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PROTON MAGNETIC RESONANCE STUDIES OF 9-(β-D-XYLOFURANOSYL)ADENINE 3′,5′-CYCLIC MONOPHOSPHATE AND 9-(β-D-ARABINOFURANOSYL)ADENINE 2′,5′-CYCLIC MONOPHOSPHATE*†

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ABSTRACT

A detailed ^{1}H 220-MHz n.m.r. study of 9-(β-D-xylofuranosyl)adenine 3′,5′-cyclic monophosphate (3′,5′-xylo-cAMP, **1**) and 9-(β-D-arabinofuranosyl)adenine 2′,5′-cyclic monophosphate (2′,5′-ara-cAMP, **2**) in D_2O solution is described. The sugar-ring conformations in **1** and **2** are shown to be ^3E and ^2E, respectively, and the phosphate rings are in a chair form. An unusual $^4J_{P,H}$ coupling of 2.4 Hz is observed between H-4′ and phosphorus in **1** and a vicinal $J_{P,H}$ of 30.8 Hz between H-5′ and phosphorus in **2**. This latter coupling verifies a similar value found previously in the ara-cytidine analog of **2**. A comparison of the conformational properties of cyclic nucleotides having fused phosphate and sugar rings has been made, together with an assessment of the use of the Karplus constants in such ring-systems.

INTRODUCTION

Adenosine 3′,5′-cyclic monophosphate (cAMP) has long been known to be of great biological importance, particularly with respect to its function as a secondary messenger[1]. More recently, cGMP and cCMP have been shown to possess interesting biological properties[2–4] and the 3′,5′-cyclic monophosphates of arabinonucleotides have been synthesized and shown to possess antitumor and antiviral activity[5]. All of these 3′,5′-cyclic monophosphates contain a 6-membered phosphate ring fused *trans* (1,2) to a 5-membered sugar ring, producing a rigid system that has been extensively studied by X-ray[6] and n.m.r. methods[7–9].

The nucleoside 2′,3′-cyclic monophosphates contain a 5-membered phosphate

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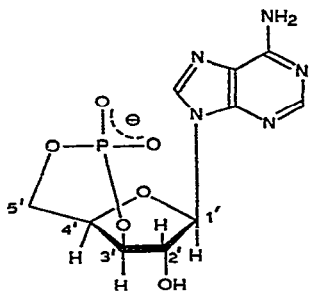
ring fused *cis* (1,2) to a 5-membered sugar ring that is not rigid but exists as a mixture of rapidly interconverting, puckered conformations[10]. Very recently, α-nucleoside 3′,5′-cyclic monophosphates have been studied by n.m.r. spectroscopy[11,12], and it has been shown that, despite the aforementioned rigidity of the sugar–phosphate ring-systems, a distortion from the favored sugar conformation of the β anomers (^3E) into a $^3T_2$ conformation occurs, because of a repulsive interaction between the 2′-hydroxyl group and the base. Lee and Sarma have investigated 2,2′-anhydro-nucleoside 5′-monophosphates as other examples of rigid ring-systems[9]. Prior to this work, the conformation of 2′,5′-ara-cCMP (3) in solution had been studied in some detail[8,13]. Conflicting conformations of the phosphate ring in 3 had been proposed and an extremely large, vicinal $^3J_{P,H}$ coupling-constant[8] of 31.0 Hz had been noted. An X-ray diffraction study and re-evaluation of the solution properties have now helped to resolve this conflict[14].



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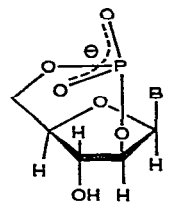

2 B = adenine
3 B = cytosine

In order to assess the effect of phosphate ring-size on the conformation of cyclic nucleotides, further investigations of cyclic adenine nucleotides having different pentose moieties were undertaken. This report describes the conformational properties of 3′,5′-xylo-cAMP[15] [a molecule that possesses a 6-membered phosphate ring fused *cis* (1,2) to a 5-membered sugar ring] and of 2′,5′-ara-cAMP[15] [a molecule that possesses a 7-membered phosphate ring fused *cis* (1,3) to a 5-membered sugar ring].

RESULTS AND DISCUSSION

Signal assignments and n.m.r. parameters. — The 220-MHz spectra, together with the assignments, of 0.02M solutions of 3′,5′-xylo-cAMP and 2′,5′-ara-cAMP at 20° and pD 7.4 are shown in Figs. 1 and 2. As expected, the base and anomeric-proton resonances occur downfield from the HDO signal (4.88 p.p.m., not shown in Figs. 1 and 2), whereas the remaining pentose-ring protons appear upfield from HDO. One exception is H-3′ of 2′,5′-ara-cAMP, which is shifted significantly downfield, to 5.375 p.p.m. The origin of this downfield shift will be discussed in a later section. The pentose-ring region of the 3′,5′-xylo-cAMP spectrum is shown in Fig. 3 (lower) on an expanded scale, together with the phosphorus-decoupled spectrum

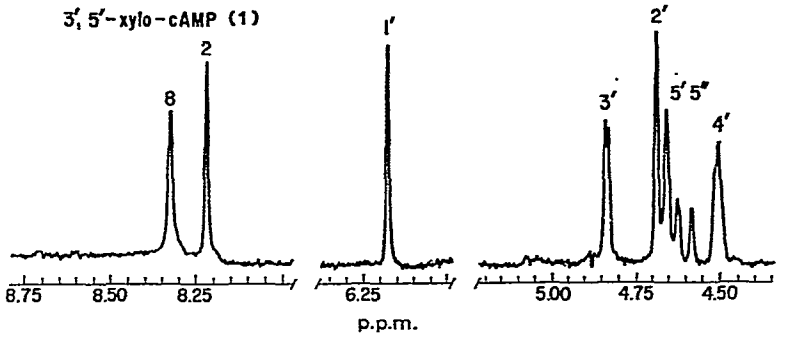

Fig. 1. 220-MHz Proton magnetic resonance spectrum of 3′,5′-xylo-cAMP (1), 0.02M, pD 7.4, 20°.

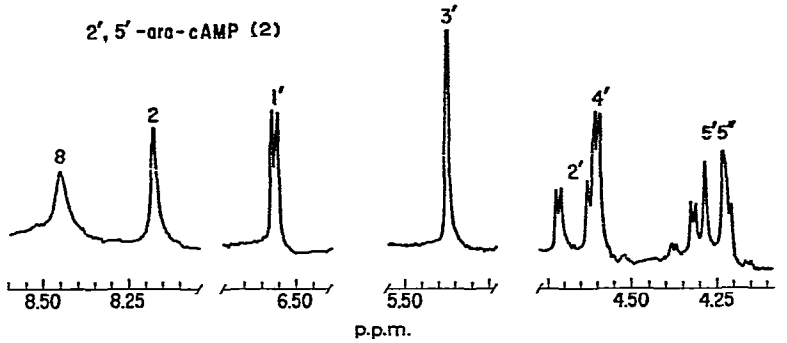

Fig. 2. 220-MHz Proton magnetic resonance spectrum of 2′,5′-ara-cAMP (2), 0.02M, pD 7.4, 20°.

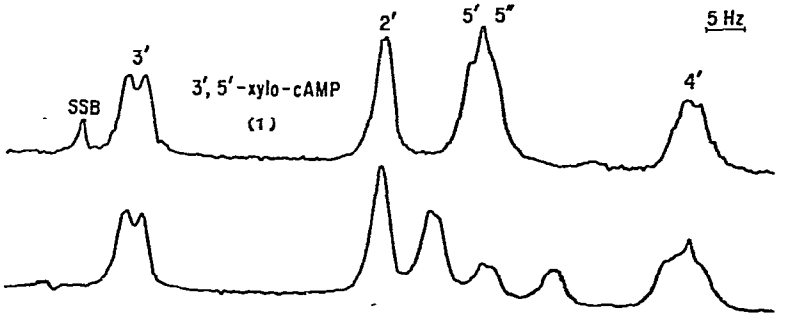

Fig. 3. The 4.90–4.40-p.p.m. spectral region of 1 at 220 MHz (20°); undecoupled spectrum (lower), phosphorus-decoupled spectrum (upper).

(upper). Because of the near magnetic equivalence of H-5′ and H-5″ at 20°, the spectrum of 3′,5′-xylo-cAMP was also measured at a higher temperature (45°). As is demonstrated in Fig. 4 (upper), the increase in temperature causes an increase in the separation of the H-5′ and H-5″ signals, thus permitting accurate determination of the coupling constants in the cyclic phosphate ring ($J_{4',5'}$, $J_{4',5''}$, $J_{5',P}$, $J_{5'',P}$, and $J_{4',P}$). The portion of the 2′,5′-ara-cAMP spectrum in the 4.24–4.66-p.p.m. range is shown in Fig. 5 (middle) on an expanded scale, together with the phosphorus-

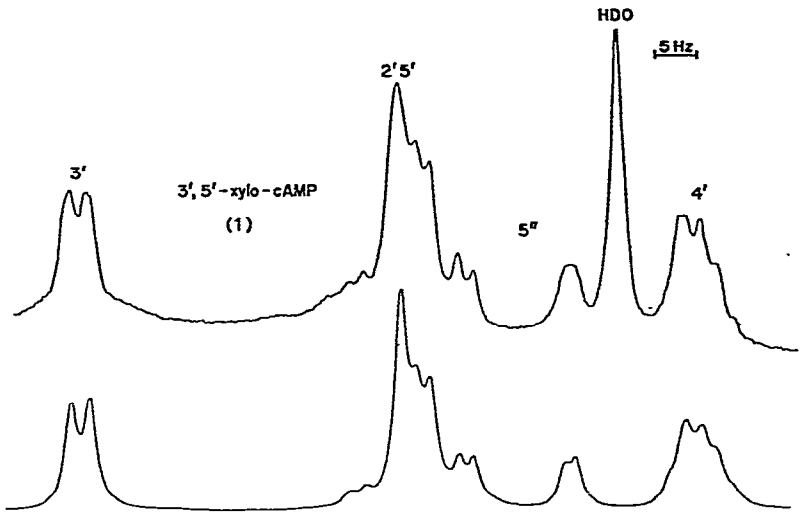

Fig. 4. The 4.90–4.40-p.p.m. spectral region of **1** at 220 MHz (45°); observed spectrum (upper), simulated spectrum (lower).

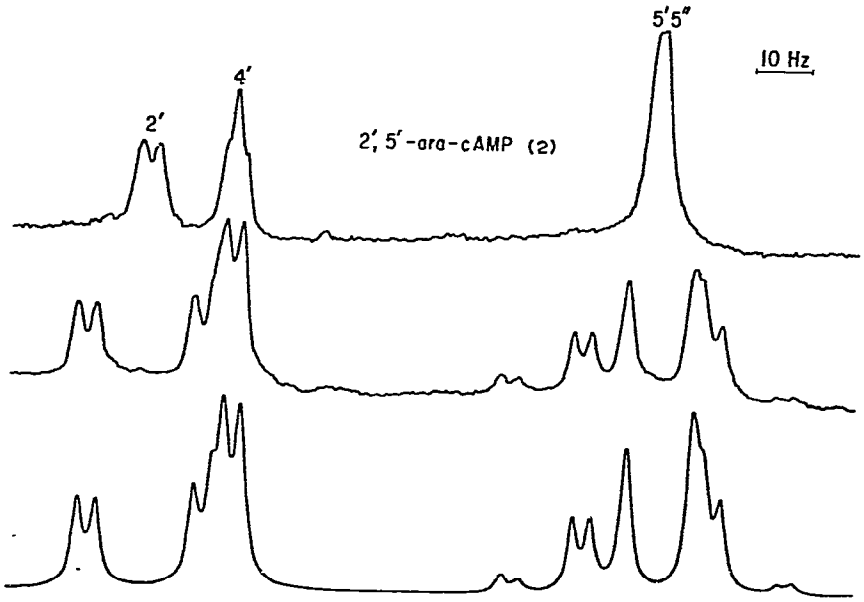

Fig. 5. The 4.80–4.10 p.p.m. spectral region of **2** at 220 MHz (20°); undecoupled spectrum (middle), simulated spectrum (lower), phosphorus-decoupled spectrum (upper).

decoupled spectrum (upper). Although H-5', H-5" have nearly identical chemical shifts in 2',5'-ara-cAMP, as in 3',5'-xylo-cAMP, it was possible to determine the coupling constants for the cyclic phosphate ring accurately without recourse to spectral measurement at higher temperature. Partial computer-simulated spectra

TABLE I

^{1}H-N.M.R. PARAMETERS AND CALCULATED DIHEDRAL ANGLES OF 3',5'-XYLO-cAMP (1) AND 2',5'-ARA-cAMP (2)

Proton	δ^a		*Coupling*	J^b (ϕ^c)	
	1	2		1	2
8	8.325	8.460	1'2'	0.0 (90°)	3.2 (52°)
2	8.221	8.184	2'3'	0.4 (99°)	0.0 (90°)
1'	6.170	6.567	3'4'	2.2 (58°)	0.0 (90°)
2'	4.687	4.663	4'5'	2.0 (60°)	3.5 (50°)
3'	4.832	5.375	4'5"	1.5 (64°)	0.0 (90°)
4'	4.564	4.594	5'5"	−13.5 (gem)	−12.8 (gem)
5'	4.645	4.259	1'P	—	—
5"	4.615	4.246	2'P	—	20.4 (143°)
			3'P	<0.3 (90°)	—
			4'P	2.4[d]	—
			5'P	1.9 (60°)	30.8 (180°)
			5"P	21.6 (165°)	4.3 (60°)

[a]Measured in p.p.m. (±0.005) from TSP; [b]±0.2 Hz; [c]See text for Karplus relations used; [d]±0.4 Hz.

for 3',5'-xylo-cAMP and 2',5'-ara-cAMP are shown in Fig. 4 (lower) and Fig. 5 (lower), respectively. Although not shown, the 3',5'-xylo-cAMP spectrum was also simulated at 20°. The final chemical-shift and spin–spin coupling-constant data at 20° are summarized in Table I.

A number of points stand out from the n.m.r. data. The anomeric proton (H-1') of **1** is shielded by ~0.4 p.p.m. relative to that of **2**, presumably because of enhanced shielding by the *cis*-oriented 2'-hydroxyl group in the former. The effect of the -OH orientation on the chemical shift of a neighboring (vicinal) proton has been discussed in detail by others[16]. As expected, the chemical shift of H-1' in **1** is very similar to that found in the normal β-adenosine 3',5'-cyclic phosphate[7–9] and 0.4 p.p.m. upfield compared to that of α-adenosine 3',5'-cyclic phosphate[12].

Examination of the H-2' chemical shift in **2** shows that this proton resonates upfield by approximately 0.4 p.p.m. relative to the same resonance in 2'-AMP, again as a consequence of shielding by the *cis*-oriented 3'-hydroxyl group. This magnitude (~0.4 p.p.m.) of the downfield shift of a proton upon reorientation of a vicinal OH group from *cis* to *trans* appears to be a general phenomenon for sugar-proton resonances of nucleosides[16–17]. Hence, it follows that, at least in the present cases, any contribution to the difference in the chemical shifts of H-1' from changes in the glycosyl torsion-angle in **1** and **2** is minimal; that is, the orientation of the adenine base relative to the pentose rings is similar in the two cyclic nucleotides.

Observation of the H-3' resonances shows a marked deshielding (0.543 p.p.m.) in **2** relative to **1**. This large downfield shift has been noted previously for 2',5'-ara-cCMP[8,13] and has been interpreted as being due to the close proximity of the phos-

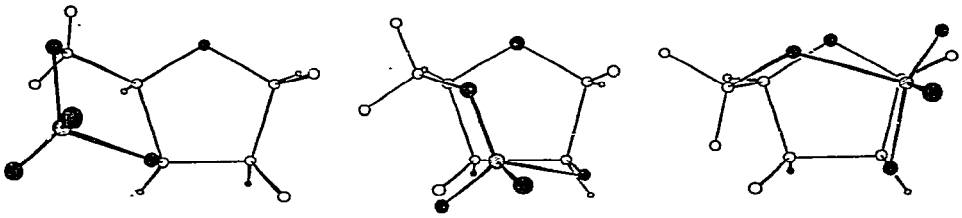

Fig. 6. (*a*) Conformation of **1** in solution; (*b*) and (*c*) possible conformations of **2** in solution[8].

phate group. Such a deshielding can be explained from the p.m.r. data by only one conformation (see later)[13].

A significant coupling between H-4′ and the phosphorus atom occurs in **1** and was verified by phosphorus decoupling (see Fig. 2). This long-range coupling ($^4J_{P,H}$ = 2.4 Hz) is particularly unusual, as neither H-4′–C-4′–C-5′–O-5′–P nor H-4′–C-4′–C-3′–O-3′–P lie in a planar, zig-zag conformation ("W-rule"). However, a few exceptions to the "W-rule" for proton–proton couplings have been noted in the literature[18]. It should be noted that, in the favored conformation of **1** [see Fig. 6(a)], the phosphorus atom is "above" the sugar ring and the C-4′–H-4′ bond lies in a direct line across the 6-membered phosphate ring with the "back-side" of the phosphorus atom. This alignment does not occur in the ribonucleoside 3′,5′-cyclic monophosphates.

Interesting trends are also found for the H-5′ and H-5″ resonances*. As a result of the rigid nature of the cyclic phosphate rings, the two protons on C-5′ (H-5′a and H-5′b) are stereochemically non-equivalent (this is also the case for the α- and β-ribonucleoside 3′,5′-cyclic phosphates[9,12]). In **1**, H-5′a is situated equatorially in the six-membered phosphate ring and is *trans* to the phosphorus atom (see later). This proton is the upfield (H-5″) of the two methylene proton resonances and is readily assigned from its large $J_{P,H}$ coupling constant (21.6 Hz). The H-5′b proton is situated axially and gives rise to the downfield (H-5′) resonance.

In the case of **2**, the most interesting observation is the extremely large $^3J_{P,H}$ coupling constant (30.8 Hz) for one of the 5′-methylene protons. This phenomenon has been noted before[8] for 2′,5′-ara-cCMP, but this is the only other precedent (previous to the 2′,5′-ara-cCMP study, the largest vicinal $^3J_{P,H}$ value observed for phosphate derivatives having saturated substituents had[19] been ~24 Hz). There are two conformations of the cyclic phosphate ring that can reasonably account for all the vicinal P–O–C–H coupling constants (see later); these are shown in Figs. 6(b) and 6(c). In either conformation, H-5′a resonates downfield (H-5′) and is oriented *trans* to the phosphorus atom. Although the orientations of H-5′a and H-5′b relative

*Throughout the discussion, we have assigned the methylene protons at C-5′ as H-5′a and H-5′b (*gauche* and *trans*, respectively, to the sugar oxygen atom) and the resonances as H-5′ (downfield) and H-5″ (upfield).

to the sugar oxygen atom are similar in **1** and **2** (namely, in both cases, H-5'a is always *gauche* to the sugar-ring oxygen atom and H-5'b is always *trans*), in **1**, H-5'a resonates upfield of H-5'b, whereas in, **2** H-5'a resonates downfield of H-5'b. These chemical-shift changes are only slight, but the rigidity of the ring systems makes the assignments reliable. The crossover may be due to slight changes in the orientation of the phosphate between **1** and **2** and/or the difference in strain in two different ring-systems (see later).

Conformation of the sugar rings. — By using the Karplus relation for J_{HH} proposed by Altona and Sundaralingam[20] as modified by Lee and Sarma[9], the dihedral angles were calculated from the observed coupling-constants and are shown in Table I. It may be seen that the conformation of the xylose ring in **1** is best described as ^3E (similar to that found in the ribonucleoside 3',5'-cyclic phosphates[7-9]) and that for **2** as ^2E. The puckering amplitude in both **1** and **2** is approximately the same, and is comparable to that found in the acyclic mononucleotides[21] and in the β-nucleoside 3',5'-cyclic monophosphates[7-9]. This suggests that the sugar rings in all of these cases are relatively free of strain, which is in contrast to the increased puckering amplitude found in the α-nucleoside 3',5'-cyclic monophosphates[12]. Thus, these two, conformationally restricted, cyclic mononucleotides (**1** and **2**) have sugar conformations approximating the "pure" ^3E (for **1**) and ^2E (for **2**) and allow the direct measurement of $J_{1',2'}$ and $J_{3',4'}$ for these two "pure" conformers. These values have been obtained previously by semi-empirical calculations[20,21] and experimentally by the use of the rigid anhydronucleosides 2,2'-anhydro-1-β-D-arabinofuranosyluracil and 2,2'-anhydro-1-β-D-ribofuranosyluracil[9]. The experimental data contained herein (see Table I) are seen to be in excellent agreement with predicted values.

Conformations of the phosphate rings. — By using the Karplus relation with the constants reported by Lee and Sarma[9] for $J_{P,H}$, the dihedral angles in **1** were calculated from the coupling constants and are shown in Table I. The phosphate ring is in a chair form and would be expected to be relatively unstrained, because of the *cis* fusion of the phosphate and sugar rings [Fig. 6(a)]. Inspection of Dreiding models indicates the possibility of a second chair-form for the phosphate ring, having the sugar ring in the $_3E$ conformation. The latter may be ruled out immediately from the observed small coupling between H-3' and phosphorus.

Because of the unusually large $J_{P,H\text{-}5'}$ coupling in **2** (30.8 Hz), it is not possible to use the suggested Karplus relation[9] for $J_{P,H}$ to determine the conformation of the cyclic phosphate ring. Use of the relation led to an incorrect choice[8] for the conformation of the cyclic-phosphate ring in the structurally related 2',5'-ara-cCMP (**3**). The crystal structure of **3** shows[14] large distortions from normal for angle C-5'–O–P (124.0°) and for angle P–O–C-2' (127.2°), and these distortions are almost certainly the origin of the large $J_{P,H\text{-}5'}$ value. Kainosho and coworkers have recently derived an empirical Karplus curve, based on a correlation between observed coupling-constants and the dihedral angles obtained from the X-ray diffraction data in this ring system[14]. Examination of models shows that there are four possible conformations of the seven-membered phosphate ring[8]. If one assumes that the observed

30.8-Hz coupling (see Table I) is that for an antiperiplanar orientation of proton and phosphorus (~180° dihedral angle), the criteria for the choice of a conformation in solution are (*i*) 180° dihedral angle between H-5'a (or H-5'b) and phosphorus, and (*ii*) 130–150° angle between H-2' and phosphorus that gives rise to the 20.4-Hz coupling. In addition, the two H-5'a, H-5'b protons must be oriented approximately *gauche–gauche* to H-4'. On the basis of these specifications, two of the four possible conformers (Figs. VIIA and VIId in ref. 8) may be eliminated. The two remaining phosphate-ring conformations that accommodate the n.m.r. coupling-constant data are both in a chair form and are shown in Figs. 6(b) and 6(c).

The conformation depicted in Fig. 6(c) can be eliminated for two reasons: (*i*) the downfield shift of H-3' can only be reasonably explained by a deshielding due to the phosphate group, and this requires close proximity of the phosphate group and H-3', as is the case for the conformation in Fig. 6(b); (*ii*) steric interactions between the base and the phosphate would be expected to destabilize the conformation in Fig. 6(c). The latter conformation is identical to that suggested by Wechter[13], and by Kainosho and coworkers for 3, in their corrected assignments[14].

By using dihedral angles of 180 and 60° (estimated from Dreiding models) for H-5'a–C-5'–O-5'–P and H-5'b–C-5'–O-5'–P, respectively, the constants in the Karplus relation ($J = a\cos^2\theta - b\cos\theta$) for[9,22] vicinal H–P coupling in the seven-membered ring are computed to be $a = 26.3$ and $b = 4.5$. These values were subsequently used in calculating a dihedral angle of 143° for the observed coupling (20.4 Hz) between H-2' and P. This is in good agreement with the angle (137°) observed in the crystalline state[14].

In summary, the sugar conformations of cyclic nucleotides having 5-, 6-, and 7-membered phosphate rings are distinctly affected by the phosphate-ring size and the nature of the ring fusion. The *cis*-(1,2)-fused, 5-membered ribonucleoside 2'-3'-cyclic monophosphates are not rigid and the sugar exists in a ${}^2E \rightleftarrows {}^3E$ equilibrium[10]. The 6-membered β-ribonucleoside 3',5'-cyclic monophosphates are *trans*-(1,2)-fused, producing a rigid, somewhat strained, bicyclic system having a ${}^3E$ sugar conformation[7–9]. In contrast, the repulsive interaction between the 2'-OH group and the base that occurs in the α-ribonucleoside 3',5'-cyclic monophosphates produces[12] a sugar conformation in the range ${}_2T^3$ to ${}^3T_2$.

In comparison, the 6-membered 3',5'-xylo-cAMP having a *cis*-(1,2) ring-fusion is relatively strain-free and the sugar conformation is ${}^3E$. In the case of the 7-membered 2',5'-ara-cAMP, with a *cis*-(1,3) ring-fusion, the sugar conformation is ${}^2E$ and large distortions from normal bond-angles have been observed in the phosphate ring[14]. These distortions cause large deviations from the expected Karplus relations. Thus, it must be reiterated that conformational analyses on cyclic mononucleotide derivatives having distorted bond-angles and various degrees of strain in the fused ring-systems should be made with extreme care.

EXPERIMENTAL SECTION

Materials and methods. — The 3',5'-xylo-cAMP (**1**) and 2',5'-ara-cAMP (**2**)

were prepared as described previously[15] from 9-(β-D-xylofuranosyl)adenine[23] and 9-(β-D-arabinofuranosyl)adenine (purchased from Pfanstiehl) respectively. Samples for n.m.r. studies were re-purified by DEAE-cellulose column chromatography prior to use. The NH_4^+ salts of the cyclic nucleotides were lyophilized once from "100%" D_2O and then dissolved in "100%" D_2O, and the solutions adjusted to 0.02M concentration and pD 7.4 (pD = pH-meter reading +0.4). A trace of sodium 4,4-dimethyl-4-silapentanoate-*2,3,3,3-d*$_4$ (TSP) was added to the sample and served as an internal reference.

Measurement of spectra. — ^1H N.m.r. spectra were recorded in the Fourier-transform mode on a Varian HR-220 spectrometer coupled to a Nicolet FT accessory and data system. Spectra were measured at 20 ±2°, or at a higher temperature (45°) by using the Varian variable-temperature accessory. ^{31}P Decoupling experiments were performed with a Schomandl ND 100 M generator set at a decoupling frequency near 89 MHz. Initial sets of chemical shifts and coupling constants were obtained directly from the observed spectra and were subsequently refined by computer simulations by using the Nicolet 6-spin ITRCAL programs. Chemical shifts are reported relative to internal TSP with an accuracy of ±0.001 p.p.m.

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