the copper chromophore. The substitution of an imidazole nitrogen with that of NCS^- might only negligibly influence the energy of the d–d transitions; the decreased rhombicity observed in the ESR spectrum of the adduct with respect to the spectrum of the native enzyme would only suggest a more regular square-pyramidal geometry in the adduct. The proposed equatorial coordination in the copper enzyme is consistent with the larger contact effect observed with respect to the model system, in which NCS^- occupies an axial position.

Finally there are evidences of additional binding of thiocyanate, at concentrations equal or larger than 1 M, to the water coordination position; however, in such concentrated solutions the results may be altered by the occurrence of conformational changes in the protein.

Influence of Thiocyanate on Inhibitor Binding. In order to check the possibility for superoxide dismutase to accommodate two different anions in the coordination sphere of copper, we carried out NMR experiments in the presence of both thiocyanate and azide ions. The data in Figure 5 indicate that N_3^- displaces both the water molecule and the thiocyanate ion at the same time; there is no evidence of mixed ligand adducts, since the two titration curves in Figure 5 overlap all over the azide/enzyme concentration ratios. From the present experiments the apparent affinity constant of azide for the enzyme–thiocyanate adduct has been estimated to be $15 \pm 5\ M^{-1}$. These values are considerably smaller than the affinity values of N_3^- for the free enzyme.[9,10] This is consistent with a binding competition between the two anions. An analogous titration of the $N^{13}CS$ relaxation with cyanide shows a similar reduction in the cyanide inhibitor affinity constant[9] ($\sim 2 \times 10^3\ M^{-1}$), again indicating competition between cyanide and thiocyanate. Although the latter ion occupies a different binding site, steric effects as well as a decrease in the net positive charge on the metal may account for its detatchment upon addition of inhibitors.

Azide 10^{-2} M is reported to be capable of protecting the enzyme against inactivation by H_2O_2, its inhibiting action being effective only at higher concentrations.[32] It had been suggested that N_3^- at concentrations smaller than 10^{-2} M replaces a histidine ligand giving rise to a still active CuN_4OH_2 chromophore; the present data do not further clarify the N_3^- behavior.

Conclusions

Anionic ligands like cyanide, azide, and halides bind superoxide dismutase in a 1:1 ratio by displacing the bound water molecule. They are also capable of inhibiting the enzymatic activity. The thiocyanate ion does not displace the water molecule and does not inhibit the catalytic activity; nevertheless it does bind the copper ion without any major change in the coordination polyhedron, possibly through displacement of a bound histidine. The moiety CuN_4OH_2, which is present in both the native and the NCS^-–ligated enzymes, is apparently essential for the catalytic mechanism independently of the type of nitrogen.

Two binding sites at the copper ion in the enzyme are therefore evidenced, the choice of any of the two depending on the nature of the inhibitor.

Acknowledgment. Thanks are expressed to Professor Luigi Sacconi for providing NMR facilities. This research is part of a CNR-NSF joint program, contribution No. 8000316.

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Conformational Characteristics of Rigid Cyclic Nucleotides. 3. The Solution Conformation of β-Lyxonucleoside Cyclic 2′,5′- and 3′,5′-Monophosphates and of α-Arabinonucleoside Cyclic 2′,5′-Monophosphates. Implications for Evaluation of the Solution Properties of Nucleoside Analogues[1]

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Abstract: A detailed ^1H 220-MHz NMR study has been made of 1-(β-D-lyxofuranosyl)uracil cyclic 3′,5′-monophosphate (**1a**, 3′,5′-cLUMP), 1-(β-D-lyxofuranosyl)-5,6-dihydrouracil cyclic 3′,5′-monophosphate (**1b**, 3′,5′-cLDHUMP), 1-(β-D-lyxofuranosyl)uracil cyclic 2′,5′-monophosphate (**2a**, 2′,5′-cLUMP), 1-(β-D-lyxofuranosyl)-5,6-dihydrouracil cyclic 2′,5′-monophosphate (**2b**, 2′,5′-cLDHUMP), and 9-(α-D-arabinofuranosyl)adenine cyclic 2′,5′-monophosphate (**3**, α-2′,5′-cAAMP) in D_2O solution. Conformational analyses showed the cyclic 2′,5′-phosphates (**2a,b** and **3**) to exhibit sugar conformations in the range of ^2E whereas the cyclic 3′,5′-phosphates (**1a,b**) showed a preference for the ^3E to ^3_4T conformation. The unusually large $^3J_{PH}$ coupling of ~31 Hz which had previously been observed for $J_{5'P}$ in 9-(β-D-arabinofuranosyl)adenine cyclic 2′,5′-monophosphate (**2c**, 2′,5′-cAAMP) and for 1-(β-D-arabinofuranosyl)cytosine cyclic 2′,5′-monophosphate (**2d**, 2′,5′-cACMP) was again apparent in **2a,b** but not in **3** even though the sugar rings are in the same conformation in all five compounds. This difference is attributed to a steric interaction between the β-oriented base and the cyclic 2′,5′-phosphate ring in **2a–d**, which is not present in **3** where the base is oriented α; this allows the phosphate ring to take up a less-strained conformation to that in **2a–d**. The cyclic phosphate rings of the compounds described in this study fix the sugar rings into a particular conformation, precluding the $^2E \rightleftharpoons {}^3E$ conformer equilibrium usually found in acyclic mononucleotides. In the cases of **1a** and **2a**, this permits an accurate evaluation of the solution properties of the parent lyxonucleoside and the conformations of lyxouridine and lyxoadenosine are shown to be different from a $^3E/^3_4T \rightleftharpoons {}^2_1T/^2_3T$ equilibrium predicted earlier for lyxouridine. Furthermore, a detailed NMR examination has been made of the **2a** → **1a** isomerization in aqueous solution, and a mechanistic rationale is proposed, based on the conformations of the sugar and phosphate rings in **1a** and **2a**.

The conformational properties of flexible nucleoside and nucleotide derivatives have been shown to be best represented in solution as a dynamic equilibrium between various conformers.[3,4] For example, the sugar conformation in most ribo- and deoxy-

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ribonucleosides and mononucleotides is usually expressed as a $^2E \rightleftharpoons {}^3E$ equilibrium, and epimeric structural modifications in such molecules have been shown to affect the position of this equilibrium.[5-7] Furthermore, such structural changes in a flexible nucleotide may change the nature of the equilibrium such that it is no longer $^2E \rightleftharpoons {}^3E$ or indeed, a two-state equilibrium may not adequately describe the situation in solution in these instances.[7-9] Other conformational equilibria which may exist for flexible nucleoside derivatives in aqueous solution include rotation about the C4′–C5′ ($\psi_+ \rightleftharpoons \psi_- \rightleftharpoons \psi_\alpha$),[10] C5′–O5′ ($\phi_\alpha \rightleftharpoons \phi_- \rightleftharpoons \phi_+$),[10] and C1′–N1 or –N9 (χ_{CN}, anti $\rightleftharpoons$ syn) bonds.[4]

An approach with considerable merit for defining specific conformational features utilizes derivatives in which the conformational equilibria are precluded due to the formation of a rigid ring system. In this regard, two types of ring systems have been used, namely, cyclic monophosphates[1,11-15] and sugar-base intramolecular cyclonucleosides.[11,16-18]

β-Ribonucleoside cyclic 3′,5′-monophosphates contain a six-membered phosphate ring fused trans (1,2) to a five-membered sugar ring, and this produces a rigid bicyclic system with the sugar held in a ^3E to $_4T^3$ conformation;[11,12] in addition, the conformation about C4′–C5′ is extremely close to ψ_-.[11] Transposition of the base produces α-nucleoside cyclic 3′,5′-monophosphates and in this instance the sugar pucker is shifted to $^3T_2$ because of repulsive interactions between the 2′-hydroxyl and the base.[1,19] Other rigid cyclic mononucleotides which have been rigorously examined by NMR methods include 9-(β-D-xylofuranosyl)adenine cyclic 3′,5′-monophosphate[13] (**1c**) which possesses a six-membered phosphate ring fused cis (1,2) to a five-membered sugar ring rigidly held in the ^3E conformation and the cyclic 2′,5′-monophosphates of 1-(β-D-arabinofuranosyl)cytosine (**2d**)[14,15,20] and 9-(β-D-arabinofuranosyl)adenine (**2c**)[13] which possess a seven-membered phosphate ring fused cis (1,3) to a five-membered sugar ring rigidly held in the ^2E conformation. One of the novel features associated with **2c,d** was the unusually large $^3J_{PH}$ of ~31 Hz found for the coupling between H(5′) and phosphorous.[13-15] Kung et al.[14] have reported a crystal structure analysis of **2d** and have shown that the bond angles in the seven-membered phosphate ring at C2′, C4′, C5′, O2′, and O5′ are somewhat larger relative to the normal tetrahedral angle. In particular, the C2′–O2′–P and C5′–O5′–P bond angles were shown to be 127.2 and 124.0°, respectively. The appreciable strain introduced into the seven-membered ring by these large bond angles has been suggested as the source of the unusual $^3J_{PH}$ coupling mentioned above.[14] Kung et al. have described[14] a Karplus curve for $^3J_{PH}$ in strained seven-membered rings by using the dihedral angles obtained from the crystal structure of **2d** and the couplings measured from the NMR spectrum. In order to ascertain whether these ring strains are an inherent feature of seven-membered phosphate rings fused cis (1,3) to a five-membered ring, it appeared of interest to examine 2′,5′-cLUMP (**2a**) and α-2′,5′-cAAMP (**3**) which have the same ring fusion as **2c,d** but with different epimeric substitution at C3′ and C1′. In addition, 2′,5′-cLDHUMP (**2b**), the analogue of **2a** having a saturated aglycone, was also evaluated. It was also of interest to examine 3′,5′-cLUMP (**1a**) and 3′,5′-cLDHUMP (**1b**) and to compare them with the xylosyl derivative **1c** in order to evaluate epimeric changes at C2′ on the bicyclic ring structure obtained by fusing a six-membered phosphate ring cis (1,2) to a five-membered sugar ring.[13]

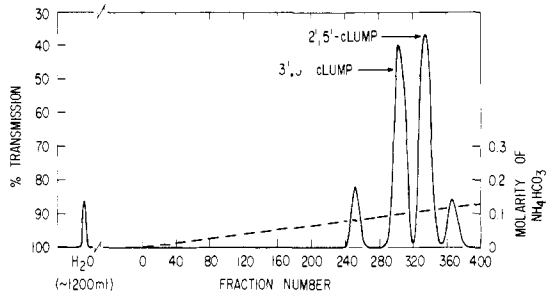
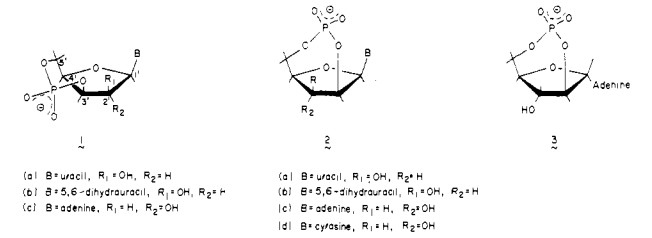


Figure 1. Separation of 3′,5′-cLUMP (**1a**) and 2′,5′-cLUMP (**2a**) on DEAE-Sephadex A-25 using a linear gradient of ammonium bicarbonate. For conditions, see Experimental Section.



Previous workers[21,22] have synthesized **1a** and **2a** and have shown that isomerization occurs in acid or base; however, they were not able to unequivocally distinguish between them. It was felt that a detailed NMR analysis would accomplish this and that a conformational analysis would provide insights into the mechanism of isomerization.

Finally, with the NMR analyses of **1a** and **2a** complete, it is now possible to evaluate accurately the solution conformational equilibrium present in the parent lyxonucleoside. Such an appraisal has proved difficult in the past because of the all-cis arrangement of the sugar protons and the uncertainty as to which vicinal correlation to use.[23a] While this manuscript was in preparation, Ekiel et al. described the crystal structure and NMR conformational analyses of selected lyxonucleosides.[23b] The work described herein presents a different evaluation of the sugar proton coupling data for lyxouridine and lyxoadenosine and arrives at different conclusions as regards their solution conformations.

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Experimental Section

A. Synthesis. UV spectra were recorded on a Beckman Model 25A spectrophotometer. Melting points were measured by using a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were determined by Galbraith Laboratories, Inc. TLC was performed on Eastman Chromatogram Sheet (cellulose 13254 with 6065 fluorescent indicator) by using 2-propanol–H_2O–concentrated NH_4OH (7:2:1) as solvent and 2540-A UV light for detection of spots. Electrophoresis was effected by using a Camag Thin-Layer Electrophoresis (after Pastuska) apparatus with the above-mentioned cellulose sheets and 0.05 M ammonium bicarbonate, pH 8, as buffer. Lyxouridine,[24a] xylouridine,[24b] and α-araAMP[25] were prepared by literature methods, and lyxoadenosine was a gift of Professor M. J. Robins. Phosphorylations were carried out by the general procedure of Yoshikawa and Kato,[26] using freshly distilled phosphorous oxychloride in triethylphosphate.

1-(β-D-Lyxofuranosyl)uracil Cyclic 3′,5′-Monophosphate (1a)[21,22] and 1-(β-D-Lyxofuranosyl)uracil Cyclic 2′,5′-Monophosphate (2a).[21,22] Lyxouridine[24] (200 mg, 0.82 mmol) was added to a cooled (0–5 °C), stirred solution of freshly distilled $POCl_3$ (0.3 mL) in triethyl phosphate (3.0 mL). The reaction was stirred at 0–5 °C for 4.5 h and then was added to 0.5 M NH_4HCO_3 (pH 8, 250 mL). This solution was extracted with Et_2O (3 × 100 mL), and the aqueous layer was evaporated to dryness several times from H_2O before being dissolved in a small volume and applied to a Whatman DE-52 column (HCO_3^- form, 6.2 × 35.0 cm). Elution was initially with H_2O (2650 mL, which contained 0.01 mmol, as determined by UV) and then with a linear gradient of H_2O (4 L) to 0.1 M NH_4HCO_3 (pH 8, 4 L), followed by 0.2 M NH_4HCO_3 until completion; 15-mL fractions were taken. Fractions 291–368 gave 0.20 mmol of **1a** (plus some **2a**); fractions 369–406 gave 0.11 mmol of **2a**; and fractions 511–610 gave 0.32 mmol of material which comigrated with 5′-UMP on TLE. Total yield of nucleotides was 77%. Re-chromatography of fractions 291–368 gave pure **1a** (0.17 mmol) and more of **2a** (0.02 mmol). A separate preparation on a 0.41-mmol scale utilized DEAE-Sephadex A-25 (HCO_3^- form, 5.0 × 39.0 cm) and a linear gradient of H_2O (3.5 L) to 0.15 M NH_4HCO_3 (3.5 L). In this instance, base-line separation of **1a** and **2a** was achieved with one column (see Figure 1). Identification of **1a** and **2a** was accomplished by comparison of the acid and base stabilities with the literature,[21,22] by TLC, TLE (both comigrated with cUMP) and particularly NMR (see Results).

9-(α-D-Arabinofuranosyl)adenine Cyclic 2′,5′-Monophosphate (3). This was prepared essentially by using the procedure previously described for the β-anomer[27] and for other cyclic monophosphates.[28] 9-(α-D-Arabinofuranosyl)adenosine 5′-monophosphate[25] (free acid, 100 mg, 0.29 mmol) and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine[29a] (86 mg, 0.29 mmol) were dissolved in pyridine (20 mL) containing enough H_2O to cause dissolution. This material was evaporated to dryness in vacuo and then dried by repeated evaporation from dry pyridine. Finally, after concentration to 50 mL this solution was added dropwise over a period of $4^1/_2$ h to a refluxing solution of dicyclohexylcarbodiimide (118 mg, 0.57 mmol) in dry pyridine (25 mL). After additional reflux for $2^1/_2$ h, the reaction mixture was evaporated to dryness and H_2O (40 mL) and Et_2O (20 mL) were added to the residue. Insoluble dicyclohexylurea was filtered off, and the layers in the filtrate were separated. The aqueous layer was concentrated and placed on a Whatman DE-52 column (HCO_3^- form, 4.0 × 14.0 cm). After a water wash (840 mL), the product (0.26 mmol, 90%) was eluted by using a linear gradient of H_2O (1 L) to 0.1 M NH_4HCO_3 (pH 8, 1 L). The product was characterized by TLC and TLE (comparison with cAMP and 5′-AMP) and by ^1H NMR. An analytical sample was obtained by dissolution of the ammonium salt of **3** in EtOH–H_2O (1:1, 5 mL) and addition of 1 M HCl until the pH was 2. After being left overnight in the refrigerator, the crystals were filtered off and dried in vacuo; mp 209 °C (dec). Anal. Calcd for $C_{10}H_{12}N_5O_6P_1 \cdot 1.75H_2O$: C, 33.29; H, 4.33; N, 19.41; P, 8.59. Found: C, 33.62; H, 4.20; N, 19.04; P, 8.50.

1-(β-D-Lyxofuranosyl)-5,6-dihydrouracil Cyclic 3′,5′-Monophosphate (1b). Compound **1b** was prepared by a modification of the hydrogenation method described earlier for the reduction of cUMP and α-cUMP.[29b] Compound **1a** (NH_4^+ salt, 0.0125 mmol) was dissolved in H_2O (0.3 mL), and EtOH (2.7 mL) was added. To this solution was added 5% Rh on Al_2O_3 (12 mg), and H_2 was bubbled through the suspension for 6 h. After centrifugation, the supernatant (plus 2 × 2 mL washings with H_2O) was applied directly to a Whatman DE-52 column (HCO_3^- form, 1.8 × 7.0 cm). After elution with H_2O (200 mL), the product was eluted with 0.1 M NH_4HCO_3 (pH 8, 200 mL). The salt was removed by repeated evaporation from H_2O, and the product was pure by TLC (detection by spraying with 5% H_2SO_4 in EtOH and charring) and NMR.

1-(β-D-Lyxofuranosyl)-5,6-dihydrouracil Cyclic 2′,5′-Monophosphate (2b). This was prepared as described above for **1b**, starting with **2a**.

B. ^1H NMR Studies. Ammonium salts of the cyclic nucleotides were lyophilized once from "100% D_2O" and dissolved in "100%" D_2O, and the solutions were adjusted to 0.02 M concentration and pD 6–8 (pD = meter reading + 0.4). A trace amount of 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ sodium salt (TSP) was added to the samples and served as an internal reference.

^1H NMR spectra were recorded in the Fourier transform mode on a Varian HR 220 spectrometer equipped with a Nicolet FT accessory and 20K data system. The spectra were measured at 20 ± 2 °C (except where stated), and chemical shifts are reported relative to internal TSP. In instances where the HDO peak overlapped extensively with certain sugar proton resonances, the 180–τ–90° pulse sequence was used to eliminate the HDO line in the spectrum. ^{31}P-decoupling experiments were performed by using a Schomandl ND 100 M generator set at the appropriate ^{31}P-decoupling frequency. Computer simulation of spectra was achieved by using a Nicolet 1080 computer and the ITRCAL simulation programs to give final coupling constants with an accuracy of ±0.2 Hz (except where stated) and chemical shifts ±0.005 ppm.

C. Isomerization Study. To a 0.02 M solution of **2a** in D_2O (1 mL) was added 20% DCl (50 μL), the final pD being 0.50. This solution was heated at 52 °C and the sample examined by ^1H NMR at regular intervals. Complete isomerization to **1a** was obtained after $3^1/_2$ h, with a $t_{1/2}$ of ~40 min. Under the same conditions, the ^1H NMR spectrum of **1a** remained unchanged.

Results

A. Synthetic Aspects. The preparation of **1a** and **2a** was accomplished by application of the Yoshikawa et al. phosphorylation procedure[26] by using lyxouridine[24] as a substrate. Previous preparations of **1a** and **2a** have involved (i) a five-step synthesis starting from 1-(5′-*O*-benzoyl-β-D-lyxofuranosyl)uracil[21] and proceeding via classical blocking–deblocking, phosphorylation, and cyclization steps to produce a mixture of **1a** and **2a** or (ii) a several-step synthesis using classical blocking groups, preparation of the appropriate phosphite, and oxidation to the phosphates **1a** and **2a** using hexachloroacetone.[22] The procedure described herein gave **1a** and **2a** in one step in reasonable yields from the readily available lyxouridine,[24] and separation was achieved on DEAE-Sephadex (Figure 1).

The 5,6-dihydro derivatives **1b** and **2b** were synthesized readily from **1a** and **2a** by catalytic hydrogenation over rhodium on alumina.[29b]

α-cAAMP (**3**) was synthesized by the general procedure of Khorana and co-workers by using the 5′-mononucleotide 4-morpholine carboxamidine salt[28] as starting material. The product was obtained in high yield (90%) after ion-exchange chromatography.

B. Identification of 1a and 2a. After TLC and TLE had shown the phosphorylation products **1a** and **2a** to be cyclic phosphates, ^1H NMR was utilized to assign the structures. Unequivocal identification of **1a** and **2a** was obtained by comparison of their spectra with the corresponding xylosyl cyclic 3′,5′-mononucleotide **1c**[13] and the arabinosyl cyclic 2′,5′-monophosphates **2c,d**.[13-15] In particular, the $^4J_{4',P}$ coupling of ~2 Hz found in **1c**[13] is also noted in **1a,b**, and the unusually large $^3J_{5',P}$ coupling of ~31 Hz found in **2c,d**[13-15] is also noted in **2a,b**. Also, the remaining sugar proton couplings (after taking into account the epimeric transposition between **1a,b** and between **2a,c** or **2d**) are in excellent agreement with the assignment.

C. Signal Assignments and NMR Parameters. The 220-MHz spectra of 0.02 M solutions of **1a,b**, **2a,b**, and **3** in D_2O are shown in Figure 2. A summary of coupling constants and chemical shifts derived by computer simulation is given in Table I. In addition, the final parameters for the nucleosides lyxouridine, lyxoadenosine,

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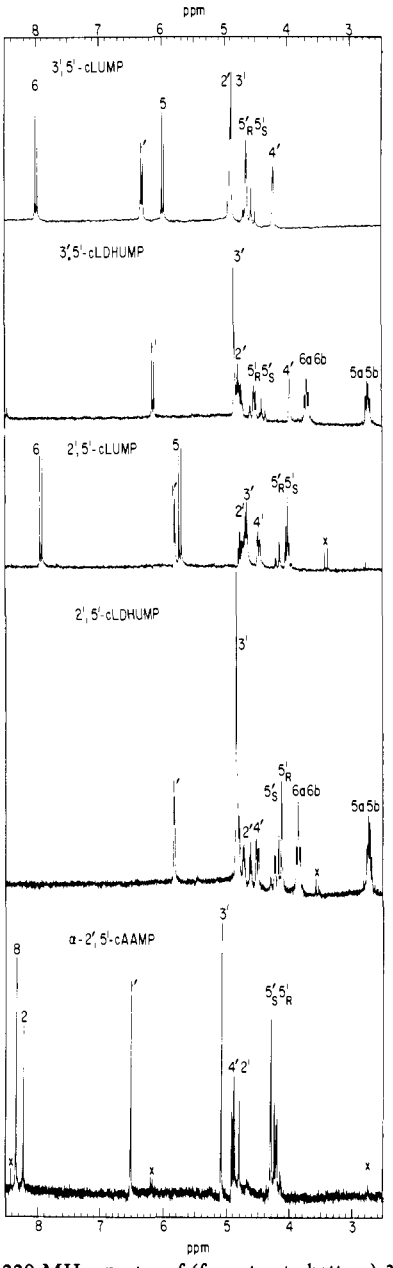


Figure 2. The 220-MHz spectra of (from top to bottom) 3′,5′-cLUMP (**1a**), 3′,5′-cLDHUMP (**1b**), 2′,5′-cLUMP (**2a**), 2′,5′-cLDHUMP (**2b**), and α-2′,5′-cAAMP (**3**). All recorded in D_2O at 0.02 M concentration, 20 °C at pD 6–8.

and xylouridine are listed in Table II. As expected, the base-protons of **1a**, **2a**, and **3** and the anomeric (H1′) proton resonances of all the compounds occur downfield of the HDO signal. The base protons of the saturated aglycone present in **1b** and **2b** are readily identified in the upfield region of the spectrum (2.7–3.87 ppm downfield of TSP). The remaining sugar protons lie in the usual range, i.e., 4.13–4.86 ppm downfield of TSP.

As a result of the rigid nature of the cyclic phosphate rings in **1–3**, the two diastereotopic methylene protons at C5′ are stereochemically nonequivalent (similar observations have been made for the α- and β-ribonucleoside cyclic 3′,5′-monophosphates[1,11]). In this paper we shall refer to the pro-S proton as $H5'_S$ and the pro-R as $H5'_R$. With use of the nomenclature of Davies,[4] these are identical with $H_{5'}{}^1$ and $H_{5'}{}^2$, respectively. It should be noted that in all the cases, $H5'_S$ is gauche to the sugar ring oxygen (O4′) and $H5'_R$ is trans. The *resonances* for these protons are described by the conventional method of H5′ (downfield) and H5″ (upfield).

A comparison of the chemical shifts of selected protons for various pairs of compounds leads to several interesting observations, some of which are critical to the evaluation of the overall

Table I. NMR Parameters[a] and Computed Dihedral Angles for **1a,b**, **2a,b**, and **3**

Chemical Shifts[b] (δ)

compd	H1′	H2′	H3′	H4′	$H5'_S$	$H5'_R$	H5(2)	H6(8)
3′,5′-cLUMP (**1a**)	6.250	4.846	4.825	4.163	4.532	4.599	5.909	7.927
2′,5′-cLUMP (**2a**)	5.964	4.861	4.857	4.609	4.232	4.164	5.883	8.097
3′,5′-cLDHUMP (**1b**)	6.135	4.742	4.796	3.968	4.454	4.577	2.746 (5a), 2.700 (5b)	3.711 (6a), 3.676 (6b)
2′,5′-cLDHUMP (**2b**)	5.817	4.663	4.817	4.506	4.180	4.137	2.756 (5a), 2.697 (5b)	3.874 (6a), 3.836 (6b)
α-2′,5′-cAAMP (**3**)	6.494	4.841	5.064	4.864	4.259	4.223	8.207	8.323

Coupling Constants[c] (J_{ij})

compd	1′,2′	2′,3′	3′,4′	4′,5′S	4′,5′R	5′S,5′R	2′,P	3′,P	4′,P	5′S,P	5′R,P	5a,5b	5a,6a	5a, 6b	5b,6a	5b,6b	6a,6b
3′,5′-cLUMP (**1a**)	6.5	4.3	2.2	1.2	1.9	−13.7	2.6	0	~2	21.4	1.1			8.15 (5,6)			
2′,5′-cLUMP (**2a**)	3.6	4.5	7.8	3.5	0.4	−13.5	24.4	−0.8	−0.2	30.5	4.5			8.20 (5,6)			
3′,5′-cLDHUMP (**1b**)	7.4	4.8	2.1	1.5	1.7	−13.6	2.5	0	2.2	21.2	1.6	−17.5[d]	6.7	5.4	5.7	8.9	−13.5[d]
2′,5′-cLDHUMP (**2b**)	2.9	4.1	7.8	3.3	0	−13.8	23.0	0	0	30.3	4.7	−17.1[d]	6.3	5.8	6.2	8.4	−13.5[d]
α-2′,5′-cAAMP (**3**)	0.0	0.4	0.0	0.6	2.9	−12.9	26.3	0	0	8.3	25.7						

Calculated Dihedral Angles[e] (θ_{ij})

compd	1′,2′	2′,3′	3′,4′	4′,5′S	4′,5′R	2′,P	3′,P	4′,P	5′S,P	5′R,P
3′,5′-cLUMP (**1a**)	32	46	59	66	61	...	90	164	66	
2′,5′-cLUMP (**2a**)	50	44	23	50	75	145	...	180	60	
3′,5′-cLDHUMP (**1b**)	26	43	59	64	62	...	90	163	63	
2′,5′-cLDHUMP (**2b**)	54	47	23	52	90	145	...	180	60	
α-2′,5′-cAAMP (**3**)	90	75	90	72	54	180	...	60	180	

[a] 0.02 M solutions in D_2O at pD 7.0. [b] Shifts in δ from TSP ±0.005 ppm. [c] Couplings in Hz ± 0.2 Hz. [d] ±0.5 Hz. [e] Angles in degrees, see text for the Karplus expressions used.

Table II. NMR Parameters[a,b] for 1-(β-D-Xylofuranosyl)uracil (xyloU), 1-(β-D-Lyxofuranosyl)uracil (lyxoU), and 9-(β-D-Lyxofuranosyl)adenine (lyxoA)

Chemical Shifts[c] (δ)

	H1′	H2′	OH2′	H3′	OH3′	H4′	H5′	H5″	OH5′	H5(2)	H6(8)
xyloU (D_2O)	5.817	4.307		4.275		4.435	3.984	3.970		5.849	7.916
lyxoU (D_2O)	6.179	4.628		4.379		4.187	3.997	3.946		5.846	7.952
lyxoU (Me_2SO-d_6)	6.026	4.340	5.479	4.053	5.278	3.835	3.701	3.588	4.776	5.565	7.877
lyxoA (D_2O)	6.372	4.852		4.458		4.298	4.055	3.992		8.243	8.393

Coupling Constants[d] (J_{ij})

	1′,2′	2′,3′	OH2′,2′	3′,4′	OH3′,3′	4′,5′	4′5″	OH5′,5′	OH5′,5″	5′,5″	5,6	% ψ_+[f]
xyloU (D_2O)	1.5	1.7		3.6		5.0	6.1			*e*	8.1	27
lyxoU (D_2O)	6.4	4.9		3.8		4.3	6.9			−11.7	8.1	26
lyxoU (Me_2SO-d_6)	6.8	4.7	6.2	3.6	3.6	4.7	6.3	5.8	5.4	−11.6	8.1	28
lyxoA (D_2O)	6.9	5.0		3.7		4.2	7.1			−12.1		25

[a] 0.02 M solutions in the solvents indicated. [b] See also ref 23. [c] Shifts in δ from TSP ±0.005 ppm. [d] Couplings in Hz ± 0.2 Hz. [e] Not measurable. [f] % $\psi_+ = [13.7 - (\Sigma J_{4'5'} + J_{4'5''})]100/9.7$.

Table III. Chemical Shift Differences between the Anomeric Pair 2c[a] and 3 [$\Delta\delta(\delta_\alpha - \delta_\beta)$]

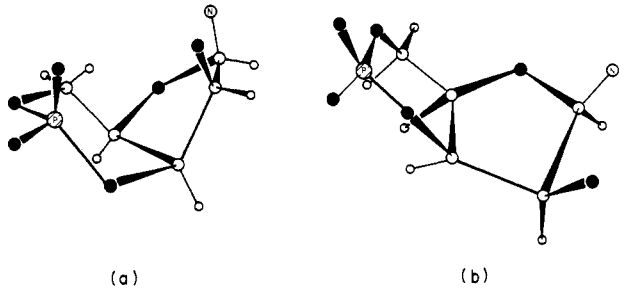
H8	H2	1′	2′	3′	4′	5′S ($H_{5'}$′)[b]	5′R ($H_{5'}$″)[b]
−0.137	0.023	−0.073	0.178	−0.311	0.270	0.00	−0.023

[a] Data for 2c taken from ref 13. [b] See text for discussion on assignments of the methylene hydrogens at C5′.

conformational properties of the molecules under investigation. Consideration of the chemical shifts of the protons in the anomeric pair of **2c**[13] and **3** (Table III) shows that the H1′ of the α-anomer **3** is 0.073 ppm upfield of H1′ in **2c**. Previous work[1,13,30] on the diamagnetic shielding of H1′ by a vicinal, cis-oriented oxygen indicates that H1′ in **3** should be shielded by much more, 0.3–0.5 ppm, relative to **2c**. In addition, the marked deshielding of H3′ in the β-anomer **2c** is not apparent in **3**. These two facts are critical to an evaluation of the phosphate ring conformation in **3** (vide infra). Finally, H4′ is deshielded by 0.27 ppm in **3** relative to **2c**. This is in accord with other work[31] which has shown a similar deshielding of H4′ in α-anomers due to a closer proximity of H4′ and the base ring when they are both on the same side of the sugar ring.

The chemical shifts of H1′ and H4′ in **1a**, **2a**, **1b**, and **2b** (see Table I) show interesting trends. In particular, it should be noted that H4′ is *deshielded* by 0.446 and 0.538 ppm, respectively, in the cyclic 2′,5′-phosphates **2a**,**b** relative to the cyclic 3′,5′-phosphates **1a** and **1b**. In addition, H1′ is *shielded* by 0.286 and 0.318 ppm, respectively, in the cyclic 2′,5′-phosphates **2a**,**b** relative to the cyclic 3′,5′-phosphates **1a**,**b**. It should also be noted that H4′ is shielded by ~0.25 ppm in going from xylouridine to lyxouridine (Table II). The implications of these shifts with regard to conformational changes about the glycosyl bond C1′–N1 will be discussed in a later section.

Several coupling constants are also worth noting at this point. The exceptionally large vicinal $^3J_{5',P}$ of ~31 Hz found in compounds **2c**,[13] **d**[14,15] was again apparent in compounds **2a**,**b** but *not* in the α-anomer **3**. In addition, the unusual $^4J_{PH}$ coupling of ~2.5 Hz between H4′ and phosphorus in **1c**[13] was also present in the lyxonucleoside cyclic 3′,5′-phosphates **1a**,**b**. Furthermore, a long-range $^4J_{PH}$ coupling of ~2.5 Hz was noted between H2′ and phosphorus in **1a**,**b**. With regard to the vicinal HH couplings, the *increase* in $J_{1',2'}$ from 6.5 to 7.4 Hz upon saturation of the uracil base ring in the cyclic 3′,5′-phosphates should be noted at this point and contrasted with a *decrease* in $J_{1',2'}$ (from 3.6 to 2.9 Hz) upon saturation of the aglycone in the cyclic 2′,5′-phosphates.



(a) (b)

Figure 3. Possible conformations of the lyxonucleoside cyclic 3′,5′-monophosphates **1a** and **1b**.

Discussion

A. Selection of Vicinal Correlations. In any NMR study of molecular conformations, the choice of vicinal correlation for translating the observed coupling constants into dihedral angles is critical. For the nucleoside and nucleotide series, the electronegativity of the substituents at C1′, C2′, C3′, and C4′, as well as their epimeric orientation, is obviously of great importance and has been discussed extensively elsewhere.[1,4,23a,32,33] In addition, theoretical calculations have indicated that the rotameric orientation of the sugar hydroxyls can affect the *J* values.[23a] However, the information currently available is insufficient to permit incorporation of all the above parameters and a well-established practice is to utilize a single approximate expression for all the sugar fragments.[1,4,11,13] For nucleic acid components, the $^3J_{HH}$ vs. θ_{HH} correlation proposed by Altona and Sundaralingam[32] and modified by Lee and Sarma[11] has been used extensively, including studies on the structurally related xylosyl and arabinosyl cyclic phosphates **1c** and **2c**.[13] This relationship has been shown in the case of **2c** to correlate well with the crystal structure data.[13,14] Accordingly, we have chosen to utilize this same vicinal correlation for $^3J_{HH}$ couplings in this series and the calculated dihedral angles are shown in Table I. Recent work by Shugar and co-workers[23] has described a theoretical approach by using the INDO–SCF–MO method for calculating dihedral angles from cisoidal $^3J_{HH}$ couplings, and they have shown, as expected, that such relationships are dependent to some degree on the glycosyl torsion angle, orientation of the hydroxyl groups, and the sugar pucker. Since nonbonded interactions [either repulsive (steric) or attractive (hydrogen bonding)] between cis-oriented substituents on a nucleoside sugar ring are expected to be the predominant factor in determining the conformation in solution, it is interesting to note the well-documented failure of the INDO scheme to account

(30) (a) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Am. Chem. Soc.*, **92**, 4088 (1970); (b) M. J. Robins, J. R. McCarthy, Jr., R. A. Jones, and R. Mengel, *Can. J. Chem.*, **51**, 1313 (1973); (c) C. K. Fay, F. B. Grutzner, L. F. Johnson, S. Sternhell, and P. W. Westerman, *J. Org. Chem.*, **38**, 3122 (1973); (d) T. Nishimura and B. Shimizu, *Chem. Pharm. Bull.*, **13**, 803 (1965).

(31) See ref 1 and references therein.

(32) (a) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972); (b) C. Altona and M. Sundaralingam, *ibid.*, **95**, 2333 (1973).

(33) D. B. Davies and S. S. Danyluk, *Biochemistry*, **13**, 4417 (1974).

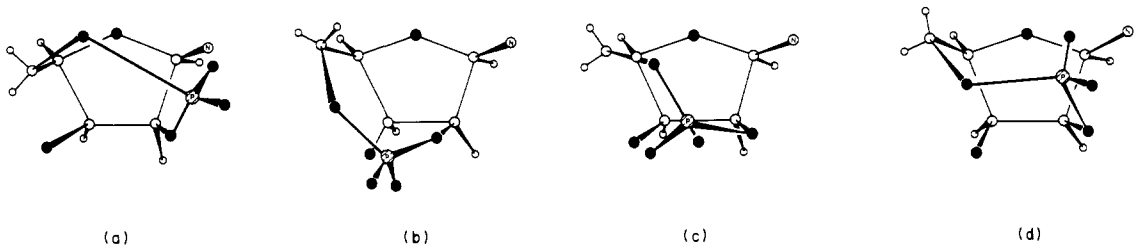


Figure 4. Possible conformations of the lyxonucleoside cyclic 2′,5′-monophosphates **2a** and **2b**. Epimeric transpositions at C3′ and C1′ give the possible conformations of the α-arabinonucleoside cyclic 2′,5′-monophosphate **3**.

correctly for nonbonded interactions.[34]

The choice of $^3J_{PH}$ for the nucleotides under study required careful evaluation since this relationship has been shown to be particularly susceptible to ring strain and bond angle distortions in nucleoside cyclic phosphates.[13-15] The chair forms of the six-membered phosphate rings in a cis 1,2 ring fusion with the five-membered sugar ring in compounds **1a–c** (see Figure 3) are not expected to contain undue strain,[13] and this is emphasized by the antiperiplanar arrangement of P and H5″ in **1a,b** (and, also in **1c**[13]), giving rise to a "normal" value of $^3J_{PH}$ of 21 Hz.[11] Accordingly, the J_{PH} vs. θ_{PH} relationship proposed by Lee and Sarma[11] was utilized in calculating the values of θ_{PH} shown in Table I for compounds **1a,b**. This relationship has been used successfully for the structurally related **1c**[13] as well as the α- and β-nucleoside cyclic 3′,5′-monophosphates.[1,11]

Because of the unusually large $^3J_{PH}$ coupling of ~30 Hz between P and H5′ in **2a,b**, it is not possible to use the same relationship for these two compounds.[13-15] The three couplings involved (2′,P, $5'_S$,P, and $5'_R$,P) are almost identical with those obtained previously for **2c**,[13]**d**.[14,15] Accordingly, the vicinal correlation proposed by MacCoss et al. for **2c**,[13] which gave excellent agreement with the X-ray data,[14] was used in this instance for **2a,b**.

The above-mentioned $^3J_{PH}$ of ~30 Hz was not apparent in **3**, even though it possesses the same ring sizes and ring fusion as **2a–d**. The conformational ramifications of this observation will be discussed later. Two large $^3J_{PH}$ couplings of ~26 Hz (2′,P and $5'_R$,P) indicate both H2′ and $H5'_R$ are antiperiplanar to the P, and Dreiding models (see later) indicate the third angle ($\theta_{P5'_S}$) to be ~60°. With use of these data, along with the measured J values, the constants in the standard Karplus expression ($J = a\cos^2\theta - b\cos\theta$) for the vicinal $^3J_{PH}$ coupling in the seven-membered ring of **3** are computed to be $a = 28.4$ and $b = -2.4$. This can be compared with the earlier Lee and Sarma correlation where $a = 18.1$ and $b = 4.8$.

One particular advantage of the use of the cyclic phosphates described in this study in determining ^{31}P–^1H "Karplus" expressions is the fact that in each instance, in any of the conformationally allowed chair or boat forms (see Figures 3 and 4), at least one of the sugar protons *must* be oriented antiperiplanar to the phosphorus. Since this orientation is expected to give the largest possible coupling,[35] one is able to make an immediate evaluation of the appropriate vicinal correlation to be used.

B. Conformational Aspects of the Cyclic Phosphate Rings. 1. The Six-Membered Rings in Compounds 1a–c. Inspection of Dreiding models indicates two possible chair forms of the six-membered phosphate rings in **1a,b**. These are depicted in Figure 3. The two possible boat forms of the phosphate rings (structures not shown) can be immediately eliminated since, in one case, the predicted θ_{PH}'s would be ~180, ~60, and ~60° and, in the other case, they would be ~180, ~180, and ~60°. Such combinations of dihedral angles are obviously not present (see Table I). It is of interest to note that the chair form depicted in Figure 3a locks the sugar into a $_3$E conformation and that depicted in Figure 3b fixes it into a ^{3}E conformation. In both cases, the orientation about C5′–O5′ is such that the phosphorus is trans to one of the protons at C5′ ($H5'_S$) and gauche to the other ($H5'_R$). However, the chair form in Figure 3a can be ruled out since it places the phosphorus and H3′ in an antiperiplanar orientation; thus a $^3J_{PH}$ of >20 Hz would be predicted. The observed value of zero for both **1a,b** is in excellent agreement with the dihedral angle of ~90° predicted for the chair form shown in Figure 3b. Furthermore, the long-range $^4J_{PH}$ of ~2.5 Hz between H2′ and phosphorus is most likely to occur when those atoms are arranged in a planar, zig-zag array ("W"-rule). Such an alignment occurs in the conformation depicted in Figure 3b but not in that depicted in 3a. Finally, the sugar conformation of $^3E/^3_4T$ (vide infra) and a similar phosphate ring conformation found in the closely related xylosyl derivative **1c**[13] make the conformation shown in Figure 3b unequivocal.

Of particular note is that the coupling constants in the phosphate rings in **1a,b,c** (i.e., $J_{3',4'}$, $J_{4',5'}$, $J_{4',5''}$, $J_{5',P}$, $J_{5'',P}$, and $J_{3',P}$) are essentially the same in all three cases, indicating that neither nature of the base (purine, pyrimidine, or saturated pyrimidine) nor the epimeric orientation of the 2′-hydroxyl (i.e., xylosyl or lyxosyl sugar) affects the conformation of this six-membered phosphate ring fused cis 1,2 to a five-membered sugar ring.

2. The Seven-Membered Phosphate Rings in Compounds 2a,b and 3. The three possible chair forms of the seven-membered phosphate rings are shown in Figures 4a,c,d, along with the one possible boat form in Figure 4b. With use of the definitions of Kung et al.,[14] conformations **4a–d** correspond to chair-P(δ), boat-P(δ), chair-P(γ), and chair-P(β), respectively. All four conformations lock the sugar into a ^{2}E pucker. With regard to the lyxosyl cyclic 2′,5′-phosphates **2a,b**, all the couplings in the seven-membered ring which have the same epimeric relationship to arabino derivatives **2c,d** (i.e., $J_{2',P}$, $J_{5',P}$, $J_{5'',P}$, $J_{4',5'}$, and $J_{4',5''}$) are almost identical in all four compounds **2a–d**, thus indicating similar conformations. Comparison of the calculated dihedral angles (Table I) with Dreiding models immediately eliminates the boat conformation shown in Figure 4b (since $\theta_{2'P}$ in that conformation is ~100–110°) and the chair conformation depicted in Figure 4d (since this requires that $\theta_{2',P} \simeq \theta_{5'_R,P} \simeq 180°$). This leaves the conformations shown in Figures 4a,c as both fitting the coupling data. In the case of arabino derivatives **2c,d**, the selection of the conformer in Figure 4c as the preferred conformation was based on the pronounced deshielding of H3′ due to the close proximity of the phosphate group in Figure 4c but not in Figure 4a and the expected destabilizing steric interactions between the phosphate and the base in Figure 4a but not in Figure 4c.[13] Since in the lyxo derivatives **2a,b** the transposition of the hydroxyl group at C3′ precludes the close proximity of H3′ and the phosphate, the above-mentioned deshielding of H3′ is not expected and, indeed, is not observed. In this instance, the preferred choice of the conformer in Figure 4c over that in Figure 4a is again based on the expected destabilizing steric interactions between the base and the phosphate ring in Figure 4a. Furthermore, the virtually identical coupling constants in the phosphate rings of **2a,b** with those in **2c,d** makes the selection of the conformer depicted in Figure 4c essentially unequivocal. It should be noted that the conformation of **2d** as shown in Figure 4c has been verified by X-ray crystallographic data.[14]

In contrast, the coupling data for α-cAAMP (**3**) (see Table I) leads to somewhat differing conclusions as regards the confor-

(34) (a) M. J. D. Dewar and G. P. Ford, *J. Am. Chem. Soc.*, **101**, 5558 (1979); (b) A. R. Gregory and M. Przybylska, *ibid.*, **100**, 943 (1978); (c) A. R. Gregory and M. N. Paddon-Row, *ibid.*, **98**, 7521 (1976); (d) A. R. Gregory, *Jerusalem Symp. Quantum Chem. Biochem.*, **6**, 23 (1974).

(35) (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

mation of this seven-membered phosphate ring. The first observation is that the large ~30-Hz $^3J_{PH}$ coupling found in **2a–d** is not present in this instance and that $J_{2',P} \simeq J_{5'_R,P} \simeq 26$ Hz. If one assumes that these two couplings are *both* the result of an antiperiplanar arrangement of phosphorus and hydrogen, then only one conformer of the phosphate ring, that depicted in Figure 4d, is possible (note that for **3**, epimeric transposition at C1′ and C3′ is required relative to the β-lyxo structures shown in Figure 4d). Further verification of the phosphate ring structure as shown in Figure 4d as being the preferred conformer is obtained by consideration of the chemical shift of H3′. Critical to the selection of the conformer depicted in Figure 4c for β-arabino cyclic 2′,5′-nucleotides **2c,d** was the pronounced downfield shift of H3′ due to the close proximity of the phosphate group. In the α-anomer **3**, the chemical shift of H3′ is shielded by 0.311 ppm relative to its β-anomer **2c** (see Table II), thus indicating a more distal relationship between H3′ and the phosphate group, as is present in the conformer shown in Figure 4d. It should be noted that in most instances when H3′ is oriented endo to C5′ (i.e., in the ribo and arabino series), then the anomeric transposition from β to α results in a *downfield* shift of H3′. Inspection of the Dreiding models for the structure depicted in Figure 4c shows the third phosphorus–hydrogen dihedral angle ($\theta_{5'_S,P}$) to be ~60°, and this was utilized to calculate the constants in the "Karplus" expression described earlier for this particular seven-membered phosphate ring fused cis 1,3 to a five-membered sugar ring.

The reason for the conformational changes in the phosphate ring in this bicyclic fused system now becomes evident. In the β-anomers of the lyxo and arabino series (**2a–d**), close steric contact between the aglycone at C1′ and the 2′–5′-bridging phosphate ring precludes the chair form of the phosphate ring as depicted in Figure 4d. Consequently, the phosphate ring takes up the alternative chair form shown in Figure 4c. However, even in this instance, in order to facilitate the presence of the aglycone, the bond angles in the phosphate ring at C2′, C4′, C5′, O2′, and O5′ are somewhat enlarged (particularly the bond angles C2′–O2′–P and C5′–O5′–P of 127.2 and 124.0°, respectively[14]), leading to appreciable ring strain and unusually large $^3J_{PH}$ values.[13-15] Neither the nature of the base (purine,[13] pyrimidine,[14,15] or saturated pyrimidine[36]) nor the epimeric substitution at C3′[36] affects this situation. Upon removal of this base–phosphate ring interaction by transposition of the aglycone to the α-anomer, as in **3**, the phosphate ring relaxes back to the preferred chair form depicted in Figure 4d. This occurs with a reduction in the bond angles and a consequently less-strained ring, as is evidenced by more normal values of $^3J_{PH}$ for an antiperiplanar orientation. It should be noted that the conformation as shown in Figure 4d is equivalent to the chair-P(β) form,[37] and our data would indicate that in this bicyclic ring system (seven-membered phosphate ring fused cis 1,3 to a five-membered sugar ring), the order of stability, in the absence of complicating steric factors, would appear to be chair-P(β) > chair-P(γ) in accordance with the predictions of Kung et al.[37] Thus, the highly strained phosphate rings present in **2a–d** are a feature of the particular nucleotide analogues under consideration and are *not* an inherent feature of seven-membered phosphate rings fused cis (1,3) to a five-membered ring. It is interesting to note that the monocyclic, unsubstituted tetramethylene phosphate prefers the chair-P(δ) conformer (equivalent to that depicted in Figure 4a), at least in the crystalline state.[38]

C. Conformational Aspects of the Sugar Rings. Inspection of Dreiding molecular models and examination of the calculated ϕ_{HH} dihedral angles for the sugar ring of the cyclic 3′,5′-phosphates **1a,b** (Table I) shows, in both instances, the sugar to be fixed into the ^{3}E to $^3_4$T (phase angle $P = 18$–36°) conformational range, with a puckering amplitude (τ_m) of ~45°. This best fit was obtained by comparing the derived values (Table I) with those predicted from the Altona–Sundaralingam approach.[32b] The largest deviations appear in $\theta_{3',4'}$ (~14°) and $\theta_{2',3'}$ (~10°), and this would appear to be a reflection on the overall accuracy of the vicinal correlation at this point on the curve and/or the effect of increased electronegativity on $J_{3',4'}$ and $J_{2',3'}$ by introduction of the phosphate group at C3′. It should be noted that an increase of only ~1 Hz in these two couplings would bring the experimentally derived $\theta_{3',4'}$ and $\theta_{2',3'}$ into extremely close agreement with the calculated values for ^{3}E/$^3_4$T. Such an electronegativity correction on $J_{3',4'}$ has been suggested by Davies.[4] In these lyxosyl derivatives, the dihedral θ_{HH} angles $\theta_{1',2'}$, $\theta_{2',3'}$, and $\theta_{3',4'}$ correspond to the endocyclic torsion angles τ_1, τ_2, and τ_3, assuming no distortion of the tetrahedral angles about the sugar carbon atoms. A similar approach for **2a,b** and **3** shows all three to be fixed into the ^{2}E conformation ($P \approx 162°$), **2a,b** having a puckering amplitude (τ_m) of 45° and **3** having a τ_m of 40°. For **2a,b**, the largest deviations from the predicted values are found for $\theta_{1',2'}$ (7–11°), again, possibly indicating the effect of phosphate electronegativity.

Thus, it appears that the sugar pucker preferred, in the six-membered phosphate rings fused cis 1,2 to the five-membered sugar rings (**1a–c**), is ^{3}E in all three cases, irrespective of the nature of the aglycone or the epimeric orientation at C2′. Similarly, for the seven-membered phosphate ring fused cis (1,3) to a five-membered sugar ring (**2a–d** and **3**), the sugar pucker is locked into a ^{2}E conformation and is invariant with the nature of the aglycone, epimeric changes at C3′ or C1′, and even the nature of the phosphate ring conformation (vide supra).

D. Conformational Aspects about the Glycosyl Bond (χ_{CN}). Determination of quantitative, self-consistent χ_{CN} values by NMR methods has long presented a challenging problem.[4,7] However, comparison of chemical shifts in structurally related compounds can provide a speculative, but not definitive, evaluation of the glycosyl torsion angle. With regard to the series of compounds in this study, it is of interest to observe the chemical shift trends of H4′ and H1′ in compounds **1a,b** and **2a,b** (Table I) and between xyloU and lyxoU (Table II). It should be noted that in going from the cyclic 3′,5′-phosphate **1a** to the 2′,5′-cyclic phosphate **2a**, H4′ is deshielded by 0.45 ppm while H1′ is shielded by 0.29 ppm. A similar, slightly more marked effect (0.54 ppm for H4′ and 0.32 ppm for H1′) is apparent in the dihydro series **1b** and **2b**. This indicates that the functionality primarily responsible for these shift changes is the carbonyl function at C2 and not the C5=C6 double bond in **1a** and **2a**. Such an observation is in good agreement with theoretical calculations.[39] One explanation for the shift trends observed relates to a different glycosyl torsion angle in the cyclic 3′,5′-phosphates **1a,b** as opposed to the cyclic 2′,5′-phosphates **2a,b**. Namely, for **1a**, a χ_{CN} of ~60°[40] (estimated from Dreiding models) which places the base in the expected anti conformation[4,7] also places H1′ in the plane of the C2=O2 bond and in a deshielding region. Such an orientation also places H4′ ~2 Å out of the same deshielding plane. Upon introduction of the cyclic 2′,5′-phosphate ring in **2a**, the steric interactions between the phosphate ring and the base become apparent (vide supra) and a change of χ_{CN} to ~15° would lead to a relaxing of these energetically unfavorable interactions and at the same time would place H4′ in the plane of the C2=O2 bond (thus deshielding H4′ in the **2a** relative to **1a**) while moving H1′ out of the plane (thus shielding H1′ in **2a** relative to **1a**). It should be noted that similar trends would be expected for the syn conformations $\chi_{CN} \simeq 240°$ for **1a** and ≃195° for **2a**, and these cannot be immediately ruled out due to the larger effect on the more distant H4′ than on H1′ (for the anti orientations). Similar arguments relating to the positioning of H4′ and H1′ relative to the plane of the C2=O2 bond can be made for the dihydro derivatives **1b** and **2b**.

A further argument for a glycosyl bond angle change in going from **1a,b** to **2a,b** can be obtained from the change in $J_{1',2'}$ upon saturation of the uracil ring. With the sugar pucker fixed in the ^{3}E conformation in both cases, $J_{1',2'}$ in the dihydro derivative **1b**

(36) This work.
(37) See ref 14, p 5476 for conformation definitions of the chair forms of a seven-membered phosphate ring and for a discussion on their relative stabilities.
(38) C. H. Coulter, *J. Am. Chem. Soc.*, **97**, 4084 (1975).
(39) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **65**, 171 (1977).
(40) For the definition of χ_{CN} used, see M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).

is 0.9 Hz *greater* than in the unsaturated case **1a**. Conversely, with the sugar fixed in the ^{2}E conformation, $J_{1',2'}$ in the dihydro derivative **2b** is 0.7 Hz *smaller* than in the unsaturated **2a**. If these coupling changes were due solely to a change in the electronegativity of the substituent at C1′, then one would expect both to change in the same direction. A difference in χ_{CN} between the cyclic 3′,5′-phosphate (**1b**) and the cyclic 2′,5′-phosphate (**2b**) would lead to a different orientation of the lone pair of electrons on the sp^3-hybridized N1 and the H1′–C1′–C2′–H2′ coupling path for $J_{1',2'}$. It should be noted that pronounced glycosyl torsion angle changes between the α- and β-anomers of 5,6-dihydrouridine cyclic 3′,5′-monophosphate have been postulated previously.[1]

Examination of the chemical shift data for the nucleosides xyloU and lyxoU (Table II) shows H4′ to be *shielded* by 0.25 ppm in the latter. Since a cross-ring diamagnetic shielding by the 2′-hydroxyl would be expected to *deshield* H4′ in the lyxoU relative to the xyloU, a change in χ_{CN} (H4′ in the plane of the base ring, i.e., $\chi_{CN} \approx 15°$, for the xyloU but out of the plane in lyxoU) would explain the observed shifts, particularly since the sugar pucker is approximately the same in both cases (see later). Such a change in χ_{CN} might be expected due to a steric interaction between the cis-oriented 2′-hydroxyl and the uracil base in the lyxoU. Similar interactions have been discussed earlier in α-ribo and β-arabino nucleoside derivatives.[1,19,41,42] A reciprocal shift change in H1′, as noted in the lyxo cyclic phosphates (vide supra), cannot be used in this instance to confirm the χ_{CN} change, since in the xylo case the diamagnetic shielding of the vicinal, cis-oriented 2′-hydroxyl is expected to shield H1′ by ~0.3–0.5 ppm[1,30] relative to the lyxonucleoside and thus complicate the situation. A shielding of 0.36 ppm is observed (see Table II).

E. Isomerization of Lyxonucleoside Cyclic 2′,5′- and 3′,5′-Monophosphates. Previous workers[21,22] have synthesized **1a** and **2a** and shown that one isomerized to the other in acid or base; they were not able to distinguish between the two isomers. The detailed conformational analyses described herein now allow a rational explanation. With use of NMR to monitor the isomerization, the cyclic 2′,5′-phosphate **2a** was shown to isomerize completely in $3^1/_2$ h, with a $t_{1/2}$ of ~40 min to the 3′,5′-isomer **1a** at 52 °C, at a pD of 0.50. No change could be detected in **1a** over a similar period. Inspection of molecular models indicates that with the phosphate ring in the conformation depicted in Figure 4c and with the sugar ring in the ^{2}E conformation (see earlier), then the 3′-hydroxyl is now ideally situated for nucleophilic attack at the phosphorus atom. Such an orientation is not possible with the cyclic 3′,5′-nucleotide **1a** having the phosphate ring in the conformation shown in Figure 3b and the sugar in the ^{3}E conformation (see earlier). In this instance, the 2′-hydroxyl and the phosphorus are not in close proximity and also are not correctly aligned for the nucleophilic attack to take place.

F. Solution Conformation of Lyxonucleosides. Despite much work on the naturally occurring ribo- and deoxyribonucleosides,[4] NMR studies on other pentofuranosyl epimers has been somewhat restricted.[4,5,7,23,42-44] One reason for this has centered around an inability to use directly the Altona–Sundaralingam approach[32] since epimeric transpositions preclude the direct use of $J_{1',2'} + J_{3',4'}$ and $J_{2',3'}$ to determine pseudorotational parameters. In addition, the different epimeric relationships of the sugar ring protons cast some doubts as to which vicinal correlation to use in these instances.[23] Examination of the data in Tables I and II indicates that the sugar ring coupling constants ($J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$) for lyxoU and lyxoA in D_2O are almost identical with those of the conformationally invariant **1a**. The only exception is $J_{3',4'}$, which has a value of 3.8 Hz in the nucleoside and 2.2 Hz in **1a**. The smaller value in **1a** may be due to some extent to the electronegative phosphate group attached to C3′;[4] in addition, the slope of the "Karplus" curve at this point is such that a large change in coupling reflects only a small change in dihedral angle. For example, the standard "Karplus" expression used in the conformational analysis of **1a–c**, **2a–c**, and **3**[11] (see earlier) predicts a difference of only 10° in $\theta_{3',4'}$ for J values of 2.2 Hz (59°) (i.e., in **1a**) and 3.8 (49°) (i.e., in lyxoU). On the basis of these observations, it is tempting to suggest that **1a** and the lyxonucleosides, lyxoU and lyxoA, have essentially the same conformation, i.e., $^3E/^3_4T$ (P = 18–36). However, due to the all-cis arrangement of the sugar protons in lyxonucleosides, one cannot rule out the possibility of the conformation being $_3E/^4_3T$ (i.e., P = 198–216) or, indeed, a conformation equilibrium between $^3E/^3_4T \rightleftharpoons {}_3E/^4_3T$, since in both of these conformations the sugar proton dihedral angles are identical. The presence of a conformational equilibrium that includes a conformer from the S hemisphere centered on ^{2}E (i.e., P = 162)[23b] can be discounted since the couplings of $J_{1',2'}$ and $J_{3',4'}$ for a pure ^{2}E contributor would be 3.6 and 7.8 Hz, respectively (i.e., the values for **2a**, see Table I). Thus even small contributions from such a conformer would lead to a marked decrease of the observed $J_{1',2'}$ and a marked increase of the observed $J_{3',4'}$. This conclusion is in contrast to that arrived at by Ekiel et al., who describe a "$^3E/^3_4T \rightleftharpoons {}^2_1T/^2_3T$" conformer equilibrium, based on essentially the same coupling data. However, the predicted $J_{1',2'}$ and $J_{3',4'}$ values for the "$^2_1T \cdots {}^2_3T$" conformation (4.5–5 and 6.5 Hz, respectively[23b]) again are so different from the observed values that even small contributions from such a conformer would cause significant deviation of the observed values from those noted for **1a**. Comparison of the observed $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ for the conformationally invariant **1a** ($^3E/^3_4T$) and **2a** (^{2}E) with those predicted by Ekiel et al. on theoretical grounds (see Figure 8 in ref 23b) shows exceptionally good agreement for these two conformations. Indeed, such calculations[23b] predict *only* the $^3E/^3_4T$ conformations to have $J_{3',4'}$ as low as ~3.8 Hz (the only other minimum value for $J_{3',4'}$ is ~6 Hz and it is found at $P \simeq$ 216, i.e., ^4_3T[23b]) and so indicate perhaps a singular preference for a $^3E/^3_4T$ conformation in solution for lyxonucleosides. It is interesting to note that X-ray data show a $_3T^2$ conformation for lyxoU in the crystalline state[23b] which is extremely close to the other conformational possibility $_3E/^4_3T$ indicated by our data (vide supra). However, the pronounced intermolecular hydrogen bonding observed in the X-ray study[23b] could well be an important factor governing the conformation in the crystalline state.

The data for xyloU indicate a high preference for the ^{3}E conformer (compare the data in Table II with the values for the locked-in cyclic 3′,5′-phosphate **1c**[13]), and, as for lyxoU, the exocyclic rotamer populations show no preference for ψ_+ (only 27%). This observation has been rationalized by the cis relationship of the 5′-CH_2OH and the 3′-OH destabilizing the ψ_+ rotamer.[23b,43] Such a low ψ_+ population tends to rule out the possiblity of hydrogen bonding between the 5′- and 3′-hydroxyls as a stabilizing factor for the $^3E/^3_4T$ conformer.[23b] In addition, the $J_{OH2',2'}$, $J_{OH3',3'}$, $J_{OH5',5'}$, and $J_{OH5',5''}$ coupling data for lyxoU, observed when the spectrum was obtained in DMSO-d_6 (see Table II), indicate essentially free rotation about the C5′–O5′ and C2′–O2′ bonds, and a preference for the two gauche conformers about the C3′–O3′ bond.[4,45] This is the same situation which exists in the β-ribo series[45] and further indicates no preference for intramolecular hydrogen bonding. Hydrogen bonding between the 5′-hydroxyl and the 2′-hydroxyl has been observed at alkaline pH in arabinonucleosides and for 3′-*O*-methyl-lyxoC,[23b] but not for the neutral forms.[23b,44] One factor, previously overlooked, for stabilizing the $^3E/^3_4T$ conformer in xylosides is that in this conformation, the dipoles in the C2′–OH and C3′–OH bonds and in the C2′–OH and C1′–N bonds are aligned as close as possible to the energetically favored antiperiplanar arrangement. At this time, it is difficult to rationalize a singular preference for $^3E/^3_4T$ in the lyxo series; however, the absence of a conformer equilibrium may be predictable on the grounds that it would require the energetically unfavorable eclipsing of substituents (–CH_2OH at C4′, –OH's

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at C3′ and C2′, and the base at C1′) on all four of the sugar-ring carbons upon passing from one conformer to another.

Summary

Evidence has been presented that the sugar pucker (^{3}E) and the phosphate ring conformation for a six-membered phosphate ring fused cis 1,2 to a five-membered sugar ring are both invariant to the nature of the β-C1′ substituent and the epimeric orientation of a hydroxyl group at C2′. Similarly, for a seven-membered phosphate ring fused cis 1,3 to a five-membered pentose sugar, the sugar pucker (^{2}E) and the phosphate ring conformation are both invariant to the nature of the β-C1′ substituent and to the epimeric orientation of a hydroxyl group at C3′. However, in this same bicyclic ring system, release of the unfavorable steric interactions between the base and the phosphate ring, by transposition of the base to the α-configuration, causes a change in the phosphate ring conformation to a more energetically favored chair form, whereas the sugar pucker remains invariant (^{2}E). The conformational information from the NMR data has permitted unequivocal identification of the cyclic 2′,5′- and 3′,5′-monophosphates of lyxouridine for the first time and, in addition, has provided a rational mechanistic explanation for the isomerization of the 2′,5′-isomer to the 3′,5′-isomer. In the light of the analyses for these conformationally fixed cyclic phosphates, a reevaluation of the coupling data for the free nucleosides lyxouridine and lyxoadenosine indicates that they exist in solution at ambient temperature either in a ^{3}E/$^3_4$T or a $_3$E/$^4_3$T conformation (or in an equilibrium between these two conformers), in contrast to a previous interpretation of the NMR data.

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Communications to the Editor

Electrocatalytic Reduction of Carbon Dioxide by Using Macrocycles of Nickel and Cobalt

Sir:

The reduction of carbon dioxide, this planet's most abundant source of carbon, is an important objective in the development of alternative fuel sources. This reduction requires both the action of a catalyst and energy input to be viable. The former is a result of the large overpotential associated with the direct electrochemical reduction of CO_2, while the latter results from the fact that CO_2 is the stable carbon end product of metabolism and other combustions. Equation 1 shows the half-cell reaction for the two-

$$CO_2 + 2e^- + 2H^+ = CO + H_2O \quad (1)$$

electron reduction of CO_2 to CO. The standard potential for this reaction is −0.10 V while at pH 7 the potential becomes −0.52 V. The direct electroreduction of CO_2 on various metal electrodes in both aqueous and nonaqueous media has been reported by numerous authors.[1-4] However, these direct electroreductions have required potentials more negative than ca. −2 V vs. SCE. In this paper, we describe an *indirect* electrochemical reduction of CO_2 which involves the initial reduction of metal complexes and their subsequent reaction with CO_2. This approach facilitates reducing CO_2 at potentials closer to the thermodynamic values. The metal complexes are thus redox-activated catalysts.

The catalysts employed were the tetraazamacrocyclic complexes (**1**–**5**) of cobalt and nickel. These are listed in Table I, along with the cell potentials applied during the electrocatalytic reactions. The preparation and electrochemical behavior of these complexes in nonaqueous solvents have been previously reported.[5] Each complex undergoes uncomplicated reversible or quasi-reversible one-electron transfers in dry, nonaqueous solvents. Evidence for CO_2 reduction was obtained from controlled potential coulometry (cpc) experiments performed in a gas-tight electrolysis cell under an exclusively carbon dioxide atomosphere.[6] The concentration of catalyst ranged from 1 to 2.5 mM in these experiments, and the solvent systems used were either acetonitrile/water or water only. Gas chromatographic analysis was used to determine the composition of the gases above the electrolysis solution during and after each run.[7] Formate was analyzed for either by esterfication to methyl formate or by dehydration to CO, each followed by GC analysis. In a typical run, 9×10^{-5} mol of **2** in 75 mL of acetonitrile/water (1:2 v/v) was electrolyzed under CO_2 at ambient room temperature. After 1.1 h, 82 C had been passed, corresponding to 8 e^- per cobalt complex, and GC analysis revealed 1.8×10^{-4} mol of CO and 1.8×10^{-4} mol of H_2 as the only products, corresponding to a current efficiency of 94%.

The results for these electrolysis experiments, which were each performed at least six times, are shown in Table I. Compounds **1**, **2**, and **3** display current efficiencies of greater than 90% and rates of catalysis in terms of electrons passed per complex per hour ranging from 2 to 9 at ambient room temperature. Longer term electrolyses performed with complexes **1** and **2** underscore the catalytic nature of the observed reactions. For example, an electrolysis with 8.8×10^{-5} mol of complex **2** produced 5.1×10^{-3} mol of H_2 and 1.4×10^{-3} mol of CO after 19 h of electrolysis under a CO_2 atmosphere, corresponding to 164 turnovers or electrons passed per catalyst complex, and a current efficiency of 96%. Similar results were obtained from other runs with both **1** and **2**.

The nature of the catalytic reduction process was investigated further by several additional cpc experiments, the results of which are summarized as follows: (1) No carbon monoxide was detected when electrolyses were performed under a CO_2 atomosphere at potentials as negative as −1.6 V vs. SCE in the absence of the catalysts. (2) No carbon monoxide was detected when complexes

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(6) The gas-tight cell was a 250-mL three-necked 14/20 round-bottomed flask equipped with two side arms. The working electrode in all the experiments was mercury. The connection to the Hg was made through a Pt wire inserted through the bottom of the flask. The cell volume was 270 mL of which 185 mL was occupied by gases.
(7) Molecular hydrogen and carbon monoxide were determined on a 2 ft × $^1/_4$ in. column of molecular sieves 5A and a 12 ft × $^1/_4$ in. column of Poropak Q at 43 °C; carbon dioxide was determined on the Poropak column under the same conditions.
(8) Turnover numbers are calculated from the mol of electrons passed per mol of catalyst. Current efficiencies are calculated from the ratio of mol of product detected to the mol of product expected on the basis of a two-electron reduction of CO_2 and the measured number of C passed during the run.

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