Carbon-13 NMR in conformational analysis of nucleic acid fragments. 4. The torsion angle distribution about the C3'-O3' bond in DNA constituents

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ABSTRACT
Carbon-13 and proton NMR spectra of a series of oligodeoxynucleotides (d(CT), d(CC), d(TA), d(AT), d(CG), d(GC), d(AG), d(AAA), d(TATA) and d(GGTAAT)) were measured at various temperatures. The three coupling constants that are related to the magnitude of backbone angle $\varepsilon$ ($J(C4'-P)$, $J(C2'-P)$ and $J(H3'-P)$) are analyzed in terms of a three-state equilibrium about this bond.

Two $\varepsilon$ (trans) angles occur, which differ in magnitude depending on the conformation (N or S) of the adjoining deoxyribose ring. The S-type deoxyribose ring is associated with a smaller $\varepsilon$ (trans) angle: $\varepsilon(t,S) = 192^\circ$. The N-type deoxyribose ring is associated with a larger $\varepsilon$ (trans) angle $\varepsilon(t,N) = 212^\circ$. The third rotamer participating in the conformational equilibrium, is a gauche(-) ($E(-)$) conformer and occurs exclusively in combination with the S-type sugar ring ($E(-,S) = 266^\circ$). Within the limits of experimental error, the magnitude of these three angles appears to be independent of the particular base sequence, except in the case of d(CG) where a slightly larger $\varepsilon(t,S)$ angle ($197^\circ$) is indicated.

A simple equation is proposed which may be used to calculate the population of $\varepsilon(t,S)$ conformer in cases where only $J(H3'-P)$ is known.

INTRODUCTION

Carbon-13 NMR spectroscopy is rapidly becoming an indispensible supplement to $^1$H NMR studies aimed at understanding fine details of conformational behaviour of oligoribonucleotides. In previous papers from our laboratories it was shown, first of all, that heteronuclear $^{13}$C-$^1$H chemical shift correlation spectroscopy affords an unambiguous assignment of $^{13}$C resonances of ribonucleic acid dimers and trimers. That study also revealed a good correlation between the chemical shifts of the $^{13}$C atoms of the ribose ring and the sugar conformational equilibrium. Subsequently, a reparametrization of the Karplus equations for vicinal NMR coupling constants in CCOP (eq. 1) and HCOP (eq. 2) fragments was introduced:

$$\begin{align*}
^{3}J_{CCOP} &= 6.9\cos^2\phi - 3.4\cos\phi \cdot 0.7 \\
^{3}J_{HCOP} &= 15.3\cos^2\phi - 6.1\cos\phi \cdot 1.6
\end{align*}$$

The parameters for equations (1) and (2) were derived simultaneously from a data set of coupling constants $^{3}J_{\text{CCOP}}$ and $^{3}J_{\text{HCOP}}$ of RNA fragments. Special attention was paid to pinpointing the magnitude of the backbone angle $\varepsilon$ (C4'-C3'-O3'-P) in the $\varepsilon^t$ and $\varepsilon^-$ conformational ranges. At this point of our investigations the question arose whether or not the parameters of eqns. (1) and (2) were applicable to DNA fragments. From a comparison of 3',5'-cyclic AMP with 3',5'-cyclic dAMP, and from a detailed analysis of couplings obtained from d(TpA) at a number of temperatures, it could be concluded that a 2' oxygen substituent does not affect the C2'-P coupling to a measurable degree.

From many previous studies on RNA nucleotide fragments it is known that the conformational preferences about $\varepsilon$ are closely correlated to the actual conformation of the 3'-O attached ribose ring: when the sugar adopts the N-type form, the torsion angle $\varepsilon$ is strictly confined to the $\varepsilon^t$ domain. From $^1$H and $^{13}$C NMR experiments on RNA model compounds the exact magnitude of $\varepsilon^t$ was found to vary with the base-sequence involved: the C-C sequence displayed a relatively large value of $\varepsilon^t$ (226°) whereas for the A-U sequence a relatively small value of $\varepsilon^t$ (214°) was derived. An average value of $\varepsilon^t = 219^\circ$ was determined, in good agreement with the value of $\varepsilon^t = 218^\circ$, which is the average value from available X-ray data on RNA dimers. When the ribose ring reverts to the S-type conformation, the 3'-phosphate is able to swing over to the other side of the C3'-H3' bond, resulting in a blend of $\varepsilon^t$ ($\varepsilon^t \sim 277^\circ$) and $\varepsilon^-$ rotamers.

The situation is different for DNA constituents. In a DNA helix the deoxyribose conformational equilibrium is biased strongly toward the S-type form. Nevertheless, the preferred rotamer about the C3'-O3' bond is $\varepsilon^t$. At this point it is of interest to note that the magnitude of the angle $\varepsilon(t,S)$ in deoxynucleotides (for example ca. 195° in d(TpA) or 191° in the dodecamer CGCGAATTCCGCG) appears to be rather smaller than the value of $\varepsilon(t,N)$ (~219°) in RNA constituents. Now the question arises as to the origin of this difference. Two possibilities come to mind:

1. $\varepsilon(t,N)$ in RNA is primarily affected by the 2'-hydroxyl group. If this were true, then one would expect that in DNA $\varepsilon(t,N) = \varepsilon(t,S)$ and the coupling constant data can be interpreted in terms of a simple two-state equilibrium $\varepsilon^t/\varepsilon^-$. In fact, this line of thought was followed in our previous communication concerning the case of d(TpA).

2. The magnitude of $\varepsilon^t$ in DNA is determined by the conformation adopted by the sugar ring. In other words, one would expect that N-type deoxyribose conformers are associated with high $\varepsilon^t$ values ($\varepsilon(t,N) > 210^\circ$), whereas the S-type deoxysugars might display $\varepsilon^t$ magnitudes in a lower range (for example $\varepsilon(t,S) <$
200°). Because a third conformer (ε−) makes its appearance in the equilibrium blend when a stacked species reverts to a random coil form, a three state conformational model (in terms of ε(t,S), ε(t,N) and ε−) is called for.

The present study was undertaken in order to shed more light upon the conformational characteristics of the backbone angle ε of DNA oligonucleotides in aqueous solution. 13C-31P and 1H-31P vicinal coupling constants were measured for a series of oligodeoxynucleotides d(AAA), d(AG), d(AT), d(TA), d(CC), d(CT), d(CG) and d(GC) at three or more temperatures each. Care was taken to include a minimum of two examples of each possible sequence of purines and pyrimidines: d(pur-pur), d(pur-pyr), d(pyr-pur) and d(pyr-pyr).

MATERIALS AND METHODS

NMR samples

The compounds studied in this work were synthesized via a modified photodiester approach1. D2O solutions of samples were prepared with nucleotide concentrations ranging from 20-50 mM. A 5 mm NMR tube containing 0.4 ml of solution was used for d(TA) and d(CG). 10 mm tubes containing 1.2 ml of solution were used for the remaining samples. A trace of EDTA was added in order to neutralize paramagnetic contaminations, tetramethylammonium chloride served as an internal reference. The pH of the samples was adjusted to 7.5 ± 0.5 (meter reading).

One-dimensional NMR spectra

13C NMR spectra were recorded on a Bruker WM-300 spectrometer operating at 75.5 MHz and on a Bruker WM-200 spectrometer operating at 50.3 MHz. In both cases a 13C probe with 31P decoupling facilities was used. 13C spectra were acquired on 8K datapoints using a two-level decoupling sequence with 1s relaxation delay between observation pulses. During acquisition 0.5-1.0 W decoupling power was employed. FIDs were multiplied by a Gaussian window and the Fourier transformation was carried out on 128K datapoints.

300 MHz 1H-NMR spectra were recorded on a selective 1H probe with 31P decoupling facilities. 1H-31P coupling constants were determined from the difference between 1H spectra and 31P-decoupled 1H spectra. Special care was taken to adjust the 31P decoupling power and decoupling frequency in order to bring about complete decoupling and at the same time avoid sample heating.

During 13C acquisition the true sample temperature was determined from the chemical shift difference $\delta_{\text{HDO}} - \delta_{\text{TMA}}^{15}$ by recording a 1H spectrum on the decouple coil immediately after each 13C run. This internal temperature calibration is essential as it was found previously that a small temperature increase (0-5°C) of the sample may occur as a result of 1H decoupling3.
Two-dimensional NMR spectroscopy

The spectra of d(TpA), d(ApG), d(CpC), d(TATA) and d(GGTAAT) were assigned by means of two-dimensional heteronuclear chemical shift correlation spectroscopy\textsuperscript{16,17}, using the pulse sequence and phase cycling proposed by Bax\textsuperscript{18}. Time domain spectra consisted of 64 datapoints ($t_1$-dimension) and 2K datapoints ($t_2$-dimension). Before Fourier transformation a phase shifted ($1/6\pi$) sine square window was applied and FIDs were zero-filled to 512 and 4K data-points, respectively.

Nomenclature

The proposed IUPAC-IUB nomenclature\textsuperscript{19} is used in this work, i.e. backbone torsion angles are labelled $\alpha$-$\zeta$ starting with $\alpha$ at the 5' terminal P-O5' bond (see Figure 1). Carbon atoms are numbered according to Figure 2.

RESULTS AND DISCUSSION

Assignment of Carbon-13 spectra

The assignment of carbon NMR spectra of deoxydinucleoside monophosphates is relatively straightforward. A number of carbon resonances is split due to coupling to phosphorus. In a dinucleoside monophosphate these resonances are C4'(1), C3'(1), C2'(1), C5'(2) and C4'(2). C3'(1) and C5'(2) have geminal couplings to phosphorus which are invariant with temperature (~5.3-5.6 Hz) and as such are of least interest. However, C4'(1) is a sensitive probe for the conformational situation around $\varepsilon$ and therefore this signal should be discriminated from C4'(2). This can be achieved by considering the magnitude of $^3J_{C4'-P}$ at high temperature: it was pointed out in a previous paper\textsuperscript{5} that the signal with the largest coupling to phosphorus at high temperature should be assigned to C4' of the 3' terminus, whereas the doublet with the smaller splitting should be assigned to the C4' of the 5' terminus. The C2' signals, finally, resonate at high field and can be recognized easily. In a few selected cases, notably d(AG),
d(CC), d(TA), d(TATA) and d(GGTAAT), the correctness of the assignment was substantiated by means of two-dimensional heteronuclear chemical shift correlation spectroscopy.

The determination of J(HCOP) and J(CCOP)

Proton decoupled $^{13}$C spectra of each compound were recorded at three or more temperatures. $^3J_{CP}$ values were directly determined from the spectra. In addition, $^1$H spectra and $^{31}$P decoupled $^1$H spectra were recorded at temperatures corresponding as close as possible to those selected for the $^{13}$C spectra. $^{31}$P-$^1$H coupling constants were determined by comparing the width of the H3' multiplet in the coupled and in the $^{31}$P decoupled spectra. In some cases (d(TA) and d(AAA), d(TATA) and d(GGTAAT)) $^{31}$P-$^1$H coupling constants were available from computer simulations carried out previously $^{20,21}$. In some instances it was not feasible to measure $^{31}$P-$^1$H coupling constants at the same temperature as the $^{13}$C couplings, because of overlapping multiplets or overlap of the residual HDO peak. In those cases $^1$H-$^{31}$P couplings were determined by interpolation between bracketing values: one measured at higher temperature and one at lower temperature. All relevant couplings are listed in Table 1. In some cases, notably d(AAA), d(TATA) and d(GGTAAT), the measurements were limited to the couplings displayed by the 5' terminal residue 1 because the C4' signals of the remaining residues 2, 3,...... etc. appear as pseudotriplets and this precludes a straightforward analysis. Moreover, signal overlap and/or small splittings prevented the measurement of $^3J_{C2'-P}$ in these three compounds.

Conformation around C3'-O3' ($\epsilon$)

At first sight three classical rotameric ranges around the C3'-O3' bond (g $^+$, t, g $^-$) should be taken into consideration in the conformational analysis of $\epsilon$. However, the wealth of information extracted from X-ray diffraction studies$^{34}$, as well as from a lanthanide-induced chemical shift investigation$^7$, shows that the 3'-phosphate group strictly avoids to adopt the g $^+$ rotamer ($\epsilon^+ \sim 60^\circ$). In
Table 1: \( \langle J(C4'P), J(C2'P) \rangle \) and \( \langle J(H3'P) \rangle \) (in Hz) as a function of temperature (°C) of the 5' terminal residues of all DNA constituents used in this work.

<table>
<thead>
<tr>
<th>T</th>
<th>d(AG) C4'P C2'P H3'P</th>
<th>d(CG) C4'P C2'P H3'P</th>
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<th>d(GC) C4'P C2'P H3'P</th>
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<th>d(TA) C4'P C2'P H3'P</th>
<th>d(AAA) C4'P C2'P H3'P</th>
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<td>5 10.3 4.5</td>
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<td>11 10.0 4.8</td>
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<td>21 9.9 4.8</td>
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<td>43 8.9 5.4</td>
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<tr>
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<td>30</td>
<td>7.9 6.1</td>
<td>92 6.6 3.6 6.8</td>
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<tr>
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<th>d(GGTAAT) C4'P C2'P H3'P</th>
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<td>8.6 5.7</td>
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</tr>
<tr>
<td>47</td>
<td>7.2 6.3</td>
<td>7.3</td>
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</tbody>
</table>

In fact, the \( \epsilon^+ \) conformer never has been observed in the solid state and may therefore be excluded safely from consideration (Figure 3).

In the introductory part of the present communication it is explained that some care should be exercised in the interpretation of the coupling constants of DNA oligonucleotides measured along the C3'-O3' bond, viz. \( \langle J(C4'P), J(C2'P) \rangle \) and \( \langle J(H3'P) \rangle \). In particular, one should be aware of the possibility that the usual two-state conformational approach - in terms of an \( \epsilon^+/\epsilon^- \) equilibrium blend - may turn out to be too simple when the value of \( \epsilon^+ \) changes with changing conformation of the adjacent deoxyribose ring.
In the following, two strategies will be employed for the determination of \( \varepsilon \) values and corresponding conformational populations. It will be shown that the two-state approach, although applicable to each individual compound, fails to yield an overall consistent picture of the rotational preferences about the C3'-O3' bond in DNA constituents.

**Two-state analysis of \( \varepsilon \) rotamers**

Taking three measured coupling constants into account, the two-state equations can be written:

\[
\begin{align*}
J(H3'P,obs) &= p^tJ(H3'P)^t + (1-p^t)J(H3'P)^- \\
J(C4'P,obs) &= p^tJ(C4'P)^t + (1-p^t)J(C4'P)^- \\
J(C2'P,obs) &= p^tJ(C2'P)^t + (1-p^t)J(C2'P)^- 
\end{align*}
\]

where \( p^t \) stands for the mol fraction of \( \varepsilon^t \) rotamer and the superscripts \( t \) and \( - \) refer to the corresponding pure \( \varepsilon^t \) and \( \varepsilon^- \) states, respectively. Under the usual assumption of trigonal projection symmetry (Figure 3) the following relations are valid:

\[
\begin{align*}
torsion\ angle\ C2'-C3'-O3'-P &= \varepsilon - 120^\circ \\
torsion\ angle\ C4'-C3'-O3'-P &= \varepsilon \\
torsion\ angle\ H3'-C3'-O3'-P &= 240^\circ - \varepsilon 
\end{align*}
\]

From these relations, in combination with eqs. (1), (2) and (3)-(5), the magnitude of \( \varepsilon^t \) and \( \varepsilon^- \) as well as the position of the \( \varepsilon^t/\varepsilon^- \) equilibrium at all experimental temperatures can be calculated in an iterative least-squares procedure, given a set of \( ^3J \) values. The calculations were carried out for each compound separately; the resulting (apparent) \( \varepsilon^t \) and \( \varepsilon^- \) angles are listed in Table 2, together with the calculated \( \varepsilon^t \) population at the highest and lowest experimental temperature used in each case. For reasons explained below we also show the corresponding population of S-type sugar \( p_S \) conformation as determined from the width of the H2'' signal according to the sum rule\(^2\), eq. (9):

\[
p_S = (17.8 - J_{1''2''} - J_{2''3''})/10.9
\]

Scrutiny of Table 2 reveals some peculiar features. The purine-purine oligonucleotide sequences d(AAA) and d(AG) appear to display smaller \( \varepsilon^t \) angles (~ 191°) than do the pyrimidine-pyrimidine sequences (~ 203°), whereas the mixed pyr-pur and pur-pyr compounds show intermediate values. Although a
Table 2: Results of the two-state analyses of the vicinal C-P and H-P couplings of 8 different DNA dimers. Apparent(*) backbone torsion angles ε(t) and ε(-) are shown as well as calculated trans populations 100x p(t) at two extreme temperatures. The corresponding populations of S-type sugar conformation are also given, p(S)×100.

<table>
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<tr>
<th>compound</th>
<th>ε(t)</th>
<th>ε(-)</th>
<th>T(°C)</th>
<th>p(t)</th>
<th>p(S)</th>
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<td>5</td>
<td>97</td>
<td>100</td>
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<td>d(AG)</td>
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<tr>
<td>d(AT)</td>
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<td>262</td>
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<td>d(CG)</td>
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<td>76</td>
</tr>
<tr>
<td>d(CC)</td>
<td>203</td>
<td>269</td>
<td>76</td>
<td>64</td>
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</tr>
<tr>
<td>d(CT)</td>
<td>204</td>
<td>272</td>
<td>21</td>
<td>75</td>
<td>73</td>
</tr>
</tbody>
</table>

(*) The numbers given should be regarded as artefacts because the two-state approach is too simple, see text.

sequence-dependent ε^t angle would not come as a surprise, attention should be paid to the following. Concomitant with the increase of ε^t angle in going from pur-pur to pyr-pyr sequences, the ε^- angle also increases from ~255° to ~270°. Since the ε^- rotamer is associated with the unstacked or random-coil forms, this result appears highly unlikely. In the first place one cannot imagine a likely mechanism whereby the base sequence would affect ε^- to such a large extent. Secondly, it is noted (Table 2) that the lower range of ε^t values (d(AAA), d(AG), d(TA)) appears to be associated with high populations of S-type sugar conformations (even approaching 100% purity at low temperatures) and vice versa: high ε^t angles, as in d(CG), d(CC) and d(CT), apparently correlate with decreased S-type populations and hence with an increase of N-type species. Most likely, the latter correlations should be considered as artefacts introduced by the incorrect limitations inherent to the two-state approach. In the following section it will be shown that these apparent anomalies disappear when the conformational analysis is carried out in terms of a three-state equilibrium.

Three-state analysis of ε rotamers

A two-state equilibrium is characterized by the feature that a plot of two observables, for example J_{C4'P} vs. J_{H3'P}, yields a straight line when the temperature is varied, whereas in the case of a three-state situation the plotted points will lie within the confines of a triangle^{28}. A plot of a selection of the data of Table 1 is given as an example (Figure 4). Figure 4 as well as similar
Figure 4: Plot of $J(H3'P)$ vs. $J(C4'P)$ for the following compounds: d(AAA): $\times$, d(TA): $+$, d(CC): $\circ$, d(CT): $\Delta$. The corners of the triangle represent the values of $J(C4'P)$ and $J(H3'P)$ in pure e$(t,N)$, e$(t,S)$ and e$(-,S)$ conformers as derived below (see text).

plots (not shown) for other compounds indeed strongly suggest the existence of a three-state equilibrium. The question as to which particular states participate in the equilibrium blend can be answered by the observation mentioned in the previous section: the two-state calculations reveal that, as the N-type sugar conformational populations increase in going from pur-pur to pyr-pyr sequences, the resulting $\varepsilon^t$ values appear to increase concomitantly. Thus, two different $\varepsilon^t$ angles are indicated: one $\varepsilon(t,S)$ in the lower range, associated with an S-type deoxyribose ring as found in B-DNA and another one $\varepsilon(t,N)$ in the higher range, associated with an N-type sugar conformation as occurs in A-DNA and A-RNA.

From this point onwards use was made of a computer program (written in APL$^{15}$) which allows one to carry out an iterative least-squares minimization of the quantity $J_{\text{obs}} - J_{\text{calc}}$. A large set of experimental coupling constants can be used as input data and the program searches for the "best" combination of the angles $\varepsilon(t,S)$, $\varepsilon(t,N)$ and $\varepsilon(-,S)$. The corresponding populations $p(t,S)$, $p(t,N)$ and $p(-,S)$ are also computed. It should be remembered that, since $p(t,S) + p(t,N) + p(-,S) = 1$, only two of these represent independent variables. In order to avoid possible pitfalls due to undetected minima, a number of pilot calculations was performed first. In these calculations angles $\varepsilon(t,S)$ and $\varepsilon(t,N)$ were in turn (and stepwise) constrained to adopt a series of predetermined values. In this way conformational space was scanned for $\varepsilon(t,N)$ varying from 200° to 240° and $\varepsilon(-,S)$ varying from 250° to 270°. Only a single -but fairly shallow- minimum was found. A remarkable feature was readily apparent in all
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Table 3: Results of the three-state analysis of C-P and H-P couplings of 7 different DNA dimers. Observed and calculated couplings are shown. $\epsilon(t,N) = 212^\circ$ and $\epsilon(t,S) = 192^\circ$ and $\epsilon(-,S) = 266^\circ$, except for d(CpG), see text. $\Delta = J(\text{obs}) - J(\text{calc})$ (in Hz), the overall rms: 0.14 Hz.

<table>
<thead>
<tr>
<th>compound</th>
<th>T</th>
<th>J(H3'P)</th>
<th>J(C4'P)</th>
<th>J(C2'P)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>obs</td>
<td>calc</td>
<td>obs</td>
</tr>
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(*) $\epsilon(t,S) = 197^\circ$, rms = 0.16 Hz.

calculations that were carried out using input values that gave low root-mean-square (rms) deviations: the rotamer population $p(t,N)$, produced by the least-squares calculation, was - within the limits of error - invariably equal to the population of N-type conformation ($p_N$) of the attached deoxyribose ring. Note that $p_N$ was determined independently from the H2" signal (eq. 9), $p_N + p_S = 1$. Therefore, in the final step the number of independent variables was drastically reduced by fixing each $p(t,N)$ to the value of each corresponding percentage of N-type sugar ring, i.e. determined for the same sequence at the same temperature, viz. Table 2. It was also clear that the computed coupling constants of d(CG) fitted less well to the experimental ones. For these reasons the final calculations were carried out as follows: a data set, consisting of 51 coupling constants of the six dimers d(AG), d(TA), d(AT), d(GC), d(CC) and d(CT) was used as input (see Table 3) and the populations $p(t,S)$ as well as the values of the three $\epsilon$ angle were refined (20 independent variables vs. 51 observables). The iteration rapidly converged and yielded $\epsilon(t,S) = 192 \pm 2^\circ$. 

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Table 4: C-P and H-P coupling constants (Hz) calculated for the three pure rotamers $\varepsilon(t,N) = 211.7^\circ$ and $\varepsilon(t,S) = 192.3^\circ$ and $\varepsilon(-,S) = 266.0^\circ(\ast)$$

<table>
<thead>
<tr>
<th>angle</th>
<th>$J(P-H3')$</th>
<th>$J(P-C4')$</th>
<th>$J(P-C2')$</th>
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<tr>
<td>$\varepsilon(t,S) = 192.3$</td>
<td>4.45</td>
<td>10.60</td>
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<tr>
<td>$\varepsilon(t,N) = 211.7$</td>
<td>8.11</td>
<td>8.58</td>
<td>0.81</td>
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<tr>
<td>$\varepsilon(-,S) = 266.0$</td>
<td>8.50</td>
<td>0.97</td>
<td>8.26</td>
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</table>

\(\ast\) In order to avoid rounding-off errors, angles are shown to 0.1°, and coupling constants to 0.01 Hz. The last digit is not significant.

$\varepsilon(t,N) = 212 \pm 4^\circ$ and $\varepsilon(-,S) = 266 \pm 3^\circ$, overall rms deviation 0.14 Hz, maximum deviation 0.3 Hz. The excellent agreement between $J_{\text{obs}}$ and $J_{\text{calc}}$ is shown in Table 3.

The 12 available couplings of d(CG) were treated in a separate least-squares minimization. In this run $\varepsilon(t,N)$ and $\varepsilon(-,S)$ were kept fixed at the previously calculated values and $\varepsilon(t,S)$ was refined, together with the population $p(t,S)$. For $\varepsilon(t,S) = 197^\circ$ an excellent fit between $J_{\text{obs}}$ and $J_{\text{calc}}$ was obtained (rms = 0.16 Hz). These results are also displayed in Table 3.

In the foregoing derivation of limiting couplings (see Table 4) the data on three compounds, for which $^3J_{C2'P}$ could not be determined experimentally, were necessarily left out of consideration. These data concern the 5' terminal residues in the higher oligomers d(AAA), d(TATA) and d(GGTAAT). Now we may revert the procedure and test whether or not a good fit between observed and calculated $^3J_{C4'P}$ and $^3J_{H3'P}$ can be obtained using the previously established limiting couplings as input and $p(t,S)$ as the only independent variable. As before, $p(t,N)$ was set equal to the population of N-type sugar conformation established earlier\textsuperscript{20-23} at various temperatures. It turns out that the three-state analysis indeed yields an excellent fit (rms 0.11-0.19 Hz). The resulting rotamer populations are also collected in Table 5.

The results of the trimer d(AAA) are of special interest because this compound is the only one in the present series for which the thermodynamic parameters of the stack/unstack equilibrium have been measured from temperature-dependent CD spectra\textsuperscript{20} and a comparison of the population $p(t,S)$ with the known population of base-stacked single-helical species (Table 7 in ref\textsuperscript{20}) can be made. In the case of d(AAA) $p(t,S)$ drops from 0.98 to 0.55 on raising the temperature from 5 °C to 76 °C. At the same time the population of base-base stack drops from 0.87 to 0.33. It is seen that at all temperatures the population of stacked species is significantly less than $p(t,S)$. This finding may be taken to mean that the random-coil form permits $\varepsilon$ to adopt both the $\varepsilon(t,S)$ and $\varepsilon(-,S)$ rotamers. Hence, one should be careful to equate neither $p(t,S)$, nor the sum $p(t,S) * p(t,N)$, to the population of stacked species. From the
Table 5: Results of the three-state analysis of C-P and H-P couplings of all DNA constituents represented in Table 1, showing the calculated rotameric populations (× 100) \( p(t,S) \), \( p(t,N) \) and \( p(\sim,S) \) at various temperatures (*).

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<th>compound</th>
<th>( T(°C) )</th>
<th>( p(t,S) )</th>
<th>( p(t,N) )</th>
<th>( \Delta p(t) )</th>
<th>( p(\sim,S) )</th>
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(*) As explained in the text, the population \( p(t,N) \) was constrained to be equal to the N-type sugar conformational population.  
(**) \( \epsilon(t,S) = 197^* \)
Comparison of solid state data with solution data. Mean $\epsilon$ values are listed for a number of DNA oligomers, with N-type ribose rings (first column) and for a number of DNA oligomers with have S-type sugar rings (second column).

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<th>S sugars</th>
</tr>
</thead>
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<tr>
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<td>Arnott A82 fiber</td>
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<td>pATAT, residue 3</td>
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<tr>
<td>mean</td>
<td>207</td>
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<tr>
<td>solution data</td>
<td>212 (*)</td>
</tr>
</tbody>
</table>

(*) this work

- admittedly meagre- available data one gains the impression that roughly 40% of the total random coil population occurs as $\epsilon^\circ$.

Comparison of $\epsilon$ angles in solution with solid state data

A comparison of the presently derived $\epsilon$ angles with those obtained earlier from X-ray diffraction data is now in order. Unfortunately, the number of available X-ray structures is relatively small. Table 6 gives the mean values of torsion angle $\epsilon$ for a number of solid state structures. The $\epsilon$ angles are divided into three classes, corresponding to those derived in this work, i.e. $\epsilon(t)$ angles which are associated with N-type sugar ring are listed in the left-hand column (A-type helices) and $\epsilon(t)$ angles which are associated with S-type sugar rings are listed in the right-hand column (B-helices). To the best of our knowledge only in one case the $\epsilon(-)$ conformation has been observed in crystal structures of oligodeoxynucleotides: viz. in the unstacked molecule d(pTpT)$_3$ (E(-) = 255°. This value compares favourably with the value of 266° derived in the present work. Returning to Table 6, one notes that the solid state data and the solution data display similar trends, i.e. N-type sugar rings are associated with larger $\epsilon(t)$ values than S-type sugar rings. Moreover, the mean values of the solid state data agree surprisingly well with those obtained in the present work. Finally, it should be pointed out that most of the solid state data refer to double helices, whereas the present work was based upon a blend of single-helices and random coil forms. It is thus indicated that the duplex structure does not im-
Information from \( J(H3'-P) \)

At present a far greater number of proton spectra of DNA constituents have been analyzed with respect to \( J(H3'-P) \) than is the case for \( ^{13}\text{C} \) spectra with respect to \( J(C4'-P) \) and \( J(C2'-P) \). Is it possible to utilize the present results for a semiquantitative interpretation of \( J(H3'-P) \) in terms of \( \varepsilon(t,S) \) when the carbon-13 spectrum has not been measured? The answer is affirmative, provided a rough estimate of the S-type deoxyribose population is available, e.g. from the width of the H2\(^\prime\) signal (eqn. 13) or from the coupling pattern displayed by the H1\(^\prime\) resonance\(^{25}\). With the aid of the limiting couplings of Table 4 the following relation between \( p(t,S) \) and the observed coupling \( J(H3'-P) \) can be deduced, eq. (10)

\[
p(t, S) = \frac{(C - J(H3'-P))}{4.05}
\]

where \( C \) is a parameter that varies linearly with the time-average sugar conformational population distribution: from 8.48 Hz for a pure S-type sugar to 8.09 Hz for a pure N-type sugar conformation. A value of \( C = 8.4 \) would suffice for most practical applications (60 - 100 % S-type sugar). It stands to reason that maximum accuracy requires the use of both proton-phosphorus and carbon-phosphorus coupling constant data.

**SUMMARY AND CONCLUSIONS**

The three observable vicinal couplings along the C3'-O3' backbone angle \( \varepsilon \), viz. \( J(C4'-P) \), \( J(C2'-P) \) and \( J(H3'-P) \), of six different deoxydinucleoside monophosphates were measured at several temperatures each. The total set of 51 couplings was used to test a model with three well-defined \( \varepsilon \) angles: \( \varepsilon(t, S) \), \( \varepsilon(t, N) \) and \( \varepsilon(-, S) \), where \( S \) and \( N \) indicate the conformation of the adjoining deoxyribose ring. The overall agreement (rms difference 0.14 Hz) lends strong support to the correctness of the three-state model: \( \varepsilon(t, S) = 192^\circ, \varepsilon(t, N) = 212^\circ, \varepsilon(-, S) = 266^\circ. \) The model also applies to 5' terminal nucleotidyl residues in several higher oligomers. Only one compound, d(CG), appears to require a slightly (5\(^\circ\)) different backbone angle \( \varepsilon(t, S) \).

The N-type deoxyribose ring conformer is associated with the \( \varepsilon(t, N) (212^\circ) \) rotamer; the combination \( \varepsilon(-, N) \) appears forbidden. In the case of d(AAA) the population of base-stacked species is definitely less than the \( \varepsilon(t, S) \) population. The randomly coiled (destacked) state appears to permit the existence of both \( \varepsilon(t) \) and \( \varepsilon(-) \) rotamers in significant amounts.

A simple equation is proposed by means of which the population \( \varepsilon(t, S) \) can be deduced from \( J(H3'-P) \), provided that a rough estimate of the sugar conformational equilibrium population is available.
As was mentioned in the Introduction, RNA model-compounds show a distinct base-sequence dependency of the magnitude of $\epsilon^t$. In the present model derived for deoxy oligonucleotides such a base sequence dependency was implicitly ignored. For the time being this approach is justified since the present dataset does not allow for the inclusion of such an effect on $\epsilon(t,S)$. Nevertheless the aberrant $\epsilon^t$ value found for the C-G dimer may indicate that a small base-sequence dependency of $\epsilon(t,S)$ occurs. Further investigations on this subject are currently being undertaken in this laboratory.

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REFERENCES

2. Abbreviations used in this study: NMR, nuclear magnetic resonance; EDTA, ethylenediaminetetraacetic acid.
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31. Data taken from ref. 27.