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Crystal and Solution Structure of 2′,5′-Arabinosylcytidine Monophosphate. Influence of POC Bond Angles on the Proton–Phosphorus Vicinal Coupling Constants in the P–O–C–H Fragments

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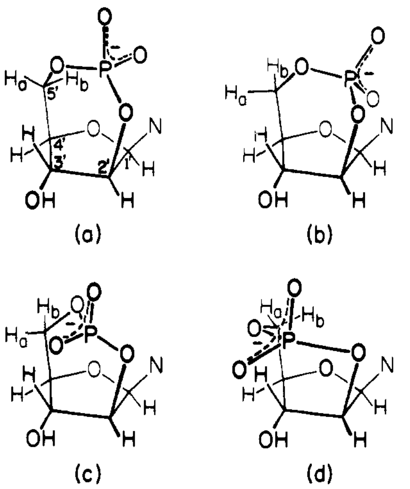
Abstract: The crystal structure of 2′,5′-arabinosylcytidine monophosphate (I) has been analyzed by x-ray diffraction methods. The arabinose ring is 2′-endo, as in solution. Coupling constants measured for I in solution can be explained on the basis of the structure found in the crystal, indicating that the molecule maintains the same conformation in both states. Strain in the seven-membered phosphate ring leads to greatly enlarged P–O–C angles, which may explain the unusually large phosphorus–proton coupling constants, J_{POCH}, reported earlier. We conclude that J–ϕ curves proposed earlier, describing the empirical relationship between coupling constants and dihedral angles ϕ, should not be used for uncharacterized systems and we propose a tentative, revised curve for seven-membered cyclic phosphates. We also discuss other conformational features of seven-membered phosphate rings, and introduce a convenient notation for describing the various conformers.

Cyclic arabinosyl-2′,5′-cytidine monophosphate (2′,5′-*ara*-cCMP; I) was first synthesized in 1969 by Wechter[2a] as a step toward further understanding of the structure–function relationship of cyclic nucleotides and of the effect of phosphate anisotropy upon the ^{1}H NMR chemical shift of organophosphorus compounds. Wechter's analysis of the proton NMR spectrum of I indicated that the phosphate group strongly deshields H(3′) of the arabinose ring, resulting in a downfield shift of about 1 ppm with respect to the chemical shift of H(3′) in arabinosylcytidine (*ara*-C) itself. This deshielding effect was attributed to a molecule conformation in which $H_{3'}$ is in close proximity to the phosphate group. More recently, Kainosho and Ajisaka[2b] reexamined the proton NMR spectrum of I in further detail and concluded that the conformation of I tacitly assumed by Wechter[2a] might be incorrect. From considerations of molecular models, Kainosho and Ajisaka arrived at four possible conformations of the sugar–phosphate ring systems in I (Figure 1) and on the basis of empirical relationships between coupling constants and torsion angles they favored conformation (a) (Figure 1) for I. In the course of this analysis, however, Kainosho and Ajisaka[2b] noted an unusual vicinal phosphorus–proton coupling constant, J_{POCH}, in the spectrum



(a) (b) (c) (d)

Figure 1. Four possible conformations of the phosphate moiety in I.

Table I. Crystal Data; Cyclic 2',5'-Arabinosylcytidine Monophosphate, $C_9H_{12}N_3O_7P$

Monoclinic	$C_9H_{12}N_3O_7P$
Space group $P2_1$	fw = 305.19
a = 9.415 (1) Å	$Z = 2$
b = 10.348 (1) Å	$F(0,0,0) = 316$
c = 6.491 (1) Å	$\rho_{\text{measd}} = 1.680$ g cm^{-3}
β = 109.29 (1)°	$\rho_{\text{calcd}} = 1.698$ g cm^{-3}
V = 596.9 Å^3	$\mu = 94.3$ cm^{-1} (for Cu Kα radiation)
λ(Cu Kα) = 1.5418 Å	R = 0.034 (1506 data points)

of I, which raised questions concerning the validity of the empirical relationships.

In an effort to clarify the situation and to explain the unusual coupling constant, we have carried out a crystal-structure analysis of I. Our results confirm conformation (c), Figure 1, for I, as originally inferred by Wechter.[2a] They also suggest that the unusual coupling constant J_{POCH} is probably due to bond-angle distortions in the seven-membered phosphate ring.

Experimental Section

A sample of I was kindly furnished by Dr. Wechter. Crystals (colorless plates) suitable for x-ray studies were obtained by slow diffusion of an HCl solution into an aqueous alkaline solution of I. Preliminary Weissenberg photographs showed them to be monoclinic; systematic absences (0k0 with k odd) indicated the space group $P2_1$. The unit-cell dimensions were determined by a least-squares fit to the 2θ values of 12 high-angle reflections measured on a quarter-circle diffractometer using Cu Kα radiation. The density was measured by flotation in a mixture of carbon tetrachloride and 1,2-dibromoethane. Crystal data are given in Table I.

A crystal approximately 0.5 × 0.3 × 0.05 mm in size was used for data collection. Intensities were measured using θ-2θ scans on a Datex-automated General Electric three-circle diffractometer equipped with a copper-target x-ray tube, Ni filter, scintillation counter, and pulse-height analyzer; backgrounds were counted for 30 s at each end of the scan, and the scan rate was 1° per min. Data were collected to a 2θ value of 150°, with the scan width varying from 2.0° at 2θ = 5° to 3.0° at 2θ = 150°. The take-off angle was 3°. A check reflection ($\overline{2}\overline{2}0$) was recorded every 30 reflections and two other check reflections ($21\overline{1}$, $1\overline{6}\overline{4}$) were recorded every 60 reflections; the variations in their intensities were well within the assigned variances. The recorded intensities were corrected for Lorentz and polarization effection, but not for absorption (μ = 24.5 cm^{-1}). The variances of the intensities, $\sigma^2(I)$, were based on counting statistics plus the term $(0.02S)^2$, where S is the scan count. The total number of independent reflections was 1507, of which only one had net intensity less than zero and another 23 had intensities (I) less than $3\sigma(I)$.

Determination and Refinement of the Structure. The structure was solved by direct methods by the application of MULTAN.[3] The resulting E map yielded the positions of all the heavy atoms except C(3) and O(7), which were located from a subsequent electron density map. A structure-factor calculation based on this model yielded an R index, $\Sigma||F_c| - |F_o||/\Sigma|F_o|$, of 0.213. The structure was refined by full-matrix least-squares minimization of the quantity $\Sigma w(F_c^2 - f_o^2)^2$, the weights w being equal to $\sigma^{-2}(F_o^2)$. The atomic scattering factors for C, N, O, and P were taken from International Tables for X-Ray Crystallography[4] and for H from Stewart, Davidson, and Simpson.[5]

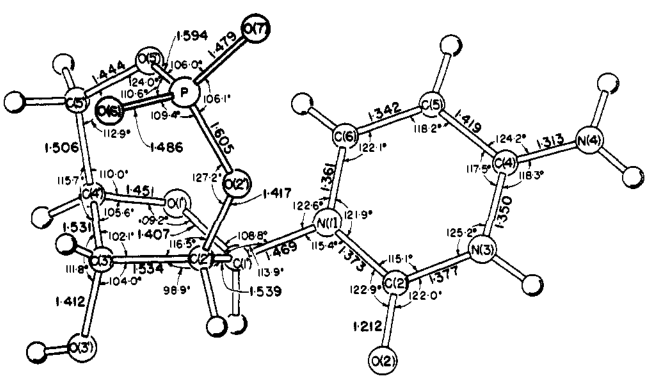


Figure 2. Bond distances and angles involving the heavier atoms.

After several cycles of isotropic refinement of the nonhydrogen atoms (R = 0.088), 8 of the 12 hydrogen atoms were readily located from a difference map. Anomalous scattering terms for P, O, and N[6] were introduced at this time in order to permit confirmation of the absolute configuration of the molecule. Structure factors were calculated for the 177 pairs of enantiomeric reflections (hkl and $\overline{h}\overline{k}\overline{l}$) which had been measured. The observed differences $\Delta F_o = |F_o(hkl)| - |F_o(\overline{h}\overline{k}\overline{l})|$ agreed in sign with the calculated differences in 141 cases. Therefore, the absolute configuration is confirmed to be the D enantiomer (Figure 1).

After further refinement, the four remaining hydrogen atoms were located from a difference map. In the final cycles of refinement in which 230 parameters were adjusted, two matrices were collected; the coordinates of the 20 heavier atoms (P, C, N, O) were in one matrix, while the scale factor, a secondary extinction coefficient,[7] the anisotropic temperature parameters of the heavy atoms, and the coordinates and isotropic temperature parameters of the 12 hydrogen atoms were in the second. After four cycles, no parameter shifted by more than 0.6σ and the refinement was considered converged. The final value of the goodness-of-fit $[\Sigma w(F_o^2 - F_c^2)^2/(N - p)]^{1/2}$ for N = 1507 reflections and p = 230 parameters was 3.4; the R index was 0.034 for the 1506 reflections with net intensities greater than zero. The relatively large goodness-of-fit reflects primarily the high values of the weights w resulting from the excellent counting statistics; there is no obvious pattern to the weighted residuals $\sqrt{w}(F_o^2 - F_c^2)$. Final parameters are given in Table II.

Proton NMR Measurements. Proton NMR spectra were run on a Varian XL-100 using Fourier transform equipment. I was dissolved in D_2O at a concentration of 0.2 M with 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) as an internal standard. In order to measure the lanthanide-shifted spectrum, solid europium(III) nitrate was added portionwise directly to the NMR sample tube to a final Eu^{3+} concentration of 0.8 M. For further details of the analysis of the spectrum, see ref 2b.

Results and Discussion

Bond Distances and Angles in Solid State. The final positional and thermal parameters are listed in Table II; bond distances and angles involving the C, N, O, and P atoms are given in Figure 2. The estimated standard deviations in the bond lengths are: P-O, 0.002 Å; C-O and C-N, 0.003 Å; C-C, 0.004 Å; in the angles, about 0.2-0.3°.

The molecule is a zwitterion with the cytosine ring protonated at N(3) and the negative charge shared equally by two oxygen atoms O(6) and O(7). The dimensions of the pyrimidine ring are in close agreement with values compiled by Mandel[8] for other protonated cytosine species, and those of

Table II. Final Atomic Parameters[a]

	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(1)	47 412 (26)	734 (26)	31 149 (35)	272 (11)	241 (12)	288 (11)	−11 (10)	155 (9)	−14 (9)
C(2)	49 213 (30)	7 930 (29)	14 408 (41)	256 (13)	263 (14)	259 (12)	6 (12)	96 (10)	13 (11)
O(2)	41 363 (26)	17 154 (26)	6 523 (35)	412 (13)	365 (13)	420 (11)	155 (11)	196 (10)	124 (10)
N(3)	60 515 (26)	3 798 (25)	6 904 (35)	271 (12)	281 (14)	268 (10)	23 (10)	128 (9)	58 (10)
C(4)	69 418 (29)	−6 559 (28)	14 531 (41)	228 (13)	228 (14)	285 (12)	−10 (11)	98 (10)	0 (11)
N(4)	79 649 (27)	−9 415 (26)	5 557 (40)	299 (13)	264 (13)	389 (13)	33 (11)	178 (11)	57 (11)
C(5)	67 238 (35)	−13 544 (34)	32 066 (46)	381 (16)	278 (15)	363 (14)	63 (14)	184 (12)	105 (14)
C(6)	56 278 (36)	−9 658 (32)	39 622 (48)	397 (17)	287 (16)	355 (14)	33 (13)	197 (13)	73 (12)
C(1′)	35 053 (32)	4 816 (29)	38 778 (43)	289 (14)	260 (15)	307 (12)	−20 (12)	184 (11)	−39 (11)
C(2′)	19 653 (32)	−897 (29)	25 500 (42)	273 (14)	266 (15)	269 (11)	14 (11)	139 (10)	−8 (11)
C(3′)	11 723 (31)	433 (31)	42 591 (45)	281 (14)	256 (14)	309 (12)	11 (12)	168 (11)	−46 (11)
O(3′)	8 087 (26)	13 692 (24)	42 069 (35)	468 (14)	282 (12)	484 (13)	86 (10)	280 (11)	−3 (10)
C(4′)	24 264 (34)	−3 219 (31)	63 689 (41)	316 (15)	325 (15)	249 (12)	−12 (12)	149 (11)	−57 (11)
C(5′)	25 027 (35)	−17 283 (34)	69 917 (43)	406 (17)	371 (18)	266 (12)	10 (13)	177 (12)	−34 (12)
O(1′)	38 187 (23)	669 (25)	60 466 (29)	290 (11)	477 (14)	248 (9)	−68 (10)	120 (8)	−53 (10)
O(2′)	21 380 (23)	−13 615 (22)	18 618 (29)	384 (11)	254 (10)	286 (8)	−43 (10)	202 (8)	−57 (9)
O(5′)	28 686 (23)	−25 571 (24)	54 496 (29)	400 (12)	336 (12)	297 (9)	83 (10)	150 (8)	−28 (10)
O(6′)	2 418 (22)	−27 974 (24)	27 270 (31)	339 (11)	275 (10)	403 (10)	3 (10)	179 (8)	22 (10)
O(7′)	25 656 (26)	−37 163 (25)	20 293 (37)	427 (13)	313 (12)	462 (12)	24 (11)	266 (10)	−97 (11)
P	18 591 (7)	−26 899 (*)	29 474 (9)	278 (4)	231 (3)	266 (3)	8 (3)	146 (2)	−32 (3)

	x	y	z	B
H(N3)	629 (6)	81 (6)	−37 (8)	5.6 (1.3)
H(N4)	815 (6)	−48 (5)	−41 (7)	4.3 (1.0)
H′(N4)	865 (5)	−161 (5)	105 (6)	3.1 (0.8)
H(5)	744 (4)	−204 (4)	374 (5)	2.1 (0.7)
H(6)	552 (7)	−132 (7)	505 (10)	7.2 (1.6)
H(1′)	334 (5)	146 (4)	361 (6)	3.0 (0.8)
H(2′)	140 (5)	64 (5)	126 (8)	4.1 (1.0)
H(3′)	34 (4)	−42 (4)	403 (5)	1.4 (0.6)
H(O3′)	44 (8)	149 (7)	495 (12)	9.5 (1.9)
H(4′)	226 (4)	19 (4)	754 (6)	2.4 (0.7)
H(5′A)	326 (5)	−187 (5)	847 (7)	3.7 (0.9)
H′(5′B)	143 (4)	−191 (4)	708 (6)	2.6 (0.8)

[a] Values for the heavier atoms are $\times 10^5$; coordinates for the hydrogen atoms are $\times 10^3$. The anisotropic temperature factors are of the form $\exp[-2\pi^2(h^2a^{*2}U_{11} + \cdots + 2klb^*c^*U_{23})]$. The y parameter of the P atom was held fixed to define the origin.

Table III. Details of the Hydrogen Bonds D–H···A

Donor (D)	H	Acceptor (A)	D···A, Å	Angle D–H···A, deg
N(3)	H(N3)	O(7′)[a]	2.684 (3)	157 (5)
N(4)	H(N4)	O(7′)[a]	2.795 (4)	138 (4)
N(4)	H′(N4)	O(6′)[b]	2.876 (3)	166 (4)
O(3′)	H(O3′)	O(6′)[c]	2.643 (3)	165 (8)

[a] At $1 - x, \frac{1}{2} + y, -z$. [b] At $1 + x, y, z$. [c] At $-x, \frac{1}{2} + y, 1 - z$.

the arabinose ring agree well with values compiled by Bunick and Voet[9] for arabinonucleosides. The lengthening of the C(1′)–C(2′) bond by about 0.02–0.03 Å relative to the same bond in ribonucleosides or ribonucleotides, which presumably reflects the uncomfortably close contact O(2′)···N(1) (2.749 Å in I) characteristic of arabinose bases, has been noted previously.[10]

Appreciable strain within the seven-membered ring system is evidenced by enlargement of the bond angles at C(2′), C(4′), C(5′), O(2′), and O(5′). The angles C(2′)–O(2′)–P and C(5′)–O(5′)–P, 127.2 and 124.0°, are the largest yet observed in nucleotides. Typical values of C–O–P angles in six-membered cyclic phosphate rings, such as those in cyclic 3′,5′-mononucleotides, are in the range 111–118°.[11–16] Recently Coulter[17] reported C–O–P angles of 121.5 and 121.7° in the seven-membered ring of tetramethylenephosphoric acid, appreciably smaller than the values we find here. Possible effects of the implied stereochemical stress on the interpretation of the NMR spectra will be discussed later.

Molecular Packing and Hydrogen Bonding. A stereoscopic view[18] of the crystal structure is shown in Figure 3, and the details of the hydrogen bonds are given in Table III. All of the hydrogen bonds are intermolecular, and all involve the highly electronegative oxygen atoms of the phosphate group as acceptors. The two bonds to O(7) involve nitrogen atoms N(3) and N(4) of the same molecule, and hence are appreciably nonlinear.

Molecular Conformation in the Crystal. A stereoscopic view of the molecule is shown in Figure 4. This is the first nucleotide derivative containing a seven-membered phosphate ring to have been characterized by diffraction methods. The seven-membered ring can be conveniently described in terms of three approximately planar (within 0.05 Å) groupings of atoms P–O(2′)–C(2′)–C(3′), P–O(5′)···C(4′)–C(3′), and O(5′)–C(5′)–C(4′). The first and third of these groups are folded to opposite sides of the second group, by dihedral angles of 65.5 and 52.1°, so as to result in a chair conformation.

Torsion angles are listed in Table IV. The overall conformation of the arabinose phosphate moiety of I is very similar to (c) in Figure 1. Thus, the C(4′)–C(5′), C(5′)–O(5′), and C(2′)–O(2′) torsion angles differ by an average of only 7° from the values estimated from molecular models (Table VI), while the agreement is very poor, particularly if the signs of the estimated torsion angles are correctly assigned for the other three conformers discussed by Kainosho and Ajisaka[2b] (Figure 1).

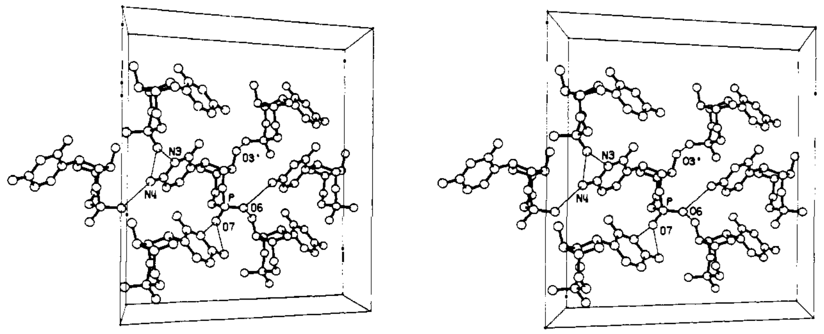


Figure 3. A stereoview[18] of the crystal structure. The view is along *c*, with the *b* axis vertical.

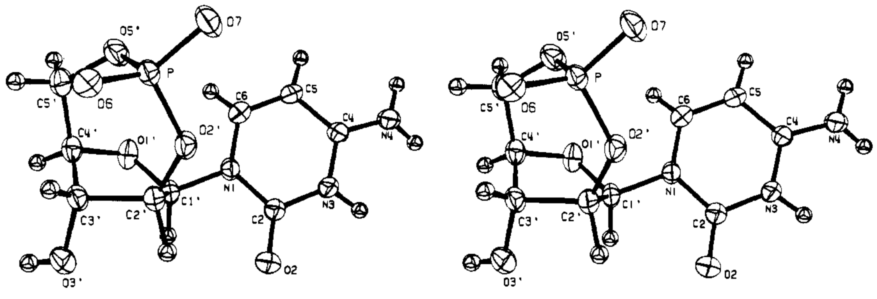


Figure 4. A stereoview[18] of the molecule.

Table IV. Some Torsion Angles[a]

Angle	Deg
Arabinose Ring	
C(6)-N(1)-C(1')-O(1')	25.9
C(3')-C(2')-C(1')-O(1')	37.5
C(4')-C(3')-C(2')-C(1')	−39.7
O(1')-C(4')-C(3')-C(2')	30.2
C(1')-O(1')-C(4')-C(3')	−6.7
C(2')-C(1')-O(1')-C(4')	−19.9
O(5')-C(5')-C(4')-O(1')	−54.6
Phosphate Ring	
O(2')-C(2')-C(3')-C(4')	78.4
P-O(2')-C(2')-C(3')	−11.4
O(5')-P-O(2')-C(2')	−53.7
C(5')-O(5')-P-O(2')	73.4
C(4')-C(5')-O(5')-P	−61.2
C(3')-C(4')-C(5')-O(5')	64.9
C(2')-C(3')-C(4')-C(5')	−91.6
$\phi_{\text{P-H}}$ and $\phi_{\text{H-H}}$	
P-O(2')-C(2')-H(2'(	−137
P-O(5')-C(5')-H(5'A)	55
P-O(5')-C(5')-H(5'B)	175
H(4')-C(4')-C(5')-H(5'A)	63
H(4')-C(4')-C(5')-H(5'B)	−53
H(4')-C(4')-C(3')-H(3')	−87
H(3')-C(3')-C(2')-H(2')	87
H(2')-C(2')-C(1')-H(1')	23

[a] Estimated standard deviations are approximately 0.3° when only the heavier atoms are involved, and about 5° when hydrogen atoms are involved.

The conformation of the arabinose ring is best described as 2'-endo, atom C(2') lying 0.64 Å from the best plane through C(1'), C(3'), C(4'), and O(1'), which are coplanar within 0.04 Å. This is essentially the same conformation predicted by Kainosho and Ajisaka[2b] on the basis of the proton-proton vicinal coupling constants $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$.

The 2'-endo conformation of the arabinose ring has also been found in crystals of the arabinonucleosides 1-β-D-arabinofuranosylcytosine (*ara*-C),[19,20] the hydrochloride of *ara*-C,[10] and in the uracil analogue *ara*-U;[21,22] the two other conformations that have been observed are 3'-endo[23] and C(1')-exo-O(1')-endo.[9] The 2'-endo conformation seems to be stabilized by intramolecular interactions between O(2') and O(5'), there being hydrogen bonds O(2')-H···O(5') in crystals of *ara*-U and *ara*-C while in the present compound the phosphate group forms a bridge. Further details of the conformation of the arabinose ring are dictated by the bite distance O(2')···O(5'). In *ara*-U, atom C(3') is slightly endo to the ring (see Table V) and in *ara*-C it is essentially in the plane of O(1'), C(1'), and C(4'); with these conformations, we calculate that the minimum O(2')···O(5') distances that can be obtained by adjusting the remaining conformational angle C(4')-C(5') are 2.70 and 2.65 Å, respectively, satisfactory for a hydrogen bond distance but too long for an O···O distance in a phosphate group. In the present compound atom C(3') switches to an exo position, attaining an O(2')···O(5') distance of 2.52 Å with a comfortably staggered conformation about the C(4')-C(5') bond (see Table IV).

As has been found in all arabinonucleosides so far studied, the conformation about the glycosidic bond is anti, the torsion angle O(1')-C(1')-N(1)-C(6) being 25.9°. This is within the range noted by Bunick and Voet[9] for arabinopyrimidines; the single arabinopurine so far studied,[9] *ara*-A, has a larger torsion angle (57.8°) which presumably reflects the lessening of steric constraints when the arabinose ring is liked to a five-membered ring. The atoms of the cytosine grouping are coplanar within 0.02 Å with the exception of C(1'), which is 0.056 Å from the plane of the six-membered ring. This slight bending of the N(1)-C(1') bond reflects the steric hindrance between the arabinose ring and the pyrimidine ring that is characteristic of arabinonucleotides.[9,10]

In Table VI is tabulated a comparison of dihedral (or, more properly, torsion) angles obtained from the x-ray analysis with those estimated from molecular models and also with those inferred from the NMR coupling constants as measured in solution.[24] The x-ray values are in satisfactory agreement with those estimated from a model of conformation (c) (Figure 1), but from none of the other three possible models. Thus, the conformation in the crystal is (c), as originally presumed by

Table V. A Comparison of the Arabinose Ring Conformation Crystals of *ara*-U, *ara*-C, and 2′,5′-*ara*-cCMP

		ara-U	*ara*-C	*ara*-cCMP
Deviations, Å[a]	C(2′)	−0.655	−0.569	−0.501
	C(3′)	−0.088	0.008	0.172
	N(1)	−0.722	−0.802	−0.831
Conformation		2′-Endo-3′-endo	2′-Endo	2′-Endo-3′-exo
O(2′)···O(5′) distance, Å		2.724	2.650	2.524
Minimum distance, Å[b]		2.704	2.646	2.443

[a] Deviations from the plane through O(1′), C(1′), and C(4′). [b] Minimum O(2′)···O(5′) distance for this conformation, calculated by varying the torsion angle about C(4′)-C(5′).

Table VI. Comparison of Torsion Angles ϕ and Coupling Constants J for Structure of 2′,5′-*ara*-cCMP (I) Together with Values Estimated for the Four Possible Molecular Conformations

Nuclei[a] ij	J_{ij}^{obsd}, Hz[b]	ϕ_{ij}^{obsd}, deg[c]	J_{ij}^{calcd}, Hz[d]	ϕ_{ij}, estimated from molecular models, deg[e]			
				a	b	c	d
1′2′	3.2	22.9	7.6	50			
2′3′	0	87.1	−0.3	80			
3′4′	0	−87.2	−0.3	−90			
4′5′a	0	63.0	1.6	40	90	60	−30
4′5′b	3.2	−53.2	3.1	−80	−30	−60	−150
2′P	22.2	−136.9	12.1	180	150	−120	−90
5′aP	4.1	55.0	2.8	180	90	60	−160
5′bP	31.0	175.5	20.8	−60	−150	180	−40

[a] For notation of the nuclei, see Figure 1. [b] Detail of the analysis of the spectrum, see ref 2b. [c] Torsion angles determined for the crystal. [d] Coupling constants calculated for the torsion angles determined by x-ray analysis, using the usual empirical ϕ-J curve.[2b] [e] All angles are crude estimates made from Drieding models, probably accurate to ±10° or so.

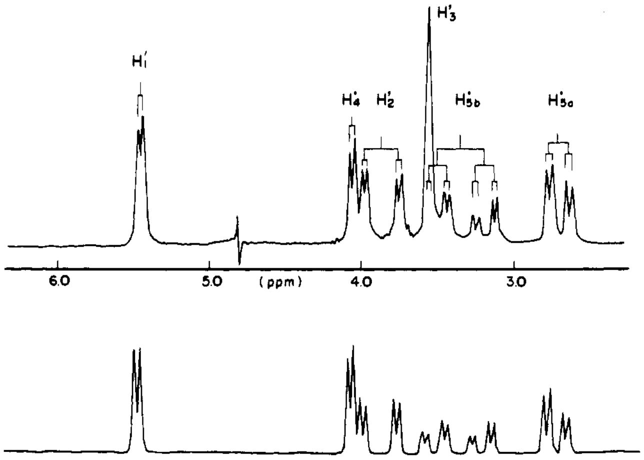


Figure 5. (top) The 100-MHz proton NMR spectrum of 2′,5′-*ara*-cCMP (0.2 M in D_2O) in the presence of 0.8 M Eu^{3+}; (bottom) simulated spectrum without H(3′). Note that the assignments of two protons at C(5′) have been revised (see ref 2b, Figure 5).

Wechter. On this basis, the assignment of resonances in the Eu-shifted NMR spectrum has been revised, the peaks identified with the two protons H(5′a) and H(5′b) having been interchanged. The revised assignments are shown in Figure 5.

This new assignment leads to much better qualitative agreement between the measured phosphorus-hydrogen coupling constants J_{POCH} and the observed dihedral angles ϕ than was obtained previously,[2b] when the assignments were based on conformation (a), Figure 1. It is now clear that all three of these coupling constants are much larger, by approximately 50%, than predicted from the J-ϕ curves derived for six-membered cyclic phosphates or for acyclic phosphates.[24-29] It seems probable that the larger J values are related to the strain in the seven-membered phosphate ring, and in particular to the enlargement of the C-O-P bond angles, but we are not able to offer a more detailed explanation. In any event, we present in Figure 6 a revised J-ϕ plot, presumably applicable to 2′,5′-cyclic phosphates. Needless to say, since this curve is based on a single compound, it should not be taken as quantitative. We emphasize, as have others before us, that use should not be made of this, or of any other, J-ϕ plot without

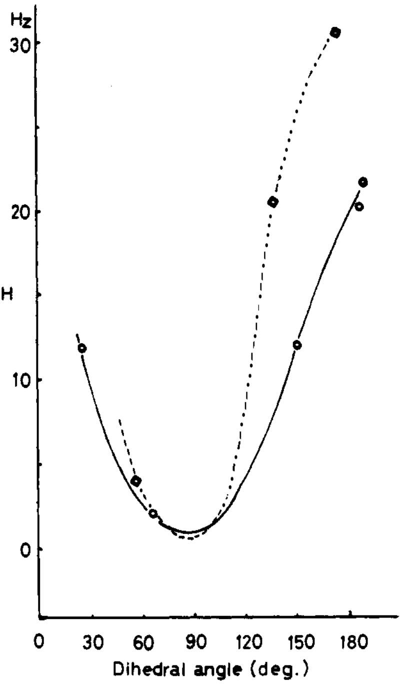


Figure 6. (dashed line) Phosphorus-proton coupling constant J_{POCH} in 1 plotted against the dihedral angles obtained for the crystal. (solid line) J_{POCH}-ϕ curve established for various unstrained phosphates.[24]

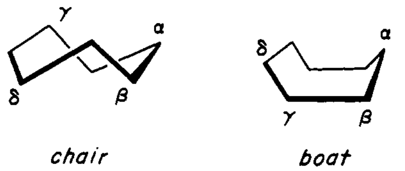


Figure 7. Two conformations of a seven-membered ring.

adequate consideration of the system to which it is being applied.

The observed proton–proton coupling constants agree well with those estimated from the observed torsion angles (Table VI) with the exception of $J_{1'2'}$. Here, the observed coupling constant of 3.2 Hz suggests a dihedral angle H(1′)-C-C-H(2′) of approximately 50°, rather than the observed value of 23°. While this discrepancy may be due, in part, to the arabinose ring assuming a somewhat different conformation in solution than it does in the crystal, in part it also reflects the relatively large uncertainty (about 7°) in the observed torsion angles due to the inability of x-ray diffraction to provide accurate coordinates for the hydrogen atoms. A better measure of this torsion angle is probably provided by the related torsion angle O(1′)-C(1′)-C(2′)-C(3′), 37.5°, and N(1)-C(1′)-C(2′)-O(2′), 35.1°, both of which have esd's of approximately 0.3°. Replacement of the remaining H-C-C-H torsion angles in Table VI with related angles involving only O and C atoms would change the value of $\phi_{2'3'}$ to about 78° but would have little effect on the remaining values.

Conformational Aspects of Seven-Membered Cyclic Phosphate Rings. Since the conformation of the seven-membered ring in I is different from that found for tetramethylenephosphoric acid,[17] II, we think it appropriate to discuss briefly some of the conformational features of such ring systems. While the conformational features of six-membered cyclic phosphates have been extensively studied,[30-34] we are aware of no such studies on seven-membered cyclic phosphates.

The conformation assumed by a seven-membered ring can be related, at least approximately, to either a boat or a chair (Figure 7). In a seven-membered cyclic phosphate the phosphorus atom can occupy any of the four nonequivalent sites α, β, γ, and δ, leading to a total of eight distinct conformers. We shall identify these eight conformers according to the position of the phosphorus atom, thus: chair-P(α), boat-P(δ), etc. This simplified notation will prove convenient in comparing one conformer with another.

The boat forms will tend to be unfavorable, since they involve approximately eclipsed conformations about three bonds (as opposed to one in the chair form) and may also entail uncomfortable contacts between substituents at the α and γ positions. While we cannot rule out the possibility that a boat conformer such as boat-P(β), which puts an oxygen atom in the α position and entails eclipsing of only two CH_2-CH_2 bonds, may turn out to be more stable than one or more of the chair forms, we shall limit our discussion to the intrinsically favorable chair conformers.

Drawings of the four chair conformers are shown in Figure 8. It seems apparent from these drawings, and even more apparent from molecular models, that the chair-P(δ) conformer should be the most stable and that the chair-P(α) conformer, which involves an eclipsed CH_2-CH_2 bond and cross-ring H(γ)···H(γ) contacts, should be the least stable; of the other two, it is our guess that the chair-P(β) conformer might be more stable than the chair-P(γ). These estimates of stability apply only to molecules such as II, where there are no additional constraints imposed by substituent groups. Compound II does, indeed, exist as the chair-P(δ) conformer in its crystals.[17] Further studies of II in solution would be of interest.

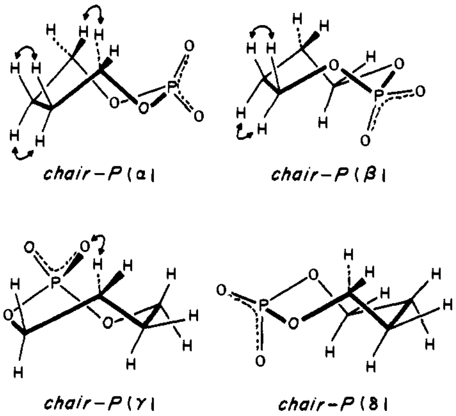


Figure 8. Four possible conformers of II. Arrows indicate the expectable steric repulsive interactions. For the notation, see text.

The conformation of the seven-membered cyclic phosphate ring in I is, of course, restricted because of steric constraints imposed by the sugar ring and the neighboring pyrimidine ring. The four possible conformations of I shown in Figure 1 relate to the seven-membered ring conformations as follows: (a) chair-P(β); (b) chair-P(δ); (c) chair-P(γ); (d) boat-P(δ). Conformation (b), which involves the stable chair-P(δ) form of the seven-membered ring, is ruled out by interactions between a phosphate oxygen atom and the cytosine ring; the conformation actually assumed, (c), involves the chair-P(γ) form of the seven-membered ring. Since this is the form found both in the crystals and in solution, it presumably achieves the optimum compromise among the various conformations of the seven-membered ring, the five-membered ring, and the glycosidic bond.

We hope that these discussions will help to emphasize the very complex conformational features of seven-membered cyclic phosphate systems and stimulate the further work that is necessary before the preferred conformations of these systems can be predicted with confidence.

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Communications to the Editor

Aromatic Substitution in the Liquid Phase by Bona Fide Free Methyl Cations. Alkylation of Benzene and Toluene

Sir:

We report the first application, concerning a typical aromatic substitution such as the liquid-phase methylation of benzene and toluene, of a new technique designed to generate *free* carbocations of specified structure in any liquid environment and to investigate their reactivity, using as a source of the charged electrophiles appropriate, tritiated precursors, dissolved at a tracer concentration within the liquid substrate(s).

The decay of a radioactive atom in tritiated methane is known from theoretical[1,2] and mass spectrometric[3] studies to yield thermal methyl cations in over 80% of the nuclear transitions. Tracer amounts of methane-t_4, prepared, purified, and analyzed as described in earlier reports,[4] were dissolved in carefully outgassed toluene or benzene–toluene mixtures at a typical specific activity of 300 μCi mL^{-1}, corresponding to a CT_4 concentration of 2.5×10^{-6} mol L^{-1}, and the samples were stored at 22 °C in the dark for 9 months in Pyrex vessels entirely filled by the liquid and equipped with a capillary neck sealed by a mobile mercury plug. After removal of the undecayed CT_4 and careful washing of the liquid with excess CH_4, the samples were analyzed by radio GLC, using a Hewlett-Packard Model 7620 A instrument equipped with a 12-m Bentone 38 + DC 200 silicone oil glass column operated at 140 °C, monitoring the activity of the effluents with a 10-mL internal flow proportional counter heated at 150 °C. The tritiated products were identified by comparing their retention volumes with those of authentic specimens under identical chromatographic conditions, and their relative yields are given in Table I.

The *absolute* overall yield, defined as the ratio of the combined activity of the products identified to the total activity of the CT_3^+ ions formed within the liquid, calculated from the initial activity and the isotopic composition of the tritiated methane (CT_4, 60.4 ± 0.5%; CHT_3, 29.4%; CH_2T_2, 8.1%; CH_3T, 2.0%), the decay rate of T, the storage time, the abundance of the methyl cations formed from the decay, and the efficiency of the detector, show that the products listed in Table I account for 50 ± 10% of the decay ions' activity, without significant differences among the systems investigated. Furthermore, the total activity of the crude liquid, after removal of the undecayed CT_4, matches almost exactly the combined activity of the products identified by GLC, excluding the presence of other tritiated species in the liquid, and suggests that the activity balance must be provided by some gaseous products, e.g., HT or partially tritiated methanes, removed together with CT_4 when the sample is outgassed.

The following discussion will be restricted to the ring methylation of the arenes, the only channel directly and exclusively related to the reactivity of CT_3^+, neglecting other processes, e.g., tritiodeprotonation of the substrate where contribution of the various charged and neutral decay fragments, formed in ~18% of the β transitions, cannot be a priori ruled out.

The methyl cation that the nuclear transition brings into being in the site formerly occupied by the methane molecule in a time very short on the chemical reactivity scale[5] *is absolutely free* since it is not associated with any counterion, its positive charge being neutralized by an electron far removed in the liquid. Moreover, although one might argue that the only unsolvated carbocations are to be found in the dilute gas state, the decay CT_3^+ ion is undoubtedly as unsolvated as conceivable for a charged species in a condensed phase. In fact, owing to the very high collision frequency with the molecules of the liquid substrate(s) and to the proven ability of the methyl ion to react at a rate approaching collision frequency even with relatively weak nucleophiles,[6] its attack of arenes is likely to

Table I

Liquid composition, mole fraction		Relative yields of products,[a] %						
Benzene	Toluene	Unknown[b]	Benzene	Toluene	Ethylbenzene	*o*-Xylene	*m*-Xylene	*p*-Xylene
	1.00	2.6		13.9	4.2	32.1	21.4	25.8
	1.00	2.7		13.8	4.5	31.5	21.2	26.3
0.50	0.50	1.5	8.0	36.0	1.5	20.7	14.3	18.0
0.09	0.91	2.1	2.0	18.4	2.8	29.9	20.0	24.8
0.91	0.09	0.5	14.0	69.6	0.2	6.1	4.3	5.5

[a] Percentage of the activity contained in each product referred to the total activity of the products isolated. Each value represents the average of the least two independent measurements. The reproducibility of the data is illustrated by comparison of the first two lines. [b] The elution volume of this product is very close to, if lower than, that of ethylbenzene.