KENNARD, O., ISAACS, N. W., MOTHERWELL, W. D. S., COPPOLA, J. C., WAMPLER, D. L., LARSON, A. C. & WATSON, D. G. (1971). *Proc. R. Soc. London Ser. A*, **325**, 401–436.

LARSON, A. C. (1978). *Acta Cryst.* B**34**, 3601–3604.

MAIN, P., WOOLFSON, M. M. & GERMAIN, G. (1971). *MULTAN. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*, Univs. of York, England, and Louvain, Belgium.

MERRITT, E. A. & SUNDARALINGAM, M. (1980). *Acta Cryst.* B**36**, 2576–2584.

MERRITT, E. A., SUNDARALINGAM, M., CORNELIUS, R. D. & CLELAND, W. W. (1978). *Biochemistry*, **17**, 3274–3278.

MULLER, F. A. & DELUKE, A. D. (1971). *Am. Crystallogr. Assoc. Abstr. Pap.* (Winter Meet.), No. D10 and private communication.

PLETCHER, J. & SAX, M. (1972). *J. Am. Chem. Soc.* **94**, 3998–4005.

PLETCHER, J., WOOD, M., BLANK, G., SHIN, W. & SAX, M. (1977). *Acta Cryst.* B**33**, 3349–3359.

RAO, S. T. & SUNDARALINGAM, M. (1969). *J. Am. Chem. Soc.* **91**, 1210–1217.

STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.

SUNDARALINGAM, M. (1966). *Acta Cryst.* **21**, 495–506.

SUNDARALINGAM, M. (1968). *Biopolymers*, **6**, 189–213.

SUNDARALINGAM, M. (1969). *Biopolymers*, **7**, 821–860.

SUNDARALINGAM, M. (1973). *Conformation of Biological Molecules and Polymers, Jerusalem Symp. Quantum Chem. Biochem.* **5**, 417–456.

SUNDARALINGAM, M. & PRUSINER, P. (1978). *Nucleic Acids Res.* **5**, 4375–4383.

SWAMINATHAN, V. & SUNDARALINGAM, M. (1972). *CRC Crit. Rev. Biochem.* **6**, 245–336.

TS'O, P. O. P. (1974). Editor, *Basic Principles in Nucleic Acid Chemistry*, Vol. 1, pp. 453–584. New York: Academic Press.

VISWAMITRA, M. A. (1979). *Curr. Sci.* **48**, 989.

VISWAMITRA, M. A., HOSUR, M. V., SHAKKED, Z. & KENNARD, O. (1976). *Nature (London)*, **262**, 234–236.

VISWAMITRA, M. A., POST, M. L. & KENNARD, O. (1979). *Acta Cryst.* B**35**, 1089–1094.

YATHINDRA, N. & SUNDARALINGAM, M. (1973). *Biopolymers*, **12**, 297–314; 2261–2277.

Acta Cryst. (1980). B**36**, 2597–2604

Calcium-Binding to Nucleotides: Structure of a Hydrated Calcium Salt of Inosine 5′-Monophosphate

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Abstract

The crystal and molecular structure of a hydrated calcium salt of inosine 5′-phosphate was determined from X-ray diffraction data. Crystals of $Ca^{2+}.C_{10}H_{11}N_4O_8P^{2-}.6{\cdot}5H_2O$ are monoclinic, space group $P2_1$, with $a = 10{\cdot}929\ (4)$, $b = 21{\cdot}315\ (2)$, $c = 8{\cdot}622\ (4)$ Å, $\beta = 98{\cdot}26\ (5)°$ and $Z = 4$. Intensity data were collected with an automated diffractometer. The structure was solved by Patterson methods and refined by least squares to $R = 0{\cdot}023$. One of the crystallographically independent nucleotides binds Ca^{2+} through atom N(7), the site that is generally used in interactions of transition metals with purine nucleotides; additional Ca^{2+} ions are directly coordinated to this nucleotide through the O(2′)–O(3′) pair of hydroxyl groups, and through an O atom of the phosphate group. The second nucleotide forms only outer-sphere, water-mediated contacts with Ca^{2+} ions.

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Introduction

Despite the general importance of interactions between alkaline-earth metals and nucleotides in biological systems, little is known about the specific factors that are involved in these interactions. Most of the current models of metal–nucleotide complexes have been based on solution and crystallographic studies of transition-metal-ion interactions with purines, pyrimidines, nucleosides and nucleotides (Phillips, 1966; Izatt, Christensen & Rytting, 1971; Frey & Stuehr, 1974; Tu & Heller, 1974; Swaminathan & Sundaralingam, 1979). Unlike the transition metals, alkaline-earth-metal ions have no vacant atomic orbitals that can be used in covalent bonding to nucleotides. Consequently, there has been a tendency to assume that ions of this type interact with nucleotides through sites that are different from those occupied by transition metals. It is generally assumed that alkaline-earth-metal ions form relatively

nonspecific electrostatic interactions with the negatively charged phosphate moieties of the nucleotides. However, the available crystallographic data for alkaline-earth-metal complexes of nucleotides suggest that alkaline-earth cations often bind to sites other than the phosphate groups (Shefter & Trueblood, 1965; Sternglanz, Subramanian, Lacey & Bugg, 1976; Hogle & Sundaralingam, 1973; Nagashima & Iitaka, 1968; Swaminathan & Sundaralingam, 1979). In this paper we describe the calcium--nucleotide interactions in the crystal structure of a hydrated calcium salt of inosine 5′-monophosphate (IMP).

Table 1. *Crystal data*

Stoichiometry	$C_{10}H_{11}N_4O_8P^{2-}.Ca^{2+}.6{\cdot}5H_2O$
Z	4
Space group	$P2_1$
a	10·929 (4) Å
b	21·315 (2)
c	8·622 (4)
β	98·26 (5)°
ρ (calculated)	1·682 Mg m^{-3}
ρ (observed)	1·68 (1) Mg m^{-3}
μ (Cu $K\bar{\alpha}$)	4·14 mm^{-1}

(The density was determined by flotation in a mixture of chloroform and dibromoethane.)

Experimental

Clear rectangular plates of calcium IMP were grown by slowly cooling an aqueous solution that contained approximately 2:1 molar quantities of calcium bromide and disodium IMP. Oscillation and Weissenberg photographs showed the crystals to be monoclinic; space group $P2_1$ was indicated by the systematic absence of reflections $0k0$ with k odd. A crystal fragment with approximate dimensions 0·35 × 0·22 × 0·12 mm was mounted on a Picker FACS-1 diffractometer with the b axis slightly inclined to the φ axes of the diffractometer. Cell parameters for use in collecting intensity data were calculated by a least-squares analysis of the observed angular settings for 12 medium-angle reflections (Cu $K\bar{\alpha}$, λ = 1·5418 Å). Intensity data were collected with the diffractometer by use of a scintillation counter, Ni-filtered Cu radiation, and a θ–2θ scanning technique. The scanning speed was 2° min^{-1} and background was counted for 20 s at each terminus of the scans. Measurements were made for each of the 3358 independent reflections having 2θ < 128°. Three reference reflections (500, 0,10,0, 004) which were measured periodically showed an overall intensity decrease of about 3% during the period of data collection. The intensities were corrected for decomposition by assuming that the rate of decomposition was linear, and was represented by the average rate displayed by the three reference reflections. More accurate values for the unit-cell parameters were determined immediately after data collection by a least-squares analysis of 2θ values for 14 high-angle reflections (Cu $K\alpha_1$, λ = 1·54051 Å). These cell parameters were not significantly different from those obtained prior to the intensity-data collection. Crystal data are given in Table 1.

Five reflections with scan counts below background level were given negative intensities, and were retained in all subsequent calculations. Intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0{\cdot}03S)^2$, S being the scan count. Intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the computer program *ORABS* (Wehe, Busing & Levy, 1962). The data were scaled by means of a Wilson (1942) plot.

One of the Ca^{2+} ions was located in a sharpened, three-dimensional Patterson map, which was calculated using only the high-angle data with $2\theta > 90°$. Coordinates for the second Ca^{2+} ion were determined from a sum-function superposition of sharpened Patterson maps translated to the symmetry-related positions for the first Ca^{2+} ion. One of the P atoms was obtained from a superposition of Patterson maps translated to the Ca positions; and the other P atom was then located in a superposition of Patterson maps translated to the Ca positions, and to the symmetry-related positions of the first P atom. The eight phosphate O atoms were found in a Fourier map phased with the two Ca^{2+} ions and the two P atoms. The remaining nonhydrogen atoms were then located in subsequent Fourier maps.

The trial structure was refined by use of a modified version of the full-matrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962; Busing, 1971). The quantity minimized was $\sum w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and the weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for Ca^{2+}, P, O, N and C were from *International Tables for X-ray Crystallography* (1962), and real and imaginary dispersion corrections for these atoms were from Cromer & Liberman (1970). The H atoms of the nucleotides, and all except six of those from the water molecules, were located in difference-Fourier maps during the latter stages of refinement. We were unable to obtain parameters for H atoms from the water molecules $W(9)$, $W(10)$ and $W(11)$, which displayed large thermal motion. Scattering factors for the H atoms were from Stewart, Davidson & Simpson (1965). Final cycles of refinement included the scale factor, k, all positional parameters, anisotropic temperature parameters for nonhydrogen atoms, isotropic temperature factors for H atoms, and Zachariasen's (1963) isotropic extinction parameter [the g' parameter, as formulated by Coppens & Hamilton (1970)]. Since limited core storage hindered

simultaneous variation of all parameters, they were divided into four blocks, which were refined in successive cycles. The parameters were divided among the four blocks as follows: nonhydrogen atoms for each of the two nucleotides constituted two of the blocks; nonhydrogen atoms for the Ca^{2+} ions and water molecules were included in a third block; and the H atom parameters were refined together in a fourth block. The extinction parameter and scale factor were included in all of the blocks.

The final R index ($\sum||F_o| - |F_c||/\sum|F_o|$) is 0·023, and the goodness-of-fit $\{[\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined$\}$ is 1·8. A final three-dimensional difference Fourier map showed a peak of 0·5 e Å^{-3} near one of the Ca^{2+} ions; there were no other peaks or troughs with magnitudes in excess of 0·3 e Å^{-3}.

Results

The heavy-atom coordinates and their estimated standard deviations are listed in Table 2.* H atom parameters are given in Table 3. The estimated errors in positional parameters are about 0·001 Å for Ca^{2+} and P; 0·003 Å for C, N and O; 0·04 Å for the H atoms of the nucleotides; and 0·06 Å for H atoms on water molecules.

The conformations of the two crystallographically independent nucleotides, which will be referred to as nucleotides *A* and *B*, are shown in Fig. 1, together with atomic thermal ellipsoids, bond lengths and bond angles. The conformations are similar to those found in other crystal structures of 5′-nucleotides (Sundaralingam, 1969; Saenger, 1973). For both IMP anions, the conformation about the glycosidic bond is *anti* (Donohue & Trueblood, 1960); the torsion angle χ_{CN}[O(1′)–C(1′)–N(9)–C(8)] is 43·9 (3)° for nucleotide *A* and 43·8 (3)° for nucleotide *B*. The best four-atom planes through the pentose rings are defined by atoms C(1′), C(3′), C(4′) and O(1′), none of which deviates by more than 0·05 Å from these planes. C(2′) is displaced from the plane, to the same side as C(5′); C(2′) is 0·54 Å from the pentose plane in nucleotide *A* and 0·57 Å in nucleotide *B*. Thus, the conformations of the ribose moieties are best described as C(2′)-*endo*. The conformation about the C(4′)–C(5′) bond is *gauche–gauche* for both nucleotides. The torsion angle φ_{OO}[O(5′)–C(5′)–C(4′)–O(1′)] is −62·5 (2)° for nucleotide *A* and −58·6 (2)° for nucleotide *B*; the

* Tables of thermal parameters, hydrogen-bond distances and angles, and structure factors have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35440 (26 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Nonhydrogen-atom parameters and their estimated standard deviations*

Values for the coordinates of Ca^{2+} ions and P atoms are multiplied by 10^5, and the other coordinates are multiplied by 10^4. The y coordinate of Ca(1) was not refined. The final value of the isotropic extinction parameter is $g' = 0{\cdot}076\ (4)$ rad^{-1}. The letters *A* and *B* correspond to the two crystallographically independent nucleotides. *W*(1)–*W*(13) are O atoms of the water molecules. $B_{eq} = 8\pi^2(U_1U_2U_3)^{1/3}$, where $U_{1,2,3}$ are the mean square displacements along the principal axes of the thermal ellipsoids.

	x	y	z	$B_{eq.}$ (Å^2)
Ca(1)	45331 (4)	0	22974 (6)	1·7
Ca(2)	31081 (5)	29607 (3)	22371 (7)	2·5
N(1)*A*	−1712 (2)	4223 (1)	3418 (3)	2·7
C(2)*A*	−2631 (3)	3867 (2)	3872 (5)	3·4
N(3)*A*	−2484 (2)	3312 (1)	4513 (4)	3·1
C(4)*A*	−1272 (3)	3142 (1)	4740 (3)	2·0
C(5)*A*	−281 (3)	3462 (1)	4340 (3)	2·2
C(6)*A*	−470 (3)	4060 (1)	3635 (3)	2·2
O(6)*A*	297 (2)	4436 (1)	3243 (3)	3·3
N(7)*A*	799 (2)	3145 (1)	4848 (4)	3·1
C(8)*A*	439 (3)	2640 (1)	5521 (5)	2·7
N(9)*A*	−814 (2)	2614 (1)	5505 (3)	1·9
C(1′)*A*	−1547 (2)	2134 (1)	6150 (3)	1·7
C(2′)*A*	−1691 (2)	1530 (1)	5195 (3)	1·6
C(3′)*A*	−1801 (2)	1042 (1)	6449 (3)	1·8
C(4′)*A*	−943 (2)	1300 (1)	7862 (3)	1·7
O(1′)*A*	−932 (2)	1974 (1)	7651 (2)	2·1
C(5′)*A*	358 (2)	1053 (2)	8129 (3)	2·1
O(2′)*A*	−2731 (2)	1592 (1)	4031 (2)	2·3
O(3′)*A*	−3053 (2)	1039 (1)	6741 (2)	2·3
O(5′)*A*	966 (2)	1169 (1)	6790 (2)	1·9
P*A*	24352 (5)	10287 (3)	69988 (7)	1·4
O(1)*A*	3103 (2)	1501 (1)	8128 (2)	2·4
O(2)*A*	2750 (2)	1090 (1)	5356 (2)	1·9
O(3)*A*	2602 (2)	363 (1)	7655 (2)	1·5
N(1)*B*	7991 (2)	2111 (1)	10756 (3)	2·1
C(2)*B*	7202 (2)	2492 (1)	9840 (3)	2·3
N(3)*B*	7496 (2)	3037 (1)	9303 (3)	2·1
C(4)*B*	8709 (2)	3172 (1)	9753 (3)	1·7
C(5)*B*	9583 (2)	2829 (1)	10699 (3)	1·8
C(6)*B*	9228 (2)	2234 (1)	11238 (3)	1·9
O(6)*B*	9872 (2)	1836 (1)	12032 (3)	2·8
N(7)*B*	10723 (2)	3133 (1)	10856 (3)	2·0
C(8)*B*	10506 (2)	3643 (1)	10016 (3)	2·0
N(9)*B*	9308 (2)	3693 (1)	9297 (3)	1·6
C(1′)*B*	8754 (2)	4181 (1)	8238 (3)	1·5
C(2′)*B*	8537 (2)	4800 (1)	9038 (3)	1·5
C(3′)*B*	8577 (2)	5260 (1)	7699 (3)	1·5
C(4′)*B*	9554 (2)	4977 (1)	6803 (3)	1·5
O(1′)*B*	9581 (2)	4310 (1)	7166 (2)	1·7
C(5′)*B*	10839 (2)	5237 (1)	7148 (3)	1·9
O(2′)*B*	7392 (2)	4790 (1)	9615 (2)	1·8
O(3′)*B*	7389 (2)	5228 (1)	6765 (2)	1·8
O(5′)*B*	11325 (2)	5136 (1)	8766 (2)	1·7
P*B*	127748 (5)	52943 (3)	92619 (7)	1·3
O(1)*B*	12972 (2)	5971 (1)	8802 (2)	2·0
O(2)*B*	13508 (2)	4853 (1)	8368 (2)	1·8
O(3)*B*	12971 (2)	5179 (1)	11006 (2)	2·0
W(1)	4472 (2)	896 (1)	631 (3)	3·0
W(2)	3060 (3)	2642 (1)	9580 (3)	3·7
W(3)	4637 (3)	3603 (1)	1264 (4)	4·6
W(4)	2593 (2)	4053 (1)	2723 (3)	3·0
W(5)	2296 (2)	2012 (1)	2983 (3)	3·4
W(6)	5180 (2)	4802 (1)	2358 (3)	3·1
W(7)	4949 (2)	868 (1)	4168 (2)	3·1
W(8)	5540 (2)	3843 (1)	8105 (4)	3·6

Table 2 (*cont.*)

	x	*y*	*z*	$B_{eq.}$ (Å²)
W(9)	4918 (3)	2252 (2)	2612 (4)	5·0
W(10)	3907 (3)	3092 (1)	5027 (3)	5·1
W(11)	4689 (3)	4511 (2)	5263 (3)	4·7
W(12)	5036 (2)	2104 (1)	6873 (3)	4·4
W(13)	2703 (3)	3676 (1)	7392 (3)	4·3

Table 3. *Hydrogen-atom coordinates*

All values have been multiplied by 10^3.

	x	*y*	*z*	*B* (Å²)
H(N1)*A*	−203 (4)	459 (2)	308 (5)	3·3 (0·8)
H(C2)*A*	−347 (3)	405 (2)	375 (4)	2·6 (0·7)
H(C8)*A*	97 (4)	231 (2)	605 (5)	3·8 (0·8)
H(C1')*A*	−239 (3)	228 (2)	617 (4)	1·6 (0·6)
H(C2')*A*	−99 (3)	146 (1)	470 (3)	1·3 (0·5)
H(C3')*A*	−158 (3)	64 (1)	614 (4)	1·5 (0·6)
H(C4')*A*	−127 (3)	118 (2)	877 (4)	2·0 (0·6)
H1(C5')*A*	76 (3)	125 (1)	904 (4)	1·5 (0·6)
H2(C5')*A*	26 (3)	55 (2)	817 (4)	2·6 (0·7)
H(O2')*A*	−281 (6)	130 (3)	319 (7)	8·0 (1·5)
H(O3')*A*	−308 (4)	77 (2)	760 (5)	3·9 (0·9)
H(N1)*B*	771 (4)	171 (2)	1117 (5)	4·9 (1·0)
H(C2)*B*	631 (3)	230 (1)	962 (3)	1·2 (0·5)
H(C8)*B*	1108 (3)	393 (2)	990 (4)	2·5 (0·7)
H(C1')*B*	800 (3)	401 (1)	771 (3)	1·4 (0·5)
H(C2')*B*	916 (2)	486 (1)	986 (3)	1·0 (0·5)
H(C3')*B*	876 (3)	564 (2)	808 (4)	2·2 (0·6)
H(C4')*B*	929 (3)	501 (2)	571 (3)	1·7 (0·6)
H1(C5')*B*	1131 (3)	502 (2)	644 (4)	2·2 (0·6)
H2(C5')*B*	1075 (3)	567 (2)	685 (5)	3·4 (0·8)
H(O2')*B*	740 (4)	500 (2)	1044 (5)	4·4 (0·9)
H(O3')*B*	732 (4)	546 (2)	613 (5)	4·6 (1·0)
H1(*W*1)	400 (5)	101 (3)	27 (6)	5·4 (1·1)
H2(*W*1)	530 (4)	100 (2)	69 (5)	4·0 (0·9)
H1(*W*2)	308 (11)	239 (7)	927 (14)	17·1 (3·9)
H2(*W*2)	291 (3)	291 (2)	904 (4)	3·2 (0·8)
H1(*W*3)	474 (5)	353 (2)	46 (6)	5·4 (1·1)
H2(*W*3)	471 (6)	396 (3)	175 (8)	8·5 (1·7)
H1(*W*4)	195 (4)	414 (2)	310 (5)	3·5 (0·8)
H2(*W*4)	275 (4)	438 (2)	222 (5)	4·4 (0·9)
H1(*W*5)	152 (4)	194 (2)	276 (5)	4·6 (0·9)
H2(*W*5)	237 (6)	193 (3)	369 (7)	7·5 (1·5)
H1(*W*6)	448 (5)	504 (3)	188 (6)	6·5 (1·3)
H2(*W*6)	595 (5)	510 (3)	214 (6)	7·1 (1·3)
H1(*W*7)	556 (4)	97 (2)	467 (5)	4·6 (0·9)
H2(*W*7)	443 (5)	104 (3)	471 (6)	6·1 (1·2)
H1(*W*8)	608 (4)	356 (2)	843 (5)	4·4 (1·0)
H2(*W*8)	515 (9)	374 (5)	700 (11)	12·5 (2·7)
H1(*W*12)	452 (4)	191 (2)	724 (5)	3·9 (0·9)
H2(*W*12)	507 (10)	207 (6)	580 (11)	18·9 (3·7)
H1(*W*13)	279 (5)	405 (3)	750 (6)	6·3 (1·2)
H2(*W*13)	194 (5)	368 (3)	705 (6)	5·8 (1·1)

torsion angle φ_{OC}[O(5')–C(5')–C(4')–C(3')] is 58·3 (3) and 61·4 (3)° for nucleotides *A* and *B*, respectively. The torsion angle P–O(5')–C(5')–C(4') is 170·7 (2)° for nucleotide *A* and 171·0 (2)° for nucleotide *B*; these values are in the range of 149–230° covered by other nucleotides (Sundaralingam, 1969).

The hypoxanthine moieties are slightly nonplanar. In nucleotide *A*, C(5) shows the largest deviation (0·05 Å) from the plane through the nine atoms of the purine ring, and O(6) and C(1') deviate from this plane by 0·06 and 0·05 Å, respectively. In nucleotide *B*, C(5) shows the largest deviation (0·04 Å) from the nine-atom purine plane, and O(6) and C(1') deviate from this plane by 0·08 and 0·11 Å, respectively. Corresponding bond lengths and angles within the two nucleotides are in excellent agreement: the overall root-mean-square differences are 0·006 Å for bond lengths and 0·8° for bond angles.

The crystal-packing and hydrogen-bonding schemes are displayed in Fig. 2. Since we were unable to locate H atoms from three of the water molecules, certain features of the proposed hydrogen-bonding scheme are somewhat tentative; distances and angles for proposed hydrogen-bonded contacts are available.* Within the crystal structure, adjacent nucleotides are joined by direct hydrogen bonds; by water-mediated hydrogen bonds; by calcium linkages; and by base-stacking interactions. The bases are stacked in the **c** direction, with the purine rings of *A* nucleotides and *B* nucleotides alternating within the stacks. The stacked bases are 3·3–3·5 Å apart, and adjacent bases are inclined about 22° relative to each other. The major stacking interactions occur between the six-membered pyrimidine rings of the neighboring bases. In addition to the base--base stacking interactions there are several close contacts (<3·2 Å) between O(1') of nucleotide *A* and atoms of the imidazole ring from nucleotide *B*; similar O(1')–purine interactions have been found in other crystal structures (Bugg, Thomas, Sundaralingam & Rao, 1971).

The Ca^{2+} ion coordination polyhedra are shown in Fig. 3. Both Ca^{2+} ions are coordinated to seven ligands, which form irregular geometrical arrays around the Ca^{2+} ions. One of the Ca^{2+} ions is coordinated only to O atoms: four from water molecules, two from ribose hydroxyl groups, and one from a phosphate group. The other Ca^{2+} ion is bound to N(7) of nucleotide *B* and to six water molecules. The Ca--water distances within these polyhedra range from 2·316 to 2·490 Å, with an average value of 2·421 Å; the calcium--hydroxyl distances are 2·407 and 2·520 Å; the calcium--phosphate contact is 2·316 Å. The Ca--N contact of 2·731 Å is somewhat longer than the 2·5–2·6 Å that one might expect from the sum of the van der Waals radii for Ca^{2+} and N (Pauling, 1960), but it is in the range of Ca–N distances that have been found in other crystal structures (Glaunsinger, White, Von Dreele, Gordon, Marzke, Bowman & Yarnell, 1978; Smith & Duax, 1976; Metz, Moras & Weiss, 1973; Bekoe, Gantzel & Trueblood, 1967).

The sites at which Ca^{2+} ions interact with the

* See previous footnote.

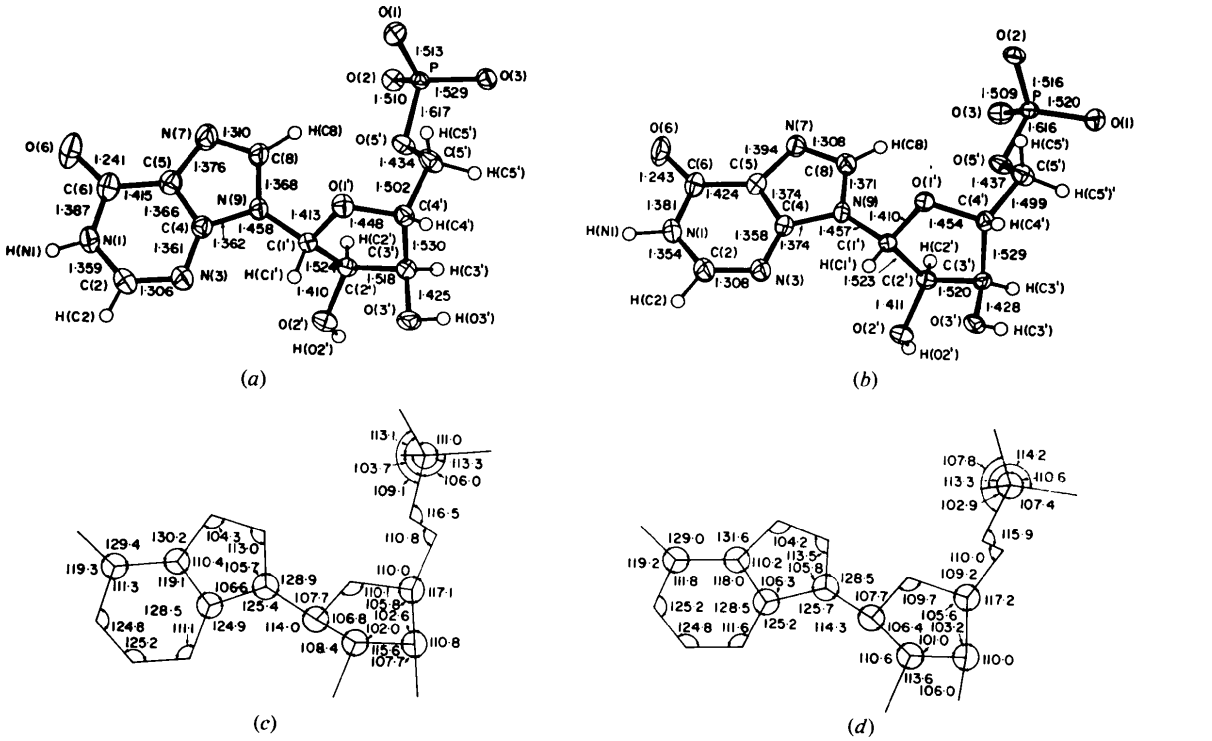


Fig. 1. Conformation and geometry of the inosine 5′-monophosphate anions: (*a*,*c*) nucleotide *A*, (*b*,*d*) nucleotide *B*. Nonhydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability. H atoms are represented by spheres corresponding to an isotropic temperature factor of 0·75 Å^2 and scaled to include 50% probability. Estimated standard deviations are about 0·004 Å and 0·2° for bond lengths and bond angles, respectively. [This drawing and Figs. 2–6 were prepared by using the computer program *ORTEP* (Johnson, 1965).]

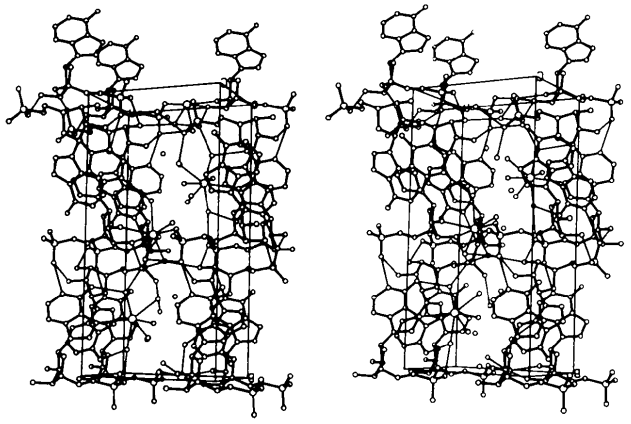


Fig. 2. Stereodrawing in which the crystal packing is viewed approximately down the *c* axis. Larger circles represent Ca^{2+} ions and P atoms, heavy lines represent covalent bonds, and light lines represent hydrogen bonds and water–calcium contacts. H atoms are not shown. All hydrogen bonds that involve water molecules, except for those water molecules coordinated to Ca^{2+} ions, have been omitted.

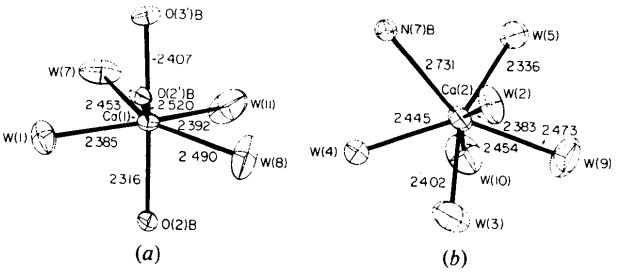


Fig. 3. Geometry of the Ca^{2+} ion coordination shells. *W*(1)–*W*(11) correspond to O atoms of water molecules. Ca^{2+} ion–ligand distances are shown. The atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50% probability. Estimated standard deviations in Ca--ligand distances are 0·003 Å.

nucleotides – either by direct bonding to ligands or by outer-sphere, water-mediated contacts – are depicted in Figs. 4 and 5. Nucleotide *A* (Fig. 4) forms no direct contacts with Ca^{2+} ions, but is involved in six (or possibly seven) Ca–water–nucleotide bridging interactions. Four of these water-mediated interactions, in which the water molecule is coordinated to the Ca^{2+} ion and is hydrogen bonded to a ligand on the nucleotide, are to phosphate O atoms; one is to the O(3′) hydroxyl group; and one is to the O(6) carbonyl group. In addition, there is a seventh possible indirect calcium interaction to O(2′) that involves water molecule *W*(9); since the *W*(9)--O(2′) contact is long (3·02 Å), and we were unable to locate the H atoms on

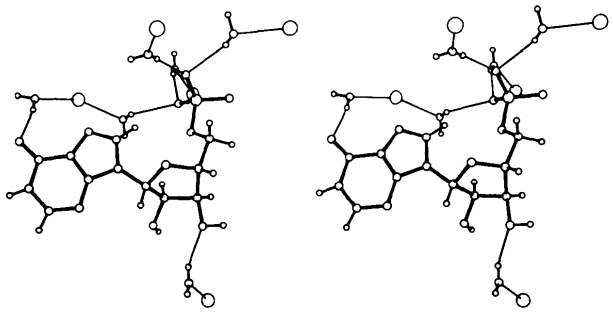


Fig. 4. Stereodrawing showing the sites at which Ca^{2+} ions interact, through water bridges, with nucleotide *A*. The largest circles represent Ca^{2+} ions and P atoms, the heavier lines represent covalent bonds, and the light lines represent water--nucleotide hydrogen bonds and calcium–ligand contacts.

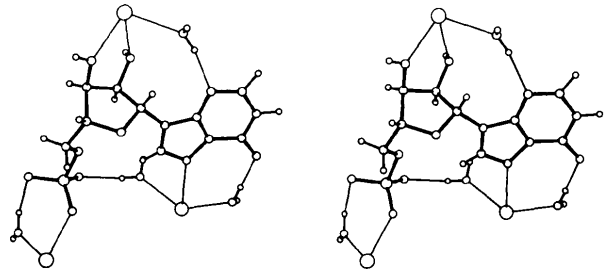


Fig. 5. Stereodrawing showing the sites at which Ca^{2+} ions interact – directly and through water bridges – with nucleotide *B*. Largest circles represent Ca^{2+} ions and P atoms, the heavier lines represent covalent bonds, and the lighter lines represent water --nucleotide hydrogen bonds and calcium–ligand contacts.

W(9), this water bridge is uncertain and will be ignored in further discussions.

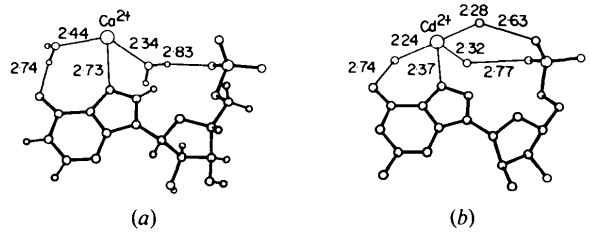
Nucleotide *B* (Fig. 5) is involved in both direct and water-mediated interactions with Ca^{2+} ions [Fig. 3(*b*)]. One Ca^{2+} ion is bound to N(7); this Ca^{2+} ion is also linked, through water molecules, to the O(6) carbonyl group and to an O atom from the phosphate group. Another Ca^{2+} ion is bound to two O atoms of the phosphate group; one of these is coordinated directly to the Ca^{2+} ion, and the second one is hydrogen bonded to a water molecule which is coordinated to the Ca^{2+} ion. Nucleotide *B* also binds a third Ca^{2+} ion, which is chelated by the O(2′)–O(3′) pair of hydroxyl groups, and is linked to N(3) through a water bridge.

Discussion

Ca^{2+} ions are directly coordinated to only one of the two nucleotides (nucleotide *B*) in this crystal structure. As shown in Fig. 5, this nucleotide directly binds Ca^{2+} ions through N(7) of the purine ring, through the hydroxyl groups of its ribose moiety, and through one of the O atoms of its phosphate moiety. It is particularly noteworthy that N(7) serves as a calcium-binding site. Solution and crystallographic studies have demonstrated that N(7) is of major importance in the binding of transition-metal ions to nucleotides, but there is little direct evidence indicating involvement of this site in interactions of alkaline-earth-metal ions with nucleotides. The general assumption has been that alkaline-earth-metal ions, which are unable to form stable covalent bonds, would not bind to the ring-nitrogen atoms of purines and pyrimidines, and would form nucleotide complexes that are quite different from those involving transition-metal ions.

The Ca interaction with N(7) in this crystal structure bears several striking similarities to those metal–N(7) interactions that have been observed in crystal structures of transition-metal complexes of IMP and of guanosine 5′-monophosphate (GMP). Crystallographic investigations have been reported for Ni^{2+} and Co^{2+} complexes of IMP (Clark & Orbell, 1974; Aoki, 1975), and for Cd^{2+}, Ni^{2+} and Mn^{2+} complexes of GMP (Aoki, 1976; DeMeester, Goodgame, Skapski & Smith, 1974; DeMeester, Goodgame, Jones & Skapski, 1974). All of those transition-metal complexes display a general pattern of metal–nucleotide interactions: the metal ions are coordinated directly to N(7), and are bound to O(6) and to the phosphate group through water bridges. The general geometry of these transition-metal–nucleotide complexes, as typified by the Cd^{2+}–GMP structure, is shown in Fig. 6, along with the corresponding calcium–IMP interactions that we find. The close similarities in the transition metal and calcium interactions are obvious. The actual contact distances are dependent upon the type of metal ion that is involved, and the transition-metal ions form two water bridges to the phosphate group, rather than the one water bridge formed by the Ca^{2+} ion; other than these differences, the calcium and transition-metal interactions at the N(7) site are remarkably similar.

It is also noteworthy that a Ca^{2+} ion is chelated by the O(2′)–O(3′) pair of hydroxyl groups from nucleotide *B* in this crystal structure. This chelation site is involved in the binding of Cd^{2+} to IMP in the crystal



(*a*) (*b*)

Fig. 6. Comparison of (*a*) the calcium–N(7) interaction with (*b*) the cadmium–N(7) interaction, as found in the crystal structure of Cd^{2+} guanosine 5′-monophosphate tetrahydrate (Aoki, 1976). The distances (Å) correspond to metal–ligand contacts, and to the donor--acceptor distances for hydrogen bonds that involve the water molecules. H atoms were not located in the cadmium structure. The cadmium–nucleotide interactions are typical of those found for other transition metals. Estimated errors in distances are 0·004 Å for the Ca^{2+} structure and 0·02 Å for the Cd^{2+} structure.

structure of $(Cd^{2+})_2(IMP)_3.12H_2O$ (Goodgame, Jeeves, Reynolds & Skapski, 1975) and it serves as a Ba^{2+} binding site in crystal structures of the barium salts of uridine 5′-phosphate (Shefter & Trueblood, 1965) and of cytidine 5′-phosphate (Hogle & Sundaralingam, 1973). As shown in Fig. 6(*a*), calcium binding to the O(2′)–O(3′) site is also accompanied by a water-bridging interaction between the Ca^{2+} ion and atom N(3) of the purine moiety. Chelation of Ca^{2+} ions by pairs of hydroxyl groups has been found in the crystal structures of several other types of calcium–carbohydrate complexes (Cook & Bugg, 1977) and these types of interactions are responsible for calcium binding to various simple sugars in aqueous solution (Rendleman, 1966). Solution studies have demonstrated that certain types of transition metals bind to the ribose moieties of nucleosides and 5′-nucleotides (Reinert & Weiss, 1969; Berger, Tarien & Eichhorn, 1972). However, the sugar residues are not usually considered in models of alkaline-earth-metal–nucleotide complexes. Considering the rather extensive evidence that alkaline-earth-metal ions display an affinity for the hydroxyl groups of sugar residues in other crystal structures and in aqueous solution, it is likely that the O(2′)–O(3′) pair of hydroxyl groups on nucleotides may be of general importance as calcium-binding sites on 5′-nucleotides.

Various solution studies have suggested that the phosphate moieties are primarily responsible for the affinity of nucleotides for alkaline-earth-metal ions (Cohn & Hughes, 1962; Hammes & Miller, 1967; Brintzinger, 1963; Happe & Morales, 1966). These solution studies have led to the conclusion that alkaline-earth-metal ions bind directly to phosphate moieties, through relatively nonspecific ionic interactions. As shown in Fig. 6(*a*) nucleotide *B* does interact directly with a Ca^{2+} ion through an O atom of its phosphate moiety. However, even this interaction is somewhat specific and is subject to considerable geometrical constraints, since the Ca^{2+} ion is also linked, *via* a water bridge, to a second O atom of the phosphate moiety. The calcium interaction with the N(7) site is probably also dependent upon the indirect water bridge that the Ca^{2+} ion forms to the phosphate group of the nucleotide. Thus it appears that the phosphate moiety might strongly influence calcium interactions with nucleotides even in situations where the Ca^{2+} ion is not in direct contact with the phosphate moiety.

Water-mediated interactions are of major importance in the binding of Ca^{2+} ions to the nucleotides in this crystal structure. All of the direct calcium interactions with nucleotide *B* (Fig. 5) are accompanied by water bridges from the calcium to additional sites on the nucleotide. However, outer-sphere interactions are of particular importance in the calcium interactions with nucleotide *A* (Fig. 4), which forms only water-mediated contacts with Ca^{2+} ions. Nucleotide *A* forms water-mediated interactions with five Ca^{2+} ions. Four of the Ca^{2+} ions are bound through water molecules to two of the O atoms on the phosphate group; one of these Ca^{2+} ions is also bound to the O(6) carbonyl group through a water molecule, resulting in a phosphate--water–calcium–water--O(6) linkage that is very similar to the one found at nucleotide *B* (Fig. 5) and those found in transition-metal–nucleotide complexes [Fig. 6(*b*)]. A fifth Ca^{2+} ion is bound to the O(3′) hydroxyl group through a water bridge. Water-mediated metal interactions with nucleotides have been observed in a number of crystal structures (Sternglanz, Subramanian, Lacey & Bugg, 1976; Shefter & Trueblood, 1965; DeMeester, Goodgame, Jones & Skapski, 1974; DeMeester, Goodgame, Skapski & Smith, 1974; Aoki, 1975, 1976) and there is evidence that similar outer-sphere complexes are common in aqueous solution (Frey & Stuehr, 1974). As can be seen in Figs. 4 and 5, these water-mediated contacts can involve appreciable steric constraints, since the bridging water molecules must simultaneously satisfy the coordination properties of the Ca^{2+} ion while forming stable hydrogen bonds to acceptor sites on the nucleotide.

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References

AOKI, K. (1975). *Bull. Chem. Soc. Jpn*, **48**, 1260–1271.
AOKI, K. (1976). *Acta Cryst.* B**32**, 1454–1459.
BEKOE, D. A., GANTZEL, P. K. & TRUEBLOOD, K. N. (1967). *Acta Cryst.* **22**, 657–665.
BERGER, N. A., TARIEN, E. & EICHHORN, G. L. (1972). *Nature (London) New Biol.* **239**, 237–240.
BRINTZINGER, H. (1963). *Biochim. Biophys. Acta*, **77**, 343–348.
BUGG, C. E., THOMAS, J. M., SUNDARALINGAM, M. & RAO, S. T. (1971). *Biopolymers*, **10**, 175–219.
BUSING, W. R. (1971). *Acta Cryst.* A**27**, 683–684.
BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1962). *ORFLS*. Report ORNL-TM-305. Oak Ridge National Laboratory, Tennessee.
CLARK, G. R. & ORBELL, J. D. (1974). *J. Chem. Soc. Chem. Commun.* pp. 139–140.
COHN, M. & HUGHES, T. R. JR (1962). *J. Biol. Chem.* **237**, 176–181.
COOK, W. J. & BUGG, C. E. (1977). *Metal–Ligand Interactions in Organic Chemistry and Biochemistry*, Part 2, edited by B. PULLMAN & N. GOLDBLUM, pp. 231–256. Dordrecht: Reidel.
COPPENS, P. & HAMILTON, W. C. (1970). *Acta Cryst.* A**26**, 71–83.
CROMER, D. T. & LIBERMAN, D. (1970). *J. Chem. Phys.* **53**, 1891–1898.

DeMeester, P., Goodgame, D. M. L., Jones, T. J. & Skapski, A. C. (1974). *Biochem. J.* **139**, 791–792.
DeMeester, P., Goodgame, D. M. L., Skapski, A. C. & Smith, B. T. (1974). *Biochim. Biophys. Acta*, **340**, 113–115.
Donohue, J. & Trueblood, K. N. (1960). *J. Mol. Biol.* **2**, 363–371.
Frey, C. M. & Stuehr, J. (1974). *Metal Ions in Biological Systems*, Vol. 1, edited by H. Sigel, pp. 51–116. New York: Marcel Dekker.
Glaunsinger, W. S., White, T. R., Von Dreele, R. B., Gordon, D. A., Marzke, R. F., Bowman, A. L. & Yarnell, J. L. (1978). *Nature (London)*, **271**, 414–417.
Goodgame, D. M. L., Jeeves, I., Reynolds, C. D. & Skapski, A. C. (1975). *Nucleic Acids Res.* **2**, 1375–1379.
Hammes, G. G. & Miller, D. L. (1967). *J. Chem. Phys.* **46**, 1533–1534.
Happe, J. A. & Morales, M. (1966). *J. Am. Chem. Soc.* **88**, 2077–2078.
Hogle, J. & Sundaralingam, M. (1973). *Am. Crystallogr. Assoc. Abstr. Pap.* (Summer Meet.), No. 196.
International Tables for X-ray Crystallography (1962). Vol. III, pp. 202–205. Birmingham: Kynoch Press.
Izatt, R. M., Christensen, J. J. & Rytting, J. H. (1971). *Chem. Rev.* **71**, 439–482.
Johnson, C. K. (1965). *ORTEP*. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.
Metz, B., Moras, D. & Weiss, R. (1973). *Acta Cryst.* B**29**, 1377–1393.
Nagashima, N. & Iitaka, Y. (1968). *Acta Cryst.* B**24**, 1136–1138.
Pauling, L. (1960). *The Nature of the Chemical Bond*, 3rd ed., p. 260. Ithaca: Cornell Univ. Press.
Phillips, R. (1966). *Chem. Rev.* **66**, 501–527.
Reinert, H. & Weiss, R. (1969). *Hoppe-Seylers Z. Physiol. Chem.* **350**, 1321–1326.
Rendleman, J. A. (1966). *Adv. Carbohydr. Chem.* **21**, 209–271.
Saenger, W. (1973). *Angew. Chem.* **12**, 591–601.
Shefter, E. & Trueblood, K. N. (1965). *Acta Cryst.* **18**, 1067–1077.
Smith, G. D. & Duax, W. L. (1976). *J. Am. Chem. Soc.* **98**, 1578–1580.
Sternglanz, H., Subramanian, E., Lacey, J. C. Jr & Bugg, C. E. (1976). *Biochemistry*, **15**, 4797–4802.
Stewart, R. F., Davidson, E. R. & Simpson, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.
Sundaralingam, M. (1969). *Biopolymers*, **7**, 821–860.
Swaminathan, V. & Sundaralingam, M. (1979). *CRC Crit. Rev. Biochem.* **6**, 245–336.
Tu, A. R. & Heller, M. J. (1974). *Metal Ions in Biological Systems*, Vol. 1, edited by H. Sigel, pp. 1–49. New York: Marcel Dekker.
Wehe, D. J., Busing, W. R. & Levy, H. A. (1962). *ORABS*. Report ORNL-TM-229. Oak Ridge National Laboratory, Tennessee.
Wilson, A. J. C. (1942). *Nature (London)*, **150**, 151–152.
Zachariasen, W. H. (1963). *Acta Cryst.* **16**, 1139–1144.

Acta Cryst. (1980). B**36**, 2604–2608

The Structures of *trans*-Dichloro(pyrrolidine)(triethylphosphine)platinum(II) and *trans*-Dichloro(*trans*-2,5-dimethylpyrrolidine)(triethylphosphine)platinum(II)

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Abstract

The structures of *trans*-[$PtCl_2(Et_3P)(C_4H_9N)$] (I) [$C_{10}H_{24}Cl_2NPPt$, monoclinic, $P2_1/c$, $a = 11{\cdot}110$ (4), $b = 11{\cdot}370$ (3), $c = 13{\cdot}167$ (6) Å, $\beta = 110{\cdot}76$ (4)°], and *trans*-[$PtCl_2(Et_3P)(C_6H_{13}N)$] (II) [$C_{12}H_{28}Cl_2NPPt$, monoclinic, $P2_1/n$, $a = 7{\cdot}608$ (3), $b = 21{\cdot}749$ (8), $c = 11{\cdot}075$ (3) Å, $\beta = 100{\cdot}56$ (2)°] have been determined. (I) and (II) are square-planar complexes of Pt^{II}; their geometries are similar, the main differences being the arrangements of the pyrrolidine ligand with respect to the $PtCl_2(Et_3P)$ moiety, and the conformations assumed by the puckered five-membered rings. The structures have been refined to $R = 0{\cdot}045$ (I) and 0·040 (II).

Introduction

Recently, the structures of *trans*-[$PtCl_2(Et_3P)$(*cis*-2,3-dimethylpyrrolidine)] (III) and *trans*-[$PtCl_2(Et_3P)$(*cis*-2,4-dimethylpyrrolidine)] (IV) were determined by X-ray analysis to assign *cis* or *trans* configurations to the products of the Pt-promoted cyclization of the unsaturated amines $CH_2{=}CH{-}CH(CH_3){-}CH_2CH_2{-}NH_2$ and $CH_2{=}CH{-}CH_2{-}CH(CH_3){-}CH_2{-}NH_2$

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