the numbering of the out-of-plane atoms. The superscript denotes that the deviation is on the same side (endo) of the plane as C(5′), while the subscript denotes the deviation is on the opposite side (exo) of C(5′). Priority is given to the atom that exhibits the primary[12] pucker and it appears before the letter, while the atom showing the secondary[11] pucker follows the letter.[14] While the results are based on X-ray data, the conformational designations would apply in general, *e.g.*, to results based on other data, such as nmr.

The twist (T) "half-chair" (Table II and Figure 3) is certainly the predominantly occurring conformation in α nucleosides. A similar conformational preference was found for the β nucleosides.[4] However, there is an important difference between the preferred twist conformations found in the α and β nucleosides. In the β nucleosides the C(3′)-endo–C(2′)-exo ($^3T_2$) [or C(3′)-endo] and C(2′)-endo–C(3′)-exo ($^2T_3$) [or C(2′)-endo] conformations were found to be preferred, while the opposite (or enantiomeric) conformations C(2′)-exo–C(3′)-endo ($_2T^3$) [or C(2′)-exo] and C(3′)-exo–C(2′)-endo ($_3T^2$) [or C(3′)-exo] and a new conformation C(4′)-endo–C(3′)-exo ($^4T_3$) [or C(4′)-endo] occur in the α nucleosides. A noteworthy feature is that the $_2T^3$ and $_2E$ conformations are exhibited by the ribosides only, while the $_3T^2$ and $^4T_3$ conformations are exhibited by the deoxyribosides. The only compound that exhibits a conformation ($^3T_2$) that is commonly found in the β nucleosides is vitamin B_{12} coenzyme, Table II. It should be pointed out here that, with the exception of the 3′,5′ cyclic nucleotides,[15] the C(2′)-exo and C(4′)-endo conformations have not so far been observed for the β nucleosides. The characteristic conformational properties of the α nucleosides on one hand and the β nucleosides on the other are mainly attributable to the differences in the steric interaction between the base and the sugar.

For the purposes of relating proton magnetic resonance coupling constants to the conformations of nucleosides in solution it is important to consider the torsion angles involving the furanoside ring hydrogen atoms. These angles are given in Table III for the structures where the hydrogen atoms have been determined by X-ray diffraction. In Table IV the torsion angles involving the furanoside ring bonds are given because they give the most precise description of the conformation of a ring.[11,12]

Acknowledgment. I am grateful to the National Institutes of Health of the United States Public Health Service for research support through Grant GM 17378.

(14) The nomenclature used here is a modification of the original proposal (L. C. Cross, private communication) by the British Carbohydrate Nomenclature Committee. If the deviations of the atoms from the three-atom plane are identical within the errors of the experiment then the furanoside ring possesses a symmetrical half-chair conformation. The displaced atoms are then shown on the same side of the letter T (see Table II).

(15) Cyclic 3′,5′-adenosine monophosphate (K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, **159**, 206 (1968)); cyclic 3′,5′-uridine monophosphate (C. Coulter, *Acta Crystallogr., Sect. R*, **25**, 2055 (1969)); 5′-methylene analog of cyclic 3′,5′-adensosine monophosphate (M. Sundaralingam and J. Abola, *Nature (London)*, in press).

Proton Magnetic Resonance Study on Adenine Dideoxynucleoside Monophosphate with Emphasis on the Furanose Conformation[1a-c]

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Abstract: Spectral assignment of the base protons, H-1′,H-2′, and H-2″, of both the dAp and pdA portions of dApdA has been made. The results indicate that dApdA has an anti,anti right-handed conformation with extensive base–base interaction. Four coupling constants, $J_{1'-2''}$ (cis), $J_{1'-2'}$ (trans), $J_{2'-3'}$ (cis), and $J_{2'-3'}$ (trans), of the furanose of both the dAp and pdA portions of dApdA, 3′-dAMP, and 5′-dAMP were determined by first-order analysis of 220-MHz spectra and 100-MHz spectra. The data show that the coupling constants between the dAp and pdA portions of dApdA are similar to those of 3′-dAMP and 5′-dAMP, while the coupling constants of 3′-dAMP and 5′-dAMP are not the same. Through the application of the Karplus equation, four dihedral angles, $\phi_{1'-2''}$ (cis), $\phi_{1'-2'}$ (trans), $\phi_{2'-3'}$ (cis), and $\phi_{2'-3''}$ (trans), were determined. These analyses suggest that the furanose conformation for 3′-dAMP and dAp in dApdA is that of C(2′)-endo (envelope) or C(2′)-endo–C(3′)-exo (twisted form), while the furanose conformation for 5′-dAMP and pdA in dApdA is that of a rapid equilibrium between C(2′)-endo and C(3′)-endo. From the temperature and solvent studies of the $J_{1'-2'}$ values of 5′-dAMP and dApdA, it is suggested that the formation of intermolecular stacks of 5′-dAMP or the formation of intranucleotidyl stacking of dApdA does not cause a change in the furanose conformation.

Proton magnetic resonance has been used successfully to study the conformation and the interaction of nucleosides, nucleotides, and dinucleotides in aqueous solution.[3-6] However, most of these stud-

(1) (a) Part IV of a series entitled "Studies of the Conformation and Interaction of Dinucleoside Mono- and Diphosphates." (b) This work was supported in part by a grant from the National Science Foundation (GB-8500) and a grant from the National Institutes of Health (GM 16066-03). (c) Presented in part at 160th National Meeting of the American Chemical Society, Chicago, Ill., 1970.

(2) American Cancer Society, Postdoctoral Fellow.

(3) P. O. P. Ts'o, N. S. Kondo, R. K. Robins, and A. D. Broom, *J. Amer. Chem. Soc.*, **91**, 5625 (1969); P. O. P. Ts'o, M. P. Schweizer,

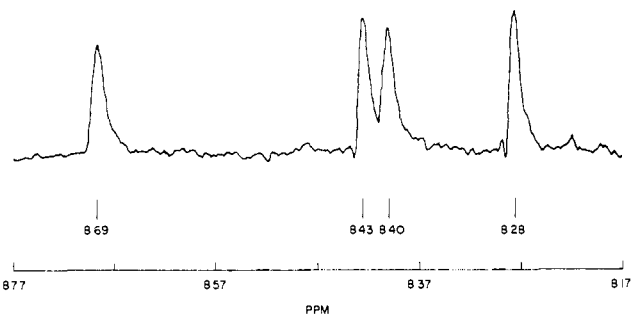

Figure 1. The pmr spectrum of the base protons of dApdA, measured at 100 MHz and 28°, in D_2O, 0.05 *M*, and pD 6.3.

ies have dealt with ribonucleoside derivatives and few studies have been made with deoxyribonucleoside derivatives.[7,8] Also, most of the information obtained from these studies concerns only the base protons and H-1′ proton. Recently, several complete analyses of the furanose ring proton spectra have appeared;[6,9,10] again, the main emphasis is on the studies of ribonucleosides and ribonucleotides. Similarly, at the dinucleotide level, investigations of the furanose conformation of the nucleotidyl units in the ribosyl dimers have extended only to the measurement of the coupling constants, $J_{1'-2'}$, and subsequent estimation of the dihedral angle $\phi_{1'-2'}$. Such information is insufficient to describe the furanose ring conformation in the dimer accurately. Furthermore, the simple question of assignment of the chemical shifts of the two H-1′ protons to the proper nucleotidyl units (Np or pN) in a homodimer is not an easy one to be answered with certainty.[4] In addition, measurements and assignments of the H-2′ to the H-5′,5″ proton resonances are often difficult since these spectral lines usually overlap in a relatively narrow region (4–5 ppm from TMS capillary) and are poorly resolved. Moreover, these proton signals are frequently obscured by the residual HDO peak when D_2O is used as solvent. Nevertheless, proper assignment of the chemical shifts of the two H-1′ protons in the dimer is of primary importance for the determination of the handedness of the screw axis of the dimer. As shown in later paragraphs, the pmr data concerning the H-2′,H-2″ protons provide valuable new knowledge about the backbone of the dinucleotides.

In this paper, a pmr study of dApdA is presented. A simple but effective procedure has been established for the assignment of the H-1′ in dinucleoside monophosphates, particularly in homodimers. Besides the base protons, we also present complete analyses of the H-2′ and H-2″ proton spectra of 3′-dAMP, 5′-dAMP, dAprA, and dApdA. The data indicate that dApdA also has an anti,anti, right-handed conformation with extensive base–base interaction. The endo H-2′ is located in the interior of the stack and is the most shielded proton in the dimer. Contrary to the ribosyl dimer, rAprA, the stacking of dApdA has little effect on the conformation of the furanose of the 3′-nucleotidyl unit (Ap) in the dimer.

Results and Discussion

Spectral Assignment of the Base Protons in dApdA and the General Conformation of the Dinucleoside Monophosphate. The pmr spectrum of the base protons of dApdA shows four distinct peaks at 8.28, 8.40, 8.43, and 8.69 ppm in D_2O at 28°, 0.05 *M* (Figure 1). Upon heating this sample in D_2O for 4 hr at about 80°, the intensities of the peaks at 8.40 and 8.69 ppm had diminished considerably due to deuterium exchange. From this exchange experiment, these two resonances can be assigned to the H-8 protons.[4] These two H-8 protons were further distinguished from each other by synthesizing dApdA with a deuterium substituted in the H-8 position of the pdA portion of the dimer. The 8.69-ppm resonance was absent in the spectrum of this selectively deuterated compound, indicating that this peak belongs to the H-8 of the pdA portion. The remaining 8.40-ppm resonance is then assigned to the H-8 of the dAp residue in the dimer. In the following section, it is shown that the H-8 resonance of the pdA portion is broadened in the presence of Mn^{2+}. This observation indicates that the nucleotidyl unit of -pdA is in the anti conformation.[4,6a] Furthermore, as shown in the following paper,[11] in the neutral, phosphoalkyl triester of dApdA (dAp(R)dA), the H-8 of pdA portion is the only proton which is shifted upfield as compared to the same proton in dApdA. This is due to the removal of the deshielding effect exerted by the negatively charged phosphate group on the H-8 when the nucleotide is in the anti conformation.[4,12] These two observations, *i.e.*, the broadening effect of Mn^{2+} on H-8 and the upfield shift of H-8 due to alkylation of the phosphate group, establish that the nucleotidyl unit of pdA in dApdA is in the anti conformation.

The proper assignment of the H-2 resonances depends on the consideration of the conformation of the dimer including the preceding conclusion that the pdA residue in dApdA is in the anti conformation. If both nucleotidyl units (5′ and 3′) are in the anti conformation, as suggested by the studies on the ribosyl dimers[4] and mononucleotides,[12] then the H-2 of the dAp portion should always be much more shielded than the H-2 of the pdA portion, regardless of whether the chirality of the dimer is right-handed or left-handed. This follows since the H-2 of the dAp- is always located inside the stack where it is shielded by the five-membered adenine ring of the pdA portion irrespective of the handedness of the screw axis of the dimer. On the other hand, if the 3′-nucleotidyl residue (dAp) is in the syn conformation, while the 5′-nucleotidyl residue (pdA) is in the anti conformation, then both the

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Table I. Chemical Shifts[a] and Dimerization Shifts ($\Delta\delta_D$)[b] of the Base Protons, H-1′ Protons, and H-2′,2″ Protons of Dideoxyadenosine Monophosphates at 28°, pD 6–7 (TMS Capillary)

	pdA portion					dAp portion				
	H-8	H-2	H-1′	H-2′ (endo)[c]	H-2″ (exo)[c]	H-8	H-2	H-1′	H-2′ (endo)[c]	H-2″ (exo)[c]
dApdA	8.78	8.55	6.76	3.21	2.92	8.49	8.41	6.57	2.55	2.84
5′-dAMP[d]	8.985	8.70	6.96	3.14	2.95					
3′-dAMP						8.785	8.68	6.93	3.16	3.05
$\Delta\delta_D$	0.205	0.15	0.20	−0.06	−0.03	0.295	0.27	0.36	0.61	0.21

[a] δ values of base protons and H-1′ protons were obtained by extrapolation to infinite dilution; in ppm relative to TMS capillary. [b] $\Delta\delta_D = \delta_{pN'} - \delta_{NpN'}$ for 5′ residue and $\Delta\delta = \delta_{Np} - \delta_{NpN'}$ for 3′ residue. [c] H-2′ (endo) proton is defined as trans to the H-1′ proton and H-2″ (exo) proton is defined as cis to the H-1′ proton. These δ values are at 0.05 *M* concentration since it is not possible to study these protons at a lower concentration. However, it has been shown that the δ values of the H-2′,2″ protons are not much concentration dependent in deoxyadenosine.[36] It is also expected that the δ values of H-2′,2″ in dApdA are only mildly concentration dependent. [d] These values are slightly different from those published from our laboratory[12] owing to a better extrapolation at lower concentration.

H-2 from the dAp residue and the H-2 from the pdA residue will be located outside of the stack, again regardless of the handedness of the dimer. In such a situation, both H-2 protons would be shielded by the neighboring unit rather slightly and to an approximately equal extent. As shown in Figure 1 and discussed in the subsequent paragraph (Table I), the two H-2 protons have different chemical shifts and one H-2 is much more shielded by the neighboring unit than the other. In following the above reasoning, it is concluded that both nucleotidyl units (5′ and 3′) in the dimer must be in the anti conformation and the H-2 resonance located at a higher field (8.28 ppm) belongs to the H-2 of dAp residue and the H-2 resonance located at lower field (8.43 ppm) belongs to the H-2 of the pdA residue in the dimer.

The chemical shifts of the four base protons of dApdA, the chemical shifts of the two base protons of 5′-dAMP and 3′-dAMP, at infinite dilution, 28°, pD 5.9, are all listed in Table I. From these δ values, the dimerization shifts, $\Delta\delta_D$, can be computed and are also shown in Table I. These rather large magnitudes of the dimerization shifts indicate an extensive interaction between the bases in the dApdA. A comprehensive study on the dApdA conformation in comparison with the rAprA, rApdA, and dAprA, investigated concomitantly by pmr, CD, and uv hypochromicity will be the subject of a later paper in this series.[13]

As implied in the foregoing discussion, data on the base proton resonances do not provide pertinent information about the handedness of the screw axis of the stack. The relevant pmr information is to be derived from the data on the two H-1′ protons and H-2′, 2″ protons to be discussed thoroughly in the following sections. Suffice it to state here that the data clearly indicate that the screw axis of the dApdA is right-handed, similar to that for the rAprA and to the helical axis for DNA. This conclusion is in accord with the ORD[14] and CD[13,15,16] spectra of dApdA which are very similar to those of the right-handed rAprA. In summary, the general conformation of dApdA in D_2O solution is shown by the pmr study to be an anti,anti, right-handed stack with extensive base–base overlapping interaction.

Spectra and Assignments of the H-1′ Protons in Adenine Dinucleoside Monophosphates. The assignment of the two H-1′ in the adenine dinucleoside monophosphates was carried out by an approach similar to that developed by Chan and Nelson[6a] for the assignment of the two H-8 protons in rAprA. This approach is based on the observation that certain divalent metal ions, such as Mn^{2+}, are bound mainly to the phosphate group of the nucleotides at low concentrations of the ions and nucleotides.[17–20] If the divalent ion is paramagnetic ion, such as Mn^{2+}, it will tend to broaden the resonances of the nearby protons through the dipole–dipole interaction. This interaction is extremely distance dependent (proportional to $(\mu^2/r^6)\tau_c$, where r is the distance between the paramagnetic ion and the proton, μ is the magnetic moment, and τ_c is the correlation time). Since the distance of the H-8 of prA residue to the phosphate group where the Mn^{2+} ion would be located is closer than the H-8 of rAp residue when the nucleotidyl units are in the anti conformation, the H-8 of prA residue will be broadened preferentially over the H-8 of rAp residue as observed in the presence of dilute Mn^{2+} solution.[6a] The assignment of the H-1′ protons is based on the same procedure, except now the effect is reversed. The H-1′ of 3′-dAMP or (3′-rAMP) is now closer to the PO_4–Mn^{2+} group than the H-1′ of 5′-dAMP (or 5′-rAMP). Therefore, as shown in Figure 2A and 2B, in the presence of increasing concentrations of Mn^{2+}, the line width of H-1′ of 3′-dAMP and 3′-rAMP is being broadened to a greater extent than the line width of H-1′ of 5′-dAMP and 5′-rAMP. The effect of Mn^{2+} on the H-1′ of 2′-rAMP is the greatest, as expected, owing to the close proximity of the H-1′ to the PO_4–Mn^{2+} group located at 2′-OH (Figure 2B).

The H-1′ resonances of dApdA and of rAprA are shown in Figure 3A and 3B, respectively. For rAprA there are two doublets: one is centered at 6.26 ppm and the other at 6.38 ppm. The line widths of the upfield set are more readily affected by the presence of Mn^{2+} (Figure 3A) and, therefore, are assigned to the H-1′ of

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Table II. A Comparative Study on the H-1′, H-2′, and H-2′′ Protons of the Deoxynucleotidyl Portion of Certain Adenine Mono- and Dinucleoside Monophosphates

	H-1′[a,c,d]				H-2′[b] (endo)[c]		H-2′′[b] (exo)[c]	
	δ		J		δ		δ	
	3′	5′	3′	5′	3′	5′	3′	5′
dApdA	6.495 (q)	6.674 (t)	8.3 5.4	6.5[e]	2.608	3.166	2.798	2.981
dAprA	6.542 (q)	6.320 (d)	8.0 5.5	5.7	2.766		2.956	
rApdA[f]	6.246 (d)	6.755 (t)	4.0	6.0[e]		3.030		2.909
3′-dAMP	6.890 (t)		6.8[e]		3.159		3.047	
5′-dAMP		6.890 (t)		6.8[e]		3.140		2.946

[a] Measured at 100 MHz, in D_2O, 0.05 *M*, pD 6–7, and 28°. [b] Measured at 220 MHz, in D_2O, 0.05 *M*, pD 6–7, and 28°. H-2′′ is cis to H-1′ and H-2′ is trans to H-1′. [c] The chemical shifts are given in parts per million from external TMS capillary. [d] The coupling constants are in hertz. [e] $J = {}^1/_2 (J_{1'-2'} + J_{1'-2''})$. [f] 0.02 *M*.

the rAp residue by this procedure. This assignment is in accord with the previous assignment based on the comparison of rAprA and rAprPu (rPu = ribosylpurine nucleoside).[4] For the H-1′ spectrum of dApdA, there is a pseudo triplet at low field and a quartet at higher field (Figure 3B, Table II). In the presence of

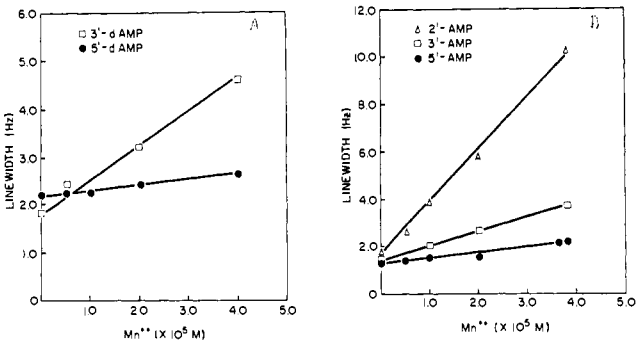

Figure 2. The changes of the line width of the H-1′ proton of (A) deoxyribonucleotides and (B) ribonucleotide in the presence of manganese(II) measured at 100 MHz and 28°, in D_2O, 0.05 *M*, and pD 6 ~ 7.

increasing concentration of Mn^{2+}, the high-field quartet became preferably broadened and, therefore, is assigned to the H-1′ of the dAp portion (Table II); thus, the low-field pseudo triplet is assigned to the H-1′ of the pdA portion (Table II). This assignment is strongly supported by the study on the dAprA and rApdA synthesized in our laboratory and to be reported in detail in the following paper in the series.[13] The H-1′ resonance of dAp portion in dAprA is a quartet and the H-1′ resonance of the pdA portion in rApdA appears as a triplet (Table II), while the H-1′ protons of the ribonucleotidyl portion of the dAprA and rApdA appear as doublets as expected (Table II). The splitting pattern and the chemical shifts of the H-1′ resonances of the deoxynucleotidyl portion in dAprA and rApdA are very similar to the corresponding 3′ and 5′ portion of dApdA (Table II), indicating the assignment made from the Mn^{2+} binding experiment is correct.

As shown in the above section, the chemical shift assignment of the H-1′ resonances in dinucleoside monophosphates is very important for understanding of the conformation of dimers in solution. In our previous studies on dimers, the H-1′ were assigned by comparative studies with other pertinent compounds, such as the comparison among dApdA, rApdA, and dAprA (Table II). This very tedious procedure can now be replaced by the Mn^{2+}-broadening procedure described in this section, which appears to be direct,

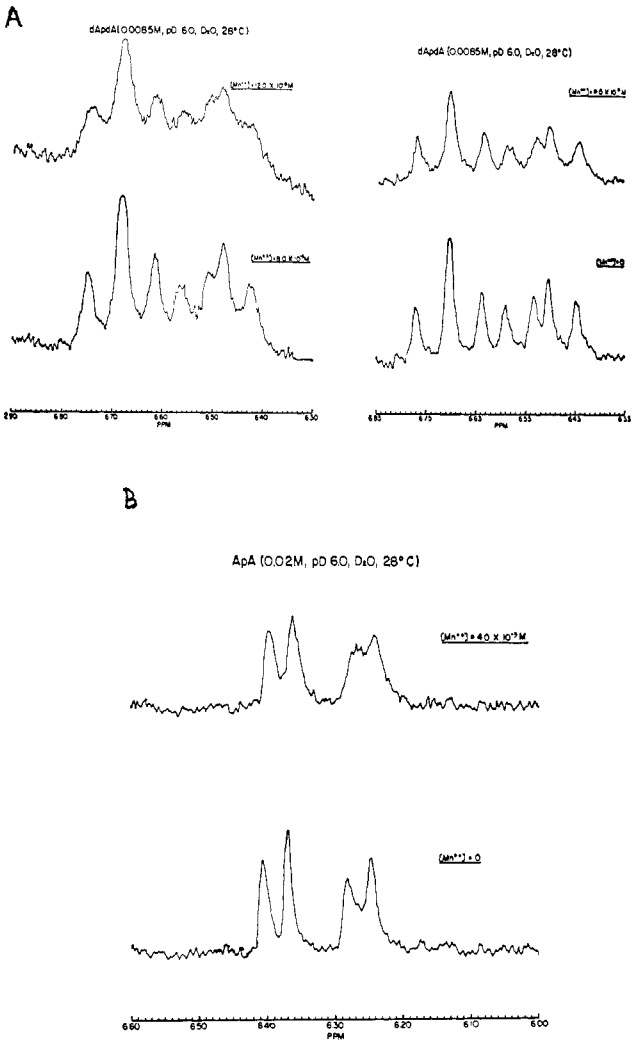

Figure 3. The pmr spectra of the H-1′ protons of (A) dApdA and (B) in rAprA in the presence of 0 to 12 × 10^{-5} *M* manganese(II) measured at 100 MHz and 28°, in D_2O, (A) 0.0085 *M*, (B) 0.2 *M*, and pD 6.0.

simple, and reliable. In the following section, it is shown that the assignment of H-1′ by this procedure is very helpful in studies on the H-2′, H-2′′ resonances.

The above discussion has confirmed the assignment of the two H-1′ protons tabulated in Table I. Therefore, the dimerization shift of H-1′ in dAp- (0.36 ppm) is almost twice as large as that of H-1′ in -pdA (0.20 ppm). From a consideration of the molecular model, these data clearly indicate that on the average, the dApdA stack is predominantly right-handed as stated in the preceding section.

Spectra Analysis and the Assignment of the H-2′ and H-2″ Protons. The H-2′ and H-2″ pmr spectra of 5′-dAMP, 3′-dAMP, rApdA, dAprA, and dApdA were recorded at 220 MHz (0.05 *M*, pD 7.0, and at 20°). The spectra are presented in Figures 4 and 5. In addition, these compounds were also studied at 100 MHz (Table II); comparison of spectra recorded at 100 MHz and at 220 MHz has been very helpful in the analyses of these resonances. The H-2′ and H-2″ proton resonances of 3′-dAMP, 5′-dAMP, and the dAp portion of dAprA can be treated as the AB part of an ABMX spin system. The H-2′, H-2″ resonances of dApdA can be treated as two AB parts of two ABMX spin systems, if proper assignment to the two residues (3′ and 5″) in the dimer can be made.

The chemical shifts and coupling constants were calculated by first-order analysis. The best parameters are given in Tables II and III. The upfield H-2″ res-

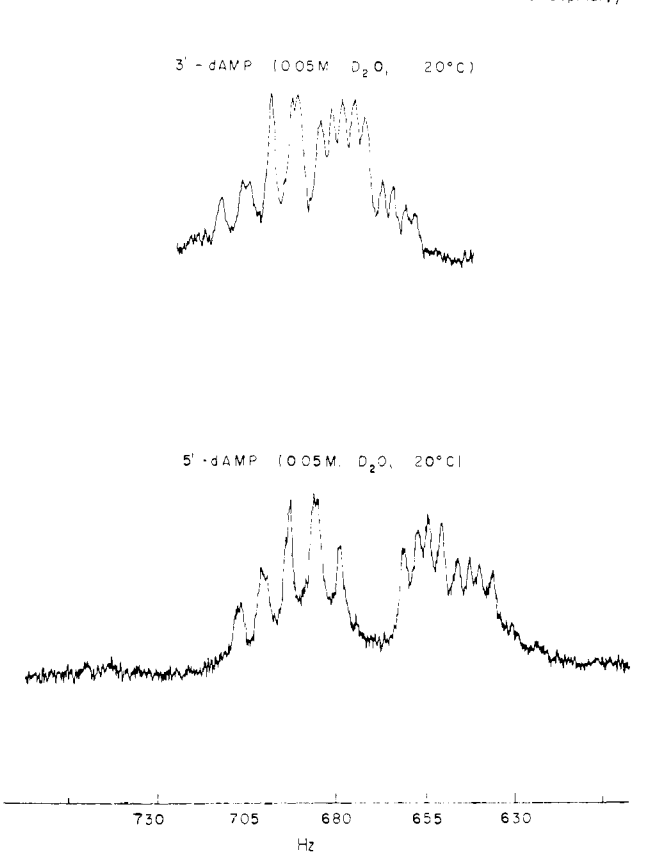

Figure 4. The pmr spectra of the H-2′ and H-2″ protons of 3′-dAMP and 5′-dAMP measured at 220 MHz and 20°, in D_2O, 0.05 *M*, and pD 6~7.

Table III. The Coupling Constants of Mono- and Dideoxynucleoside Monophosphates[a-c]

	$J_{1'-2''(cis)}$		$J_{1'-2'(trans)}$		$J_{2'-3'(cis)}$		$J_{2''-3'(trans)}$	
	3′	5′	3′	5′	3′	5′	3′	5′
dApdA	5.6	6.6	8.8	6.7	5.5	6.7	2.2	4.0
dAprA	5.7		8.7		5.5		2.0	
3′-dAMP	6.0		8.1		6.3		2.9	
5′-dAMP		6.6		6.9		5.8		3.8

[a] Measured at 220 MHz, in D_2O, 0.05 *M*, pD 6–7, and 20°. [b] The coupling constants are given in hertz. [c] The coupling constants are calculated directly from the spectra (Figures 4 and 5) for the AB part of the ABMX spin system by using a first-order approximation (see E. D. Becker in "High Resolution NMR," Academic Press, New York, N. Y., 1969, p 149). $\Delta\delta_{AB}/\delta_{AB}$ is about 3.3 except for 3′-dAMP, which is about 2.0 at 220 MHz. The calculation was also repeated for the spectra taken at 100 MHz, except for 3′-dAMP, whose lines were not well resolved at 100 MHz. The coupling constants obtained from both the 100-MHz spectra agreed with those obtained from the 220-MHz spectra. The error was estimated to be ±0.2–0.3 Hz.

onance of 5′-dAMP is assigned to proton cis to the H-1′ based on the shielding effect by the neighboring 3′-OH group on the H-2″. This type of shielding by a cis hydroxyl group was also observed in ribonucleotides.[4,12,21] Substitution of a phosphate group at the 3′-hydroxyl position may be expected to affect the C-2′ protons either by an inductive effect or by a field effect or both. An inductive effect should perturb both H-2′ and H-2″ protons to about the same extent while a field effect should cause the proton (H-2″) cis to the phosphate to be more perturbed. Comparison of the δ values of H-2′, H-2″ of 3′-dAMP to those of 5′-dAMP (Table II) indicates that phosphorylation of the 3′-hydroxyl position shifts the high-field proton from 2.946 ppm downfield to 3.047 ppm (about 0.1 ppm) and shifts the low-field proton from 3.140 ppm to only 3.159 ppm (about 0.02 ppm). From these observations and the above reasoning, the resonances at 3.047 ppm and 2.946 ppm are assigned to the H-2″ (exo,cis to the H-1′) protons of 3′-dAMP and 5′-dAMP, respectively; and the resonances at 3.159 ppm and 3.140 ppm are assigned to the H-2′ (endo,trans to the H-1′) protons of 3′-dAMP and 5′-dAMP, respectively. Attempts to assign the H-2′ and H-2″ of 3′-dAMP and 5′-dAMP by the Mn^{2+}-binding experiment were not successful. This may be expected upon examination of the three dimensional models which indicate the difference in distance between the H-2′ and the H-2″ to the phosphate group in these two mononucleotides is small.

The analysis of the four H-2′,2″ protons in dApdA is a formidable task. This task has been accomplished through the following approaches: (1) comparison of the spectra of dApdA to the spectra of the deoxynucleotidyl portions of dAprA and rApdA as well as to the spectra of 3′-dAMP and 5′-dAMP; (2) comparison of the $J_{1'-2'}$ and $J_{1'-2''}$ coupling constants observed both in the H-1′ proton spectra and in H-2′,2″ proton spectra; (3) consideration of the differences in the shielding effects of the neighboring adenine ring on the H-1′ *vs.* H-2″ as well as on the same protons in the dAp residue *vs.* those in pdA residue.

The upfield signals at 2.55 ppm and 2.84 ppm of the dApdA are very similar to the H-2′ and H-2″ signals of the deoxynucleotide portion of dAprA, while the downfield signals at 2.92 and 3.21 resemble more

(21) H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston, and D. R. Trentham, *Tetrahedron*, 22, 705 (1966).

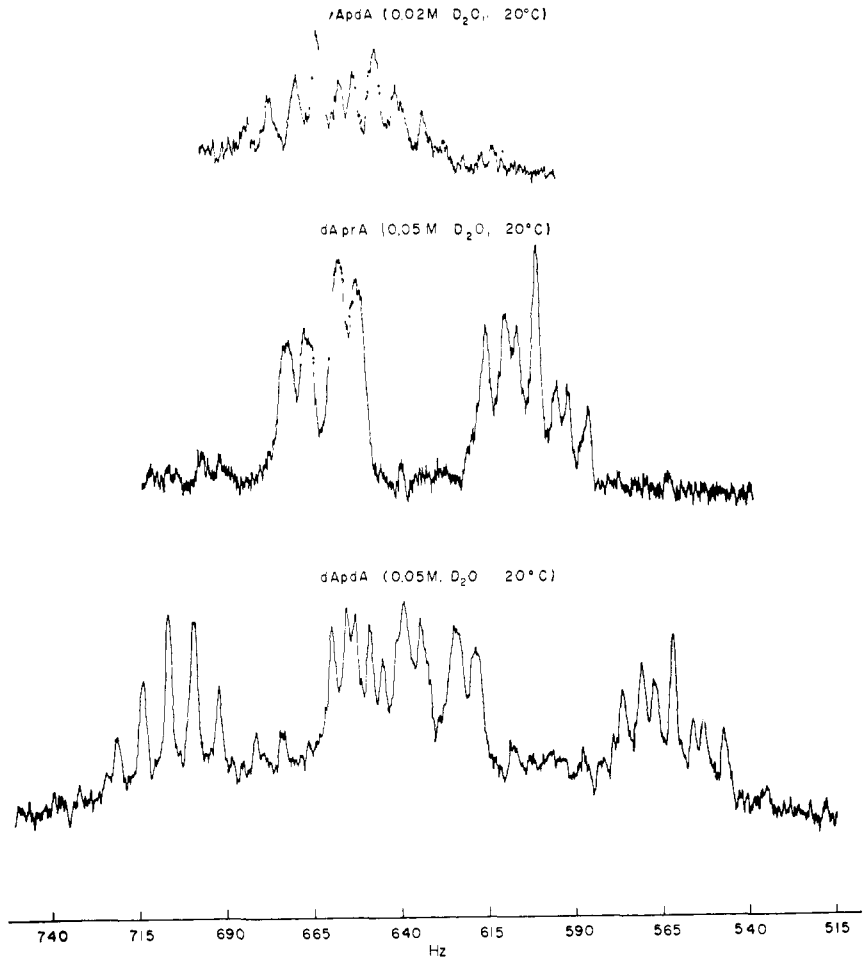

Figure 5. The pmr spectra of the H-2′ and H-2′′ protons of dApdA, dAprA, and rApdA measured at 220 MHz and 20°, in D_2O, and pD 6~7.

the H-2′ and H-2′′ signals of the deoxynucleotide portion of rApdA (Figures 4, 5, Tables I and II). These observations suggest that the two upfield resonances belong to the dAp portion and the two downfield resonances belong to the pdA portion of dApdA. Furthermore, the H-1′ resonances of the dAp portion in dApdA and dAprA appear as quartets with coupling constants of 8.7 and 5.6 Hz. The same coupling constants are also found in the upfield resonances at 2.55 and 2.84 ppm, respectively. The larger coupling constant can be assigned to trans coupling, or $J_{1'-2'}$, and the smaller coupling constant to cis coupling, or $J_{1'-2''}$[22] (caution must be made since trans coupling could be smaller than cis coupling in other furanose derivatives, depending upon the conformation of the furanose ring[23,24]). Hence, the upfield proton resonance (2.55 ppm) can be readily assigned as H-2′ (trans to the H-1′) and the lower field proton resonance (2.84 ppm) as H-2′′ (cis to the H-1′ proton). Thus, the spectral positions of H-2′ and H-2′′ in dAp- of the dimer are reversed from those of 3′-dAMP; this is due to the large shielding effect of the neighboring adenine ring to the endo H-2′ in the dimer.

(22) E. O. Bishop in "Annual Review of NMR Spectroscopy," Vol. I, E. F. Mooney, Ed., Academic Press, New York, N. Y., 1968, p 91.
(23) T. D. Inch and P. Rich, *J. Chem. Soc. C*, 1784 (1968).
(24) L. D. Hall, P. R. Steiner, and C. Pedersen, *Can. J. Chem.*, **48**, 1155 (1970).

As for the two resonances of the pdA residue in dApdA, the 3.21 ppm signal is assigned to the H-2′ (endo) and the 2.92 ppm to H-2′′ (exo) in accordance with the order assigned in the preceding section for 5′-dAMP and with -pdA in rApdA. From the consideration of the molecular models (Corey–Pauling–Koltun and Kendrew models), the H-2′ and H-2′′ of the pdA residue are located outside of the stack. This expectation is confirmed by the very small $\Delta\delta$ values for these two protons shown in Table I. Therefore, the resemblance of the order for the H-2′ and H-2′′ of the pdA residue to that of the 5′-dAMP is to be expected, since the shielding effect by the neighboring residue in the dimer is minimal.

The preceding two sections have shown that the conformation of dApdA is an anti,anti, right-handed stack and there is extensive interaction between the two adenine bases in the dimer. Inspection of the Corey–Pauling–Koltun and Kendrew models of dApdA which were built according to these conformational guide lines show that the H-2′ (endo) of the dAp residue is located in a region strongly shielded by the five-membered adenine ring of the pdA residue, while the shielding from the neighboring unit to the H-2′′ (exo) should be much less since this H-2′′ is located below the plane of the furanose ring. This situation would be reversed if the stack is left-handed, that is why the proper assignment of the H-1′ protons is so important. There-

fore, the large $\Delta\delta_D$ value (0.61 ppm) of H-2′ (endo) *vs.* the $\Delta\delta_D$ value (0.21 ppm) of H-2″ (exo) of dAp- is in agreement with the assignment and the expectation from the model.

The Conformation of Furanose Ring. Pmr spectroscopy is the method most commonly used in the study of the furanose conformation in solution. Since the early sixties, many workers[6a,9,25-27] have made substantial contributions to the determination of the furanose conformation of the nucleosides and nucleotides by measuring the vicinal coupling constant, especially the $J_{1'-2'}$, and by utilizing the Karplus equation[28,29] with empirical constants to relate the observed vicinal coupling constant to the dihedral angle of the H–C–C–H system. However, the Karplus equation is subjected to the influence of other factors such as substitution effects.[29,30] Moreover, it is a very difficult task to determine the overall conformation from measurement of a single vicinal coupling constant, especially in the flexible furanose ring system. Very often, the preferred conformation, as determined by the Karplus method, does not permit exclusion of other possible conformations. Nevertheless, a proper interpretation of the coupling constants should lead to valuable information about the furanose conformation of the nucleotides. In the application of the Karplus equation, the J_0 used in our calculation is 9.3 Hz for $0° \leq \phi \leq 90°$ and 10.4 Hz for $90° \leq \phi \leq 180°$, as suggested by Abraham, *et al.*, for 1,2-*O*-isopropylidine-α-D-xylohexofuranose derivatives.[31] From the Karplus equation, each coupling constant could lead to two dihedral angles, one below 90° and the other between 90 and 180°. The choice between these two possible dihedral angles for a H–C–C–H system has to be dependent on the knowledge about the steric constraint obtained by examination of molecular models.

Based on the observed coupling constants listed in Table III, the four dihedral angles of the H-1′, H-2′, H-2″, and H-3′ for 3′-dAMP and 5′-dAMP are calculated. As mentioned in the above paragraph, only the preferred angles are shown in Table IV after inspection of Kendrew models and studies from previous reports.[6c,9] The only problem is the choice of $\phi_{2''-3'}$ (trans). This angle can be either smaller than 90° or larger than 90°, depending upon puckering at the 3′-carbon atom; $\phi_{2''-3'}$ is smaller than 90° if C-3″ is in the exo position, and is larger than 90° if C-3′ is in the endo position.[32] A theoretical calculation of the dihedral angles for twenty possible conformations by Smith and Jardetzky[9] indicates that the minimum value of $\phi_{2''-3'}$ is 60° and the maximum value is 180°. In the 60° situation, the C(3′)–H(3′) bond bisects the angle between the C(2′)–H(2′) bond and C(2′)–H(2″) bond with the furanose in a C(2′)-endo–C(3′)-exo conformation, while in the 180°, the C(3′)–H(3′) bond lies outside of the angle between the C(2′)–H(2′) bond and C(2′)–H(2″) bond with the furanose in a C(3″)-endo–C(2′)-exo conformation. Based on Abraham's J_0 value in the Karplus equation, our calculation of $\phi_{2''-3'}$ give values of 130 and 50° for 5′-dAMP, and 125 and 55° for 3′-dAMP. For 5′-dAMP, we prefer 130° rather than 50° because the latter is smaller than the minimum theoretical angle (60°); thus, the adaptation of a 50° angle would require the furanose ring to be severely twisted around C2′–C3′. We can also examine this question from the standpoint of the deviation from the sum or difference obtained from the idealized stereochemical model; the sum ($\phi_{2'-3'}$ (cis) + $\phi_{2''-3'}$ (trans)) or the difference ($\phi_{2'-3'}$ (cis) − $\phi_{2''-3'}$ (trans)) should be equal to 120°.[9] The smaller the deviation from 120°, the more favorable is the calculated angle considered from the standpoint of stereochemistry. In the case of 5′-dAMP, both 130° or 50° angles give the same extent of deviation (30°, see Table IV), therefore a choice cannot be made on that basis.

Table IV. Calculated Dihedral Angles for dApdA,[a] 5′-dAMP,[a] and 3′-dAMP,[a] and for the Four Plausible Furanose Conformations[b,c]

	$\phi_{1'-2''}$ (cis), deg	$\phi_{1'-2'}$ (trans), deg	$\phi_{2'-3'}$ (cis), deg	$\phi_{2''-3'}$ (trans), deg
dAp-(3′)	40	160	40	60 (120)
pdA-(5′)	30	145	30	130 (50)
5′-dAMP	35	145	40	130 (50)
3′-dAMP	35	155	35	55 (125)
C-2′-endo–C-3′-exo	45	165	60	60
C-2′-endo	45	165	45	75
C-3′-exo	15	135	45	75
C-3′-endo	15	105	45	165

[a] The coupling constants used for the calculation of the dihedral angles are from Table III. [b] The data are from ref 9. [c] The calculation of the dihedral angles from the Karplus equation is based on the *J* values obtained from ref 31.

For 3′-dAMP, the choice between 125° and 55° is very difficult. Again, the deviation from the sum or the difference obtained from the idealized model is 30° for either angle, thus offering no help in this respect. As described in later paragraphs, the choice between the two calculated $\phi_{2''-3'}$ (trans) angles for dAp- and -pdA is more definitive based on the extent of deviation (smaller the better) from the sum or difference obtained from the idealized stereochemical model. Since the coupling constants of dAp- and -pdA in dApdA are similar to those of 3′-dAMP and 5′-dAMP, respectively, we assumed that the furanose conformations in dAp- and -pdA are similar to those of 3′-dAMP and 5′-dAMP. Based on this assumption and the following discussion on the dApdA, the value of 55° for $\phi_{2''-3'}$ (trans) in 3′-dAMP is favored (Table IV). It should be noted that regardless of the validity of the application of the Karplus equation, and of Abraham's J_0 value on this particular conformational analysis, the experimentally observed coupling constants (Table

(25) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 222 (1960).
(26) C. D. Jardetzky, *ibid.*, **84**, 62 (1962).
(27) For a review, see P. O. P. Ts'o in "Fine Structure of Proteins and Nucleic Acids," S. Timasheff and G. Fasman, Ed., Marcel Dekker Inc., New York, N. Y., 1970, p 49.
(28) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(29) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).
(30) G. E. Maciel, J. W. McIver, Jr., N. S. Ostlund, and J. A. Pople, *ibid.*, **92**, 4497 (1970).
(31) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLanchlan, *J. Chem. Soc.*, 3699 (1962).
(32) Exo position is defined as the out of plane atom (carbon) located opposite to the C-5′ atom; endo position is defined as the out of plane atom located on the same side of the C-5′ atom. The plane in the furanose ring is defined by the O-1′, C-1′ and C-4′ atoms.

Table V. Temperature and Solvent Effect on Chemical Shifts[a] and Coupling Constants[b] of the H-1′ in Monophosphate Mono- and Diribo- and -deoxyribonucleotides

	5′-dAMP		5′-AMP		dApdA				rAprA			
					dAp		pdA		rAp		prA	
T, °C	J	δ	J	δ	J	δ	J	δ	J	δ	J	δ
6	6.6[c]	6.804			8.8, 5.6[f]	6.457	6.4[f]	6.605	2.5[h]	6.20	2.0[h]	6.31
28	6.8[c]	6.897	5.6[e]	6.51	8.5, 5.5[f]	6.535	6.4[f]	6.718	3.2[h]	6.29	3.5[h]	6.41
60			5.6[e]	6.62	7.9, 6.0[f]	6.669	6.7[f]	6.860	4.5[h]	6.43	4.1[h]	6.555
70	6.8[d,g]											
82			5.5[e]	6.63								
92	6.7[d,g]											
DMSO-d_6	6.8[c]	6.788	5.5[e]	6.28	7.9, 5.7[f,i]	6.682	6.1[f,i]	6.721	6.8[e]	6.23	5.2[e]	6.29

[a] δ is in parts per million relative to TMS capillary. [b] J is in hertz. [c] This work, concentration is 0.05 *M*. [d] From Dr. J. L. Alderfer in our laboratory, private communication. [e] Ref 4a. [f] Concentration is 0.025 *M*. [g] Concentration is 0.01 *M*. [h] Ref 4b. [i] The values cannot be measured accurately due to the overlapping of the two H-1′.

III) did indicate a difference in the furanose conformation between the 3′-dAMP and 5′-dAMP and a similarity between the furanose conformation in dAp- and in -pdA and those in 3′-dAMP and 5′-dAMP, respectively.

The overall furanose conformation of 5′-dAMP and 3′-dAMP can be described on the basis of a comparison between the four adopted dihedral angles of these two nucleotides to those computed from 20 possible conformations of deoxyribonucleotides. In Table IV, the dihedral angles from the four most plausible conformations are listed. The comparison in Table IV suggests that the furanose conformation for 3′-dAMP is that of C(2′)-endo (envelope form) or C(2′)-endo–C-(3′)-exo (twisted form); and for 5′-dAMP is that of a rapid equilibrium between C(2′)-endo and C(3′)-endo. Recently, a complete analysis of all the coupling constants of the furanose of uridine and β-pseudouridine has been made[33] and this study indicated that the ribose conformation of these two nucleosides exists in a rapid equilibrium between various puckered forms. Based on the observed $J_{1'-2'}$ (average) value of 6.4 cps for deoxyuridine, Prestegard and Chan suggested that the conformation of the furanose in this deoxynucleoside is 2′-endo.[5b]

The four dihedral angles for the furanose conformation of the dAp and the pdA portion in dApdA were computed as described above for the mononucleotides and are shown in Table IV. Again, there are two choices for $\phi_{2''-3'}$ (trans). For the dAp portion, the 60° angle is favored since the deviation (20°) from the sum or difference (120°) of the two $\phi_{2'-3'}$ (cis) and $\phi_{2''-3'}$ (trans) angles obtained from the idealized stereochemical model is smaller for the 60° angle (40° + 60° = 100°) than the deviation (40°) for the 120° angle (120° − 40° = 80°). For the -pdA portion, however, the 130° angle is favored since the deviation (20°) from sum or differences (120°) obtained from the idealized model is smaller for the 130° angle (130° − 30° = 100°) than the deviation (40°) for the 50° angle (50° + 30° = 80°). Also, the value of 60° is the minimum value allowed for the $\phi_{2''-3'}$ (trans) from the work of Smith and Jardetzky[9] mentioned in the preceding paragraph; therefore the value of 50° for -pdA can also be rejected on this ground. As discussed above, the determinations on the dihedral angle $\phi_{2''-3'}$ (trans) for dAp- and -pdA have affected the choice for the same $\phi_{2''-3'}$ (trans) for 3′-dAMP and 5′-dAMP. The data in Table III and the calculation shown in Table IV indicate that the furanose conformations of dAp- and -pdA in dApdA are closely similar to those of 3′-dAMP and 5′-dAMP, respectively. This is a very important conclusion, since this is certainly not the case for rAprA.

Perturbation to the Furanose Conformation When the study was carried out in dilute nucleotide solutions, variation in temperature had little effect on the value of $J_{1'-2'}$ for 5′-rAMP and on the $J_{1'-2'}$ (average) for 5′-dAMP as shown in Table V. The same conclusion was reached previously by Hruska and Danyluk for 3′-rAMP and 5′-rAMP and by Blackbourn, *et al.*, on uridine.[34] Similarly, change of solvent from D_2O to DMSO-d_6 exerts no effect in the $J_{1'-2'}$ value for 5′-rAMP or in the $J_{1'-2'}$ (average) value for 5′-dAMP. Also, adenosine in pyridine-d_5, D_2O, and DMSO-d_6 has the same $J_{1'-2'}$ values of 6.0 ± 0.1 Hz. On the other hand, Prestegard and Chan[5b] reported that the $J_{1'-2'}$ values of uridine and 5′-rUMP in aqueous solution can be decreased by the addition of salts (0 to 1.5 *M*) such as $Mg(ClO_4)_2$ and $NaClO_4$. However, such a phenomenon was not observed for 2′-deoxyuridine.[5b]

Effects of concentration of the nucleotides on the $J_{1'-2'}$ (or $J_{1'-2'}$ average) values of 5′-dAMP and 5′-rAMP are shown in Table VI. As reported previously from our laboratory,[4a] $J_{1'-2'}$ of 5′-rAMP decreases at higher concentration.[35] This phenomenon was attributed to the influence of the neighboring molecules in the stack formation, since addition of purine (1 *M*) can also cause the lowering of the $J_{1'-2'}$ value in a manner similar to the increase by the concentration of 5′-rAMP.[4a] However, the situation for 5′-dAMP

Table VI. Effect of the Nucleotide Concentration on the $J_{1'-2'}$ Coupling Constants of 5′-dAMP and 5′-AMP[a]

	5′-dAMP		5′-AMP[b]	
Concentration, *M*	$J_{1'-2'}$, Hz[c]	δ[d]	$J_{1'-2'}$, Hz	δ[d]
0.05	6.7	6.897	5.6	6.51
0.4	6.7	6.764	5.1	6.405
0.85	6.8	6.716	4.7	6.35
0.05 + purine (1)	6.6	6.435	5.2	6.14

[a] Temperature is 28°, pD 6–7. [b] From ref 4a. [c] $J_{1'-2'} = \frac{1}{2}(J_{1'-2'} + J_{1'-2'})$. [d] δ is in parts per million relative to TMS capillary.

(33) B. J. Blackburn, A. A. Grey, and I. C. P. Smith, *Can. J. Chem.*, **48**, 2866 (1970).

(34) F. E. Hruska and S. S. Danyluk, *J. Amer. Chem. Soc.*, **90**, 3266, (1968).

(35) It was noted in ref 5b that such a phenomenon was also reported in the Ph.D. thesis of J. H. Nelson, California Institute of Technology, 1969, p 79.

is completely different. Under the same condition, the $J_{1'-2'}$ (average) value is not concentration dependent and is not affected by the addition of 1 *M* purine. Previous studies indicate 5′-dAMP and deoxyadenosine associate in aqueous solution to an extent comparable to that of 5′-AMP or adenosine.[12,36] The data on the δ values in Table VI confirm this conclusion. The upfield shift due to increase in concentration from 0.05 to 0.85 *M* for the H-1′ of 5′-rAMP is 0.16 ppm. The upfield shift due to the presence of 1 *M* purine for the H-1′ of 5′-dAMP is 0.46 ppm and for the H-1′ of 5′-rAMP is 0.35 ppm. These data (Table VI) clearly indicate that the formation of stack of 5′-dAMP either by itself or with purine is comparable (or at a slightly larger extent) to that of 5′-rAMP in aqueous solution. However, the formation of stacks apparently has no effect on the furanose conformation (as monitored by $J_{1'-2'}$ (average)) of 5′-dAMP, contrary to the situation observed for 5′-rAMP.

The $J_{1'-2'}$ values for the rAp and prA portions in rAprA are known to be much smaller than those of 3′-rAMP and 5′-rAMP, respectively.[4a,34] As shown in Table V, at higher temperature, these $J_{1'-2'}$ values of rAprA become larger and closer to the values observed for the mononucleotides.[20,34] When rAprA is dissolved in a destacking solvent such as DMSO-d_6,[4a] the $J_{1'-2'}$ values now are very close to those of the mononucleotides (Table V). On the other hand, the $J_{1'-2'}$ and $J_{1'-2''}$ values (Table V) for the dAp and pdA portions in dApdA are insensitive to temperature and to the change of solvent from D_2O to DMSO-d_6, and are very close to those of the 3′-dAMP and 5′-dAMP as described in the previous section. The extent of stacking in dApdA in comparison to that in rAprA is a subject of a following paper in this series.[13] Suffice to state here that the extent of stacking in dApdA is even larger than that of the rAprA and this statement is supported by comparison of the dimerization shift ($\Delta\delta_D$) reported in Table I to the respective $\Delta\delta_D$ of rAprA previously published.[4a] Such a stacking effect on the H-1′ proton for dApdA and rAprA can also be observed from the values in Table V. These observations suggest that the formation of stacks in rAprA has a large effect on the furanose conformation in the dimer, while the formation of stack in dApdA has little effect on the furanose conformation. Hruska and Danyluk[34] have reached the same conclusion previously for the rAprA. Similarly, Bangerter and Chan[5a] had observed the temperature dependence on the $J_{1'-2'}$ of the H-1′ in rAprC and rCprA and concluded that stacking affects the furanose conformation in these ribosyl dimers. In an early publication from our laboratory,[4a] while a change of $J_{1'-2'}$ of rAprA was also reported, a concern was raised whether this change of $J_{1'-2'}$ represents a change of furanose conformation. It is because at that time the concentration-dependent $J_{1'-2'}$ value of 5′-rAMP had also been found, though the $J_{1'-2'}$ value of 5′-rAMP at dilute concentration was known to be temperature independent and solvent independent (at least from water to DMSO). We felt that unless the properties of the monomers are well understood, the interpretation of the dimer data would not be on a firm ground. The present comparative studies between the rAprA and dApdA at both monomer and dimer level have clarified these issues and indicate that the early interpretation[34,5a] about the stacking effect of the bases on the furanose conformation in these ribosyl dimers is correct.

(36) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **89**, 3612 (1967).

Conclusion

Spectral assignment of the base protons, H-1′, H-2′, and H-2″, of both dAp- and pdA portions in dApdA has been made. The assignment of the two H-8 protons was assisted by selective deuterio substitution at the H-8 position; the assignment of the two H-1′ protons was assisted by the distance-dependent relaxation effect of Mn^{2+} bound to phosphate group; and the assignment of the two pairs of H-2′ and H-2″ protons was assisted by the comparison with the spectra of dAprA and rApdA. This study indicates that dApdA also has an anti,anti right-handed conformation with extensive base–base interaction. In this respect, dApdA closely resembles rAprA, but may have even a greater base–base overlap as will be discussed in a future publication.

Four coupling constants, $J_{1'-2''}$ (cis),(trans), $J_{2'-3'}$ (cis), and $J_{2''-3'}$ (trans), of the furanose of both the dAp and pdA portions in dApdA and of 3′-dAMP and 5′-dAMP were determined by first-order analysis of 220-MHz spectra and 100-MHz spectra. The data show that the coupling constants between the dAp and pdA portions in dApdA are similar to those of 3′-dAMP and 5′-dAMP, although the coupling constants of 3′-dAMP and 5′-dAMP are not the same, especially $J_{1'-2'}$ (trans) and $J_{2''-3'}$ (trans). Through the application of the Karplus equation and the J_0 values of Abraham, *et al.*,[31] four dihedral angles, $\phi_{1'-2''}$ (cis), $\phi_{1'-2'}$ (trans), $\phi_{2'-3'}$ (cis), and $\phi_{2''-3'}$ (trans), were determined with the aid of the sterochemical analysis of Smith and Jardetzky.[9] The choice between two possible angles for the $\phi_{2''-3'}$ (trans) is tentative in the case of the mononucleotides but is more certain for the nucleotidyl units in the dimer. These analyses suggest that the furanose conformation for 3′-dAMP and dAp- in dApdA is that of C(2′)-endo (envelope form) or C(2′)-endo–C(3′)-exo (twisted form), while the furanose conformation for 5′-dAMP and -pdA in dApdA is that of a rapid equilibrium between C(2′)-endo and C(3′)-endo.

In dilute solution, the values of $J_{1'-2'}$ of 5′-rAMP and of $J_{1'-2'}$ (average) of 5′-dAMP are insensitive to temperature variation as well as insensitive to the change of solvent from D_2O to DMSO-d_6 (or to pyridine-d_5 as well in the case of adenosine). However, the $J_{1'-2'}$ of 5′-rAMP in aqueous solution is sensitive to the concentration of nucleotides or to addition of purine (1 *M*), while under the identical condition the $J_{1'-2'}$ of 5′-dAMP is insensitive to both nucleotide concentration or the presence of purine. At the dimer level, the $J_{1'-2'}$ values of rAprA are much smaller than those of the corresponding mononucleotides at room temperature, increase at elevated temperature, and approach those of the mononucleotides when dissolved in a destacking solvent such as DMSO-d_6. On the other hand, the values of $J_{1'-2'}$ (cis) and $J_{1'-2''}$ (trans) of dApdA are not sensitive to temperature or to the change of solvent from D_2O to DMSO-d_6. As mentioned before, the four coupling constants in dApdA are very similar to those of the corresponding mononucleotides at room temperature. These data indicate that the formation of intermolecular

stacks of the 5′-rAMP or the formation of intranucleotidyl stacking of rAprA causes a change in the furanose conformation of the nucleotidyl unit. Change in furanose conformation in ribosyl dinucleoside monophosphates has been observed previously.[4a,5a,34] On the contrary, in this communication we reported that the formation of intramolecular stacks of 5′-dAMP or the formation of intranucleotidyl stacking of dApdA *does not* cause a change in the furanose conformation. This appears to be a fundamental difference in stereochemistry between the backbone of the ribosyl dinucleotide and that of the deoxyribosyl dinucleotide which can be manifested even at the mononucleotide level upon stacking. The reason for the existence of such a difference remains to be investigated. Comparative studies by pmr[37] and by CD[37,38] on ribosyl dimers and on 2′-*O*-methyl ribosyl dimer such as 2′-*O*-methyl-Ap-2′-*O*-methyl-A show that these two types of dimers have similar properties. This observation suggests that the difference between the ribosyl dimer and the deoxyribosyl is not due to the hydrogen-bonding property of the 2′-OH group. Bangerter and Chan[5a] have proposed that the nonbonded repulsion between the 2′-hydroxyl group in the rNp portion of the ribosyl dimer to the base and to the ether oxygen of the furanose in the prN portion of the dimer may provide a steric hindrance to stacking in ribosyl dinucleoside monophosphate. Such a hindrance, if existed, should be considerably reduced in the situation of deoxyribosyl dinucleoside monophosphate.

Experimental Section

The following compounds were purchased from Sigma Chemical Co., St. Louis Mo.: 5′-dAMP, 3′-AMP, and 5′-AMP. Adenylyl-(3′–5′)-adenosine (rAprA) was obtained from Zellstoffabrik, Mannheim, Germany. The synthesis of 2′-deoxyadenylyl-(3′–5′)-2′-deoxyadenosine (dApdA)[11] and 2′-deoxyadenylyl-(3′–5′)-adenosine (dAprA)[13] will be published elsewhere. Deuterium oxide (99.8% purity) was obtained from Diaprep Inc., Atlanta, Ga. Manganeous chloride ($MnCl_2 \cdot 4H_2O$) was obtained from Fischer Scientific Co.

The proton magnetic resonance spectra were obtained on Varian HA-100 and HR-220 spectrometers. Chemical shifts were measured from an external TMS capillary. A Varian C-1024 computer was used with HA-100 to enhance the signal to noise ratio. The spectra obtained from HR-220 were recorded by a single scan. Ultraviolet spectra were recorded on a Cary 15 spectrometer.

Paper chromatography was carried out on Whatman No. 1 paper by the descending technique with solvent A, 2-propanol–concentrated ammonium hydroxide–H_2O (7:1:2, v/v), and solvent B, 1-propanol–concentrated ammonium hydroxide–H_2O (50:10:35, v/v). Paper electrophoresis was carried out for 30 min with a voltage gradient of 40 V/cm on Whatman 3MM paper, with triethylammonium bicarbonate buffer (pH 7.8, 0.05 *M*) using a Savant power supply and a unit built to specification of Savant AV-5000-3.

2′-Deoxyadenosine 3′-Phosphate (3′-dAMP). *N*-Benzoyl-5′-*O*-di-*p*-methoxytrityldeoxyadenosine[39] (890 mg, 1.3 mmol) and dipyridinium β-cyanoethyl phosphate (2.6 mmol) were dried by repeated evaporation with anhydrous pyridine (four 1-ml portions). The resulting gum was treated with *p*-toluenesulfonyl chloride (764 mg, 3.9 mmol) in 3 ml of dry pyridine for 7 hr at room temperature. The reaction mixture was cooled to 0°, concentrated ammonium hydroxide was added, and the solution was stored at room temperature for 3 days. The solvents were evaporated and the residue was treated with 10 ml of 80% acetic acid for 4 hr at room temperature. Following evaporation of solvents, the residue was treated with a solution containing 10 ml of pyridine and 10 ml of 2 *N* NaOH for 10 min at 0°. The reaction solution was neutralized with Dowex 50X resin in the pyridinium form. The resin was filtered and washed with 50% aqueous ethanol. The combined filtrate and washings were evaporated to dryness. The residue was taken up in 20 ml of water and extracted with chloroform (three 20-ml portions). The aqueous solution was concentrated to ~2 ml and applied to a DEAE-cellulose column (2 × 30 cm) in bicarbonate form. The column was eluted with a linear gradient of ammonium bicarbonate (0.0 *M* → 0.25 *M*; 2 l. total volume). Material which eluted with 0.075 *M* salt was collected, and the solvents were evaporated. The residue was exhaustively evaporated with water to remove buffer. The resulting material was rechromatographed on DEAE-cellulose as described above to remove a trace of contaminating *p*-toluenesulfonic acid. The nucleotide material was isolated as above, and after removal of buffer, was lyophilized from water. The resulting **2′-deoxyadenosine 3′-phosphate** diammonium salt weighed 200 mg (42%): uv, λ_{max} 259 mμ, λ_{min} 226 mμ; paper chromatography, R_f^A 0.08, R_f^B 0.41; paper electrophoresis, R_m^{dpA} 1.10.

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