Conformational analysis of oligoarabinonucleotides. An NMR and CD study*

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ABSTRACT.

A 500 and 300 MHz proton NMR study of the series of oligoarabinonucleotides 5'aAMP, 3'aAMP, aA-aA, $(aA-)_2aA$ and $(aA-)_3aA$ is presented. In addition, circular dichroism is used to study the stacking behaviour of aA-aA. The complete ^{1}H-NMR spectral assignment of the compounds (except the tetramer) is given. Proton-proton and proton-phosphorus coupling constants, obtained by computer simulation of the high-field region of the spectra, yield information on the conformation of the arabinose rings (N- or S-type) and on the intramolecular stacking properties of the dimer and the trimer. The monomers 5'aAMP and 3'aAMP exhibit a preference for N- and S-type sugar conformation, respectively. It is shown that the dimer aA-aA at low temperature prefers a mixed stacked state of the type aA(S)-aA(N). In the trimer the aA(2)-aA(3) fragment exhibits a conformation similar to that found in the dimer, whereas the aA(1) residue prefers to adopt S-type sugar and has some tendency to stack upon residue aA(2).

INTRODUCTION.

The solution conformation of arabinonucleosides has been a subject of study for many years, mainly because of the biological significance of these compounds as antimetabolites[1,2].

A few years ago it was thought[3] that the arabinose sugar ring in arabinonucleosides did not conform to the usual two-state N/S conformational model of five membered sugar rings[4,5], but an improved analysis of the coupling constants[6] demonstrated conclusively the two-state character of the arabinose conformational equilibrium. In agreement with this finding a recent survey[7] of crystallographic data shows that eight out of the nine crystal structures of arabinonucleosides studied exhibit either an N- or S-type conformation.

Recalculation[6] of the N/S molar ratio from published couplings[3] of a series of 2'-, 3'- or 5'methylated arabino-A nucleosides in neutral aqueous solution revealed a fairly con-

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sistent picture: compounds methylated at the 2'- or 5'-hydroxyls (or both) display slight preference for an N-type five membered ring, whereas methylation at the 3'-oxygen invariably causes a shift toward the S-type conformation, i.e the conformation in which both O-2' and O-3' assume pseudo-axial positions. One suspects a fine-tuned interplay between steric and stereo-electronic factors to be at work here. This suspicion is strengthened because a correlation also appears to exist between the N/S equilibrium position and the rotamer population around the C4'-C5' bond(γ)[3]: N-type conformation is accompanied by a γ^+ rotamer.

Substituents located at C-3' have different effects as compared to substituents at O-3' : $-ND_2$, -Cl, $-N_3$ and -Br appeared to induce N-type conformation, whereas a 3'-F substituted arabinose ring prefers S-type[8]. However, these effects should not be compared directly to the effect of $3'-OCH_3$ and $3'-OPO_2^{2-}$, since the substituents studied by Klimke et al.[8] are attached to the C-3' atom, whereas the methyl- and phosphate-group mentioned above are both connected to the O-3' atom.

Few experimental data on arabinonucleotides are currently available. The crystalstructure of 5'aCMP[9] was shown to exhibit the N-type sugar conformation. In a theoretical study[10] it was predicted that the presence of a phosphate group at the 5'-position induces preference for N-type sugar.

Base stacking in arabinose containing dinucleotides was studied by Brahms et al.[11] and by Maurizot et al[12]. Both studies led to the conclusion that the presence of an arabinose unit as 5'-linked residue (-paX) does not impede stacking, whereas such a unit present as 3'-linked residue (aXp-) in a dinucleoside monophosphate has ruinous effects on stacking properties. In the series: ApaC, UpaC, aCpA, aCpU and aCpaC, the first two compounds were found to be good "stackers", whereas the other ones showed very little stacking tendency[11,12].

In recent studies, carried out by the Leiden group, attention was paid to the influence of the nature of the furanose residue (ribose[13], 2'-deoxyribose[13], 3'-deoxyribose, xylose (Doornbos, J. & Altona, C., unpublished)) and the type of phosphate linkage (3'-5' or 2'-5')[14] on the conformation of the sugar ring and the base stacking behaviour of oligo-adenine

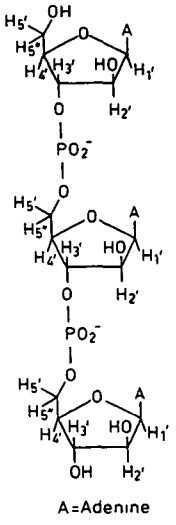

Figure 1. Structure of aA-aA-aA.

nucleotides. A common property of these oligomers is the presence of mixed stacked states, i.e. stacks containing a combination of N-type and S-type sugar puckers side-by-side, e.g. S-N or N-S.

The present paper is concerned with the conformation of 5'aAMP, 3'aAMP, aA-aA, $(aA-)_2aA$ (Fig. 1) and $(aA-)_3aA$, studied by means of ^{1}H-NMR spectroscopy and circular dichroism.

MATERIALS AND METHODS.

The compounds aA-aA, $(aA-)_2aA$ and $(aA-)_3aA$ were synthesized by J.L.B., H.L. & J.L.I. (unpublished results) and generously placed at the disposal of the Leiden group. The monomers aA and 5'aAMP were purchased from Serva Feinbiochemica (Heidelberg, G.F.R.). The synthesis of 3'aAMP was accomplished (J.v.W., G.V. & J.H.v.B.) by using a recently developed phosphorylation technique[15].

In the preparation of the NMR samples the compounds were treated with DOWEX cation-exchange resin (Na^+-form) to yield the sodium salts, lyophilized three times from 99.75% ^2H_2O and finally dissolved in 99.95% ^2H_2O. The p^{2}H of the solutions of the dimer, trimer and tetramer was adjusted to 7.4 (meter reading); the p^{2}H of the solutions of 5'aAMP and 3'aAMP was adjusted to 5.2 (meter reading), at which p^{2}H the phosphate

group occurs in the monoionized state. Samples were made up to a concentration of 20 mM dimer, 13 mM trimer and 6 mM tetramer. The monomers 5'aAMP and 3'aAMP were studied at a concentration of 40 mM and 80 mM, respectively. A trace of tetramethylammonium chloride (Me_4NCl) was added to the samples as an internal chemical shift reference; chemical shifts are given relative to the methyl peak of Me_4NCl. FT-NMR spectra were recorded on a Bruker WM-500 spectrometer (spectral width 4000 Hz, 8 K datapoints) and on a Bruker WM-300 spectrometer (spectral width 2500 Hz, 8 K datapoints); both spectrometers were connected to an ASPECT-2000 computer. The FID's were multiplied by an appropriate Gaussian function[16] to enhance the resolution, zero-filled to 32 K and Fourier-transformed.

CD spectra were taken in a 1-cm pathlength quartz-cell on a CNRS-Roussel-Jouan III dichrographe (Jobin-Yvon, France) at 1, 11, 43 and 69°C. Correction for baseline imperfections was made by subtracting the average of three baselines from each spectrum. Further technical details are given elsewhere[17]. The dimer aA-aA(Na^+) was dissolved in "Millipore"-filtered water, pH 7. An ultraviolet absorption spectrum was taken on a Cary-14 spectrophotometer at 20°C, O.D. = 0.71 at 259 nm. Circular dichroism is given in molar units per base. In the calculation of the CD an estimated hypochromicity value of 10% was used, which is somewhat smaller than found for well-stacking adenine dinucleotides, e.g. ApA and $m_2^6Apm_2^6A$[13].

RESULTS AND DISCUSSION.

Conformational notation.

In this paper the α-ζ notation[18] for backbone torsion angles is used (Scheme 1). The usual Klyne-Prelog convention[19] of torsion angle notation is adhered to.

$$P \overset{\alpha}{-} O5' \overset{\beta}{-} C5' \overset{\gamma}{-} C4' \overset{\delta}{-} C3' \overset{\varepsilon}{-} O3' \overset{\zeta}{-} P$$

Scheme 1

The nucleotide units, protons and torsion angles are numbered from the 5'- to the 3'-end, e.g. HO-5'aA(1)-aA(2)3'-OH. The H-5' and H-5" resonances are assigned according to Remin and Shugar[20], i.e. H5" is taken to resonate upfield from H5'

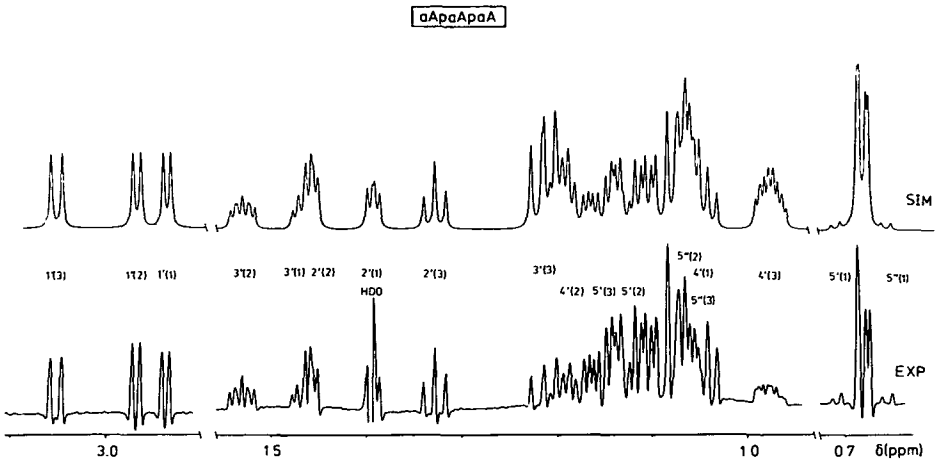

Figure 2. NMR spectrum of aA-aA-aA (13 mM in $^{2}H_2O$, 25°C, p^{2}H = 7.4). EXP = experimental spectrum, SIM = computer simulation.

Spectra.

The arabinosyl proton signals in the NMR spectra of the monomers, dimer and trimer were assigned by extensive decoupling experiments. The groups of protons belonging to the respective residues in the dimer and trimer could be distinguished by the presence or absence of ^{1}H-^{31}P coupling in the H-3', H-5' and H-5" signals. Most resonances in the spectrum of the tetramer $(aA-)_3aA$ eluded definite assignment because of considerable crowding of peaks in the 4',5',5" region. Exceptions are the H-1'(4), H-2'(4) and H-3'(4) signals, which could be unambiguously assigned by decoupling experiments. By selective irradiation of the four H-1' signals four separate H-2' signals were located and by irradiation of these H-2' resonances the four H-3' signals were found. Three of these H-3' signals appear as multiplets, due to spin coupling with a phosphorus atom, whereas the H-3'(4) resonance shows up as a triplet and can therefore be assigned unambiguously.

Signals of the H-2 and H-8 protons were distinguished by their different H-D exchange behaviour, i.e. after measurements at high temperature the intensity of H-8 signals is decreased relative to the intensity of H-2 signals, due to ^{1}H-^{2}H exchange

Table 1. Chemical shifts in ppm relative to Me_4NCl[a].

arabinose protons	5'aAMP at 19°C	3'aAMP at 23°C	aA-aA at 25°C	aA-aA-aA at 25°C
	δ	δ	δ A(1)	δ A(1)
1'	3.208	3.206[b]	2.929	2.882
2'	1.408	1.444[b]	1.404	1.384
3'	1.237[b]	1.463	1.430	1.447[b]
4'	1.000[b]	1.080	1.058	1.076[b]
5'	1.053	0.790	0.673	0.688
5"	0.980	0.749	0.657	0.676
			δ A(2)	δ A(2)
1'			3.059	2.891
2'			1.359	1.452
3'			1.227	1.514
4'			0.961	1.182
5'			1.150	1.132
5"			1.042	1.074
				δ A(3)
1'				2.984
2'				1.307
3'				1.199
4'				0.955
5'				1.172
5"				1.042
base protons				
H-2	4.978	4.878	4.821	4.719
H-2			4.780	4.632
H-2				4.618
H-8	5.250	5.176	5.082	5.014
H-8			4.993	5.000
H-8				4.933

a) δ(Me_4NCl) is 3.18 ppm relative to δ(DSS).

b) Could not be determined exactly because of overlapping peaks.

with the solvent. No attempt was made to assign these base--proton signals to specific residues.

NMR spectra of the monomers, the dimer, and the trimer were computer simulated using programme LAME. Fig. 2 shows part of the spectrum of aA-aA-aA and its computer simulation. Chemical shift and coupling constant data, obtained via the computer simulations, are given in Tables 1 and 2, respectively. CD spectra of aA-aA, taken at various temperatures, are shown in Fig. 3.

Table 2. Coupling constants (Hz) for aA residues at various temperatures.

Fragment	Coupling constant	monomers		dimer			trimer		tetramer
		3'aAMP							
		2°C	53°C	0°C	25°C	65°C	1°C	45°C	27°C aA(1)
aAp-	1'2'	4.4	4.3	3.4	3.6	4.1	3.4	3.9	3.5
	2'3'	3.6	a	1.4[a]	2.1	2.8	1.4	2.6	
	3'4'	4.3	a	2.4[a]	2.9	3.7	2.3	2.2	
	4'5'	3.3	3.5	3.5	3.8	3.8	3.6	4.3	
	4'5"	5.9	5.2	6.5	5.8	5.7	6.1	5.5	
	5'5"	-12.5	-12.6	-12.5	-12.5	-12.6	-12.5	-13.0	
	3'P	7.8	a	5.7[a]	6.2	6.5	6.8	6.9	
									aA(2) aA(3)
-paAp-	1'2'						3.8	4.0	3.5 3.5
	2'3'						2.1	2.6	
	3'4'						3.6	3.5	
	4'5'						3.0[a]	3.1	
	4'5"						5.0[a]	6.1	
	5'5"						-11.7	-11.7	
	5'P							5.5	
	5"P							5.9	
	3'P						5.7	6.8	
	4'P							0.7	
		5'aAMP							
		2°C	47°C						aA(4)
-pA	1'2'	6.0	5.6	6.1	6.1	5.9	6.0	5.8	5.6
	2'3'	6.5	5.9	6.4	6.3	5.9	6.4	6.0	6.0
	3'4'	6.5	6.1	7.7	7.5	7.0	7.4	7.0	7.0
	4'5'	4.5		2.0	2.2	2.8	1.9[a]	2.7	
	4'5"	3.8[a]		4.4	4.7	5.6	3.6	4.7	
	5'5"	-12.8		-12.0	-12.0	-12.1	-11.7	-11.9	
	5'P	4.5		4.5	5.0	5.2	4.5[a]	5.0	
	5"P	5.5		4.2	5.0[a]	5.7	3.8	4.9	
	4'P	a		1.8[a]	1.9[a]	1.0	2.2	2.0	

a) Overlapping signals precluded exact determination.

Conformation of the arabinose ring.

The pseudorotation parameters[4,5] describing the conformation of the arabinose ring (ϕ_m, P) and the mol fraction N-conformer (X_N) are calculated from the coupling constants $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ by use of the iterative computer program PSEUROT[21]. The calculation is based upon a recently developed Karplus-type relation[22]. This relation describes the dependency of the three bond H-C-C-H coupling constant on (i) the proton--proton torsion angle (ϕ_{HH}) and (ii) the electronegativity and

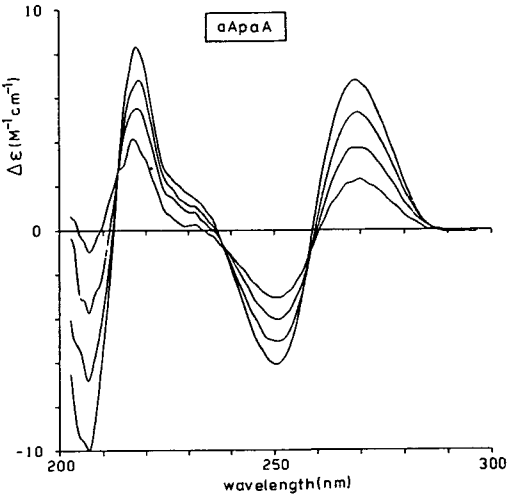

Figure 3. CD spectra of aA-aA, recorded at four (1, 22, 43, and 69°C) experimental temperatures. The spectrum with the largest amplitude was taken at the lowest temperature.

(iii) orientation of the substituents (R_1-R_4) in the HR_1R_2C--CR_3R_4H fragment in question. The correlations between ϕ_{HH} and the parameters Φ_m and P (Eqn. 1) were taken from ref. 6. The observed coupling constants are interpreted as a weighted average of the couplings in the pure N- and S-conformer (Eqn. 2).

$$\phi_{1'2'} = 3.3^{\circ} + 1.102\ \Phi_m \cos(P-144) \qquad (1a)$$

$$\phi_{2'3'} = 120.2^{\circ} + 1.109\ \Phi_m \cos P \qquad (1b)$$

$$\phi_{3'4'} = -124.9^{\circ} + 1.095\ \Phi_m \cos(P+144) \qquad (1c)$$

where ϕ_{ij} represent the H-C-C-H torsion angles and P and Φ_m have their usual meaning[4,5].

$$J_{obs} = X_N J_N + (1-X_N) J_S \qquad (2)$$

where X_N is the mol fraction N-type conformer.

The PSEUROT program was used in the following way. Sets of coupling constants of the monomers and the trimer measured at two different temperatures and the dimer at three different temperatures were treated together in the PSEUROT calculation. The -paA fragments were treated separately from the aAp- and -paAp- fragments. Since -paA fragments exhibit preference for N-conformer, the parameters belonging to the minor form, P_S

Table 3. N-type conformer population in aA residues calculated from the vicinal couplings with the aid of program PSEUROT[a]. (Temperature in °C)

	araA	5'aAMP		3'aAMP		aA-aA				$(aA-)_2aA$			$(aA-)_3aA$	
t	23	2	47	2	53		2	25	65		0	45		25
%N	64[b]	72	66	33	35	aA(1)	10	17	28	aA(1)	9	23	aA(1)	13
						aA(2)	79	77	71	aA(2)	22	25	aA(2)	13
										aA(3)	77	71	aA(3)	13
													aA(4)	70

a) $P_N = 16^\circ$, $P_S = 158^\circ$, $\Phi_N = 41^\circ$, $\Phi_S = 31^\circ$.

b) Coupling constants taken from ref. 23.

and Φ_S, were first constrained to assume values characteristic of aA nucleosides, 158.8° and 30.4°, respectively[6]. The resulting calculated parameters for the major N-conformer were in turn used as constrained values in the pseudorotation analysis of the aAp- and -paAp- residues which exhibit an outspoken bias toward the S-conformation. The parameters for the S-type now obtained were again applied to the analysis of the aAp- fragments as fixed parameters. After three of these cycles the pseudorotation parameters did not show further changes. The results are compiled in Table 3. A good agreement between observed and calculated coupling constants was found (r.m.s. = 0.15 Hz).

It is seen from Table 3 that the N/S distributions are only slightly temperature dependent. The calculated values of P and Φ_m occur within the ranges established from a large number of crystal structures of nucleic acid constituents[7]. An interesting feature of the series of arabinocompounds is the strong bias toward S-type conformation of the aAp- and -paAp- residue (65-91% S, at low temperature) and the preference for N-type in -paA residues (66-79% N, at low temperature). It appears that a phosphate group located at the 3'-position induces a preference for S-type conformation in the sugar ring, whereas a phosphate group at the 5'-position does not significantly affect the N/S equilibrium, as is seen from comparison with the parent compound aA[23] (Table 3). The peculiar effect of a phosphate group located at O-3' is also exerted by a methyl group

at this position (vide supra).

The calculated Φ_N and Φ_S values (Table 3) deserve some comment. The value deduced for Φ_N (~ 40°) appears consistently greater than Φ_S (~ 30°). Similar results were previously obtained by de Leeuw and Altona[6]. However, in the previous analysis equal values of Φ_N and Φ_S (36°) were selected, since both combinations of Φ values resulted in equal r.m.s. values (0.2 Hz) and there was no a priori reason to believe that the ring puckering coordinate depended on the conformation (N or S) adopted[7]. In the present analysis similar observations were made. In the mol fraction range $0.3 < X_N < 0.7$, wherein most of the data used in ref. 6 occur, both combinations of Φ_N and Φ_S give similar r.m.s. values. However, in the extreme cases, listed in the present data set (Table 3), the combination $\Phi_N = 41°$ and $\Phi_S = 31°$ yields a smaller r.m.s. value (0.15 Hz) as compared to the combination $\Phi_N = \Phi_S = 36°$ (0.30 Hz). On the basis of these r.m.s. values, one cannot exclude the possibility that the S-type arabinose ring in aqueous solution is somewhat flattened ($\Phi = 31°$) in comparison with the N-type ring ($\Phi = 41°$). However, this selective flattening is not observed in the solid state[7].

The dependency of the N/S equilibria upon base stacking will be discussed in a following section.

Conformation around C3'-C4'-C5'-O5'(γ) and C4'-C5'-O5'-P(β).

The relative populations of the gauche$^+$, trans and gauche$^-$ conformers along the C4'-C5' bond were calculated[24] from the observed coupling constants $J_{4'5'}$ and $J_{4'5''}$. The results are shown in Table 4. The calculated relative populations of the γ^t and γ^- conformers depend on the stereochemical assignment of H-5' and H-5", a reversed assignment roughly reverses the amount of γ^t and γ^- conformers. It is seen from Table 4 that, with the chosen assignment, the γ^t rotamer is generally preferred rather than the γ^- rotamer. This finding accords with energy considerations, since in the γ^t situation an energetically favourable gauche relationship exists between the O-5' and O-1' atoms. For this reason, the usual assignment[20] of the H-5' and H-5" signals was made. The small $J_{4'5'}$ and $J_{4'5''}$ values observed in residues A(2) of the dimer and A(3) of the trimer at low temperature, indicate[25] that the magnitude of the γ^+

Table 4. *Rotamer distribution around γ and β (temperature in °C).*

rotamer		γ^+		γ^t		γ^-		β^t	
3'aAMP	t	2	53	2	53	2	53		
	%	43	49	47	39	9	12		
5'aAMP	t	2		2		2		2	
	%	55		20		25		74	
dimer	t	2	65	2	65	2	65	2	65
aA(1)	%	36	42	50	40	14	18		
aA(2)	%	72	43	28	39	0	19	80	69
trimer	t	12	45	12	45	12	45	12	45
aA(1)	%	39	39	45	36	16	25		
aA(2)	%	55	43	41	46	4	11	68	66
aA(3)	%	77	63	23	31	0	6	80	74

angle is about 46° instead of 52° as assumed in earlier work on ribose molecules[24]. Therefore, in the present calculations on aA derivatives a γ^+ torsion angle of 46° was used. Table 4 exhibits the following details. At low temperature γ^+ is preferred in the 3'-OH terminal residues of the dimer and trimer (~ 75%) and also, to a smaller extent, in 5'aAMP (55%). In the other residues γ^+ and γ^t are practically equally populated (40-50%), whereas the γ^- rotamers occurs only to a minor amount (< 25%). At elevated temperature the preference for γ^+ vanishes. The observed trend is in agreement with the results of Yathindra and Sundaralingam[10] who predicted, from theoretical calculations, that a γ^+ conformer is more preferred in an N-type than in an S-type sugar ring (cf. Table 3).

The implications of the behaviour of γ for the understanding of the stacking properties of aA oligomers will be treated in the corresponding section, vide infra.

The amount of trans conformer around β is calculated from a newly revised sum rule[25] (Eqn. 3):

$$\%\beta^t = \frac{23.9-\Sigma'}{18.9} \times 100 \qquad (3)$$

where $\Sigma' = J_{5'P} + J_{5''P}$.

The β^t rotamer is much preferred in the aA(3) residue of the trimer and the aA(2) residue of the dimer at low temperature (80%, Table 4) and slightly less so in the aA(2) residue of the trimer (68%) and in 5'aAMP (74%).

Conformation around C3'-O3'(ε).

The 3'-phosphate group in an S-type arabinosyl unit has sterical surroundings similar to that of the 3'-phosphate group in a deoxyribosyl residue. In both compounds the position at C-2', adjacent to the phosphate, is occupied by a hydrogen atom, whereas in a ribosyl residue an oxygen atom is present at this position. Moreover, the remarkably high S-content of arabino 3'phosphates in question is similarly encountered in oligodeoxynucleotides. The $J_{3'P}$ values - which monitor the conformational behaviour of ε - observed in the arabinose dimer and trimer, display values between 5.7 and 6.9 Hz (Table 1), which is well within the range of coupling constants observed in deoxyoligomers[26]. The temperature dependency of the $J_{3'P}$ coupling constants in residues aA(1) of the dimer and aA(2) of the trimer is also comparable to the case of oligodeoxyribonucleotides[26], i.e. $J_{3'P}$ values tend to decrease when the temperature is lowered. Therefore, it appears reasonable to assume the presence of a large amount of trans conformer around ε in the residues in question at low temperature, as was deduced for dA-dA[26]. The torsion angle ε will presumably adopt a value close to 188°, as was calculated in a series of deoxyribonucleotides[25], rather than a value close to 208° as calculated for a series of ribonucleotides[25].

CD spectra.

The CD of aA-aA (Fig. 3) exhibits the characteristic exciton type spectrum found in homonucleotide dimers. As was shown before, the phenomenon of base stacking in a dimer is monitored by the intensity change of CD effect. It is not the absolute value of the circular dichroism but its dependency on the temperature that gives information about the stacking behaviour of the molecule studied. In the spectra of aA-aA the intensity of the long-wavelength peak at 70°C has dropped to about a quarter of the value observed at 0°C; this is similar to the decrease observed in the spectra of ApA and $m_2^6Apm_2^6A$[13] and means that a

considerable change of base stacking occurs in this temperature range. A closer scrutiny of the CD spectra reveals two important features: (a) the existence of well-defined isodichroic points; (b) the λ_{max} and λ_{min} values are independent of temperature. These features are indicative for two-state stack/unstack equilibrium behaviour[27] (Altona, C. & Gijsman, P., unpublished).

Stacking properties of the oligomers.

Stacking in oligonucleotides is reflected by several variables, e.g. temperature-dependent NMR chemical shifts, coupling constants, and circular dichroism. Information extracted from chemical shift changes should be considered with some care, since intermolecular association effects become non-negligible at nucleotide concentrations used in NMR experiments[28]. In the present study, mainly coupling constants and circular dichroism data are utilized to obtain information about conformational details and base stacking.

The dimer aA-aA shows a strong preference for S-type ring pucker in the aA(1) fragment and for N-type in the aA(2) fragment at low temperature. Upon increase of temperature the tendencies towards N- or S-type become less obvious but remain present. The aA(1) and aA(2) units of the dimer have similar characteristics as are displayed by the monomers 3'aAMP and 5'aAMP, i.e. preferences for S-type and N-type sugar conformation are indicated, respectively. The respective preferences occur more outspoken in the dimer at low temperature than in the monomers. Temperature changes induce only a relatively small shift in the N/S equilibria of the residues aA(1) and aA(2). However, the rotamer population around $\gamma(2)$ and the intensity of the CD spectrum are strongly affected as the temperature is changed. The amount of $\gamma^+(2)$ conformer increases from 43% at 65°C to 72% at 0°C (Table 4), and the intensity of the CD increases by about four times in this temperature range. These two observations yield strong indications of the presence of stacked states in aA-aA, since a right-handed stack requires a γ^+ torsion angle and gives rise to a CD spectrum of the exciton type, as observed (Fig. 3). The arabinodinucleotide appears to be a "good stacker" and an aA(S)-aA(N) type stack is indicated. The favoured combination of a γ^+ torsion angle

with an N-type arabinose ring was observed before[3,8]. In the S-type arabinose ring the γ^+ rotamer appears disfavoured because of steric repulsion between the O-2'(up) atom and the phosphate group. Modelbuilding shows that this repulsion is absent in the N-conformation. From Tables 3 and 4 it is seen that the preferred combination γ^+-N is also reflected in the monomer data. The exact 1:1 correlation between the population of the γ^+ torsion angle and N-type conformation proposed by Klimke et al.[8] in arabinonucleosides does not appear to be valid for the arabinonucleotides presently studied. For example, in residue aA(2) of the dimer the amount of N-type sugar decreases only from approximately 80% to 70% when the temperature is raised from 2°C to 65°C, whereas the amount of γ^+(2) rotamer falls from about 70% to 40%. Therefore, it is concluded that the decrease of the γ^+(2) population upon increase of temperature is a consequence of destacking, whereas in the aA(2) residue N-type conformation remains favoured according to the inherent preference of arabinofuranose rings unsubstituted at the 3'-position. Summarizing, the preferred geometry of the low-temperature (stacked) state of the dimer aA-aA can be described by S-ε^t-β^t--γ^+-N. In our earlier study of A2'-5'A a method was presented whereby a crude estimate of the stacking population can be deduced from the backbone and sugar population[14]. Reasoning along similar lines we now obtain roughly 50% of stacked dimer aA-aA at 2°C. This conclusion is supported by the observation of differential shieldings $\Delta\delta$, i.e. the upfield shifts displayed by the dimer proton signals in comparison with the signals of the corresponding monomers, measured at 25°C. For H-1'(1) and H-1'(2) one finds $\Delta\delta$ values of 0.28 and 0.15 ppm respectively. Differential shieldings are also observed in the case of the base protons. As the H-2 and H-8 protons of the dimer have as yet not been assigned to particular residues, no definite $\Delta\delta$'s can be given. However, depending upon the assignment, $\Delta\delta$ of H-2 protons amounts to 0.06 - 0.2 ppm or 0.16 - 0.10 ppm; for H-8 protons these values are 0.15 - 0.26 ppm or 0.17 - 0.18 ppm.

The trimer aA-aA-aA shows preference for S-type conformer in units aA(1) and aA(2), 3'-terminal unit aA(3) again prefers the N-conformer. It is clear that the presence of a 3'-phosphate

group causes a strong shift of the N/S equilibrium towards the S-type, and that this tendency completely controls the conformation of the 5'terminus and of all internal residues in a poly(aA) chain. In all respects the 3'-terminal residues aA(2)--aA(3) in the trimer and, as far as can be seen, also aA(3)--aA(4) in the tetramer, behave in virtually the same way as does the dimer. From the data collected in Tables 2, 3 and 4 it can be inferred that the preferred geometry of the stack again can be described by the sequence S-ε^{t}-β^{t}-γ^{+}-N; its population can be estimated as roughly 50%.

The presence of significant amounts of one (or more) stacked state(s) at the 5'terminal aA(1)-aA(2) of the trimer can be deduced from the chemical shift behaviour of base and H-1' protons of residue aA(1), Table 1. Although the base protons have not been assigned, a comparison of the H-2 and H-8 shifts seen in the trimer with those displayed by 3'aAMP clearly shows differential shieldings $\Delta\delta$ of the order of 0.16 - 0.26 ppm for the H-2 resonances and 0.16 - 0.24 ppm for the H-8 resonances. Comparison with the shifts of 5'aAMP would make $\Delta\delta$ even larger. H-1'(1) is shielded by 0.32 ppm with respect to the corresponding monomer. Now the question as to the preferred geometry or geometries of the aA(1)-aA(2) fragment in the trimer arises. Table 3 reveals a surprisingly high content of S-type arabino sugar conformation (91%) in residue aA(1) at low temperature. This S-population content is comparable to that found in aA(1) in the dimer (90%) and appears significantly higher than that occurring in 3'-aAMP (67%). Therefore, it can be deduced that residue aA(1) partakes in one or more stacking arrangements that are characterized by aA(1) adopting S-type sugar conformation.

The next problem concerns the behaviour of the central aA(2) residue (22% N-type sugar at low temperature). The following possibilities can be envisaged (Fig. 4): type I, a continuous helical S-S-N state; type II, a aA(1)-aA(2), S-S stack; type III, a aA(1)-aA(2) S-N stack; type IV, a aA(2)-aA(3) S-N stack; and V, a completely unstacked assembly of forms. At this stage it appears impossible to give a quantitative estimate of the relative importance of the various forms I-V. However, it

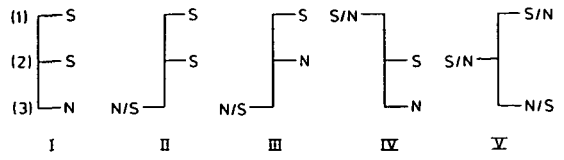

Figure 4. Schematic representation of stack types in aA-aA-aA (see text).

is suggestive to note that an approximately equal population of all five forms results in a N/S distribution (aA(1), 12% N; aA(2), 27% N; aA(3), 82% N) that is close to the observed distribution (9%, 22% and 77%, respectively). This leads to a total of 60% stack along aA(1)-aA(2) and 40% stack along aA(2)--aA(3), and this seems not unreasonable. The only difficulty connected to such a distribution of stacks lies in the low population of γ^{+}(2) and one would have to assume the possibility of a stack type that features γ^{t} instead of γ^{+}.

In the case of the arabinotetranucleotide only the $J_{1'2'}$ coupling constants could be obtained. The percentage N-type was calculated from these values in a pseudorotation analysis with ϕ_N, ϕ_S, P_N and P_S constrained at the values obtained in the analysis of the monomers, the dimer and the trimer. Again, it is evident that in aAp- and -paAp- fragments the S-type sugar pucker is preferred (87% S), whereas in the -paA unit the N-type is favoured (70% N, cf. Table 3). It is proposed that the increase in S-content in residues aA(1), aA(2) and aA(3) of the tetramer (87% S), as compared to 3'aAMP (67% S), is a consequence of considerable S-type stacking. Tentative extrapolation suggests that poly(aA) will adopt a regularly stacked helix containing S-type sugar residues.

The early conclusion[11,12], that base stacking is very weak in dimers containing a 3'-linked arabinose unit, is obviously not supported by the present work. This non-stacking behaviour exhibited by aCpA, aCpU and aCpaC[11,12] may be caused by the specific base sequence in these compounds, which have the aCp-unit as common feature. In ribonucleotides, dimers with the pyrimidine-purine sequence appear to stack to a lesser extent than do dimers with purine-purine or purine-pyrimidine sequences[25]. In the present report a considerable amount of base

stacking in an arabinodinucleoside monophosphate of the purine--purine type is deduced; this observation is in agreement with the results obtained in the studies of ribose and deoxyribose dimers having the purine-purine sequence[13,17,25]. Analogously, it is not surprising that pyrimidine-purine sequences, containing arabinosyl units, appeared to show little stacking tendency[11,12].

After completion of this work our attention was directed to a recent paper by Gioeli et al.[29] in which the synthesis and the CD spectra of $(aA-)_2aA$ at various temperatures are described. The qualitative conclusion[29] that the araA trimer is capable of existing in the stacked state is confirmed and extended by our NMR investigations.

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ABBREVIATIONS.

NMR	: Nuclear Magnetic Resonance
CD	: Circular Dichroism
ppm	: parts per million
arabinosyl	: arabino-furanosyl
aA, araA	: 9-β-D-arabinofuranosyladenine
5'aAMP	: 9-β-D-arabinosyladenine 5'-monophosphate
3'aAMP	: 9-β-D-arabinosyladenine 3'-monophosphate
aA-aA	: 9-β-D-arabinosyladenylyl(3'-5')9-β-D-arabinosyladenine
dA	: deoxyadenosine
rA	: adenosine
m_2^6A	: N^6-dimethyl-adenosine
DSS	: 2,2-dimethyl-2-silapentane-5-sulfonate(Na^+)

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