hydrogen atoms, H1′A and H3′A, are almost in this plane, both being displaced to the same side by only 0.10(3) Å and 0.30(4) Å, respectively, while C4′ is displaced by 0.780 Å in the opposite direction. The C4′*endo* envelope corresponds to the following pseudorotational parameters: phase angle P = 237.7°, amplitudes of pucker $\nu_0 = 42.9°$, $\nu_{max} = 80.3°$. It should be noted that, in calculating the angles ν_0 and ν_3, the positions of the hydrogen atoms H1′A and H3′A were used instead of the missing C2′ (Table 3a).

The side chain N9-C1′-O4′-C4′-C5′ is partially folded, the N9 and C4′ atoms being in the *gauche* (-sc) orientation, with a torsion angle of −79.5(2)° for N9-C1′-O4′-C4′, whereas C1′ and C5′ are *trans* to each other, with a torsion angle of 157.0(3)° (Table 3b).

Cyclophosphate ring. The phosphate group is part of a six-membered ring in the chair conformation. Four of the ring atoms C3′, O5′, O3′, and C5′, lie in one plane (Plane 2 in Table 4) while C4′ and P are displaced from this plane by −0.628(5) and 0.717(3) Å, respectively. The ring is symmetrical about a plane passing through the atoms C4′ and P, with the exception of the P-O2′ bond (1.497(2) Å) which is 0.025 Å longer than the P-O1′ bond (Table 2). The difference can probably be attributed to differences in the packing environments of the two oxygen atoms, O2′ being the acceptor of three hydrogen bonds, two from the two water molecules W1 and W2 and one from the NH_2 group, while O1′ is the acceptor of two hydrogen bonds (see below). The endocyclic angles in the ring are in the range 52° to 59°, close to the classical value of 60° (Table 3a). O4′ is attached to C4′ in the axial position of the cyclophosphate ring (Fig. 3).

Hydrogen bonding and packing. As may be seen from the packing diagram (Fig. 4) and the hydrogen bond distances and angles listed in Table 5, the molecules form infinite chains of hydrogen bonds via the water molecules and O2′, forming layers of linked molecules parallel to the yz-plane. This type of hydrogen bonding

Table 1
(b) Thermal parameters, U (i,j)

	U_{11} (U)	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N 1	1.82 (13)	1.66 (13)	1.99 (13)	0.07 (10)	−0.05 (10)	0.05 (10)
C 2	1.39 (14)	2.08 (15)	1.94 (14)	−0.25 (11)	−0.06 (11)	−0.19 (11)
N 2	3.60 (15)	2.10 (14)	1.76 (13)	0.47 (12)	0.06 (11)	−0.04 (11)
N 3	1.71 (12)	1.75 (12)	1.90 (12)	0.11 (10)	−0.12 (9)	0.10 (9)
C 4	1.14 (13)	1.57 (14)	1.86 (14)	−0.22 (11)	−0.22 (10)	−0.14 (11)
C 5	1.30 (14)	2.45 (15)	1.69 (14)	−0.29 (12)	−0.32 (11)	−0.10 (11)
C 6	1.08 (13)	1.98 (14)	2.04 (14)	−0.33 (11)	−0.17 (11)	−0.29 (12)
O 6	2.28 (11)	2.26 (11)	2.06 (10)	0.31 (9)	−0.11 (8)	−0.52 (8)
N 7	2.20 (13)	2.32 (14)	1.58 (12)	−0.33 (11)	−0.16 (10)	0.02 (10)
C 8	2.33 (16)	2.11 (16)	1.79 (15)	−0.50 (13)	−0.59 (12)	0.40 (12)
N 9	2.01 (13)	1.44 (12)	2.06 (12)	0.00 (10)	−0.52 (10)	0.01 (10)
C 1′	2.50 (17)	1.70 (15)	2.64 (17)	0.24 (12)	−0.49 (13)	−0.14 (12)
O 4′	2.97 (12)	1.63 (10)	2.03 (10)	−0.32 (9)	−0.61 (8)	0.03 (8)
C 4′	4.15 (19)	1.55 (16)	1.85 (15)	−0.11 (14)	−0.94 (13)	0.11 (12)
C 3′	4.90 (23)	3.48 (20)	2.21 (18)	−2.10 (17)	0.22 (16)	−0.05 (14)
O 3′	2.78 (12)	3.13 (12)	2.72 (12)	−0.50 (10)	0.23 (9)	−0.77 (9)
P	2.74 (4)	1.62 (4)	1.62 (4)	−0.10 (3)	−0.30 (3)	0.07 (3)
O 1′	5.71 (16)	2.15 (12)	2.43 (12)	0.49 (11)	−0.65 (11)	0.12 (9)
O 2′	4.01 (13)	2.33 (11)	2.00 (11)	0.03 (10)	−0.94 (9)	−0.05 (9)
O 5′	3.00 (12)	2.63 (11)	2.03 (11)	−0.52 (9)	0.22 (9)	−0.20 (9)
C 5′	3.15 (18)	2.50 (17)	2.28 (16)	0.64 (14)	−0.63 (14)	−0.55 (13)
W 1	4.20 (14)	3.36 (14)	1.89 (12)	−1.02 (12)	−0.24 (10)	−0.14 (10)
W 2	6.49 (19)	2.99 (14)	3.80 (15)	−1.56 (13)	−2.21 (13)	0.58 (12)
H 1	4.5 (11)					
H 2A	4.7 (11)					
H 2B	3.5 (10)					
H 7	5.1 (11)					
H 8	1.5 (7)					
H 1′A	1.8 (8)					
H 1′B	2.4 (8)					
H 4′	2.7 (8)					
H 3′A	4.8 (11)					
H 3′B	4.2 (10)					
H 5′A	2.5 (8)					
H 5′B	2.2 (8)					
H W1A	4.4 (11)					
H W1B	8.1 (17)					
H W2A	7.6 (15)					
H W2B	11.3 (20)					

The e.s.d's refer to the last digit printed
The anisotropic temperature factors are of the form
$T = \exp\left[-2\pi^2\left(U_{11}h^2a^{*2} + \cdots + 2U_{12}hka^*b^* + \cdots\right)\right]$.

is energetically the most favourable due to the cooperativity effect [17]. Here, the water oxygen atoms in the infinite chains act both as donors and acceptors, while the oxygen atoms O2′ act only as acceptors. In addition, there are cross-links from these infinite chains to the O6, N2 and N7 atoms of the bases, making most of the hydrogen bonds face the water channels. The molecules are also cross-linked with hydrogen bonds from the N1 and N2 atoms of the bases to the O1′ of the phosphate. The molecules pack in such a way that these hydrophilic regions, located around the twofold screw axes parallel to the *b*-axis, alternate with hydrophobic regions consisting of stacks of the guanine heterocycles, almost parallel to the yz-plane. The distances between atoms in the overlapping bases range from 3.157 Å (C6...C6) to 3.367 Å (N3...O6). This type of packing is common in nucleoside and nucleotide crystal structures [18].

All of the OH and NH hydrogen atoms are involved in hydrogen bonds. However, none of the ether oxygen atoms act as acceptors, which is not an uncommon feature. There is no CH hydrogen bond observed here, the H8...O1′ distance being too long (2.99(3) Å), even though the C8 and O1′ atoms make a close contact of 3.241(4) Å. There is also another contact of similar length (3.220(4) Å) between C1′ and O4′, the H1′B...O4′ distance being 2.48(3) Å and the C1′-H1′B.O4′ angle being 130.0(21)°.

The shortest hydrogen bond occurs between the positively charged N7 and a water molecule, the

Table 2
Final bond lengths and angles

N1-C2	1.375	(3)	C4′-C3′	1.504	(5)
N1-C6	1.406	(4)	C4′-C5′	1.506	(5)
N1-H1	0.84	(4)	C4′-H4′	0.93	(3)
C2-N2	1.331	(4)	C3′-O3′	1.460	(4)
C2-N3	1.324	(3)	C3′-H3′A	0.95	(4)
N2-H2A	0.93	(4)	C3′-H3′B	0.92	(4)
N2-H2B	0.88	(4)	O3′-P	1.5975	(22)
N3-C4	1.349	(3)	P-O1′	1.4718	(22)
C4-C5	1.375	(4)	P-O2′	1.4972	(20)
C4-N9	1.386	(3)	P-O5′	1.5956	(20)
C5-C6	1.420	(4)	O5′-C5′	1.450	(4)
C5-N7	1.394	(4)	C5′-H5′A	0.95	(3)
C6-O6	1.226	(3)	C5′-H5′B	0.94	(3)
N7-C8	1.311	(4)			
N7-H7	0.99	(4)	W1-HW1A	0.87	(4)
C8-N9	1.357	(4)	W1-HW1B	0.85	(5)
C8-H8	0.95	(3)	W2-HW2A	0.88	(5)
N9-C1′	1.468	(4)	W2-HW2B	0.94	(6)
C1′-O4′	1.396	(3)			
C1′-H1′A	0.94	(3)			
C1′-H1′B	1.00	(3)			
O4′-C4′	1.445	(3)			
C2-N1-C6	126.33	(24)	O4′-C4′-H4′	107.2	(18)
C2-N1-H1	116.8	(24)	C3′-C4′-C5′	112.5	(3)
C6-N1-H1	116.8	(24)	C3′-C4′-H4′	110.2	(18)
N1-C2-N2	116.8	(3)	C5′-C4′-H4′	109.4	(18)
N1-C2-N3	123.56	(24)	C4′-C3′-O3′	110.40	(25)
N2-C2-N3	119.68	(25)	C4′-C3′-H3′A	113.2	(21)
C2-N2-H2A	116.1	(21)	C4′-C3′-H3′B	109.1	(22)
C2-N2-H2B	116.4	(21)	O3′-C3′-H3′A	106.3	(22)
H2A-N2-H2B	126	(3)	O3′-C3′-H3′B	109.3	(21)
C2-N3-C4	111.49	(22)	H3′A-C3′-H3′B	108	(3)
N3-C4-C5	129.04	(25)	C3′-O3′-P	115.23	(21)
N3-C4-N9	124.08	(23)	O3′-P-O1′	107.58	(13)
C5-C4-N9	106.88	(23)	O3′-P-O2′	108.92	(12)
C4-C5-C6	119.99	(24)	O3′-P-O5′	102.48	(11)
C4-C5-N7	107.29	(24)	O1′-P-O2′	118.63	(12)
C6-C5-N7	132.71	(25)	O1′-P-O5′	107.34	(12)
N1-C6-C5	109.53	(23)	O2′-P-O5′	110.65	(12)
N1-C6-O6	120.75	(25)	P-O5′-C5′	116.66	(18)
C5-C6-O6	129.7	(3)	C4′-C5′-O5′	111.54	(25)
C5-N7-C8	108.08	(23)	C4′-C5′-H5′A	112.4	(18)
C5-N7-H7	127.7	(21)	C4′-C5′-H5′B	108.6	(18)
C8-N7-H7	124.1	(21)	O5′-C5′-H5′A	106.1	(18)
N7-C8-N9	110.42	(24)	O5′-C5′-H5′B	108.1	(17)
N7-C8-H8	126.9	(16)	H5′A-C5′-H5′B	110	(3)
N9-C8-H8	122.6	(16)	O4′-C4′-C3′	110.0	(3)
C4-N9-C8	107.33	(22)	O4′-C4′-C5′	107.45	(24)
C4-N9-C1′	126.27	(23)	HW1A-W1-HW1B	111	(4)
C8-N9-C1′	126.36	(23)	HW2A-W2-HW2B	97	(4)
N9-C1′-O4′	111.97	(23)			
N9-C1′-H1′A	108.5	(17)			
N9-C1′-H1′B	105.3	(16)			
O4′-C1′-H1′A	112.4	(17)			
O4′-C1′-H1′B	106.6	(17)			
H1′A-C1′-H1′B	111.8	(24)			
C1′-O4′-C4′	115.14	(22)			

N7...W1 distance being 2.610(3) Å. This is in agreement with the fact that strong hydrogen bonds are generally observed between electronegative atoms that are positively or negatively charged. The hydrogen bonds are mostly linear, with the largest deviation from linearity occuring in W2-HW2B...O6, with an angle of 147(4)°. This hydrogen bond is also one of the weakest in this structure, with the HW2B...O6 distance being 2.04(6) Å. The only weaker one is N2-H2B...O2′ with an H2B...O2′ distance of 2.16(4) Å.

Solution conformation of DHPG-cMP

The conformation of the cyclic phosphate ring of DHPG-cMP in neutral aqueous medium was derived from the vicinal coupling constants ^{1}H-^{1}H and ^{1}H-^{31}P which, with the aid of appropriate parametrization of the Karplus relationship, are related to the torsion angles in the ^{1}H-C-C-^{1}H [19] and ^{1}H-C-O-^{31}P [20] fragments.

The measured NMR parameters (Table 6) demonstrate that the six-membered cyclic phosphate ring in solution is in a chair form, with the phosphorus and C4′ carbon displaced in opposite directions from the plane of the ring, and with O4′ axial with respect to the ring, as in the solid-state (see above). The endocyclic angles in the ring are about 50°, hence deviating slightly more from the classical value of 60° than in the crystal structure (Table 3).

Comparison with acyclonucleosides and cGMP

The crystal structure of DHPG-cMP is now compared with that of the structural analogue cGMP sodium tetrahydrate [15] and of some acyclonucleosides, *viz.* DHPG, DHPAde [21] and Acyclovir [22]. All the appropriate torsion angles are shown in Tables 3a, b.

The orientation of the base relative to the 'sugar' moiety in DHPG-cMP ($\chi = 93.1°$) is similar to those found in other acyclonucleosides, viz. 104.3° and −107.5° in the two molecules of DHPAde, and −90.5° in molecule C of ACV, where the side chain is fully

Table 3
(a) Comparison of torsion angles (°) of sugar moiety and phosphate ring with those of cyclic GMP

Glycosyl angle and sugar moiety	DHPG-cMP	cGMP
χ [O4′ N 9 C 1′ C 4]	93.1 (3)°	78 °
υ_0 [here H1′A C1′ O4′ C4′]	42.9 (17)	−18.4
υ_1		−9.3
υ_2		31.7
υ_3 [here O4′ C4′ C3′ H3′A]	54.8 (22)	−44.3
υ_4	−80.3 (3)	38.7
P	237.7	42.6
Puckering	~^{4}E	$_{4}T^{3}$
Phosphate ring conformation	**DHPG-cMP**	**cGMP**
C 3′ O 3′ P O 5′	54.2 (2)°	44.2°
C 4′ C 3′ O 3′ P	−59.1 (2)	−59.8
C 5′ C 4′ C 3′ O 3′	55.5 (2)	68.5
C 3′ C 4′ C 5′ O 5′	−53.3 (2)	−60.5
P O 5′ C 5′ C 4′	54.8 (2)	53.6
O 3′ P O 5′ C 5′	−52.2 (2)	−44.3

Table 3
(b) Comparison of torsion angles (°) of the side chain with those of other acyclo nucleosides

Side chain	DHPG-cMP	DHPG	DHP-Ade		ACV		
			A	B	A	B	C
O 4′ C 1′ N 9 C 4 [χ]	93.1 (3)	69.7	104.3	−107.5	−76.5	−74.4	−90.5
	−89.7 (3)				97.3	104.3	91.4
N 9 C 1′ O 4′ C 4′	−79.5 (2)	−152.1	−68.0	70.0	−76.9	−66.3	−173.3
C 1′ O 4′ C 4′ C 5′	157.0 (3)	157.0	−135.5	137.8	173.2	−176.2	−171.9
O 4′ C 4′ C 3′ O 3′	−64.3 (2)	*gauche*	61.4	−52.6			
O 4′ C 4′ C 5′ O 5′	67.8 (2)	*trans*	61.5	−61.9	60.6	73.5	−174.4
C 5′ C 4′ C 3′ O 3′	55.5 (2)		−61.1	62.3			
C 3′ C 4′ C 5′ O 5′	−53.3 (2)		−174.2	−178.8			

Table 4
Weighted least-squares planes

Plane 1		Plane 2		Plane 3	
	Distance (Å)		Distance (Å)		Distance (Å)
N 1	−0.006 (3)	C 3′	0.027 (5)	C 1′	0.000 (4)
C 2	0.021 (3)	O 3′	−0.007 (3)	O 4′	0.000 (3)
N 3	−0.002 (3)	O 5′	0.007 (3)	C 3′	0.000 (6)
C 4	−0.002 (3)	C 5′	−0.017 (4)	C 4′	−0.780 (4) *
C 5	0.007 (3)	C 4′	−0.628 (5) *	H 1′A	0.10 (3) *
C 6	−0.017 (3)	P	0.717 (3) *	H 3′A	0.30 (4) *
N 7	0.013 (3)	χ^2	56.0	χ^2	0
C 8	0.002 (3)				
N9	−0.011 (3)				
N 2	0.053 (4) *				
O 6	−0.040 (3) *				
C 1′	−0.079 (4) *				
χ^2	124.7				

* Atoms excluded from the calculation of the plane.
Equations of the planes:
1. 6.2409 (17)X + 3.636 (8) Y + 1.380 (13)Z = 6.529 (4) Å.
2. 3.236 (6)X + 0.253 (24)Y + 16.464 (10)Z = 4.987 (24).
3. −4.325 (15)X + 8.715 (22)Y − 0.82 (4)Z = 5.00 (3).
Dihedral angle between planes A and B:

A	B	Angle (°)
1	2	56.15 (8)
1	3	112.75 (17)
2	3	111.03 (14)

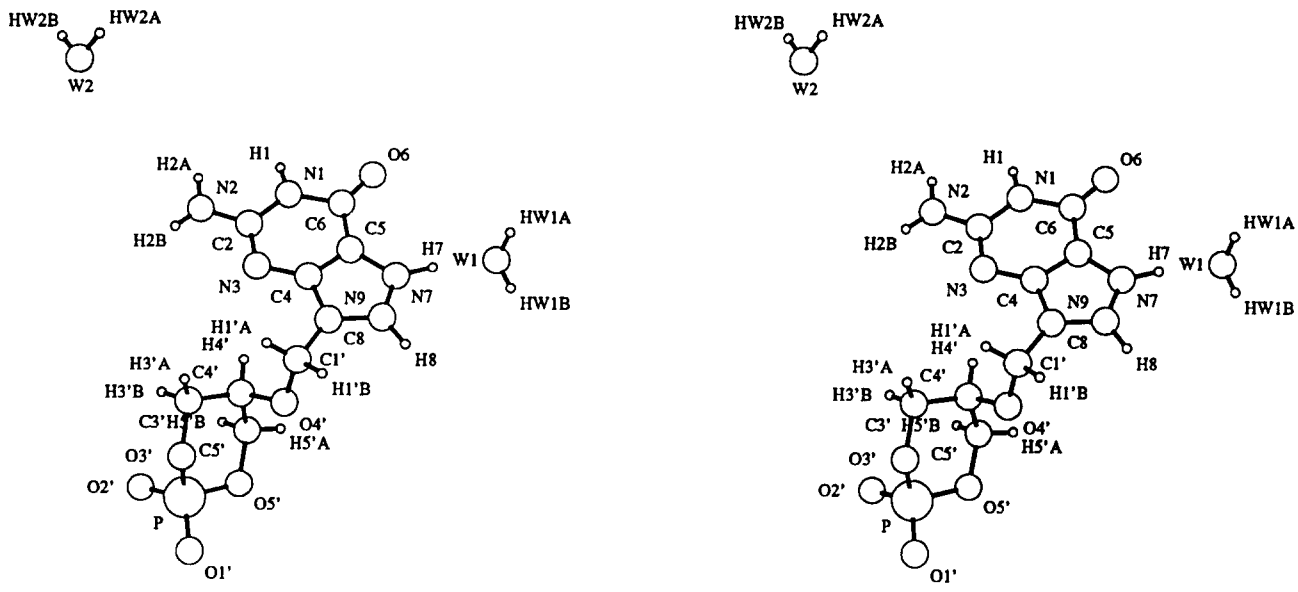

Fig. 2. Stereoscopic view of the solid-state structure of DHPG-cMP.

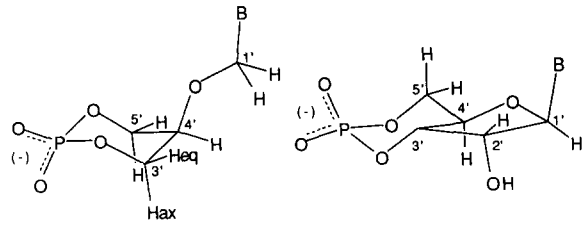

Fig. 3. Comparison of conformations of the cyclic phosphate rings of DHPG-cMP (left) and cGMP (right).

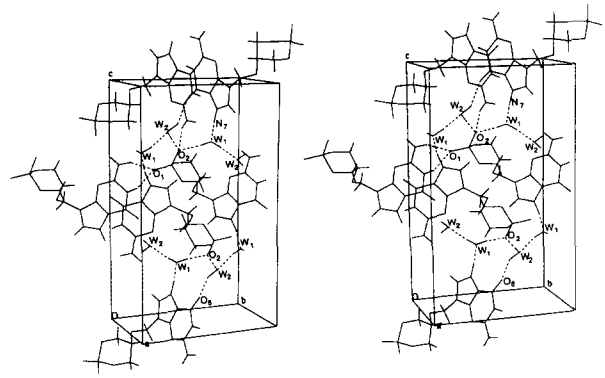

Fig. 4. Stereoscopic view of the packing and hydrogen bonding of DHPG-cMP in the solid state.

extended. However, somewhat smaller values, 78° in cGMP, −76.5° and −74.4° in molecules A and B of ACV, and 69.7° in DHPG, have been observed; the latter values correspond more closely to the typical *syn* conformation (sc).

The torsion angle of −79.5(2)° for the N9-C1′-O4′-C4′ region of the side chain of DHPG-cMP is fairly close to the values in DHPAde (−68.0° and 70.0°) and to those in molecules A and B of ACV (−76.9° and −66.3°), but different from that in DHPG (−152.1°) and in molecule C of ACV (−173.3°) where it is *trans*. The conformation about O4′-C4′ is extended, with C1′ and C5′ close to *trans* to each other. The deviation of 23.0° from the ideal extended chain is the same as that observed in DHPG, while a larger deviation of 44° is observed in DHPAde. In ACV this part of the chain is fully extended with smaller, 4–8°, deviations. The ^{4}E conformation of the sugar part of DHPG-cMP, taken as the whole ring, is opposite (in the pseudorotational sense) to that of cGMP: $_4T^3$ (C4′*exo*, C3′*endo*), P = 42.6° and ν_{max} = 44.3°. The latter is typical for nucleoside 3′:5′-cyclic phosphates [23].

The most significant difference between the structures of DHPG-cMP and cGMP is that O4′ is attached to the cyclic phosphate ring in the axial position in the former, whereas it is equatorially oriented in the latter (Fig. 3). Hence the vicinal oxygen atoms in DHPG-cMP all exhibit *gauche* orientations about the C3′-C4′(−64.3(2)°) and C4′-C5′ (67.8(2)°) bonds. In cGMP, on the other hand, they are *trans* to each other. The cyclophosphate rings in both molecules are in the chair conformations, but there is much more stress in cGMP due to fusion of the ribose and cyclophosphate rings. This is manifested by an increased puckering in the C3′, C4′ half and a flattening in the P,O5′ half of the phosphate ring. There are hence much greater variations in the torsion angles in cGMP, viz. from 44.2° to 68.5°.

In contrast to cGMP there is considerable conformational freedom in DHPG-cMP, viz. about the three bonds N9-C1′, C1′-O4′, and O4′-C4′, as in other acyclonucleosides [10]. The differences between the conformations of DHPG-cMP and cGMP are reflected in the only minimal larger distance between the aglycon and the cyclic phosphate ring in the former (see below), and the marked difference in the relative orientations between the aglycon and the cyclic phosphate rings in the two compounds (Fig. 5).

There are a number of similarities between the hydrogen bonds in the solid-state structures of DHPG-cMP and cGMP, even though the latter is a sodium tetrahydrate. In both structures the O6 and N7 atoms are hydrogen bonded to different water molecules in the water channel. Another similarity is the way the equatorial O1′ of the phosphate is the acceptor of two hydrogen bonds from the base, one from N2 and the

Table 5
Hydrogen-bond distances (Å) and angles (°)

Donor atom	Acceptor atom	Position of acceptor atom	Distance D...A	Distance H...A	Angle (°)	
W1	O2′	$x-\frac{1}{2}, 1\frac{1}{2}-y, z-\frac{1}{2}$	2.735 (3)	1.87 (4)	W1-HW1A · · · O2′	174 (4)
W1	W2	$1-x, 1-y, -z$	2.722 (4)	1.89 (5)	W1-HW1B · · · W2	166 (5)
W2	O6	$x-\frac{1}{2}, \frac{1}{2}-y, \frac{1}{2}+z$	2.877 (3)	2.04 (6)	W2-HW2B · · · O6	147 (4)
W2	O2′	$x-1, y-1, z$	2.785 (3)	1.91 (5)	W2-HW2A · · · O2′	178 (4)
N1	O1′	$x, y-1, z$	2.744 (3)	1.97 (4)	N1-H1 · · · O1′	153 (3)
N2	O1′	$x, y-1, z$	2.808 (3)	1.96 (4)	N2-H2A · · · O1′	150 (3)
N2	O2′	$1\frac{1}{2}-x, y-\frac{1}{2}, \frac{1}{2}-z$	2.984 (3)	2.16 (4)	N2-H2B · · · O2′	157 (3)
N7	W1	x, y, z	2.610 (3)	1.63 (4)	N7-H7 · · · W1	168 (3)

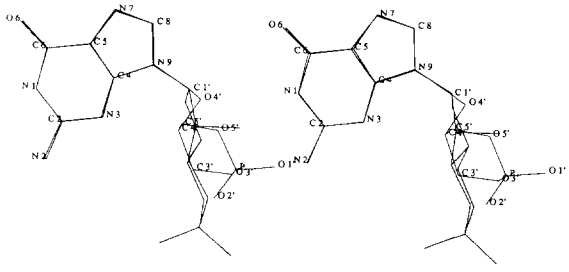

Fig. 5. Stereoscopic view of the superposition of the solid-state structures of DHPG-cMP and cGMP. Atoms in the DHPG-cMP molecule are numbered.

other from N1. These two hydrogen bonds form a six-membered ring consisting of the atoms H1, N1, C2, N2, and H2A from one molecule, and O1′ from a neighbouring one. The axial oxygen atom O2′ of the phosphate is the acceptor of three hydrogen bonds, two of them from water molecules, and the third one from the second hydrogen of the NH_2 group. In cGMP the equivalent oxygen atom (O7) is also the acceptor of three hydrogen bonds, but all from three different water molecules.

Biological aspects

Numerous acyclonucleoside analogues, and their phosphates and phosphonates, exhibit substrate/inhibitor properties in various enzyme systems [1]. The initial finding of Schaeffer et al. [24] that the adenine analogue of ACV is a substrate of adenosine deaminase led to the development of potent acyclonucleoside reversible inhibitors of this enzyme [25], and subsequently to the clinicaly effective ACV as an inhibitor of herpesvirus replication [26]. Various acyclonucleosides are good inhibitors of purine nucleoside phosphorylase [27] and uridine phosphorylase [28], and the pyrophosphate of ACV is the most effective inhibitor of human purine nucleoside phosphorylase [29,30]. This testifies to the fact that the acyclic chains may mimic the 'upper' and/or 'lower' portion of the pentafuranose ring, leading to 'recognition' by the appropriate enzymes.

DHPG-cMP was the first example of the cyclic phosphate of an acyclonucleoside which, by virtue of its broad-spectrum antiviral activity [3], must necessarily be recognized by some constituent(s) of infected cells. In this context it should be noted that DHPG-cMP is a structural analogue of cGMP, a key intracellular mediator of extracellular signals, which interacts with at least three types of receptor proteins, viz. cGMP-dependent protein kinases, cGMP-regulated cyclic nucleotide phosphodiesterases and cGMP-regulated ion channels [31].

A number of viral protein kinases, with properties that differ samwhat from their cellular counterparts, have been unequivocally identified [32]; but, as yet, none which is cAMP- or cGMP-dependent. On the other hand, as pointed out above (see Introduction), there are now numerous examples of the modulation of viral replication by cAMP and cGMP [1], the most recent being HIV [8]. These effects may be related to the regulation by cGMP of intracellular signals by stimulation of hydrolysis of cAMP, with a consequent decrease in cAMP-dependent protein phosphorylation [31]. Antagonistic effects between cAMP and cGMP, in modulation of viral replication, have also been noted with several viruses [1].

Comparison of the structures of DHPG-cMP and cGMP may provide information on possible involvment of the former in the complex system of protein phosphorylation, which involves such enzymes as cGMP-dependent protein kinase, guanylyl cyclase and cGMP-dependent phosphodiesterases [33]. For example, conformational analyses have been conducted on model cyclic phosphates, including hypotetical transition-state analogues in the active centre of phosphodiesterases, in attempts to elucidate the mechanism of enzymatic hydrolysis of cAMP [34].

The differences in the solid-state conformations of the cyclic phosphate rings and the sugar moieties between DHPG-cMP and cGMP (see above) result in differing relative orientations of the planes of the cyclic phosphate rings and aglycon (Fig. 5). Suprisingly, the distance between the aglycon and the cyclic phosphate ring in DHPG-cMP (evaluated from the N9-P distance) is only 0.08 Å greater than in cGMP, so that, despite the differences in conformation of the cyclic phosphate rings in the two compounds, the flexibility of the acyclic

Table 6
Values of ^1H chemical shifts (ppm ± 0.005 *vs.* internal TSP) and ^1H-^1H and ^1H-^{31}P coupling constants (±0.1 Hz) for DHPG-cMP in neutral ^2H_2O at 30°C

H8	H1′ [a]	H3′eq	H3′ax	H4′	H5′eq	H5′ax
7.960	5.630	4.245	4.375	3.835	4.245	4.375

J (^1H,^1H)							J (^1H,^{31}P)				
3′ax,3′eq	3′eq,4′	3′ax,4′	4′,5′eq	4′,5′ax	5′ax,5′eq	3′ax,5′eq	3′eq,P	3′ax,P	4′,P	5′eq,P	5′ax,P
−12.8	2.0	2.0	2.0	2.0	−12.8	1.2	19.9	3.6	1.9	19.9	3.6

As in Fig. 3, ax = axial, eq = equatorial.
[a] Coalescence of two proton signals.

chain in DHPG indicates possible reasonable overlapping of the two structures.

It would clearly be desirable to study the substrate/inhibitor properties of DHPG-cMP vs. cGMP-dependent kinases, as well as cyclases and cyclic nucleotide phosphodiesterases. We have elsewhere examined the behaviour of the cyclic phosphates of several acyclonucleosides against a number of nucleolytic enzymes. Whereas two other acyclonucleoside cyclic phosphates are substrates for specific cyclic nucleotide phosphodiesterases from beef heart and bovine brain, DHPG-cMP is inactive as a substrate or inhibitor. It is, however, a weak substrate for the relatively non-specific cyclic nucleotide phosphodiesterase from higher plants [35]. A counterpart of the latter has been identified in mammalian cells [36], and is probably the enzyme responsible for the slow rate of hydrolysis of DHPG-cMP in such cells [6,13]. We have also found that DHPG-cMP cannot replace cGMP in a cGMP-dependent protein kinase from yeast, and does not inhibit the latter enzyme (Jakubowicz, T. and Shugar, D., unpublished). This may be due to the fact that DHPG-cMP is a closer structural analogue of 2′-deoxy-cGMP, and the latter is only 1% as active as cGMP with cGMP-dependent protein kinases from a variety of cellular systems [37]. Extension of such studies to other kinases, particulary those in infected cells, is clearly called for.

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