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# Studies of the Conformation and Interaction in Dinucleoside Mono- and Diphosphates by Proton Magnetic Resonance*

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ABSTRACT: Proton magnetic resonance data (100 Mc) on 15 nucleosides and nucleotides and on 25 dinucleoside mono- and diphosphates (23 (3′–5′)-ribosyl dimers, TpT, and (2′–5′)-ApC) are reported. The data consist of the chemical shifts, the peak assignments, the line widths, and the coupling constants of all the base protons and the H-1′ protons, observed at two temperatures, 28–30 and 60°, and at varying concentrations in $D_2O$. These data were analyzed on the basis of two basic guide lines established in studies on the monomers, *i.e.*, the shielding effect of the ring current on the neighboring bases and the specific deshielding effect of the 5′-phosphate on the H-8 proton of purine nucleotides and the H-6 proton of the pyrimidine nucleotides. From these studies, a general conformational model for all the dimers is constructed. In this model the nucleosidyl units all have the *anti* conformation with respect to the sugar–base torsion angle, and the turn of the (3′–5′) screw axis of the stack is right handed.

The general features of this model derived mainly from the data from the ribosyl dimers are similar to those of the single strand in the DNA helix. Within the temperature range of 30–60°, no major change in the ribose conformation or the sugar–base torsion angle of the dimer can be detected. The data suggest that the main effect of temperature elevation on the conformation of the dimer probably comes from the rotation of the phosphorus–oxygen bond (–P–O–) in the ester linkage.

The significance of the conformation of dinucleoside mono- or diphosphates has been well recognized as indicated by the extensive studies on this subject by several techniques from various laboratories. The importance of this problem can be envisaged from two points of view. Firstly, when the monomers are linked together covalently in varying degrees of polymerization, the properties of the dimer must have the closest relationship to those of the monomer as compared with other members in the series. The conformation of the dimer is relatively simple and perhaps can be known to a high degree of completeness and precision. This knowledge is obtained usually through a careful and systematic comparison between the monomer and the dimer. From such a study, the influence of a neighboring unit exerted on the linked monomer may be deduced. Secondly, the dimer can be viewed also as the fundamental unit for the building of the polymer. The

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trimer can be considered as the joining of two dimers with a common neighboring unit, and the tetramer as the joining of three dimers with two common neighboring units, etc. This approach has been adopted successfully in calculating certain optical properties of the trimers, oligomers, and polymers from those of the monomers (Cantor and Tinoco, 1965; Cantor *et al.*, 1966). The underlying condition for the success of this approach is that the properties observed as indications of an interaction must be mainly those of the nearest neighbors only. In other words, the interaction observed must be predominantly that of a very short distance (such as those dependent inversely upon $r^2$ or $r^3$, etc.), and the interaction between the *non-neighboring* monomeric units can be conveniently and safely neglected.

For the past 7 years, our laboratory has studied the properties, the interaction, and the conformation of bases, nucleosides, and nucleotides by a variety of techniques. Through the application of proton magnetic resonance, the mode of association of these monomeric units in aqueous solution was established. These compounds associate to form vertical stacks with the neighboring bases overlapping each other. In this study, the shielding effect of the ring current from the $\pi$ electrons of the bases, especially the purines, was clearly demonstrated. This phenomenon becomes one of the most useful guidelines for the proton magnetic resonance studies of the shielding effect exerted by the neighboring unit in the stack on various protons; reliable models have been constructed to describe in considerable detail the geometry of the nucleoside or nucleotides in the stack (Schweizer *et al.*, 1965; Broom *et al.*, 1967; Schweizer *et al.*, 1968). The next important finding in the proton magnetic resonance studies of the monomers is the specific deshielding effect of the 5′-phosphoryl group (not the 3′- or 2′-phosphates) on the H-8 proton (and not H-2) of the 5′-purine nucleotides and on the H-6 proton (and not H-5) of the 5′-pyrimidine nucleotides (Schweizer *et al.*, 1968). This deshielding effect is largest when the phosphate is in the dianion form, less when in the monoanion form, and least as the monomethyl ester. This observation not only established that the 5′-nucleotides with respect to the sugar–base torsion angle, $\phi_{CN}$, are in the *anti* conformation, but also provides the necessary understanding of the phosphate effect exerted on chemical shifts of protons in the dimer. We have collected high-quality spectra on various dimers from an 100-Mc instrument as early as 1965 (Schweizer *et al.*, 1966). However, not until the recent establishment of the above two guiding principles from the studies of the monomers could a conformational model for the dimer be proposed which has the general features to explain practically all existing proton magnetic resonance data in a unifying manner.

In this paper, we have reported the proton magnetic resonance data on 15 nucleosides and nucleotides and on 25 dinucleoside mono- and diphosphates at varying concentrations in $D_2O$ and at two temperatures, 28–30 and 60°. The data consist of the chemical shifts, the peak assignments, the line widths, and the coupling constants of all the base protons and the H-1′ protons. Based on these data, a general conformational model for all the dimers is constructed. In this model, the nucleosidyl units all have the *anti* conformation and the turn of the (3′–5′) screw axis of the stack is right handed. The general features of this model derived mainly from the ribosyl dimer are similar to those of the single strand in the DNA helix.

## Experimental Section

*Instrumentation.* Nuclear magnetic resonance spectra were recorded with a Varian Associates HA-100 spectrometer operating on the sweep frequency mode. Probe temperatures were regulated by a Varian V-6057 variable temperature accessory and monitored by observing the splitting in methanol and ethylene glycol. Chemical shifts were measured from an external tetramethylsilane capillary and are reliable to better than ±0.005 ppm. Extrapolated infinite dilution values are measured with an accuracy of about 0.02 ppm. No bulk susceptibility corrections have been made. A Varian C-1024 computer of average transients was used to enhance the signal intensity for dilute solutions.

*Procedures.* The method of deuterium exchange was used to assist in distinguishing between H-8 proton and H-2 proton resonances in ApA. A solution of 0.004 M ApA in $D_2O$ was heated at 90° for 2 hr at pD 7.4. Approximately 50% of the H-8 protons were exchanged under this condition.

Determination of apparent pH values in $D_2O$ solvent was made with a Model 22 pH meter from Radiometer, Copenhagen. To obtain pH values, the equation pD = meter reading +0.4, of Glasoe and Long (1960), was used.

For paper chromatography, Whatman No. 40 paper was employed. Solvent I was 5% $KH_2PO_4$ saturated with isoamyl alcohol and solvent II was 5% $NaH_2PO_4$ saturated with amyl alcohol. For thin-layer chromatoggraphy, Eastman "chromagram" silica gel sheets or cellulose-coated glass plates were used. The following solvents were used: A, 2-propanol–concentrated ammonium hydroxide–$H_2O$ (7:1:2, v/v); B, 2-propanol–0.5 M ammonium acetate (5:2, v/v, pH 6); C, ethanol–0.5 M ammonium acetate (5:2, v/v, pH 3.8; D, isobutyric acid–$H_2O$–concentrated ammonium hydroxide (4:2:0.004, v/v); and E, 1-butanol (saturated with $H_2O$). For paper electrophoresis, Whatman No. 3MM paper was used in an apparatus consisting of a Savant power supply and a unit built to specifications of Savant Model HV 5000-3.

The solutions of mononucleotides were prepared in molal concentration while the solutions of dinucleotide monophosphates were prepared in molar concentration. The concentrations are based on the molecular weight of the monomer or the dimer; thus a 0.02 M solution of ApA is 0.04 M in adenosine. Although the weighing error is probably less than 1%, the accuracy of the reported concentrations is about 5% due to varying moisture content.

*Materials.* When possible, commercially available compounds of the highest degree of purity were used without further purification.

The following compounds were purchased from Sigma Chemical Co., St. Louis, Mo.: 5′-AMP, 5′-GMP, 5′-CMP, 5′-TMP, adenosine, 3′-AMP, uridine, 2′(3′)-CMP, cytidine, thymidine, AMP-2′:3′-cyclic phosphate (A>*p*), deoxycytidine. California Corp. for Biochemical Research, Los Angeles, Calif., supplied: 5′-UMP, 3′(2′)-UMP, 5′-CMP, 5′-TMP, 5′-GMP, 2′(3′)-GMP, 5′-AMP, UpG, ApU, UpU, A2′p5′C, and ApC. UpA, ApG, GpA, ApA, GpC, CpG, GpU, UpG, CpU, UpC, and GpG were obtained from Zellstofffabrik, Waldhof, Mannheim, Germany. Gallard-Schlesinger, Long Island, N. Y., was the source of ApA, CpA, CpG, and CpC. The following compounds were obtained from Miles Chemical Co., Elkhart, Ind.: UpG, ApAp!, and UpUp (2′,3′). ApAp (2′,3′) was obtained from the alkaline partial hydrolysate of poly A after fractionation through a DEAE-cellulose column.

Adenylyl-(3′–5′)-purine, ApPu, was a gift from Dr. Hartmut Follmann, University of Iowa, Iowa City, Iowa. A gift of pUpU from Dr. Marshall Nirenberg, the National Heart Institute, Bethesda, Md., and a gift of pApA from Dr. Leon A. Heppel, Department of Biochemistry, Cornell University, are deeply appreciated.

The purity of all dinucleoside monophosphates was checked by thin-layer chromatography and also in some cases by paper chromatography and paper electrophoresis. Those samples with more than a trace of impurity were rejected.

The following compounds were synthesized in our own laboratory. *5′-O-Phosphorylthymidylyl-(3′–5′)-thymidine* (*pTpT*) was prepared according to the method of Khorana and Vizsolyi (1961), in which pyridinium 5′-thymidylic acid and pyridinium 3′-*O*-acetylthymidine 5′-phosphate are condensed, using dicyclohexylcarbodiimide in anhydrous pyridine to form oligonucleotides of thymidylic acid. After basic hydrolysis of the acetyl group the polymerization mixture was separated by chromatography on DEAE, and the pTpT obtained was characterized by paper chromatography and paper electrophoresis (43 V/vm, 45 min, 0.05 M Tris-HCl buffer, pH 7.5) mobility relative to 5′-TMP = 0.94. Thin-layer chromatography in solvents A, C, and D revealed $R_F$ 0.17, 0.31, and 0.11.

*Thymidylyl-(3′–5′)-thymidine* (*TpT*) was synthesized according to the procedure of Gilham and Khorana (1958), in which 5′-*O*-tritylthymidine is condensed with 3′-*O*-acetylthymidine 5′-phosphate using dicyclohexylcarbodiimide. Paper electrophoresis (43 V/cm, 45 min, 0.05 M Tris-HCl buffer, pH 7.5) mobility relative to 5′-TMP = 0.49. Thin-layer chromatography in solvents A, C, and D revealed $R_F$ 0.58, 0.59, 0.25.

*5′-O-Tritylthymidylyl-(3′–5′)-thymidine* (5′-*O*-Tr-TpT) was prepared using Gilham and Khorana's procedure for TpT, but the acid hydrolysis step for detritylation is omitted, yielding 5′-*O*-tritylthymidylyl-(3′–5′)-thymidine. 5′-*O*-Tr-TpT was separated from the other components of the reaction mixture (including Tr-TpT and 5′-TMP) using DEAE chromatography, with a linear 0.01–0.25 M $NH_4HCO_3$ gradient used as eluent. The desired product was normally eluted last under these conditions. After concentration and repeated evaporation to remove $NH_4HCO_3$, it was checked by thin-layer chromatography and electrophoresis and verified as 5′-*O*-TrTpT through comparison with Gilham and Khorana's values. Paper electrophoresis (43 V/cm, 100 min, 0.02 M NaOAc–HOAc buffer, pH 5) mobility relative to 5′-TMP = 0.36. Thin-layer chromatography in solvent A, C, D, and E revealed $R_F$ 0.70, 0.86, 0.54, and 0.07.

*Thymidylyl-(3′–5′)-thymidine 3′-phosphate* (*TpTp*) was obtained by phosphorylating the 5′-*O*-TrTpT (ammonium salt) prepared as described above. The method of Tener (1961) using β-cyanoethyl phosphate was followed. The authenticity of the TpTp thus prepared was established by thin-layer chromatography and paper electrophoresis (0.05 M ammonium acetate buffer, pH 4.1, 36 V/cm, 70 min) against 5′-TMP. TpTp has the same mobility as pTpT relative to pT, 1.29, confirming the results of Gilham and Khorana (1958) and Khorana and Vizsolyi (1961). Thin-layer chromatography in solvent A revealed $R_F$ 0.26.

## Results and Discussion

### *I. Pertinent Data on the Mononucleotides*

The chemical shifts of the base protons and the H-1′ protons of the mononucleosides and mononucleotides at 30 and 60° are given in Table IA, and the coupling constants, $J_{H\text{-}1'\text{-}H\text{-}2'}$, at 30 and 60°, are given in Table IB. The chemical shifts of the base protons of the pyrimidine compounds are not concentration dependent (Schweizer *et al.*, 1965). As for GMP (same for either the 3′- or 5′-nucleotide) the concentration dependence of H-8 (0.01 ppm or less from 0.05 to 0 M) and of H-1′ (0.04–0.05 ppm from 0.05 to 0 M) is also relatively small. The concentration dependence over a wider range (0.1–0.9 M) was reported by us previously (Schweizer *et al.*, 1968). On the other hand, the concentration dependence of the chemical shifts of the base protons and H-1′ protons of adenine compounds is very steep (Broom *et al.*, 1967; Schweizer *et al.*, 1968). When a special effort was made to measure the chemical shifts of the AMP at very low concentration, we were able to extrapolate these δ values linearly to infinite dilution as shown in Figure 17A,B.

The first important observation from Table IA is that with the present external reference system (tetramethylsilane capillary), the differentials between the δ value at 30° and the δ value at 60° are not identical for different protons from the same compound or from different compounds. For the H-2 protons, the H-5 protons, and many H-1′ protons the differential is about 0.1 ppm, a value similar to that observed for the $CH_2$ and $CH_3$ protons of ethanol (0.02 M) in $D_2O$ (Table I). This value of differential (0.1 ppm) is considered to reflect mainly the change of the bulk magnetic susceptibility of the medium of these two temperature levels. The temperature differential, however, is considerably less for the H-6 protons of the pyrimidine compounds (only around 0.04 ppm) and for some of the H-8 of the purine compounds as well as for some

TABLE I

| Compound | Concn (*m*) | Temp (°C) | H-8 | H-2 | H-6 | H-5 | H-1′ |
|---|---|---|---|---|---|---|---|
| A. Chemical Shifts of Base Protons and H-1′ Protons of Mononucleosides and Mononucleotides (pD 6.3) at 30 and 60° (Parts per Million from Tetramethylsilane Capillary) | | | | | | | |
| Adenosine | 0.00 | 60 | 8.821 | 8.765 | | | 6.616 |
| Adenosine | 0.00 | 30 | 8.753 | 8.671 | | | 6.495 |
| | | | 0.068 | 0.094 | | | 0.121 |
| 5′-AMP | 0.00 | 60 | 9.005 | 8.795 | | | 6.656 |
| 5′-AMP | 0.00 | 30 | 8.925 | 8.685 | | | 6.557 |
| | | | 0.080 | 0.110 | | | 0.099 |
| 3′-AMP | 0.00 | 60 | 8.864 | 8.785 | | | 6.620 |
| 3′-AMP | 0.00 | 30 | 8.777 | 8.685 | | | 6.546 |
| | | | 0.087 | 0.100 | | | 0.074 |
| 2′-AMP | 0.00 | 60 | 8.853 | 8.796 | | | 6.695 |
| 2′-AMP | 0.00 | 30 | 8.770 | 8.659 | | | 6.600 |
| | | | 0.083 | 0.137 | | | 0.095 |
| 5′-GMP | 0.00 | 60 | 8.594 | | | | 6.430 |
| 5′-GMP | 0.00 | 30 | 8.546 | | | | 6.350 |
| | | | 0.048 | | | | 0.080 |
| 2′,3′-GMP | 0.00 | 60 | 8.500 | | | | 6.448 (3′) |
| 2′,3′-GMP | 0.00 | 30 | 8.430 | | | | 6.387 (3′) |
| | | | 0.070 | | | | 0.061 |
| Uridine | 0.045 | 60 | | | 8.320 | 6.408 | 6.396 |
| Uridine | 0.045 | 33 | | | 8.284 | 6.314 | 6.331 |
| | | | | | 0.036 | 0.094 | 0.065 |
| 3′-UMP | 0.048 | 60 | | | 8.350 | 6.420 | 6.440 |
| 3′-UMP | 0.048 | 31 | | | 8.311 | 6.324 | 6.365 |
| | | | | | 0.039 | 0.096 | 0.075 |
| 5′-UMP | 0.045 | 60 | | | 8.483 | 6.469 | 6.484 |
| 5′-UMP | 0.045 | 28.5 | | | 8.441 | 6.385 | 6.412 |
| | | | | | 0.042 | 0.084 | 0.072 |
| Cytidine | 0.048 | 60 | | | 8.296 | 6.553 | 6.391 |
| Cytidine | 0.048 | 33 | | | 8.251 | 6.459 | 6.313 |
| | | | | | 0.045 | 0.094 | 0.078 |
| 5′-CMP | 0.046 | 60 | | | 8.472 | 6.622 | 6.502 |
| 5′-CMP | 0.046 | 29 | | | 8.430 | 6.534 | 6.411 |
| | | | | | 0.042 | 0.088 | 0.091 |
| 2′,3′-CMP | 0.042 | 60 | | | 8.338 (3′) | 6.580 | 6.447 (3′) |
| 2′,3′-CMP | 0.042 | 28.5 | | | 8.287 (3′) | 6.487 | 6.367 (3′) |
| | | | | | 0.051 | 0.093 | 0.080 |
| Deoxycytidine | 0.055 | 60 | | | 8.296 | 6.553 | 6.391 |
| Deoxycytidine | 0.055 | 30 | | | 8.246 | 6.460 | 6.315 |
| | | | | | 0.050 | 0.093 | 0.076 |
| | | | | | | Methyl | |
| Thymidine | 0.470 | 60 | | | 8.108 | 2.399 | 6.765 |
| Thymidine | 0.470 | 28.5 | | | 8.064 | 2.313 | 6.706 |
| | | | | | 0.044 | 0.086 | 0.059 |
| 5′-TMP | 0.430 | 60 | | | 8.224 | 2.399 | 6.817 |
| 5′-TMP | 0.430 | 28.5 | | | 8.197 | 2.344 | 6.774 |
| | | | | | 0.027 | 0.055 | 0.043 |

TABLE I (*Continued*)

| | | | $CH_2$ | $CH_3$ |
|---|---|---|---|---|
| EtOH | 0.02 | 60 | 4.145 | 1.693 |
| EtOH | 0.02 | 28.5 | 4.064 | 1.593 |
| | | | 0.081 | 0.100 |

| | 30° | 60° |
|---|---|---|
| B. The Coupling Constant, $J_{H\text{-}1'\text{-}H\text{-}2'}$,[a] in Mononucleotides at 30 and 60° | | |
| 3′-AMP | 5.8 | 5.6 |
| 5′-AMP | 5.6 | 5.5 |
| 3′-GMP | 5.5 | 5.5 |
| 5′-GMP | 5.8 | 5.8 |
| 3′-CMP | 3.3 | |
| 5′-CMP | 3.3 | 3.3 |
| 3′-UMP | 4.6 | 4.8 |
| 5′-UMP | 4.5[c] | 5.0 |
| 5′-TMP | 7.3[b] | 6.7 |
| 2′-AMP | 5.9 | 5.9 |

[a] The accuracy of the values is about ±0.02 cps. [b] $J_{H\text{-}1'\text{-}H\text{-}2'} = (1/2)(J_{H\text{-}1'\text{-}H\text{-}2'} + J_{H\text{-}1'\text{-}H\text{-}2''})$. [c] The low resolution of the spectral peaks leads to a larger error, probably ±0.4 cps.

H-1′ protons. As a general rule, the temperature differential for the H-6 proton is about half of that for the H-5 proton of the same pyrimidine compound, and the temperature differential for the H-8 is about 70–80% of that for the H-2 proton of the same adenine compound. This phenomenon most likely is due to a greater solvent–solute interaction of the water molecules with the H-6 and H-8 protons than with the H-5 proton or H-2 proton at room temperature. This notion receives strong support from experiments comparing the $\delta$ values of nucleosides in $D_2O$ (infinite dilution) to that in dimethyl sulfoxide (0.04 M) (Schweizer, 1968; Ts'o *et al.*, 1969). All the values are shifted to higher field in dimethyl sulfoxide in comparison with those in $D_2O$ (tetramethylsilane capillary), but the values for this dimethyl sulfoxide induced shift ($\Delta\delta_{\text{dimethyl sulfoxide}}$) are dissimilar for different protons. For uridine, the $\Delta\delta_{\text{dimethyl sulfoxide}}$ (parts per million) for H-6 is (0.05), H-5 (0.33), H-1′ (0.22), and H-5′ and H-5″ (0.3). For adenosine, the $\Delta\delta_{\text{dimethyl sulfoxide}}$ for H-8 is (0.14), H-2 (0.30), H-1′ (0.24), and H-5′ and H-5″ (0.3). For the methyl proton of methanol (0.4 M) and of acetone (0.4 M), the $\Delta\delta_{\text{dimethyl sulfoxide}}$ is 0.25 and 0.22 ppm. These data again indicate that the $\Delta\delta_{\text{dimethyl sulfoxide}}$ for the H-6 proton and H-8 proton is much less than those for other protons. This observation suggests that dimethyl sulfoxide molecules are hydrogen bonded to a certain degree with the H-6 and H-8 protons and the hydrogen bonding keeps the chemical shifts of these two protons at a relatively low-field position. Interaction of H-8 adenine and guanine with dimethyl sulfoxide has also been noted previously by Katz and Penman (1966). Therefore, we interpret that the small temperature differentials of the H-6 (pyrimidine) and H-8 (purine) protons are due to a weak hydrogen bonding between these protons and the water molecules at low temperature. This weak hydrogen bonding diminishes at 60°, thus moving the chemical shifts of these protons upfield. This contribution of the shielding effect counteracts the deshielding effect from the change of bulk magnetic susceptibility at higher temperature. The net result is that the chemical shifts of these protons are less sensitive to temperature elevation because of these counteracting factors.

The second important observation from Table IA is presented in Table II. As discussed fully in our previous publication, the 5′-phosphate group but not the 3′-phosphate group specifically deshields the H-8 (purine) and the H-6 (pyrimidine) protons of the nucleotides (Schweizer *et al.*, 1968). Furthermore, in order for this phosphate-deshielding effect to occur, the nucleotides have to be in the *anti* conformation (Schweizer *et al.*, 1968; Danyluk and Hruska, 1968). Therefore, the differential in chemical shift of the H-8 proton of the 5′-nucleotides to that of the 3′-nucleotide in the case of a purine compound, or the differential in the chemical shift of the H-6 proton of the 5′-nucleotide to that of the 3′-nucleotide in the case of a pyrimidine compound, is an index of the sugar–base torsion angle $\phi_{CN}$ (Donohue and Trueblood, 1960), which defines the *anti* or *syn* conformation. As shown from the computed values in Table II, the same differentials in the $\delta$ values for the H-8 or H-6 protons between the 5′-nucleotides and the 3′-nucleotides are obtained at both 30 and 60°. We conclude from these data, therefore, that the sugar–base torsion angles, $\phi_{CN}$, of these 5′-nucleotides must be very much the same at both 30 and 60°; thus, the 5′-nucleotides still assume the same *anti* conformation at 60° as at 30°. This is the second important observa-

tion, which is pertinent to the interpretation of the proton magnetic resonance data of the dinucleoside monophosphates at 60°.

The third important observation is from Table IB, which contains the values of the coupling constants, $J_{H\text{-}1'\text{-}H\text{-}2'}$, of most of the mononucleotides at 30 and 60°. The values of AMP differ slightly from those in the early work of Jardetzky (1962), but are close to those recently reported by Hruska and Danyluk (1968a,b), probably because of the improved quality of the spectra obtained from 60- and 100-Mc instruments. Similar to the situation in nuclosides (Jardetzky and Jardetzky, 1960; Jardetzky, 1960), the pyrimidine nucleotides all have significantly lower values of $J_{H\text{-}1'\text{-}H\text{-}2'}$ coupling constants than the purine nucleotides (Table IB). In a recent review (Ts'o *et al.*, 1969), we have discussed

TABLE II: Difference in Chemical Shift ($\Delta\delta_{5'\text{-}3'}$, ppm) between 5'-Nucleotides and 3'-Nucleotides at 30 and 60°—an Indication of *anti* Conformation. $\Delta\delta_{5'\text{-}3'} = (\delta_{5'\text{-nucleotide}}) - (\delta_{3'\text{-nucleotide}})$, ppm.[a]

| Nucleotide | Concn (*m*) | Temp (°C) | H-8 | H-2 | H-6 | H-5 | H-1' |
|---|---|---|---|---|---|---|---|
| AMP | 0[b] | 30 | 0.148 | 0 | | | 0.011 |
| | 0 | 60 | 0.141 | 0.01 | | | 0.036 |
| GMP | 0[b] | 30 | 0.116 | | | | 0.038 |
| | 0 | 60 | 0.094 | | | | 0.018 |
| CMP | 0.045 | 30 | | | 0.143 | 0.047 | 0.044 |
| | 0.045 | 60 | | | 0.134 | 0.040 | 0.055 |
| UMP | 0.045 | 30 | | | 0.130 | 0.060 | 0.047 |
| | 0.045 | 60 | | | 0.133 | 0.049 | 0.044 |
| TMP[c] | 0.430 | 30 | | | 0.133 | 0.03 ($CH_3$) | 0.070 |
| | 0.430 | 60 | | | 0.116 | 0 | 0.052 |

[a] Values of the $\delta$ 5'-nucleotide and $\delta$ 3'-nucleotides are from Table I. [b] Extrapolation to infinite dilution. [c] $\delta$ value of the thymidine is used as substitute for the $\delta$ value of 3'-TMP.

FIGURE 1 (top row, left): The Corey–Pauling–Koltun model of ApA in the "*anti,anti*, right-handed" conformation. The left side is the front view with the (3'–5') screw axis advancing forward, and the right side is the back view with the screw axis retreating backward, from the plane of the paper. The base and H-1' protons are designated.

FIGURE 2 (top row, right): The Corey–Pauling–Koltun model of ApGpA in the "*anti,anti*, right-handed" conformation. The (3'–5') screw axis is advancing forward.

FIGURE 3 (second row, left): The Corey–Pauling–Koltun model of ApCpA in the "*anti,anti*, right-handed" conformation. The (3'–5') screw axis is advancing forward.

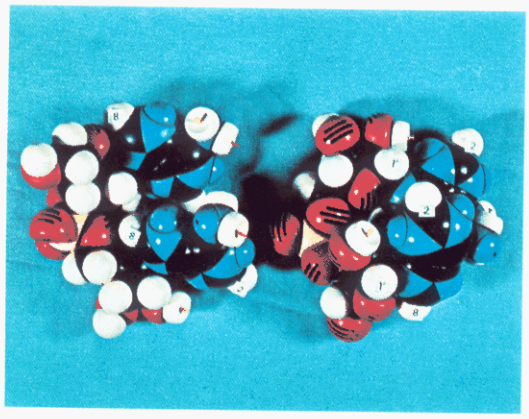
FIGURE 4 (second row, right): Corey–Pauling–Koltun model of CpUpC in the "*anti,anti*, right-handed" conformation. The (3'–5') screw axis is advancing forward.

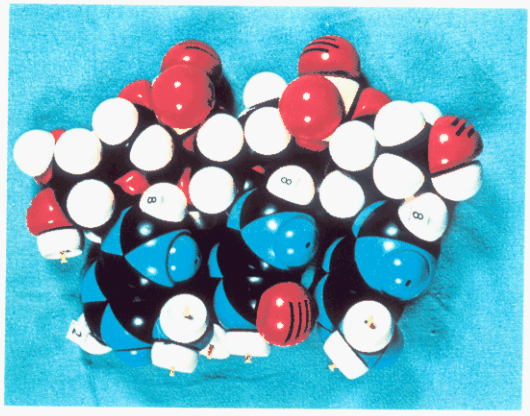
FIGURE 5 (third row, left): The Corey–Pauling–Koltun model of (2'–5')ApC in the "*anti,anti*, right-handed" conformation. The right side is the front view with the (3'–5') screw axis advancing forward, and the left side is the back view with the screw axis retreating backward from the plane of the paper.

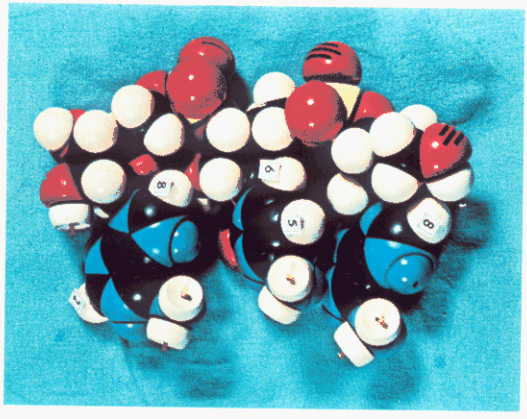
FIGURE 6: The Corey–Pauling–Koltun models of ApApAp. (A; third row, right) With the nucleotidyl units in "*anti*" conformation. The model at the right is right handed and the model at the left is left handed. The base protons of the first (Ap-) unit are depicted. As shown, the bases of both models are progressing in a counterclockwise rotation. For the right-handed model, the (3'–5') screw axis is advancing upward from the plane of the photo; for the left-handed model, the (3'–5') screw axis is retreating downward into the plane of the photo. (B; bottom row, left) The screw axes of both models are now advancing upward, however. At the left, the bases of left-handed model are progressing in a clockwise rotation, while at the right, the bases of the right-handed model are progressing in a counterclockwise rotation.

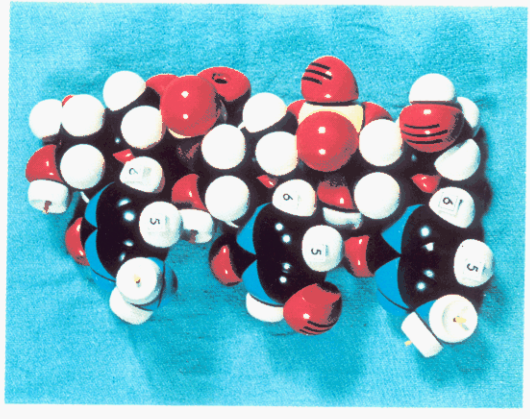
FIGURE 7 (bottom row, right): The Corey–Pauling–Koltun models of the arabinosylcytidyl-(3'–5')-arabinosylcytidylic acid (aCpaC) and CpC. All nucleotidyl units are in the "*anti*" conformation, and the 2'-OH groups are depicted. The model at the center is CpC in the right-handed conformation. The model at the left is aCpaC in the left-handed conformation and the model at the right is aCpaC in the right-handed conformation.

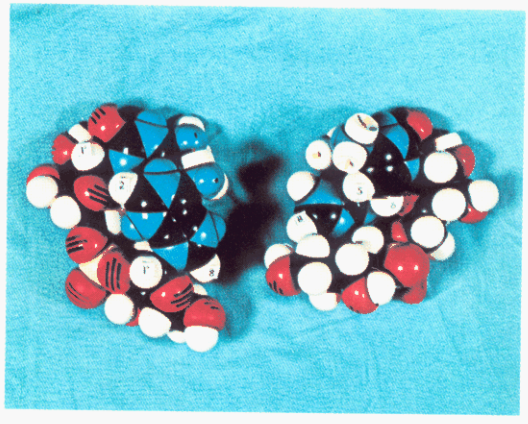
In Figures 2–4 the page should be rotated 90° counterclockwise for the proper prospective.

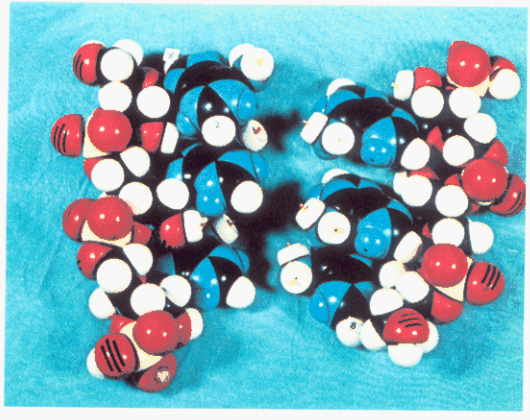


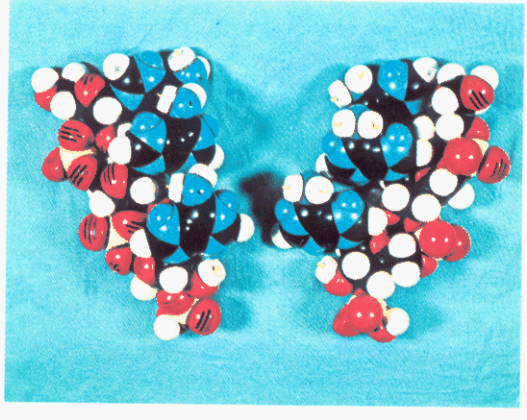


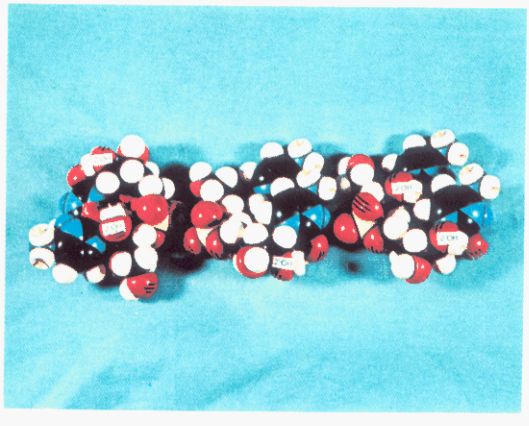












the implication of these data together with the proposal of Jardetzky. Therefore, this point will not be elaborated further here. It is also noteworthy that the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of all these nucleotides are relatively unaffected by temperature within the range of 30–60°, in agreement with the observation of Hruska and Danyluk (1968a) on AMP. As shown in a later paragraph, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of AMP and GMP are concentration dependent and are related to the influence of the neighboring compounds in the stack. This observation is very important for the interpretation of these $J$ values of the dinucleotides. At present, it is most reasonable to conclude that the conformation of the ribose ring of the mononucleotides, especially with respect to the C-2′ and C-1′ carbon, is unaffected by temperature in the 30–60° range.

*II. Spectral Data and Assignments of Chemical Shifts of the Dinucleoside Mono- and Diphosphates at 28–30°*

The chemical shifts of the base and H-1′ protons of 16 ribosyl dinucleoside monophosphates, TpT, and (2′–5′)-ApC at 0.02 M, 30°, are given in Table III. Values at lower concentration (0.002–0.004 M) are listed also for ApG, GpA, ApA, GpU, and UpG. The spectral peaks for GpG are not observable due to broadening at 30° even at a low concentration of 0.004 M, but were observable at 60° as reported later. The values for the coupling constant of the H-1′ and H-2′ protons, $J_{H\text{-}1'\text{-}H\text{-}2'}$, for 13 dinucleoside monophosphates (pD 7.4, 30°) are given in Table IV. For illustration, the spectra of UpC, CpU, ApC, and CpA are shown in Figure 8.

TABLE III: Chemical Shifts of Base and H-1′ Protons of Dinucleotide Monophosphates (0.02 M, $D_2O$, pD 7.4, 28°).[e]

| Dinucleoside | $A_{H\text{-}8}$ | $G_{H\text{-}8}$ | $A_{H\text{-}2}$ | $C_{H\text{-}6}$ | U, $T_{H\text{-}6}$ | $C_{H\text{-}5}$ | $U_{H\text{-}5}$ | H-1′ (1) | H-1′ (2) |
|---|---|---|---|---|---|---|---|---|---|
| ApC | 8.75 | | 8.55 | 8.13 | | 6.11 | | 6.45 (A) | 6.16 (C) |
| CpA | 8.82 | | 8.63 | 8.11 | | 6.24 | | 6.51 (A) | 6.13 (C) |
| ApU | 8.75 | | 8.60 | | 8.18 | | 6.06 | 6.47 (A) | 6.18 (U) |
| UpA | 8.83 | | 8.63 | | 8.15 | | 6.18 | 6.51 (A) | 6.13 (U) |
| ApG (0.02 M) | 8.66 | 8.325 | 8.52 | | | | | 6.34 (A) | 6.23 (G) |
| (0.004 M)[a] | 8.67 | 8.345 | 8.56 | | | | | 6.365 (A) | 6.24 (G) |
| GpA (0.02 M) | 8.74 | 8.31 | 8.58 | | | | | 6.49 (A) | 6.10 (G) |
| (0.004 M)[a] | 8.76 | 8.32 | 8.62 | | | | | 6.51 (A) | 6.12 (G) |
| ApA (0.02 M) | 8.64 (5′)<br>8.60 (3′) | | 8.50 (5′)<br>8.36 (3′) | | | | | 6.38 (5′) | 6.25 (3′) |
| (0.004 M) | 8.685 (5′)<br>8.65 (3′) | | 8.57 (5′)<br>8.44 (3′) | | | | | 6.41 (5′) | 6.29 (3′) |
| GpC | | 8.44 | | 8.23 | | 6.13 | | 6.31 (C) | 6.29 (G) |
| CpG | | 8.43 | | 8.16 | | 6.31 | | 6.31 (G) | 6.19 (C) |
| GpU (0.02 M) | | 8.405 | | | 8.27 | | 6.17 | 6.32 (G) | 6.32 (U) |
| (0.004 M) | | 8.415 | | | 8.26 | | 6.17 | 6.325 (G) | 6.325 (U) |
| UpG (0.02 M) | | 8.45 | | | 8.175 | | 6.21 | 6.32 (G) | 6.20 (U) |
| (0.004 M) | | 8.45 | | | 8.175 | | 6.21 | 6.32 (G) | 6.20 (U) |
| CpU | | | | 8.31 | 8.34 | *b* | *b* | *b* | *b* |
| UpC | | | | 8.35 | 8.33 | 6.45 | 6.28 | 6.365 (C) | 6.26 (U) |
| CpC | | | | 8.34 (5′)<br>8.29 (3′) | | *b* | *b* | *b* | *b* |
| UpU | | | | | 8.34 (5′)<br>8.33 (3′) | | 6.32 (5′)<br>6.29 (3′) | 6.37 (5′) | 6.31 (3′) |
| TpT | | | | | 8.12 (5′)<br>8.08 (3′) | | 2.32 ($CH_3$) | 6.75 (5′) | 6.665 (3′) |
| (2′–5′)-ApC | 8.72 | | 8.40 | 7.765 | | 6.11 | | 6.59 (A) | 5.98 (C)[c] |
| GpG | | *d* | | | | | | *d* | *d* |

[a] Values measured at 0.002 M are the same as 0.004 M. [b] Unable to discern. [c] Singlet resonance; $J_{1'\text{-}2'}$ not visible. [d] At both 0.02 and 0.004 M, the spectral peaks are not observable due to broadening. [e] Chemical shifts are in parts per million.

TABLE IV: The Coupling Constant, $J_{H\text{-}1'\text{-}H\text{-}2'}$, in Dinucleoside Monophosphate [a] (0.02 M, pD 7.4, 30°).

| | A | G | C | U |
|---|---|---|---|---|
| ApC | 3.7 | | 2.3 | |
| CpA | 3.9 | | 3.0 | |
| ApU | 4.5 | | | 2.6 |
| UpA | 4.5 | | | 4.5[b] |
| ApG | 4.0 | 4.3 | | |
| GpA | 4.5 | 3.8 | | |
| ApA | 3.2 (5′)<br>2.9 (3′) | | | |
| GpC | | 4.1 | 3.2 | |
| CpG | | 4.7 | 3.6 | |
| GpU | | 3.8 | | 3.8 |
| UpG | | 4.9 | | 4.9 |
| (2′–5′)-ApC | 6.8 | | <1 | |
| GpG (60°)[c] | | 4.6 (5′)[c]<br>5.0 (3′) | | |

[a] The peaks of the H-1′ protons of CpU, UpC, CpC, and UpU are not sufficiently discernible for the determination of the $J$ value to within ±1 cps. [b] The accuracy is estimated to be ±0.5 cps. [c] Measurement was made from spectrum at 60°, since the spectral peaks are not observable at 30° due to broadening. This tentative assignment was made with the assumption that the H-1′ of the 5′-nucleotidyl group is located downfield as in the case of ApA.

*a. Assignments of the Base and H-1′ Protons of the Heterodinucleoside Monophosphates.* Based on the knowledge from our systematic investigation of the mononucleoside and mononucleotides as presented in Table I in the above section, assignments for the base protons are relatively straightforward for the dinucleotides containing two different bases, XpY. The assignments for the H-1′ protons, however, are more tentative. The assignments of the H-1′ protons were made using the following procedures. (1) Since the coupling constants, $J_{H\text{-}1'\text{-}H\text{-}2'}$, of the purine nucleotides (A or G) are larger than that of the pyrimidine nucleotides (C or U) (Table IB), it is assumed that this is also true for the nucleotidyl units in the dinucleotides. Thus, the H-1′ protons having significantly larger $J_{H\text{-}1'\text{-}H\text{-}2'}$ values are assigned to the A or G nucleotides in ApC, CpA, ApU, GpC, and CpG. (2) Since the chemical shift of the H-1′ of AMP (6.55) is at a substantially lower field than the H-1′ of other nucleotides (6.35–6.40, Table IA), it is assumed that this is also true for the adenine nucleotidyl unit in the dinucleotides. Thus, the H-1′ protons at lower field are assigned to A in ApC, CpA, ApU, UpA, ApG, and GpA. Assignments for ApC, CpA, and ApU by this second procedure agree with that by the first procedure. The H-1′ proton of the 2′-AMP is located at a very low field due to the deshielding effect of the neighboring phosphate (Schweizer *et al.*, 1968) and thus the H-1′ proton for the 2′-AMP unit in (2′–5′)-ApC can easily be assigned. The H-1′ proton of the 5′-CMP unit in (2′–5′)-ApC appears to be a singlet with the half-width of 2.5 cps. This finding will be discussed further in a later section. (3) The above two procedures leave the H-1′ protons for GpU, UpG, and UpC unassigned (H-1′ protons for the CpU are undiscernible). In the case of GpU, the two H-1′ protons have the same chemical shifts (6.32, Table III) and the same coupling constant (Table IV); therefore, no assignment is needed. It is interesting to note the H-1′ protons of the CMP and GMP in GpC also have very similar chemical shifts (6.31 and 6.29). Therefore, it is reasonable to assume the $\delta$ value of H-1′ protons of CpG will be similar to that of UpG. Since the doublet at 6.31 has been assigned to H-1′ of G and the doublet at 6.19 to the H-1′ of C in the spectrum of CpG, by analogy the doublet at 6.32 is assigned to G and the doublet at 6.20 to U in the spectrum of UpG. In the case of UpC, again analogy is taken of this spectrum to that of UpU, since the H-1′ protons in the UpU spectrum have been assigned by other procedures as shown in the following section. Thus, the H-1′ proton at the lower field (6.35) is assigned to the 5′-C and the H-1′ proton at the higher field (6.26) is assigned to the 3′-U in the spectrum of UpC.

*b. Assignments of the Base and H-1′ Protons of the Homodinucleotides.* The problem of assignment of the base and H-1′ protons of the dinucleotides containing the same base, *i.e.*, XpX, is challenging and formidable. Through careful comparative studies with other pertinent compounds, it is felt that most of the assignments can be made with a high degree of certainty.

1. ApA. The pertinent information for the assignment is presented in Table VA and the spectrum is presented in Figure 9. Heating at 90° in $D_2O$ for 2 hr eliminated the two low-field peaks (around 8.69 and 8.65). This result indicates that these two peaks are the H-8 protons which can be exchanged with deuterium upon heating in $D_2O$ (Schweizer *et al.*, 1964; Bullock and Jardetzky, 1964). The assignment of these two H-8 protons to the 5′- or 3′-nucleotyl unit in the dimer was achieved by the comparison of the chemical shifts of pApA to ApA. (This comparison had to be carried out near pD 4.9, the pD of the sample of pApA which was too small for adjustment of the pD of the solution). As shown in Table V, one of the H-8 peaks was shifted downfield by about 0.10 ppm, while the spectral positions of the other three peaks remain more or less unchanged. There is one structural requirement and one conformational requirement in order for the deshielding effect of the phosphate group on the H-8 proton of the adenine to take place (Schweizer *et al.*, 1968, and above section). The phosphate group has to be in the 5′ position of the adenosine and the 5′-nucleotide has to have the *anti* conformation. This comparative study in Table VA, therefore, un-

ambiguously assigns the peak at 8.81 position as that of H-8 of the (pAp-) unit in pApA. In addition, the (pAp-) unit must have the *anti* conformation. It is reasonable to assume that the (-pA) unit in ApA also has the *anti* conformation; therefore, the H-8 proton at the lower field is assigned to the 5'-nucleotide (-pA) which has about the same $\delta$ value (8.75) as that for the (-pA) in pApA (8.74). This assignment is further supported by the comparative study on (3'–5')-adenosine–ribosyl purine monophosphate (ApPu). The three very low field protons are those of the H-6, H-2, and H-8 of the 5'-purinyl riboside unit based on our knowledge of purine riboside (Broom *et al.*, 1967). The H-8 proton of the (Ap-) unit in ApPu is almost identical with the higher field H-8 proton in ApA. This comparison strongly suggests that the higher field H-8 proton belongs to the (Ap-) unit and the low-field H-8 proton belongs to the (-pA) unit in ApA, thus supporting the above assignment of H-8 from the comparative study of pApA. In addition, the $\delta$ value (8.48) of the adenosine H-2 proton in ApPu is much closer to the high-field H-2 proton (8.44) than to the low-field H-2 proton (8.57) in ApA. This comparison strongly suggests that the high-field H-2 proton (8.44) in ApA should be assigned to the 3'-(Ap-) unit. This small downfield shift of the spectral position (from 8.44 to 8.48) is to be expected in comparing the H-2 of ApA to ApPu, since the stacking tendency of adenosine is higher than that of purine riboside (Broom *et al.*, 1967). This assignment of the low-field H-2 proton

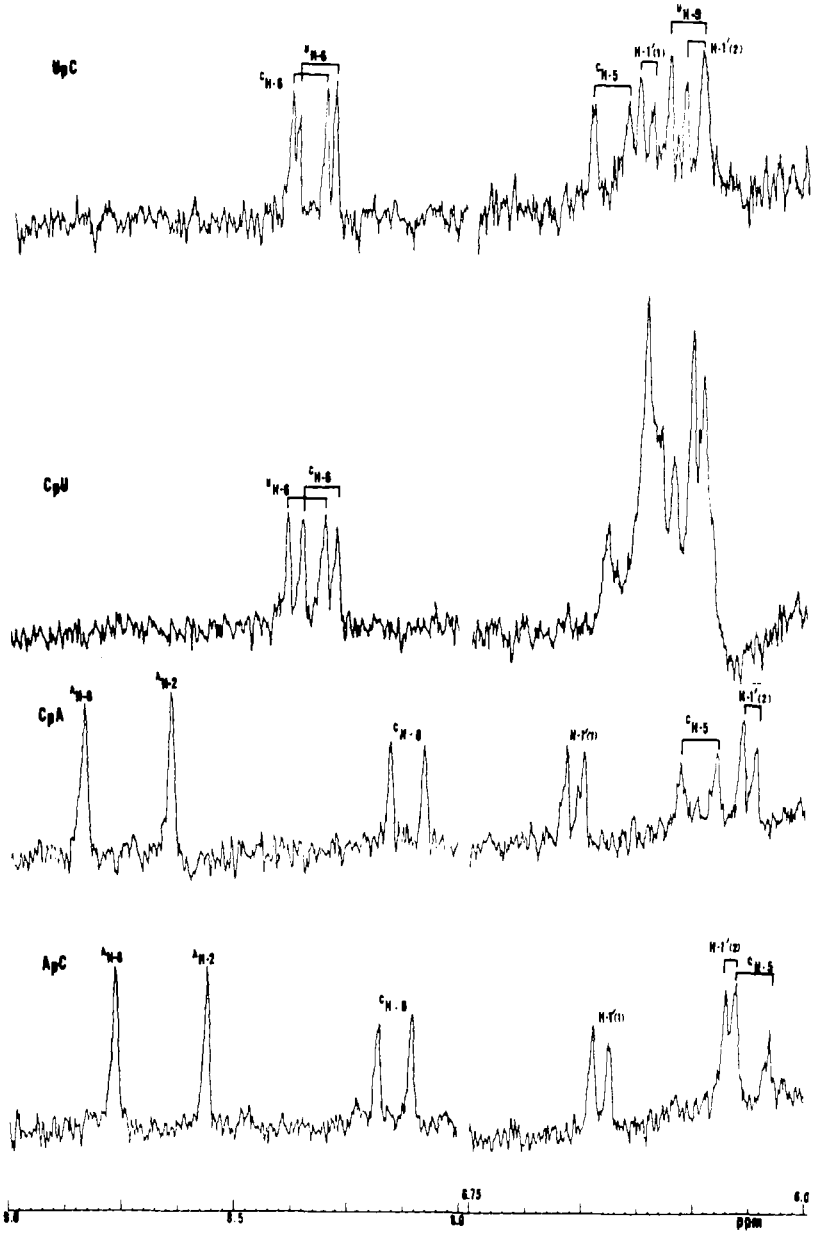



FIGURE 8: The proton magnetic resonance spectra from a 100-Mc instrument of UpC, CpU, CpA, and ApC in $D_2O$, 0.02 M (with reference to the dimer), pD 7.4, 28°. The assignment of the H-1' protons is given in Table III.

TABLE V: Comparative Studies on Nucleoside Phosphates for the Assignment of Chemical Shifts (Tetramethylsilane Capillary, parts per million)

| | Concn (*m*) | H-8 (5′) | H-8 (3′) | H-2 (5′) | H-2 (3′) | H-1′ (5′) | H-1′ (3′) |
|---|---|---|---|---|---|---|---|
| A. Adenosine Phosphates (pD 7.4, 28°) | | | | | | | |
| ApA,[a] pD 4.7 | 0.004 | 8.75 | 8.72 | 8.65 | 8.51 | | |
| pApA,[a] pD 4.9 | 0.0044 | 8.74 | 8.81 | 8.63 | 8.49 | | |
| ApA | 0.004 | 8.69 | 8.65 | 8.57 | 8.44 | 6.41 | 6.29 |
| ApPu | 0.004 | 9.07 (Pu) | 8.66 (A) | 9.33 (Pu)<br>9.37 (H-6, Pu) | 8.48 (A) | 6.60 (Pu) | 6.32 (A) |
| ApA-2′:3′-p (cyclic-p) | 0.013 | 8.69 | 8.59 | 8.52 | 8.48 | 6.67 | 6.21 |
| (2′,3′)-ApAp | 0.01 | 8.77 | 8.64 | 8.57 | 8.48 | 6.55 6.43 | 6.27 |
| | 0.004 | 8.77 | 8.64 | 8.57 | 8.50 | 6.54 6.47 | 6.27 |

| | H-6 (5′) | H-6 (3′) | H-5 (5′) | H-5 (3′) | H-1 (5′) | H-1 (3′) |
|---|---|---|---|---|---|---|
| B. Uridine Phosphates (0.01 M, pD 7.0–7.2) | | | | | | |
| UpU | 8.33 | 8.32 | 6.32 | 6.30 | 6.36 | 6.32 |
| pUpU | 8.34 | 8.49 | 6.35 | 6.38 | 6.38 | 6.42 |
| pU[b] | 8.44 | | 6.38 | | 6.42 | |
| Up[b] | | 8.32 | | 6.32 | | 6.37 |

| | H-6 (5′) | H-6 (3′) | $CH_3$ |
|---|---|---|---|
| C. Thymidine Phosphates | | | |
| TpT (0.018 M, pD 5.4) | 8.12 | 8.09 | 2.32 |
| pTpT (0.016 M, pD 6.1) | 8.11 | 8.19 | 2.33 |
| TpTp (0.01 M, pD 6.4) | 8.14 | 8.09 | 2.33 |
| pT[c] | 8.20 | | 2.34 |
| Thymidine[c] | | 8.07 | 2.31 |

[a] The original pD of the pApA sample was at 4.9 and the sample is too small in amount for the adjustment of the pD. Therefore, the comparative study was carried out at this pD. [b] Values from Table IA. [c] Values from Table IA.

to the (-pA) unit and the high-field H-2 proton to the (Ap-) unit in ApA is also firmly supported by other conformational studies on ApA as discussed in later sections.

Comparison between the spectrum of ApA and that of ApPu also provides information for the assignment of H-1′ protons of ApA (Table VA). The H-1′ proton located at a very low field position (6.60) is readily assigned to the (-pPu) unit since the H-1′ proton of the purine riboside is located at a much lower field position (6.70) than the H-1′ proton of adenosine (6.50) (Broom *et al.*, 1967). The $\delta$ value of the H-1′ proton (6.32) of the (Ap-) unit in ApPu is very close to the high-field H-1′ proton (6.29) of the ApA; therefore, this high-field H-1′ proton is assigned to the (Ap-) unit in the ApA. This assignment is completely confirmed in the comparative studies with ApA-2′:3′ cyclic phosphate (ApA-2′:3′-p) and with (2′,3′)-ApAp (Table VA). In the case of the cyclic dinucleotides, one of the H-1′ protons is shifted significantly downfield (6.67) because of the deshielding of the adjacent phosphate linked to the 2′-OH group. This H-1′ proton must belong to the (pA-2:3p) unit, and the remaining H-1′ proton is that of the (Ap-) unit. The $\delta_{H\text{-}1'}$ value of the (Ap-) unit (6.21) in ApA-2′:3′-p is closer to one of the H-1′ protons (6.29) of ApA than the other (6.41); therefore, the H-1′ proton at 6.29 is assigned to the (Ap-) unit in ApA. In the case of (2′,3′)-ApAp, the two H-1′ protons at the lower field position (6.54 and 6.47) have only about half of the intensity as that of the higher field H-1′ proton (6.27). The intensities of these two low-field protons are about equal to each other. The high-field H-1′ proton of full intensity is that of the (Ap-) unit

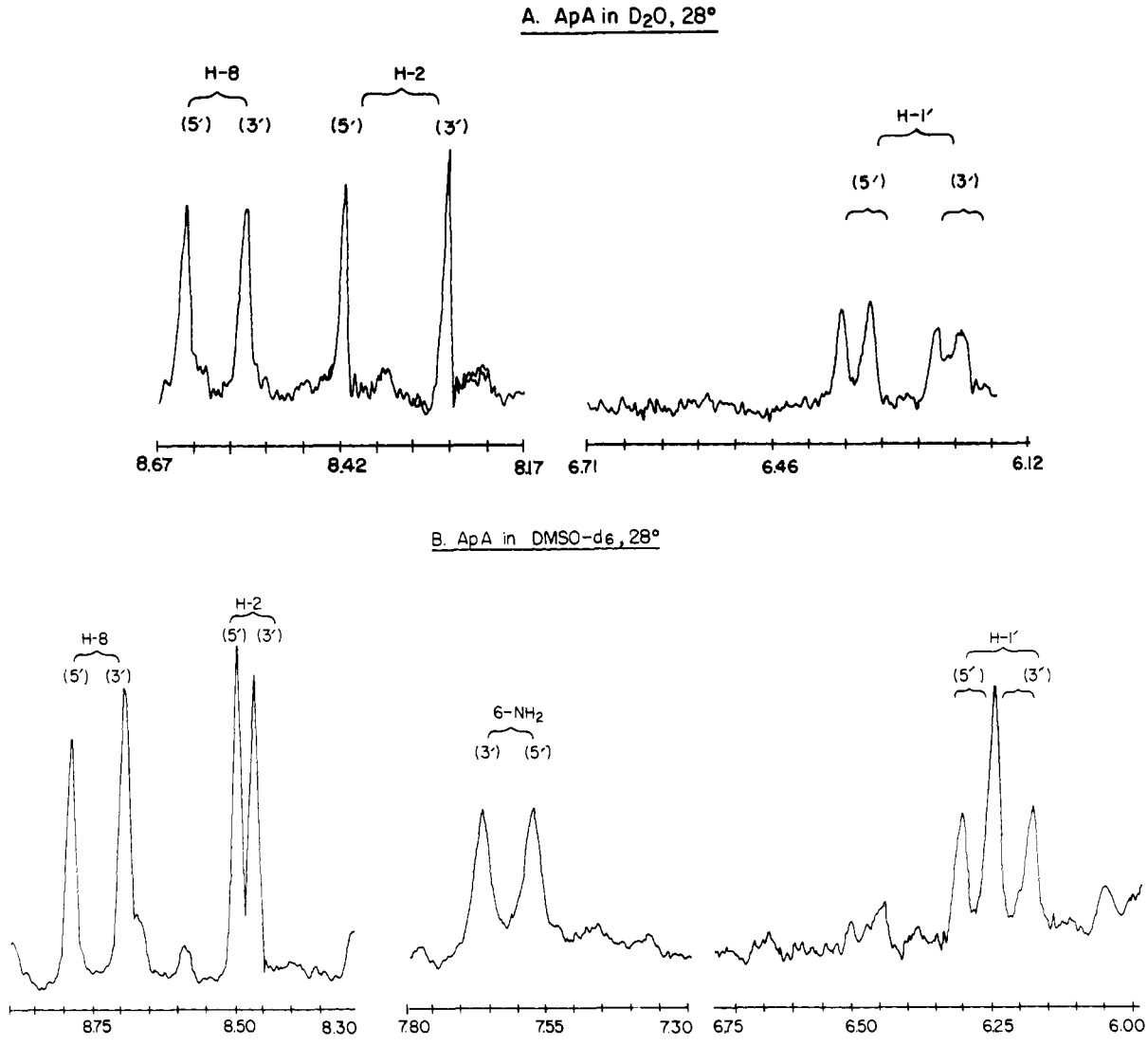



FIGURE 9: Proton magnetic resonance spectra (100 Mc). (A) Of ApA in $D_2O$, 0.068 M, pD 7.4, 28°. (B) Of ApA in dimethyl sulfoxide-$d_6$, 0.02 M, 28° (12 scans). Before dissolving in dimethyl sulfoxide-$d_6$, the sample was first dissolved in $H_2O$, neutralized with NaOH to pH 7.7, and then lyophilized to dryness.

in the (2′,3′)-ApAp, and its value is very close to that of the H-1′ proton assigned to the (Ap-) in ApA, thus supporting the assignment. The splitting of the low-field H-1′ proton is due to the presence of both (-pA2′p) and (-pA3′-p) units in the (2′,3′)-ApAp. The H-1′ proton at the field position of 6.55 must belong to the (pA2′-p) unit and the H-1′ proton at the field position of 6.47 must belong to the (-pA3′-p) unit, and this $\delta$ value is very close to that of the (-pA) unit in ApA, thus again supporting the assignment. The chemical shifts of the base protons of ApA-2′:3′-p will be discussed in a later publication and the base proton chemical shifts of (2′,3′)-ApAp will be considered in later sections of this paper.

Our assignment of the base protons of ApA-2′:3′-p is in agreement with that recently proposed by Hruska and Danyluk (1968b). However, in their paper, the experimental data and the rationale for their assignment were not included and were referred to a future publication. Same assignments for the base protons of ApA were also obtained by Chan and Nelson (1969) from a different approach.

2. UpU. Table VB provides the information necessary for the assignment. The differences in $\delta$ values of the two H-6 protons and the H-5 protons are very small. One of the H-6 protons, and to a much lesser extent, one of the H-5 protons, is shifted significantly downfield when a phosphate group is added to the 5′-OH end in forming pUpU. As described in our previous publication (Schweizer *et al.*, 1968) and in the previous section, the deshielding of the H-6 proton (and to a much lesser extent, the H-5 proton) by a phosphate group requires this phosphate to be linked at the 5′-OH position and the 5′-nucleotide to have the *anti* conformation. These requirements, therefore, assign the low-field H-6 proton (8.49) and the low-field H-5 proton (6.38) to the (pUp-) unit in pUpU. Since the (pUp-) unit in pUpU is in the *anti* conformation, it is

reasonable to assume that the (-pU) unit in UpU is also in the *anti* conformation. Therefore, the H-6 proton (8.33) and the H-5 proton (6.32) located at slightly lower field are assigned to the (-pU) unit in UpU. Their $\delta$ values are also closer to the values of the protons from the (-pU) unit in pUpU.

We have also examined a sample of (2′,3′)-UpUp. However, because of the scarcity of the material, the purity of this sample was not vigorously examined. Three H-6 protons were found, having $\delta$ values of 8.34, 8.33, and 8.30. The intensities of the two protons located at higher field are equal, but are half of that of the low-field H-6 proton (8.34). These H-6 protons can not be assigned with certainty at present, though it is tempting to assign the H-6 proton located at 8.34 to the (Up-) unit and the two H-6 protons of half intensity to the ((2′,3′)-pUp) unit because of the pattern of intensity distribution. Nevertheless, the absence of a H-6 proton resonance located at very low field (lower than 8.35) in the spectrum of (2′,3′)-UpUp clearly indicates the specific deshielding effect of the 5′-phosphate in pUpU on the H-6 proton (8.49) of the (pUp-) unit.

The two H-1′ protons of UpU are separated by 0.04 ppm (6.36 and 6.32). It is interesting to note that the H-1′ proton of pU (6.41) is located downfield from the H-1′ proton of Up (6.37) by 0.04 ppm, (Table I). Therefore, the low-field H-1′ proton (6.36) is assigned to the (-pU) unit and the high-field H-1′ proton (6.32) is assigned to the (Up-) unit in the UpU.

3. TpT. The pertinent information for the assignment is presented in Table VC. The H-6 protons of the TpT are separated by about 0.03 ppm, while the methyl protons are all in one sharp peak. For the pTpT, one of the H-6 protons is shifted downfield by about 0.10 ppm while the other remains unchanged. This is the same situation as that discussed above in the comparative study on UpU *vs.* pUpU. In order for this situation to take place, the added phosphate group has to be attached to the 5′ position of the thymidine and the nucleoside has to be in the *anti* conformation. This argument assigns the low-field H-6 proton (8.19) to the (-pTp) unit in the pTpT. Indeed, this value of the H-6 proton of (-pTp) unit is very close to that (8.20) of pT, the 5′-thymidylic acid. Addition of phosphate to the 3′-OH end of TpT in forming TpTp brings about very little change in the original spectrum of TpT. This result demonstrates the specific deshielding effect of adding the phosphate to the 5′-OH end of the dinucleoside monophosphate. All these observations strongly suggest that the thymidine units in the TpT are all in *anti* conformations. For this reason, the low-field proton of the H-6 in TpT is assigned to the (-pT) unit and the H-6 proton at a slightly higher field position is assigned to the (Tp-) unit.

TpT has been studied by proton magnetic resonance techniques previously by Chan *et al.* (1966) and by McDonald *et al.* (1967). The magnetic nonequivalence of the H-6 protons was not noted by Chan *et al.* presumably because of the lower degree of resolution of the 60-Mc instrument. In the paper of McDonald *et al.* their attention was focused on the methyl group resonance of the TpT.

A comment here on the general procedure of assigning the H-8 or the H-6 protons by comparing XpX with pXpX is in order. Generally, the deshielding effect of adding a 5′-phosphate (0.1–0.15 ppm) is much larger than the difference (0.01–0.04 ppm) in $\delta$ values of the H-8 or H-6 protons between the (Xp-) unit and (-pX) unit in the XpX. The major reason is that the added 5′-phosphate in pXpX is a *monoester*, while the 5′-phosphate in XpX is a *diester*. The difference in deshielding effect between the 5′-methyl ester and the 5′-phosphate *monoanion* upon the H-8 of 5′-AMP is about 0.08 ppm, and this difference for the H-6 of 5′-CMP or 5′-UMP is about 0.04–0.05 ppm (Schweizer *et al.*, 1968). The difference in deshielding effect between the 5′-methyl ester and the 5′-phosphate *dianion* is even larger. This is the reason why the deshielding effect of the 5′-phosphate in pUpU is so large (0.17 ppm) since this compound was measured at pD 7.0. Other considerations based on conformational factors will be discussed in later sections.

### *III. The Variation in Line Width and the Effect of Concentration on the Chemical Shifts*

The line widths of the base protons of ten dinucleoside monophosphates at 0.02 M, 28–30°, are shown in Table VI. The line widths of the base protons from these ten dimers are narrow. In our limited experience, we have not yet encountered any commercial samples or lots of preparation of these ten dimers which gave large line widths in their spectra. The line widths of the base protons of six other dimers are shown in Table VII.

TABLE VI: Line Widths (cycles per second) of the Base Protons of Ten Dinucleoside Monophosphates (28–30°, 0.02 M).[a]

| | H-8 | H-2 | H-6 | H-5 |
|---|---|---|---|---|
| CpC | | | 1.3 | *b* |
| UpU | | | 2.0 | 2.0 |
| CpU | | | 1.2[c] | *b* |
| UpC | | | 1.1[c] | 1.7[c] |
| ApC | 1.4 | 1.4 | 1.3 | *b* |
| CpA | 1.7 | 1.6 | 1.5 | 1.8 |
| ApU | 1.4 | 1.5 | 1.4 | 1.7 |
| UpA | 1.7 | 1.7 | 1.3 | *b* |
| CpG | 2.5 | | 1.3 | *b* |
| (2′–5′)-ApC | 1.5 | 1.5 | 1.3 | 1.3 |

[a] For TpT, the two H-6 protons are located too closely together for the accurate determination of line width. For GpG, the proton signals are too broad to be detected. At 60°, the line width of the H-8 proton of GpG is 3.5 cps. The accuracy of the line-width measurement is $\pm 0.2$ cps. Samples of these dimers encountered so far all have narrow line width. [b] The H-5 proton peaks are insufficiently discernible from the H-1′ proton peaks. [c] Same line width for both U and C protons.

TABLE VII: Line Widths (cycles per second) of the Base Protons of Six Dinucleoside Monophosphates (28–30°, 0.02 M).[a]

| | | H-8 | H-2 | H-6 | H-5 |
|---|---|---|---|---|---|
| ApA | Sample 1 [b] | 1.8 (5′) | 1.6 (5′) | | |
| | | 1.6 (3′) | 1.4 (3′) | | |
| | Sample 2 | 13 (5′ and 3′ merged) | 7 (5′) | | |
| | | | 5.5 (3′) | | |
| GpA | Sample 1 | 2.0 (A) | 2.0 (A) | | |
| | | 2.6 (G) | | | |
| | Sample 2 | 2.5 (A) | 2.5 (A) | | |
| | | 7.5 (G) | | | |
| ApG | Sample 1 | 3.6 (A) | 2.5 (A) | | |
| | | 5.6 (G) | | | |
| | 1 + 0.005 M ETDA | 1.7 (A) | 1.6 (A) | | |
| | | 1.6 (G) | | | |
| | Sample 2 | 15–20 (all peaks merged) | | | |
| | 2 + 0.008 M ETDA | 1.9 (A) | 1.7 (A) | | |
| | | 1.7 (G) | | | |
| UpG | Sample 1 | 20–30 | | 2.5 | *c* |
| | Sample 2 | 3.5 | | 1.7 | 1.8 |
| | Sample 3 | 2.5 | | 1.7 | 1.8 |
| GpU | Sample 1 | 12 | | 1.3 | *c* |
| | Sample 2 | 2.5 | | 2.0 | 2.3 |
| GpC | Sample 1 | 4.5 | | 1.4 | 1.8 |
| | Sample 2 | 4.0 | | 2.0 | 2.0 |
| | 2 + 0.008 M ETDA | 1.8 | | 1.8 | 1.8 |

[a] The accuracy of the line width is about ±0.3 cps. Different samples of these dimers gave different line widths. [b] The line width of base protons in the spectra of sample 1 of ApA is unchanged from 0.004 to 0.07 M. [c] The separation of the H-1′ proton and the H-5′ proton of U is poor.

We have observed variation in line widths from different commercial preparations of these dimers. For example, one commercial preparation of ApA gave spectra of very narrow line width, while the spectra of another preparation had very broad line width (Table VII). A similar situation was also experienced for samples of GpA, ApG, UpG, GpU, and GpC. In every case, the H-8 proton of G in the dimer had a much wider line width than the other base protons. These situations clearly indicate the presence of a contaminant(s) in those samples which showed broad spectral lines. Obviously, the prime suspects are paramagnetic metal ions. This suspicion was confirmed when it was found that addition of EDTA to these samples would lead to dramatic sharpening of these broad spectral lines. For example, the H-8 proton of G in a sample of ApG and in a sample of GpC had line widths twice as large as those of the other base protons in the dimers (Table VII). After addition of EDTA to these samples, all the spectral lines are sharpened, especially the H-8 protons of G, which now have the same line width as the other base protons. The chemical shifts of these protons are the same in different preparations of a dimer; within experimental accuracy the $\delta$ values are not related to the line widths.

This situation is well known for the monomers. Cohn and Hughes (1962) were the first to report the preferential broadening of the H-8 proton of ATP by $Cu^{2+}$ and $Mn^{2+}$ in $D_2O$. Eichhorn *et al.* (1966) have shown that the H-8 line width of dGMP is most sensitive to the broadening effect of $Cu^{2+}$, the H-8 line width of dAMP next, while the line widths of the H-2 of dAMP, of the H-6 of dGMP, and of the H-6 of dTMP are not very sensitive to the presence of $Cu^{2+}$. A similar conclusion was reached by Wang and Li (1966) in their study of the binding of $Zn^{2+}$ to purine and by Sternlicht *et al.* (1965) in their study of the binding of $Mn^{2+}$, $Ni^{2+}$, and $Co^{2+}$ to ATP. The reason for this phenomenon is that the protons (H-8) near the binding site of the metal (N-7) are relaxed by the paramagnetic ions and their proton magnetic resonance lines are thus broadened. In a preliminary communication, Inoue and Aoyagi (1967) reported that the line width of H-8 of the (G) unit in UpGp was very wide (54 cps), then that of G in CpGp was next, and that of G in ApGp was the least. However, their spectrum of ApGp shows only two peaks (instead of three peaks in our ApG spectrum; see Table II) and these peaks were not uniquely assigned. The spectra were measured at 0.2 M concentration and no study

on the concentration dependence was reported. These authors interpret their observations in terms of specific *intramolecular* interaction resulting from internal stacking. In view of our data and our experience shown in Table VII, the possibility of the influence of paramagnetic metal ion(s) which might be present in these samples has to be considered.

The situation for GpG is quite different. At 28–30°, all the protons of the GpG are too broad to be detected at 0.02 or 0.004 M concentration (Table III). Addition of 0.01 M EDTA to the sample did not bring about the appearance of the proton peaks. This broadening phenomenon, therefore, is most likely due to intermolecular association. The protons of GpG are detectable at 60°, and will be discussed in section V.

Over the concentration range from infinite dilution to 0.02 M, no significant concentration dependence upon chemical shifts has been observed for all the dimers, except for GpA, ApG, and ApA (Table III). The dependence of chemical shifts for GpA and ApG at this range is small but definitely measurable. On the other hand, the $\delta$ values of the base protons and the H-1 protons of ApA are shifted upfield noticeably upon increase in concentration, yet the line widths of these protons of ApA are not changed by an increase of 17-fold of concentration from 0.004 to 0.07 M (Table VII). The concentration dependence of the chemical shifts of ApA is shown in Figure 10. This shielding effect of increasing concentration of ApA is similar to that observed for the adenosine mononucleotides (Schweizer *et al.*, 1968). In Table VIII, the concentration dependence $\Delta\delta$ of the chemical shifts of the base protons and H-1′ protons of ApA is compared with that of 5′-MeAMP. For both the dimer and the monomer, the H-2 proton has the largest value of $\Delta\delta$. The $\Delta\delta$ of the H-8 is the next largest and the $\Delta\delta$ of H-1′ is the smallest for ApA; while for the monomer, the values of $\Delta\delta$ of H-8 and that of H-1′ are about the same. It should be noted also that the maximal value of $\Delta\delta$ for ApA is reached at about 0.1 M concentration (Figure 10) while the maximum value of $\Delta\delta$ for 5′-MeAMP is not reached until about 0.3 M (Schweizer *et al.*, 1968). Both ApA and 5′-MeAMP have about the same maximal $\Delta\delta$ value for their H-2 protons, and one of the H-8 (3′) protons of ApA also has the same maximal value as the H-8 proton of 5′-MeAMP (Table VIII). On the other hand, both H-1′ protons of the ApA have distinctly lower maximal $\Delta\delta$ as compared with that of the H-1′ proton of the monomer. These data (Table VII) clearly indicate that ApA molecules also associate significantly to form stacks as the monomeric AMP in aqueous solution of moderate concentration. The geometrical arrangement of the adenosine and AMP molecules in a stack with respect to each other in aqueous solution has been previously discussed in considerable detail (Broom *et al.*, 1967; Schweizer *et al.*, 1968). The model for the stack of these monomers indicates that the six-membered ring of one AMP is partially overlapping the six-membered ring of its nearest neighbor in the stack, and similarly the five-membered ring of this AMP molecule is partially overlapping the five-membered ring of its nearest

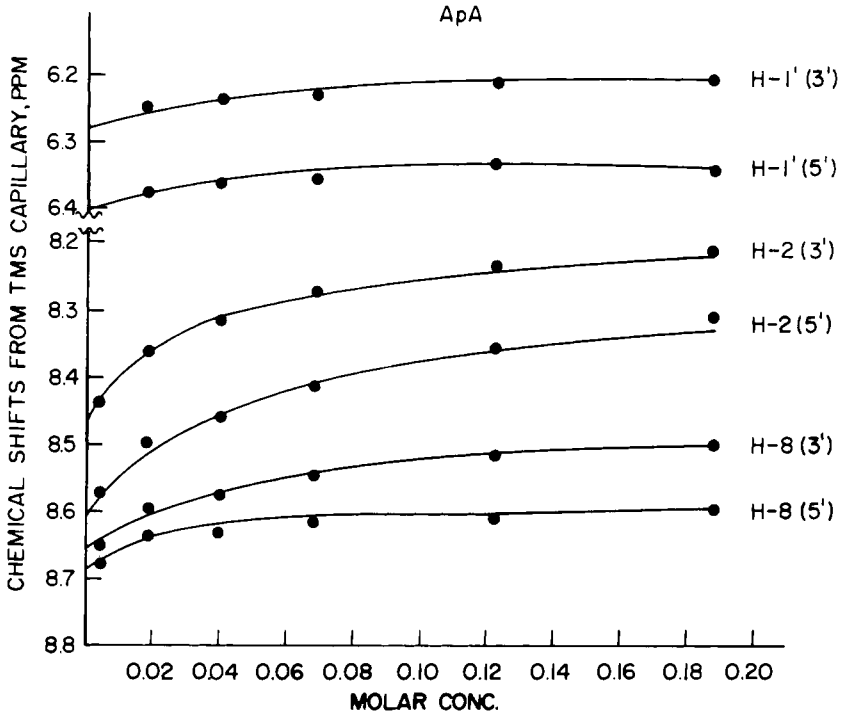



FIGURE 10: The concentration dependence of the chemical shifts of the base and H-1′ protons of ApA in $D_2O$, pD 7.4, 28°. The solution concentration with reference to the dimer for the 0.12 and 0.19 M samples were determined by ultraviolet absorption, and the concentrations for the other samples were determined by direct weighing.

TABLE VIII: Comparative Studies on the Concentration Dependence of Chemical Shifts for Protons of ApA and 5′-MeAMP.[a]

| | Concn (*m*) | H-8 (5′) | H-8 (3′) | H-2 (5′) | H-2 (3′) | H-1′ (5′) | H-1′ (3′) |
|---|---|---|---|---|---|---|---|
| ApA | 0.0 | 8.69 | 8.66 | 8.61 | 8.47 | 6.41 | 6.29 |
| | 0.188 | 8.60 | 8.50 | 8.31 | 8.21 | 6.34 | 6.21 |
| | | Δδ 0.09 | 0.16 | 0.30 | 0.26 | 0.07 | 0.08 |
| 5′-MeAMP | 0.0 | 8.88 | | 8.71 | | 6.605 | |
| | 0.188 [b] | 8.78 | | 8.49 | | 6.49 | |
| | | Δδ 0.10 | | 0.22 | | 0.115 | |
| | 0.30 [b] | 8.73 | | 8.41 | | 6.45 | |
| | | Δδ 0.15 | | 0.31 | | 0.16 | |

[a] Chemical shift from tetramethylsilane capillary in parts per million. [b] From Schweizer *et al.* (1968).

neighbor. The data in Table VIII, therefore, suggest that the geometrical arrangement of one (A) unit in the ApA to its nearest neighboring (A) unit from another ApA molecule in the stack must be rather similar to the geometrical arrangement of the AMP molecules in the stack of the monomers, with the exception that the H-1′ protons of the ApA are probably farther away from the diamagnetic influence of the neighboring adenine rings. From stereochemical consideration, this explanation of the lower maximal value of $\Delta\delta$ for H-1′ is rather reasonable.

One of the most interesting observations in Table VIII is that the $\Delta\delta$ value for the H-8 (3′) proton is significantly larger than that for the H-8 (5′) proton of ApA. This observation can be explained on the basis of the stacked conformation of ApA proposed and described fully in a later section. This stacked model of ApA (or any dinucleotide, XpY) is built from two conformational requirements: (1) each nucleoside unit has an *anti* conformation; and (2) the (3′–5′) screw axis has the right-handed turn. In this *anti*, right-handed stack of ApA, the H-8 (5′) proton is pointing toward the phosphate group, participating as an inner part of the cleft between two parallel bases, and is almost completely surrounded. On the other hand, the H-8 (3′) proton is located outside of the cleft, is completely open on one side, and is only partially surrounded from the other side by its own ribosyl group. Therefore, the H-8 (3′) is much more accessible than the H-8 (5′) to the approaching base from the other ApA molecule. To a lesser extent, the H-2 (5′) of ApA has a larger $\Delta\delta$ value than that of the H-2 (3′) (Table VII). In this *anti*, right-handed model of the stacked ApA, the H-2 (3′) is shielded on one side by the adenine base of the 5′-(pA) unit, while the H-2 (5′) is not shielded from either side. This difference in the concentration dependence of the base protons of ApA in association to form stacks is considered to be a support for the *anti*, right-handed model.

The effect of concentration on the spectrum of ApA has also been studied by Chan and Nelson (1968). Their basic observations are essentially in agreement with ours.

Association of certain dimers, trimers, and tetramers in aqueous solution at the concentration range of 0.005–0.01 M (total residue concentration) in aqueous solution has been studied by optical rotatory dispersion techniques (Jaskunas *et al.*, 1968). They found no association of ApC, GpU, or an equimolar mixture of ApC and GpU in 0.5 M NaCl at 1°. Self-association of ApGpC and GpGpC was observed in 0.01 M $MgCl_2$ at 1°. Jaskunas *et al.* detected no association of ApApApA, while association of ApA is observed here. It is likely that proton magnetic resonance may be more sensitive than optical rotatory dispersion in detecting the association of oligomers by stacking. There is also another interesting possibility. The self-association by stacking of the oligomer $(X)_n$ is likely first to increase with the degree of polymerization, *n*, to a certain value of *n*; then may decline as the value of *n* continues to increase. From this consideration of the "end effect," it is possible that ApA may have a greater tendency to self-stacking than ApApApA. Through optical rotatory dispersion and ultracentrifuge studies, Jaskunas *et al.* also concluded that GpGpC and GpCpC associate to form complexes of higher order. The strong tendency of GMP or dGMP to associate in forming a helical structure has been known through the work of several laboratories (Gellert *et al.*, 1962; Miles and Frazier, 1964; Sarkar and Yang, 1965). Scheit *et al.* (1967) compared the chemical shifts of H-8 and H-2 of (-pdA) in 5′-*O*-alkyl-pdT-pdTpdA with the chemical shifts of the corresponding protons of 5′-dAMP measured at 0.1 M concentration. The H-2 of (-pdA) in the trimer was found to be at lower field than the H-2 of AMP by about 0.14 ppm, while the H-8 protons have about the same $\delta$ value in both compounds. Based on these observations, they proposed that there is a hydrogen bonding between the T and A of these trimers in solution. They did not realize that at 0.1 M concentration, the H-2 proton of 5′-dAMP is shielded upfield by about 0.15 ppm and the H-8 proton by about 0.08 ppm as compared with those values extrapolated to infinite dilutions, because of self-stacking. Therefore, the $\delta$ value of H-2 of the (-pdA) in the trimer is about the same as that of the

5′-dAMP at infinite dilution, while the $\delta$ value of H-8 of the (-pdA) in the trimer is about 0.08 ppm *upfield* in comparison with the $\delta$ value of H-8 of 5′-dAMP. As discussed in great detail in the previous section (II) on assignment and in the following section (IV) on conformation, this upfield shift of the H-8 of (-pdA) is due to the reduction of the deshielding effect of the 5′-phosphate group on the H-8 proton when the phosphate group is transformed from a monoester in 5′-AMP to a diester in the trimer. Therefore, there is no evidence from proton magnetic resonance at present for the occurrence of hydrogen bonding of base pairs in aqueous solution of dimers or trimers.

### *IV. Conformation*

The observations presented in the above sections provide sufficient data for describing the general features of dinucleoside monophosphate conformation in aqueous solution at 28–30°. For the sake of presentation, a model will first be constructed as a working hypothesis. It will be shown in this section that all the chemical shift data of the dimers can be explained on the basis of this model together with the proton magnetic resonance data of the monomers in a unifying manner.

This model is constructed with two conformational requirements. The first one is that all the nucleosidyl units in the dinucleoside monophosphates have the *anti* conformation. There are three reasons to adopt this requirement: (1) As shown in our previous publication (Schweizer *et al.*, 1968) and in section I, all the 5′-nucleotides and thus most likely all the nucleosides have the *anti* conformation. (2) As shown in Table V, section II, it can be concluded that the (pAp-) unit in pApA, the (pUp-) unit in pUpU, and the (pTp-) unit in pTpT all have the *anti* conformation. (3) All the X-ray data and stereochemical analysis based on X-ray data indicated that most of the purine and pyrimidine nucleosides and nucleotides exist in the solid state with the *anti* conformation, and that there is a substantial rotational barrier between the *syn* and *anti* conformations, and a rapid conversion between them is unlikely (Kraut, 1965; Haschemeyer and Rich, 1967; Sasisekharan *et al.*, 1967; Davies, 1967).

The second conformational requirement for this model is that the (3′–5′) screw axis of all the dinucleoside monophosphates have a right-handed turn in their stacked form. The strongest support for this principle so far is from the experimental and theoretical study of the dinucleoside monophosphates by optical rotatory dispersion (Warshaw and Tinoco, 1965; Bush and Tinoco, 1967). It is noteworthy that the conformation of the stacked dimer according to this model is similar to that of a segment of the strand in the helical DNA; both have a right-handed turn for the (3′–5′) axis and have the nucleotidyl units in the *anti* conformation.

There are two basic guidelines from the proton magnetic resonance studies on the mononucleotides which are pertinent in relating the proton magnetic resonance data to the conformation. The first guideline is that with the nucleoside in the *anti* conformation, the 5′-phosphate group will specifically deshield the H-8 proton but not the H-2 proton of the purine nucleosides, and will deshield the H-6 proton and much less the H-5 proton of the pyrimidine nucleosides. The 3′-phosphate group has no effect on the base protons. However, since the phosphate diester has less deshielding effect than the monoester (Schweizer *et al.*, 1968), the phosphate group of the dinucleoside monophosphate will have a smaller influence on the base protons than the phosphate of the 5′-mononucleotide as discussed in section II. The second guideline is that on a time average basis if a proton is closely located beneath or above the ring of a base, this proton will be shielded by the ring current of this base ring. The ring-current intensities of various base rings relative to benzene have been calculated by Giessner-Prettre and Pullman (1965) as follows: adenine, six-membered ring (0.9); adenine, five-membered ring (0.7); guanine, five-membered ring (0.7); guanine, six-membered ring (0.2); cytosine (0.2); and uracil (0.05). Therefore, the shielding effect of the six-membered ring of adenine is the largest and that of the uracil ring is the smallest. The studies on the concentration dependence of the chemical shifts of various bases, nucleosides, and nucleotides due to stacking in aqueous solution have amply supported this conclusion (Chan *et al.*, 1964; Jardetzky, 1964; Schweizer *et al.*, 1965, 1968; Broom *et al.*, 1967). These calculated ring-current values for various bases have been useful also for the computation of the relative chemical shifts of the purine and the nucleosides (Ts'o *et al.*, 1969).

Two procedures are adopted for the analysis of the chemical shift data of the dinucleoside monophosphates. The first procedure is to compare the values of the 5′-nucleotidyl unit or the 3′-nucleotidyl unit in the dimer with the values of the corresponding 5′-mononucleotides or 3′-mononucleotides. This difference is termed dimerization shift ($\Delta\delta_D$, usually negative) which reflects the shielding of this proton by the neighboring nucleotidyl unit in the dimer. The second procedure is to compare the $\delta$ values and the $\Delta\delta_D$ values of the dimer XpY *vs.* that of the dimer YpX. A comparative study on the effect of sequence was found to be most demonstrative and convenient in relating the value of $\delta$ and $\Delta\delta_D$ to the model through the guidelines established in the previous proton magnetic resonance work. In addition, our experience has shown that an examination of the molecular model XpYpX is the best mean to compare the difference due to sequence between the dimer XpY and YpX. In the trimer of XpYpX, the X unit and the Y unit can be viewed as being a 3′ unit (Xp- or Yp-) or a 5′-unit (-pX or -pY) simultaneously. Two kind of models are presented, one of which is the color photo of the space-filling Ealing CPK models, and the other kind is a schematic presentation of arrangements of the base protons. The dimensions of the bases are those determined by X-ray diffraction (described in "Polynucleotides"; Steiner and Beers, 1961). Either type of model is constructed with the two conformational requirements cited above, *i.e.*, nucleoside unit in *anti* conformation, and the

TABLE IX: Comparative Study on the Effect of Sequence on the Chemical Shifts ($\delta$) and Dimerization Shifts ($\Delta\delta_D$) of the Base and H-1′ Protons of the Dinucleoside Monophosphates[a] (28–30°, pD 5.9; in parts per million, all Negative Unless Otherwise Indicated). For 5′-Nucleotidyl Unit[b] $\Delta\delta_D = \delta_{pY} - \delta_{XpY}$ and for 3′-Nucleotidyl Unit[b] $\Delta\delta_D = \delta_{Xp} - \delta_{XpY}$.

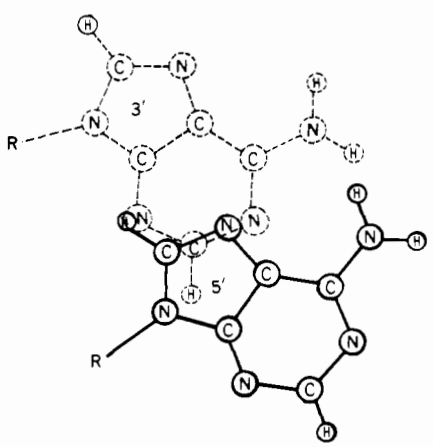
| | A or G | | | | | | C, U, or T | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-8 | | H-2 | | H-1′ | | H-6 | | H-5 | | H-1′ | |
| | $\delta$ | $\Delta\delta_D$ | $\delta$ | $\Delta\delta_D$ | $\delta$ | $\Delta\delta_D$ | $\delta$ | $\Delta\delta_D$ | $\delta$ | $\Delta\delta_D$ | $\delta$ | $\Delta\delta_D$ |
| ApC | 8.75 | 0.03 | 8.55 | 0.14 | 6.45 | 0.09 | 8.13 | 0.30 | 6.11 | 0.42 | 6.16 | 0.25 |
| CpA | 8.82 | 0.11 | 8.63 | 0.06 | 6.51 | 0.05 | 8.11 | 0.18 | 6.24 | 0.25 | 6.13 | 0.24 |
| (2′–5′)-ApC | 8.72 | 0.05[c] | 8.40 | 0.26[c] | 6.59 | 0.02[c] | 7.77 | 0.66 | 6.11 | 0.42 | 5.98 | 0.43 |
| ApU | 8.75 | 0.03 | 8.60 | 0.09 | 6.47 | 0.08 | 8.18 | 0.26 | 6.06 | 0.33 | 6.18 | 0.23 |
| UpA | 8.83 | 0.10 | 8.63 | 0.06 | 6.51 | 0.05 | 8.15 | 0.16 | 6.18 | 0.14 | 6.13 | 0.24 |
| GpC | 8.44 | +0.01 | | | 6.29 | 0.10 | 8.23 | 0.20 | 6.13 | 0.40 | 6.31 | 0.10 |
| CpG | 8.43 | 0.12 | | | 6.31 | 0.04 | 8.16 | 0.13 | 6.31 | 0.18 | 6.19 | 0.17 |
| GpU[d] | 8.42 | 0.01 | | | 6.33 | 0.06 | 8.26 | 0.18 | 6.17 | 0.22 | 6.33 | 0.08 |
| UpG[d] | 8.45 | 0.10 | | | 6.32 | 0.03 | 8.18 | 0.13 | 6.21 | 0.11 | 6.20 | 0.17 |
| CpU | | | | | | | 8.31 (C)<br>8.34 (U) | +0.03 (C)<br>0.10 (U) | *e* | *e* | *e* | *e* |
| UpC | | | | | | | 8.35 (C)<br>8.33 (U) | 0.08 (C)<br>0.02 (U) | 6.45 (C)<br>6.28 (U) | 0.08 (C)<br>0.04 (U) | 6.37 (C)<br>6.26 (U) | 0.04 (C)<br>0.11 (U) |
| ApG[d] | 8.67 (A)<br>8.35 (G) | 0.11 (A)<br>0.20 (G) | 8.52 | 0.17 | 6.34 (A)<br>6.24 (G) | 0.21 (A)<br>0.11 (G) | | | | | | |
| GpA[d] | 8.76 (A)<br>8.32 (G) | 0.17 (A)<br>0.11 (G) | 8.62 | 0.07 | 6.51 (A)<br>6.12 (G) | 0.05 (A)<br>0.27 (G) | | | | | | |
| ApA[f] | 8.69 (5′)<br>8.66 (3′) | 0.24 (5′)<br>0.12 (3′) | 8.61 (5′)<br>8.47 (3′) | 0.07 (5′)<br>0.22 (3′) | 6.41 (5′)<br>6.29 (3′) | 0.15 (5′)<br>0.26 (3′) | | | | | | |
| CpC | | | | | | | 8.34 (5′)<br>8.29 (3′) | 0.09 (5′)<br>0 (3′) | *e* | *e* | *e* | *e* |
| UpU | | | | | | | 8.34 (5′)<br>8.33 (3′) | 0.10 (5′)<br>+0.02 (3′) | 6.32 (5′)<br>6.29 (3′) | 0.07 (5′)<br>0.03 (3′) | 6.37 (5′)<br>6.31 (3′) | 0.04 (5′)<br>0.06 (3′) |
| TpT | | | | | | | 8.12 (5′)<br>8.08 (3′) | 0.08 (5′)<br>0.02 (3′) | (Methyl)<br>2.32<br>(3′ and 5′) | 0.02 (5′)<br>0 (3′)[g] | 6.75 (5′)<br>6.66 (3′) | 0.02 (5′)<br>0.05 (3′)[g] |

[a] The $\delta$ values for the XpY are from Table III. [b] The $\delta$ values for the Xp or pY are from Table I. The accuracy of the $\Delta\delta_D$ is about $\pm 0.01$ ppm. [c] The $\delta$ values for the 2′-AMP were obtained from our previous paper (Schweizer *et al.*, 1968). [d] At 0.004 M concentration (Table III). [e] Spectral peaks undiscernible. [f] At infinite dilution (Table VIII). [g] The $\delta$ values of thymidine (Table I) are used in substitution for the $\delta$ values of 3′-TMP.

helical turn is right handed. The $\delta$ value and the $\Delta\delta_D$ values of the base protons and the H-1′ protons of 18 dimers are presented in Table IX.

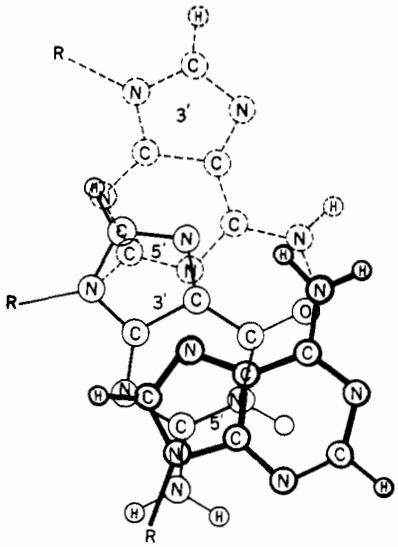
The schematic model of ApA is shown in Figure 11 and the photos of both the front view and the back view of the Ealing CPK model of ApA are presented in Figure 1. These models depict that the H-8 of the (Ap-) and the H-2 of (-pA) are far away from the bases of the neighboring unit and should have small values of $\Delta\delta_D$. The H-2 of the (Ap-) is located beneath the five-membered ring of (-pA) and should be shielded substantially with a large $\Delta\delta_D$ value. The H-8 of (-pA) should be located downfield from the H-8 of (Ap-) because of the deshielding effect of the phosphate group nearby. The $\Delta\delta_D$ value of the H-8 of (-pA) should be also much larger than the $\Delta\delta_D$ of H-8 of (Ap-) for two reasons: (1) H-8 of (-pA) is much closer to the base rings of the neighboring unit; (2) in comparison with the $\delta$ values of the 5′-AMP, the deshielding effect of the phosphate is reduced because of the transformation of a monoester phosphate group in a monomer to a diester phosphate group in a dimer as discussed above. Furthermore, as shown from the back view of the ApA (Figure 1), the H-1′ proton of (Ap-) is more directly under the base ring of the neighboring unit, while the H-1′ of (-pA) is farther away from the neighboring base ring. All these predictions about the $\delta$ values and the $\Delta\delta_D$ values from this model of ApA are confirmed in Table IX. Thus, the $\delta$ values of six protons (two H-2 protons, two H-8 protons, and two H-1′ protons) of ApA in comparison with the $\delta$ values of these six protons of the corresponding monomeric units have strongly substantiated these two conformational requirements employed in building this model.

Similar reasoning can be applied to relate the model of ApGpA (Figures 12 and 2) to the proton magnetic resonance data. The H-8 of (-pG) in ApG should be at lower field than the H-8 of (Gp-) in GpA because of the proximity of the 5′-phosphate group. The $\Delta\delta_D$ value of the H-8 of (-pG) in ApG should have a larger




ApA

FIGURE 11: A schematic presentation of the front view of the bases of ApA in the "*anti,anti*, right-handed" model. The (3′–5′) screw axis is advancing forward from the plane of the paper.




Ap Gp A

FIGURE 12: A schematic presentation of the front view of the bases of ApGpA in the "*anti,anti*, right-handed" model for the interpretation of the dimer data of the ApG and GpA. The H-2 proton of (AP-) in ApG is directly under the N-9 of (-pG). The (3′–5′) screw axis is advancing forward. The base with broken line is in back, and the base with the heavy line is in front.

value than that of the H-8 of (Gp-) in GpA because of the closer proximity of the (5′) H-8 to the neighboring ring and the transformation of the monoester to diester of the phosphate group. The H-1′ of (Gp-) in GpA is again more shielded than the H-1′ of (-pG) in ApG, a condition identical with that in ApA. As for the protons in the (A) units, the H-2 of (-pA) in GpA is much less shielded than the H-2 of (Ap-) in ApG. However, the shielding of H-2 of (Ap-) by (-pG) in ApG is less (0.17 *vs.* 0.22) than the shielding of the H-2 of (Ap-) by the (-pA) unit in ApA, presumably reflecting the reduction of ring current in the six-membered ring in G as compared with the six-membered ring in A. The H-8 of (-pA) in GpA and the H-1′ of (Ap-) in ApG again have a larger $\Delta\delta_D$ value than their corresponding sequential counterpart, *i.e.*, H-8 of (Ap-) in ApG and the H-1′ of (-pA) in GpA, as shown in the model. The $\Delta\delta_D$ for the H-8 of (-pA) in GpA is reduced (0.17 *vs.* 0.24) as compared with the same proton in ApA indicating that this proton is likely to be influenced by the six-membered ring of its neighboring base, the ring current of which has been reduced when it is G instead of A. On the other hand, the $\Delta\delta_D$ for the H-1′ of (Ap-) in ApG is increased (0.21 *vs.* 0.15) as compared with the same proton in ApA, indicating this proton is under the influence of the five-membered ring of its neighbor; these data suggest that the H-1′ of (Ap-) is closer to the five-membered ring of (-pG) in ApG than to that of (-pA) in ApA, probably a reflection of greater folding in the stack. This possibility of a larger extent of base overlapping in ApG as compared with ApA is also supported by the observation that the $\Delta\delta_D$ of H-8 of (-pG) in ApG is *less* than the $\Delta\delta_D$ of H-8 of pA in ApA. This is suggestive that the H-8 of

(-pG) is closer to the five-membered ring of the neighboring (Ap-) and the H-8 of (-pA) is closer to the six-membered ring of the neighboring (Ap-). A larger extent of the overlapping of (-pG) over (Ap-) is needed in order for the (5′) H-8 proton to be close to the five-membered ring instead of the six-membered ring of the neighboring (Ap-).

The same way may be followed to analyze the proton magnetic resonance data of the purine–pyrimidine dimers such as ApC *vs.* CpA. As shown in the models (Figures 13 and 3) the H-6 of (-pC) is located at lower field than the H-6 of (Cp-) because of the adjacent phosphate group. The H-6 of (-pC) has a much larger $\Delta\delta_D$ value (0.30) than the H-6 of (Cp-) because of the closer proximity to the six-membered ring of (Ap-) and of the transformation of the phosphate group from monoester to diester. The $\Delta\delta_D$ value (0.42) of the H-5 of (-pC) is even larger, because this proton is located right on the top of the six-membered ring of (-pA). As shown in the model, the H-5 of (Cp-); on the other hand, is much less shielded by the (-pA); therefore, the value of $\Delta\delta_D$ (0.25) is much less than that of (-pC). Nevertheless, the $\Delta\delta_D$ value of the H-5 of (Cp-) is not small, indicating in a time average basis, the H-5 of (Cp-) is shielded to a certain extent by the neighboring (-pA). The H-1′(s) of (Cp-) and (-pC) are shielded to about the same extent by the neighboring A unit. This is consistent with the model though not shown in the front view (Figure 3). As shown in Table IX, the same situation is found for the H-1′ (s) of (Up-) and (-pU) in UpA and ApU. As for the protons of the (A) units, they have low $\Delta\delta_D$ values, reflecting the diminished ring-current effect of the neighboring pyrimidine. The H-8 of (-pA) is located downfield from the H-8 of (Ap-), again due to the deshielding influence of the

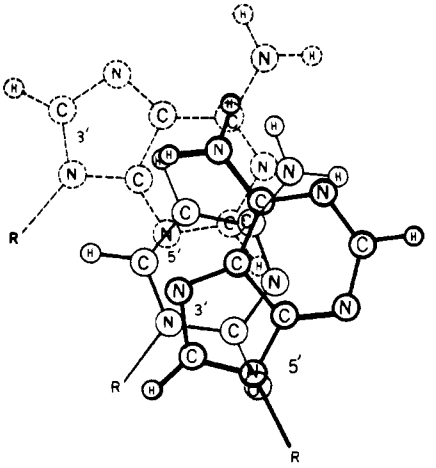



FIGURE 13: A schematic presentation of the front view of the bases of ApCpA in the "*anti,anti*, right-handed" model for the interpretation of the dimer data of ApC and CpA. The (3′–5′) screw axis is advancing forward. Base with the broken line is in back and the base with the heavy line is in front.

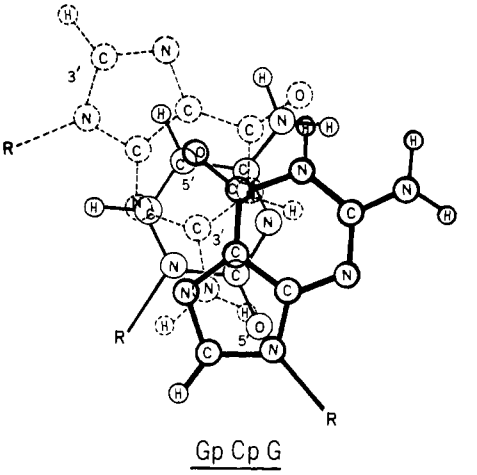



FIGURE 14: A schematic presentation of the front view of the bases of GpCpG in the "*anti,anti*, right-handed" model for the interpretation of the dimer data of GpC and CpG. The (3′–5′) screw axis is advancing forward.

phosphate group nearby. The H-8 of (-pA) also has a larger $\Delta\delta_D$ value than the H-8 of (Ap-) as expected because of the phosphate transformation. The H-2 of (Ap-) is shielded specifically by the (-pC); as shown by the model, this H-2 proton is located right under the cytosine ring.

Analysis of the proton magnetic resonance data of ApU *vs.* that of UpA is strictly similar to the analysis performed for ApC *vs.* CpA, mindful of the fact that the ring-current effect of U is even less than that of C.

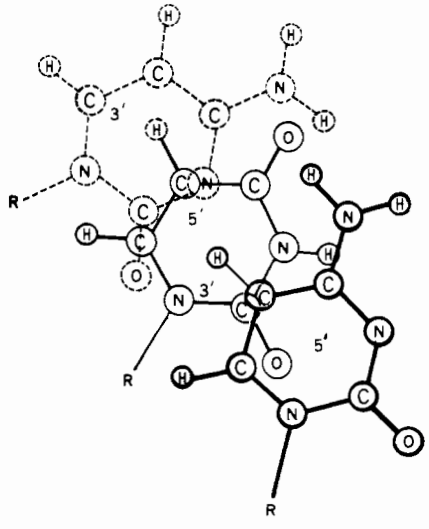
Analysis of the proton magnetic resonance data of GpC *vs.* that of CpG again follows the same pattern as the analysis of ApC *vs.* CpA. It should be remembered, however, that the six-membered ring of G has much less ring-current effect than the six-membered ring of A though the ring-current effects of the five-membered ring of these two bases are calculated to be roughly comparable. Therefore, in order for the H-5 of (-pC) in GpC to have a $\Delta\delta_D$ value (0.40) as that for the H-5 of (-pC) in ApC (0.42), the H-5 of (-pC) must be in the dimagnetic five-membered ring of the (Gp-). This situation requires an extent of overlapping of C over G in GpC larger than that in ApC (Figure 14). The $\Delta\delta_D$ value of H-1′ of (-pC) (0.10) is less than that of H-1′ of (Cp-) (0.17). This difference suggests that the H-1′ of (-pC) is more under the influence of the six-membered ring (less ring-current effect) and the H-1′ of (Cp-) is more under the influence of the five-membered ring (more ring-current effect).

The analysis of GpU *vs.* UpG is strictly analogous to that for the GpC *vs.* CpG. The extent of overlapping of the U over G in UpG must be less than that of C over G in CpG, since the H-5 of (Up-) has a smaller value of $\Delta\delta_D$ (0.22).

The analysis of proton magnetic resonance data of the pyrimidine dimers in terms of a conformation model is done with much less certainty. It is simply that in the absence of strong ring-current effect, the spatial orientation of the neighboring unit relative to the proton of interest cannot be surely ascertained. A model of

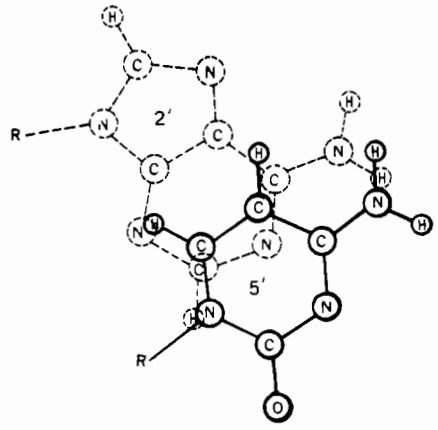
CpUpC built with these two conformational requirements is shown in Figures 4 and 15. The H-6 of (-pC) in UpC is located downfield from the H-6 of (Cp-) in CpU, because of the deshielding effect of the 5′-phosphate in proximity. This is true for all the pyrimidine dimers, indicating that all the nucleotidyl units have the *anti* conformation. The H-6 of (-pU) is probably shielded to a certain extent by the (Cp-) in CpU as shown in the model, since this H-6 proton is located only slightly downfield from the H-6 of (Up-) in UpC. The larger $\Delta\delta_D$ value of H-5 of (-pC) than that of the H-5 of (Up-) in UpC, and the larger $\Delta\delta_D$ value of H-1′ of (Up-) than that of the H-1′ of (-pC) in UpC, all support the model shown in Figure 4. Spectra of UpU and TpT all show the same tendency in this comparison, even though the values of the $\Delta\delta_D$ are in general very small.

The proton magnetic resonance data of (2′–5′)-ApC is very interesting especially in comparison with the naturally occurring (3′–5′)-ApC (Table IX). The H-2 of (Ap-2′), the H-6 of (-pC), and the H-1′ of (-pC) all have much larger values of $\Delta\delta_D$, indicating these protons are much more shielded by their neighboring units than their corresponding protons (H-2 of Ap-, H-6 and H-1′ of -pC) in the (3′–5′)-ApC. These data strongly support the model of (2′–5′)-ApC as shown in Figures 5 and 16. As presented, the H-6 of (-pC) is right over the six-membered ring of the (Ap-2), thereby having the largest $\Delta\delta_D$ value (0.66) ever observed for the dimer (Table IX). The back view of the (2′–5′)-ApC (Figure 5) also shows that the H-1′ of (-pC) is located very near to the six-membered ring of (Ap-2′), thus providing the reason for the large $\Delta\delta_D$ value (0.43) observed. All the proton magnetic resonance data indicate that the overlapping of C over A is much more extensive in (2′–5′)-ApC than that in (3′–5′)-ApC. The same conclusion is drawn when one compares




CpUpC

FIGURE 15: A schematic presentation of the front view of the bases in CpUpC.




(2′–5′)ApC

FIGURE 16: A schematic presentation of the front view of the bases in (2′–5′)-ApC.

Figure 13 with Figure 16, and compares Figure 3 with Figure 5.

This sequence effect on the proton magnetic resonance spectra of the dimers has also been observed before by McDonald *et al.* (1967) in their study on the $\delta$ values of the thymidine methyl proton in the dideoxynucleoside monophosphates, TpdX and dXpT (X = T, C, A, and G). They presented a dApTpdA model constructed in accordance with the stacking relationship of the bases in a strand of the helical DNA. Based on this schematic model of this trimer, they could interpret the data by stating "the methyl group of thymine is quite close to the adenine ring on the 5′ side and is quite remote from the adenine ring at the 3′-neighbor position." It should be emphasized that the geometrical relationship of the bases in a strand of the helical DNA consists of the same two conformational requirements adopted for our model, *i.e.*, "the nucleosidyl units in *anti* conformation" and "the helical turn in *right handedness*." Their observation of the $CH_3$-proton shielding of T in dApT but much less in TpdA is strictly analogous to our observation of the shielding of H-6 proton of U (or C) in ApU (or ApC) but much less in UpA (or CpA). (Some confusion may arise from the terminology employed in these two papers. The (Ap-) unit in ApU is described here as the 3′-AMP unit by us, and the (dAp-) unit in dApT was described as the adenine on the 5′ side of the thymidine by McDonald *et al.* (1967)). Therefore, their observation on the methyl proton of T in TpdX and dXpT also supports our model constructed for the ribosyl dimers. Chan and Nelson (1968) have also concluded from their studies in ApA that this dimer has the *anti* conformation and right-handed turn.

The sequence effect on the optical rotatory dispersion patterns of the dinucleoside monophosphates was first reported by Warshaw and Tinoco (1966). The most dramatic difference was observed between GpA *vs.* ApG. A certain degree of success has been acheived

by the theoretical computation of the optical rotatory dispersion patterns based on geometric relationships of the bases of a strand in the DNA helix, to account for the sequential effect observed (Bush and Tinoco, 1967). As discussed above, this "DNA model" is basically the same as that proposed here. The sequence effect on the circular dichroism patterns of the dimer has been reported by Brahms *et al.* (1967). The difference in the circular dichroic spectra between the sequential isomers XpY and YpX is very demonstrable, though the difference in ultraviolet absorption spectra is very small.

The titration properties of UpA *vs.* ApU as well as UpU and ApA have been investigated by spectrophotometric methods (Simpkins and Richards, 1967). The pH values at half-ionization were found to be different between UpA and ApU; this difference was interpreted to indicate that ApU has a greater tendency to stack than the UpA. It is interesting to note that, according to our dimer model (Figures 13 and 3; ApCpA is essentially the same as ApUpA), the uracil is overlapped almost exclusively with the six-membered ring of A in ApU, but is overlapped mainly with the five-membered ring of A in UpA.

Fluorescence and phosphorescence of dimers have been studied by Eisinger *et al.* (1966), by Gueron *et al.* (1966), and by Helene and Michelson (1967). These groups have reported that notable differences in fluorescence spectra existed between the sequence isomers, such as ApU *vs.* UpA, ApC *vs.* CpA, and ApG *vs.* GpA. Gueron *et al.* have found that the electron paramagnetic resonance intensity of the adenine, measured upon illumination, differs between ApC and CpA.

Our conclusion about the conformation of (2′–5′)-ApC based on the proton magnetic resonance study is

TABLE X: Chemical Shifts of Base and H-1′ Protons of Dinucleoside Monophosphates at 60° (0.02 M, $D_2O$, pD 7.4).[e]

| Dinucleoside | $A_{H-8}$ | $G_{H-8}$ | $A_{H-2}$ | $C_{H-6}$ | U, $T_{H-6}$ | $C_{H-5}$ | $U_{H-5}$ | H-1′ (1) | H-1′ (2) |
|---|---|---|---|---|---|---|---|---|---|
| ApC | 8.83 | | 8.715 | 8.275 | | 6.37 | | 6.57 (A) | 6.36 (C) |
| CpA | 8.91 | | 8.77 | 8.20 | | 6.42 | | 6.62 (A) | 6.30 (C) |
| ApU | 8.83 | | 8.74 | | 8.29 | | 6.26 | 6.58 (A) | 6.34 (U) |
| UpA | 8.91 | | 8.77 | | 8.23 | | 6.33 | 6.62 (A) | 6.29 (U) |
| ApG | 8.75 | 8.47 | 8.68 | | | | | 6.48 (A) | 6.36 (G) |
| GpA | 8.85 | 8.395 | 8.73 | | | | | 6.59 (A) | 6.26 (G) |
| ApA (0.02 M) | 8.81 (5′)<br>8.72 (3′) | | 8.69 (5′)<br>8.61 (3′) | | | | | 6.54 (5′) | 6.40 (3′) |
| (0.004 M) | 8.83 (5′)<br>8.74 (3′) | | 8.71 (5′)<br>8.64 (3′) | | | | | 6.55 (5′) | 6.43 (3′) |
| GpC | | 8.50 | | 8.32 | | 6.37 | | 6.42 (C) | 6.38 (G) |
| CpG | | 8.52 | | 8.22 | | 6.45 | | 6.40 (G) | 6.33 (C) |
| GpU | | 8.48 | | | 8.34 | | 6.35 | 6.43 (G) | *a* |
| UpG | | 8.52 | | | 8.25 | | 6.35 | 6.43 (G) | 6.34 (U) |
| CpU | | | | 8.34 | 8.37 | 6.52 | 6.39 | *a* | *a* |
| UpC | | | | 8.39 | 8.34 | *a* | *a* | 6.47 (C) | 6.385 (U) |
| CpC | | | | 8.39 (5′)<br>8.325 (3′) | | *a* | | *a* | *a* |
| UpU | | | | | 8.38 (5′)<br>8.35 (3′) | *a* | | *a* | *a* |
| TpT | | | | | 8.15 (5′)<br>8.11 (3′) | | 2.37 ($CH_3$) | 6.825 (5′) | 6.76 (3′) |
| A2′p5′C | 8.81 | | 8.59 | 7.92 | | 6.27 | | 6.675 (A) | 6.135 (C)[b] |
| GpG (0.004 M) | | 8.35[c]<br>8.37[c] | | | | | | 6.49 (5′)[d] | 6.23 (3′)[d] |

[a] Unable to discern. [b] Singlet resonance; $J_{1'-2'}$ not visible. [c] Only one spectral peak for both H-8 protons at either 0.02 or 0.004 M. [d] This tentative assignment was made with the assumption that H-1′ of the 5′-nucleotidyl group is located downfield as in the case of ApA. [e] Chemical shifts from tetramethylsilane capillary, ppm.

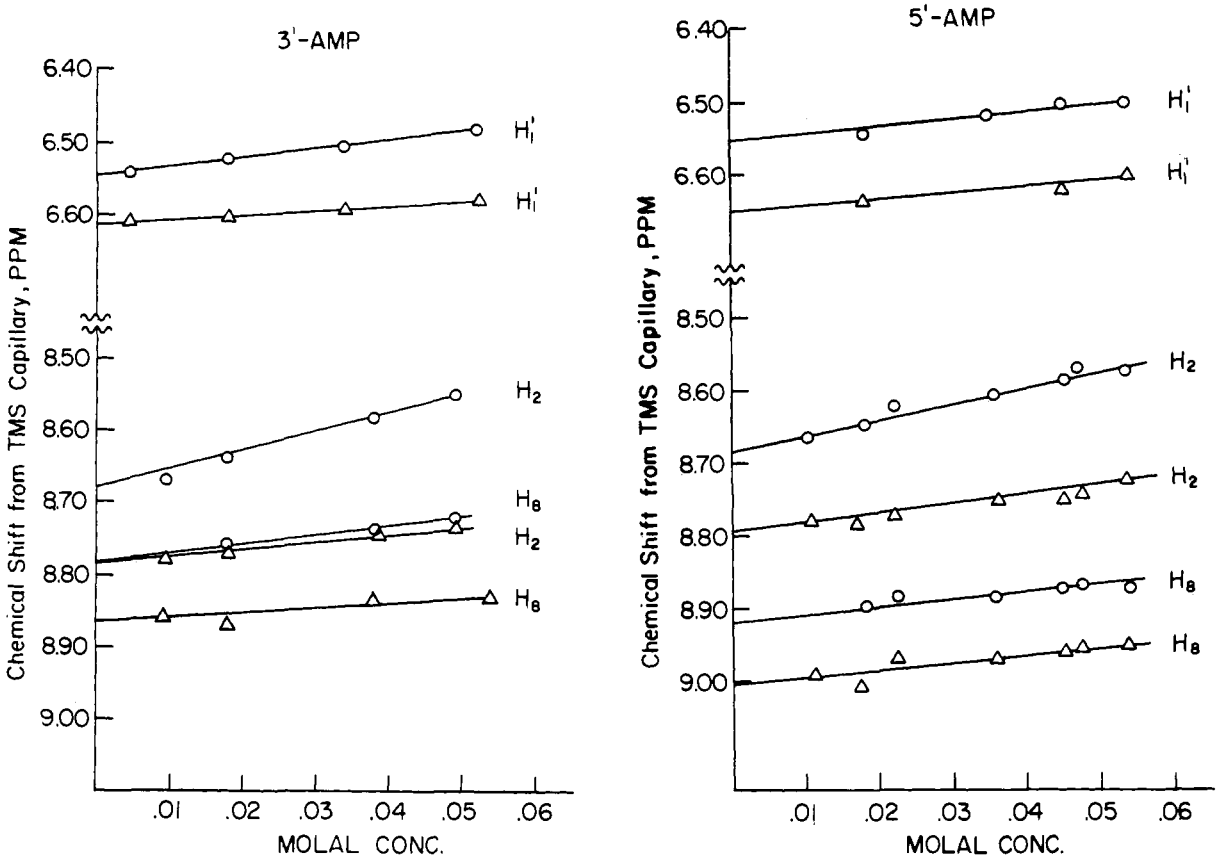



FIGURE 17: Concentration dependence studies. (A, left) Of the chemical shifts of the H-8, H-2, and H-1′ protons of 3′-AMP in $D_2O$, pD 6.3, at 30° (—○—○—) and at 60° (—△—△—). (B, right) Of the chemical shifts of the H-8, H-2, and H-1′ protons of 5′-AMP in $D_2O$, pD 6.3, at 30° (—○—○—) and at 60° (—△—△—).

quite different from that by Brahms *et al.* (1967) based on circular dichroism study. This point will be discussed at a later section (VII) after the presentation of the temperature data.

### *V. Effect of Temperature*

The chemical shifts of the base protons and H-1′ protons of 18 dinucleoside monophosphates at 60° are reported in Table X. The value of the dimerization shift at 60° ($\Delta\delta_D^{60°} = [\delta_{Xp}^{60°}]$ or $[\delta_{pY}^{60°}] - [\delta_{XpY}^{60°}]$, usually negative) can be obtained from the comparison of the $\delta$ values of the dimers (XpY) at 60° with the $\delta$ values of corresponding mononucleotidyl units (Xp or pY) at 60° reported in Table IA. The difference between the dimerization shift at 28–30° ($\Delta\delta_D$) and the dimerization shift at 60° ($\Delta\delta_D^{60°}$) is termed as the "temperature differential" ($D_T = \Delta\delta_D - \Delta\delta_D^{60°}$, usually negative), which is a corrected value for the temperature effect on the chemical shifts. The values for dimerization shift at 28–30° ($\Delta\delta_D$, Table IX), the values of the dimerization shift at 60° ($\Delta\delta_D^{60°}$), and the values of $D_T$ for all the dimers are presented in Table XI. Four major conclusions can be reached from these comparative studies.

(a) As discussed in section I, the differentials in the $\delta$ values for the H-8 or H-6 protons between the 5′-nucleotides and the 3′-nucleotides at 60° are the same as those at 30° (Table II). It was concluded, therefore, that the 5′-nucleotides still assume the same *anti* conformation at 60° as at 30°. This conclusion, by itself, already strongly suggests that the 5′-nucleotidyl units in the dimer at 60° may also have the same *anti* conformation as at 30°; together with the data in Table XI, this conclusion also provides the necessary argument to support this suggestion. This reasoning is most easy to demonstrate with the pyrimidine dimers because of the absence of the ring-current effect in shielding. According to the data in Table XI (or Table IX), for dimers containing U or C, the largest $\Delta\delta_D$ value for a given dimer usually is that of the H-6 proton of the (-pC) or (-pU) unit in the dimer. As explained in section II, this apparent shielding effect originates from the comparison of the deshielding effect of the 5′-phosphate *monoester* of the mononucleotide on the sensitive H-6 proton to the deshielding effect of the 5′-phosphate *diester* of the 5′-unit in the dimer. It is known that the deshielding effect of the diester is about 0.07–0.10 ppm less than that of the monoester (Schweizer *et al.*, 1968). Therefore, this is the source of the $\Delta\delta_D$ value of the H-6 proton. In the case that the 5′-nucleotidyl units in the dimers take a *syn* conformation or oscillate rapidly at 60°, then the sensitive H-6 protons will sway away from the deshielding influence of the 5′-phosphate. As a result, the $\Delta\delta_D^{60°}$ value will become *larger* (up to 0.06–0.10 ppm), since it is now certain that

TABLE XI: Comparative Study on the Dimerization Shifts of the Base and H-1′ Protons of the Dinucleoside Monophosphates at 30° ($\Delta\delta_D$)[a] and at 60° ($\Delta\delta_D^{60°}$)[a] (parts per million). I, $\Delta\delta_D^{60°} = \delta_{pY}^{60°} - \delta_{XpY}^{60°}$ or $\Delta\delta_D^{60°} = \delta_{Xp}^{60°} - \delta_{XpY}^{60°}$, and II,[b] $D_T = \Delta\delta_D - \Delta\delta_D^{60°}$.

| | A or G | | | | | | | | | C, U, or T | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-8 | | | H-2 | | | H-1′ | | | H-6 | | | H-5 | | | H-1′ | | |
| | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ | $\Delta\delta_D$ | $\Delta\delta_D^{60°}$ | $D_T$ |
| ApG (0.004 M) | (A) 0.11 | 0.09 | 0.02 | 0.17 | 0.11 | 0.06 | 0.21 | 0.14 | 0.07 | | | | | | | | | |
| | (G) 0.20 | 0.12 | 0.08 | | | | 0.11 | 0.07 | 0.04 | | | | | | | | | |
| GpA (0.004 M) | (A) 0.17 | 0.16 | 0.01 | 0.07 | 0.07 | 0 | 0.05 | 0.06 | +0.01 | | | | | | | | | |
| | (G) 0.11 | 0.11 | 0 | | | | 0.27 | 0.19 | 0.08 | | | | | | | | | |
| ApA[d] (0.004 M) | (5′) 0.24 | 0.18 | 0.06 | 0.08 | 0.09 | 0.01 | 0.15 | 0.11 | 0.04 | | | | | | | | | |
| | (3′) 0.12 | 0.12 | 0 | 0.22 | 0.15 | 0.07 | 0.26 | 0.20 | 0.06 | | | | | | | | | |
| CpC | | | | | | | | | | (5′) 0.09 | 0.08 | 0.01 | c | c | c | c | c | c |
| | | | | | | | | | | (3′) 0 | 0.02 | +0.02 | c | c | c | c | c | c |
| UpU | | | | | | | | | | (5′) 0.10 | 0.10 | 0 | 0.07 | c | c | 0.04 | c | c |
| | | | | | | | | | | (3′) +0.02 | 0 | 0.02 | 0.03 | c | c | 0.06 | c | c |
| | | | | | | | | | | | | | | (Methyl) | | | | |
| TpT | | | | | | | | | | (5′) 0.08 | 0.08 | 0 | 0.02 | 0.03 | +0.01 | 0.02 | 0 | 0.02 |
| | | | | | | | | | | (3′) 0.02 | 0 | 0.02 | 0.01 | 0.03 | +0.02 | 0.05 | 0 | 0.05 |
| GpG[e] | (5′) | 0.24 | | | | | | −0.07 | | | | | | | | | | |
| | (3′) | 0.15 | | | | | | 0.23 | | | | | | | | | | |
| ApC | 0.03 | 0.03 | 0 | 0.14 | 0.08 | 0.06 | 0.09 | 0.06 | 0.03 | 0.30 | 0.20 | 0.10 | 0.42 | 0.25 | 0.17 | 0.25 | 0.14 | 0.11 |
| CpA | 0.11 | 0.10 | 0.01 | 0.06 | 0.03 | 0.03 | 0.05 | 0.03 | 0.02 | 0.18 | 0.14 | 0.04 | 0.25 | 0.16 | 0.09 | 0.24 | 0.15 | 0.09 |
| (2′–5′)-ApC | 0.05 | 0.04 | 0 | 0.26 | 0.21 | 0.05 | 0.02 | 0.02 | 0 | 0.66 | 0.55 | 0.11 | 0.42 | 0.35 | 0.07 | 0.43 | 0.37 | 0.06 |
| ApU | 0.03 | 0.03 | 0 | 0.09 | 0.05 | 0.04 | 0.08 | 0.04 | 0.04 | 0.26 | 0.19 | 0.07 | 0.33 | 0.21 | 0.12 | 0.23 | 0.14 | 0.09 |
| UpA | 0.10 | 0.10 | 0 | 0.06 | 0.03 | 0.03 | 0.05 | 0.03 | 0.02 | 0.16 | 0.12 | 0.04 | 0.14 | 0.09 | 0.05 | 0.24 | 0.15 | 0.09 |
| GpC | +0.01 | 0 | +0.01 | | | | 0.10 | 0.07 | 0.03 | 0.20 | 0.15 | 0.05 | 0.40 | 0.25 | 0.15 | 0.10 | 0.08 | 0.02 |
| CpG | 0.12 | 0.07 | 0.05 | | | | 0.04 | 0.03 | 0.01 | 0.13 | 0.12 | 0.01 | 0.18 | 0.13 | 0.05 | 0.18 | 0.13 | 0.05 |
| GpU | 0.01 | 0.02 | +0.01 | | | | 0.06 | 0.02 | 0.04 | 0.18 | 0.14 | 0.04 | 0.22 | 0.12 | 0.10 | 0.08 | | |
| UpG | 0.10 | 0.07 | 0.03 | | | | 0.03 | 0 | 0.03 | 0.13 | 0.10 | 0.03 | 0.11 | 0.07 | 0.04 | 0.17 | 0.10 | 0.07 |
| CpU | | | | | | | | | | (C) +0.03 | 0 | −0.03 | | | | | | |
| | | | | | | | | | | (U) 0.10 | 0.11 | −0.01 | c | c | c | c | c | c |
| UpC | | | | | | | | | | (C) 0.08 | 0.08 | 0 | 0.08 (C) | c | c | 0.04 | 0.03 | 0.01 |
| | | | | | | | | | | (U) 0.02 | 0.01 | 0.01 | 0.04 (U) | c | c | 0.11 | 0.06 | 0.05 |

[a] All values of dimerization shifts ($\Delta\delta_D$ or $\Delta\delta_D^{60°}$) are negative unless otherwise indicated. $\Delta\delta_D$ values are from Table IX. $\delta_{pY}^{60°}$ values and $\delta_{Xp}^{60°}$ values are from Table I, and $\delta_{XpY}^{60°}$ values are from Table X. [b] All values of $D_T$ are negative unless otherwise indicated. [c] The peaks in the spectra are not sufficiently discernible. [d] The $\Delta\delta_D$ values of ApA are from infinite dilution (Table IX). [e] The spectrum of GpG at 30° is not observable due to broadening (Table III).

5′-mononucleotides at 60° have the same *anti* conformation as at 30°. In Table XI, the $\Delta\delta_D$ values and the $\Delta\delta_D^{60°}$ values are practically the same for the H-6 of the (-pC), (-pU), (-pT) units in CpC, UpU, CpU, UpC, and TpT. These results show that the (-pC), (-pU), and (-pT) units in the dimer must have the same *anti* conformation at 60° as that at 30°. The same arguments can be applied to the (-pA) units in UpA and CpA. The $\Delta\delta_D$ observed for the H-8 protons of the (-pA) unit in these two dimers again is not due to the shielding by the neighboring C or U which is negligible. The $\Delta\delta_D$ values came from the reduction of the deshielding effect of the phosphate diester in the dimer as compared to that of the phosphate monoesters in the monomers. Since the $\Delta\delta_D^{60°}$ and the $\Delta\delta_D$ of the H-8 in (-pA) units of UpA and CpA have the same values (Table XI), the (-pA) unit in the dimer must have the same *anti* conformation at 60° as that at 30°. This argument cannot be applied directly to the (-pA) or (-pG) units in ApA, ApG, or GpA, since the $\Delta\delta_D$ values of the H-8 of these 5′-purinyl units partially comes from the shielding of the neighboring (Ap-) or (Gp-) units in the dimers. Therefore, the $\Delta\delta_D^{60°}$ values are lower than the $\Delta\delta_D$ values for the H-8 of these (-pA) or (-pG) units in the purine dimers, since the neighboring 3′-purinyl units will be farther away at a higher temperature. Similarly this argument cannot be applied to the H-6 proton of (-pC) or (-pU) in the ApC, ApU, GpC, and GpU dimers, since part of the $\Delta\delta_D$ values of these H-6 protons comes from the shielding effect of the neighboring 3′-purinyl units and low values of $\Delta\delta_D^{60°}$ are expected. A small extent of lowering of the $\Delta\delta_D^{60°}$ values of H-8 of (-pG) in CpG and UpG was observed also (Table XI). In no case, however, was there found an increase of the $\Delta\delta_D^{60°}$ value in comparison with the $\Delta\delta_D$ value, which would be an indication of a shifting away from the *anti* conformation of the 5′ (-pY) units in the dimers at 60°. Though all these data are directly pertaining only to the conformation of the (-pY) units in the dimers at 60°, it is most reasonable to propose that the same holds true also for the 3′ (Xp-) units in the dimers. Therefore, the first conclusion is that at 60° the nucleosidyl units in the dimer most likely have the same *anti* conformation as at 30°. The work of McDonald *et al.* (1967) on the methyl proton resonance in DNA also supports this conclusion. It was stated that "even at 90° in the single-stranded DNA polymer, thymine bases maintain a stacked relationship with respect to purine 5′ neighbors that is similar to that of helical DNA." As explained in section IV, the geometrical arrangement of the bases in the helical DNA is in the *anti* conformation.

(b) The second question concerns the effect of temperature on the ribose as shown by the value of $J_{H\text{-}1'\text{-}H\text{-}2'}$, the coupling constants between the H-1′ and H-2′ protons. The data in Table IB indicate that, in the range of 30–60°, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of all the nucleotides studied are independent of temperature. The same phenomenon was observed for AMP by Hruska and Danyluk (1968a) over the range of about 20° to abouts 80°. On the other hand, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values for (-pA) and (Ap-) in ApA are, respectively, 3.2 and 2.9 at 30° and 4.1 and 4.5 at 60°. These values agree exactly to those reported by Hruska and Danyluk (1968a). In this communication, these authors have reported the temperature dependence of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of ApA, ApA-2′-3′-p, and GpA. They have related the increase of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of ApA to the decrease of base

TABLE XII: Effect of Stacking on the Coupling Constant, $J_{H\text{-}1'\text{-}H\text{-}2'}$, of 5′-AMP.[a]

| Concn (M) | Temp (°C) | $J_{H\text{-}1'\text{-}H\text{-}2'}$ | H-1′[b] | A H-8[b] | A H-2[b] | Pu H-6[b] | Pu H-2[b] | Pu H-8[b] |
|---|---|---|---|---|---|---|---|---|
| 5′-AMP (0.05) | 28–30 | 5.6 | 6.51 | 8.87 | 8.57 | | | |
| 5′-AMP (0.40) | 28–30 | 5.1 | 6.405 | 8.75 | 8.345 | | | |
| 5′-AMP (0.85) | 28–30 | 4.7 | 6.35 | 8.64 | 8.22 | | | |
| 5′-AMP (0.05) and purine (1.0) | 28–30 | 5.2 | 6.14 | 8.50 | 8.01 | 8.96 | 8.86 | 8.66 |
| 5′-AMP (0.05) | 60 | 5.4 | 6.62 | 8.95 | 8.75 | | | |
| 5′-AMP (0.85) | 60 | 5.0 | 6.51 | 8.82 | 8.50 | | | |
| 5′-AMP (0.05) and purine (1.0) | 60 | 5.3 | 6.33 | 8.63 | 8.30 | 9.20 | 9.06 | 8.80 |
| 5′-AMP (0.05) | 82 | 5.5 | 6.63 | 8.94 | 8.77 | | | |
| 5′-AMP (0.85) | 82 | 5.1 | 6.55 | 8.83 | 8.58 | | | |
| 5′-AMP (0.05) and purine (1.0) | 82 | 5.5 | 6.38 | 8.87 | 8.41 | 9.29 | 9.16 | 8.87 |

[a] The accuracy of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value is ±0.1 cps at pD 6.3. [b] Chemical shifts from tetramethylsilane capillary, ppm.

stacking upon increasing temperature. They interpreted this to mean that the base stacking has influenced the conformation of ribose, stating: "The temperature dependence of $J_{H\text{-}1'\text{-}H\text{-}2'}$ for the dimers, therefore, indicates a conformational change of the ribose group from the $C_{3'}$-endo form at low temperatures." The mechanism by which the ribose conformation is affected by stacking, however, was not proposed. While we totally agree with the experimental observation about the change of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of ApA and the reduction of the stacking of the bases upon increasing temperature, we cannot, at present, view the interpretation proposed by Hruska and Danyluk without a great deal of reservation. We feel that the change of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of ApA in this special instance may not necessarily reflect the change of the ribose conformation for the following reasons: (1) as shown in Table XII, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of 5'-AMP is concentration dependent, lowering from 5.6 at 0.05 M to 5.1 at 0.4 M and to 4.7 cps at 0.85 M, 28–30°. While the $J$ value of the AMP at low concentration is temperature independent as described above, the $J$ value of the AMP at high concentration is temperature dependent. As the temperature is being increased, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ of the 5'-AMP at high concentration approaches that at low concentration. The shielding of the H-1' proton at high concentration by the neighboring AMP is also reflected in the upfield shift of the H-1' proton (Table XII). These data clearly implicate the stacking effect and the shielding effect of the ring current of the neighboring base on the value of the $J_{H\text{-}1'\text{-}H\text{-}2'}$. In dimethyl sulfoxide-$d_6$, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of the 5'-AMP (0.04 M sodium salt) is 5.5 cps, within experimental error, the same as that in aqueous solution. It is very difficult to visualize how the ribose conformation of the AMP will not be changed by raising the temperature from 20 to 80° or by switching from an aqueous medium to a dimethyl sulfoxide medium but can be changed by merely being overlapped by another molecule of AMP in the stacks. Therefore, it appears that the concentration dependence of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of AMP is more likely related to the shielding effect through the ring current of the nearby base than the alteration of the ribose conformation. Such a concentration dependence of $J_{H\text{-}1'\text{-}H\text{-}2'}$ was not found for uridine ($4.3 \pm 0.1$) or cytidine ($3.5 \pm 0.1$) at a range of 0.1–0.7 *m*, even though these pyrimidine nucleosides do associate at this high concentration level (Ts'o *et al.*, 1963). (2) Though the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values are difficult to obtain with sufficient accuracy, especially those of the pyrimidine dimers, some of them can be measured. The $J_{H\text{-}1'\text{-}H\text{-}2'}$ values of UpG (5.0 for G and 4.8 for U) and those of GpC (4.0 for G and 3.2 for C) at 60° are virtually the same as those at 30° within experimental errors. Therefore, apparently the ribose conformation of these two dimers is not affected within this temperature range. Also, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of A in ApU, UpA, ApG, and GpA are all about the same ($4.5 \pm 0.3$) at both 60 and 30°. (The $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of (-pA) in GpA was reported by Hruska and Danyluk to be 4.3 (71°), 4.2 (55°), 4.0 (35°), and 3.0 (4°).) Therefore, it is difficult to comprehend that while the ribose conformation of (-pA) or (Ap-) unit in ApA will be changed a great deal by raising the temperature from 30 to 60°, yet the ribose conformation of these same A units in ApU, UpA, ApG, and GpA will be much less affected by the same elevation of temperature. These data are suggestive that in these instances the change of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ values may not be related to a change of ribose conformation, but rather be related to the shielding effect of the neighboring units in the dimer. (3) Comparison of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of 5'-CMP (3.3, Table IB) and that of the (-pC) in (2'–5')-ApC is of interest. Only a singlet was found for the H-1' of the (-pC) unit in (2'–5')-ApC indicating the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of the (-pC) unit in the dimer must be less than 1 cps (Table IV). If this change of $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of 5'-CMP to the (-pC) unit in the dimer is to be interpreted as a direct revelation of the change of the ribose conformation, such a change would be rather large. (The dihedral angle between the H-1' and H-2' protons will have a minimum change of 30–40° according to the Karplus equation for vicinal coupling (Karplus, 1959)). It is rather puzzling why such a large change of ribose conformation would occur when a 5'-CMP is attached to the AMP at the C-2' position, since the attachment to the AMP at the C-3' position for the formation of the (3'–5')-ApC dimer only changes the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value from 3.3 to 2.3 cps. On the other hand, as shown in Table IX and discussed in section IV, the H-1' proton of the (-pC) unit in the (2'–5')-ApC is very much shielded by the neighboring (2'-pA), having a value of $\Delta\delta_D$ of 0.43 ppm. (4) At pD 6.3 and room temperature, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ value of 5'-GMP in 0.01–0.025 M is $6.0 \pm 0.2$ cps. In 0.045 M at pD 6.3 and room temperature, the 5'-GMP solution forms a gel. When this solution returned to room temperature after warming, the $J$ value was measured to be 5.6 cps just before the onset of gelation. The value of the chemical shift of H-1' was 6.35 for all these solutions. At pD 8.0 and room temperature, 5'-GMP does not form a gel even in 0.4 M solution, the $J$ value is 5.3, and the H-1' is at 6.30. These studies on 5'-GMP indicate that there is a slight reduction of $J$ value in concentrated solutions of 5'-GMP (barely above the experimental error for the 5.6 cps). Under these conditions, however, very extensive interactions of GMP take place as indicated by the gel formation and by other previous studies (Gellert *et al.*, 1962; Sarkar and Yang, 1965). Though no quantitative comparison has been made, it is accepted generally that the self-association of GMP is much more extensive than that of AMP; yet the reduction of $J$ value of GMP due to association is rather modest. These are the reasons that the change of $J_{H\text{-}1'\text{-}H\text{-}2'}$ values in these instances may not be related directly to the change of ribose conformation. There are, however, some experimental findings indicating the change of the $J_{H\text{-}1'\text{-}H\text{-}2'}$ may not be directly related to the extent of the shielding of the H-1' protons either. In Table XII, the H-1' proton of 5'-AMP (0.05 M) is shown to be highly shielded (0.37 ppm) in solution containing 1 M purine. Under this condition, at 30°, for instance, the $J_{H\text{-}1'\text{-}H\text{-}2'}$ of AMP is reduced (from 5.6 to 5.2) but not as extensively as that (to 4.7) in 0.85 M AMP solution, even though the H-1' of

the AMP in 0.85 M solution (6.35) is not as shielded as the H-1′ of the AMP (6.14) in 1 M solution of purine. Similar results are obtained at 60 and 28°. The $J_{H\text{-}1'\text{-}H\text{-}2'}$ of uridine (0.10 M) was found also to be decreased from about 4.5–4.1 cps in the presence of 0.11 M purine (Bangerter and Chan, 1968). Therefore, in this case, the extent of reduction of $J_{H\text{-}1'\text{-}H\text{-}2'}$ value does not correspond directly to the extent of shielding of the H-1′ proton (or the base as well) by the influence of the neighboring molecule in the stacks through the ring-current effect. Also, in the situation of 0.4 M 5′-GMP at pD 8.0, the reduction of $J$ value (about 0.5 cps) is accompanied only by a very small shielding of H-1′ (about 0.05 ppm). Obviously more theoretical and experimental investigation is needed for a more thorough understanding of this phenomenon.

Nevertheless, we conclude that since the ribose conformation (strictly speaking, the dihedral angle between the H-1′ and the H-2′ protons) of all the mononucleotides, of at least two dimers (UpG and GpC), and of (A) units in four dimers (UpA, ApU, ApG, and GpA) is not changed detectably within the temperature range of 30–60° as indicated by the insensitivity of their $J_{H\text{-}1'\text{-}H\text{-}2'}$ values to temperature; therefore, the ribose conformation of all the dimers most likely is not significantly affected by temperature within this range. We cannot exclude the possibility that, as a special case, there is a change of ribose conformation of ApA within this temperature range (30–60°), as indicated by the significant change of the $J_{H\text{-}1'\text{-}H\text{-}2'}$. However, several experimental observations suggest that there could well be another interpretation of this change of $J_{H\text{-}1'\text{-}H\text{-}2'}$ value. In this particular situation, this change observed may not be directly related to a change of the ribose conformation.

(c) The effect of temperature on the chemical shifts of the protons after a proper correction is shown by the value of $D_T$, the temperature differential. As shown in Table XI, the values of $D_T$ for all the pyrimidine dimers are very small, indicating their chemical shifts are essentially insensitive to temperature. This is to be expected since their values for $\Delta\delta_D$ and $\Delta\delta_D^{60°}$ (the dimerization shifts at 30 and 60°) are generally small, because of the absence of shielding through ring-current effects by neighboring units. The large $\Delta\delta_D$ values observed (around 0.10 ppm) for these dimers can be found only for the H-6 protons of the 5′ units (-pC or -pU). As discussed several times before, these $\Delta\delta_D$ values came from the reduction of the shielding effect of the phosphate monoester when transformed into a diester in the dimer. Therefore, these values are not temperature sensitive. This same situation, of course, also exists for all the H-6 protons of the 5′-pyrimidinyl units (-pC or -pU) and all the H-8 protons of the 5′-purinyl units (-pA or -pG), though it is not necessarily so obvious. The reason is that the shielding of some of these protons as indicated by $\Delta\delta_D$ and $\Delta\delta_D^{60°}$ partially came from the ring-current effect of the neighboring units. Under this condition, the shielding will be temperature sensitive as shown by the value of $D_T$. For example, the $D_T$ of the H-8 of (-pA) in UpA or CpA is 0 or 0.01, while $D_T$ of H-8 of (-pA) in ApA is 0.06. Evidently, the H-8 of (-pA) in ApA is shielded by the neighboring unit and the H-8 of (-pA) in UpA or CpA is not shielded by the neighboring unit (because of the lack of ring current) as depicted by the description and the models shown in Figures 1, 3, 11, and 13.

In fact, this is the situation indicated throughout the temperature study. The proton strongly shielded by the neighboring units, as indicated by the $\Delta\delta_D$ value and by the models, is the proton which has a large value of

TABLE XIII: Chemical Shifts of Base Protons, H-1′ Protons, and 6-Amino Protons of Adenosine, AMP, and ApA in Dimethyl Sulfoxide-$d_6$.[d]

| | H-8 | | H-2 | | H-1′ | | 6-$NH_2$ | |
|---|---|---|---|---|---|---|---|---|
| | (5′) | (3′) | (5′) | (3′) | (5′) | (3′) | (5′) | (3′ |
| Adenosine in $D_2O$, 30°, 0 M | 8.75 | | 8.67 | | 6.50 | | | |
| Adenosine in dimethyl sulfoxide-$d_6$, 30°, 0.04 M | 8.70 | | 8.49 | | 6.23[a] | | 7.67 | |
| | 0.05 | | 0.18 | | 0.27 | | | |
| 5′-AMP in dimethyl sulfoxide-$d_6$, 30°, 0.02 M | 8.85 | | 8.49 | | 6.28[b] | | 7.66 | |
| 3′-AMP in dimethyl sulfoxide-$d_6$, 30°, 0.02 M | | 8.72 | | 8.48 | | 6.28[c] | | 7.66 |
| | | 0.13 | | 0.01 | | 0 | | 0 |
| ApA in dimethyl sulfoxide-$d_6$, 0.02 M, 30° | 8.78 | 8.70 | 8.50 | 8.48 | 6.29[b] | 6.23[c] | 7.59 | 7.67 |
| 60° | 8.84 | 8.80 | 8.63 | 8.61 | | | 7.52 | 7.57 |
| 75° | | | | | | | 7.34 | 7.34 |

[a] The $J_{H\text{-}1'\text{-}H\text{-}2'}$ value for adenosine in dimethyl sulfoxide is 6.0 cps. [b] The $J_{H\text{-}1'\text{-}H\text{-}2'}$ value for the H-1′ of the 5′-AMP is 5.5 and of the (-pA) in ApA is 5.2 cps. [c] The $J_{H\text{-}1'\text{-}H\text{-}2'}$ value for the H-1′ of the 5′-AMP is 6.1 and of the (Ap-) in ApA is 6.8 cps. [d] Chemical shifts from tetramethylsilane capillary, ppm.

$D_T$, which indicates a large effect of temperature on chemical shift. For the purine dimers, those protons having $D_T$ value of 0.06–0.08 are the H-2 protons of the 3′ units (Ap- in ApG or in ApA), the H-8 protons of the 5′ units (-pA in ApA, and -pG in ApG), and the H-1′ protons of the 3′ units (Ap- in ApA or in ApG, and Gp- in GpA). For the purine–pyrimidine mixed dimers, the protons having a large $D_T$ value (0.10–0.17) are the H-5 protons and to a lesser extent the H-6 protons and the H-1′ protons of the 5′-pyrimidinyl units (-pC in ApC or GpC, and -pU in ApU or GpU). The $D_T$ values also indicate that the influence of the 3′-A is much larger than the influence of the 3′-G as expected from ring-current argument. Similarly, the $D_T$ values also indicate that the shielding effect of the pyrimidinyl units on their purine neighbors is small, the largest being on the H-2 protons (0.06–0.05 $D_T$ values) as anticipated from the models (Figures 3 and 13). In summary, the results of the temperature study as indicated by the $D_T$ values support the models proposed in section IV.

(d) For the dimers containing purine nucleosides, the values of $\Delta\delta_D^{60°}$ indicate that the neighboring units in these dimers are still much shielded by those purinyl units at 60°, such as the H-5 of (-pC) in ApC or GpC, and H-2 of the (Ap-) in ApA, etc. The most striking example is that of (2′–5′)-ApC. The comparison of the $\Delta\delta_D$ and the $\Delta\delta_D^{60°}$ of this (2′–5′) dimer indicates that increase in temperature from 28–30 to 60° has only changed the shielding effect slightly, a lowering of only 15–25% of the dimerization shift values. These results are in agreement with the observations that the response of the optical properties of the dimers to a given temperature elevation is about the same at 60° as that at room temperature (Leng and Felsenfeld, 1966; Brahms *et al.*, 1967; Davis and Tinoco, 1968). Davis and Tinoco also showed the nonequivalence of the two H-2 protons of ApA in 25% LiCl at 90°, a situation developed from the shielding effect of the neighboring units. Thus, a much more severe condition is needed in order to reduce the interaction of the neighboring units to a minimum. Treatment at much higher temperature is not practical; it may lead to hydrolysis and the exchange of the H-8 proton of purine bases in $D_2O$. The obvious answer to this problem is to study the properties of the dimers in organic solvents.

### *VI. The Spectra of Adenosine, AMP, and ApA in Dimethyl Sulfoxide*

The early study in our laboratory on the properties of nucleic acid in organic solvents had shown that DNA and RNA dissolved in dimethyl sulfoxide essentially have lost all their secondary structure as indicated by having the same sign (negative) of Cotton effect as that from the sum of the mononucleotides, while denatured DNA at 90° still has a positive Cotton effect (Helmkamp and Ts'o, 1961).

In Table XIII, the chemical shifts of the base protons, H-1′ protons, and 6-amino protons of adenosine, AMP, and APA in dimethyl sulfoxide-$d_6$ are shown. It is of interest first to compare the $\delta$ value of the protons of adenosine in $D_2O$ with that in dimethyl sulfoxide-$d_6$ (Table XIII). As discussed in section I, our previous study has shown that the upfield shift of the $\delta$ values of the methyl proton of methanol (0.4 M) and of acetone (0.4 M from $D_2O$ to dimethyl sulfoxide) are respectively 0.25 and 0.22 ppm (Ts'o, *et al.*, 1969). The origin of this solvent upfield shift from $D_2O$ to dimethyl sulfoxide with the present external reference system (tetramethylsilane capillary) is probably the change of bulk magnetic susceptibility and the change of weak solute–solvent interaction. The values of $\Delta\delta$ of H-2 (0.18) and H-1′ (0.27) of adenosine observed from the $D_2O$–dimethyl sulfoxide transfer are in this magnitude (0.2–0.3 ppm) and, therefore, can be considered as having the same origin as the $\Delta\delta$ of the methylene proton of methanol and acetone. However, the $\Delta\delta$ (0.05) of the H-8 proton of adenosine in this $D_2O$–dimethyl sulfoxide transfer is distinctly smaller, indicating a bigger change of solute–solvent interaction in the opposite direction. The H-8 proton of purine nucleosides is known to be more "acidic" than other protons. It appears, therefore, that the "acidic" H-8 proton is strongly hydrogen bonded to the receptors in dimethyl sulfoxide solvent and is shifted to lower field (Ts'o *et al.*, 1969). This is most likely the reason why the solvent upfield shift of H-8 is small. The same reasoning is adopted in section I for the explanation of the comparatively smaller temperature shift observed in $D_2O$ for the H-8 of purine and H-6 of pyrimidine compounds.

The 5′-AMP, the 3′-AMP, and adenosine in dimethylsulfoxide-$d_6$ have the same values for their H-2, H-1′, and 6-$NH_2$ protons but not for the H-8 protons (Table XIII). The H-8 proton of 5′-AMP is located at 0.13 ppm lower field than the H-8 protons of 3′-AMP or adenosine. Following the argument presented in section I, and in our previous publication (Schweizer *et al.*, 1968), this result must be due to the specific deshielding effect of the 5′-phosphate group on the H-8 proton in 5′-AMP. These data, therefore, indicate that the 5′-AMP also has the *anti* conformation in dimethyl sulfoxide.

The spectrum of ApA in dimethyl sulfoxide-$d_6$ is shown in Figure 9B and the $\delta$ values of its protons are given in Table XIII. As indicated, the $\delta$ values of the H-8 and H-2 protons of the (Ap-) unit and the $\delta$ values of the H-2 and H-1′ protons of the (-pA) unit are essentially identical with those of the corresponding monomers. In other words, the values of the dimer shift ($\Delta\delta_D^{\text{dimethyl sulfoxide}}$) for these protons are essentially nil, indicating the absence of the influence of these neighboring groups. The H-8 proton of (-pA) is about 0.07 ppm upfield from the H-8 of 5′-AMP. This value of $\Delta\delta_D$ is expected from the reduction of the deshielding effect of the phosphate group when transformed from a monoester to a diester. Therefore, the H-8 proton of the (-pA) is unlikely to be shielded by the neighboring unit also. The H-1′ proton of the (Ap-) unit is the only proton in the dimer which is slightly shielded; however, the $\Delta\delta_D^{\text{dimethyl sulfoxide}}$ value is only 0.05 ppm as compared to $\Delta\delta_D^{30°}$ of 0.26 ppm and to $\Delta\delta_D^{60°}$ of 0.20 ppm (Table XI). In $D_2O$, the H-8, H-2, and the H-1′ protons in ApA are normally measured. In dimethyl sulfoxide-$d_6$, the 6-$NH_2$ protons can also be studied and the

observation on these protons is interesting. The 6-$NH_2$ protons of one nucleotidyl unit are shielded upfield by about 0.07 ppm while the 6-$NH_2$ protons of the other unit have the same $\delta$ value as the monomer (Figure 9B and Table XIII). From the conformational model, we tentatively assign the upfield 6-$NH_2$ protons to be those of the (-pA) unit. The difference in $\delta$ values between these two groups of 6-$NH_2$ protons diminished as the temperature of the dimethyl sulfoxide-$d_6$ solution was elevated, and became nil at about 75°. From these results, it is concluded that when dissolved in dimethyl sulfoxide at 28–30°, most of the base protons and the H-1′ protons of ApA are not influenced by the neighboring units. A small degree of shielding of the H-1′ proton and the 6-$NH_2$ proton of the (Ap-) unit can still be detected, however. The residual shielding of these protons by the neighboring unit disappears when the temperature of the dimethyl sulfoxide solution is elevated to above 60°. Under this condition, as far as the method of proton magnetic resonance is concerned, the individual nucleotidyl unit in ApA becomes totally free from the influence of the neighboring unit in the dimer.

### *VII. Concluding Remarks*

The molecular models presented above are mainly for the purpose of demonstrating the general conformational features of these dimer molecules. These are the time average, approximate models. Nevertheless, starting with this model and, perhaps, with only a slight adjustment, one can obtain a stacked conformation which will, at least semiquantitatively, satisfy the requirement of the proton magnetic resonance data on the base protons and the H-1′ protons of all the dimers so far studied. The proton magnetic resonance technique potentially, however, is capable of providing a more precise description of the conformation of the dimers containing purines, especially adenine. More theoretical and experimental work in this direction is forthcoming, especially on model compounds which have a fixed conformation.

As described above, the proton magnetic resonance data are in complete accord with an "*anti,anti*, right-handed" model. However, the question still remains: can these data be explained by models built from other conformational requirements? The specific deshielding of H-6 proton of pyrimidine (Py) and H-8 proton of purine (Pu) by the 5′-phosphate in the dimers, the shielding of the H-2 of the (Ap-) but not the H-2 of the (-pA) in the ApA, and the preferential shielding of the H-5 and the H-6 protons of the pyrimidine in the PupPy dimer but not in the PypPu dimer, etc., are the conclusive evidences indicating that the nucleotidyl units in the dimers must *not* be in the *syn* conformation.

On the other hand, the answer to the question about the direction of the (3′–5′) screw axis, *i.e.*, right handed or left handed, is more complex. The (3′–5′) screw axis of a right-handed dimer is rotating counterclockwise and advancing forward from the plane of the paper, while the (3′–5′) screw axis of a left-handed dimer is either rotating counterclockwise but retreating backward from the plane of the paper, or when examined in another direction, is rotating clockwise and advancing forward from the plane of the paper. These two views for comparing right-handed and left-handed stacks are shown in Figure 6A,B for the ApApAp, a trinucleotide. The simplest way to compare these two types of stacks is to maintain the rotation of the (3′–5′) screw axis as counterclockwise, but having the axis advancing forward for the right-handed stack and the axis retreating backward for the left-handed stack. Thus, in the right-handed stack, the base of the 3′-nucleotidyl unit (Xp-) is shielded by the base of the 5′-nucleotidyl unit (-pY) in front, while in the left-handed stack, the base of (Xp-) is shielded by the base of (-pY) at the back. Examination of the Corey–Pauling–Koltun models of all the purine–pyrimidine mixed dimers such as ApC, ApU, GpC, and GpU, and their sequential isomers, indicate that the conformation of the left-handed stack is not in agreement with all the proton magnetic resonance data. Three examples are cited here for illustration. (1) The left-handed model (with *anti* nucleotidyl units) requires that the H-1′ protons of pyrimidine in the 3′-nucleotidyl units (Pyp-) are to be very much more shielded than the H-1′ protons in the (-pPy) units. Such a case is not found in the data of ApC *vs.* CpA or the data of ApU *vs.* UpA, for the H-1′ protons of the pyrimidine units are shielded (the $\Delta\delta_D$ value) to about the same extent, regardless of the position in the dimer (Table XI). For the dimers containing G and C or U, the H-1′ protons of (Pyp-) are more shielded than the H-1′ protons of (-pPy); however, this situation can be explained by both the left-handed or the right-handed model. (2) The left-handed model requires that the H-1′ protons of the pyrimidine 3′-nucleotidyl units are to be much more shielded than the H-5 of the same unit. Such a situation was not found in CpA and CpG. Again, this situation does exist in UpA and UpG, which, however, can also be explained by both models. (3) Finally, the left-handed model is not compatible with the large shielding observed for H-2 of ApC, H-5 of ApC, ApU, GpC, and GpU, and should predict a much larger shielding of H-1′ of (Pyp-) than that observed for CpA, UpA, CpG, and UpG. Examination of the Corey–Pauling–Koltun models of the purine dimers also indicates that the conformation of the left-handed stack is not in quantitative agreement with the proton magnetic resonance data, even though the evidence here is not as decisive as that of the purine–pyrimidine dimers. The left-handed model predicts a less extensive shielding (smaller $\Delta\delta_D$ values) than that observed for the following protons: H-8 of (-pA) in ApA, H-1′ of (-pA) in ApA, H-2 of (Ap-) in ApG, and perhaps the H-8 of (-pA) in GpA. Also, the left-handed model predicts a more extensive shielding (larger $\Delta\delta_D$ values) than observed for the H-1′ protons of the 3′-nucleotidyl groups (Pup-) in the dimer. As for the pyrimidine dimers, because of the absence of the ring current, the existing proton magnetic resonance data provides no decisive information about the extent of the stacking of the bases or the direction of the turn of the stacks. As for the (2′–5′)-linked dimer, (2′–5′)-ApC, the extensive shielding of the H-1′ of (-pC) is conclusively

against a model of left-handed stack. There is another form of left-handed stack, in which the H-2′, H-3′, and H-5′ (5″) protons of the pentose of one residue face these same protons of the other residue in a nearly folded conformation. The proton magnetic resonance data on the dimers containing purines definitely exclude the existence of such a stacked form.

Two recent papers on the arabinosyl pyrimidine dimers may have a direct implication on the question of the direction of the turn of the stack of the dimers (Maurizot *et al.*, 1968; Adler *et al.*, 1968). In comparing the circular dichroism and other optical data of the monomers and the dimers, as well as the temperature dependence of their optical properties, these authors concluded that arabinosylcytidyl-(3′–5′)-arabinosylcytidylic acid (aCpaC) has very little ordered structure, especially in comparison with CpC. Maurizot *et al.* (1968) ascribed this loss of ordered structure of aCpaC to the reduction of the interaction of the 2′-OH group to phosphate; they considered that the distance between the 2′-OH group to the phosphate is greater for the 3′-arabinosyl nucleotide than that for the 3′-ribosyl nucleotide. Brahms *et al.* (1967) previously proposed that the hydrogen bonding between the 3′-linked 2′-OH group and the phosphate oxygen "definitely restricts rotational freedom of the phosphodiester linkage." Adler *et al.* (1968), working on similar experiments, came to an opposite conclusion. They have made a very important, additional observation as control; they observed that the dCpdC has more ordered structure than aCpaC. Though the aCpaC still has a 2′-OH group which is sterically capable of interacting with the 3′-phosphate group, yet this arabinose compound has less ordered structure than the deoxyribose compound, dCpdC, which has no 2′-OH group at all. Together with other arguments, Adler *et al.* (1968) concluded that "a stabilizing hydrogen bond between the 2′-hydroxyl group and a phosphate oxygen seems unlikely." It appears to us that the 2′-OH group located at a transposition to the H-1′ proton as in the arabinosyl nucleotide has a *negative* contribution to the stacking of the dimer. This obstruction to the stacking of the dimer apparently exists only when the *trans* 2′-OH is located in the 3′-nucleotidyl units. In comparing ApaC *vs.* aCpA, and aCpU *vs.* UpaC, Maurizot *et al.* (1968) reported that the circular dichroism spectra of the dimers containing 3′-aracytidyl units (aCp-) are much less temperature dependent in comparison with those of the corresponding sequential isomers which contain 5′-aracytidyl units (-paC). We propose that the obstruction of the *trans* 2′-OH group at the 3′-arabinosyl nucleotidyl unit may be caused by the steric hindrance of the 2′-OH group to the close contact of two bases in a right-handed stack. As shown in the model of aCpaC in Figure 7, the *trans* 2′-OH group of the 3′ unit (aCp-) is situated between two bases in a right-handed model while the *trans* 2′-OH group of the 5′ unit (-paC) is located outside the stack. In a left-handed model (Figure 7), however, both the *trans* 2′-OH groups of the (aCp-) and the (-paC) units are situated outside of the stack. For the verification of this hypothesis, it is important to study comparatively the dimers containing 2′-*O*-methylarabinosyl 3′-nucleotides to those containing 2′-deoxyribosyl 3′-nucleotides and to those containing 2′-*O*-methylribosyl 3′-nucleotides. For the right-handed conformation, the present hypothesis predicts that the dimers containing 2′-*O*-methylarabinosyl 3′-nucleotides should have much less ordered structure than those containing either the 2′-deoxyribosyl nucleotides or the 2′-*O*-methylribosyl nucleotides, even though there is no intramolecular interaction with the 2′-OH group in all three types of compounds. Conversely, for the left-handed conformation, the present hypothesis predicts that the dimers containing 2′-*O*-methylribosyl 3′-nucleotides should have much less ordered structure than those containing deoxyribosyl or 2′-*O*-methylarabinosyl 3′-nucleotides. These experiments may provide the conclusive evidence in deciding the direction of the turn of the (3′–5′) screw axes of the dimers in the stack.

What are the physicochemical reasons for the preference of the dimers for the "*anti*, right-handed" conformation? Two theoretical calculations have been made on the energy level of the "*syn*" conformation *vs.* that of the "*anti*" conformation (Tinoco *et al.*, 1968; Jordan and Pullman, 1968). Their calculations indicate that the preferred conformation for adenosine, cytidine, and uridine is "*anti*" and the preferred conformation for guanosine is "*syn*." Experimentally, 5′-GMP in solution was shown also to have the *anti* conformation as described above (Schweizer *et al.*, 1968). Their computed results showed that the difference between the energy levels of the "*syn*" and "*anti*" conformation is bigger for the pyrimidine nucleosides than for the purine nucleosides. One major reason for the unfavorable energy level for the *syn* conformation could be the repulsion between the lone pair of electrons of the O-4′ ether oxygen of the pentose and the lone pair of electrons of the 2-keto oxygen of the pyrimidine, or the lone pair of the N-3 nitrogen of the purine. The problem concerning the rotational barrier between the *syn* and the *anti* conformation is currently under investigation in our laboratory. Unfortunately, this hypothesis cannot be easily verified by studying the analogs. For instance, the change of the O-4′ ether oxygen into a carbon atom will introduce two protons to this position, which will provide a steric hindrance. Such an analog does exist for adenosine, *i.e.*, aristeromycin which has a cyclopentane ring in place of the furanose ring, otherwise is identical with adenosine. The X-ray study on a crystal of aristeromycin indicated that this compound has a conformation analogous to the *anti* conformation of adenosine (Kishi *et al.*, 1967). A change of the N-3 nitrogen of purine into a carbon atom as in the case of 3-deazaadenosine will also bring in the steric hindrance of the added proton to this position. As for the pyrimidine nucleotides study on the ribosyl 4-oxopyrimidine derivatives will be of value to this problem. It is an interesting and challenging endeavor to consider why the (3′–5′) screw axes of the dimers prefer a right-handed turn over a left-handed turn. The Corey–Pauling–Koltun models suggest a very likely steric hindrance from the H-1′ anomeric protons

and also a possible hindrance from the 2′-OH group. It appears that dimers containing 2′-*O*-methylribosyl 3′-nucleotide (2′-*O*-methyl-Xp-) would have more difficulties in forming a left-handed stack because of the obstruction of the 2′-*O*-methyl group. Depending upon the rotational freedom of the C-2′–O-2′ bond, slight steric hindrance may also exist for forming the right-handed stack. As for the (2′–5′) dimers, no obvious steric hindrance can be detected which may prevent these compounds from forming a left-handed stack. Indeed, examination of the Corey–Pauling–Koltun models provides no clue as to why the (2′–5′) dimer prefers the right-handed conformation over the left-handed conformation at least in the case of (2′–5′)-ApC. Therefore, the possible existence of left-handed stack of the (2′–5′) dimer cannot be disregarded on a prior grounds. This possibility perhaps has not received sufficient consideration in the past. Fuller *et al.* (1965) have stated that through the model building study on the B form of DNA, "it is not easy to eliminate a left-handed structure." This same conclusion was reached by Haschemeyer and Rich (1967): "The question can not be settled by simple model building, as we have found that a left-handed DNA model can be constructed, apparently without unfavorable interatomic contact distances."

Comparison of the proton magnetic resonance data of the dimers obtained at 28–30° to those at 60° indicates that the nucleotidyl units remain mainly in the *anti* conformation and there is probably no change of the ribose conformation. The shielding of the base and the H-1′ protons by its neighbor in a dimer, nevertheless, is substantially reduced. What is the mode of the motion of a dimer in response to a perturbation by temperature change? Two valuable conformational analyses on nucleosides and nucleotides based on stereochemical considerations such as bond angles and allowable interatomic distances have appeared recently (Haschemeyer and Rich, 1967; Sasisekharan *et al.*, 1967). The work of Haschemeyer and Rich is mainly on the steric barriers to rotation about the glycosyl bond, while the work of Sasisekharan *et al.* is on the allowed conformations of the monomeric unit in a polynucleotide chain. Based on a hard sphere model, they have analyzed quantitatively the allowable positions for the three internal rotations of the D-ribose unit along the backbone denoted as $\Theta$ 1 about O-5′–C-5′, $\Theta$ 2 about C-5′–C-4′, and $\Theta$ 3 about C-3′–O-3′. They found that the allowed regions for the rotation of these bonds are rather limited, especially for the $\Theta$ 3. No result on the study of the rotation of the O-3′–P-3 bond (angle $\phi$) and the O-5′–P-5 bond (angle $\chi$) was presented, however. Examination of Corey–Pauling–Koltun models indicates that there is very little restriction on the rotation of the O-5′–P-5 bond or even the O-3′–P-3 bond. Starting from a stacked conformation, a complete rotation around the O-5′–P-5 bond (most likely the O-3′–P-3 bond as well) will bring back the original stacked position with little hindrance from the consideration of a hard sphere model. This stereochemical situation is in accord with the basic assumption of the "torsional oscillator model" proposed for the treatment of the temperature-dependent optical properties (Davis and Tinoco, 1968; Glaubiger *et al.*, 1968). It is important to note that the transformation of a right-handed stack to a left-handed stack (or *vice versa*) involves mostly rotation of the unrestricted O-5′–P-5 bond and the O-3′–P-3 bond, and perhaps with a very slight adjustment (10–30°) of the $\Theta$ 1, $\Theta$ 2, and the $\phi_{CN}$ (glycosyl bond rotation) bond angle. These models suggest that the rotational barrier for the interconversion between right-handed stack and the left-handed stack for the *dimer* is rather low. Of course, this situation for the polynucleotides or even for the oligonucleotides is *not* the same. For the polymer, in order to have a successful conversion of the helical turn, all these steps have to take place cooperatively.

The proton magnetic resonance data (Table XI) shows the extensive shielding of the base and H-1′ proton of C and the H-2 proton of A by the neighboring units in (2′–5′)-ApC. In fact, the shielding of these three protons in (2′–5′)-ApC is about twice as much as those in (3′–5′)-ApC at both 30 and 60°. These data indicate that the overlapping between A and C is much more extensive in the (2′–5′) dimer than that in the (3′–5′) dimer. A different conclusion was reached by Brahms *et al.* (1967). They concluded that while (3′–5′) dimers form stacks at low temperature and neutral pH, "the 2′–5′ derivatives ApC, ApA, and CpC (also deoxy GpG) are essentially in a disordered conformation in the range of temperature used, *i.e.*, from −20 to 80°." The reasons for this conclusion were based on the observations that in 4.7 M KF–0.01 M Tris, (2′–5′)-ApC and other (2′–5′) dimers have much lower circular dichroism values than their corresponding (3′–5′) isomers, and also the temperature dependence of circular dichroism spectra of (2′–5′) dimers is less than those of the (3′–5′) dimers at the temperature range of −20 to 80°. The maximum extinction coefficients of (2′–5′)-ApC and of (3′–5′)-ApC were reported to be the same (Brahms *et al.*, 1967). There are at least two other alternant explanations for the circular dichroism results which may bring these data in agreement with the proton magnetic resonance observations. The first possible explanation is that the angle between the transition moments of A and C differs between the (3′–5′) dimer and the (2′–5′) dimer. According to the exciton theory the rotational strength is a trigonometric function of this angle; therefore, the circular dichroism spectra of the stack of the (2′–5′)-ApC can conceivably have a smaller intrinsic rotational strength than that of the stack of the (3′–5′)-ApC. We have invoked this argument previously to explain the difference in the optical rotatory dispersion spectra of poly dA *vs.* poly rA at neutral pH (Ts'o *et al.*, 1966). Since the rotational strength of the unstacked form is low also, the temperature dependence of the (2′–5′)-ApC will tend to appear much smaller than that of the (3′–5′) isomers. An appropriate theoretical analysis of the slopes in the $\Delta\epsilon_{max}$ *vs.* temperature plot of these two dimers is not yet feasible, since the data on the (2′–5′) dimer is scarce and the temperature-insensitive zones for the $\Delta\epsilon_{max}$ at both high and low range of temperature have not been reached (Figure 8, Brahms

*et al.*, 1967). Another possible explanation is that a certain percentage of the population of the stacks of the (2′–5′)-ApC has the left-handed conformation while other molecules have the right-handed conformation. The plausibility of the existence of left-handed stacks of the (2′–5′) dimers was discussed above. Since the sign of the rotational strength of the left-handed stack is opposite that of the right-handed stack, the algebraic sum of the rotational strength will be smaller for a population of dimer molecules with both left-handed and right-handed conformations. At high temperature, the population of the stacked dimers of both conformations will diminish to reduce further the rotational strength of the solution. As stated before, though the proton magnetic resonance data of (2′–5′)-ApC favors the right-handed stack as a predominant conformation, the existence of a small percentage of left-handed stack cannot be ruled out. Brahms *et al.* (1967) have realized that there is no steric restriction for the (2′–5′) dimers to have a stacked conformation. They attributed the difference observed between the (2′–5′) dimer and the (3′–5′) dimer to the loss of the 2′-OH group in the case of the (2′–5′) dimer. In their view as stated above, the internal hydrogen bond between the 2′-OH group and the phosphate oxygen will stabilize the stacked conformation of the dimer. They claimed that "in 2′–5′-dinucleoside phosphates (and in deoxy GpG), the absence of the 2′-hydroxyl group does not allow the formation of this hydrogen bond." However, in the case of the (2′–5′) dimer, there is a 3′-OH group left. It appears that the remaining 3′-OH group in the (2′–5′) dimer can form a hydrogen bond equally well with 2′-linked phosphate group. The role of the remaining 3′-OH group in the (2′-5′) dimers was not mentioned by Brahms *et al.* (1967). It appears that it is inappropriate to equate the (2′–5′) dimers to the deoxy dimers as reasoned by Brahms *et al.* (1967). The circular dichroism temperature profile for (2′–5′)-CpC was found not to be the same as that for the (3′–5′)-dCpdC (Adler *et al.*, 1968).

It is of importance to obtain proton magnetic resonance data on the trinucleoside diphosphates and to compare the observed values with those computed from the data on the dimers. In effect, such an investigation will determine whether or not the trimer models employed successfully here for the analysis of the dimer data can also be used to correlate the data on the trimers. Successful calculation of the optical properties of the trinucleoside diphosphates from those of the dimers has been achieved previously by Cantor and Tinoco (1965). We have obtained the proton magnetic resonance spectra of the homotrinucleoside diphosphates of adenine and uracil (ApApA and UpUpU).

The values of the chemical shifts of the base protons of the trimers can be computed from the data of the related dimers based on an "*anti*, right-handed" conformation for the stack. This approach hopefully will lead to a more thorough understanding of the conformation of the single-stranded polynucleotides through the studies of the dimers and the trimers.

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