Solution Conformations of Some Acyclo Nucleoside and Nucleotide Analogues of Antiviral Acyclonucleosides, and Their Substrate/Inhibitor Properties in Several Enzyme Systems

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Acyclonucleosides, Phosphates, Cyclic Phosphates, Solution Conformations, Substrate/Inhibitor Properties

Chemical and enzymatic procedures have been employed for the preparation of various phosphorylated derivatives of the acyclonucleoside 9-(1,3-dihydroxy-2-propoxymethyl)adenine, an analogue of the active antiviral agent 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG). In combination with the previously reported 2',3'-seco nucleosides and their phosphates and cyclic phosphates (Stolarski et al., Z. Naturforsch. 41c, 758–770, 1986), this made available a broad class of acyclonucleosides and nucleotides, the acyclic moieties of which are capable of mimicing the ribose and 2'-deoxyribose rings.

The solution conformations of the foregoing were determined with the aid of H, 13C and 31P NMR, and compared with those of DHPG and 9-(hydroxyethoxymethyl)guanine (Acyclovir, ACV). Particular attention was devoted to conformations about C-O bonds in different acyclic fragments, which demonstrated well-defined differences between 2',3'-seco derivatives on the one hand (conformational “rigidity”) and derivatives with DHP and AC acyclic chains on the other (rotation about the C(1')-O(4') bond). The overall results are in good general agreement with reported crystal structures, and are compared with those obtained by quantum mechanical calculations.

The conformational features of the various compounds are also discussed in relation to their substrate and/or inhibitor properties in a number of enzyme systems, including adenosine deaminase, phosphodiesterases, nuclease P1, 3'-nucleotidase and herpes virus type 1 thymidine kinase.

Abbreviations: AC, 2-hydroxyethoxymethyl; DHP, 1,3-dihydroxy-2-propoxymethyl; seco, 2',3'-seco or 1,5-dihydro-4-hydroxy-5-methyl-3-oxapentyl-2-[R]; ACV, Acyclovir or AcycloG, 9-(2-hydroxyethoxymethyl)guanine; ACVMP, monophosphate of AC; AC-Cyt, 1-(2-hydroxyethoxymethyl)cytosine, with similar connotations for AC-Aden, AC-Uri; DHPG, 9-(1,3-dihydroxy-2-propoxymethyl)guanine; DHP-Ade, adenine analogue of DHPG; DHPGMP, monophosphate of DHPG; DHP-AdeMP, monophosphate of DHP-Ade; DHP-Ade-diP, 3',5'-diphosphate of DHP-Ade; DHP-Ade-3':5'-cMP, the 3':5'-cyclic monophosphate of DHP-Ade; secoA, 2',3'-secoadenosine or 9-(1,5-dihydro-4-hydroxy-5-methyl-3-oxapentyl-2-[R])adenine, with similar connotations for other acyclonucleosides; secoG, monophosphate of secoG, and similarly for seco-5'-GMP and seco-5'-AMP; secoC-3':5'-cCMP, 2',3'-seccotidin-3':5'-cyclic phosphate, and similarly for secoA-3':5'-cCMP; TK, thymidine kinase; cPDase, cyclic nucleotide phosphodiesterase.

For purposes of simplicity, the abbreviated terms are used in the text, with the carbon atoms of the acyclic chains numbered as for the corresponding carbon atoms of the pentose ring, as shown in Scheme 1.

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Introduction

Since the initial demonstration that the acyclonucleoside Acyclovir (ACV, or acycloG, 9-(2-hydroxyethoxymethyl)guanine) is a potent antiviral agent [1, 2], now licensed for clinical use, intense interest has centred about the synthesis of acyclonucleosides analogues, particularly with reference to their potential antiviral activities, see e.g. [3]. This interest has been further enhanced by the finding that the mechanism of action of ACV is due to its selective phosphorylation in infected cells by the viral-coded, but not cellular, thymidine kinase (TK), followed by its phosphorylation by cellular enzymes to the triphosphate, which is in turn a selective inhibitor of the viral DNA polymerase [4, 5].

Amongst the multitude of acyclonucleoside analogues now available by synthetic routes, some exhibit significant antiviral activities, in a number of instances as a result of their specific or selective activities in viral-coded enzyme systems [6]. Purine and pyrimidine acyclonucleosides and acyclonucleotides have also proven to be very potent inhibitors of purine and pyrimidine nucleoside phosphorylases [7, 8].

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One common feature of many acyclonucleosides is the fact that the acyclic moieties may mimic some of the structural features of the intact pentose rings of normal nucleosides (Scheme 1). It appears reasonable to assume that the conformations of these acyclic moieties must play some role in interaction with the enzyme systems involved in nucleoside and nucleotide metabolism. This prompted us to initiate studies on the conformational properties of such compounds, including the long known (and in some instances readily available) 2',3'-seco nucleosides and nucleotides [9, 10]. While the exceptional flexibility of such systems is such that they may exhibit marked conformational changes during interaction with a given enzyme, a prerequisite for such studies is some knowledge of their conformational properties in the free form, extended in the present investigation to additional analogues: 2-hydroxyethoxy-methyl (AC) and 1,3-dihydroxy-2-propoxymethyl (DHP).

**Materials and Methods**

Melting points (uncorr.) were measured on a Boetius microscope hot stage.

Thin-layer chromatography was performed with Merck (Darmstadt, GFR) F₅₄₄ cellulose sheets, using the following solvent systems (v/v): (A) isopropanol: water:25% NH₄OH (4:4:1); (B) 1 M ammonium acetate:96% ethanol (2:5); (C) isopropanol: water:acetic acid (4:4:1); (D) isopropanol: 1% ammonium sulphate (7:3).

Pyridine was rendered anhydrous by storage over solid KOH, followed by distillation over CaH₂. POCl₃ was purified by distillation, and trimethyl phosphate by distillation under reduced pressure. Dowex resins were products of Bio-Rad (Richmond, CA., USA). Sephadex from Pharmacia (Uppsala, Sweden), and bacterial alkaline phosphatase from Koch-Light (Bucks, UK). 9-(2-hydroxyethoxy-methyl)guanine (ACV, acycloG) was a product of Wellcome (UK) and ACVMP was prepared as previously described [11]. 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) was a kind gift of Dr. J. G. Moffatt. Other products were commercial, reagent grade.

9-(1,3-dihydroxy-2-propoxymethyl)adenine (DHP-Ade) was prepared according to Ogilvie et al. [12] by condensation of 6-chloropurine, obtained by chlorination of hypoxanthine according to Beaman and Robins [13], with 1,3-dibenzoxy-2-propanol (kindly provided by Dr. M. Draminski). The product, m.p. 195–196 °C, compared to reported values of 193–195 °C [12] and 195–197 °C [14], was chromatographically homogeneous in 4 solvent systems (Table I).

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHP-Ade</td>
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<td>0.53</td>
<td>0.73</td>
<td>0.48</td>
</tr>
<tr>
<td>DHP-Ade-3' : 5'-cMP</td>
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<td>0.29</td>
<td>0.56</td>
<td>0.28</td>
</tr>
<tr>
<td>(R,S)-DHP-AdeMP</td>
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<td>0.08</td>
<td>0.53</td>
<td>0.22</td>
</tr>
<tr>
<td>DHP-Ade-diP</td>
<td>0.49</td>
<td>0.00</td>
<td>0.41</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table I. \( R_f \) values of DHP-Ade and its nucleotides on cellulose sheets with various solvent systems.

Scheme 1. Showing formal possible structural resemblance of: (a) AC-derivatives (B = guanine, R = H, PO₃⁻), (b) DHP-derivatives (B = guanine, adenine, R = H, PO₃⁻), (c) 2',3'-seco-derivatives (B = any base, R = H, PO₃⁻), (d) DHP-Ade-3' : 5'-cMP (B = adenine), (e) 2',3'-seco-nucleoside-3' : 5'-cMP (B = any base), to the corresponding parent nucleosides and nucleotides. The numbering system of the carbon atoms of the acyclic chains has been maintained to correspond to those of the pentose ring in a nucleoside.
2',3'-seco derivatives of nucleosides and nucleotides were prepared as previously described [9, 10].

(R,S)-monophosphate of DHP-Ade (DHP-AdeMP). DHP-Ade (290 mg, 1.2 mmol) was phosphorylated with the wheat shoot phosphotransferase system [11] and isolated on a 3.5 x 24 cm column of 200/400 mesh Dowex 1 x 8 (HCOO\(^{-}\)). An initial water wash removed unreacted nucleoside (8,600 OD\(_{260}\) units). Subsequent elution with a 0–1 m linear gradient of HCOOH (1.5 x 1.5 l) gave the nucleotide at about 0.48 m. Unreacted nucleoside was recycled by the foregoing procedure, and the combined products brought to dryness several times from water to remove formic acid. The product was crystallized from water in the form of small white plates, yield 181 mg (47%), m.p. 215–216 °C (preceded by signs of decomposition at 185 °C), free of inorganic phosphate, and chromatographically homogeneous (Table I).

DHP-Ade-3' :5'-cyclic phosphate was prepared by cyclization of 96 mg (0.3 mmol) DHP-AdeMP as previously described for 2',3'-secoA-3':5'-cMP [9], and the product crystallized from aqueous ethanol as the free acid in the form of white needles m.p. 285–286 °C (dec.), yield 66%. The product was chromatographically homogeneous (Table I), and fully resistant to alkaline phosphatase.

Chemical phosphorylation of DHP-Ade. To a suspension of 240 mg (1 mmol) DHP-Ade in 8 ml trimethylphosphate at 0 °C was added, in 5 portions of 50 µl over a period of 2.5 h, with stirring, of POCl\(_3\) (total 250 µl, 2.8 mmol). Stirring was continued for 1.5 h, following which the mixture was added to 50 ml of icd water, and the whole brought to neutrality with NaHCO\(_3\). The mixture was stirred intensively at room temperature for 30 min to remove CO\(_2\), and then loaded on a 3.5 x 26 cm column of DEAE Sephadex A-25 (HCOO\(^{-}\)). A water wash removed 400 OD\(_{260}\) units (2.7%) of unreacted nucleoside. Elution was then conducted with a 0–1 m linear gradient of triethylammonium carbonate (2 x 2 l), with collection of 19-m1 fractions. The first peak (fractions 40–55, 66% relative to starting nucleoside) was identified as the cyclic phosphate; the second (fractions 65–75, 9.5%) consisted of the monophosphate of DHP-Ade; and the third (fractions 106–121, 21.5%) was the DHP-Ade-3',5'-diphosphate. The monophosphate, DHP-AdeMP, was contaminated with inorganic phosphate, which was removed by passage through Dowex 1 x 8 (HCOO\(^{-}\)), to give a product with m.p. and other properties identical with those of the product obtained by enzymatic phosphorylation.

NMR spectroscopy

All spectra were recorded on a Bruker 270 AM instrument at a temperature of 30 °C.

\(^1\)H NMR spectra were run on solutions at a concentration of 0.05 M. Chemical shifts, to an accuracy of ±0.005 ppm, were measured relative to internal Me\(_4\)Si for solutions in (C\(_2\)H\(_2\))SO, and relative to internal 2,2,3,3-tetadeutero-3-(trimethylsilyl)-propane, sodium salt (TSP) for solutions in \(^2\)H\(_2\)O. Coupling constants \(^1\)H-\(^1\)H were determined to an accuracy of ±0.1 Hz.

\(^13\)C NMR spectra were run on 0.3 M solutions at a frequency of 67.93 MHz. Chemical shifts are relative to internal dioxane. The values of \(^1\)H-\(^13\)C coupling constants were determined either directly from multiple splitting of the \(^13\)C signals; or, in the case of a large number of coupled protons, by successive decoupling of each of these. Varying decoupling intensities were employed, and allowance made for the influence of the decoupling signal for a given proton on the values of \(^1\)H-\(^13\)C coupling constants with other protons. Values of \(^1\)H-\(^13\)C coupling constants were estimated to an accuracy of ±0.3 Hz.

\(^31\)P NMR spectra were recorded at a frequency of 109.35 MHz, under conditions similar to those for \(^1\)H NMR spectra.

Results and Discussion

Enzymatic phosphorylation of DHP-Ade was carried out with the wheat shoot phosphotransferase system [11] as previously described for 2',3'-seco nucleosides [9, 10]. This led to one product, identified as a monophosphate by its chromatographic mobility (Table I), by \(^1\)H and \(^31\)P NMR spectroscopy (Table II), and its quantitative conversion to DHP-Ade (Table I) by treatment with alkaline phosphatase.

The prochiral 3'- and 5'-hydroxymethyl groups of DHP-Ade are magnetically equivalent because of the symmetry of the system (Tables II and III; see also below). Phosphorylation of one of these leads to chirality, the \(R\) and \(S\) enantiomers being esterified at the 3' and 5' positions, respectively. Phosphorylation of one of these should lead to asymmetry and separation of the signals of the two groups. Since no such
Table II. \(^1\)H chemical shifts (in ppm vs internal Me₄Si in (C\(^2\)H₅)₂SO, and vs internal TSP in \(^2\)H₂O) and \(^13\)C chemical shifts (in ppm vs internal dioxane) for acyclo nucleoside and acyclo nucleotide analogues.

<table>
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<th>Analogue</th>
<th>Solvent</th>
<th>H(2)</th>
<th>H(8)</th>
<th>H(1')</th>
<th>H(2')</th>
<th>H(3')</th>
<th>H(3')</th>
<th>H(4')</th>
<th>H(5')</th>
<th>H(5')</th>
</tr>
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<tbody>
<tr>
<td>ACV</td>
<td>((C^2)H(^5))SO</td>
<td></td>
<td></td>
<td>7.81</td>
<td>5.35</td>
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<td>-</td>
<td>-</td>
<td>3.47</td>
<td>3.47</td>
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<tr>
<td>ACV</td>
<td>(^2)H₂O</td>
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<td></td>
<td>7.95</td>
<td>5.53</td>
<td></td>
<td>-</td>
<td>-</td>
<td>3.68</td>
<td>3.68</td>
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<tr>
<td>ACVMP</td>
<td>(^2)H₂O</td>
<td></td>
<td></td>
<td>7.96</td>
<td>5.55</td>
<td></td>
<td>-</td>
<td>-</td>
<td>3.77</td>
<td>3.98</td>
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<tr>
<td>DHPG</td>
<td>((C^2)H(^5))SO</td>
<td></td>
<td></td>
<td>7.79</td>
<td>5.44</td>
<td></td>
<td>-</td>
<td>3.43</td>
<td>3.30</td>
<td>3.54</td>
</tr>
<tr>
<td>DHPG</td>
<td>(^2)H₂O</td>
<td></td>
<td></td>
<td>7.38</td>
<td>5.60</td>
<td></td>
<td>-</td>
<td>3.64</td>
<td>3.55</td>
<td>3.78</td>
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<tr>
<td>DHP-Ade</td>
<td>((C^2)H(^5))SO</td>
<td></td>
<td></td>
<td>8.16</td>
<td>8.24</td>
<td>5.65</td>
<td>-</td>
<td>3.43</td>
<td>3.31</td>
<td>3.61</td>
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<td>DHP-Ade (^2)H₂O</td>
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<td>8.26</td>
<td>5.76</td>
<td>-</td>
<td>3.65</td>
<td>3.56</td>
<td>3.79</td>
<td>3.65</td>
<td>3.56</td>
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<tr>
<td>((R,S))-DHP-AdeMP (^2)H₂O</td>
<td>8.23</td>
<td>8.34</td>
<td>5.83</td>
<td>5.80</td>
<td>-</td>
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<td>4.24</td>
<td>4.37</td>
<td>3.88</td>
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<td>5.99</td>
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<td>3.77</td>
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</tr>
<tr>
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<td>5.86</td>
<td>4.03</td>
<td>4.00</td>
<td>3.86</td>
<td>3.75</td>
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<td>3.81</td>
<td>3.71</td>
<td>3.86</td>
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<td></td>
</tr>
<tr>
<td>SecoC-3'5'-eMP (^2)H₂O</td>
<td>6.28</td>
<td>8.06</td>
<td>5.97</td>
<td>3.83</td>
<td>3.83</td>
<td>4.41</td>
<td>4.41</td>
<td>3.87</td>
<td>4.14</td>
<td>4.38</td>
</tr>
</tbody>
</table>

C(2) C(4) C(5) C(6) C(8) C(1') C(2') C(3') C(4') C(5')

\(^a\) The signals of both H(1') protons in analogues with the AC and DHP acyclic chains, and of both H(4') protons in the AC analogues, exhibit identical chemical shifts.

Separation was observed, whereas the protons at C(1') do exhibit magnetic non-equivalence (Table II), the product of phosphorylation must be a race-mate with comparable populations of \(R\) and \(S\), consistent with the known lack of specificity of the wheat shoot enzyme [11]. By contrast, the herpes virus coded thymidine kinase specifically phosphorylates only the 5'-CH₂OH to give the \(S\) enantiomer [15], but this is not at the moment applicable on a preparative scale because of the small quantities of enzyme available.

It is of interest, however, that the yield of DHP-AdeMP from a single run (~ 45%) was considerably higher than for phosphorylation of a seco nucleoside, 2',3'-seco cytidine (5% phosphorylation of the 5'(3') hydroxyls + 2.5% of the 2'-OH).

Chemical phosphorylation. As anticipated from previous findings with seco nucleosides [9, 10], treatment of DHP-Ade with POCl₃ in trimethylphosphate yielded three homogeneous products (see Scheme 2), isolated by column chromatography. The first, chromatographically identical with the product of cyclization of DHP-AdeMP with dicyclohexylcarbodiimide (see Materials and Methods), resistant to alkaline phosphatase, and containing only one phosphate as indicated by intensity of the \(^31\)P signal, was identified as the 3':5'-cyclic phosphate, further confirmed by enzymatic opening of the cyclic phosphate ring (see below). The second product (in order of elution from the column) was chromatographically identical with the product of enzymatic phosphorylation of DHP-Ade (see above), with the same m.p., and with \(^1\)H and \(^31\)P NMR spectra identical to those of the product of enzymatic phosphorylation, must be \((R,S)\)-DHP-AdeMP. The third, also chromatographically homogeneous, with a lower mobility on cellulose than the monophosphate (Table I), and exhibiting the presence of 2 phosphate groups by \(^31\)P NMR, magnetically identical (Table II), must be the 3',5'-diphosphate of DHP-Ade, additionally con-
firmed by its quantitative conversion by alkaline phosphatase to DHP-Ade via an intermediate with the \( R_f \) of DHP-AdeMP (Table I).

Particularly noteworthy is the high yield of the cyclic phosphate (\( \sim 65\% \)), even higher than the yields of the 3'':5'-cyclic phosphates of seco adenosine and cytidine by the same procedure, 50–55% [9, 10]. This is in striking contrast to the results of Tolman and MacCoss [16], who phosphorylated DHP-Ade with POCl\(_3\) in triethylphosphate and found, as the major products, the \((R,S)\)-monophosphate (30%) and the 3',5'-diphosphate (34%). Another report from the same laboratory [17] on analogous phosphorylation of DHPG gave, as the main product, the 3',5'-diphosphate (73%), and only 7% of the 3':5'-cyclic phosphate. It is conceivable that these low yields, or absence, of the cyclic phosphates are due to the use of triethylphosphate, as against trimethyl phosphate in our procedure.

Subsequently Prisbe et al. [18] improved the foregoing procedure by solubilization of DHPG in acetonitrile with stannic chloride, and then phosphorylating with pyrophosphoryl chloride. This gave the cyclic phosphate in 50% yield, and the cyclic phosphate of DHP-Ade in 57% yield. It was concluded that the enhanced solubility of the acyclonucleosides, presumed due to formation of a \( \sigma \)-complex between the heterocyclic base and stannic chloride, was responsible for the enhanced yields of the cyclic phosphates. This interpretation does not account for the difference between our results and those of Tolman and MacCoss [16]. An alternative interpretation is the ability of stannic chloride to form a complex with cis-diols, facilitating their etherification, the mechanism of which has been elucidated [19]. Since the 3'- and 5'-hydroxyls of DHPG (and DHP-Ade) may form isopropylidene derivatives [20] it is possible that these two hydroxyls may also form a complex with stannic chloride, and that this facilitates formation of the cyclic phosphate. In view of the widespread interest in acyclonucleosides and their phosphorylated products, this problem is deserving of further investigation.

The high yields of the 3':5'-cyclic phosphates obtained by POCl\(_3\) phosphorylation of seco nucleosides [10] and compounds like DHP-Ade are, at first sight, rather unexpected, since this phosphorylating agent is the most widely employed for high-yield synthesis of nucleoside 5'-phosphates [21]. There are, however, some interesting relevant exceptions. POCl\(_3\) treatment of xylofuranosyladenine in trimethylphosphate led to a 38% yield of the 3':5'-cyclic phosphate [22]; it is consequently worth noting that the solid state structure of DHPG (and presumably of DHP-Ade and of seco nucleosides) exhibits the 3'-CH\(_2\)OH group in the “up”, xylo, form [23]. Marumoto et al. [24] found that treatment of 8-bromo-2-chloroadenosine with trichloromethylphosphorodichloride led, via an intermediate nucleoside 5'-trichloromethylphosphonate, to a 40% yield of the 3':5'-cyclic phosphate.

**Enzymatic hydrolysis of DHP-Ade-3':5'-cyclic phosphate**

It was previously shown that secoC-3':5'-cMP is resistant to hydrolysis of the cyclic phosphate ring by
beef heart cyclic nucleotide phosphodiesterase [10]. This was confirmed by the finding that seco-A-3':5'-cCMP exhibits no biological activity in such mammalian systems (B. Jastorff, personal communication). We did, however, find that both seco-C-3':5'-cCMP and seco-A-3':5'-cCMP are substrates of higher plant (potato tuber) cyclic nucleotide phosphodiesterase, with opening of the cyclic phosphate rings to give the monophosphates, at a rate about 10% that for cAMP [10]. We now find, on application of the same procedure, that DHP-Ade-3':5'-cyclic phosphate is an even better substrate of the plant enzyme, which converts it to the monophosphate at a 5-fold higher rate than for the seco nucleoside cyclic phosphates. This may be of physiological significance since, as we have previously pointed out, mammalian systems contain a non-conventional cyclic nucleotide phosphodiesterase [25] with properties strikingly similar to that of our plant enzyme.

**Enzymatic hydrolysis of acyclonucleotides**

Rye grass 3'-nucleotidase is known to readily dephosphorylate 3'-AMP, but not 3'-dAMP. It relatively rapidly hydrolyses DHP-Ade-diP to DHP-AdeMP (probably the 5'), and then slowly to DHP-Ade. (R,S)-DHP-AdeMP is slowly hydrolyzed, presumably to the 5'-phosphate.

By contrast, nuclease P1, which is also a 3'-nucleotidase and readily dephosphorylates 3'-AMP, but not 3'-dAMP, is inactive vs seco-5'-AMP, seco-2'-CMP and seco-5'-CMP, as well as DHP-AdeMP and DHP-3',5'-diphosphate.

**Conformational analysis**

Conformation about C-C bonds

For the compounds herein examined (Scheme 1), the acyclic moieties may mimic to different extents the pentose moiety via the O(4') oxygen and the different number of hydroxymethyl groups at C(1') and C(4').

Analysis of the vicinal 1H-1H coupling constants (Table III), and application of a modified Karplus relationship [26], leads to the conformations about C(1')-C(2'), C(3')-C(4') and C(4')-C(5'). As previously shown for purine seco nucleosides and nucleotides [9], rotation about C-C bonds results in significant populations of all allowable gauche and trans conformers (Scheme 3), with a tendency for predominance of the gauche form. The same situation prevails for the acyclic analogues embraced in this study. For the cytosine seco congeners the population of the g- form is about 40%, and the g+ and t forms each about 30%, about the C(1')-C(2') bond; as well as comparable populations of 30–35% for all

**Table III. Values of coupling constants 1H-1H and 1H-31P, as well as of some vicinal coupling constants 1H-13C, employed for determination of conformational properties of acyclonucleoside and acyclonucleotide analogues.**

<table>
<thead>
<tr>
<th>Analogue</th>
<th>Solvent</th>
<th>J(1'H, 1'H)</th>
<th>J(1'H, 31P)</th>
<th>J(1'H, 13C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1',2' 1',2' 2',2' 3',3' 3',4' 3',4' 4',5' 4',5' 5',5' 5',5'</td>
<td>3',P 3',P 5',P 5',P 1',4' 1',4'</td>
<td>1',4' 1',4'</td>
</tr>
<tr>
<td>ACV</td>
<td>(C(^2)H(_3))SO</td>
<td>- - - - - - - - a a b b - - - - -</td>
<td>3.3 4.2 3.1 5.1</td>
<td></td>
</tr>
<tr>
<td>ACVMP</td>
<td>(^2)H(_2)O</td>
<td>- - - - - - - - - - -</td>
<td>6.6 6.6 3.2 4.1 3.4 4.6</td>
<td></td>
</tr>
<tr>
<td>DHPG</td>
<td>(C(^2)H(_3))SO</td>
<td>11.3 4.8 5.6 4.8 5.6 11.3 - - - - -</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>DHP</td>
<td>(^2)H(_2)O</td>
<td>12.4 4.1 6.1 6.1 12.4 - - - - -</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>DHP-Ade</td>
<td>(C(^2)H(_3))SO</td>
<td>11.4 4.8 5.8 4.8 5.8 11.4 - - - - -</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>DHP-AdeMP</td>
<td>(^2)H(_2)O</td>
<td>12.3 4.0 6.2 4.0 6.2 12.3 - - - - -</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>(R,S)-DHP-AdeMP</td>
<td>(^2)H(_2)O</td>
<td>12.4 3.8 5.7 4.5 6.0 11.2 6.0 6.0 6.0 6.0</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>DHP-Ade-diP</td>
<td>(^2)H(_2)O</td>
<td>11.2 4.4 4.4 4.4 4.4 11.2 5.4 6.0 5.4 6.0</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>DHP-Ade-3':5'-cCMP</td>
<td>(^2)H(_2)O</td>
<td>12.8 2.3 2.1 2.1 2.1 12.8 19.9 3.8 19.9 3.8</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>SecoA</td>
<td>(C(^2)H(_3))SO</td>
<td>5.7 5.7 5.7 5.7 5.7 5.7 5.7 5.7 5.7</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>SecoC</td>
<td>(C(^2)H(_3))SO</td>
<td>5.4 5.0 12.2 12.2 3.5 3.5 12.2 12.2 3.5 3.5 3.5 3.5</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>Seco-GMP</td>
<td>(^2)H(_2)O</td>
<td>5.1 5.9 12.2 12.2 3.3 3.3 4.5 4.5 5.0 5.0 5.0 5.0</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>Seco-CMP</td>
<td>(^2)H(_2)O</td>
<td>4.9 4.9 4.9 4.9 4.9 4.9 4.9 4.9 4.9 4.9 4.9 4.9</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
<tr>
<td>Seco-5'-cCMP</td>
<td>(^2)H(_2)O</td>
<td>4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8 4.8</td>
<td>3.2 4.2 5.3 4.5</td>
<td></td>
</tr>
</tbody>
</table>

a Signal overlapping precluded measurements of coupling constants.
b This is a "deceptively simple" ABX system, hence only the mean vicinal coupling AX and BX were measured.
^ An additional coupling constant, 11.2 Hz, is observed between the non-equivalent protons at C(1')H (see Table II).
^ Values only approximate because of signal overlapping.
three forms about C(3')-C(4') and C(4')-C(5'). For the DHP analogues it is not possible to independently determine the conformer populations about C(4')-C(5') and C(3')-C(4'), due to the symmetry of these systems, as a result of which $\delta H(5') = \delta H(3')$ and $\delta H(5") = \delta H(3")$ (see Table II). The mean populations of the three conformers about these bonds are similar (30–40%), and independent of the solvent. In the case of ACVMP the $g^+$ form about C(4')-C(5') predominates (50%).

From the vicinal couplings $^1$H-$^1$H and $^1$H-$^{31}$P, the conformation of the 3':5'-cyclic phosphate ring in the seco and DHP analogues appears to be in the "chair" form with O(4') oriented gauche relative to the ring oxygen (cf. ref. [9]). The chair conformation of the cyclic phosphate ring in the seco and DHP analogues differs from the chair conformation in conventional 3':5'-nucleotides in that, in the latter, O(4') adopts the orientation equatorial trans relative to the ring oxygens [27, 28]. This is a consequence of steric effects involving the 5-membered sugar ring (Scheme 4).

Conformation about glycosidic bond

Conformations about the glycosidic bond of seco-5'-CMP and secoC-3':5'-cMP may be estimated from the values of the coupling constants between H(1') and the pyrimidine C(2) and C(6) (Table III), with the aid of the parametrization of the Karplus relationship proposed by Davies et al. [29], and based on several simplified assumptions. For both compounds there is a syn-anti dynamic equilibrium (Scheme 5) between the glycosidic torsion angles $\chi_{anti} = 290^\circ$ (and/or 190°) and $\chi_{syn} = 110^\circ$ (and/or 10°), with a preference for the conformation anti (population 65–70%).

In the case of purine nucleosides and nucleotides there are no appropriate Karplus relationships which link the $^1$H-$^{13}$C coupling constants with the dihedral angles of the fragments H(1')-C(1')-N(9)-C(8) and H(1')-C(1')-N(9)-C(4). An additional difficulty with congeners of the AC and DHP types derives from the fact that the spectra provide only the mean values of the coupling constants of carbon atoms with the two magnetically equivalent H(1') protons. For normal nucleosides and nucleotides, however, the populations of the syn and anti conformers may be deduced from the chemical shifts of the pentose protons and carbons [30]. Extension of the foregoing to a comparison of the values of the carbon-proton coupling constants in normal nucleosides and nucleotides (Stolarski et al., submitted to Life Science Advances) with the corresponding values for the acyclic analogues, renders possible evaluations of the syn and anti populations of the latter. In this way it was established that the purine acyclo analogues ex-
Conformations about the O(4')-C(1') and C(4')-O(4') bonds

Resolution of the conformations about C-O bonds is a prerequisite for establishment of the overall structure of acyclonucleosides, and the extent to which they mimic the structure of the pentose ring in normal nucleosides and nucleotides. The difficulties associated with determination of these conformations are due simply to the fact it is possible to measure only one $^1$H-$^{13}$C coupling constant, either because of the presence of substituents at C(1') and C(4'), or the symmetry of the system, which leads to identical chemical shifts for the pairs of protons at C(1') and C(4'). There is also the lack of an appropriate Karplus relationship to link the $^1$H-$^{13}$C coupling constants with the dihedral angles in the fragments H(1')-C(1')-O(4')-C(4') and H(4')-C(4')-O(4')-C(1'). An approximation to the required relationship may be obtained from measurements of $^1$H-$^{13}$C coupling constants in ether type fragments [31, 32], from which a dihedral angle of $60^\circ$ corresponds to a coupling constant of about 3 Hz, and an angle of $180^\circ$ to 9-11.5 Hz. It may also be assumed that the molecules adopt conformations close to the classical gauche and trans about a C-O bond, as shown for the examined acyclic fragments in Scheme 6. Crystallographic data [33] also point to possible deviations of up to $20^\circ$ from the typical angles $60^\circ$ and $180^\circ$.

The conformations of the acyclic fragments of secoA and secoA-3':5'-cMP in $^2$H$_2$O were previously determined [9]. We now examine secoA in (C$_2$H$_5$)$_2$SO, as well as seco-5'-GMP, seco-5'-CMP and secoC-3':5'-cMP in order to determine the dependence of the conformation, if any, on the nature of the base and the solvent. For all the seco derivatives the coupling constants are comparable, with J[C(1'),H(4')] = 3.5-4.5 Hz and J[C(4'),H(1')] = 2.5-3.2 Hz, pointing to very similar conformations. Similar conformations may be assumed for secoC and seco-5'-AMP (for which the $^{13}$C spectra were not recorded) from the similarity of their $^1$H spectra. The conformation $g^+$ or $t$, or a dynamic equilibrium between both, may exist about the C(1')-O(4') bond (Scheme 6), corresponding to an angle between H and C of $60^\circ$. Solid-state data for seco-DRB [33] point to predominance of the conformer $g^+$ with an extended carbon chain. The forms $t$ and $g^-$ may also exist about the bond C(4')-O(4'), both with extended carbon chains. Solid state data suggest predominance of the form $t$. Deviations of coupling constants from 3 Hz may be due to rotation of about $20^\circ$ in the range of the conformation $t$, as in one of the independent molecules in the seco-DRB crystal unit, or, alternatively, to a low participation (up to 20%) of the form $g^+$, although we consider the latter less likely.

For analogues with the DHP acyclic chain, deviations of the values of the coupling constants $J[C(1'),H(4')]$ from those typical for the form $t$ about the C(4')-O(4') bond (3 Hz) are even more pronounced than for the seco derivatives (Table III). In addition, and independently of solvent, they exhibit magnetic equivalence of the 3'- and 5'-hydroxymethyls, i.e. $\delta H(3') = \delta H(5')$, $\delta H(3') = \delta H(5')$, and $\delta C(3') = \delta C(5')$ (see Table 1). This, in conjunction with rotation about the C(1')-C(4') bond (see below)
points rather to a dynamic equilibrium deviating to a small extent from the classical \( t \) and \( g^+ \) forms, and likely involvement of the form \( g^- \) (~ 30%), which would lead to symmetry of the system about the plane through the C-O bond. The mean value of the coupling constants \( J(C(4'),H(1')) \) for both H(1') protons is 4.5 Hz, which does not correspond to any unique conformation about the C(1')-O(4') bond either with angles of 60° or 180°, or angles deviated from the latter two up to 30°. There is rather a dynamic equilibrium of the forms \( t \) and \( g \) (because of symmetry, the forms \( g^- \) and \( g^- \) are equivalent) with comparable populations of 50%.

Similarly, for analogues with the AC chain the \( g \) and \( t \) forms are in dynamic equilibrium about C(1')-C(4'), with a somewhat higher population for the \( g \) conformer (60–70%). By contrast, the conformer \( t \) about C(4')-O(4') is predominant. Deviations from classical forms do not exceed 10°, and participation of the form \( g \) does not exceed 10%. These results are consistent with solid-state data for ACV [34], where all three independent molecules in the unit cell exhibit the conformation \( t \) about C(4')-O(4'), but differ in conformation about C(1')-C(4'), viz. two extended forms of type \( t \), and one of type \( g \).

**Comparisons of conformations with different acyclic chains**

The foregoing results emphasize in a more quantitative manner the more pronounced flexibility of acyclonucleosides as compared to that of nucleosides and nucleotides. The former exhibit dynamic equilibrium of all allowable conformations (with comparable populations) about the C-C and the glycosidic C-N bonds. A certain degree of “rigidity”, however, exists about the C-O bonds. The extended form about C(4')-O(4'), with transoidal location of carbons, is predominant in all the acyclo compounds. There is, on the other hand, a significant difference in conformations about C(1')-O(4') between the seco analogues and those with a DHP or AC chain. Similar to the latter, the predominant form is an extended one with a transoidal orientation of C(2') and C(4') (gauche orientation of C(4') and N); whereas \( g \) and \( t \) forms exhibit comparable populations for the latter. This is most likely due to the CH\(_2\)OH group at C(1') of the seco congeners and its repulsive interaction with the remainder of the chain. The nature of the base, and of the solvent, appear to have only minimal, if any, effects. Solid-state results (even in the absence of any for those with a DHP chain) are fully consistent with our solution data.

Quantum mechanical calculations for ACV and AC-Cyt (Wesolowski and Geller, submitted) and secoG and DHPG (Wesolowski et al., in preparation), using the forcefield approach, lead to the inference that the acyclic moieties possess a large number of conformational minima in the range up to 16 kJ/mol relative to the global minimum (with 14 differing conformations for ACV). Conformational flexibility prevails about both C-C and C-O bonds. In general there is a more marked preference for the gauche type forms about C(1')-C(4') and C(4')-O(4'). These result in twisting of the acyclic chains, so that they are in closer proximity to the base, notwithstanding that the trans type forms show up at about 4 kJ/mol above the global minimum for the analogues with an AC, DHP and seco chain. The stabilization ascribed to interaction of the twisted acyclic chains with the base may be due to the fact that such calculations are conducted for a molecule in a “vacuum”. In polar solvents, such as water or (CH\(_3\))\(_2\)SO, such intramolecular interaction would be strongly reduced, leading to the extended, zig-zag, conformations of the acyclic chains.

**Biological aspects**

Consideration of the role of the conformations of acyclonucleosides in biological systems, particularly in enzymatic reactions, must necessarily take account of the following general findings from the foregoing investigation:

(a) In contrast to nucleosides and nucleotides, the solution conformation of the acyclic chain of an acyclonucleoside or acyclonucleotide is virtually independent of the nature of the aglycone.

(b) The conformation of a 2',3'-seco chain differs significantly from chains such as AC or DHP, due to the role of the 2'-OH.

(c) The conformation of the 3':5'-cyclic phosphate ring in acyclic chains such as DHP or 2',3'-seco is different from that in nucleoside-3':5'-cyclic phosphates (see Scheme 4).

The antiviral activity of acyclonucleoside analogues in HSV-infected cells is dependent on their prior phosphorylation by viral thymidine kinase (TK). Crystallographic and NMR data have shown that the mutual orientation of the 5'-OH and 3'-OH groups is of key importance in the phosphorylation
step [35]. Relevant to this is the observation that 5-methoxymethyl-1-(2'-deoxy-β-D-lyxofuranosyl) uracil [36] in which the cisoidal hydroxyl groups are close to each other, is not phosphorylated by viral TK; whereas 5-methoxymethyl-2'-deoxyuridine, in which the two hydroxyls are widely separated, is a substrate.

The presence of the 3'-OH nonetheless facilitates phosphorylation of the 5'-OH; and phosphorylation of DHPG by viral TK yields only one of the monophosphates with the type S configuration about C(4'), corresponding to the configuration of the 5'-monophosphate of a nucleoside. The rate of phosphorylation of DHPG is 6-fold that for ACV, which lacks the second hydroxyl at C(4'), while acycloguanosine with three primary hydroxyls at C(4') is also a substrate [15]. This points to the requirement by the viral TK of structural similarity of the acyclic fragment about C(4') to the parent pyrimidine nucleoside substrate, e.g. thymidine or deoxycytidine [2].

By contrast, substituents at C(1') more profoundly affect interaction with the HSV-1 TK. Neither 2',3'-secoG, nor the ACV analogue with a methyl at C(1'), are substrates, and are only weak inhibitors [2]. It should be noted that the acyclic chains AC and DHP may be regarded as analogues of the deoxyribofuranose ring (Scheme I), whereas the 2',3'-seco chain does not quite correspond to the ribofuranose ring, and its conversion to a form more closely resembling the latter would require considerable energy (T. Wesolowski et al., in preparation).

It should also be noted that, apart from a requirement for structural similarity of the acyclic moiety to that of the pentose ring, the nature of the aglycone is equally important, e.g. the best substrate is 2'-deoxyctydine, although uridine and araU are better substrates than 2'-deoxyuridine [2]. ACV and DHPG are good substrates, whereas 2'-deoxyguanosine is inactive, as are also AC-Cyt and DHP-Cyt, although DHP-Ura is phosphorylated at 14% the rate for thymidine [2].

A similar behaviour is manifested by some adenine acyclonucleosides in the adenosine deaminase system, in studies including a broad class of adenosine analogues with modified sugar rings. Most of the acyclonucleoside analogues, including secoA and some derivatives, and those with alcoholic chains of varying lengths and number of OH groups, exhibited only inhibitor properties with \( K_i \) values in the range \( 10^{-4} - 10^{-5} M \) [37–39]. By contrast, AC-Ade and DHP-Ade were weak substrates with \( K_m \) values of 1.3 x 10^{-4} M and 1.9 x 10^{-3} M, and \( V_{max} \) about 2% that for adenosine [37, 12]. These results were rationalized in terms of a “tight-fit” model for the furanose ring at the active site of the enzyme [39], with a lack of bulk tolerance for substituents at C(1').

This reasoning led to exclusion as a substrate of secoA, as well as psicofuranin and 9-β-D-fructofuranosyl adenines. The inhibitory properties of secoA were interpreted in terms of possible adoption (on binding to the enzyme) of a conformation such that the C(2')-OH interacts with the specific binding site for inhibitor hydroxyls, notwithstanding that the solution conformation of secoA was then not known. This model now appears fairly reasonable, even though it does not fully explain some subsequent observations e.g. (a) most acyclo analogues bind to the enzyme, but are not hydrolyzed despite the fact that the acyclic chains are capable of adopting a conformation resembling that of the sugar ring; (b) 8:5'- (5)-cycloadenosine, containing the intact sugar ring, with the 5'-OH in the fixed \( t \) orientation, and the base in the fixed anti conformation, is an inhibitor but not a substrate; (c) the second epimer, 8:5'- (R)-cycloadenosine, with the 5'-OH in the g' conformation, is neither substrate nor inhibitor, whereas inhibitory properties are displayed by the carbocyclic analogue of adenosine, and a congner with no, or only one, hydroxyl groups [39]; introduction of additional hydroxyls, including the 5'-OH, confers substrate properties [40]. The “tight-fit” model therefore involves binding of substrates in a manner differing from that for inhibitors, and, e.g. \( K_i \) for DHP-Ade is an order of magnitude lower than \( K_m \) for adenosine [12]. In contrast to inhibitors, binding conditions which permit of deamination require the presence of at least two hydroxyls appropriately oriented, e.g. a ring hydroxyl and the 5'-OH, or a 5'-OH and 3'-OH.

We have elsewhere reported that the 3':5'-cyclic phosphates of seco-adenosine and cytidine are not substrates of beef heart cAMP phosphodiesterase. This has been more extensively investigated by Prof. B. Jastroff (personal communication), who examined seco-adenosine-3':5'-cyclic phosphate in the binding assay to cAMP-dependent protein kinase; no binding was detected at concentrations as high as 0.5 M, and it was concluded that the conformational
freedom, especially in the 2'-OH region, is too high, bearing in mind the stereochemistry at the 2', 3' and 5' regions of cAMP essential for biological activity [4].

It is consequently of interest that the 3':5'-cyclic phosphates of seco-adenosine and seco-cytidine are hydrolyzed by higher plant cPDase, albeit at only 10% the rate for cAMP. We have now found that the rate of hydrolysis of the 3':5'-cyclic phosphate of DHP-Ade is increased 5-fold relative to the 3':5'-cyclic phosphate of secoA [10]. One obvious conclusion is that the plant enzyme does not possess the stringent requirements for the cyclic phosphate ring conformation exhibited by the mammalian enzymes. In the light of the controversy regarding the physiological significance of the plant enzyme [42], the foregoing observations strongly imply that the latter does not emulate in higher plants the specific physiological functions of cAMP phosphodiesterases in mammalian systems.

Particularly interesting is the specificity of rye grass 3'-nucleotidase, which hydrolyzes ribonucleoside, but not 2'-deoxyribonucleoside-3'-phosphates. This enzyme slowly hydrolyzes only one of the epimers of DHP-AdeMP, presumably the R, with the phosphate at the 3'-position, notwithstanding that this is a 2'-deoxynucleotide analogue. By contrast, the 3',5'-diphosphate of DHP-Ade, also a 2'-deoxy-nucleotide analogue, is initially more rapidly hydrolyzed to the monophosphate (presumably the S epimer), and then slowly to the nucleoside. A possible interpretation of this is that the presence of two phosphate groups enhances binding to the enzyme and that, following hydrolysis of the 3'-phosphate, the enzyme rotates the 5'-phosphate about the C-O bond to the 3'-position, with resultant slow hydrolysis.

Acknowledgements

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[36] This is the nomenclature employed by the authors. Actually this should be 1-(2'-deoxy-β-D-threo-pentofuranosyl)-5-methoxy-methyluracil.