Acknowledgment. This research was supported in part by the Energy Research and Development Administration under Contract EY-76-C-02-1198, in part by a grant from the Research Corporation, and in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society.

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Conformational Characteristics of Rigid Cyclic Nucleotides. 2. The Solution Conformation of α-Nucleoside 3′,5′-Cyclic Monophosphates and the Role of the 2′-Hydroxyl Group[1]

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Abstract: The first detailed study has been made of the 220-MHz NMR spectra of α-adenosine 3′,5′-cyclic monophosphate (I, α-cAMP), α-uridine 3′,5′-cyclic monophosphate (II, α-cUMP), α-cytidine 3′,5′-cyclic monophosphate (III, α-cCMP), α-deoxyadenosine 3′,5′-cyclic monophosphate (IV, α-cdAMP), α-5,6-dihydrouridine 3′,5′-cyclic monophosphate (V, α-cDHUMP), and β-5,6-dihydrouridine 3′,5′-cyclic monophosphate (VI, β-cDHUMP) in D_2O solution. Analyses of the spectra of I–III were aided by the use of europium chloride as a shift reagent. A conformational analysis showed the sugar moieties of I–III to exhibit a conformation in the range ${}_2T^3$ to ${}^3T_2$ with an unusually high distortion from planarity, in contrast to the β anomers which prefer ${}^3E$ to ${}_4T^3$ and the acyclic mononucleotides which show a ${}^2E \rightleftarrows {}^3E$ equilibrium. This change in the preferred conformation is attributed to a repulsive interaction between the 2′-hydroxyl and the base. Removal of the 2′-hydroxyl group eliminates this interaction and causes a relaxation to a less strained system. This is clearly demonstrated in the sugar ring conformation of IV which exhibits a ${}^3E$ to ${}_4E$ pucker and a puckering amplitude that is less than in the ribo series. Hydrogenation of the pyrimidine ring of II and β-cUMP gave the 5,6-dihydro products V and VI. VI exhibits preference for a ${}^3E$ to ${}_4T^3$ ribose ring conformation and in the case of V the 2′-hydroxyl–base interaction is markedly reduced owing to the increased flexibility of the aglycon. This results in a relaxation of the sugar ring conformation from the ${}_2T^3$ to ${}^3T_2$ in I–III back toward the ${}^3E$ to ${}_4T^3$ conformation found in the β anomers. Saturation of the base is not as effective as removal of the 2′-hydroxyl group in relieving the strain in these rigid systems. The phosphate ring is found to be in a flattened chair form in all cases. A detailed discussion is presented for chemical shift differences for particular protons in anomeric pairs.

In recent years, the conformational properties of nucleoside and nucleotide derivatives have been extensively studied by x-ray[3] and NMR methods.[4] The conformational properties of the flexible molecules such as nucleoside monophosphates and nucleoside 2′,3′-cyclic monophosphates have been shown to be best represented in solution as a dynamic equilibrium between various conformers.[4] For example, the sugar conformation in such derivatives is usually expressed as a ${}^2E \rightleftarrows {}^3E$ equilibrium, and structural modifications to the molecule have been shown to affect the position of this equilibrium.[5,6]

An approach with considerable merit for defining specific conformational features utilizes derivatives in which this equilibrium is eliminated by the formation of a rigid ring system. This allows a simpler evaluation of particular intramolecular interactions[7] and of the shielding properties of hydroxyl groups on the sugar ring protons. The β-nucleoside 3′,5′-cyclic monophosphates are of biological importance,[8] and structurally they contain a six-membered phosphate ring fused trans (1,2) to a five-membered sugar ring. This produces a rigid bicyclic system with the sugar held in a ${}^3E$ to ${}_4T^3$ conformation.[7,9] Other rigid cyclic nucleotides which have been rigorously examined by NMR methods include 9-(β-D-xylofuranosyl)adenine 3′,5′-cyclic monophosphates[10] [possessing a six-membered phosphate ring fused cis (1,2) to a five-membered sugar ring] and the 2′,5′-cyclic monophosphates of 1-(β-D-arabinofuranosyl)cytosine[9b,11] and 9-(β-D-arabinofuranosyl)adenine[10] [possessing a seven-membered phosphate ring fused cis (1,3) to a five-membered sugar ring]. Another series of derivatives which are structurally rigid is the anhydronucleosides, and these too have been well studied by NMR methods.[7,12]

A question of some significance is the influence of the 2′-

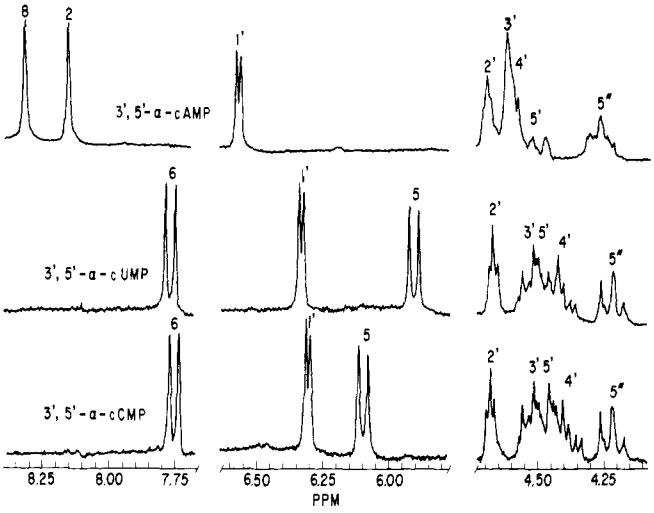


Figure 1. The 220-MHz spectra of α-cAMP (top), α-cUMP (middle), and α-cCMP (bottom) in D_2O, 0.02 M concentration, at 20 °C and pH 7.

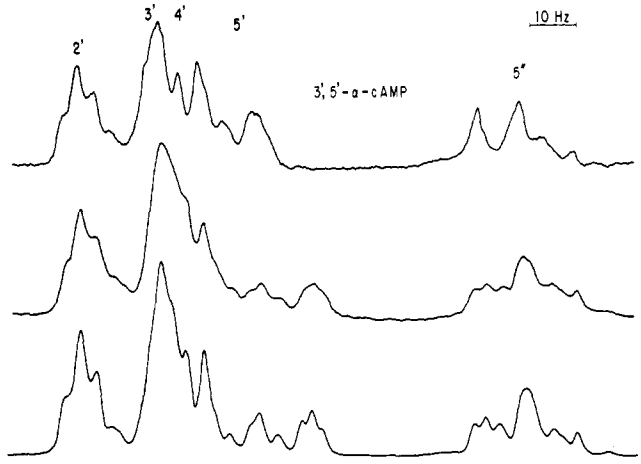
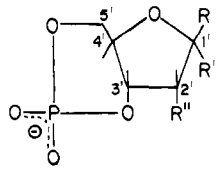


Figure 2. The 220-MHz spectra of the ribose ring region of α-cAMP in D_2O, 0.02 M concentration at 20 °C and pH 7; observed (middle), phosphorus decoupled (upper), and computer simulated (lower).

hydroxyl group on the conformational properties when oriented cis to the base[5,6] (e.g., in β-arabino- or α-ribonucleosides). In arabinoadenosine, a change in the conformer populations of the $^2E \rightleftarrows {}^3E$ equilibrium (from ~65% ^2E for adenosine to ~25% ^2E for arabinoadenosine[5b]) has been used to quantitate this interaction. However, for arabinocytidine Remin et al. calculated a 50% ^2E conformer population.[5a] Similarly, the interaction between the base and the 2′-hydroxyl group in the α anomers of ribonucleoside derivatives has been examined by observation of the changes in the ribose ring conformer populations.[6,13]

The α-nucleoside 3′,5′-cyclic monophosphates provide an ideal system for observation of this vicinal interaction in sugar rings since it should be manifested as a distinct change in the puckering mode of a rigid system as opposed to a shift in the conformational equilibrium of a flexible system. In the β-arabinonucleoside 3′,5′-cyclic monophosphates, the 2′-hydroxyl was considered to present a potential barrier to rotation about the glycosyl bond;[14] however, no change in sugar pucker (relative to the ribo derivatives) was noted.



(I) R=H, R′=adenine, R″=OH
(II) R=H, R′=uracil, R″=OH
(III) R=H, R′=cytosine, R″=OH
(IV) R=H, R′=adenine, R″=H
(V) R=H, R′=dihydrouracil, R″=OH
(VI) R=dihydrouracil, R′=H, R″=OH

Recent studies[15] utilizing the anomeric coupling constants ($J_{1'2'}$) of α- and β-nucleoside 3′,5′-cyclic monophosphates, to produce a "geometry-only" specific method for the determination of the anomeric configuration of ribonucleosides, indicated that differences in the sugar conformation between the α and β anomers exist. This prompted the present study of a full analysis of various α-nucleoside 3′,5′-cyclic monophosphates at 220 MHz in which we have examined the conformational changes which occur with different 2′ substituents and with different degrees of flexibility in the base.

Experimental Section

The nucleoside 3′,5′-cyclic monophosphates were synthesized as described elsewhere[15b,16] and were repurified by DEAE-cellulose column chromatography prior to use. Ammonium salts of the cyclic nucleotides were lyophilized once from "100%" D_2O, dissolved in "100%" D_2O, and the solutions adjusted to 0.02 M concentration and pD 7.4 (pD = meter reading + 0.04). 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. All of the spectra were measured at 20 ± 2 °C and chemical shifts are reported relative to internal TSP with an accuracy of ±0.005 ppm. ^{31}P decoupling experiments were performed using a Schomandl ND 100 M generator set at the appropriate ^{31}P decoupling frequency, ~89 MHz.

Because of extensive overlap of signals, spectra of I, II, and III were also obtained in the presence of europium chloride.[9b,16] The pD of the solution containing the cyclic nucleotide was first adjusted to 1.0 and successive portions of $EuCl_3$ were added until the ^1H NMR signals were sufficiently resolved to allow a complete determination of the coupling constants. Using these coupling constants and the chemical shifts from the unshifted spectra as initial parameters for iterative simulations, the spectra in D_2O (unshifted) were fully analyzed employing a Nicolet 1080 computer and the ITRCAL simulation programs. Final simulations were performed on an IBM 370/195 computer using NMRIT and NMREN programs.

Results

Figure 1 shows the 220-MHz spectra for 0.02 M solutions of the α-nucleoside 3′,5′-cyclic monophosphates I-III in D_2O. All of the spectra are characterized by well-resolved base and anomeric H(1′) signals in the region 5.9-8.3 ppm downfield from TSP. The remaining sugar proton resonances are concentrated within a narrow range (ca. 0.5 ppm) from 4.218 to 4.715 ppm with the overlap of signals greatest for α-cAMP. The signals in I-III were assigned with the aid of $EuCl_3$ shifted spectra and ^{31}P decoupling experiments. After addition of $EuCl_3$, the signals for the ribose ring portion were sufficiently well resolved to make the assignments and computer simulation straightforward.

To obtain the coupling constants in the unshifted spectra at pD 7.4, the initial values were measured from the $EuCl_3$ shifted spectra and refined by simulation. Chemical shifts and coupling constants were adjusted to obtain the best fit to the observed spectra. In all cases, neither the low pD nor the addition of $EuCl_3$ significantly affected the coupling constants[9b,16] (±0.2 Hz). The partial ^1H NMR spectrum (sugar protons) of α-cAMP at pD 7.4 is shown in Figure 2 (middle) along with the ^{31}P decoupled (upper) and simulated (lower) spectra. This simulated spectrum was calculated utilizing the chemical shifts and coupling constants shown in Table I. Similar analysis procedures were followed for II and III, and the NMR parameters are also listed in Table I.

The 220-MHz spectrum of α-deoxyadenosine 3′,5′-cyclic monophosphate is shown in Figure 3. Measurement of the coupling constants and chemical shifts was aided by phos-

Table I. NMR Parameters for the α-Nucleoside 3',5'-Cyclic Monophosphates and for β-cDHUMP

	δ (ppm from TSP)								
	8(6)	2(5)	1'	2'	2"	3'	4'	5'	5"
α-cAMP (I)	8.302	8.137	6.571	4.715		4.645	4.612	4.560	4.286
α-cUMP (II)	7.755	5.901	6.332	4.673		4.524	4.408	4.497	4.220
α-cCMP (III)	7.746	6.097	6.309	4.680		4.515	4.394	4.491	4.218
α-cdAMP (IV)	8.487	8.262	6.537	3.068	2.918	4.650	4.383	4.410	4.228
α-cDHUMP (V)	3.609	2.636	5.918	5.172		4.395	3.923	4.455	4.209
	3.753	2.636							
β-cDHUMP (VI)	3.506	2.741	5.845	4.509		4.318	4.045	4.468	4.200
	3.548	2.759							

	J_{ij} (Hz), ϕ_{ij}, and τ_i																	
	1'2'	1'2"	2'3'	2'2"	2"3'	3'4'	4'5'	4'5"	5'5"	5a5b	5 6	6a6b	1'P	2'P	3'P	4'P	5'P	5"P
α-cAMP (I)	3.6		3.9			10.2	4.7	10.4	−9.4						1.8		21.5	1.8
α-cUMP (II)	3.5		3.8			10.0	4.8	10.2	−9.6						2.1		21.3	1.9
α-cCMP (III)	3.5		4.0			10.2	4.7	10.1	−9.4						2.0		21.3	1.8
ϕ_{ij}	50°		48°			159°	43°	160°	gem						62°		165°	62°
τ_i	50°		−48°			39°												
α-cdAMP (IV)	6.8	7.9	6.6	−12.5	11.0	9.7	4.6	10.5	−9.4						2.0		20.8	2.2
ϕ_{ij}	23°	135°	24°	gem	148°	155°	44°	163°	gem						62°		161°	60°
τ_i	23°		−24°			35°												
α-cDHUMP (V)	5.0		4.9			9.7	4.9	10.4	−9.6	*a*	*b*	−14.5			2.1		20.8	2.1
ϕ_{ij}	41°		42°			155°	42°	162°	gem	gem	*b*	gem			60°		161°	60°
τ_i	41°		−42°			35°												
β-c-DHUMP (VI)	~0.5		5.3			9.8	4.6	10.2	−9.7	*a*	*c*	−12.4			2.0		21.0	1.8
ϕ_{ij}	102°		40°			156°	44°	160°	gem		*c*	gem			62°		162°	62°
τ_i	18°		−40°			36°												

[a] Owing to the near magnetic equivalence of H(5a) and H(5b), it was not possible to determine J_{5a5b} accurately. [b] $J_{5,6}$ = 6.9, 6.3, 6.5, and 6.5 Hz. [c] Owing to the near magnetic equivalence of H(5a) and H(5b) and of H(6a) and H(6b), it was not possible to determine $J_{5,6}$ accurately.

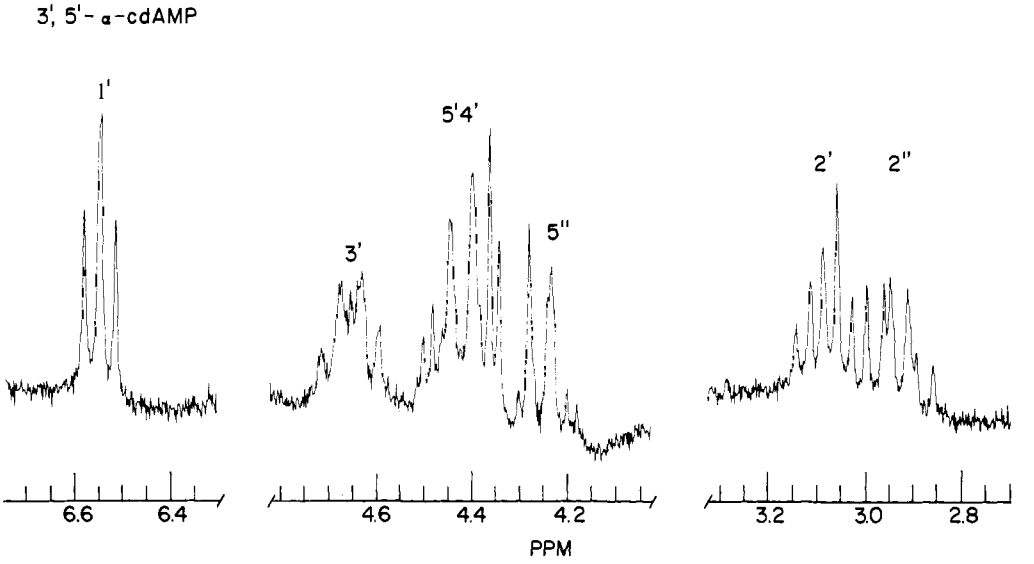


Figure 3. The 220-MHz spectrum of the sugar protons of α-cdAMP in D_2O, 0.02 M concentration, at 20 °C and pH 7.

phorus decoupling experiments and computer simulation. As expected, the H(2') and H(2") resonances[17] appear upfield in the 3-ppm region with a 0.15-ppm relative shift difference between the two resonances. The assignment of these two protons as shown in Figure 3 was determined on the basis of earlier work[16] utilizing the bandwidth change upon the addition of lanthanide ion. The low-field signal is due to the proton [H(2')] which is located cis to H(3') and H(2") gives rise to the high-field resonance. A full account of the chemical shifts of H(2') and H(2") in 2'-deoxynucleoside 3',5'-cyclic monophosphates has been recently described.[16] The spectrum of IV was also recorded after the addition of $EuCl_3$ (not shown), but this data was not required for the full analysis of the unshifted spectrum.

The spectra for the α- and β-5,6-dihydrouridine 3',5'-cyclic monophosphates were also sufficiently well resolved and did not require addition of $EuCl_3$ (Figure 4). Interesting features of the α-5,6-dihydrouridine 3',5'-cyclic monophosphate spectrum include the significant nonequivalence (~0.15 ppm) of the two H(6) signals and the 0.7 ppm downfield shift of the H(2') signal relative to the β anomer.

Discussion

A. Conformational Aspects of the Cyclic Phosphate and Sugar Rings. Since the coupling constants for the α-ribonucleoside 3',5'-cyclic monophosphates I-III (Table I) are nearly identical, it is reasonable to conclude that the conformations of the cyclic phosphate and ribose rings are similar in these

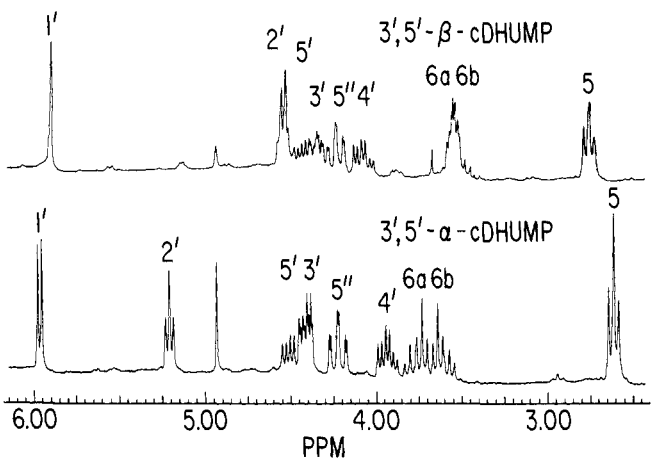


Figure 4. The 220-MHz spectra of β-cDHUMP (upper) and α-cDHUMP (lower) in D_2O, 0.02 M concentration, at 20 °C and pH 7.

three cases. Apparently, the nature of the base does not cause any marked changes in the conformational properties of the fused cyclic phosphate-ribose ring systems. Thus, for I-III, an averaged value for each coupling constant was used in calculating the dihedral angles (ϕ_{ij}). The Karplus relations, proposed by Lee and Sarma[7] for vicinal J_{PH} and J_{HH}[18] couplings, were utilized for computing the P-O-C-H and H-C-C-H dihedral angles in compounds I-III, V, and VI, and the data are listed in Table I. The parameters derived for the relation $J_{HH} = A\cos^2\theta - B\cos\theta$ to describe proton-proton coupling in the ribose ring cannot be used to calculate the dihedral angles about C(1')-C(2') and C(2')-C(3') in the deoxyribose ring of IV because of the differences in electronegativities of the two ring systems.[18] It has been postulated that removal of the 2'-hydroxyl can cause differences as much as 0.5 Hz.[7,19] In order to derive values of A and B for α-cdAMP (IV), the following procedure was used. Using a range of values for $\phi_{1'2'}$ and $\phi_{1'2''}$ ($0 < \phi_{1'2'} < 90°$ and $\phi_{1'2''} = \phi_{1'2'} + 120°$) and the observed $J_{1'2'}$ and $J_{1'2''}$, a series of Karplus coefficients was generated. A similar series was then generated for $\phi_{2'3'}$ and $\phi_{2''3'}$ ($0 < \phi_{2'3'} < 90°$ and $\phi_{2''3'} = \phi_{2'3'} + 120°$) using $J_{2'3'}$ and $J_{2''3'}$. The coefficients that were in closest agreement between the two series ($A = 11.5 \pm 0.3$ and $B = 3.2 \pm 0.7$) were then utilized to compute the dihedral angles for IV shown in Table I. These same values when used to calculate dihedral angles for the β-deoxynucleoside 3',5'-cyclic monophosphates gave little deviation (±10°) from published values.[7,9]

While the use of Karplus relation to generate absolute conformations in these systems is beset with uncertainties due to changes in electronegativity and/or strain,[16] it is established practice to employ their use for comparison purposes within a series of structurally related compounds.[6,20]

1. Conformation of the Cyclic Phosphate Rings. The coupling data (Table I) show that the conformation of the cyclic phosphate ring is essentially constant throughout the series I-VI. Neither the nature of the base nor the anomeric configuration have any significant effect on the properties of this ring. Furthermore, the data indicate that the conformation of the cyclic phosphate ring is best described as a chair form (Figure 5a) in which the P is gauche to H(3') and the upfield axially oriented H(5'')[21] but trans to the low field equatorially oriented H(5'), H(4') being trans to H(5'') and gauche to H(5'). While there are small deviations in the dihedral angles predicted for ideal chair conformations, a slight flattening of the phosphate ring will accommodate these angles.[7] These deviations from an ideal chair could also be explained by the presence of molecular strains imposed by the trans fusion of the six-membered phosphate and five-membered sugar rings and/or the uncertainties in the values of the constants used in the Karplus relation.

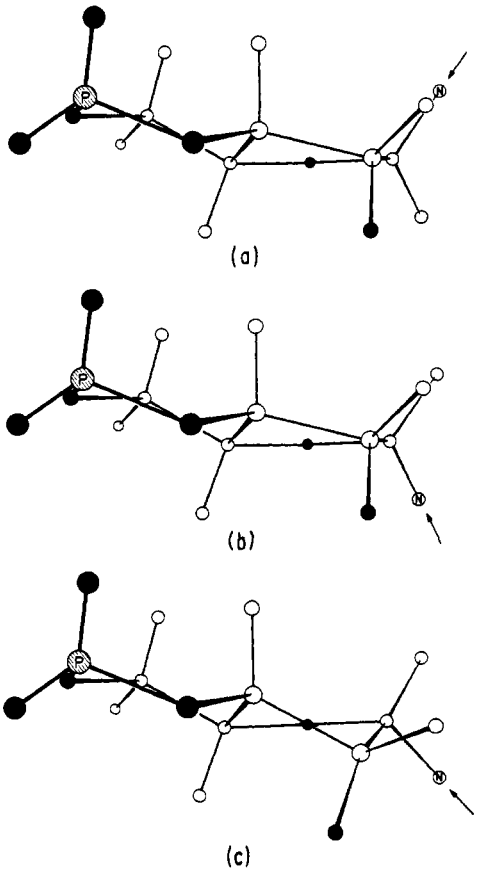


Figure 5. Possible conformations of the ribose and cyclic phosphate rings in nucleoside 3',5'-cyclic monophosphates: (a) β anomers, ^3E sugar pucker; (b) α anomer, ^3E sugar pucker; (c) α anomer, ^2_3T sugar pucker. In all cases the cyclic phosphate ring is shown in a chair form.

The proposed flattening of the cyclic phosphate ring in aqueous solution is in accord with the crystal structure data for β-nucleoside 3',5'-cyclic monophosphates[22] where the largest puckering occurs about the C(3')-C(4') bridging linkage between the two ring systems. It has been suggested[22a] from x-ray data that the phosphate ring is more flexible than the furanose ring; a possibility that is not inconsistent with the observed coupling constants which can also accommodate a dynamic equilibrium between an "ideal" and a "flattened" chair.

2. Conformation of the Sugar Rings. A close examination of the dihedral angles for the α-ribonucleoside 3',5'-cyclic monophosphates (I-III) shown in Table I and of molecular models shows a highly strained sugar ring with a conformation comparable to that predicted by Pitzer and Donath for "maximum distortion" in a cyclopentane ring.[23] The smaller value of $J_{2'3'}$ in the α relative to the β anomers (3.9 Hz compared to ~5 Hz[7,9]) indicates that the distortion from planarity in I-III is greater than in the corresponding β series; the distortion about C(1')-C(2') ($\tau_1 = 50°$) being approximately 10° greater than that about C(3')-C(4') ($\tau_3 = 40°$). Using the nomenclature proposed by Altona and Sundaralingam,[24a] the conformation in I-III is best described as ^2_3T [C(3')-endo, C(2')-exo][25] with a possible bias toward $_2T^3$ (see Figure 5c). This is significantly different from the ^3E to $_4T^3$ conformation (Figures 5a and b) computed for the β isomers[7,9,22] or the $^2E \rightleftarrows {}^3E$ equilibrium mixture found in the acyclic nucleoside monophosphates.[19,26]

Further examination of Table I indicates the conformation of the deoxyribose ring in α-cdAMP (IV) to be best described as ^3E to $_4E$ with a puckering amplitude (as estimated from $J_{2'3'}$) similar to that found in the deoxy β anomers[9,16] (see

Table II. Chemical Shift Differences between the α and β Anomeric Pairs of Nucleoside 3′,5′-Cyclic Monophosphates

	$\Delta\delta(\delta_\alpha - \delta_\beta)$								
	8(6)	2(5)	1′	2′	2″	3′	4′	5′	5″
cAMP[a]	+0.098	−0.063	+0.454	+0.027		−0.070	+0.275	+0.005	−0.063
cUMP[a]	+0.093	+0.015	+0.488	+0.113		+0.108	+0.164	−0.038	−0.099
cCMP[a]	+0.047	+0.001	+0.466	+0.190		+0.116	+0.128	−0.054	−0.114
cdAMP[a]	+0.214	+0.078	+0.026	+0.159	+0.109	+0.282	+0.323	−0.068	−0.081
cDHUMP	+0.103	−0.123	+0.073	+0.663		+0.077	−0.122	−0.013	+0.009
	+0.205	−0.105							

[a] δ_β taken from ref 7 [correction factor: internal TSP − internal tetramethylammonium chloride (TMA) = 3.206 ppm]. Note, values in ref 7 are measured at 0.1 M with respect to nucleotide; the spectrum of β-cdAMP was recorded in this laboratory at 0.02 M and showed no significant changes in the chemical shifts of the sugar ring protons relative to the values quoted at 0.1 M.

Figures 5a and 5b). It is interesting to note that reversing the assignments of H(2′) and H(2″) and recalculating the dihedral angles, after generating the appropriate Karplus coefficients, leads to a similar choice of sugar conformation. In the β-deoxynucleoside 3′,5′-cyclic monophosphate series[7,9,16] where $J_{1'2'}$ (~1.6 Hz) and $J_{1'2''}$ (~8.4 Hz) differ significantly, the assignments can be made unequivocally from the coupling constants.[16]

The situation for the α- and β-5,6-dihydrouridine 3′,5′-cyclic monophosphates (V and VI) is again different. The conformation of the β anomer (VI) is identical with that found in the unsaturated β-nucleoside 3′,5′-cyclic monophosphates (^{3}E to $_2$T^3).[7,9,22] While in both anomers the out of plane distortion (as estimated from $J_{2'3'}$) and the conformations about C(2′)-C(3′) (τ_2) and C(3′)-C(4′) (τ_3) are similar, a significant difference is observed about C(1′)-C(2′) (τ_1). In V, τ_1 is computed to be 41° and in VI it is 18°, reflecting a greater distortion about C(1′)-C(2′) in the α anomer. A full examination of the NMR parameters indicates the sugar conformation in VI to be in the range $^3_2$T to ^{3}E.

B. Comparison of the Conformational Properties of α- and β-Nucleoside 3′,5′-Cyclic Monophosphates. 1. Adenine, Cytosine, and Uracil Ribonucleoside Derivatives. Comparison of the chemical shift differences for the α- and β-anomeric pairs ($\Delta\delta = \delta_\alpha - \delta_\beta$) listed in Table II reveals a significant (~0.5 ppm) downfield shift of H(1′) in the α anomers I-III. This shift has been used previously as a criterion for the determination of anomeric configuration in nucleosides and nucleotides.[27] It has been suggested from a survey of a variety of furanosides[28a,b] that the differences in the chemical shift of H(1′) for a pair of anomers[27] is due to a difference in the diamagnetic shielding effect[28] of the 2′-hydroxyl group. In the β anomers (see Figure 5a), H(1′) is cis to the 2′-OH and is expected to experience a net shielding compared to H(1′) in the α anomers (see Figures 5b and 5c) where the corresponding orientation is trans. A further deshielding influence could originate from a difference in the glycosyl torsion angle in the two anomers, since reorientation of the base about χ_{CN} can cause either an upfield or downfield shift of H(1′) depending on whether the anomeric proton approaches the deshielding zone (in the plane) or the shielding zone (above and below the plane) of the base.[29] As there is no a priori reason why the orientation of the base relative to the ribose ring should be the same in both anomers (vide infra) such a contribution, while difficult to quantitate, cannot be completely ruled out. Attempts to estimate the preferred glycosyl torsion angle in I-III using the change of chemical shift of the base protons as a function of pH[30] were ineffective due to the distance of the phosphate group from the aglycon. A similar finding was noted by Schweizer and Robins for the β-nucleoside 3′,5′-cyclic monophosphates.[14] It should be noted that for α- and β-deoxynucleosides[31] and for α- and β-cdAMP (see Table II), the chemical shift of H(1′) does not change significantly in the two anomers, indicating that the shielding effect of the cis-oriented 2′-hydroxyl group is the predominant factor in the ribose series.

Next in order of magnitude is the deshielding of H(4′) (Table II) in the α anomers. The largest effect is seen for α-cAMP (I) in which H(4′) is deshielded by 0.275 ppm, whereas the pyrimidine derivatives (II and III) show a 0.13-0.16-ppm change. Similar observations have been made for anomeric pairs of nucleosides[31] and nucleoside acyclic monophosphates,[7] although the chemical shift of H(4′) in pseudouridine is the same in both anomers.[13] Nuclear Overhauser enhancement (NOE) experiments have shown that irradiation of H(4′) in α-nucleosides produces a 9-16% enhancement of the base proton signals [H(5), H(6) or H(2), H(8)] while no such effect could be detected in the β anomers.[32,33] This indicates that, on the average, H(4′) is in closer proximity to the base in the α anomers and so its chemical shift is more sensitive to base orientation. Shugar and co-workers have estimated that in 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-5-ethyluracil the deshielding effect (relative to the β isomer) on H(4′) due to anisotropy of C(6)-H(6) and C(5)=C(6) bonds is in excess of 0.3 ppm.[31b] For pyrimidine nucleoside derivatives, it has been argued that an anti orientation of the base ring is necessary to account for the H(4′) chemical shifts of the α anomers.[31b] However, theoretical calculations on the dependence of ribose ring proton chemical shifts on the glycosyl torsion angle (χ_{CN}) suggest that H(4′) in the α anomer is expected to appear downfield of the same proton in the β anomer for most orientations of the base.[29] For α-pyrimidine nucleosides, the maximum downfield shift occurs when H(4′) is in the plane of the base ring with the C(2)=O(2) bond pointing toward the C(4′)-H(4′) bond (χ_{CN}[34] ~150°, syn orientation). In the case of purines, the maximum downfield shift of H(4′) also occurs in an in-plane orientation when χ_{CN} is either 330° (anti) or 150° (syn).

It is also of interest to note the $\Delta\delta$ for H(5′) and H(5″). In the β anomers, H(5″) [trans to H(4′), see Figure 5] is closer to the base than H(5′), and so its chemical shift should be the most affected when the base is transposed into the α configuration. This trend is observed experimentally, i.e., $\Delta\delta_{H(5')}$ (−0.05 to +0.005 ppm) < $\Delta\delta_{H(5'')}$ (−0.063 to −0.114 ppm). The net upfield shift of H(5″) can be accommodated by this proton being in a deshielding zone of the base in the β anomer. Finally, the small (~0.5 Hz) long-range $^5J_{1'P}$ noted in the β anomers[7,9] is not detected in the α series since in this instance H(1′) does not lie in a planar zigzag coupling path from the phosphorus.[35]

The conformation of the six-membered cyclic phosphate ring is almost identical in both the α and β anomers and, as noted above, is best described as being in a "flattened" chair form.[7] However, a pronounced difference in the conformation of the ribose rings is apparent. The β anomers favor an ^{3}E to $_4$T^3 puckering mode[7,9,24] with a puckering amplitude similar to that found in the 5′-mononucleotides.[19] This contrasts with the $_2$T^3 to ^{3}T$_2$ mode and increased puckering amplitude found in the α anomers (vide supra). In view of the relative conformational invariability of the cyclic phosphate ring and the expectation that any perturbation of the sugar by a base-phosphate in-

teraction is minimal, due to their large separation (vide supra),[14] then changes in the sugar ring in going from the β to α anomer can be attributed to an interaction(s) between the base and the sugar. Of the various possibilities,[6] a repulsive interaction between the cis-oriented base and OH(2′) in the α anomers seems most likely. There are two types of interaction which could lead to the changes observed. The first is a repulsive electrostatic (dipole-dipole) interaction which is expected to be at a maximum when the C(2′)-O(2′) and C(1′)-N(9) bonds are oriented parallel and at a minimum when perpendicular to each other.[36] The second is a steric repulsion (contact interaction) between OH(2′) and the base [N(3) in the case of the purines and O(2) in the pyrimidines], for certain orientations of the base that brings them into close contact (vide infra). In the β series the OH(2′) is situated too distant (trans to the base) for any such steric interaction, although Kainosho and Ajisaka[9b] have attributed a "repulsive interaction" as being responsible for forcing the H(1′)-C(1′)-C(2′)-C(3′)-O(3′)-P nuclei into a nearly coplanar zigzag array. The change from ^{3}E (Figure 5b) to the range $_2T^3$-$^3T_2$ (Figure 5c) in the α series leads to an increased distance between OH(2′) and the base and a change toward a perpendicular orientation of the two dipoles, thus minimizing these repulsive interactions. It should be noted that a similar interaction would be expected to occur in 3′,5′-ara-cAMP where again the OH(2′) and base are situated cis to each other. Such a possibility, leading also to a change from the ^{3}E pucker, cannot be completely ruled out from the NMR data available.[14] In an NMR study[6] of the α and β anomers of NMN and NMNH, it was shown that a similar repulsive interaction exists for α-NMNH leading to a change in sugar conformation from 2′-endo to 2′-exo as a result of the β to α transition. In the case of α-NMN, an intramolecular attraction was postulated to exist between the positively charged nicotinamide ring and OH(2′) in order to explain the predominance of the 2′-endo conformer.

Further evidence for a strong repulsive interaction between the 2′-hydroxyl and the base has been obtained from theoretical calculations.[37] Using the PCILO method, and assuming a ^{3}E sugar conformation, it was possible to show the existence of large energy barriers to rotation about the glycosyl bond in the α anomers, the maxima being at values of χ_{CN} corresponding to close contact between the OH(2′) and the N(3) of the purine bases or O(2) of the pyrimidines. These barriers almost completely disappeared when the same calculations were made assuming a $^3T_2$ sugar conformation. Similar energy barriers are observed for β-ara-cAMP if a ^{3}E conformation is assumed.

2. Adenine Deoxyribonucleoside Derivatives. The chemical shifts of H(1′) in α- and β-cdAMP are very similar (see Table II), further verifying that the shielding effect (~0.4 ppm) characteristic of the cis oriented vicinal OH(2′) in the β anomers of the ribo series is removed when this hydroxyl is replaced by hydrogen. Moreover, δH(1′) and δH(3′) in IV are similar to those in I, indicating that a trans oriented vicinal hydroxyl group has a minimal effect on the chemical shift of a neighboring proton.

As in compounds I-III, H(4′) of IV is significantly deshielded (0.323 ppm) relative to the β anomer and the same explanation can be used (viz., the closer proximity of the base to H(4′) in the α anomer). By the same token, H(3′) is expected to be shielded in a β to α transition, as is observed ($\Delta\delta$ −0.282 ppm). In contrast, II and III show a downfield shift of H(3′) and I shows only a slight (0.070 ppm) upfield shift. It should be emphasized that in the ribo series the interpretation of these results is complicated by the fact that contributions to the chemical shift differences can occur because of different ribose ring conformations in the two anomers, in addition to any possible reorientation of the base. This situation does not occur in IV since the sugar conformations are essentially the same in both α and β anomers[9,16] (^{3}E to $_4$E). A suggestion that β-cdAMP exists in an "unusual ^{3}E ⇄ ^{0}E equilibrium"[7] has recently been shown to be in error[16] because of incorrect analysis of virtual coupling effects.

Examination of molecular models shows that the location of H(2′) [or H(2″)] relative to the base in a β-deoxynucleoside derivative is identical with that of H(2″) [or H(2′)] in the α anomer, assuming that no change in the sugar conformation occurs. If there is no change in the orientation of the base in the β to α transition then one would expect $\Delta\delta$H(2′) ≃ −$\Delta\delta$H(2″). Experimentally, both protons are observed to be deshielded in the α anomer (0.11-0.26 ppm). This is the most direct evidence of different orientations of the base in the α and β anomers. In contrast, H(2′) of thymidine and 5-ethyldeoxyuridine moves downfield and H(2″) moves upfield in the β to α transition, leading to the suggestion[31] that no significant reorientation of the base occurs in these two cases.

3. 5,6-Dihydrouracil Derivatives. Catalytic hydrogenation of the 5,6 double bond in α-cUMP and β-cUMP to produce V and VI, respectively,[15b] introduces several new aspects. Anisotropy effects of the C(5)=C(6) bond are removed, the dipolar character of the C(1′)-N(1) bond may be significantly reduced, and the overall flexibility of the aglycon is increased with N(1) now being sp^3 hybridized and having a lone pair of electrons not directly involved in bonding. All of these alterations coupled with the possibility of glycosyl torsion angle changes are expected to produce chemical shift changes of various sugar protons. Thus, a comparison of shift effects for unsaturated and saturated aglycons (II and V) is not feasible; however, such comparisons between the α and β anomers V and VI can be made since both bear the same aglycon.[6,20]

The most dramatic chemical shift change observed is the pronounced (ca. 0.7 ppm) downfield shift of H(2′) in the α anomer. It should be noted that in VI, H(2′) is orientated cis to an aliphatic nitrogen [N(1)] possessing a lone pair of electrons [in contrast to the more aromatic nature of N(1) in α- and β-cUMP]. This cis orientation would be expected to shield H(2′) in VI relative to V in a similar fashion[28c] to the shielding of H(1′) by the 2′-hydroxyl in the β anomers;[27] in fact, an $-NH_2$ group has been shown to shield a cis oriented proton by as much as 1.17 ppm relative to one oriented trans.[28c] It is interesting to note that for α- and β-NMNH, which also possess an unshared pair of electrons at N(1), the $\Delta\delta$ of H(2′) is much less (ca. 0.1 ppm);[6] however, the dihydrouracil and dihydronicotinamide rings have other functionalities which can affect the magnitude of the chemical shifts of the sugar protons, namely, C(2)=O and C(4)=O in the former and C(2)=C(3), C(5)=C(6), and C(3)-CO-NH_2 in the latter.

Further examination of the data in Table II shows that for compounds V and VI, the relative deshielding of H(1′) in the α anomers, relative to the β, is much less (0.073 ppm) than in the unsaturated case (ca. 0.5 ppm). Since the relative shielding of H(1′) by the 2′-hydroxyl in VI compared to V should be approximately the same as for the unsaturated anomeric pair, and as the anisotropy due to the C(5)=C(6) bond is now absent, it follows that either H(1′) in V is shielded relative to H(1′) in VI by the C(2)=O group to compensate for the loss of shielding from the trans oriented OH(2′), or H(1′) in VI is deshielded relative to H(1′) in V by the C(2)=O group. NMR[38a] and x-ray diffraction[39] studies on the β isomer of 5,6-dihydrouridine have shown the base to be in a predominantly anti conformation (χ_{CN} = 57.1-65.5°[39]) which places H(1′) in a deshielding zone of the C(2)=O group.[40] The chemical shift of H(1′) in 5,6-dihydrouridine is within 0.2 ppm[38a] of that of H(1′) in VI. If it is assumed that VI adopts a similar conformation about the glycosyl bond (χ_{CN} ≃ 60°), then in V the C(2)=O must take up an orientation in which H(1′) is shielded by ca. 0.4 ppm relative to VI. This indicates

a marked difference (ca. 90°[40]) in χ_{CN} between V and VI. Thus, in going from the α anomer (V) to the β anomer (VI), the shielding effect of the 2′-hydroxyl group on H(1′) is compensated for by the base taking up a different conformation which places H(1′) in a deshielding zone of the C(2)=O group, resulting in only a small observed change in chemical shift. It is interesting to note that these particular orientations of the base are two of the possible conformers that would be predicted by consideration of the anomeric effect.[41] This latter effect utilizes the minumum amount of lone-pair orbital overlap between the sugar ring oxygen and the C(1′) substituent to rationalize preferred sugar conformations in various glycosides.[41] However, since little is known at this time of the dynamic conformational properties of the dihydrouracil ring[42] (inversion rates, etc.), it would not be prudent to eliminate the possibility of other conformers on this basis alone. Previously, the anomeric effect has been used to rationalize conformational preferences in the sugar ring for bases with varying electronic structures.[41] However, for the α and β anomers of NMN and NMNH, it was reported that the anomeric effect did not have a determining role in defining the sugar conformation.[6]

In the case of H(4′), the resonance appears upfield by 0.127 ppm in the α (V) compound relative to the β (VI) anomer, and this is opposite to the trend observed in the unsaturated derivatives. However, as noted above, distinct changes in the glycosyl torsion angle between V and VI can play a critical part in the chemical shift differences.[40]

One further point of interest is the nonequivalence of H(6a) and H(6b) shifts in both V and VI. Since H(6a) and H(6b) are much closer to the sugar moiety than H(5a) and H(5b), this effect would be expected to be much less for the latter protons and these appear as a single triplet in both anomers.

The sugar ring in VI is best described as ^{3}E and is similar to the β anomers having an unsaturated base.[7,9] A comparison of V and VI (Table I) indicates the out of plane distortion as given by τ_2 and τ_3 to be approximately the same. However, τ_1 is 23° greater in V than in VI and approximates to a sugar conformation in the range $^2_3$T to ^{3}E. This difference in conformations for the two anomers is much smaller in magnitude than is the case for the cyclic nucleotide anomeric pairs containing an unsaturated more rigid base (vide supra). Thus, in this instance, the pronounced repulsive interaction between OH(2′) and the base in the α anomer is significantly reduced, presumably owing to the increased flexibility of the base introduced by saturation of the C(5)=C(6) bond and the presence of a tetrahedral aliphatic nitrogen at N(1). This results in a relaxation of the strained $_2$T^3 to ^{3}T$_2$ conformation normally found in compounds I-III (containing rigid unsaturated bases), back toward the ^{3}E to $_4$T^3 conformation of the β anomers.

Summary

Direct evidence has been presented that the OH(2′) group plays an important role in governing the overall conformation of α-nucleoside 3′,5′-cyclic monophosphates. Namely, it has been shown that (1) the presence of the OH(2′) group causes an increase in the distortion from planarity of the sugar ring, i.e., transition from the ribo to the deoxyribo leads to a flattening of the ring, (2) the steric interaction between OH(2′) and the base is minimal in the β anomers but increases significantly in the α anomers leading to an increasingly strained system in which the ribose ring adopts a $^2_3$T conformation which is highly distorted, (3) replacement of OH(2′) by a hydrogen (oxy → deoxy transition) in the α anomers relaxes the repulsive interaction and allows the ribose ring to return to its "normal" ^{3}E to $_4$E puckered mode, and (4) hydrogenation of the pyrimidine ring partially relaxes the interaction due to the increased flexibility of the base fragment, as is reflected by the ribose ring adopting a conformation in the range $^2_3$T to ^{3}E. The latter method apparently is not as effective in removing the OH(2′)-base interaction as is the transition from ribo to deoxyribo. The evidence is consistent with all 3′,5′-cyclic mononucleotides adopting a basically rigid $_4$E to $_2$T^3 sugar conformation, the precise conformation within this range being dependent upon the interactions which are introduced due to changes in sugar substitution.

Acknowledgments. This work was supported by the U.S. Energy Research and Development Administration, the National Research Council of Canada (A5890), and the National Cancer Institute of Canada.

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Gas-Phase Spectroscopy of Protonated Hexamethylbenzene and Hexamethyl(Dewar benzene)

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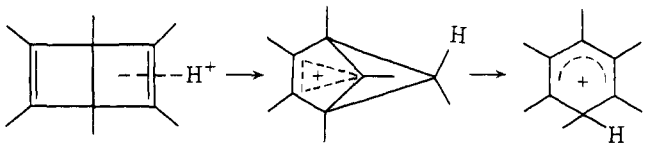
Abstract: Techniques of photodissociation spectroscopy indicate that the gas-phase protonated hexamethyl(Dewar benzene) cation rearranges within less than a few seconds to the protonated hexamethylbenzene structure. The spectrum of protonated hexamethylbenzene is analogous to those of other gas-phase protonated methylbenzenes, and is similar in peak position (with a small blue shift) and intensity to the solution spectrum of hexamethylbenzenium ion. The proton affinities of the ^1L_b and ^1L_a excited states of hexamethylbenzene are apparently higher by 30 and 28 kcal, respectively, than that of the ground state.

Hogeveen and Volger[1] found that in solution protonated hexamethyl(Dewar benzene) ion I rearranges to the bicyclic structure II at low temperature, and that II rearranges irreversibly to protonated hexamethylbenzene III at temperatures



I II III

near room temperature with an Arrhenius activation energy of 24.3 kcal. The various rearrangements and possible cation structures issuing from initial formation of I have been the subject of extensive investigation and discussion.[2] It is clear that III represents the most thermally stable of structures I–III, but the potential surface has a local minimum for II which can allow its observation under appropriate conditions.[3] The question of whether I or II can be observed as a stable isolated species under gas-phase conditions is of interest in understanding the rearrangement processes occurring in this potential surface. The new technique of photodissociation spectroscopy[4] provides an approach uniquely well suited to obtaining structural information about gas-phase ions, and was found to be readily applied to this case.

Experimental Section

In the photodissociation spectroscopic technique,[4] the ionic species of interest is generated and trapped in the ion cyclotron resonance cell. Under illumination by monochromatic light photodissociation of the ion may occur, and is observed by standard techniques of ion cyclotron resonance. The photodissociation cross section plotted as a function of wavelength is the photodissociation spectrum of the ion under observation, and is characteristic of the structure of the ion.[5]

In the "time resolved" photodissociation technique,[6] the photodissociation of a trapped population of ions is followed as a function of time after turning on the light source. A spectroscopically homogeneous ion population decays in smoothly exponential fashion toward the baseline; while if some of the ions are nondissociating at the given wavelength, the decay curve will tend to level off at a nonzero value. This method can clearly reveal situations where the ion population comprises two or more distinct structures.

Hexamethyl(Dewar benzene) photodissociation spectra were obtained with a pulsed ICR spectrometer technique[7] at pressure of $\sim 2 \times 10^{-8}$ Torr, ion trapping times of about 5 s, and ionizing electron energy of 30 eV. The protonated parent ion is rapidly formed by proton transfer from $(M - 1)^+$ and other primary ions, and no added acid was necessary. Although the photodissociation spectrum of the parent radical cation would be of interest, no conditions were found for which the abundance of parent ion signal was greater than the expected ^{13}C isotope peak from $(M - 1)^+$. Time-resolved spectra were obtained in the steady-state trapped ion ICR mode used in previous work.[8]

Spectra of protonated hexamethylbenzene were obtained with the same pulsed-ICR technique at $\sim 5 \times 10^{-8}$ Torr and 30 eV. It was necessary to add a trace of water as proton source to protonate the neutral molecule.

The absolute cross sections were obtained by reference to the known photodissociation cross section of toluene[8] at 400 nm.

Results and Discussion

Photodissociation spectra are shown in Figure 1 for both protonated hexamethylbenzene and the ion obtained by protonation of hexamethyl(Dewar benzene). (In addition to the peak shown in the figure, protonated hexamethylbenzene also showed a photodissociation peak near 285 nm having a peak cross section of the order of 1×10^{-17} cm^2. In the hexamethyl(Dewar benzene) case, the spectral region below 300 nm was obscured by extraneous photochemistry which we were unable to eliminate.) The position and intensity of the strong photodissociation peak at 385 nm is identical within experimental uncertainty for the two species, which constitutes nearly conclusive evidence for their structural identity. Since there is little doubt that the spectrum obtained for protonated hexamethylbenzene is that of structure III, it is evident that the I ions formed by initial protonation rearrange within a few seconds to III.

The homogeneity of the population of ions from protonated hexamethyl(Dewar benzene) was checked by time-resolved photodissociation at 4000 and 3700 Å, and it was found that