Carbohydrate Research, 92 (1981) 21–36
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

CONFORMATIONAL PARAMETERS OF THE CARBOHYDRATE MOIETIES OF α-ARABINONUCLEOSIDES

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(Received September 30th, 1980; accepted for publication, December 23rd, 1980)

ABSTRACT

The conformations of the sugar moieties of a number of α-D- and -L-arabinofuranosyl nucleosides in solution have been investigated, largely with the aid of a statistical procedure developed for this purpose. The overall results demonstrated the existence, for a broad range of analogs, of a two-state, conformational equilibrium, N⇌S (or ${}^{2}E \rightleftharpoons {}^{3}E$). As between different analogs, there is a large variation in the relative populations of these two states that is related to the nature of the aglycon, the type of protecting groups on the sugar hydroxyl groups, and, to a much smaller extent, the solvent system. There is a striking correlation between the conformation of the sugar and the orientation of the exocyclic, hydroxymethyl group. Both for purine and pyrimidine nucleosides, a preponderance of the *gauche-gauche* rotamer of the exocyclic group is associated with the type-N state of the furanose ring, whereas, with the type-S state, the *gauche-gauche* rotamer population is virtually nonexistent. A comparison with X-ray diffraction data for 9-α-D-arabinofuranosyladenine demonstrated that, as for other classes of nucleosides, the solid-state conformation corresponds to one of the states participating in the equilibrium in solution.

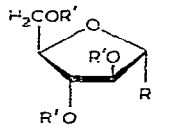
INTRODUCTION

Although nucleotide residues having the α-anomeric configuration are not found in natural nucleic acids, there are a number of examples of α-nucleosides and α-nucleotides in the free state, or as constituents of smaller molecules in living cells[1,2], indicating that these are substrates and, possibly, inhibitors in some enzymic systems. In fact, the α anomers of hexofuranosyladenines are weak substrates of adenosine deaminase[3], some α-nucleoside 5′-phosphates are weak substrates of 5′-nucleotidase[4,5], and 5-formyl-α-uridine is a sufficiently good, noncompetitive inhibitor of the DNA-dependent, RNA polymerase of *Escherichia coli* to permit of the affinity labelling of the enzyme[6].

Current, widespread interest in the properties of α-nucleosides stems largely from the significant antimetabolic (antitumor and antiviral) properties exhibited by some of them[7–10], *e.g.*, the α anomers of a number of benzimidazole nucleosides[11].

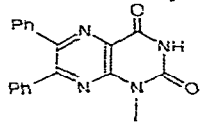
Undoubtedly the most striking illustration is 2′-deoxy-6-thio-α-guanosine, which is readily phosphorylated by kinase(s) in some tumor cells[12], and is sufficiently active against experimental tumors[13] to have been selected for clinical trials[14]. This has raised some question as to the presumed, gross, structural dissimilarity between α and β anomers, and has led to the synthesis of the anomers of 3′-(hydroxymethyl)-branched 2′-deoxynucleosides of 6-thioguanine; in agreement with expectations, the anomers were found to be equally effective as inhibitors of the growth of WI-L2 human lymphoblastoid cells, and even more effective in this regard than the parent 2′-deoxy-6-thio-α-guanosine[15].

Among the D-arabinofuranosyl nucleosides, the β anomers of which are potent, and clinically employed, therapeutic agents, the α anomer (**1**) of 9-D-arabinofuranosyl-adenine (AraA) has been shown to exhibit appreciable, antiviral activity[9]. Furthermore, whereas 9-β-D-arabinofuranosyl-8-azaadenine exhibits cytotoxic activity[9], it was found to be inactive as an antiviral agent[7-9]; in contrast, the corresponding α anomer (**2**) has been reported by two groups to display such activity to an appreciable extent.

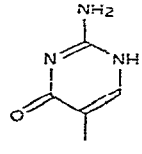


1 R = adenin-9-yl, R′ = H

2 R = 8-azaadenin-9-yl, R′ = H

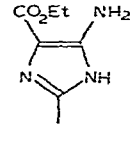


6,7-Diphenyllumazin-1-yl

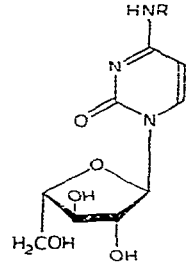


ψ-isocytosin-1-yl

7 R = 5-ethyluracil-1-yl, R′ = H

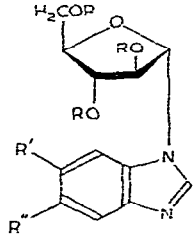


5-amino-4-(ethoxycarbonyl) imidazol-2-yl



3 R = H

4 R = PhCO



8 R = R′ = R″ = H

9 R = Ac, R′ = R″ = H

10 R = H, R′ = R″ = Cl

11 R = H, R′ = R″ = Br

12 R = H, R′ = Br(H), R″ = H(Br)

Because substrate and inhibitor properties of a given class of compounds are dependent, among other factors, on conformational parameters, we previously reported the results of an investigation on the interrelationships of the conformational parameters and equilibrium conformational states for a variety of β-D-arabinofuranosyl nucleoside analogs, and showed how these may be applied to examination of possible correlations between conformation and biological activity[16]. It was noted at that time that the correlations observed between conformational parameters did not appear applicable to several α anomers of the corresponding β-nucleosides. We have now extended these preliminary observations, in part with data culled from the literature, and present here a detailed account of the conformational parameters and equilibrium conformational states for a number of α-D- and -L-arabinonucleosides, including some having biological activity.

EXPERIMENTAL

Compounds. — We are indebted to Dr. J. A. Montgomery for samples of α-D-AraA (**1**) and α-D-Ara-8-azaA[17] (**2**), and to Dr. T. Kulikowski for a sample of 1-α-D-arabinofuranosyl-5-ethyluracil (α-D-Ara-5-EtU)[18] (**7**). The synthesis of the α-D-arabinofuranosylbenzimidazoles has been described[19]. Debenzoylation of 1-α-L-arabinofuranosyl-N^4-benzoylcytosine (α-L-Ara-N^4-BzC; **4**) in acetic acid yielded α-L-AraC (**3**). For the other compounds used in this investigation, we are indebted to those investigators listed in the references in Table I for supplying the experimental spectra; these were subjected to simulation and analysis as described.

Solvents. — These included D_2O (>99.9 mol% D), $(CD_3)_2SO$ (>99.8 mol% D), NaOD (40% in D_2O, >99 mol% D), and DCl (99.5 mol% D), all obtained from Merck (Darmstadt, GFR). Fully deuterated chloroform was from the IBJ Radioisotope Centre (Świerk, Poland).

N.m.r. spectra. — These were recorded with Jeol-100 and Varian-100 instruments, as well as with a Bruker-90 spectrometer operating in the Fourier-transform mode, usually for solutions at a concentration of 0.15–0.20M. In the case of α-D-Ara-8-azaA (**2**) in D_2O, the concentration was 0.02M.

Calculations. — The conformations of the sugar moieties were analyzed with the aid of a special program, KONFOR, for a two-state model, using the pseudorotational model to derive the ring geometry[20]. The spectra were simulated with the aid of the program LAOCOON III. Orthogonal regressions were calculated with the aid of the program[21] MINCON.

RESULTS

The values of the coupling constants, obtained from analysis of the simulated spectra of the various compounds, are listed in Table I. Because of the limited solubility of some of the compounds in water, it was also necessary to employ other solvents. From Table I, it will be noted that, for α-D-AraA (**1**), change from D_2O

TABLE I

VALUES OF COUPLING CONSTANTS (IN Hz) FOR α-ARABINONUCLEOSIDES[a]

Point number	*Compound*	*Compound number*	*Solvent, and remarks*	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	*Source of spectral data (reference)*
1	α-D-AraA	1	D_2O, neutral	4.8	5.7	6.0	3.0	5.0	—
2			D_2O, pD ~ 14	4.5	4.9	5.4	2.4	4.9	—
3			D_2O, protonated	4.6	5.2	6.4	3.4	4.8	—
4			$(CD_3)_2SO$	4.8	5.4	6.2	3.7	4.9	—
5	α-D-Ara-8-azaA	2	D_2O, protonated	5.0	5.3	[b]	3.0[c]	4.0[c]	—
6			$(CD_3)_2SO$	5.9	7.0	8.6	2.3	4.6	—
7	α-L-AraC	3	D_2O, neutral	3.7	4.3	5.0	3.5	5.6	—
8			D_2O, pD ~ 14	3.2	3.9	4.3	2.8	4.0	—
9			$(CD_3)_2SO$	3.0	3.2	4.0	4.7[d]		—
10	α-L-Ara-N^4-BzC	4	$(CD_3)_2SO$	2.0	2.4	2.9	5.4[d]		—
11	α-D-Ara-6,7-Ph_2-lumazine	5	$(CD_3)_2SO$-$(CD_3)_2CO$-D_2O	6.5	7.4	9.2	2.1	4.3	22
12	α-D-Ara-ψ-isoC	6	D_2O	6.1	5.8	[b]	[b]	[b]	23
13	α-D-Ara-5-EtU	7	$(CD_3)_2SO$	4.4	4.7	4.7	3.8	4.5	19
14			D_2O, protonated	5.5	5.3	6.0	2.6	5.2	—
15	α-D-Arabz[e]	8	$(CD_3)_2SO$	5.3	5.0	6.3[c]	3.0[c]	3.7[c]	—
16			D_2O, protonated	4.3	4.0[c]	[b]	[b]	[b]	—
17	Ac_3-α-D-Arabz	9	$CDCl_3$	2.1	1.9	3.0	6.3	4.6	—
18	α-D-Ara-5,6-Cl_2bz	10	$(CD_3)_2SO$	4.9	5.1	5.4[c]	3.9[c]	4.4[c]	—
19	α-D-Ara-5,6-Br_2bz	11	$(CD_3)_2SO$	4.7	4.5	5.7	3.4	4.8	—
20	α-D-Ara-5(6)-Brbz	12	$(CD_3)_2SO$	5.3	4.7	6.0	3.4	4.7	—
21	See formula	13	$CDCl_3$	1.8	1.5	1.3[c]	[b]	[b]	24

[a]Accuracy of measurements is 0.1–0.2 Hz for coupling constants for the sugar ring, and ~0.3 Hz for the couplings of protons of the exocyclic group. [b]Not identified. [c]Accuracy ~0.5 Hz. [d]In this instance, it proved possible to determine only the average of $J_{4',5'}$ and $J_{4',5''}$. [e]Benzimidazole = bz.

to $(CD_3)_2SO$ results in only small, but still significant, changes in coupling constants. This factor will be considered in more detail later.

Sugar conformations. — For this class of compounds, all three proton–proton, vicinal coupling-constants of the pentofuranose ring are transoidal, a situation particularly useful for conformational analyses, as (*a*) their range of variation is broader than for cisoidal coupling-constants, and (*b*) their period of variability, as a function of the phase angle of pseudorotation (*P*), is 360°, so that there are only two conformations for which the values of the coupling constants are equal, whereas there are four in the case of cisoidal coupling-constants.

Nonetheless, conformational analysis of the α-arabinonucleosides proved to be

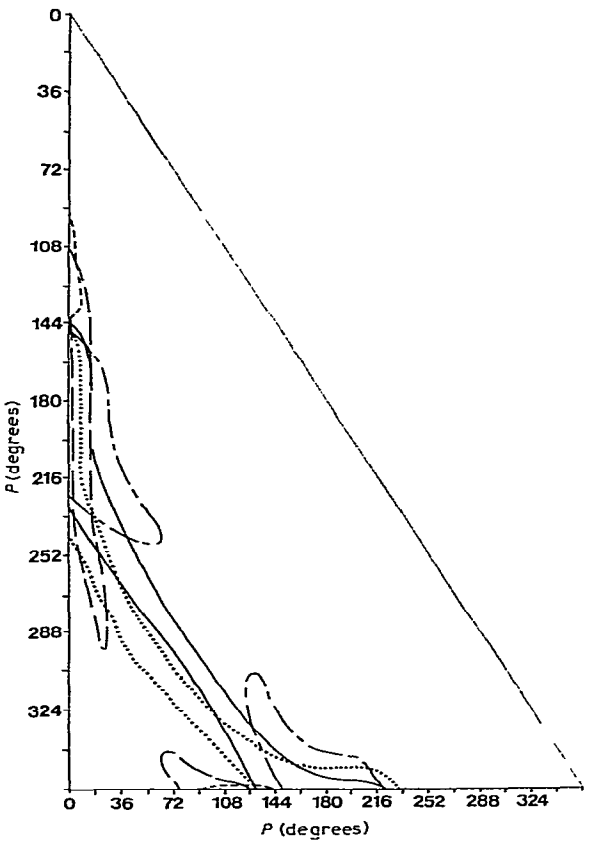


Fig. 1. Acceptable conformational regions of the sugar moiety, from calculations for individual α-arabinonucleosides. [Key: (———) α-D-Ara-N^4-benzoyl C; (– – –) α-D-Ara-6,7-diphenyllumazine; (. . .) α-D-AraA, neutral; (——) α-D-AraC, neutral; and (— — —) α-D-Ara-8-azaA. For α-D-Ara-N^4-BzC and α-D-AraC, calculations were performed by using the coupling-constant values of their L forms. The "acceptable regions" are those embraced within the curves shown. As indicated in the text, different parametrizations of the Karplus relation were adopted for the individual bonds, as follows: $J_{1',2'} = 9.0\cos^2\phi - 0.5\cos\phi - 0.3$; $J_{2',3'} = 9.85\cos^2\phi - 0.55\cos\phi - 0.3$; and $J_{3',4'} = 10.5\cos^2\phi - 1.2\cos\phi$. Changes in parametrization within the range of those normally applied to nucleosides do not essentially change these results. An amplitude of ring puckering of 40° was adopted.

considerably more complex than for the corresponding β-ribonucleosides. For the latter, the experimental values of $J_{2',3'}$ alone suffice to delineate two narrow, conformational ranges centered about the states ^3E and ^2E. By contrast, calculations for the α-arabinonucleosides indicated that (*a*) experimental values of the vicinal coupling-constants for the individual compounds do not correspond to a *unique* conformation, or even to a narrow, conformational range (of the order of 90° of the phase angle of pseudorotation) corresponding to an averaging of the values of the coupling constants at the energy minimum, and (*b*) apart from those compounds for which *all three* vicinal coupling-constants of the sugar moiety are large [α-D-Ara-6,7-diphenyllumazine (**5**) and α-D-Ara-8-azaA (**2**)], or small [α-L-Ara-N^4-BzC (**4**) and 1-(2,3,5-tri-*O*-acetyl-α-Ara)benzimidazole (**9**)], it is possible, for each value of P, to find another phase angle of pseudorotation distal from it by more than 90°, such that, to a good approximation, there is, in solution, an apparent equilibrium between these two conformations. Such calculations were performed for all possible pairs, P_1 and P_2, by varying P in 6-degree steps. Optimal populations were found for which the sum of the squares of the deviations of the calculated from the experimental values of $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ was minimal. Good pairs selected were those for which the value of this sum, with an optimal choice of populations, did not exceed 0.5 Hz^2. The results of such calculations for five selected compounds are listed in Fig. 1.

Unequivocal determination of the conformation of the glycosyl group of α-arabinonucleosides, without any *a priori* premise as to the existence of two equilibrium states, as in the case of β-ribonucleosides, may be more readily achieved by an examination, not of individual compounds, but of entire groups; this renders possible supplementary analyses of mutual relationships between the values of coupling constants. A fundamental assumption in such an approach is acceptance of the existence, for *all* of the compounds of a given group, of an equilibrium between two

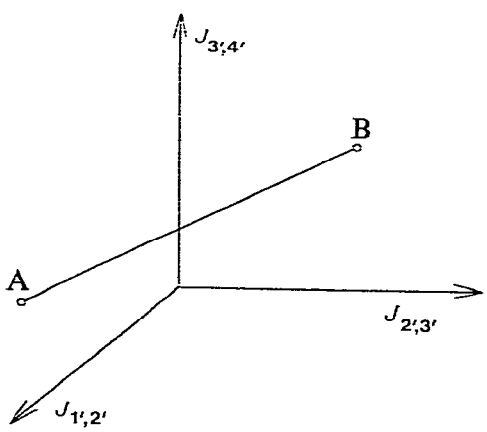


Fig. 2. Two-state equilibrium model, in three-dimensional space, of the coupling constants for the furanosyl group.

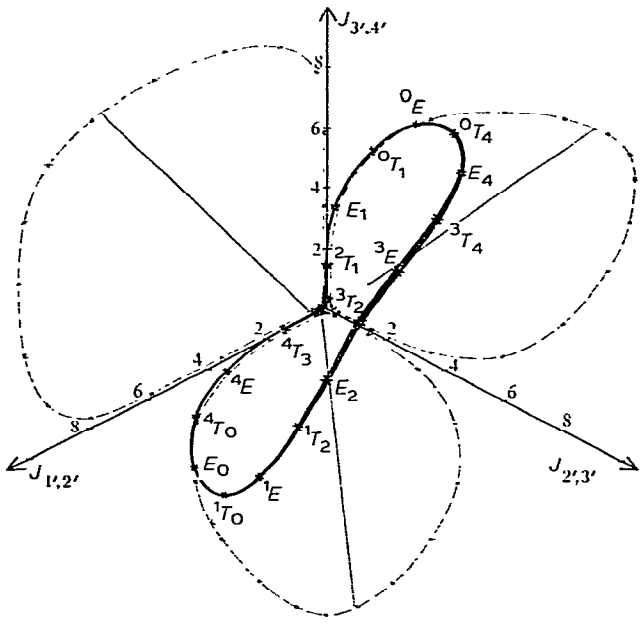


Fig. 3. Pseudorotational curve in ^3J space for the sugar ring of α-arabinonucleosides. (The three straight lines are the projections on the coordinate planes of the straight line in the three-dimensional space obtained from the experimental points.)

similar, conformational states of the sugar moiety. Such an assumption has been shown to be reasonable* in the case of β-ribonucleosides[25].

If there is a two-state, conformational equilibrium, A⇌B, of the glycosyl group in solution, then, in a three-dimensional representation of the coupling constants (J), the points representing the individual sets of three coupling constants should fall along the line AB (see Fig. 2). The experimental results are consistent with this hypothesis, *viz.*, the correlation coefficients between the coupling constants are $\rho(J_{1',2'}, J_{2',3'}) = 0.95$, $\rho(J_{1',2'}, J_{3',4'}) = 0.97$, and $\rho(J_{2',3'}, J_{3',4'}) = 0.94$. From the experimentally determined points, the parameters of the line AB may be calculated quite simply by linear, orthogonal regression. For 18 such points, we find $J_{1',2'} = 7.5 - 0.7t$, and $J_{2',3'} = 8.4 - 0.8t$, on the assumption** that $J_{3',4'} = 10.0 - t$, where t is a parameter that may be related to the populations of the two states. The calculated sum of the squares of the deviations from the linear relation amounted to 3.81 Hz^2, and hence, it is reasonably consistent with the assumption of a two-state, conformational equilibrium for the group of α-arabinonucleosides. Because, for several of the compounds, only two values of coupling constants, $J_{1',2'}$ and $J_{2',3'}$,

*In a number of instances, accurate values of P and τ_m for both states have been evaluated for individual compounds. It has been shown[26] that the accuracy of such results is, in large measure, a consequence of the method applied, and is not necessarily a reflection of the actual state of affairs.
**Such an assumption corresponds to (*a*) the choice of the value $t = 0$ for $J_{3',4'} = 10.0$ Hz. and (*b*) definition of the scale t. As this assumption is arbitrary, the relationship of the value of t to the conformational populations requires separate consideration.

were available and, for several, the accuracy of the measured value of $J_{3',4'}$ was, in some instances, lower than for the other two, an independent calculation of the orthogonal-regression line was conducted in two dimensions. The results were virtually identical with those obtained for the three-dimensional case.

From the Karplus relation, with the parametrization* of Altona and Sundaralingam[27], and determination of the values of the dihedral angles on the basis of the pseudorotational model, the values of the three coupling constants for the arabinofuranosyl ring, as a function of the phase angle of pseudorotation, may be determined. In a three-dimensional portrayal of the coupling constants, this leads to a continuous curve representing the pseudorotational cycle** for the α-arabinonucleosides (see Fig. 3).

The linear relationship just described, obtained from the experimental values of the coupling constants, should, in the three-dimensional J space, intersect at two points with the curve representing the pseudorotational cycle. These points correspond to the two states between which there is an equilibrium in solution. From these, their populations may then be calculated.

From calculations performed for all of the analogs, it may be seen that, in the range of high values of the coupling constants, the straight line derived from the experimental points does not precisely intersect the pseudorotational curve; however, as the deviation from the intersection point is small, this may be ascribed to the approximate nature of the model. Better agreement is obtained by modification of the Karplus relation (see the Discussion). For one of the extremal states, the values of all three coupling constants are close to zero. This corresponds to a conformation in the range ${}^2E \cdots {}^2T_3 \cdots E_3$. For the second, $J_{3',4'} > J_{2',3'} > J_{1',2'}$, with a fairly high value for $J_{3',4'}$ ($\sim$10 Hz) corresponding to the conformation ${}^3T_2 \cdots {}^3E$ (see Fig. 3). These results indicate that the conformational equilibrium for α-arabinonucleosides in solution proceeds between two states that are similar to those for β-ribonucleosides.

To evaluate the populations of the type N and S states (or DN and DS for L compounds‡), it may be assumed that, for the S-type state, $J_{1',2'} = 0.5$ Hz, $J_{2',3'} =$

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*From among those applied to nucleosides, this parametrization was selected because it gives large values of the coupling constants. The choice of another type of parametrization would not significantly affect the results, apart from a certain shift in populations in favor of the type-N state.

**It should be noted that this is only an approximation, because of (*a*) the approximate nature of the Karplus relation, (*b*) the assumption of a unique amplitude of puckering for all conformations of the sugar moiety, and (*c*) the assumption that the pseudorotational model satisfactorily describes the conformational continuum of the pentofuranose ring.

‡According to nomenclature rules, a change in the configuration of a nucleoside from D to L results in an N-type to S-type (and S to N) conformational change. For example, in the D configuration, a ${}^3E$ conformation means that C-3′ is displaced to the same side of the ring as the exocyclic, -CH_2OH group, but, in the L configuration, the displacement is in the opposite direction relative to -CH_2OH (for a general definition of the upper side of a furanoid ring, see ref. 28). Because a group of nucleosides is discussed that includes both D and L enantiomers, DN or DS conformations for L configurations will be referred to as corresponding to the N or S conformations of the D enantiomers. For example, $P_{DN} = 26\%$ for α-L-Ara-N^4-BzC (see Table II) means that α-D-Ara-N^4-BzC would have $P_N = 26\%$, but, actually, α-L-Ara-N^4-BzC has 74% of the N-type state.

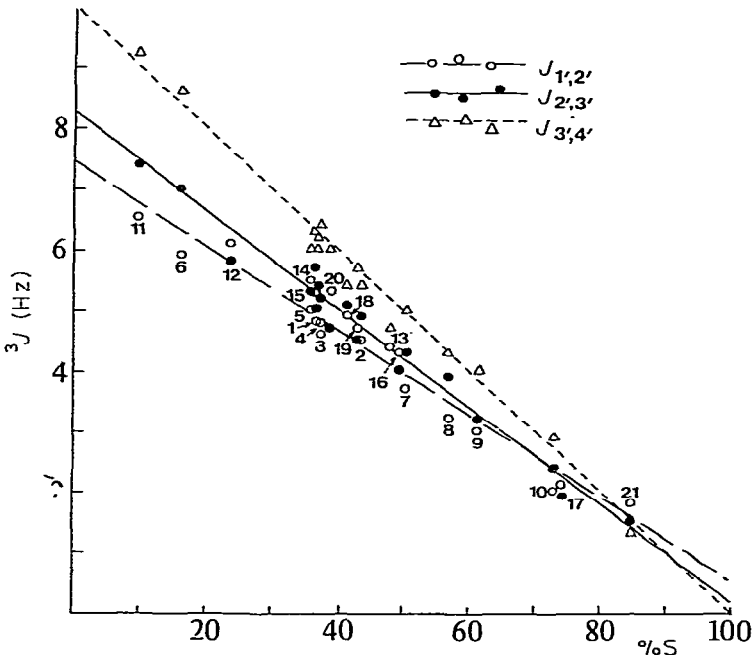

Fig. 4. Relationship between values of coupling constants and populations of the N and S states of the sugar ring of α-arabinonucleosides. (For each nucleoside, three experimental points, corresponding to the values of $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$, are on a vertical line. The values of $J_{2',3'}$ and $J_{3',4'}$ were taken as zero for the S state, and as not exceeding 10 Hz for the N state. As these assumptions are somewhat arbitrary, particularly the latter, the populations calculated may be subject to systematic error, evaluated as not exceeding 10%.)

$J_{3',4'} = 0$ Hz, whereas for the N-type state, the corresponding values are, respectively, 7.5, 8.3, and 10.0 Hz (see Fig. 4). The populations obtained in this way are listed in Table II. In general, the results obtained from the different coupling-constants yield population values that agree within ~10%. The only serious departure (~20%) occurs for α-D-Ara-ψ-isoC (**6**); however, this analog is a *C*-nucleoside, which may affect the value of $J_{1',2'}$, and the accuracy of the coupling constants for it was also lower (of the order of 0.3–0.5 Hz) than for the other compounds.

Orientation of sugar hydroxyl groups. — A comparison of the values of the coupling constants $J_{H\text{-}C\text{-}O\text{-}H}$ (listed in Table III) with those for ribonucleosides[29] and β-arabinonucleosides[30] demonstrates that, as for the latter, OH-5′ of the α-arabinonucleosides exhibits approximately comparable populations of the three classical rotamers, g′g′, t′g′, and g′t′.

The orientation of OH-2′ is similar to that for β-ribosides and β-arabinosides, *viz.*, ~33% of the *trans* rotamer and ~66% of the *gauche*, and is weakly correlated with the conformation of the sugar moiety. A much clearer correlation prevails in the case of OH-3′, for which the value of $J_{H\text{-}C\text{-}O\text{-}H}$ among the α-arabinonucleosides exhibits appreciable variability; for α-D-AraA (**1**) and α-D-Ara-8-azaA (**2**), it points to a population of ~33% *trans*, whereas, for α-L-Ara-N^4-BzC (**4**), the value of this

TABLE II

CALCULATED POPULATIONS OF THE TYPE-*N* STATE OF THE SUGAR RING, AND OF THE *gauche-gauche* ROTAMER OF THE EXOCYCLIC 4′-CH_2OH GROUP, FOR VARIOUS α-ARABINOFURANOSYLNUCLEOSIDES

Point number	*Compound*	*Compound number*	P_N (%)	$P_{gauche-gauche}$ (%)
1[a]	α-D-AraA	**1**	63	50
2			56	57
3			62	48
4			62	44
5	α-D-Ara-8-azaA	**2**	63	60
6			82	61
7	α-L-AraC	**3**	49[b]	39
8			43[b]	62
9			38[b]	36
10	α-L-Ara-N^4-BzC	**4**	26[b]	22
11	α-D-Ara-6,7-Ph_2-lumazine	**5**	89	66
12	α-D-Ara-ψ-isoC	**6**	75	[c]
13	α-D-Ara-5-EtU	**7**	53	47
14			65	52
15	α-D-Arabz[d]	**8**	64	63
16			50	[c]
17	Ac_3-α-D-Arabz	**9**	25	21
18	α-D-Ara-5,6-Cl_2bz	**10**	59	47
19	α-D-Ara-5,6-Br_2bz	**11**	58	48
20	α-D-Ara-5(6)-Brbz	**12**	62	49
21	See formula	**13**	16	[c]

[a]The numbering of the compounds corresponds to that in Table I. [b]P_{DN} (see text). [c]$J_{4',5'}$ and $J_{4',5''}$ were not measurable, because of overlapping signals. [d]Benzimidazole = bz.

TABLE III

VALUES OF VICINAL COUPLING-CONSTANTS (IN Hz) WITH HYDROXYL PROTONS IN α-ARABINONUCLEOSIDES, IN $(CD_3)_2SO$

Nucleoside	$J_{2',2'-OH}$	$J_{3',3'-OH}$	$J_{5',5'-OH}$
α-AraA	5.4	5.1	5.4[a]
α-Ara-8-azaA	6.0	5.5	5.3; 6.2
α-Ara-N^4-BzC	4.6	3.4	5.3[a]

[a]Mean value.

coupling constant is appreciably lower, and the *trans*-rotameric population, calculated on the basis of the equations of Davies and Danyluk[29] is only 13%. This is related to the conformation of the sugar moiety in this analog, which exhibits a very marked tendency to assume the S-type state. With such a sugar conformation, there is close contact between OH-3′ and the aglycon. A similar situation prevails for

α-"deoxyribo"nucleosides when the sugar moiety is in the S-type conformation, and, in fact, this has been proposed as the cause of the flattening of the sugar ring in such compounds in the solid state[31].

Orientation of the exocyclic, 4'-CH_2OH group. — The orientation of this group in α-arabinonucleosides is of particular interest, in that, in these compounds, there is no direct interaction between this group and the heterocyclic base. Its possible correlation with the sugar conformation should be of special significance.

The populations of the *gauche-gauche* rotamer (see Table III) were calculated from the relation

$$P_{g-g} = \frac{13 - \Sigma}{10}, \quad \text{where} \quad \Sigma = J_{4',5'} + J_{4',5''}.$$

Fig. 5 exhibits the interdependence between the orientation of the exocyclic group and the sugar conformation. Bearing in mind the extensive data on the effects of solvent on the orientation of the exocyclic group[32], the observed interdependence of the two is unusually striking. This may, in part, be fortuitous, as the number of experimental points is not large. It will, on the other hand, be noted that an identical correlation prevails both for purine and pyrimidine nucleosides, as might have been

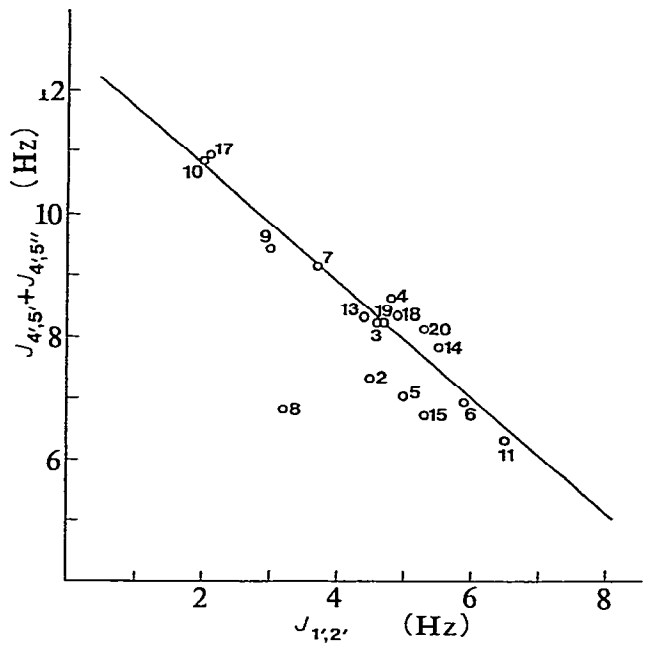

Fig. 5. Dependence of $J_{1',2'}$, representing the sugar conformation [population of N-type state = $(J_{1',2'} - 0.5)/7.5$], on the sum $J_{4',5'} + J_{4',5''}$, linearly related to the *gauche-gauche* rotamer population. [The continuous, straight line was obtained by the orthogonal-regression procedure, using the coupling constants listed in Table I. Not included in the calculations are the values for α-AraC and α-AraA in alkaline medium, and compounds for which the measurements of coupling constants were subject to appreciable error (those accompanied by the letter "c" in Table I).]

anticipated from the absence of direct contact between the exocyclic group and the base.

From Fig. 5, it may be seen that the *gauche-gauche* rotamer is absent for those compounds having a type-S sugar conformation. With the type-N conformation, the populations of the *gauche-trans* and *trans-gauche* rotamers may attain values up to 30%.

The behavior of α-L-AraC (3) in strongly alkaline medium (point 8 in Fig. 5) differs slightly from that of the other compounds, in that, at high pD, there is an increase in the *gauche-gauche* population concurrent with only minor modification of the sugar conformation. This may be due to appearance of a low population of molecules having an intramolecular hydrogen-bond, 5′-OH···O-2′$^-$; however, formation of such a bond is less favored than for the β-arabinonucleosides. A somewhat similar, but even less pronounced, effect may be noted for α-D-AraA (**1**).

DISCUSSION

Comparisons with other classes of furanose nucleoside. — The demonstration that, in solution, α-arabinofuranosyl nucleosides exhibit a type N⇌S conformational equilibrium of the sugar moiety, in conjunction with previous data for ribo-, β-arabino-[16], β-xylo-[33], and β-lyxo-[34] furanosylnucleosides, points to the general appearance of the furanosyl group of the nucleosides in a conformational equilibrium between states of type N (close to ^3E) and S (close to ^2E). Certain departures from this rule have been noted only for some β-arabinonucleosides[16]. Such conformations were proposed for furanosides even prior to the extensive development of conformational analysis by Bishop and Cooper[35], who concluded, solely from a consideration of the interaction between "eclipsed groups" on neighboring carbon atoms, that the most stable conformations should be those in which C-2′ or C-3′, or both, are forced out of the plane of the ring.

It is difficult to specify whether there is any real difference in equilibrium states between the different groups of compounds in solution. It is instructive to conduct a comparison with the β-arabinonucleosides, in view of the identical situation about the bonds with the transoidal couplings $J_{2',3'}$ and $J_{3',4'}$. When compared with the β anomers, the correlation between these coupling constants for the α-arabinonucleosides is more pronounced, so that there is little doubt as to the validity of the two-state model. The N-type state for both groups of compounds does not differ in the phase angle of pseudorotation by more than 18°, which, for the β anomers, is shifted in the direction of $^3T_2$ and, for the α anomers, somewhat in the direction of $^3T_4$.

Similarly, the correlation between the conformation of the sugar and the orientation of the exocyclic 4′-CH_2OH group is better for the α- than for the β-arabinonucleosides. The fact that the *gauche-gauche* rotamer is absent for the S-type state is, therefore, a consequence of interactions within the sugar ring itself. In the β anomers, there is an additional, weak influence of the base on the populations of the exocyclic group.

The β-xylonucleosides differ from the corresponding α-arabinonucleosides by the change in orientation of the exocyclic, 4′-CH_2OH group (and the transition from the D to L configuration). If β-D-XylA (for which there is a very strong tendency for a type-N conformation of the sugar) is now compared with α-D-AraA (which also exhibits, to a somewhat smaller extent, a strong tendency for the type-N conformation), some inference as to the role of the exocyclic group in determining the pentofuranosyl conformation may be derived. If this group has no effect, the compounds should exhibit reverse conformations. This is not, however, the case, thus confirming the role of the interaction between OH-3′ and the exocyclic group on the observed conformation of xylonucleosides[33] in solution.

Conformation of the sugar moieties of individual analogs. — From Tables I and II, it is evident that even the small number of α-arabinonucleosides here investigated embraces a very broad range of sugar conformations, *viz.*, from ~15 to almost 90% of type N, and testifies to the conformational variability of this class of compound, in solution, under the influence, in particular, of the nature of the base, of substitution of the sugar hydroxyl groups, and of the nature of the solvent. This is in striking contrast to the situation for 2′-deoxynucleosides (which exhibit a tendency to assume the S state, and for which the influence of the heterocyclic base is small) and for β-xylonucleosides (favoring the N state, and only slightly affected by the nature of the aglycon). For β-ribonucleosides and β-arabinonucleosides, also, only a major modification of the sugar moiety (such as replacement of a hydroxyl group by some other substituent) leads to a clear-cut change in conformation.

Particularly noteworthy is the unusually high population (~80%) of the N state for α-D-Ara-8-azaA (**2**) in $(CD_3)_2SO$, as compared to 60% of the type-N state for α-D-AraA (**1**). The low solubility of the former in D_2O precluded a similar comparison for this solvent. However, for both β-ribosyl and β-arabinosyl nucleosides, there is no essential difference in conformation between adenine and 8-azaadenine derivatives in aqueous medium[36].

The populations of the type-N state in α-D-AraA (**1**) and α-L-AraC (**3**)* are not far removed from 50%, that for **3** being somewhat lower, as with β-arabinonucleosides[16]. The situation here is, consequently, the reverse of that for β-ribonucleosides.

The lowest populations of the type-N state appear in the α-arabinonucleosides having protected hydroxyl groups (compounds **9** and **13**). The difference in population, relative to the parent nucleosides having free hydroxyl groups, is very marked (*cf.*, compounds **8** and **9**).

Whereas, for 5-α,β-D-ribofuranosylpyrimidines, the type N and S states exhibit comparable populations[37,38], an unusually large effect of the aglycon on the sugar conformation prevails for the corresponding 5-α,β-D-arabinofuranosylpyrimidines[23].

The difference in populations between the two anomeric forms is very large,

*In the case of α-L-AraC, this is the population of DN, which corresponds to the N population of α-D-AraC (see previous footnote).

the β anomer exhibiting a 10% population of the N state, and the α anomer, ~75%. An even higher population of the type-N state, close to 100%, prevails for the α anomer of 2,4-diamino-5-D-arabinofuranosylpyrimidine[39].

Accuracy of the conformational analysis of the sugar moiety. — Notwithstanding the approximate character of the pseudorotational curve depicted in Fig. 3, its contour permits of formulation of several concrete proposals. The conformational states ${}^2E\cdots E_3$ are very closely located in the ${}^3J$ space. As, in solution, one of the conformational states lies within this particular range, it is not feasible, on the basis of vicinal coupling-constants alone, to assign this state with an accuracy better than ${}^4T_3\cdots{}^2E$. However, in the vicinity of the second of the states observed in solution (type N), the distances between neighboring E and T states are appreciable. Nonetheless, the experimental straight line does not precisely intersect the pseudorotational curve. The situation is improved by abstaining from the use of identical parametrizations of the Karplus relation for all three relevant bonds on the sugar ring. From Fig. 3, it is clear that the calculated value of $J_{3',4'}$ must be raised, relative to those for $J_{2',3'}$ and $J_{1',2'}$ (by ~20%). Likewise, for β-ribonucleosides, the differences in the parametrization of the Karplus relation between $J_{1',2'}$ and $J_{3',4'}$ provide the simplest confirmation for the differences observed in the values of the coupling constants for the N-type state, $J_{1',2'} = 0$ and $J_{3',4'} = 10.7$ Hz, and the type-S state, for which[25] $J_{1',2'} = 9.1$ and $J_{3',4'} = 0$ Hz. It should be emphasized that, because of the applied method of *independent* assignment of the values of the coupling constants for the states between which equilibrium prevails in solution, a change in parametrization does not affect the populations of the calculated, N and S states listed in Table II.

Calculations of the cisoidal coupling-constants for the pentofuranosyl moieties of nucleosides predict higher values[40] for $J_{3',4'}$ than for $J_{1',2'}$ and $J_{2',3'}$, due to the influence, on $J_{3',4'}$, of the exocyclic carbinol group. It is conceivable that this group similarly affects transoidal couplings.

Possible, intramolecular hydrogen-bonding. — Reference to this was made in connection with the results on the orientation of the exocyclic, 4'-CH_2OH group. The intramolecular hydrogen-bond 5'-OH···O-2'$^-$ readily arises in β-arabinonucleosides in strongly alkaline medium, where the sugar hydroxyl group(s), particularly OH-2', ionize[41]. Such hydrogen bonding is more limited in analogs of β-LyxC, depending on the possibility of ionization[34] of the "up" OH-3'. There is, consequently, no well defined criterion regarding the ability to form, or not to form, such bonds in solution. In the α-arabinonucleosides, the increase in the *gauche-gauche* population by 10–20% in alkaline medium, and the accompanying, minor modifications in the conformation of the sugar moiety, may reflect the appearance of states with, and without, such intramolecular hydrogen-bonding, shifted in favor of the latter. Apart from possible interference by the solvent itself, clarification of this question would require some knowledge of the acidity of OH-2', which, in β-arabinonucleosides, is the most acidic hydroxyl group[42].

Comparison with crystallographic data. — During the course of this investigation, a report appeared on the solid-state structure of α-D-AraA (**1**), which was

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18 T. KULIKOWSKI, Z. ZAWADZKI, D. SHUGAR, J. DESCAMPS, AND E. DE CLERCQ, *J. Med. Chem.*, 22 (1979) 647–653.
19 Z. KAZIMIERCZUK, L. DUDYCZ, R. STOLARSKI, AND D. SHUGAR, *Z. Naturforsch. Teil C*, 35 (1980) 30–35.
20 R. STOLARSKI, A. POHORILLE, L. DUDYCZ, AND D. SHUGAR, *Biochim. Biophys. Acta*, 610 (1980) 1–19.
21 *CERN 6600*, Computer Library, Sec. D 504 and 505, CERN, Geneva.
22 W. HUTZENLAUB, K. KOBAYASHI, AND W. PFLEIDERER, *Chem. Ber.*, 109 (1976) 3217–3227.
23 U. REICHMAN, C. K. CHU, I. WEMPEN, K. A. WATANABE, AND J. J. FOX, *J. Heterocycl. Chem.*, 13 (1976) 933–935.
24 G. BARNATHAN, T. HUYNH DINH, A. KOLB, AND J. IGOLEN, *Eur. J. Med. Chem. Chim. Ther.*, 11 (1976) 67–72.
25 D. B. DAVIES, *Prog. Nucl. Magn. Reson. Spectrosc.*, 12 (1978) 135–225.
26 F. E. EVANS AND R. H. SARMA, *J. Biol. Chem.*, 249 (1974) 4754–4759; I. EKIEL, J. ANTOSIEWICZ, A. JAWORSKI, AND D. SHUGAR, in press.
27 C. ALTONA AND M. SUNDARALINGAM, *J. Am. Chem. Soc.*, 95 (1973) 2333–2344.
28 Conformational Nomenclature, *Eur. J. Biochem.*, 111 (1980) 295–298.
29 D. B. DAVIES AND S. S. DANYLUK, *Can. J. Chem.*, 48 (1970) 3112–3117.
30 J. G. DALTON, A. L. GEORGE, F. E. HRUSKA, T. N. MCGAIG, K. K. OGILVIE, J. PEELING, AND D. J. WOOD, *Biochim. Biophys. Acta*, 478 (1977) 261–273.
31 A. POHORILLE, Ph. D. Thesis, University of Warsaw (1978).
32 W. GUSCHLBAUER, T. D. SON, M. BLANDIN, AND J. C. CATLIN, *Nucleic Acid Res.*, 1 (1974) 855–864.
33 I. EKIEL, E. DARŻYNKIEWICZ, L. DUDYCZ, AND D. SHUGAR, *Biochemistry*, 17 (1978) 1530–1536.
34 I. EKIEL, E. DARŻYNKIEWICZ, G. I. BIRNBAUM, AND D. SHUGAR, *J. Am. Chem. Soc.*, 101 (1979) 4724–4728.
35 C. T. BISHOP AND F. P. COOPER, *Can. J. Chem.*, 41 (1963) 2743–2758.
36 C. H. LEE, F. E. EVANS, AND R. H. SARMA, *J. Biol. Chem.*, 250 (1975) 1290–1296.
37 A. A. GREY, I. C. P. SMITH, AND F. E. HRUSKA, *J. Am. Chem. Soc.*, 93 (1971) 1765–1769.
38 C. K. CHU, I. WEMPEN, K. A. WATANABE, AND J. J. FOX, *J. Org. Chem.*, 41 (1976) 2793–2797.
39 C. K. CHU, U. REICHMAN, K. A. WATANABE, AND J. J. FOX, *J. Med. Chem.*, 21 (1978) 96–100.
40 A. JAWORSKI AND I. EKIEL, *Int. J. Quantum Chem.*, 16 (1979) 615–622.
41 M. REMIN, E. DARŻYNKIEWICZ, A. DWORAK, AND D. SHUGAR, *J. Am. Chem. Soc.*, 98 (1976) 367–376.
42 E. DARŻYNKIEWICZ, H. SIERAKOWSKI, AND D. SHUGAR, *Z. Naturforsch. Teil C*, 30 (1975) 565–570.
43 S. J. CLINE AND D. HODGSON, *Biochim. Biophys. Acta*, 563 (1979) 540–544.
44 M. SUNDARALINGAM, *J. Am. Chem. Soc.*, 93 (1971) 6644–6647.