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Short Communication

Interconversion and Hydrolysis of 1-[(2′*S*)-2′,3′-Dihydroxypropyl]cytosine Analogues of Isomeric Dinucleoside Monophosphates, 2′,5′-CpA and 3′,5′-CpA

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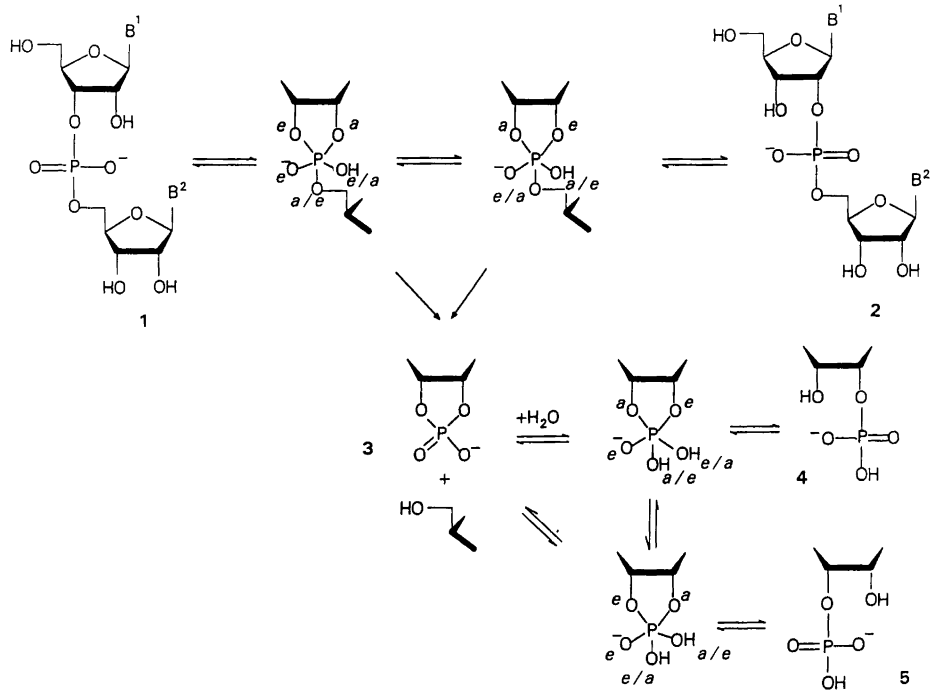
Dinucleoside 3′,5′-monophosphates (**1**), the fragments of ribonucleic acids (RNA), undergo in aqueous acid two concurrent reactions: (i) phosphodiester hydrolysis and (ii) intramolecular transesterification to the 2′,5′-isomer.[1] Under neutral conditions the latter reaction predominates, whereas in aqueous alkali only hydrolysis takes place. Both reactions proceed through a common pentacoordinated phosphorane intermediate obtained by an intramolecular nucleophilic attack of the 2′-hydroxy group on phosphorus (Scheme 1; apical and equatorial ligands on phosphorus indicated by *a* and *e*).[1–3] We now report on the corresponding reactions of 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine-3′-*O*-phosphorylyl-(3′,5′)-adenosine (**6**), an acyclic analogue of **1**. In addition, the



Scheme 1.

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reactions of the initial and final hydrolysis products of **6**, *viz.* 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine 2′,3′-cyclic monophosphate (**8**), 3′-monophosphate (**9**) and 2′-monophosphate (**10**) (Scheme 2) have been studied. It is known that these kinds of acyclic analogues of dinucleoside monophosphates and nucleoside 2′,3′-cyclic monophosphates are rather stable towards enzymic hydrolysis.[4,5] For example, the pancreatic RNAase A catalysed hydrolyses of **6** and **8** are 10^5 and 10^3 times slower than those of the natural substrates, respectively.[5] For this reason the kinetics of the non-enzymic reactions are of considerable interest.

Fig. 1 shows the time-dependent product distribution for the hydrolysis of the acyclic analogue, **7**, of 2′,5′-CpA in aqueous hydrogen chloride (1 mol dm^{-3}) at 363.2 K. As seen, the compound is isomerized to the 3′,5′-diester (**6**) concurrently with its hydrolysis to adenosine and a mixture of the monoesters, **9** and **10**. Depurination, i.e., hydrolytic cleavage of adenine from **7**, competes strongly with these reactions. The first-order rate constants for the hydrolysis and isomerization were observed to be $k_3 = 4.0 \times 10^{-3}$ s^{-1} and $k_{-1} = 1.0 \times 10^{-3}$ s^{-1}, respectively, the rate of depurination being comparable to that of isomerization. For the hydrolysis and isomerization of a closely related dinucleoside monophosphate, 2′,5′-UpA (**2**; B^1 = uracil, B^2 = adenine), the corresponding rate constants have been reported to be $k_3 = 2.0 \times 10^{-2}$ s^{-1} and $k_{-1} = 5.4 \times 10^{-3}$ s^{-1}.[1] Accordingly, the reactions via the phosphorane intermediate appear to be only moderately slower than those of ribonucleoside derivatives, as long as the attacking nucleophile is the primary hydroxy group (3′-OH). In contrast, the reactions that proceed by an initial attack of the secondary hydroxy group (2′-OH) are too slow to compete with depurination; disappearance of the 3′,5′-isomer, **6**, was not accompanied by a detectable accumulation of either **7** or adenosine.

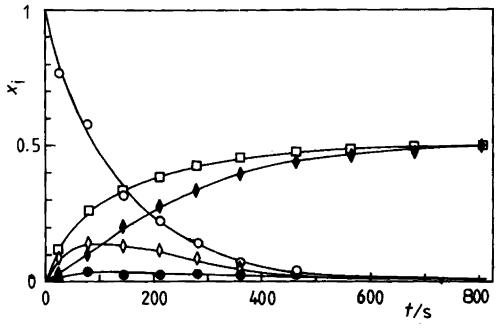
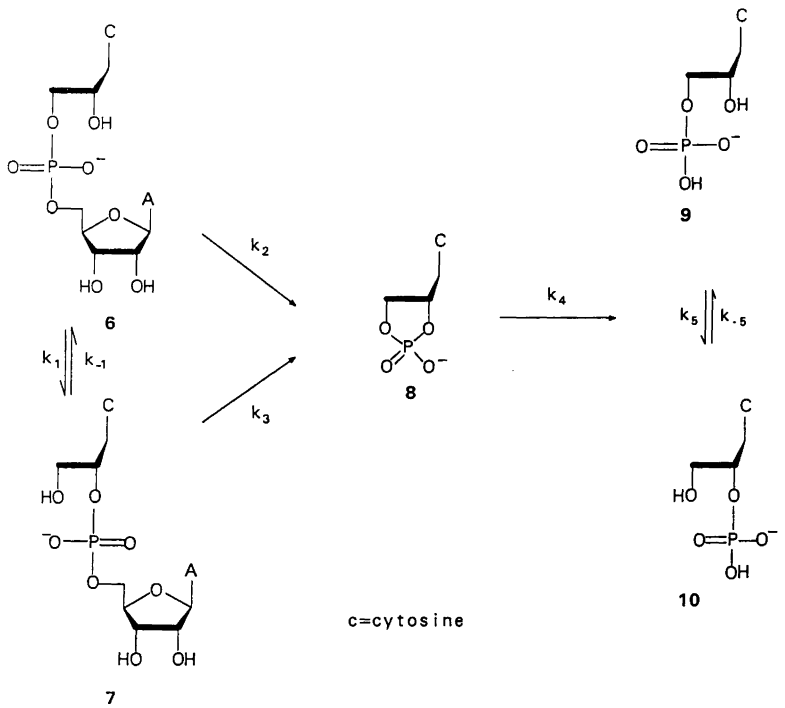


Fig. 1. Time-dependent product distribution for the reactions of the acyclic analogue of 2′,5′-CpA (**7**) in aqueous hydrogen chloride (1 mol dm^{-3}) at 363.2 K. Notation: **7** (○), **6** (●), adenosine (◇), adenine (◆), and **8**–**10** and depurinated **6** + **7** (□).

While the hydrolysis of **7** in aqueous acid is almost as fast as that of dinucleoside monophosphates, the situation is drastically changed on going to alkaline conditions, where the attacking intramolecular nucleophile is an oxyanion instead of hydroxy group.[6] The first-order rate constants obtained for the alkaline hydrolysis of **6** and **7** were 3.4×10^{-6} s^{-1} and 2.9×10^{-5} s^{-1} ([OH$^-$] = 0.1 mol dm^{-3}, T = 333.2 K), respectively. These values are considerably smaller than those reported[1] for hydrolysis of 3′,5′-UpA and 2′,5′-UpA (**1** and **2**;



Scheme 2.

B^1 = uracil, B^2 = adenine) under identical conditions, *viz.* $1.71 \times 10^{-3}\ s^{-1}$ (3′,5′-UpA) and $1.98 \times 10^{-3}\ s^{-1}$ (2′,5′-UpA). Since the reactivity difference is much larger than in aqueous acid, a considerable part of it may be expected to result from the fact that the secondary hydroxy functions of dinucleoside monophosphates are more acidic than the hydroxy group in either **6** or **7**, and hence the mole fraction of the reactive ionic form, having the attacking hydroxy group deprotonated, is greater with the ribo compounds. Comparison of the hydrolysis rates of **6** and **7** reveals that the reaction proceeding by an attack of the primary 3′-hydroxy group is again faster than that involving participation of the secondary 2′-hydroxy function. This is consistent with the greater acidity of the primary hydroxy group. Hydrolysis of the 3′,5′-isomer is 500 times slower than the hydrolysis of 3′,5′-UpA. The rate retardation is thus comparable to the 1000-fold deceleration detected in the enzymic hydrolysis.[5]

Hydrolysis of the cyclic monophosphate of 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine (**8**), i.e., the initial hydrolysis product of **6** and **7**, was studied separately. It has been shown previously that hydrolysis of five-membered cyclic phosphodiesters is strongly exothermic[7] and exceptionally rapid, approximately 10^6 times faster than the hydrolysis of acyclic esters.[8] This enhanced reactivity has been attributed to the relief of ring strain on going from the initial state to the pentacoordinated transition state obtained by a nucleophilic attack of water or hydroxide ion on phosphorus.[9,10] Evidently replacing the ribofuranose ring with an acyclic structure has only a moderate effect on the strain of the cyclic monophosphate. Under acidic conditions **8** is hydrolytically almost as unstable as nucleoside 2′,3′-cyclic monophosphates, the first-order rate constants obtained in aqueous hydrogen chloride ($[H^+] = 0.1\ mol\ dm^{-3}$) at 323.2 K being $6.6 \times 10^{-3}\ s^{-1}$ with **8** and $7.9 \times 10^{-3}\ s^{-1}$ with **3** (B = cytosine). In aqueous alkali the reactivity difference is almost one order of magnitude; at $[OH^-] = 0.05\ mol\ dm^{-3}$ and 323.2 K ($I = 0.1\ mol\ dm^{-3}$ with NaCl) the rate constants obtained with **8** and **3** are $1.7 \times 10^{-4}\ s^{-1}$ and $1.6 \times 10^{-3}\ s^{-1}$, respectively. Both the acid- and base-catalysed hydrolysis of **8** yield a mixture of 3′- and 2′-monophosphates (**9** and **10**), analogously to the hydrolysis of **3**. The ratio of **[9]**/**[10]** in the initial product mixture is 0.33 in aqueous acid and 0.67 in aqueous alkali. As with 2′-*C*- and 3′-*C*-methyl-2′,3′-cUMPs,[11] the isomer having a phosphate group at the more substituted carbon predominates. This is consistent with the Westheimer's[8] concept of pentacoordinated phosphorane intermediates and transition states, according to which electronegative ligands prefer apical positions, and the entrance or departure of the ligand may take place at apical positions only. Since a primary hydroxy group is slightly more acidic than a secondary one, a primary alkoxy ligand may be expected to favour an apical position and hence leave more readily.

As mentioned above, the acid-catalysed hydrolysis of **8** initially yields a mixture of isomeric monophosphates (**9** and **10**), containing 77% the 2′-isomer (**10**). However, prolonged treatment with aqueous acid results in an interconversion of the 2′- and 3′-monophosphates, and the equilibrium mixture obtained at 363.2 K contains **10** only as a minor component (26%). Accordingly, an intramolecular transesterification takes place, the migration from the secondary 2′-hydroxy group to the primary 3′-hydroxyl being 2.8 times as fast as its reverse reaction. These results may be compared to those of Buchwald *et al.*,[12] according to which the equilibrium between 1- and 2-phosphates of propane-1,2-diol favours, under acidic conditions ($0.5\ mol\ dm^{-3}\ HClO_4$), the 1-phosphate ($K_{eq} = 1.8$), although hydrolysis of the cyclic 1,2-phosphate initially gives the 2-phosphate as the major product (molar ratio 1.7). It is also known that with 2′-*C*- and 3′-*C*-methyl-2′/3′-UMPs migration of the monophosphate group from the tertiary to secondary hydroxy group is much faster than its reverse reaction.[11] The dihydroxypropyl compounds (**9** and **10**) are interconverted more slowly than nucleoside 2′- and 3′-monophosphates (**5** and **4**). A value of $k_5 + k_{-5} = 7.3 \times 10^{-5}\ s^{-1}$ was observed for the isomerization of **9** and **10** in aqueous hydrogen chloride ($0.1\ mol\ dm^{-3}$) at 363.2 K. For comparison, values of $9.0 \times 10^{-4}\ s^{-1}$ and $1.5 \times 10^{-3}\ s^{-1}$ have been reported for the interconversion of 2′/3′-CMP and 2′/3′-UMP, respectively.[13] Migration of the phosphate group between the 2′- and 3′-hydroxy groups of **9** and **10** was not detected in alkaline solutions. The phosphomonoesters are, under these conditions, present as dianions and hence nucleophilic attack on phosphorus is retarded.

Experimental

Materials. The preparation of 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine-3′-*O*-phosphorylyl-(3′,5′)-adenosine (**6**),[14] 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine-2′-*O*-phosphorylyl-(2′,5′)-adenosine (**7**)[14] and 1-[(2′*S*)-2′,3′-dihydroxypropyl]cytosine 3′,3′-cyclic monophosphate (**8**)[15,16] has been described previously.

Kinetic measurements. Reactions were followed by the HPLC technique described previously.[17] Chromatographic separations were carried out on a Shandon Hypersil ODS column (4.6 × 250 mm, 5 μm). An acetic acid/sodium acetate buffer ($0.045/0.015\ mol\ dm^{-3}$) containing $0.1\ mol\ dm^{-3}$ ammonium chloride and 0–3% acetonitrile was employed as the eluant. The signal areas were assumed to be proportional to the concentrations, as long as the base moieties of the starting materials and all the products remained unchanged. With the dimers (**6**, **7**) the contribution of the two different base moieties on the signal areas were assumed to be additive. The hydronium-ion concentrations of the reaction solutions were adjusted with hydrogen chloride and sodium hydroxide standard solutions (J. T. Baker).

The products of the hydrolysis of the cyclic

monophosphate (**8**) were identified by ^1H and ^{31}P NMR spectroscopy. The ^{31}P chemical shifts in D_2O as ppm from phosphoric acid were 1.448 (**9**) and 0.362 (**10**). ^1H NMR of **9** (400 MHz, shifts as ppm from external Me_4Si in D_2O): δ 7.60 (H6, d, $J_{H5,H6}$ 7.3 Hz), 5.99 (H5, d), 4.06–4.12 (H2′, m, partial overlap with H1′), 4.03–4.09 (H1′, dd, $J_{H1',H2'}$ 4.0 Hz, partial overlap with H2′), 3.95–3.89 (H3′, m), 3.81–3.88 (H3″, m), 3.73 (H1″, dd, $J_{H1',H1''}$ 14 Hz, $J_{H1'',H2'}$ 8.1 Hz). ^1H NMR of **10** (400 MHz, shifts as ppm from external Me_4Si in D_2O): δ 7.68 (H6, d, $J_{H5,H6}$ 7.5 Hz), 6.05 (H5, d), 4.39 (H2′, oct, $J_{H2',P}$ 8 Hz), 4.16 (H1′, d, $J_{H1',H1''}$ 14 Hz, $J_{H1',H2'} < 2$ Hz), 3.85 (H1″, dd, $J_{H1'',H2'}$ 8.5 Hz), 3.76 (H3′, dd, $J_{H3',H3''}$ 12.3 Hz, $J_{H2',H3'}$ 4.6 Hz), 3.69 (H3″, dd, $J_{H2',H3''}$ 4.0 Hz). The assignment of the methylene protons as H′ and H″ is tentative and does not necessarily refer to the absolute configuration.

The rate constants for the hydrolysis of the cyclic monophosphate were calculated by applying the integrated first-order rate equation to the disappearance of the starting material. First-order rate constants for the interconversion of the 2′- and 3′-phosphates (**6**/**7** and **9**/**10**) were calculated by means of eqns. (1) and (2),

$$(k_1 + k_{-1})t = \ln[(1 - x_e)/(x - x_e)] \quad (1)$$

$$k_1/k_{-1} = (1 - x_e)/x_e \quad (2)$$

where x and x_e stand for the mole fraction of the 3′-isomer in the mixture of isomers at time t and at equilibrium, respectively.

The first-order rate constant for the phosphodiester hydrolysis of **7** was calculated by means of eqn. (3) where

$$\frac{[\text{Ado}]}{[7_0]} = \frac{k_3}{k_A - k_3}[\exp(-k_3 t) - \exp(-k_A t)]$$

[Ado] and stands for the concentration of adenosine at time t and $[7_0]$ for the initial concentration of the starting material. k_3 is the rate constant of phosphoester hydrolysis of **7** and k_A the rate constant of the hydrolysis of adenosine ($k_A = 8.84 \times 10^{-3}\ s^{-1}$).

NMR measurements were carried out on a JEOL GX 400 spectrometer.

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