CARBON-13 NMR SPECTRUM OF 2'-FLUORO-2'-DEOXYADENOSINE 3',5'-CYCLIC PHOSPHATE

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Abstract — 2'-Fluoro-2'-deoxyadenosine 3',5'-cyclic phosphate (3) was synthesized and its $^{13}$C nmr spectrum was measured. The sugar carbon signals can be unambiguously assigned because the C(1'), C(2') and C(3') have different $^{13}$C-$^{19}$F coupling constants. Comparison of the present data with those of other nucleoside 3',5'-cyclic phosphates gives a definitive solution to the disputed assignment of the C(3') and C(4') signals for the 3',5'-cyclic phosphate derivatives.

Nucleoside 3',5'-cyclic phosphates are known to play important roles in living organisms. Adenosine 3',5'-cyclic phosphate (Ado-3',5'-P, 4) and guanosine 3',5'-cyclic phosphate[2] are extensively studied, and are recognized as ubiquitous regulatory molecules controlling diverse metabolic process. The 3',5'-cyclic phosphate derivatives of cytidine and uridine are also found in animal cells, and are considered to regulate cell growth.[3] $^{13}$C nmr spectra of 3',5'-cyclic phosphate derivatives of four ribonucleosides and thymidine were first published by Lapper et al. in 1973.[4] During $^{13}$C nmr studies on phosphate derivatives of modified nucleosides,[5,6] we found that the published assignment of the C(3') and C(4') signals of the 3',5'-cyclic phosphates should be reversed. This proposition was based on analysis of chemical shift changes upon introduction of a 3',5'-cyclic phosphate group.[7] The definitive answer to this problem can be obtained by measuring $^{13}$C spectrum of a 3',5'-cyclic phosphate derivative with some kind of label on the C(3') or C(4'). For this purpose, the 3',5'-cyclic phosphate derivative of 2'-fluoro-2'-deoxyadenosine (2'-F-dAdo-3',5'-P, 3) was chosen by us since its C(3') has $^{13}$C-$^{19}$F coupling constant which is different from those of the C(1') and C(2').[8] Moreover, it is very interesting to examine the biological activity of
2'-F-dAdo-3',5'-P because the homopolynucleotides containing 2'-fluoro-2'-deoxy-purinenucleoside residues have shown similar biological activities to those of the corresponding natural nucleoside derivatives.\(^9,10\) We report here the synthesis of 2'-F-dAdo-3',5'-P and its \(^{13}\)C nmr spectrum which confirms our previous proposition.\(^7\)

\[
\begin{align*}
\text{i: } & \text{POCl}_3, (\text{EtO})_3\text{PO} \\
\text{ii: } & \text{DCC, Pyridine}
\end{align*}
\]

2'-Fluoro-2'-deoxyadenosine (2'-F-dAdo, 1) was synthesized by a modified procedure of Ikehara et al.\(^11\) via cycloadenosine and arabinofuranosyladenine derivatives. The overall yield of 1 from adenosine was 6%.\(^{12}\) 1 was phosphorylated with POCl\(_3\) in triethyl phosphate\(^{13}\) to give the 5'-phosphate of \(\sim(2'-F\text{-dAdo}-5'-\text{P}, 2)\. 2\) was isolated by chromatography on a column of Dowex 1 resin in 80% yield. 2'-F-dAdo-3',5'-P (3) was synthesized by intramolecular condensation of the 3'-OH and phosphate groups using dicyclohexylcarbodiimide in a relatively large volume of pyridine.\(^{14}\) 2 was isolated by chromatography on a column of DEAE-cellulose in 73% yield. This compound was characterized and identified by various techniques such as paper chromatography, paper electrophoresis, \(^1\)H nmr and \(^{13}\)C nmr. The \(^1\)H nmr spectrum shows that the coupling constant between H1' (\(\delta\), 6.45 ppm) and H2' (\(\delta\), 5.59 ppm) (\(J_{1'2'}\)) is close to 0 Hz while the \(J_{1'2}\), of 2'-F-dAdo-5'-P is 2.2 Hz. The small \(J_{1'2}\), (\(<1\) Hz) is characteristic of \(\beta\)-nucleoside 3',5'-cyclic phosphates.\(^{15}\) The \(^{13}\)C nmr data for the sugar carbons are shown in Table 1. The data of
2'-F-dAdo and its 5'-phosphate are also included. The resonances of C(1'), C(2') and C(3') are easily assigned because they have \(^{13}\text{C} - ^{19}\text{F}\) coupling constants of about 34, 190 and 16 Hz, respectively. The remaining C(4') and C(5') resonances can be easily distinguished because their chemical shift difference is usually large, and the \(^{13}\text{C} - ^{31}\text{P}\) coupling constant data of 2'-F-dAdo-5'-P confirm the assignment. Therefore, all the sugar carbon signals of 2'-F-dAdo-3',5'-P are safely assigned. The chemical shift changes in sugar carbons upon introduction of a 3',5'-cyclic phosphate group, \(\delta(3',5'-\text{cyclic phosphate}) - \delta(\text{nucleoside})\), are shown in Table II. The data for Ado-3',5'-P are also included for the purpose of comparison. The both 3',5'-cyclic phosphates show about the same changes in each carbon. These results unambiguously confirm the unusually large upfield shift of C(4') resonance of Ado-3',5'-P which has been suggested by us.\(^7\) This large upfield shift (-12 - -15 ppm), which is generally observed in 3',5'-cyclic phosphate derivatives,\(^7\) may be caused by the close contact of C(4') with the diesterified phosphate group. Since 3',5'-cyclic phosphates have a quite rigid sugar-phosphate structure, these compounds may provide a good model system for theoretical calcu-

Table I. \(^{13}\text{C}\) Chemical shifts (ppm, from external TMS) and \(^{13}\text{C} - ^{31}\text{P}\) coupling constants (Hz, in parentheses)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>C(1')</th>
<th>C(2')</th>
<th>C(3')</th>
<th>C(4')</th>
<th>C(5')</th>
</tr>
</thead>
<tbody>
<tr>
<td>2'-F-dAdo</td>
<td>86.58</td>
<td>94.05</td>
<td>69.17</td>
<td>85.00</td>
<td>61.30</td>
</tr>
<tr>
<td>2'-F-dAdo-5'-P</td>
<td>86.55</td>
<td>94.17</td>
<td>68.83</td>
<td>82.59</td>
<td>63.64</td>
</tr>
<tr>
<td>(8.1)</td>
<td></td>
<td></td>
<td>(4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'-F-dAdo-3',5'-P</td>
<td>89.42</td>
<td>91.65</td>
<td>76.72</td>
<td>71.87</td>
<td>67.61</td>
</tr>
<tr>
<td>(8.5)</td>
<td></td>
<td></td>
<td>(4.5)</td>
<td></td>
<td>(4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.8)</td>
</tr>
</tbody>
</table>

* \(^{13}\text{C}\) nmr spectra were recorded on a Hitachi R-900 (22.63 MHz) spectrometer operating in the Fourier transform mode. The nucleotides were measured in D\(_2\)O (pD 7.5) and the nucleoside in DMSO-d\(_6\).

Table II. Chemical shift changes (ppm)* in sugar carbons by the introduction of a 3',5'-cyclic phosphate group

<table>
<thead>
<tr>
<th>Compound</th>
<th>C(1')</th>
<th>C(2')</th>
<th>C(3')</th>
<th>C(4')</th>
<th>C(5')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ado-3',5'-P</td>
<td>3.30</td>
<td>-1.31</td>
<td>6.39</td>
<td>-14.35</td>
<td>5.37</td>
</tr>
<tr>
<td>2'-F-dAdo-3',5'-P</td>
<td>2.84</td>
<td>-2.40</td>
<td>7.55</td>
<td>-13.13</td>
<td>6.31</td>
</tr>
</tbody>
</table>

* \(\delta(3',5'-\text{cyclic phosphate}) - \delta(\text{nucleoside})\). The positive value represents a downfield shift.
lation of $^{13}$C shielding effects.

REFERENCES AND NOTES


12. T. Kaneyasu, unpublished result.


Received, 28th August, 1981