The influence of \( N^6 \)-protecting groups on the acid-catalyzed depuration of 2'-deoxyadenosine

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**ABSTRACT**

Kinetics for the acidic hydrolysis of several \( N^6 \)-substituted 2'-deoxyadenosines were studied in a wide pH-range. The proportions of the partial reactions proceeding via mono- and di-protonated substrates were estimated on the bases of the rate profiles obtained and the acidity constants determined spectrophotometrically for the monocations. The site of the initial protonation was established by the effects that trifluoroacetic acid exerted on the \( ^{15}N \) NMR chemical shifts. The exceptional lability of the monocations of \( N^6 \)-acyl protected compounds is suggested to result from the preferred N7 protonation.

**INTRODUCTION**

The acidic hydrolysis of 6-substituted 9-(2-deoxy-\( \beta \)-erythro-pentofuranosyl)purines has been shown to proceed by a rate-limiting departure of the protonated base moiety with a concomitant formation of a cyclic glycosyl oxocarbenium ion (Scheme 1).\(^1\) In consistence with this mechanism, the influence of polar groups at C6 on the rate of hydrolysis is only a moderate one. Electron-withdrawing substituents, for example, decrease the standing concentration of the protonated substrate, but simultaneously facilitate its heterolysis. These opposite effects generally cancel each other almost completely.\(^1\) Accordingly, it is rather surprising that \( N^6 \)-benzoyl group, employed frequently as a protecting group in the chemical synthesis of oligonucleotides, accelerates the acidic depuration by almost one order of magnitude.\(^2\) The present report is aimed to clarify mechanistically this anomaly.
RESULTS AND DISCUSSION

Fig. 1 shows the first-order rate constants obtained at different concentrations of oxonium ion for the hydrolysis of the N-glycosidic bond of several N$^6$-protected 2'-deoxyadenosines. With unprotected 2'-deoxyadenosine (1) and its N$^6$-(2-nitrophenylsulfonyl) (2) and N$^6$-(N-methyl-2-pyrrolidinediamine) (3) derivatives the reaction is apparently of first-order with respect to oxonium ion over the whole acidity range studied. The explanation for the linear pH-dependence is that the rate constants, $k_1/K_1$ and $k_2/K_2$, referring to the partial reactions via mono- and dication, are almost equal.$^{1,3}$ In contrast, N$^6$-benzoyl (4), N$^6$-(3-chlorobenzoyl) (5), N$^6$-(9-fluorenylmethoxycarbonyl) (6) and N$^6$-(2,2,2-trichloro-tert.-butyloxy carbonyl) (7) derivatives all exhibit curvilinear rate profiles passing through an inflection point at pH = pK$_A$. The shape of the curves strongly suggests that with these compounds $k_1/K_1$ is for some reason considerably larger than $k_2/K_2$.

Table 1 records the values obtained for the partial rate constants, $k_1/K_1$ and $k_2/K_2$, as described previously.$^1$ The rate constants referring to the hydrolysis via the dications are with all the compounds of the same magnitude. In contrast, the values of $k_1/K_1$ are with N$^6$-monoaoyl compounds (4-7) from 8 to 15 times larger than with 1 or 2. This difference may be explained by
Fig. 1: Rate-profiles for the hydrolysis of N^6-protected 2'-deoxyadenosines at 323.2 K.

studying the effects that addition of trifluoroacetic acid exerts on the ^15N NMR chemical shifts of various nucleosides. With unsubstituted 2'-deoxyadenosine (1) only the N1 shift is markedly influenced. In contrast, with N^6-acylated compounds (4-7) both the N1 and N7 resonances are shifted upfield, the effect on the N7 shift being with 4 and 5 even the predominant one. Accordingly, N^6-acyl groups appear to favor N7 protonation. This may partly, but not completely, result from their electronegativity, which lowers the basicity of N1 more than that of N7. For comparison, compound 2, which is slightly less basic than 4-7, is still predominantly protonated at N1. It has been suggested previously by Maki et al. that N^6-benzoyl group suppresses the amidine resonance in the pyrimidine ring and thus decreases the
Table 1: Partial rate constants for the acidic hydrolysis of N\textsuperscript{6}-protected 2'-deoxyadenosines at 323.2 K.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_1/K_1$ (dm\textsuperscript{3} mol\textsuperscript{-1} s\textsuperscript{-1})</th>
<th>$k_2/K_2$ (dm\textsuperscript{3} mol\textsuperscript{-1} s\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.027</td>
<td>0.025</td>
</tr>
<tr>
<td>2</td>
<td>0.025</td>
<td>0.043</td>
</tr>
<tr>
<td>4</td>
<td>0.39</td>
<td>0.035</td>
</tr>
<tr>
<td>5</td>
<td>0.34</td>
<td>0.042</td>
</tr>
<tr>
<td>6</td>
<td>0.24</td>
<td>0.031</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>0.046</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The partial rate constants, $k_1/K_1$ and $k_2/K_2$, are defined in Scheme 1.

electron density at N1 and increases it at N7.

The preceding findings suggest that the exceptionally facile hydrolysis of N\textsuperscript{6}-acyl-2'-deoxyadenosines through monoprotonated species results from a change in the site of initial protonation. In other words, an N7 protonated adenine ring is a better leaving-group than the N1 protonated one. This argument receives support from the known fact that (7H)adenine is more stable than its (1H)tautomer.\textsuperscript{5} At high concentrations of oxonium ion the hydrolysis takes place via the N1,N7-dication, and under such conditions the N\textsuperscript{6}-acyl derivatives are not exceptionally labile any more.

REFERENCES