

- thetical solution volume of 500 Å³.
- (36) The molecular cavity radii for the crystals of *all-trans*- and *11-cis*-retinal were calculated using the formula, $a = (3W/4\pi n)$,¹³ where W is the observed^{27,37} unit cell volume, and n is the number of molecules per unit cell (for the above crystals, $n = 4$): $a(\text{all-trans-retinal})^{37} = 4.774 \text{ \AA}$, $a(\text{11-cis-retinal})^{27} = 4.727 \text{ \AA}$.
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Proton Magnetic Resonance Studies of the Effects of Sugar Hydroxyl Dissociation on Nucleoside Conformation. Arabinosyl Nucleosides with an Intramolecular Hydrogen Bond between the Pentose O(5') and O(2')

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Abstract: Analyses have been made of the proton magnetic resonance spectra, in neutral and alkaline media, of 1- β -D-arabinofuranosylcytosine and some of its O'-methyl derivatives and of some O'-methylarabinofuranosyluracils. In particular, changes in chemical shifts of specific protons were correlated with ionization of the sugar 2' and 3' hydroxyls. In aqueous medium the neutral forms of the foregoing derivatives possess comparable populations of the forms C(2')endo and C(3')endo and of the three classical exocyclic 5'-CH₂OH conformers. For those derivatives with free 2' and 5' hydroxyls, dissociation of the "up" 2'-OH leads to an unusual modification in the sugar conformation, hitherto not encountered in solution, viz., C(2')endo-C(3')exo and gauche-gauche, accompanied, and probably constrained to this form, by formation of an intramolecular hydrogen bond with the 5'-OH as donor, 5'-OH \cdots O(2')⁽⁻⁾; this conformation is similar to that observed in the solid state for the neutral forms of arabinofuranosyluracil and arabinofuranosylcytosine. No marked changes in conformation occur when either the 2'-OH or 5'-OH is etherified, nor with cytidine. Dissociation of the 3'-OH does not lead to detectable changes in conformation of arabinonucleosides, but, in the case of 2'-O-methylcytidine leads to a preference for the form C(2')endo. The marked preference for the conformation anti is maintained on dissociation of the sugar hydroxyls. In the case of 3'-O-methylarabinofuranosyluracil, and of arabinosylcytosine, it proved possible to evaluate the pK for dissociation of the 2'-OH from the dependence of chemical shifts and coupling constants on the degree of alkalinity of the solutions. It is shown that ¹H NMR titration of sugar hydroxyls in general is a feasible procedure. The lower rate of alkali-catalyzed exchange of the pyrimidine H(5) in arabinofuranosylcytosine, relative to its 5'-O-methyl and 3',5'-di-O-methyl derivatives, is shown to be related to the intramolecular hydrogen bond in the former.

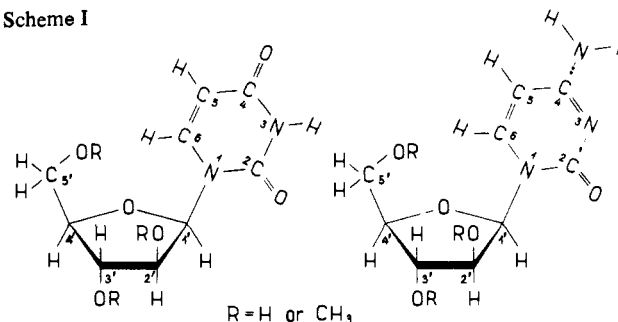
Considerable attention has been devoted to studies on the possible effects on nucleoside and nucleotide conformations of protonation and/or ionization of the heterocyclic base residues. No attempts appear to have been made to analyze the potential influence of dissociation of the sugar hydroxyls; in fact, in a ¹H NMR investigation of the influence of base ionization on the conformation of pseudouridine, Deslauriers and Smith^{2a} avoided the use of pH values above 11 so as to eliminate possible complications from sugar hydroxyl dissociation. Much earlier the ¹H NMR spectra of some nucleosides were recorded at pH values up to 14 by Jardetzky and Jardetzky,^{2b} but no attempt was made to interpret the observed effects.

It was long ago shown that dissociation of the sugar hydroxyl(s) of pyrimidine nucleosides is reflected in important modifications of the electronic absorption spectra of the heterocyclic bases,³ indicative of some type of interaction between the two. More recently, the use of the aglycone as a

uv "probe" has made possible in some instances reasonable estimates of the acidities of these hydroxyls.

During the course of some studies on the acidities of the sugar hydroxyls of some nucleosides, it was noted that very marked changes in the coupling constants of the pentose protons of araC⁴ and 3'-maraU (Scheme I) occurred under

Scheme I



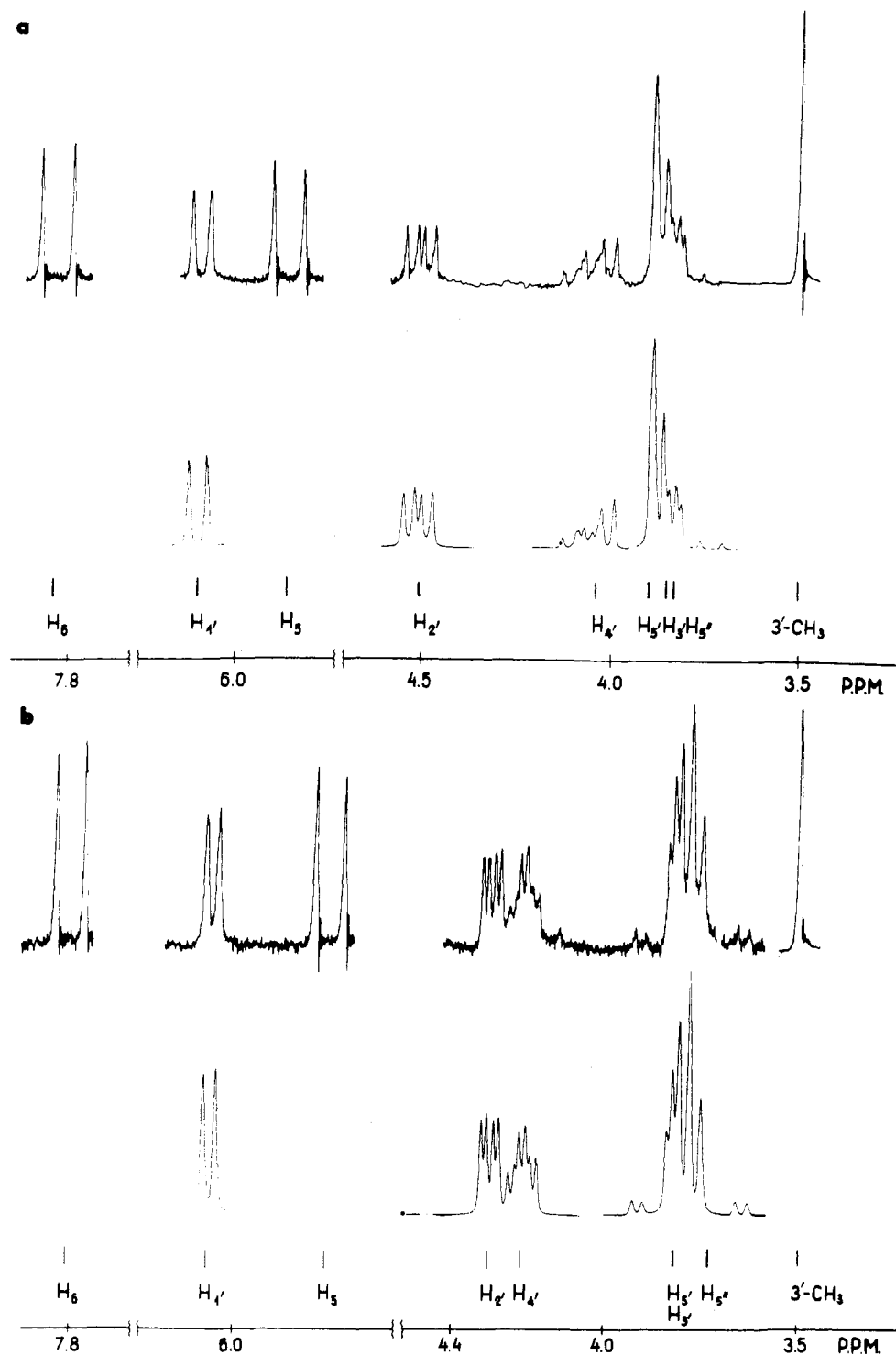


Figure 1. Experimental (upper) and simulated (lower) ^1H NMR spectra of 3'-maraU: (a) neutral form, (b) following addition of 0.18 mmol of NaOD so that 2'-OD is fully dissociated.

alkaline conditions where the sugar hydroxyls undergo dissociation, and pointing to important modifications in conformation of the arabinose ring.⁵ We present here more detailed analyses of the ^1H NMR spectra of pyrimidine arabinonucleosides, and some of their O'-methyl derivatives, in strongly alkaline aqueous medium, and the accompanying modifications in conformation. The results, particularly relevant to the conformation of the neutral form of araC, because of its known antiviral and antitumor activities,⁶ are also of interest in the development of model systems for testing the Karplus relations, in the potential use of ^1H NMR spectroscopy for titration of sugar hydroxyl acid-

ties,⁵ and in studies on the properties of sugar hydroxyls in general.

Experimental Section

The preparations of 3'-maraU, 2',3',5'-m₃araU, and araC and its O'-methylated derivatives have been elsewhere described.⁷

^1H NMR spectra were recorded with a Varian XL-100 instrument, using approximately 0.15 M solutions of nucleosides in D₂O containing 1% DSS as internal standard. All measurements were made at $24 \pm 1^\circ\text{C}$.

Analyses of the ^1H NMR spectra made use of a first-order analysis for "weakly" coupled systems. More "strongly" coupled sys-

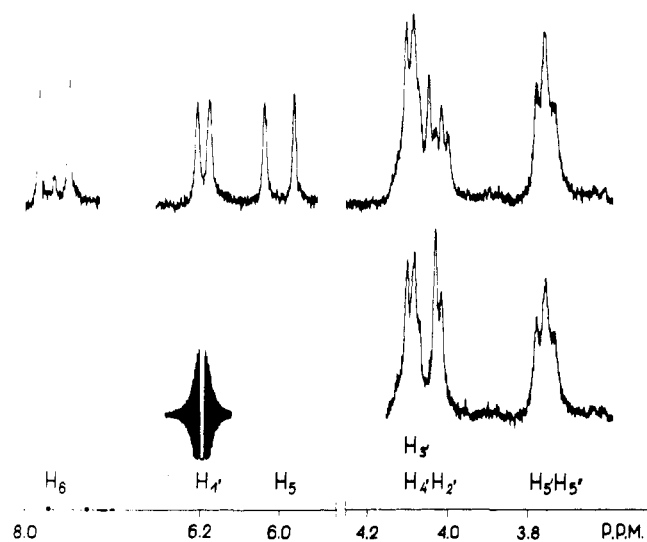


Figure 2. Experimental ^1H NMR spectrum of araC (upper) in aqueous medium following addition of 0.075 mmol of NaOD so that 2'-OD is largely dissociated. Lower spectrum shows decoupling resulting from irradiation of H(1').

tems were treated to a first approximation as AB or ABX systems. In several instances the chemical shifts and coupling constants were corrected by LAOCOON-3 simulation of systems including six spins. Spin decoupling was employed in some cases to check the identification of signals.

Solutions of 0.40 ml were brought to increasing degrees of alkalinity by the stepwise addition, with the aid of Carlsberg micropipettes, of known volumes of NaOD solution (Merck, Darmstadt, GFR, >99.7 mol % D). Alkalinity of a given solution is expressed in terms of n , the number of millimoles of added NaOD, using 1 N NaOD for additions of up to 0.3 mmol, and 10 N NaOD for larger quantities. The pD values of some of the solutions were measured with the aid of a Radiometer (Copenhagen) PHM22 instrument and associated G 220 B semimicro glass electrode. In addition to a correction for the "sodium error", a correction factor of 0.45 was added to the pH meter reading⁸ to obtain the pD in this high alkaline range, and at 24°C.

Of all the analogues examined, 3'-maraU was found to be the one most susceptible to a detailed analysis of properties in alkaline medium. One of its advantages over 3'-maraC was its much higher solubility in weakly alkaline medium. Furthermore the methyl substituent on O(3') served as a "shift reagent", strongly modifying the chemical shifts of neighboring protons, e.g., an upfield shift of H(3') by about 0.3 ppm, and downfield shifts of H(2') and H(4') by about 0.1 ppm.⁹ This effect was of particular value in analyses of the spectra in strongly alkaline medium where, e.g., the H(2'), H(3'), and H(4') signals of araC are all located within a band the width of which is less than 0.05 ppm.

Results and Discussion

Figure 1 exhibits the experimental and simulated spectra of 3'-maraU in the neutral form and in alkaline medium at a pD corresponding to total ionization of the 2'-hydroxyl. In Figure 2 are shown the experimental spectrum for araC with the 2'-OH¹⁰ fully dissociated, and the spectrum of the same sample with spin decoupling of H(1') and H(2'). Figure 3 exhibits the spectrum of the neutral form of 3',5'-m₂araC, as well as that at an alkaline pD corresponding to approximately 70–80% dissociation of the 2'-OH.

Figures 4a through 4e exhibit the dependences of the chemical shifts of the various protons of 3'-maraU, and of the associated coupling constants, on the amount n (in mmol) of NaOD added to the solutions. It will be noted that the variations observed cover three ranges of added NaOD, viz., $n_1 \sim 0.03$ mmol, $n_2 \sim 0.09$ mmol, and $n_3 \sim 1.5$ mmol, related to the sequential dissociation of the deuterons on N₃, O(2'), and O(5') (see below), with pK values of pK₁,

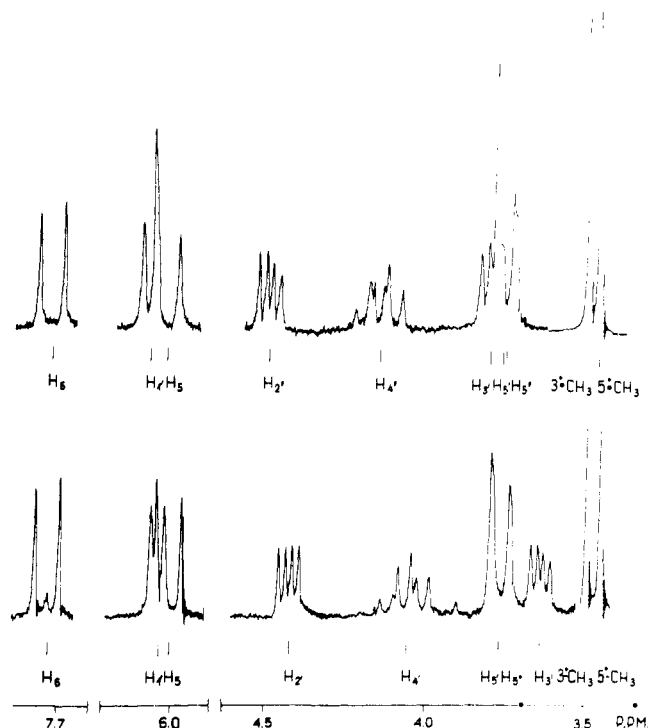


Figure 3. Experimental ^1H NMR spectra of 3',5'-m₂araC: (upper) neutral form; (lower) following addition of 0.18 mmol of NaOD so that 2'-OD is about 70–80% dissociated.

pK₂, and pK₃. Accurate determination of the pK values under these conditions is rather involved because of the relatively high concentrations ($\sim 0.15 M$) of the nucleosides (but see below). At lower nucleoside concentrations ($\sim 10^{-4} M$), these values may be determined by simple spectrophotometric titration and are respectively pK₁ ~ 9.5 in H₂O,³ pK₂ = 13.3 ± 0.15 in D₂O, pK₃ > 14.5.¹¹ Note, in particular, that pK₁ is sufficiently far below pK₂ as not to affect evaluation of the latter.

Figures 5a through 5e, in turn, exhibit the dependences of the chemical shifts of the specific protons of araC, and the associated coupling constants, on the amount n of added NaOD. In this instance the changes in chemical shifts are due to the combined effects of dissociation of the 2'-OH and 3'-OH, which partially overlap, but an approximate estimate of the pK value for the 2'-OH may be derived from an analysis of the changes in coupling constants, as described below.

Effects due to Dissociation of N(3) Proton in 3'-maraU. In the initial n_1 region, both H(5) and H(6) are shifted upfield by about 0.09 and 0.16 ppm (Figure 4a) with increasing NaOD concentration, due to modifications of electron density on C(5) and C(6) resulting from dissociation of the N(3) deuteron. For 2',3',5'-m₃araU, where only N(3)H dissociation can occur, the NaOD induced upfield shifts of H(5) and H(6) are 0.07 and 0.16 ppm (see Table I). It was previously shown that a change in electron density of 0.001 electron on a carbon atom leads to a change in the chemical shift of a proton bound to this atom of 0.004 ppm, so that the ratio (β) of the change in chemical shift to the change in electron density on the carbon is 4 ppm/electron.⁹ Application of the CNDO/2 method to calculation of electron densities on the various atoms of the uracil ring in the neutral and dissociated forms¹² showed that for the latter the electron densities on C(5) and C(6) increased by 0.016 and 0.035 electron, respectively. Relating these to the observed changes in chemical shifts of H(5) and H(6) for 2',3',5'-m₃araU (where the effects of hydroxyl ionization cannot in-

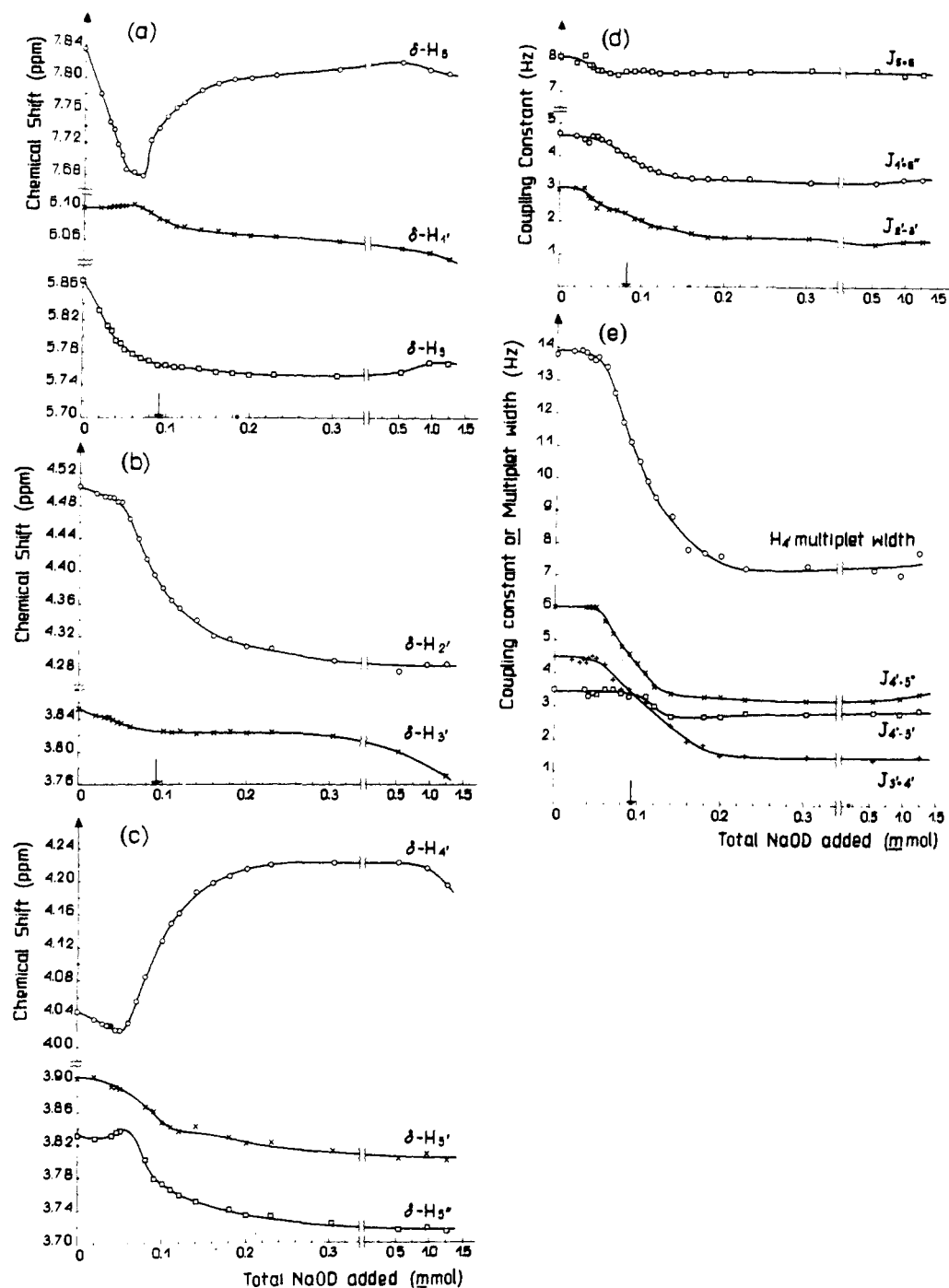


Figure 4. Dependence of chemical shifts (a, b, c) and coupling constants and H(4') multiplet width (d, e) for 3'-maraU on amount of NaOD (in mmol) added to sample. Arrows indicate amounts of added NaOD which induce one-half the total changes in chemical shifts, coupling constants, and H(4') multiplet width.

terfere) gave a value of $\beta\text{-H}(5) = 4.4$ and $\beta\text{-H}(6) = 4.6$, in reasonable agreement with the value previously reported.

Effects of 2'-OH Dissociation. (a) **Chemical Shift of H(6).** 2'-OH dissociation results in decreased shielding of H(6) in 3'-maraU by 0.1 ppm (Figure 4a). A similar effect is observed with araC (Figure 5a) and 3'-maraC,¹³ but not for 5'-maraC and 3',5'-m₂araC (Table I).

(b) **Chemical Shift of H(2').** In the case of 3'-maraU, the marked upfield shift (~ 0.2 ppm) of H(2') in the region $n_2 \sim 0.9$ mmol of added NaOD (Figure 4b) is clearly due to dissociation of the 2'-hydroxyl, which is more acidic than the 5'-hydroxyl.^{3,11,14,15} A similar effect of 2'-OH dissociation on the chemical shift of H(2') occurs also in araC (Figure 5b) and its 3'- and/or 5'-O-methyl derivatives (Table

I), and is due to the increase in negative charge on the O(2') resulting from ionization, with a concomitant increase in negative charge on H(2') and increased shielding of the latter.

(c) **Chemical Shift of H(4').** In 3'-maraU this proton is shifted downfield by 0.2 ppm as a result of 2'-OH dissociation (Figure 4c). This effect is also observed with araC (Figure 5c) and 3'-maraC (Table I), but not with 3',5'-m₂araC (Table I).

(d) **Chemical Shifts of H(5',5'').** With 3'-maraU, (Figure 4c), araC (Figure 5c), and 3'-maraC (Table I), both these protons are shifted 0.1 ppm upfield on dissociation of the 2'-OH. No such effect is noted with 5'-maraC and 3',5'-m₂araC (Table I).

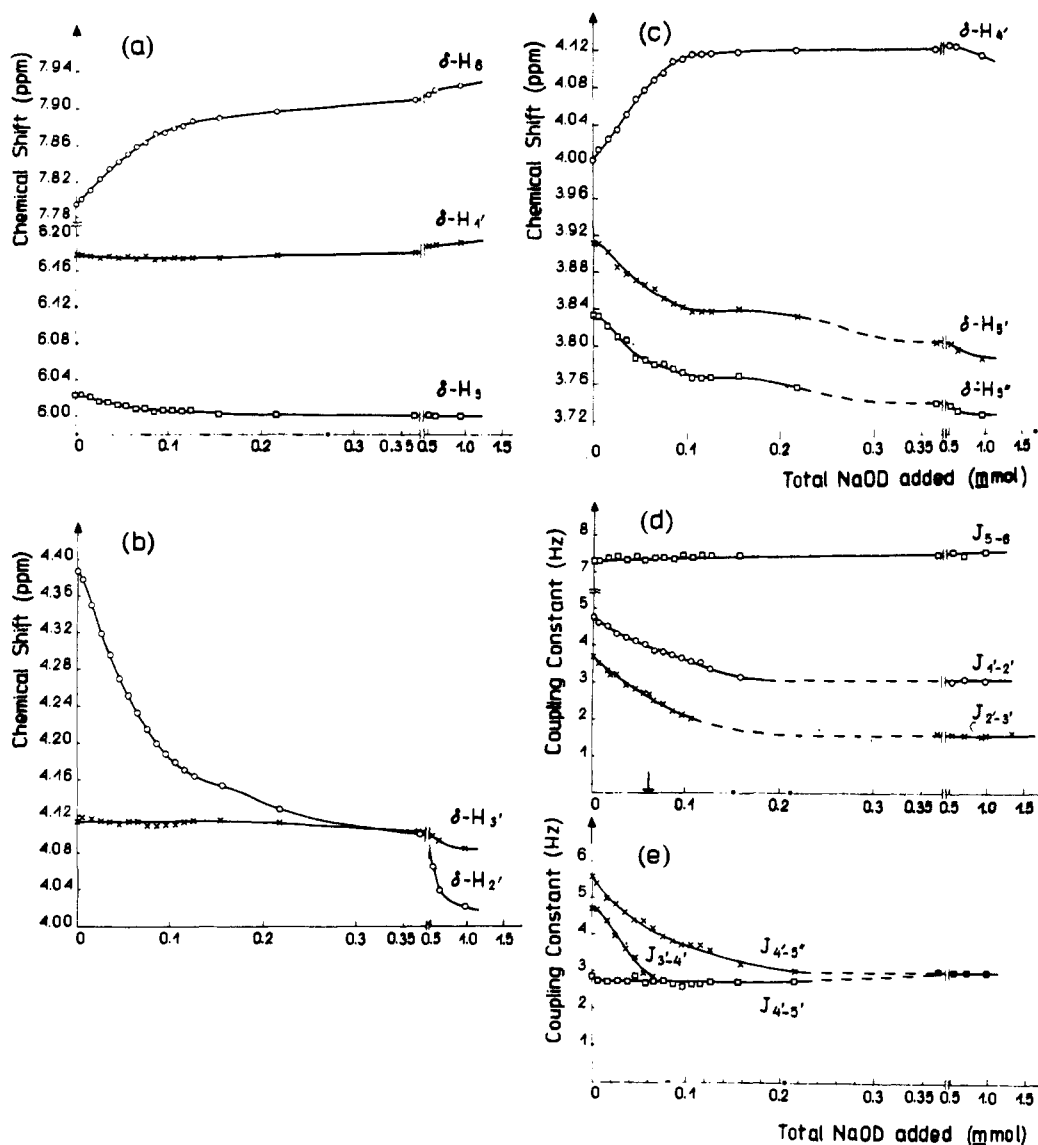


Figure 5. Dependence of chemical shifts (a, b, c) and coupling constants (d, e) for araC on amount of NaOD (in mmol) added to sample. Arrow in (d) indicates amount of added NaOD which leads to half the total change in the values of $J(1'-2')$ and $J(2'-3')$.

(e) **Chemical Shifts of Methyl Protons.** For all the O'-methyl derivatives embraced in this study, sugar hydroxyl dissociation only minimally affected the chemical shifts of the methyl protons, <0.02 ppm, as may be seen, e.g., from Figures 1 and 3.

(f) **Coupling Constants.** Figures 4d and 4e reveal very marked changes in the vicinal coupling constants of the arabinose ring protons in 3'-maraU in the n_2 region corresponding to dissociation of the 2'-OH. The values of $J(1'-2')$, $J(2'-3')$, and $J(3'-4')$ which, for the neutral form, are 4.65, 3.0, and 4.5 Hz, respectively, are 3.25, 1.4, and 1.6 following dissociation of the 2'-OH. Analogous effects are observed for araC (Figures 5d,e). These changes are not the direct consequence of charge modification on O(2') accompanying ionization, since they are not observed with 5'-maraC or 3',5'-m₂araC (Table II). They must consequently be due to modifications in conformation of the arabinose ring.

$J(2'-3')$ and $J(3'-4')$, which represent couplings between trans protons of the arabinose ring, are generally more sensitive to changes in conformation than couplings between cis protons. The low values observed, ~ 1.5 Hz, following 2'-OH dissociation, correspond approximately, from the Karplus relation,¹⁶ to dihedral angles for $\phi(2'-3')$ and

$\phi(3'-4')$ in the range $80-100^\circ$, consistent with a conformation C(2')endo-C(3')exo.

As regards $J(1'-2')$, representing coupling between cis protons (the value for which is usually about 5 Hz¹⁷), its measured value following 2'-OH dissociation in 3'-maraU and araC, 3.2 Hz (Figures 4d and 5d), represents a considerable departure from the norm. For such a value the dihedral angle $\phi(1'-2')$ would be expected, from the Karplus relation, to be fairly large, of the order of 50° , and corresponds to pronounced puckering of the arabinose ring of the form C(2')endo-C(1')exo.

Conformation of Arabinose Ring. A conformational analysis of the arabinose ring was carried out on the assumption of the existence in solution of an equilibrium between two states of the type, C(2')endo and C(3')endo, formally designated as ²E and ³E.¹⁸ With this type of model the experimentally determined coupling constants represent the weighted means of the coupling constants corresponding to each of the two classes of conformers ²E and ³E. Determination of the relative populations of the two conformational states requires, consequently, values of the coupling constants characteristic for each state. Since it is the coupling between trans vicinal protons, in this case $J(2'-3')$ and $J(3'-4')$, which is strongly dependent on the populations of

Table I. Chemical Shifts (in ppm vs. internal DSS at 24°C) of the Various Protons in araC and araU Analogues (0.15 M in D₂O) Prior to and Following Two Stepwise Additions of NaOD (in mmol) as Indicated in the Second Column^a

Analogue	Total NaOD added, mmol	H(5)	H(6)	H(1')	H(2')	H(3')	H(4')	H(5')	H(5'')
3'-maraU	0	5.869	7.836	6.099	4.504	3.852	4.042	3.904	3.832
	0.2	5.752	7.801	6.066	4.308	3.823	4.214	3.822	3.734
	1	5.768	7.811	6.044	4.284	3.781	4.213	3.804	3.715
2',3',5'-m ₃ araU	0	5.868	7.728	6.194	4.16	3.915	4.13	3.73	3.72
	0.25	5.795	7.565	6.229	4.13	3.855	4.07	3.72	3.715
	0.63	5.798	7.562	6.229	4.13	3.855	4.075	3.72	3.715
AraC	0	6.023	7.794	6.178	4.39	4.11	4.00	3.91	3.83
	0.36	6.000	7.910	6.181	4.09	4.10	4.12	3.80	3.73
	0.95	6.000	7.925	6.192	4.02	4.08	4.11	3.78	3.72
2'-maraC	0	6.034	7.734	6.285	4.10	4.18	3.96	3.89	3.785
	0.2	6.025	7.727	6.285	3.91	4.00	3.80	3.83	3.76
	0.8	6.033	7.724	6.280	3.87	3.96	3.78	3.83	3.73
3'-maraC	<i>b</i>	6.244	8.057	6.113	4.54	3.87	4.105	3.90	3.845
	0.38	5.996	7.892	6.037	4.29	3.82	4.26	3.815	3.72
	0.53	5.999	7.888	6.036	4.29	3.81	4.27	3.81	3.72
5'-maraC	0	6.029	7.720	6.177	4.37	4.07	4.07	3.78	3.73
	0.2	6.021	7.718	6.174	4.22	3.91	3.92	3.77	3.71
	0.8	6.016	7.708	6.164	4.195	3.89	3.90	3.77	3.71
2',3'-m ₂ araC	0	6.036	7.738	6.184	4.16	3.90	4.025	3.87	3.79
	0.2	6.041	7.750	6.176	4.145	3.89	4.015	3.845	3.775
	0.9	6.044	7.751	6.159	4.12	3.86	3.995	3.785	3.775
3',5'-m ₂ araC	0	6.027	7.727	6.077	4.49	3.805	4.15	3.76	3.755
	0.2	6.000	7.726	6.034	4.42	3.635	4.055	3.76	3.76
	0.9	6.001	7.713	6.017	4.405	3.59	4.03	3.755	3.75
2',5'-m ₂ araC	0	6.043	7.679	6.280	4.08	4.15	4.03	3.76	3.69
	0.2	6.028	7.653	6.286	3.89	3.99	3.86	3.77	3.65
	0.7	6.038	7.638	6.291	3.86	3.95	3.82	3.77	3.64
C	0	6.036	7.836	5.899	4.295	4.20	4.125	3.93	3.82
	0.2	6.037	7.793	5.811	4.165	4.02	3.985	3.88	3.77
	1	6.045	7.774	5.804	4.14	3.96	3.955	3.84	3.73
2'-mC	0	6.046	7.860	5.967	3.985	4.285	4.09	3.935	3.815
	0.2	6.039	7.884	5.943	3.82	4.15	4.01	3.93	3.79
	0.8	6.027	7.880	5.934	3.74	4.07	3.995	3.92	3.76

^aChemical shifts of H(5), H(6), and H(1') are given to an accuracy of 0.002 ppm and all others to an accuracy of 0.005 ppm. ^bpH 1.5 (data from ref 9).

Table II. Coupling Constants (in Hz) of Vicinal Protons in Various Analogues of araC and araU, 0.15 M in D₂O at 24°C, and the Corresponding Calculated Percentage Gauche-Gauche Populations of the Exocyclic 5'-CH₂OH Groups, and Percentage C(2')endo Population of the Arabinose Conformation in the Equilibrium C(2')endo ↔ C(3')endo

Analogue	State ^a	<i>J</i> (1'-2')	<i>J</i> (2'-3')	<i>J</i> (3'-4')	<i>J</i> (4'-5')	<i>J</i> (4'-5'')	% gauche- gauche	% C(2')endo
3'-maraU	A	4.6	3.0	4.5	3.5	6.0	30	60
	B	3.2	1.4	1.6	2.8	3.2	80	90
2',3',5'-m ₃ araU	A	4.9	3.2	5.0	4.3	6.0	20	60
	B	5.0	3.1	5.1	4.2	6.0	20	60
araC	A	4.8	3.7	4.8	2.8	5.6	45	50
	B	3.0	1.5	<i>b</i>	3.0	3.0	80	90
2'-maraC	A	4.7	4.5	5.2	2.7	5.6	50	50
	B	4.9	4.6	5.4	<i>b</i>	5.3 ^c	<i>b</i>	50
3'-maraC	<i>a</i>	4.5	2.7	4.7	3.3	6.5	30	60
	B	3.0	1.2	1.3	3.0	3.0	80	90
5'-maraC	A	4.7	3.7 ^c	<i>b</i>	3.5 ^c	5.5 ^c	40	60
	B	4.8	3.8	<i>b</i>	3 ^c	6 ^c	40	65
2',3'-m ₂ araC	A	4.7	3.0	4.7	3.8	5.9	30	60
	B	4.5	2.6	4.4	4.1	6.5	20	65
3',5'-m ₂ araC	A	4.2	2.5	4.3	4.7	5.7	20	65
	B	4.1	2.5	4.0	5.4	5.8	10	65
2',5'-m ₂ araC	A	4.8	4.7	4.7 ^c	3.2	5.6	40	50
	B	5.0	4.5 ^c	5.5 ^c	2.5 ^c	6.8 ^c	35	45
C	A	3.6	5.1	5.8	2.7	4.1	70	40
	B	4.2	5.0	5 ^c	3.0	5.4	45	45
2'-mC	A	3.6	5.1	6.2	2.8	4.2	65	35
	B	2.5	4.5	7.0	2.0	5.1	65	25

^a(A) at pD ~ 7, and (B) following addition to each sample of ~ 0.8 mmol of NaOD; ^bNonresolved. ^cAccuracy of 0.5 Hz. ^dpH 1.5 (data from ref 9).

arabinose conformers, these are the coupling constants which must be considered in studies on the equilibrium ${}^2\text{E} \leftrightarrow {}^3\text{E}$.

The values earlier proposed¹⁸ for $J(2'-3')$ of 0.5 and 8.7 Hz, and for $J(3'-4')$ of 0.6 and 7.1 Hz, for the ${}^2\text{E}$ and ${}^3\text{E}$ conformers, respectively, of araU and araC require revision, since experimentally $J(2'-3')$ is actually 0–2 Hz lower than the corresponding value of $J(3'-4')$ for the same compound (Table II; cf. ref 9). It is conceivable that the effect of the two electronegative substituents 2'-OH and 3'-OH in the case of $J(2'-3')$, relative to the effect of the single 3'-OH in the case of $J(3'-4')$, may partially account for the differences between the measured coupling constants. Undoubtedly the O'-methyl substituents affect the couplings to some extent, even when the conformation is unchanged, but this effect (see Table II) does not exceed 1 Hz.

Assuming that the Karplus relation for the coupling constants of the ribofuranose and arabinofuranose ring protons is similar, the sum $J(1'-2') + J(3'-4')$ for the ribose ring may be taken as the upper limit for the values of the couplings of trans pairs of protons. For O'-methylated cytosine ribonucleosides this sum varies over the range 9.4–10.1 Hz.⁹ For a series of nucleosides and nucleotides, Altona and Sundaralingam¹⁷ present the "best" value of this sum as 10.1 Hz. For 5'-AMP this sum is 9.5 Hz over a wide range of nucleotide concentration.¹⁹ Since the range in which one should find the lowest values of the experimental trans coupling constants cannot be narrower than 0–1 Hz, it follows that the highest values of the coupling constants should be located in the range 8.5–10 Hz.^{20–22} Furthermore, since $J(2'-3')$ in arabinosides may be additionally decreased due to the electronegative effect of the two hydroxyls 2'-OH and 3'-OH, it appears reasonable to adopt a range of 7.5–9 Hz for the highest values of $J(2'-3')$. These values were then employed to calculate the population of the ${}^2\text{E}$ conformer for the arabinonucleosides in neutral and strongly alkaline media (Table II). Analogous data are included for cytidine and 2'-mC, the calculations being based on $J(1'-2')$ and $J(3'-4')$, corresponding to the trans vicinal protons in this instance.

The neutral form of 3'-maraU will be seen to exhibit a moderate predominance (~60%) of the conformer ${}^2\text{E}$, and increases with dissociation of the 2'-OH to attain a value in excess of 90%. Similar changes in conformation are undergone by araC and 3'-maraC, the neutral forms of which exhibit ${}^2\text{E}$ conformer populations of about 50% and 60%, respectively, both increasing to over 90% in alkaline medium. For derivatives with an etherified 2'-OH or 5'-OH there is little, or no, modification of the sugar conformation in alkaline medium. These results are compiled in Table II.

Comparison of the values of the coupling constants for cytosine arabinosides in the neutral (Table II) and protonated⁹ forms points to the negligible effect of protonation of the cytosine ring on arabinose conformation.²³ Table II also shows, for both the neutral and protonated forms, the influence of individual O'-methyl substituents on the equilibrium ${}^2\text{E} \leftrightarrow {}^3\text{E}$, evaluated on the assumption that replacement of a given hydroxyl by an O'-methyl does not affect the Karplus relation: e.g., a 3'-O-methyl increases the population of the ${}^2\text{E}$ conformer (from 50% to 60%), whereas neither a 2'-O-methyl nor a 5'-O-methyl sensibly modifies the ${}^2\text{E}$ population.

Conformation of Exocyclic 5'-CH₂OH. The conformation of the exocyclic 5'-CH₂OH about the C(4')-C(5') bond may be determined from the values of the coupling constants $J(4'-5')$ and $J(4'-5'')$ as described by Blackburn et al.²⁴ and extended by Remin and Shugar.²⁵ The procedure is based on the existence of three geometrically preferred

conformers,²⁴ identification of the H(5') and H(5'') protons from the PMR spectrum,^{25–29} and application of an appropriate correction for the electronegativity of O(5'), O(4'), and C(3') to give the Karplus function with values of 9.1, 0, and 11.1 Hz for the points 0, 90, and 180°.⁹

It was previously shown⁹ that the protonated forms of cytosine arabinosides exhibit a lower population of the conformer *gauche-gauche* (20–35%) than *gauche-trans* (40–50%). For the neutral forms the three conformer populations are approximately equal, as indicated by the comparable values of $J(4'-5')$ and $J(4'-5'')$.

Figures 4e and 5e exhibit the values of these coupling constants as a function of the amount of NaOD added to samples of 3'-maraU and araC, respectively. Note, in particular, the marked decreases in the region n_2 corresponding to dissociation of the 2'-OH, indicative of a marked increase (to ~80%) in the *gauche-gauche* population at the expense of the *gauche-trans*. The *gauche-gauche* conformer populations, prior to and following virtually complete dissociation of the 2'-OH, are listed in Table II. A similar increase (to ~80%) in the *gauche-gauche* population accompanying 2'-OH ionization was noted for 3'-maraC, but not for derivatives in which the O(2') and/or O(5') were methylated (Table II).

It is pertinent at this point to emphasize that the exocyclic group conformation derived, above, for the dissociated forms of 3'-maraU and other arabinonucleosides, has only rarely been encountered in the *solution* conformation of nucleosides.²⁸

Chemical Shift of H(4'). For 3'-maraU the marked dependence of the chemical shift of H(4') on the amount of added NaOD in the region n_2 is shown in Figure 4c. With increasing dissociation of the 2'-OH, H(4') shifts downfield, mainly as a result of a change in conformation of the exocyclic 5'-CH₂OH. It has elsewhere been shown^{9,30} that, for a hydroxyl and proton which are vicinal, the proton is more shielded (by ~0.30 ppm) when it is *cis*, as compared to *trans*, to the hydroxyl. With the conformation *gauche-gauche*, H(4') is *trans* to the 5'-OH, whereas it is *cis* for the conformations *gauche-trans* and *trans-gauche*; hence decreased shielding of H(4') should accompany an increase in the *gauche-gauche* population. Dissociation of 2'-OH leads to an increase in the *gauche-gauche* population from ~30% to ~80% (Table II), with an accompanying increase in the chemical shift of H(4') of 0.17 ppm (Figure 4c), in reasonably good agreement with the anticipated increase resulting from the differing effect of the C-OH group in the conformations *cis* and *trans*, which amounts to 0.15 ppm.

The foregoing comparison takes no account of the direct influence of dissociation of the 2'-OH on the chemical shift of H(4'). The magnitude of this effect may be roughly estimated in the case of 3',5'-m₂araC, where it amounts to an upfield shift of ~0.1 ppm. But a part of this shift is presumably due to the decrease in the *gauche-gauche* population, from ~20 to ~10%, accompanying 2'-OH dissociation in 3',5'-m₂araC.

In the case of araC, alkalization of the solution leads to deshielding of H(4') by 0.11 ppm (Figure 5c), i.e., less than for 3'-maraU. This is due simply to the additional shielding effect on H(4') due to dissociation of the 3'-OH, as may be seen in the case of 2'-maraC and 2',5'-m₂araC, as well as 2'-mC (Table I).

Evidence for Intramolecular Hydrogen Bond in Solution. Conformational Data. In considering the behavior of the arabinonucleosides in alkaline medium, it is pertinent to recall the correlation earlier noted by Hruska³¹ between the conformations of the ribose ring and the exocyclic 5'-CH₂OH for a number of ribonucleosides, viz., an increase in the pop-

ulation of the conformer C(3')endo (in the equilibrium C(2')endo \rightarrow C(3')endo) is in general accompanied by an increase in the population gauche-gauche. Whereas this regularity may be extended to the neutral and protonated forms of arabinonucleosides, for which none of the sugar or exocyclic group conformers are specifically preferred,⁹ it is no longer applicable to such nucleosides, with free 2' and 5' hydroxyls, in alkaline medium.

Dissociation of the 2'-OH in 3'-maraU leads to a *simultaneous* increase (to >90%) of the sugar conformer ²E (e.g. C(2')endo) and the exocyclic conformer gauche-gauche (to >80%). We now proceed to present evidence from x-ray crystallographic studies, and the use of molecular models, to show that the foregoing satisfies the geometrical requirements for formation of a hydrogen bond between the 5'-OH and the O(2')⁽⁻⁾.³²

X-Ray Diffraction Data. Crystallographic studies³³ have shown that the neutral forms of araC and araU both possess the conformation C(2')endo and gauche-gauche, with an intramolecular hydrogen bond O(2')H...O(5'), in which O(2') is the donor, the interatomic distance O(2')—O(5') being 2.7–2.8 Å.³⁴ By contrast, the HCl salt of araC in the crystalline form is in the conformation C(2')endo, gauche-trans, so that no intramolecular hydrogen bond is either possible or observed.³⁵ Similarly 4-thio-araU in the crystal possesses the conformation C(3')endo, gauche-gauche, so that the distance O(2')—O(5') is 5.0 Å, and an intramolecular hydrogen bond is again neither possible nor observed.³⁶

Molecular Models. The necessary conformational requirements for formation of the foregoing type of intramolecular hydrogen bond, either for the neutral form in which the 2'-OH is the donor, or the dissociated form with 5'-OH as the donor, are readily placed in evidence with the aid of CPK space-filling, Dreiding, or other molecular models. It should again be emphasized that these requirements are no longer met when either the 2'-OH or 5'-OH is etherified (e.g., 2,3'-m₂araC, 3',5'-m₂araC, 5'-maraC), the resulting conformations of which, in alkaline medium, are not C(2')endo, gauche-gauche (see Table II).

Chemical Shifts of H(3'). There is an additional effect pointing to the existence of the postulated intramolecular hydrogen bond. Dissociation of the 2'-OH only slightly affects the chemical shift of H(3') in 3'-maraU (or 3'-maraC), but results in marked shielding of this proton in 3',5'-m₂araC (by ~0.22 ppm) (see Table I). It follows that in the former case there must be some factor which compensates the direct effect of 2'-OH dissociation on H(3'). The change in conformation of the arabinose ring would by itself not be expected to markedly affect the chemical shift of H(3'). However, one would expect increased shielding of H(3') by ~0.1 ppm by the exocyclic 5'-CH₂OH, the gauche-gauche population of which increases at the expense of the gauche-trans.³⁷ Consequently the change in conformation of the exocyclic group in 3'-maraU, on dissociation of the 2'-OH, does not account for the accompanying behavior of the chemical shift of H(3'). However, the effect to be expected from this change testifies to the existence of a "supplementary factor" which deshields H(3') by 0.3 ppm in 3'-maraU in alkaline medium.

Chemical Shifts of H(5') and H(5''). The upfield shifts of H(5') and H(5'') due to dissociation of the 2'-OH, in 3'-maraU, araC, and 3'-maraC, which is similar for both protons (~0.1 ppm), do not occur in the case of 5'-maraC and 3',5'-m₂araC (Table I). It is unlikely that this effect is due to a change in conformation of the exocyclic group, since the upfield shifts are the same for both protons. It is more likely that this is due to the O(5')H...O(2')⁽⁻⁾ hydrogen bond (as suggested by one of the referees, to whom we are indebted).

Table III. Changes in Chemical Shifts (in ppm $\times 10^2$) of H(6) Resulting from Introduction of a 5'-O-Methyl Substituent into araC, 2'-maraC, and 3'-maraC, in Acid Medium (data from ref 9), in Neutral Medium (data from Table I and ref 13), and in Alkaline Medium (data from Table I and ref 13)

5'-O-Methylation of to	Change in chemical shift of H(6) in		
	Acid medium	Neutral medium	Alkaline medium
araC \rightarrow 5'-maraC	8.8	7.4	21.7 (8.6) ^a
2'-maraC \rightarrow 2',5'-m ₂ araC	7.1	5.5	8.6
3'-maraC \rightarrow 3',5'-m ₂ araC	6.7	5.5	17.5 (6.9) ^a

^a Interpretation of the differences in chemical shifts between araC and 5'-maraC (and 3'-maraC and 3',5'-m₂araC) in alkaline medium must take account of the deshielding effect of the intramolecular 5'-OH...O(2')⁽⁻⁾ bond on H(6) in araC (0.131 ppm) and in 3'-maraC (0.106 ppm)¹³ (see Table I). Introduction of a 5'-O-methyl into each of these derivatives liquidates the hydrogen bond. Hence, to evaluate the *direct* effect of 5'-O-methylation on the chemical shift of H(6), these measured values must be decreased by the value for the deshielding effect of the hydrogen bond, to give the value in brackets which may be compared directly with the others in the table.

Chemical Shifts of H(6). The deshielding of H(6) by 0.1 ppm on dissociation of the 2'-OH in 3'-maraU, araC, and 3'-maraC,¹³ an effect not observed in 3',5'-m₂araC and 5'-maraC (Table I), is probably due to the same "supplementary factor" responsible for deshielding of H(3'). This "factor" should be similarly oriented relative to H(3') and H(6), and somewhat more proximal to the former proton. With the nucleoside in the conformation anti, the hydrogen bond O(5')H...O(2')⁽⁻⁾ fulfills the conditions of this "supplementary factor".

In fact dissociation of the 2'-OH leads to an upfield shift of H(3') by ~0.2 ppm, independently of the formation of a hydrogen bond. However, the distance of H(3') from the 2'-OH may be appreciably smaller than that of H(6) from the 2'-OH. This would explain why, in 5'-maraC and 3',5'-m₂araC, where dissociation of the 2'-OH occurs, the effect on H(6) is small.

General Considerations. Intramolecular hydrogen bonding to an acceptor such as O⁽⁻⁾ is not new. A classical illustration is provided by the isomeric maleic and fumaric acids, for which the ratio of the first to the second ionization constants is 20000 for the cis isomer (maleic acid) and 23 for the trans isomer, an effect interpreted in terms of the existence of a strong hydrogen bond in the maleinate anion.³⁸ The enhanced acidities of the cis hydroxyls in ribonucleosides have been interpreted in terms of hydrogen bonding such as 2'-OH...O(3')⁽⁻⁾ \leftrightarrow 3'-OH...O(2')⁽⁻⁾,³⁹ a concept which has been extended to explain the increased acidities of some hydroxyls in other sugar glycosides.⁴⁰

Conformation about Glycosidic Bond. A previous analysis of the equilibrium syn \leftrightarrow anti profited from the change in chemical shift of H(6) resulting from replacement of the 5'-OH by 5'-OCH₃ in the protonated forms of ribo- and arabinonucleosides.⁹ The shielding effect of the 5'-OCH₃ on H(6) is anisotropic in nature and should increase with an increase in the population of the conformers anti and gauche-gauche.

Table III exhibits the changes in chemical shifts of H(6) resulting from introduction of a 5'-O-methyl in araC and its 2'-O- and 3'-O-monomethyl derivatives in acid (protonated form), neutral (neutral form), and alkaline (dissociated form) media. Bearing in mind that the gauche-gauche population of 5'-O-methyl derivatives of araC varies over the range 20–40% (Table II), the marked effect (0.05–0.09 ppm) of the 5'-O-methyl on the chemical shift of H(6) clearly points to predominance of the conformation anti.

It should also be recalled (see above) that the deshielding effects accompanying formation of the intramolecular $5'\text{-OH}\cdots\text{O}(2')^{(-)}$ bond is readily interpreted on the assumption that the conformation anti is maintained in alkaline medium. This is further supported by the fact that dissociation of the $3'\text{-OH}$ and etherification of the $5'\text{-OH}$ do not modify the chemical shift of $\text{H}(1')$ (Table I). The absence of such a change argues for maintenance of the conformation anti, for otherwise a shift in the equilibrium $\text{syn} \leftrightarrow \text{anti}$ toward the form syn would lead to a marked influence of the strongly magnetically anisotropic ring carbonyl on the chemical shift of $\text{H}(1')$.^{27,41,42}

¹H NMR Titration. The changes in chemical shifts and coupling constants in $3'\text{-maraU}$ and in araC accompanying dissociation of the $2'\text{-OH}$ (Figures 4 and 5) should provide some information about the $\text{p}K$ for the latter process. In the case of $3'\text{-maraU}$, over the alkaline pD range herein employed, we are dealing essentially with the dissociation of only one hydroxyl, the $2'$. The dependence of the chemical shifts of the pentose protons sensitive to the effects of dissociation, and of the corresponding coupling constants, on the amount of added NaOD, resemble titration curves. If the millimoles of NaOD scale were replaced by a pD scale, we would have a typical titration curve. Consequently, measurement of the pD at the point on the abscissa scale corresponding to one-half the total change in chemical shifts and/or coupling constants provides, to a reasonable approximation, the $\text{p}K_{\text{D}}$ of the $2'\text{-hydroxyl}$ (Figure 4), ~ 13.3 .⁴³ The fact that this same value is obtained from the changes in chemical shifts, coupling constants $J(1'-2')$ and $J(2'-3')$, or the width of the $\text{H}(4')$ multiplet (i.e., the sum of the coupling constants of this proton with $\text{H}(3')$, $\text{H}(5')$, and $\text{H}(5'')$, which is experimentally much more accurate than the individual coupling constants for this proton) indicates that the use of coupling constants may in some instances be of particular value for determination of the $\text{p}K$ of a specific hydroxyl in the presence of an additional one with similar acidity, e.g., araC . In this case the use of chemical shifts is virtually excluded because of overlapping of the effects of dissociation of the $2'\text{-OH}$ and $3'\text{-OH}$. Dissociation of the $3'\text{-OH}$ in araC does not modify the conformation of the sugar ring (see above), so that modifications observed are due to ionization of the $2'\text{-OH}$, which is indispensable for formation of a hydrogen bond with the $5'\text{-OH}$. The points corresponding to 50% of the alkali-induced changes in $J(1'-2')$ and $J(2'-3')$ occur at 0.065 and 0.055 mmol of added NaOD, respectively (Figure 5d); the mean value is 0.060 mmol of NaOD, for which the experimentally determined pD is 13.2. The latter value is, to a first approximation, the $\text{p}K_{\text{D}}$ for dissociation of the $2'\text{-hydroxyl}$ in araC .

It is necessary once again to emphasize that the foregoing $\text{p}K$ values are at best close approximations. They do show, however, that it should be feasible to obtain analytical values for the microscopic $\text{p}K$'s of certain sugar hydroxyls.⁴⁴ It is, however, likely that such titrations may be more readily carried out by means of ¹³C NMR.

Alkali-Catalyzed H(5) Exchange. Pyrimidine nucleosides, in both acid and alkaline media, undergo facile exchange of the pyrimidine $\text{H}(5)$ via a mechanism involving reversible saturation of the 5,6 bond.⁴⁵ The rate of exchange of $\text{H}(5)$ for pyrimidine arabinosides is approximately 50-fold higher than for the corresponding ribosides, due to intramolecular catalysis by the arabinosyl "up" $2'\text{-OH}$, leading to formation of $2',6\text{-anhydro}$ intermediates in the anti conformation.^{7,45,46} Exchange of $\text{H}(5)$ for ²H may be followed by the collapse of the $\text{H}(6)$ doublet to a singlet, and the decrease in integral intensity of the $\text{H}(5)$ signal relative to the nonexchangeable $\text{H}(1')$.

Rate constants for exchange of $\text{H}(5)$ were measured for

araC , $5'\text{-maraC}$, and $3',5'\text{-m}_2\text{araC}$, each at a concentration of $\sim 0.15\text{ M}$ in 1 N NaOD in D_2O at 52°C , from the relationship

$$\ln(I_0/I) = -k \cdot t$$

where I_0 is the initial integral intensity of the $\text{H}(5)$ signal and I the value at time t . Values of I_0 and I were measured relative to $\text{H}(1')$ and $\text{H}(6)$. Possible deamination under these conditions was controlled by following changes in chemical shifts of $\text{H}(5)$ and $\text{H}(6)$ relative to those for the corresponding uracil arabinosides, and did not exceed 3–4%.

The marked preference for the conformation $\text{C}(2')\text{endo}$ of araC in alkaline medium, as a result of which the $2'\text{-O}^{(-)}$ is closer to $\text{C}(6)$ than in $3',5'\text{-m}_2\text{araC}$ and $5'\text{-maraC}$, would be expected to facilitate the exchange reaction. In fact the rate constant for $\text{H}(5)$ exchange in araC ($1.5 \times 10^{-2}\text{ min}^{-1}$) is lower than for $3',5'\text{-m}_2\text{araC}$ ($3.0 \times 10^{-2}\text{ min}^{-1}$) and $5'\text{-maraC}$ ($5.2 \times 10^{-2}\text{ min}^{-1}$). The formation of the intramolecular hydrogen bond $5'\text{-OH}\cdots\text{O}(2')^{(-)}$ in dissociated araC appears to offer a reasonable interpretation of this effect, since this would interfere with the interaction between the $\text{O}(2')^{(-)}$ and $\text{C}(6)$. Such an interpretation is consistent with the observation of Santi and Brewer,⁴⁵ who found that araU , which can form an intramolecular hydrogen bond, undergoes $\text{H}(5)$ exchange in 0.5 M NaOCH_3 in CH_3OD at 60°C at one-half the rate for $5'\text{-deoxy-araU}$, in which such hydrogen bonding is not possible.

Concluding Remarks

It is perhaps not out of place to emphasize that, although the present study was restricted to the sugar hydroxyls of pyrimidine nucleosides, its applicability and utility are probably much more widespread. We have already demonstrated its application to a purine arabinoside,³² and it appears reasonable to expect that this can be extended to studies on the acidities and other properties of the sugar hydroxyls of glycosides in general, as well as the modifications in conformation associated with sugar hydroxyl(s) dissociation. Undoubtedly the use of ¹³C magnetic resonance would even further extend the range of utility of this method, and such trials are now under way in this laboratory.

It is further pertinent to note that the $\text{C}(2')\text{endo}$ and gauche-gauche conformation of araC resulting from dissociation of the $2'\text{-OH}$ may conceivably be the extreme ²E conformer, the coupling constants of which are essential for a *fully quantitative* application of the Karplus relation with the use of the geometrical parameters from x-ray diffraction data for this conformation in the solid state.³³ Model building studies suggest that a similar model for the ³E type conformer may possibly be found with the aid of xylofuranosides; the $\text{p}K$ for sugar hydroxyl(s) ionization in $1\text{-}\beta\text{-D-xylofuranosyladenine}$, virtually identical with that for adenosine, and which Christensen et al.⁴⁷ interpreted in terms of increased hydration of the former, may equally be accounted for by intramolecular hydrogen bond formation of the type $5'\text{-OH}\cdots\text{O}(3')^{(-)}$.

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- (43) Independently of the foregoing, we have measured the pK values for dissociation of the sugar hydroxyls of some araC and araU analogues by spectrophotometric titration.¹¹ For 3'-maraU, the measured pK_D in D₂O was 13.3 ± 0.15. It should be noted that the concentration employed for titration by uv spectrophotometry is of the order of 10⁻⁴ M.
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