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Conformational analysis of the trinucleoside diphosphate 3'd(A2'-5'A2'-5'A). An NMR and CD study*

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ABSTRACT.

A 500 MHz and 300 MHz NMR study of the trinucleoside diphosphate 3'd(A2'-5'A2'-5'A) is presented. In addition, circular dichroism is used to study base stacking in the title compound. The complete 1H-NMR spectral assignment of the sugar ring proton signals is given. Information about the sugar ring (N- or S-type conformation) and about the backbone geometry along C4'-C5' and C5'-O5' bonds is obtained from the NMR coupling constants. It is shown that the trimer mainly occurs in the N-N-N stacked state at low temperatures; the presence of a minor amount of N-N-S conformational sequence is indicated.

INTRODUCTION.

The activity of 2'-5' linked oligo-adenylates in the inhibition of protein synthesis has been subject of recent studies[1-4]. It was observed that pppA2'-5'A2'-5'A (2-5A) was efficiently broken down by phosphodiesterases[5]. The analogon 3'd(A2'-5'A2'-5'A) was designed in the expectation that this compound would have similar inhibitive characteristics as the parent ribose compound, yet might be more stable from a metabolic point of view[3]. Biochemical experiments revealed that the title compound was indeed much more resistant to enzymatic digestion than the ribose analogue and that its ability to inhibit cell growth was not deteriorated[3,4].

The conformational characteristics of the mononucleoside 3'deoxy-adenosine (cordycepin, 3'dA) in various solvents have been studied by means of NMR[6]; it appeared that cordycepin strongly favours the N-type sugar conformation (C-3' endo). In the crystalline phase the sugar ring in 3'dA also exhibits the N-conformation[7].

One of our previous papers[8] dealt with the conformation of A2'-5'A2'-5'A; this compound shows base stacking interactions,

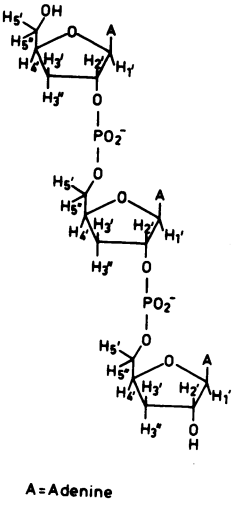

Figure 1.
Structure of 3'd(A2'-5'A2-5'A)

nevertheless, no definite preference for N- or S-type sugar was observed. The xylose analogue of the title compound, xA2'-5'xA2'--5'xA, has also been subjected to a conformational study (Doornbos, J. & Altona, C., to be published); the sugar residues in this xylose trimer appear to adopt either the N- or S-type sugar conformation, dependent on the position of the xylose unit in the trimer sequence.

In the present paper we wish to report on the conformational properties and the intramolecular base stacking tendency of 3'd-(A2'-5'A2'-5'A) (Fig. 1).

MATERIALS AND METHODS.

The title compound was synthesized by R.C. and W.P. via the phosphotriester method[9] and generously placed at the disposition of the Leiden group. The sample was treated with Dowex cation--exchange resin (Na^+ form). In the preparation of the NMR sample the compound was lyophilized three times from 99.75% ^2H_2O, after adjustment of the p^2H to 7.4, and finally dissolved in 99.95% ^2H_2O. The concentration of the sample was made up to 25 mM. NMR spectra were taken on Bruker WM-500 and WM-300 spectrometers (at 500 MHz and 300 MHz, respectively), interfaced to ASPECT--2000 computers. The WEFT pulse sequence was used in order to suppress the residual solvent peak; FIDs (8 K datapoints) were

averaged (using a spectral width of 4000 and 3000 Hz, respectively), multiplied by an appropriate Gaussian function to enhance the spectral resolution, zero-filled to 32 K datapoints and Fourier-transformed. Tetramethylammonium chloride (Me_4NCl) was used as an internal chemical shift reference ($\delta(Me_4NCl)-\delta(DSS)$ = 3.18 ppm). CD spectra were recorded at four different temperatures (1, 25, 47 and 70°C) on a CNRS Roussel-Jouan III dichrographe (Jobin-Yvon, France). Further experimental details are given by Olsthoorn et al.[10]. A quartz cell (1 cm pathlength) was used; the sample was dissolved in 'Millipore'-filtered water (pH = 7.2) to an absorbancy of 0.86 at 259 nm. The CD is given in molar units per residue. A molar absorption coefficient ε_{259}= 11,100 per residue was used[9] in the calculation of the CD.

RESULTS AND DISCUSSION.

Conformational Notation.

Throughout this paper we adhere to the recently proposed nomenclature rules[11]. A minor adaptation to the case of 2'-5' linked nucleotides is shown in Scheme 1.

$$P \overset{\alpha}{-} O5' \overset{\beta}{-} C5' \overset{\gamma}{-} C4' \overset{\delta}{-} C3' \overset{\delta'}{-} C2' \overset{\varepsilon}{-} O2' \overset{\zeta}{-} P$$

Scheme 1

It should be noted that the definition of torsion angle δ in Scheme 1 differs from the usual definition of δ in 3'-5' linked nucleotides. The nucleotide residues, the torsion angles and the H atoms will be indexed between brackets, e.g. 3'd(A(1)2'-5'A(2)-2'-5'A(3)).

Assignment of the NMR spectrum.

Assignment of the sugar protons of 3'd(A2'-5'A2'-5'A) was performed by extensive decoupling experiments at 26°C. The H-1' signals are readily assigned as a group since these are located at relatively low field as compared to the remaining sugar-proton signals. Upon irradiation of a given H-1' resonance a change in the corresponding H-2' is observed. Irradiation at the frequency of the H-2' signal leads to the detection of the H-3' and H-3" signals, and so on. The residues (1) and (3) are discriminated by the absence of ^1H-^{31}P splitting in the H-5'(1), H-5"(1) and H-2'(3) multiplets, respectively. The H-3' and H-3" signals are

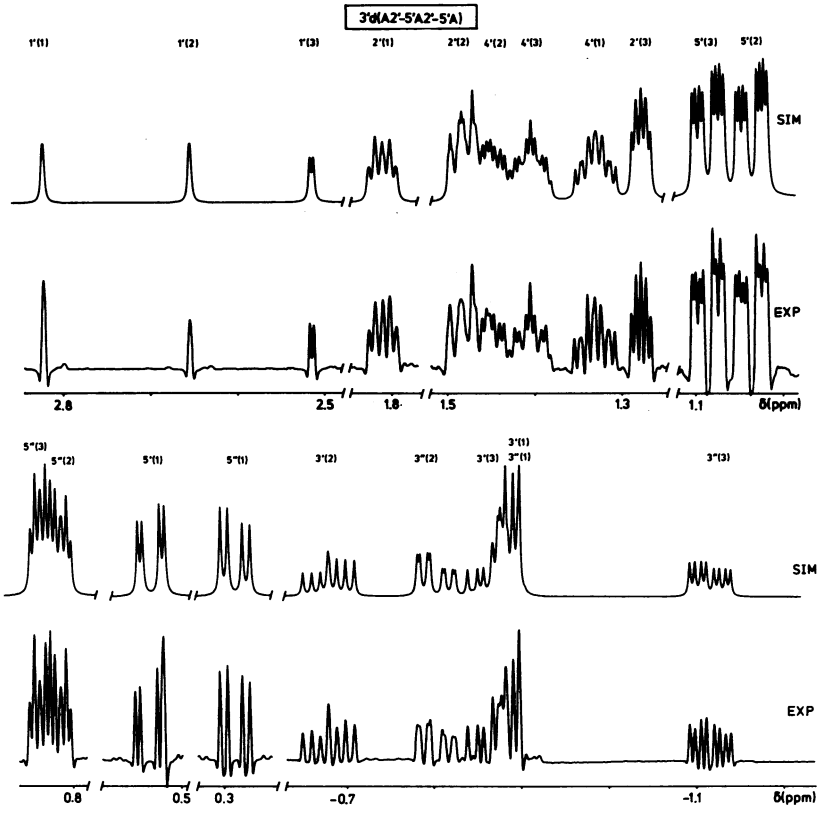

Figure 2. ^{1}H-NMR spectrum of 3'd(A2'-5'A2'-5'A) in $^{2}H_2O$ (500 MHz, 26°C, 25 mM, p^{2}H = 7.4). EXP = experimental spectrum, SIM = computer simulation.

distinguished by means of their $J_{2'3'}$ and $J_{2'3''}$ coupling constants; when the 3'dA ring adopts the N-conformation (vide infra) $J_{2'3'}$ and $J_{2'3''}$ have values of approximately 5.0 and 1.5 Hz, respectively. The H-5' and H-5" signals were assigned according to Remin and Shugar[12]. The H-2 and H-8 base proton signals are distinguished by their different T_1-relaxation times, as reflected in the reduced intensity of H-2 signals in the spectra recorded under WEFT conditions. No attempt was made to assign the base proton signals to specific residues. The chemical shifts and coupling constants of the sugar protons were determined from extensive computer simulation, by means of program LAME, of the 500 MHz spectrum recorded at 26°C (Fig. 2). The spectral data are

Table 1. Chemical shifts (ppm relative to Me_4NCl) and coupling constants in 3'd(A2'-5'A2'-5'A), 25 mM in ^2H_2O, 26°C, p^2H = 7.4.

proton \ residue	3'dA(1)	3'dA(2)	3'dA(3)	3'dA(60°C)[a]	
δ 1'	2.852	2.656	2.516	2.623	
2'	1.812	1.485	1.280	1.401	
3'	-0.882	-0.679	-0.866	-1.105	
3"	-0.886	-0.799	-1.113	-1.216	
4'	1.332	1.454	1.406	1.196	
5'	0.546	1.036	1.086	0.478	
5"	0.298	0.823	0.835	0.296	
couple				-60°C[b]	60°C[a]
J 1'2'	0.9	1.1	2.2	0.5	2.4
2'3'	5.6	5.2	5.7	4.9	6.0
2'3"	1.7	1.5	2.9	1.2	3.5
3'3"	-14.4	-14.4	-13.9		
3'4'	10.2	10.2	9.0	10.8	8.8
3"4'	5.8	5.8	6.6	5.3	6.7
4'5'	2.8	2.0	2.0	2.6	3.0
4'5"	4.3	2.6	2.8	2.7	4.7
5'5"	-12.7	-11.9	-11.7		
5'P		4.2	4.4		
5"P		2.7	2.5		
4'P		2.7	2.7		
2'P	8.5	7.8			
base protons H-2 and H-8					
δ H-2	4.916			δH-2	5.380[c]
H-2	4.668			H-8	5.510[c]
H-2	4.612				
H-8	4.818				
H-8	4.792				
H-8	4.767				

a) in ^2H_2O, 60°C, taken from ref. 6.

b) in ND_3, -60°C, taken from ref. 6.

c) in pyridine-d5, 60°C, taken from ref. 6.

listed in Table 1. In addition, 300 MHz NMR spectra were taken at 1, 15 and 77°C. As these latter served mainly to monitor the temperature-dependent behaviour of the sugars as well as that of the γ and β rotamer populations, only a partial computer simulation was carried out in order to extract $J_{1'2''}$, $J_{4'5'}$, $J_{4'5''}$, $J_{5'P}$ and $J_{5''P}$ couplings. The results are presented in Table 2 (data at 26°C are included for the sake of completeness).

Conformation of the 3'deoxyribose rings.

It was shown earlier[6,13] that the conformational equilibrium

Table 2. Coupling constants of 3'd(A2'-5'A2'-5'A) at various temperatures.

temperature (°C)		1	15	26	77
residue	couple				
3'dA(1)	1'2'	1.0	1.0	0.9	2.0
	4'5'	2.2	2.6	2.8	2.9
	4'5"	3.8	3.9	4.3	4.8
3'dA(2)	1'2'	1.0	1.0	1.1	1.5
	4'5'	a	1.7	2.0	2.3
	4'5"	2.0	2.2	2.6	3.6
	5'P	a	4.2	4.2	4.5
	5"P	2.1	2.3	2.7	4.0
3'dA(3)	1'2'	2.2	2.2	2.2	2.2
	4'5'	a	2.0	2.0	2.3
	4'5"	2.0	2.2	2.8	3.4
	5'P	a	4.1	4.4	4.5
	5"P	2.2	2.2	2.5	3.6

a) Could not be determined because of overlapping peaks.

of the 3'deoxyribose ring may be described in terms of a two-state equilibrium of N- and S-type[14] sugar rings. The conformation of an N- or S-type sugar is completely described by the pseudorotation parameters P and Φ_m[14]. The pseudorotation analysis is based on a generalized Karplus equation[15] and on the relations between P and Φ_m and the proton-proton torsion angles given in Eqn. 1a-1e. These equations incorporate the expected deviations from trigonal projection symmetry about the respective central bonds C1'-C2', C2'-C3' and C3'-C4'. The observed coupling constants represent time average values and thus are dependent on the mol fraction (x_N) of N and S conformer and on the couplings in the pure conformers as reflected in Eqn. 2.

$$\phi_{1'2'} = 124.0^\circ + 1.09\ \Phi_m \cos(P-144^\circ) \quad (1a)$$

$$\phi_{2'3'} = -2.4^\circ + 1.06\ \Phi_m \cos P \quad (1b)$$

$$\phi_{2'3"} = -122.9^\circ + 1.06\ \Phi_m \cos P \quad (1c)$$

$$\phi_{3'4'} = -121.4^\circ + 1.03\ \Phi_m \cos(P+144^\circ) \quad (1d)$$

$$\phi_{3"4'} = -0.9^\circ + 1.02\ \Phi_m \cos(P+144^\circ) \quad (1e)$$

$$J_{obs} = x_N J_N + (1-x_N) J_S \quad (2)$$

Eqns. 1a-1e are adapted to the case of a 3'deoxyribose ring. The 3'deoxyribose ring is considered to be a more or less exact mirror image of the 2'deoxyribose ring. Therefore, the same relation-

Table 3. Vicinal H-H torsion angles in 3'dA[a]. All values given in degrees.

ϕ_{HH}	calculated[b]	experimental[c]
1'2'	100.7	98.4
2'3'	31.0	32.0
2'3"	-89.4	-89.6
3'4'	-151.9	-154.4
3"4'	-31.1	-32.8

a) P = 12.5, ϕ_m = 32.3, calculated from data in ref. 7.
b) From Eqns. 1a-1e.
c) From UTAH calculation, see text.

ships between the vicinal proton-proton torsion angles as calculated from a series of 2'deoxyribose compounds[16] are used, taking into account the mirror image correspondence between 3'dA and 2'dA. The properties of the H1'-H2' and H1'-H2" combinations in 2'dA are transferred to the H3'-H4' and H3"-H4' combinations, respectively and vice versa. The same interchange was applied to the couples H2"-H3' and H2'-H3". The assumption of a mirror image relationship was born out of necessity, since no large body of crystallographic data is presently available that would allow us to derive equations from least squares regressions as was done in the earlier work[16]. However, the crystal structure of the monomer cordycepin has been published[7] and this makes it possible to perform an independent test on the accuracy of Eqns. 1a-1e, at least in the N region of pseudorotation space. The parameters P and ϕ_m of the cordycepin structure[7] were computed from the ring atom coordinates, by means of program FICON (de Leeuw, H.P.M. & Altona, C., unpublished). With the aid of program UTAH5A[17] hydrogen atoms were generated, according to standard procedures[16], onto the C-1', 2', 3', 4' and 5' carbon atom skeleton as derived from the X-ray structure analysis of 3'dA; the resulting H-H vicinal torsion angles are compared to these derived from Eqns. 1a-1e. The results are shown in Table 3. Considering the small differences ($\leq 2.5^{o}$) between the calculated and the "experimental" H-H torsion angles (Table 3), it can be concluded that Eqns. 1a-1e constitute a reasonably accurate approximation of the relation between the proton-proton torsion angles and the pseudorotation parameters P and ϕ_m.

Eqns. (1) and (2) have been incorporated into program PSEUROT[18]. In this program an initial guess of P and ϕ_m and of

Table 4. Population N-conformer[a] (%) in 3'd(A2'-5'A2'-5'A).

temperature (°C)	1[b]	15[b]	26	77[b]
residue				
3'dA(1)	100	100	98	86
3'dA(2)	100	100	98	92
3'dA(3)	80	82	82	82
3'dA	100[c]			83[d]

a) $P_N = -5^o$, $P_S = 160^o$, $\Phi_N = 37^o$, $\Phi_S = 36^o$.
b) Calculated from the value of $J_{1'2'}$.
c) Data taken from ref. 6, solvent ND_3, -60^oC.
d) Data taken from ref. 6, solvent D_2O, 60^oC.

the mol fraction N conformer (x_N) is used to compute the vicinal H-H coupling constants, which are then fitted to the observed coupling constants by computer adjustment of P, Φ_m and x_N in a least squares iteration process.

In the present pseudorotation analysis the P and Φ_m value of the S-type conformer (the minor component in the equilibrium) were constrained to adopt values of 160^o and 36^o respectively, which represent average values for the S-type ring[16]. Results of the analysis are shown in Table 4.

It is evident that the 3'deoxyriboserings in the present trimer display an overwhelming preference for the N-type conformation, in agreement with the earlier observations on the monomer 3'dA[6]. At low temperature the sugar rings of units 3'dA(1) and 3'dA(2) appear to convert completely into the N-form. The 3'dA(3) terminal residue displays the same conformational composition as does cordycepin at 60^oC (18% S-type at all temperatures). At high temperature the very high N-type preferences displayed by residues (1) and (2) decrease, but a distinct bias toward N-type remains present ($\geq$ 86% N). The difference in behaviour between the conformational equilibria of residues (1) and (2) as compared to residue (3), appears to be in line with the conformational behaviour observed in other oligonucleotides, e.g. d(A-A-A)[19] and d(T-A-A-T)[20], in which compounds a strong preference for a single conformer (S-type in these examples) was observed, except in the 3'-OH terminal residue. Thus, a consistent picture begins to emerge. With few exceptions, the

residue at the 3'-OH (or 2'-OH) terminal enjoys significantly greater conformational freedom than do the central and 5'-OH residues in nucleic acid oligomers.

Backbone conformations.

The conformational preferences of the backbone torsion angles β, γ and ε can be calculated from ^{1}H-NMR data. The rotamer distribution around C4'-C5' is calculated from the $J_{4'5'}$ and $J_{4'5''}$ coupling constants according to the guidelines introduced by Haasnoot et al.[21]. The Haasnoot procedure requires an educate guess as to the values of the γ torsion angles, γ^+ in particular[22]. For γ^+(1) the usual mononucleoside value of 52° was adopted[16]. However, the measured couplings $J_{4'5'}$ and $J_{4'5''}$ in residues (2) and (3) of the trimer point to a smaller γ^+ angle. For example in the central residue 3'dA(2) one finds (at 15°C) $J_{4'5'}$ = 1.7 Hz and $J_{4'5''}$ = 2.2 Hz. These values accord[22] well with a γ^+ angle in the range 43°-46°. Therefore, rotamer populations γ(2) and γ(3) were calculated on the basis of $\gamma^+ = 44°$. Incidentally, application of the criterion[22] that, of a pair of couplings $J_{4'5'}$ and $J_{4'5''}$, the one that increases most strongly at higher temperatures should be identified with $J_{4'5''}$ leads to complete agreement with the Remin and Shugar rule[12]. It can be seen from Table 5 that the γ^+ rotamer represents by far the most populated state. At 15°C γ(2) and γ(3) assume a virtually pure γ^+ conformation (~ 100%). At high temperature (77°C) the preference for γ^+ has dropped to about 80% in γ(2) and γ(3). The preference for γ^+ at the 5'-OH terminal γ^+(1) (71% at 15°C, 59% at 77°C) is consistently smaller than that found in the internucleotide bonds.

The amount of trans conformer around C5'-O5' (β) is calculated from Eqn. 3 [22].

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

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

The trans conformer about β is strongly preferred (92% - 93%) in β(2) as well as in β(3), cf. Table 5. A slight temperature dependence is observed: the conformational purity of β^t decreases by about ten percent when the temperature is raised from 15 to 77°C. A reasonable estimate of the low-temperature

Table 5. Rotamer population (%) around β and γ torsion angles in 3'd(A2'-5'A2'-5'A).

temperature (^{o}C)		1	15	26	77
residue	rotamer				
3'dA(1)	γ^{+}_{t}	74	71	65	59
	γ^{-}	26	28	32	37
	γ^{t}	0	1	4	5
3'dA(2)	γ^{+}_{t}	a	100	89	80
	γ^{-}	a	0	9	16
	γ^{t}	a	0	2	4
	β^{t}	a	92	90	81
3'dA(3)	γ^{+}_{t}	a	98	89	82
	γ^{-}	a	0	9	14
	γ^{t}	a	2	2	4
	β^{t}	a	93	90	84
3'dA[b] (60^{o}C)	γ^{+}_{t}				59
	γ^{-}				35
	γ^{t}				6

a) See footnote a, Table 2.
b) Data taken from ref. 6, D_2O, 60^{o}C.

value of the β^{t} angle can be obtained by application of Eqn. 4 [22].

$$\sin \Delta\beta = (J_{5''P} - J_{5'P})/23.9 \qquad (4)$$

The value of β is equal to 180^{o} + Δβ.
For both 3'dA(2) and 3'dA(3) residues at 15^{o}C one finds $\beta^{t} \approx 175^{o}$.

The conformation around the C2'-O2' bond (ε) is reflected by the coupling constant $J_{2'P}$. In principle, three rotamers (ε^{-}, ε^{+}, ε^{t}) should be considered. However, from model building considerations it becomes immediately evident that the ε^{+} rotamer can be excluded from serious consideration, since this conformation gives rise to short intramolecular contacts between nonbonded atoms. The ε^{-} and ε^{t} rotamers have similar H2'-C2'-O2'-P torsion angles and these two possibilities cannot be distinguished by the use of $J_{2'P}$ as a probe. For steric reasons the ε^{-} conformer appears to be the most probable one, as will be discussed in the next section.

The long range coupling constants $J_{4'P}(2)$ and $J_{4'P}(3)$, both 2.7 Hz, indicate the presence of a considerable amount of planar W-type coupling path H4'-C4'-C5'-O5'-P. Planarity is only present when γ^{+} and β^{t} rotamers occur simultaneously; this

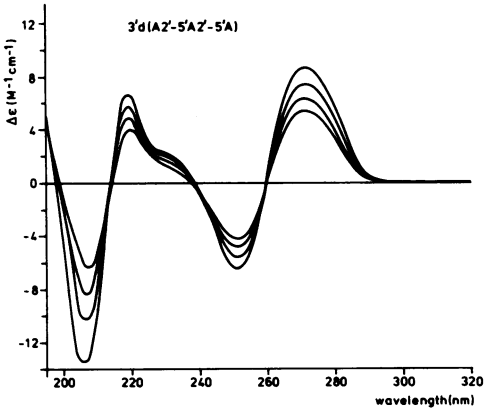

Figure 3. CD spectra of 3'd(A2'-5'A2'-5'A), taken at 1, 25, 47 and 70°C. The spectrum with the largest amplitude was taken at the lowest temperature

is the usual conformation of γ and β in a right-handed stacked state and therefore it is concluded that the molecule exhibits base stacking to a significant extent.

CD spectra.

The temperature dependence of the intensity of the CD effect in oligonucleotides is known to be a sensitive probe of intramolecular base stacking. The observed CD of 3'd(A2'-5'A2'--5'A), shown in Fig. 3, decreases (by a factor of two) with increasing temperature, i.e. to an extent comparable to the decrease in CD observed in the parent compound A2'-5'A2'-5'A in the temperature range 0-70°C [8]. In the case of the latter molecule this decrease was ascribed to the phenomenon of destacking and this was independently confirmed by NMR experiments[8]. It seems reasonable to conclude that the observed decrease in CD of the title compound in the temperature range 0-70°C reflects destacking. It is interesting to note that the band shape and the temperature dependency of the CD spectra of A2'-5'A2'-5'A [8] and of xA2'-5'xA2'-5'xA (Doornbos, J. & Altona, C. to be published) are very similar to the corresponding properties in 3'd(A2'-5'A2'-5'A).

Summary and conclusions.

The following characteristics arise from Tables 4 and 5. At

Table 6. Comparison of various 2'-5' linked adenosine trimers.

2'-5' trimer		A2'-5'A2'-5'A	xA2'-5'xA2'-5'xA	3'd(A2'-5'A2'-5'A)
Sugar conformation (5°C)	%N A(1)	57	95	100
	A(2)	63	83	100
	A(3)	56	47	82
Main stack types		various mixed stacks	N(1)-S(2)-u(3)[c] u(1)-N(2)-S(3)[c]	N(1)-N(2)-N(3) N(1)-N(2)-S(3)
Hypochromicity		21%	17%	26%
Biochemistry				
Stability[a]		1	120	30
Activity[b]		1	5	2

a) Relative stability to phosphodiesterase degradation (ref. 4).
b) Relative inhibition of DNA synthesis (ref. 4).
c) u = unstacked.

low temperature a trimer 3'd(A2'-5'A2'-5'A) conformation appears indicated which includes N-type sugar (100%, 100% and 82% N in the respective units), γ^+ rotamer (100% and 98% γ^+ in the internucleotide bonds), β^t rotamer (92% and 93% β^t in 3'dA(2) and 3'dA(3), respectively) and ε^- or ε^t rotamers. From the temperature dependence of the CD, as well as from the high conformational purities attained along the sugar phosphate backbone (vide supra), it is inferred that base stacking takes place at low temperature. According to expectations, the preferences for N-type sugar and for γ^+ and β^t rotamers decrease at higher temperatures, but remain extant. Modelbuilding shows that in a right-handed stacked arrangement ($\alpha = g^-$, $\zeta = g^-$) the torsion angle C3'-C2'-O2'-P (ε) adopts the ε^- conformation. The ε^t rotamer, when included in a stack, would cause serious steric hindrance between the bases of neighbouring residues. Therefore, we propose the presence of a stacked state in 3'd(A2'-5'A2'-5'A) which is characterized by the following features:

$$N(1) - \varepsilon^- - \beta^t - \gamma^+ - N(2) - \varepsilon^- - \beta^t - \gamma^+ - N(3)$$

Considering the amount of S-conformation (18%) present in residue 3'dA(3) the presence of a small amount of N-N-S stacked state appears probable.

At this stage a comparison between the various 2'-5' linked adenosine trimers appears appropriate. Table 6 displays a summary of some important features of the three compounds considered.
The hypochromicity of the various compounds is included in the Table since this value more or less reflects the relative base stacking tendencies. It is seen that base stacking appears most abundant in the 3'dA trimer and this observation agrees with the NMR data of the three compounds; the highest conformational purity in both sugar and backbone conformations was observed in 3'd(A2'-5'A2'-5'A). The stacked states in the three compounds are all different. At this point it is well to remember that the two major types of single-helical stacking of oligonucleotides, A form and B form, are characterized among other properties by a continuous sequence of N- and S-type sugar conformations, respectively. A stacking mode which is characterized by one or more N-S or S-N combinations is defined to be of mixed stack type. In the case of A2'-5'A2'-5'A no outspoken preference for a definite stack type was found and various types of mixed stacks were suggested[8]. In xA2'-5'xA2'-5'xA only the N-S stacked sequence appears present, i.e. N(1)-S(2) and N(2)-S(3). The 3'dA trimer seems to prefer a continuous N-N-N (A-type) stacking mode.

Biochemical parameters taken from ref. 4 are included in Table 6 in order to examine the possible existence of a clear cut correspondence between conformational preferences and biochemical properties. As far as can be seen, no such correspondence is apparent. Instead, the chemical nature of the sugar rings in the various trimers examined seems to be of some importance.

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*This paper is no.31 in the series 'Nucleic Acids Constituents'. For no.30 see previous paper

ABBREVIATIONS.

NMR : Nuclear Magnetic Resonance
CD : Circular Dichroism
ppm : part per million
3'dA : 3'-deoxyadenosine
xA : 9-β-D-xylofuranosyladenine
WEFT : Water Eliminating Fourier Transform
FID : Free Induction Decay

REFERENCES.

1. Hovanessian, A.G., Brown, R.E. & Kerr, I.M. (1977) Nature (Lond.) 268, 537-540.
2. Kerr, I.M., Brown, R.E. & Hovanessian, A.G. (1977) Nature (Lond.) 268, 540-542.
3. Doetsch, P., Wu, J.M., Sawada, Y. & Suhadolnik, R.J. (1981) Nature (Lond.) 291, 355-358.
4. Eppstein, D.A., Marsh, Y.V., Schryver, B.B., Larsen, M.A., Barnett, J.W., Verheyden, J.P.H. & Prisbe, E.J. (1982) J. Biol. Chem., 257, 13390-13397.
5. Schmidt, A., Chernajovsky, Y., Schulman, L., Federman, P., Berissi, H. & Revel, M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4788-4792.
6. Westhof, E., Plach, H. Cuno, I. & Lüdemann, H.-D. (1977) Nucleic Acids. Res. 4, 939-953.
7. Radwan, M.M. & Wilson, H.R. (1980) Acta Cryst. B36, 2185-2187.
8. Doornbos, J., den Hartog, J.A.J., van Boom, J.H. & Altona, C. (1981) Eur. J. Biochem. 116, 403-412.
9. Charubala, R. and Pfleiderer, W. (1980) Tetrahedron Lett. 21, 4077-4080.
10. Olsthoorn, C.S.M., Haasnoot, C.A.G. & Altona, C. (1980) Eur. J. Biochem. 106, 85-95.
11. Davies, D.B. & Dixon, H.B.F. (1982) IUPAC-IUB Abbreviations and Symbols for the Description of Conformations of Polynucleotide Chains, Document J.C.B.N. 14.2, final draft, March 1982.
12. Remin, M. & Shugar, D. (1972) Biochem. Biophys. Res. Commun. 48, 636-642.
13. Haasnoot, C.A.G., de Leeuw, F.A.A.M., de Leeuw, H.P.M. & Altona, C. (1981) Org. Magn. Reson. 15, 43-52.
14. a. Altona, C. & Sundaralingam, M. (1972) J. Am. Chem. Soc. 94, 8205-8212.
    b. Altona, C. & Sundaralingam, M. (1973) J. Am. Chem. Soc. 95, 2333-2344.
15. Haasnoot, C.A.G., de Leeuw, F.A.A.M. & Altona, C. (1980) Tetrahedron, 36, 2783-2792.
16. de Leeuw, H.P.M., Haasnoot, C.A.G. & Altona, C. (1980) Isr. J. Chem. 20, 108-126.
17. Faber, D.H. & Altona, C. (1977) Computers & Chemistry, 1, 203-213.
18. de Leeuw, F.A.A.M. & Altona, C. (1983) J. Comp. Chem. in the press.
19. Olsthoorn, C.S.M., Bostelaar, L.J., van Boom, J.H. and Altona, C. (1980) Eur. J. Biochem. 112, 95-110.
20. Mellema, J.R., Haasnoot, C.A.G., van Boom. J.H. & Altona, C. (1981) Biochim. Biophys. Acta, 655, 256-264.
21. Haasnoot, C.A.G., de Leeuw, F.A.A.M., de Leeuw, H.P.M. & Altona, C. (1979) Recl. Trav. Chim. Pays-Bas 98, 576-577.
22. Altona, C. (1982) Recl. Trav. Chim. Pays-Bas 101, 413-433.