Short Communication

Kinetics and Mechanisms of the Hydrolysis of the 2-Chlorophenyl Ester of Uridine 3′-Monophosphate: Comparison with the Methyl Ester

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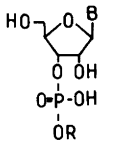
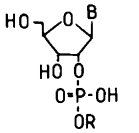
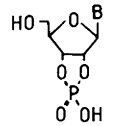
We have shown previously[1] that the monomethyl ester of adenosine 3′-monophosphate (**1a**), which may be regarded as a model compound for a dimeric fragment of ribonucleic acids, undergoes three parallel reactions in aqueous solution: (i) isomerization to the monomethyl ester of adenosine 2′-monophosphate (**3a**), (ii) hydrolysis to a mixture of adenosine 2′- (**2a**) and 3′-monophosphates (**2a**), and (iii) hydrolysis to adenine (**6**) and D-ribose 3′-monophosphate. All these reactions proceed at comparable rates under acidic conditions, while in neutral solution (pH 4–9) reaction (i) prevails, and reaction (ii) is the only one detected in aqueous alkali. Methylphosphate migration (i) and phosphodiester hydrolysis (ii) proceed *via* the same pentacoordinated intermediate, which is formed by a nucleophilic attack of the 2′-oxygen atom on phosphorus and may be decomposed in two alternative ways. Departure of the methoxy ligand gives a cyclic 2′,3′-monophosphate (**5a**), which subsequently undergoes ring-opening to a mixture of 2′- and 3′-monophosphates, whereas rupture of the P–O3′ bond results in isomerization. The present study was carried out to further the understanding of the effects that the polar nature of the esterified alcohol has on the rates of reactions (i) and (ii). For this purpose, the methoxy group in **1a** was replaced with a much better leaving group, namely 2-chlorophenoxy, which is often used as a protecting group during the chemical synthesis of oligonucleotides by the phosphotriester method.[2] Adenine base was replaced by uracil to avoid depurination as a side reaction.



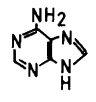
1a; B = Adenine, R = CH_3
b; B = Uracil, R = *O*-chlorophenyl
2a; B = Adenine, R = H
b; B = Uracil, R = H



3a; B = Adenine, R = CH_3
b; B = Uracil, R = *O*-chlorophenyl
4a; B = Adenine, R = H
b; B = Uracil, R = H



5a; B = Adenine
b; B = Uracil



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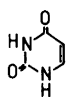

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Hydrolysis of uridine 3′-(2-chlorophenyl)phosphate (**1b**) to 2-chlorophenol and uridine monophosphates (**2b**, **4b**, **5b**) is fast compared with migration of the (2-chlorophenyl) phosphate group over the whole acidity range, in striking contrast with the hydrolysis of **1a**. Clear evidence for phosphate migration was obtained only at pH < 2, and even then the rate of this reaction was small compared with that of phosphoester hydrolysis, about 10% of the hydrolysis rate at $[H^+] = 0.01$ mol dm^{-3} and 25% at $[H^+] = 0.5$ mol dm^{-3}. As an illustrative example, a time-dependent product distribution observed at $[H^+] = 0.01$ mol dm^{-3} is depicted in Fig. 1.

The hydrolysis reaction undoubtedly proceeds by intra-

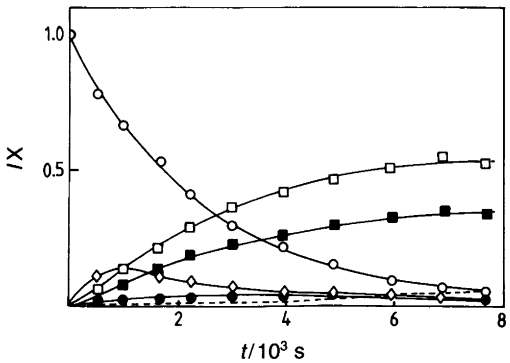

Fig. 1. Time-dependent product distribution for the hydrolytic reactions of uridine 3′-(2-chlorophenyl)phosphate (**1b**) in aqueous hydrogen chloride (0.01 mol dm^{-3}, I = 0.1 mol dm^{-3} with NaCl) at 363.2 K. Notation: Starting material (○), its 2′-isomer (**3b**, ●), 2′,3′-cUMP (◇), 2′-UMP (■), 3′-UMP (□), uridine (dotted line).

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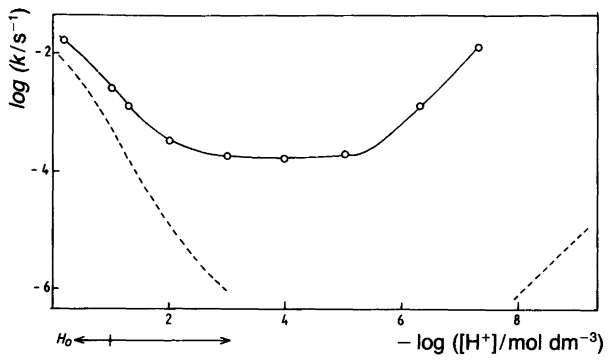


Fig. 2. The pH–rate profile of the hydrolysis of the 2-chlorophenyl ester of uridine 3′-monophosphate at 363.2 K. The rate profile reported previously[1] for adenosine 3′-methylphosphate (**1a**) is represented by a dotted line.

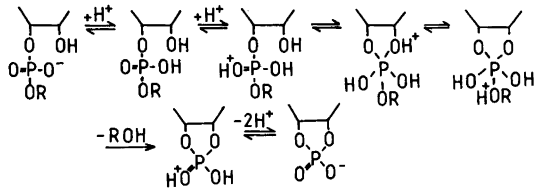
molecular participation of the 2′-hydroxy function, *i.e.* analogously to hydrolysis of the methyl ester (**1a**), since the initial reaction product was observed to be 2′,3′-cUMP (**5b**) under acidic, neutral and alkaline conditions. At pH > 3, hydrolysis of **1b** is considerably faster than that of 2′,3′-cUMP, and hence **5b** is quantitatively accumulated. No other products derived from the nucleoside moiety were observed. Under acidic conditions 2′,3′-cUMP is subsequently hydrolyzed to a mixture of 2′- and 3′-UMP (**2b**, **4b**), as seen from Fig. 1. It is worth noting, however, that the ratio of the mole fractions of **2b** and **4b** remains constant during the whole kinetic run, and is equal to that (1.5) observed for the hydrolysis of 2′,3′-cUMP to **2b** and **4b**.[3] Isomerization of **2b** into **4b**, although leading to the same equilibrium composition, is too slow to account for the product distribution observed.

Fig. 2 shows the pH–rate profile observed for the hydrolysis of **1b**. For comparison, the rate profile reported previously[1] for **1a** is included in the same figure. These two profiles differ from each other in one important respect. As discussed elsewhere in detail,[1] the observed first-order rate constant, k, for hydrolysis of **1a** may be expressed by eqn. (1) under conditions where ionization of the 2′-hydroxy group is negligible. Hydrolysis of **1b** obeys, in turn, eqn. (2). The partial rate constants are those indicated in Scheme 1. Accordingly, the methyl ester (**1a**) exhibits a

$$k = \frac{(k_a/K_a)[H^+]^2 + k_c + k_d(K_w/[H^+])}{1 + [H^+]/K_a} \quad (1)$$

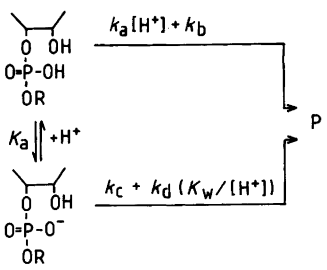
$$k = \frac{(k_a/K_a)[H^+] + k_c + k_d(K_w/[H^+])}{1 + [H^+]/K_a} \quad (2)$$

second-order rate dependence on hydronium ion concentration at pH > pK_a values of the phosphate moiety, indicating that the predominant reaction under acidic conditions is that depicted in Scheme 2. By contrast, hydrolysis of **1b** is first-order with respect to hydronium ion under

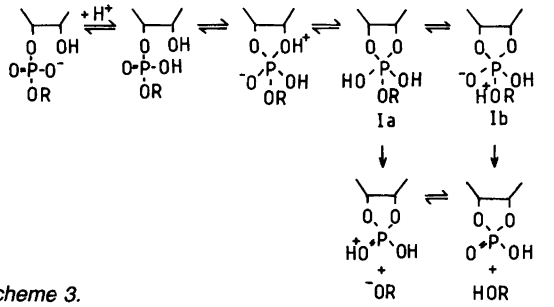


Scheme 2.

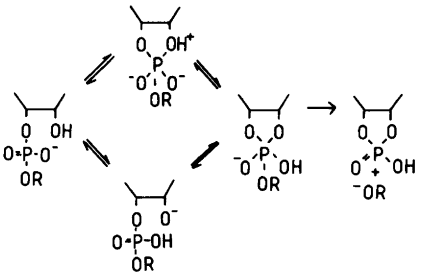
similar conditions. This means that the 2′-hydroxy group is able to attack the neutral phosphate group (Scheme 3), and hence, on the basis of microscopic reversibility, the 2-chlorophenoxy group departs either as a phenoxy ion from the neutral form of the pentacoordinated intermediate (**Ia**), or as 2-chlorophenol from the less stable, but undoubtedly more reactive, zwitterionic intermediate (**Ib**). It is also interesting to note that the terms $(k_a/K_a)[H^+]^2$ and $(k_b/K_a)[H^+]$ both contribute significantly to the hydrolysis rate of the phenyl ester of *cis*-4-hydroxytetrahydrofuran 3-monophosphate.[4] In other words, both of the routes depicted in Schemes 2 and 3 appear to be utilized, when the leaving-group is better than an alkyl group, but worse than 2-chlorophenoxy group. Generally speaking, the rate of acid-catalyzed hydrolysis is rather insensitive to the polar nature of the esterified alcohol; electron withdrawal by the leaving group, for example, facilitates nucleophilic attack of neighboring hydroxy group on the phosphorus atom and cleavage of the P–O bond, but simultaneously the protonation steps are retarded.



Scheme 1.



Scheme 3.

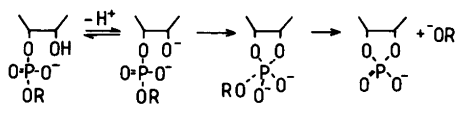


Scheme 4.

pH-Independent hydrolysis of **1b** becomes faster than the acid-catalyzed reaction at pH > 2. The points falling in the plateau range may include the contribution of buffer catalysis. In all likelihood this contribution is, however, of minor importance. The rate constants were measured at buffer concentrations lower than 0.05 mol dm^{-3}, and the results of Anslyn and Breslow[5] suggest that under such conditions the buffer-dependent rates are hardly detectable compared with buffer-independent ones.

With **1a** the spontaneous hydrolysis was too slow to be measured accurately, but its first-order rate constant was estimated to be smaller than 1×10^{-7} s^{-1} at 363.2 K. In other words, the reaction, which may be mechanistically depicted as in Scheme 4, is more than three orders of magnitude slower than with the 2-chlorophenyl ester (**1b**). This is expected, since the electronegative aryl group inductively facilitates both the attack of the neigboring hydroxy group on the phosphorus atom and heterolysis of the resulting pentacoordinated intermediate to 2-chlorophenoxide ion and 2′,3′-cUMP. It is worth noting that no phosphate migration occurs, although it is the predominant reaction with the methyl ester (**1a**) under the same conditions. Evidently the aryloxy group departs so much more readily than 2′- or 3′-oxyanions that no migration is detected.

The reactivity difference between **1a** and **1b** is even larger in alkaline solution, where the attacking nucleophile is the ionized 2′-hydroxy group and the reaction is known to proceed by the so-called in-line associative mechanism (Scheme 5),[4,6,7] *i.e. via* a pentacoordinated transition state, or *via* a pentacoordinated intermediate that cannot undergo pseudorotation. As seen from Fig. 2, **1b** is hydrolyzed 10^5 times faster than **1a**. Kinetic measurements at 298.2 K indicated that the reaction is first order with respect to hydroxide ion at least up to pH 12.



Scheme 5.

Experimental

Materials. The 2-chlorophenyl ester of uridine 3′-monophosphate (**1b**) was obtained by conventional deblocking of commercially available 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)uridine 3′-[2-chlorophenyl(2-cyanoethyl)phosphate] (Sigma). The 2-cyanoethyl group was first removed by *tert*-butylamine-catalyzed β-elimination in pyridine,[8] followed by a normal sodium hydrogencarbonate/chloroform work-up. Subsequently the acid labile 2′-*O* and 3′-*O* protecting groups were hydrolyzed in aqueous hydrogen chloride (0.01 mol dm^{-3}) at room temperature. The progress of deblocking was followed by HPLC. After 3 h the reaction mixture was neutralized with aqueous ammonia and evaporated to dryness. Deblocked **1b** was separated by column chromatography (Bondesil RP-18, 40 μm, 2.5×10 cm) from uridine monophosphates formed as by-products (< 30 %). The latter compounds and inorganic salts were eluted with water, and **1b** with an 8:92 (v/v) mixture of methanol and water. The evaporated product was homogenous by HPLC and exhibited ^{1}H NMR signals at (ppm from Me_4Si in DMSO-d_6): δ 3.62 (m, H5′, H5″), 4.03 (m, H4′), 4.12 (t, H3′), 4.56 (m, H2), 5.67 (d, J = 8 Hz, H5), 5.78 (d, J = 5 Hz, H1′); δ (2-chlorophenyl) 7.01 (t), 7.23 (t), 7.39 (d), 7.58 (d), 7.88 (d, J = 8 Hz, H6). Only one ^{31}P resonance at −4.74 ppm from phosphoric acid was detected. Uridine monophosphates, used as reference materials, were commercial products from Sigma.

Kinetic measurements. Reactions were followed by the HPLC technique described previously.[9] Chromatographic separations were carried out on a Hypersil ODS5 column (4×250 mm, 5 μm) using a 15:85 (v/v) mixture of acetonitrile and aqueous ammonium dihydrogenphosphate (0.05 mol dm^{-3}) as the eluent. Signal areas were converted into concentrations with the aid of calibration solutions of known concentrations. An integrated first-order rate equation was applied to the disappearance of **1b**, or, at pH < 2, to the disappearance of **1b** and **3b**. Hydronium ion concentrations of reaction solutions were adjusted with hydrogen chloride, and formate, acetate, triethanolamine and glycine buffers, and calculated from the pK_a values of the buffer acids under experimental conditions.[10–13]

References

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