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The Structure of the Zwitterion Inosine Cyclic 3′,5′-Monophosphate (cIMP) Monohydrate. Analysis of Torsional Flexibility in the Furano–Phosphate Moiety

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Abstract

Inosine cyclic 3′,5′-monophosphate (cIMP), which has been implicated in hormonal and regulatory control mechanisms, crystallizes with one water molecule of hydration ($C_{10}H_{11}N_4O_7P.H_2O$) in the monoclinic space group $P2_1$ ($Z = 2$) with unit-cell constants a = 6·190 (3), b = 13·090 (2), c = 9·095 (2) Å and β = 108·43 (2)°; d_o = 1·656, d_c = 1·654 g cm^{-3}. The structure was solved by the multisolution technique and refined by the least-squares method to a final R of 0·034 using 1422 intensities. The free acid of cIMP, which exists in the zwitterionic form with protonation at N(7) of the base, exhibits the *anti* conformation: χ = 18·1 (3)°, and the $^3T_4$ pucker for the ribofuranose ring: P = 27·6 (2)° and τ_m = 43·4 (2)°. The phosphate ring is in the chair conformation and exhibits the sharpest pucker at the ribose C(3′)–C(4′) bond. The *anti* orientation of the base is stabilized by hydrogen bonds from the water of hydration to the base N(3) site and the ribose O(2′) atom of the same molecule. The protonated base atom N(7) is involved in the strongest hydrogen bond of 2·521 (3) Å to a phosphate O atom of an adjacent nucleotide. The conformational variability for the phosphodiester ring, between different 3′,5′-cyclic nucleotide structures, increases as one moves from the ribose C–C bonds to the phosphate P–O bonds.

Introduction

Since the discovery that adenosine cyclic 3′,5′-monophosphate (cAMP) plays a central role in various phases of hormonal and metabolic control mechanisms (Sutherland & Rall, 1960; Robison, Butcher & Sutherland, 1971), guanosine cyclic 3′,5′-monophosphate (cGMP) has also been found to be involved in cell regulation (Hardman, Robison & Sutherland, 1971). The recognition of the importance of these cyclic nucleotides has stimulated interest in other cyclic nucleotides, in particular cIMP. cIMP has been shown to be only slightly less potent than cAMP in stimulating cAMP-sensitive protein kinase in various tissues (Corbin & Krebs, 1969). While the existence of an 'inosyl cyclase' has not been demonstrated, Ferguson & Price (1973) have, however, shown that cIMP is a deamination product of cAMP in toad bladder and that cIMP, like cAMP, mimics the effects of neurohypophysial hormones on the transport of sodium and water across the intact bladder. This structural investigation provided information on the zwitterionic character of cIMP (Sundaralingam, 1975) and precise geometrical and conformational parameters for cIMP which are compared with the other known cyclic nucleotides for which coordinates have been published.

Experimental

Large crystals of cIMP monohydrate were obtained by dissolving cIMP in hot water and then allowing the water to evaporate over a period of several days at 281 K. Intensity data were collected on an Enraf–Nonius CAD-4 diffractometer using Ni-filtered Cu $K\alpha$ radiation (λ = 1·5418 Å). Unit-cell parameters were refined by a least-squares algorithm using 25 automatically centered reflections. Of a total of 1545 unique reflections measured up to a 2θ limit of 156°, 1422 with intensities greater than $2\sigma(I)$ were used for the structure analysis. Four reflections were monitored throughout data collection for crystal decay which was found to be negligible; thus no decay correction was applied. An empirical φ absorption correction was applied to the data, as well as corrections for Lorentz and polarization effects.

Structure determination and refinement

The structure was solved by application of the multisolution tangent-formula technique using the computer program *MULTAN* (Main, Germain & Woolfson, 1970). An E map using the phases from one of the phase sets revealed the phosphate group and several ribose atoms. Subsequent Fourier syntheses yielded all remaining non-H atoms in the structure.

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Least-squares refinement of these atoms to convergence using anisotropic temperature factors brought R $[=\sum(||F_o| - |F_c||)/\sum|F_o|]$ to 0·062. A modified counting-statistics weighting scheme with the weight of each reflection proportional to $1/[\sigma^2(F) + (0{\cdot}1F_o)^2]$ was used. All H atoms were subsequently located from difference Fourier syntheses. Further refinement of all atoms, using isotropic temperature factors for the H atoms and anisotropic temperature factors for the others, reduced R to 0·034. The average and maximum shift/σ ratios for the atomic parameters are 0·02 and 0·25 respectively. The scattering factors employed for the non-H atoms were taken from Cromer & Waber (1965), while those for H were taken from Stewart, Davidson & Simpson (1965).

Results and discussion

The final positional parameters are presented in Table 1. Fig. 1 is an *ORTEP* drawing (Johnson, 1976) showing the atom numbering. All bond lengths and bond angles between non-H atoms are given in Table 2. Table 3 presents a comparison of various conformational parameters for all known 3′,5′-cyclic nucleotides for which coordinates have been published.*

Geometry

The bond lengths and angles for the base are similar to those observed for neutral inosine structures except in the vicinity of N(7) where the protonation by a phosphate group from an adjacent nucleotide drastically perturbs the geometry (Sundaralingam & Prusiner, 1978). Compared to the average values for three distinct inosine molecules (Thewalt, Bugg & Marsh, 1970; Munns & Tollin, 1970), the C(5)–N(7)–C(8) bond angle is increased by 3·8° from 103·8 to 107·6 (3)°, while the C(4)–C(5)–N(7) and N(7)–C(8)–N(9) angles are decreased from 111·3 to 107·9 (3)° and from 113·6 to 110·8 (3)°, respectively. The most significant change is observed for the C(8)–N(9) bond which is shortened from 1·367 to 1·349 (3) Å. The substituent atom C(1′) deviates significantly [0·155 (3) Å] from the least-squares plane through the nine-membered ring system ($-0{\cdot}5667X - 0{\cdot}2464Y + 0{\cdot}7862Z = 3{\cdot}130$), while the keto O(6) is slightly displaced [0·016 (3) Å] on the opposite side.

The bond lengths for the ribose and phosphate rings of cIMP (Table 2) are all within two standard deviations from the mean for all published 3′,5′-cyclic nucleotide structures. The C(4′)–O(4′) and O(4′)–C(1′) bonds of the furanoid ring are nearly equal although slightly shorter than the mean. The C(4′)–O(4′)–C(1′) bond angle of 108·3 (3)° deviates

Table 1. *Fractional positional parameters* ($\times 10^4$; $\times 10^3$ *for* H) *and isotropic thermal parameters for all atoms of cIMP*.H_2O

$$B_{eq} = \tfrac{4}{3}\sum_i\sum_j \beta_{ij}\,\mathbf{a}_i\cdot\mathbf{a}_j.$$

	x	y	z	B_{eq}/B (Å^2)
N(1)	4994 (4)	2888 (2)	6902 (2)	3·10 (5)
C(2)	6736 (5)	3450 (2)	7842 (3)	2·77 (5)
N(3)	6657 (3)	4407 (2)	8192 (2)	2·36 (4)
C(4)	4562 (3)	4813 (2)	7483 (2)	1·98 (4)
C(5)	2687 (4)	4322 (2)	6517 (2)	2·21 (4)
C(6)	2817 (5)	3262 (2)	6139 (3)	2·75 (5)
O(6)	1325 (4)	2722 (2)	5297 (2)	3·88 (5)
N(7)	952 (3)	5022 (2)	6045 (2)	2·58 (4)
C(8)	1735 (4)	5901 (2)	6693 (3)	2·51 (5)
N(9)	3932 (3)	5815 (1)	7579 (2)	2·18 (4)
C(1′)	5304 (4)	6599 (2)	8646 (2)	2·12 (4)
C(2′)	6902 (4)	7197 (2)	7944 (3)	2·30 (4)
O(2′)	8897 (3)	7496 (2)	9118 (3)	3·47 (5)
C(3′)	5438 (3)	8135 (2)	7357 (2)	2·05 (4)
O(3′)	6584 (2)	9037 (1)	7148 (2)	2·31 (4)
C(4′)	4349 (4)	8294 (2)	8629 (2)	2·12 (4)
O(4′)	3722 (3)	7290 (1)	8920 (2)	2·66 (4)
C(5′)	2394 (4)	9039 (2)	8076 (2)	2·28 (4)
O(5′)	3487 (3)	9985 (1)	7875 (2)	2·71 (4)
P	4904 (1)	10000	6671 (1)	2·03 (1)
O(1P)	6373 (3)	10928 (1)	7016 (3)	3·68 (5)
O(2P)	3311 (3)	9840 (2)	5074 (2)	3·21 (3)
O(*W*)	10872 (4)	5602 (2)	9743 (3)	4·12 (5)
H(1)	525 (6)	222 (3)	681 (4)	3·2 (7)
H(2)	802 (5)	312 (2)	826 (3)	2·1 (5)
H(7)	−63 (5)	483 (3)	531 (4)	2·6 (6)
H(8)	103 (4)	648 (2)	656 (3)	2·0 (5)
H(1′)	624 (5)	629 (3)	970 (4)	2·6 (6)
H(2′)	722 (5)	680 (3)	712 (4)	2·5 (6)
H(O2′)	978 (7)	687 (3)	929 (4)	3·7 (7)
H(3′)	431 (4)	795 (2)	642 (2)	0·4 (4)
H(4′)	566 (6)	857 (2)	960 (4)	2·3 (6)
H(5′1)	125 (5)	885 (2)	719 (3)	1·7 (5)
H(5′2)	169 (5)	913 (2)	887 (3)	1·6 (5)
H(1*W*)	1138 (5)	560 (3)	1071 (4)	2·9 (6)
H(2*W*)	956 (10)	512 (5)	936 (7)	7·8 (15)

* Lists of structure factors, anisotropic thermal parameters and a comparison of geometry for the ribose and phosphate rings of published cyclic nucleotides have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36650 (9 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

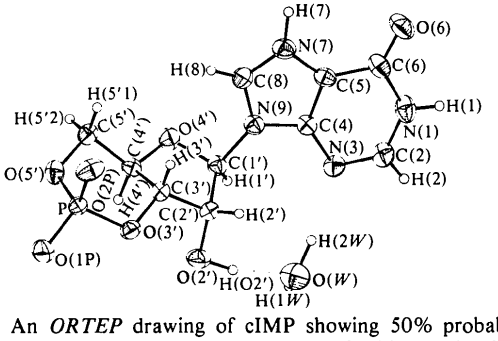

Fig. 1. An *ORTEP* drawing of cIMP showing 50% probability ellipsoids for non-H atoms and spheres of arbitrary size for H atoms.

Table 2. *Non*-H *bond lengths* (Å) *and angles* (°) *for cIMP*

N(1)–C(2)	1·361 (4)	C(2)–N(3)	1·297 (4)	N(3)–C(4)	1·361 (3)
C(4)–C(5)	1·374 (3)	C(5)–C(6)	1·438 (4)	C(6)–N(1)	1·395 (4)
C(6)–O(6)	1·221 (4)	C(5)–N(7)	1·373 (3)	N(7)–C(8)	1·314 (4)
C(8)–N(9)	1·349 (3)	N(9)–C(4)	1·379 (3)	N(9)–C(1′)	1·482 (3)
C(1′)–C(2′)	1·549 (4)	C(2′)–C(3′)	1·519 (4)	C(2′)–O(2′)	1·409 (4)
C(3′)–C(4′)	1·526 (3)	C(3′)–O(3′)	1·421 (3)	C(4′)–C(5′)	1·511 (4)
C(4′)–O(4′)	1·419 (3)	O(4′)–C(1′)	1·412 (3)	C(5′)–O(5′)	1·450 (3)
O(5′)–P	1·605 (2)	P–O(3′)	1·604 (2)	P–O(1P)	1·490 (2)
P–O(2P)	1·490 (2)				

C(2)–N(1)–C(6)	124·9 (3)	N(1)–C(2)–N(3)	126·3 (3)
C(2)–N(3)–C(4)	111·4 (3)	N(3)–C(4)–C(5)	127·3 (3)
N(3)–C(4)–N(9)	126·0 (3)	C(5)–C(4)–N(9)	106·7 (3)
C(4)–C(5)–C(6)	120·3 (3)	C(4)–C(5)–N(7)	107·9 (3)
C(5)–C(6)–N(1)	109·8 (3)	C(5)–C(6)–O(6)	128·3 (3)
N(1)–C(6)–O(6)	121·9 (3)	C(5)–N(7)–C(8)	107·6 (3)
N(7)–C(8)–N(9)	110·8 (3)	C(8)–N(9)–C(4)	107·1 (2)
C(8)–N(9)–C(1′)	126·5 (2)	C(4)–N(9)–C(1′)	125·8 (2)
N(9)–C(1′)–C(2′)	112·9 (2)	N(9)–C(1′)–O(4′)	105·9 (2)
C(2′)–C(1′)–O(4′)	108·5 (3)	C(1′)–C(2′)–C(3′)	99·5 (3)
C(1′)–C(2′)–O(2′)	110·4 (3)	C(3′)–C(2′)–O(2′)	108·9 (3)
C(2′)–C(3′)–C(4′)	101·6 (2)	C(2′)–C(3′)–O(3′)	116·8 (2)
C(4′)–C(3′)–O(3′)	110·3 (2)	C(3′)–C(4′)–O(4′)	103·2 (2)
C(3′)–C(4′)–C(5′)	109·6 (2)	C(3′)–O(3′)–P	112·4 (2)
C(4′)–O(4′)–C(1′)	108·3 (3)	C(5′)–C(4′)–O(4′)	115·0 (3)
C(4′)–C(5′)–O(5′)	103·7 (3)	C(5′)–O(5′)–P	118·3 (2)
O(5′)–P–O(3′)	104·7 (1)	O(3′)–P–O(1P)	106·5 (1)
O(3′)–P–O(2P)	109·0 (2)	O(5′)–P–O(1P)	107·2 (2)
O(5′)–P–O(2P)	109·1 (2)	O(1P)–P–O(2P)	119·4 (2)

by more than two standard deviations from the mean: 105·9 (1·2)°.

Glycosyl conformation

The only major conformational variable in 3′,5′-cyclic nucleotides is the glycosyl torsion angle: O(4′)–C(1′)–N(9)–C(8) which in cIMP is in the *anti* range: 18·1 (3)°. In contrast, the glycosyl torsion angles observed for the other cyclic nucleotides in Table 3 are in the vicinity of either 70 (*anti*) or −115° (*syn*). Both *syn* and *anti* base orientations are observed since the cyclized phosphate is far removed from the path of base rotation and thus does not influence base orientations as much as in the case of the free 5′-phosphate (Sundaralingam, 1973).

Ribose conformation

The ribofuranose conformation for cIMP is the only entry in Table 3 found in the ($^3T_4$) twist* conformation.

* Sundaralingam (1973).

Table 3. *Comparison of conformational parameters for 3′,5′-cyclic nucleotides*

The values for cIMP are given with e.s.d.'s. The last column reports standard deviations from the mean of the tabulated values.

	cIMP	(1)*	(2)	(3)	(4)	(5*A*)	(5*B*)	(6)	Mean (standard deviation)
χ [O(4′)–C(1′)–N(9)–C(8)] or [O(4′)–C(1′)–N(1)–C(6)] (°)	18·1 (3)	−125·7	75·8	−107·0	−102·4	76·9	58·4	−108·6	
Glycosyl conformation	*anti*	*syn*	*anti*	*syn*	*syn*	*anti*	*anti*	*syn*	
Phase angle of pseudorotation (*P*) (°)	27·6 (3)	36·8	44·5	51·0	42·7	42·0	47·9	48·7	42·7 (7·5)
Maximum amplitude of pseudorotation (τ_m) (°)	43·4 (2)	45·7	49·9	47·3	44·2	47·8	47·2	47·3	46·8 (2·2)
Ribofuranose pucker	$^3T_4$	^3_4T	$_4T^3$	$_4T^3$	$_4T^3$	$_4T^3$	$_4T^3$	$_4T^3$	
Ribose torsion angles (°)									
τ_0[C(4′)–O(4′)–C(1′)–C(2′)]	−7·0 (3)	−14·5	−22·5	−26·4	−18·4	−19·4	−23·5	−24·2	−19·5 (6·3)
τ_1[O(4′)–C(1′)–C(2′)–C(3′)]	−20·1 (3)	−14·1	−8·7	−3·2	−9·2	−5·5	−10·4	−5·1	−9·5 (5·5)
τ_2[C(1′)–C(2′)–C(3′)–C(4′)]	37·2 (3)	35·7	35·1	29·6	31·6	30·8	34·6	30·3	33·1 (2·9)
τ_3[C(2′)–C(3′)–C(4′)–O(4′)]	−43·5 (3)	−46·2	−49·4	−47·5	−44·3	−46·7	−48·1	−46·6	−46·5 (1·9)
τ_4[C(3′)–C(4′)–O(4′)–C(1′)]	31·3 (3)	37·5	44·5	45·6	38·7	43·2	41·4	43·6	40·7 (4·7)
Backbone torsion angles (°)									
ω' [O(5′)–P–O(3′)–C(3′)]	47·8 (2)	50·4	39·7	50·1	44·3	57·4	49·3	49·3	48·3 (5·6)
φ' [P–O(3′)–C(3′)–C(4′)]	−59·7 (3)	−63·5	−54·1	−60·0	−60·0	−66·7	−61·2	−60·9	−60·8 (3·6)
ψ' [O(3′)–C(3′)–C(4′)–C(5′)]	69·1 (3)	69·1	65·9	66·8	68·6	69·1	67·1	67·1	67·9 (1·3)
ψ [C(3′)–C(4′)–C(5′)–O(5′)]	−64·5 (3)	−64·1	−59·3	−62·9	−60·6	−61·4	−59·5	−58·9	−61·4 (2·2)
φ [C(4′)–C(5′)–O(5′)–P]	59·8 (3)	54·9	50·9	60·5	53·7	60·5	56·5	53·9	56·3 (3·6)
ω [C(5′)–O(5′)–P–O(3′)]	−51·0 (2)	−47·4	−40·3	−53·6	−44·4	−56·7	−50·5	−49·1	−49·1 (5·2)
Cremer & Pople (1975) ring-puckering parameters for P–O(5)–C(5′)–C(4′)–C(3′)–O(3′) ring									
Q (Å)	0·606 (3)	0·609	0·529	0·605	0·566	0·566	0·596	0·577	0·584 (27)
θ (°)	10·6 (2)	6·3	17·0	6·6	13·6	5·0	6·6	9·8	9·4 (4·2)
φ (°)	178 (1)	184	189	174	196	167	202	209	187 (14)

* Compound names and references: (1) 5′-Methyleneadenosine cyclic 3′,5′-monophosphonate monohydrate [C(6′)=O(5′)], Sundaralingam & Abola (1972). (2) *P*–*O*-Ethyl ester of adenosine cyclic 3′,5′-monophosphate, Cotton, Gillen, Gohil, Hazen, Kirchner, Nagyvary, Rouse, Stanislowski, Stevens & Tucker (1975). (3) 8-[(2-Aminoethyl)amino]adenosine cyclic 3′,5′-monophosphate tetrahydrate, Sheldrick & Rieke (1978). (4) Guanosine cyclic 3′,5′-monophosphate sodium tetrahydrate, Chwang & Sundaralingam (1974). (5) Uridine cyclic 3′,5′-monophosphate (two molecules in the asymmetric unit), Coulter (1969). (6) 2′-Acetyluridine cyclic 3′,5′-monophosphate benzyl triester, Depmeier & Engels (1977).

The phase angle of pseudorotation (P) (Altona & Sundaralingam, 1972) of 27·6 (3)° for cIMP is 9·2° less than the smallest value observed previously, while the amplitude of pseudorotation (τ_m) of 43·4 (2)° is significantly flattened from the mean value of 46·8°. When compared to free nucleotides, the observed pseudorotational variation in cyclic nucleotides is limited (Table 3), because of the constraints imposed by the phosphate ring.

Sugar–phosphate 'backbone' conformation

Structural studies on helical polynucleotides have indicated that these molecules strongly favor the (g^-,g^-) ($\omega' = \omega = -60°$) conformation for the phosphodiester group in right-handed helices (Sundaralingam, 1969, 1973). All published 'free' dinucleotide structures fall into this domain with the exception of UpA (Rubin, Brennan & Sundaralingam, 1972; Sussman, Seeman, Kim & Berman, 1972) and pTpT (Camerman & Fawcett, 1976) which are found in the (g^+,g^+) and (t,g^-) domains (Fig. 2). This preference for the (g^-,g^-) conformation is also exhibited by drug-intercalated dinucleotides even though the intercalated drug nearly doubles the normal base-stacking distance. Recently, two left-handed forms of DNA (Z-DNA) have been reported (Wang, Quigley, Kolpak, van der Marel, van Boom & Rich, 1981) which have different phosphodiester conformations for the repeating dinucleotide unit CpGp. Amongst the two forms of Z-DNA, the (g^+,g^+), (g^+,t), and (g^-,t) conformations are observed. In the cyclic nucleotides, however, the sugar–phosphate backbone is constrained to the normally disallowed (g^+,g^-) conformation (Fig. 2), which reverses the phosphodiester chain direction to form the cyclic phosphate ring. The *gauche* angles for φ and φ' are also markedly different from those of the oligo and polynucleotides where they are usually in the *trans* domain (Sundaralingam, 1969, 1979).

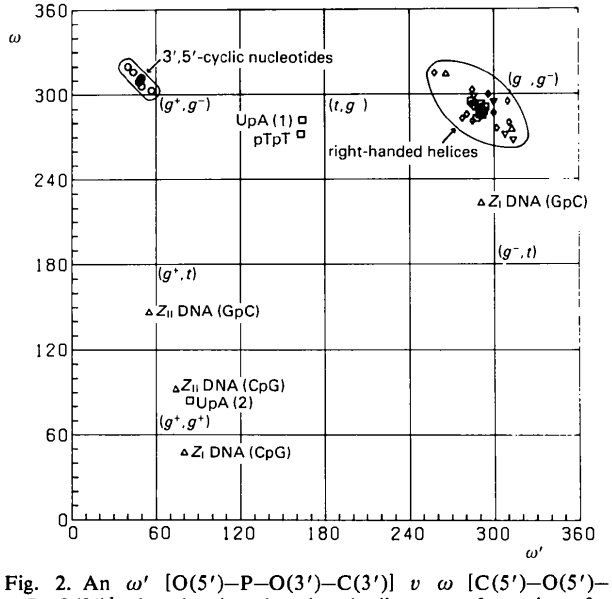

Fig. 2. An ω' [O(5′)–P–O(3′)–C(3′)] v ω [C(5′)–O(5′)–P–O(3′)] plot showing the phosphodiester conformations for a variety of phosphodiester linkages. ○, cyclic nucleotides; □, dinucleotides; ◇, drug-bound dinucleotides; △, DNA's; and ▽, RNA's. The list of references for the data in this figure has been deposited.

Torsional variability in cyclic nucleotides

Cyclization forces the phosphate ring into virtually the same conformation regardless of base variation, substitution of O(5′) by a C atom, or the presence of a substituent on one of the anionic phosphate O atoms. However, within this rigid framework there still is some variability of flexibility as measured by the standard deviations in Table 3. Considering the sugar–phosphate 'backbone' torsion angles, it is seen that ω' and ω show the greatest variability: 5·6 and 5·2° respectively, while the least variability of 1·3° is seen for ψ' [since the C(4′)–C(5′) bond is shared by both the five-membered ribose ring and the six-membered phosphodiester ring] which is closely followed by a variability of 2·2° for ψ. The two C–O bond torsion angles φ' and φ show an intermediate degree of flexibility with a standard deviation of 3·6°.

The ribofuranose ring can be similarly analyzed. The greatest variability is observed for the τ_0 torsion angle: 6·3°, closely followed by the τ_1 and τ_4 torsion angles: 5·5 and 4·7° respectively. The τ_2 (2·9) and τ_3 (1·9°) torsion angles display considerably less variability. Thus, in cyclic nucleotides the torsion angles of the bonds opposite to the fused C(3′)–C(4′) bond show the greatest flexibility.

Hydrogen bonding and molecular packing

The hydrogen-bonding scheme is illustrated in Fig. 3. There are five different hydrogen bonds (Table 4), the strongest [2·521 (3) Å] is between the protonated N(7) atom and a phosphate O atom of an adjacent molecule. In the zwitterionic 5′-methyleneadenosine cyclic 3′,5′-monophosphonate (Sundaralingam & Abola, 1972), a somewhat longer hydrogen bond (2·651 Å) was observed between the negatively charged phosphonate O(6) and the positively charged base N(1) site. Three of the five hydrogen bonds in the structure involve the water of crystallization which forms a hydrogen-bonded bridge between the 2′-hydroxyl group of the ribose and the base N(3) atom of the same molecule, while donating its other H atom to a phosphate O atom of a screw-related molecule. This hydrogen bonding is only possible for the *anti* disposition of the base and presumably stabilizes the *anti* conformation of the molecule in the crystal. Thus, although the ribose pucker ($^3T_4$) places the 2′-hydroxyl group in an unfavorable geometry to participate in a

Table 4. *Hydrogen-bond lengths and angles in cIMP*.H_2O

A–H···B	Symmetry code for B	Translation for B x	y	z	A–H	H···B	A···B	A–H···B
N(1)–H(1)···O(1P)	1	0	−1	0	0·90 (4) Å	1·82 (4) Å	2·695 (3) Å	166 (4)°
N(7)–H(7)···O(2P)	2	0	−1	0	1·03 (3)	1·58 (3)	2·521 (3)	149 (3)
O(2′)–H(O2′)···O(W)	1	0	0	0	0·97 (4)	1·79 (4)	2·743 (4)	166 (4)
O(W)–H(1W)···O(1P)	2	2	−1	2	0·84 (4)	2·14 (4)	2·933 (4)	159 (4)
O(W)–H(2W)···N(3)	1	0	0	0	1·00 (6)	2·01 (6)	2·986 (3)	166 (5)

Symmetry codes: (1) x,y,z; (2) $-x, y+\frac{1}{2}, -z$.

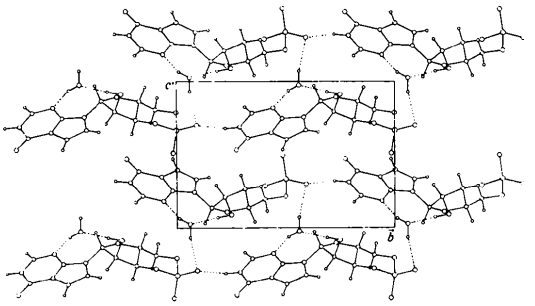

Fig. 3. A packing diagram of cIMP viewed down **a** with **b** horizontal and **c** vertical (tilted 18·43° out of the plane of the paper).

direct hydrogen bond to the base, it can be accomplished *via* a solvent water molecule. The distance between O(2′) and the base N(3) atom is 4·271 (3) Å. The water bond angle is 111 (6)° (also see Table 4).

There is no base stacking in the structure. The cyclic phosphate moieties are instead sandwiched between translation-related bases as illustrated in Fig. 3. The closest nonbonded contacts are between the anionic phosphate O(2P) and the N(3) and C(4) atoms of the base below with distances of 3·030 (3) and 3·013 (3) Å respectively. The phosphate ester O(3′) sits above the center of the π-cloud of the pyrimidine moiety of the base.

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