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Compelling evidence for a stepwise mechanism of the alkaline cyclisation of uridine 3′-phosphate esters†‡

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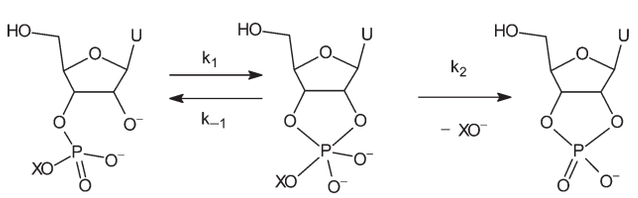
Received 7th May 2004, Accepted 15th June 2004
First published as an Advance Article on the web 5th July 2004

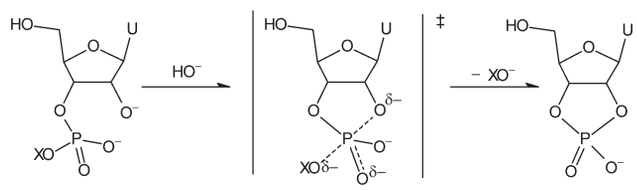
A Brønsted graph with a convex break at pK_a (Lg) = 12.58 provides compelling evidence for an intermediate in the alkaline cyclisation of uridine 3′-phosphate esters. The transient pentacoordinated oxyphosphorane dianion intermediate collapses to reactant and cyclic uridine 2′,3′-monophosphate faster than it can pseudo-rotate and isomerise to the 2′-isomer.

There is abundant evidence that nucleophilic displacement reactions at P(V) acyl (phosphyl) group centres involve stepwise mechanisms with pentacoordinated phosphorane intermediates or concerted mechanisms with phosphorane-like transition structures.[1] The demonstration of the stepwise mechanism has usually involved indirect arguments. Pentacoordinate oxyphosphoranes (analogous to the reaction intermediates) have been isolated under special conditions and their involvement as transient intermediates has been deduced from studies of the reactivity of cyclic phosphate esters[2] and ^{18}O-isotope exchange.[2b] Apart from Haake's study[3] there has been no compelling ***kinetic*** evidence for the intervention of an intermediate in any displacement reactions at a phosphyl centre.

In recent years the technique using quasi-symmetrical reactions, which demonstrates either a stepwise or a concerted process in displacement reactions,[4] has diagnosed concerted processes for some phosphyl esters.[5] A convex breakpoint in a Brønsted plot can be predicted at $pK_a^{Lg} = pK_a^{Nu}$ for those reactions involving a putative stepwise mechanism: A–Lg + Nu^- → A–Nu + Lg^- where the intermediate is transient (does not accumulate). The observation of a convex breakpoint in a Brønsted or Hammett plot (varying either Lg^- or Nu^-) is classical evidence for a stepwise process[4b,c] and would be diagnostic of an intermediate even when the displacement reaction does not conform to the criteria of a quasi-symmetrical reaction such as in the cyclisation of uridine 3′-phosphate esters [eqns. (1) and (2)].



(1)



(2)

Data[6,7] for the hydroxide ion-catalysed cyclisation of aryl and alkyl uridine 3′ phosphate esters (Supplementary Table 1†) yields a non-linear Brønsted plot (Fig. 1) when the rate constants are plotted against the pK_a's of the leaving hydroxyl groups (XOH = ArOH or ROH).[8] The observation of excellent pseudo-first order rate constants for all the substrates over the whole of the progress of the alkaline cyclisation reactions indicates that there is no accumulation of any intermediate. The non-linear Brønsted dependence provides compelling, classical, evidence for the intervention of a transient intermediate in a stepwise mechanism [eqn. (1)] and excludes a concerted process [(eqn. (2)]. The overall kinetic equation for the stepwise process (Eqn 1) is given by eqn. (3).[9]

$$k_{obs} = k_1k_2/(k_{-1} + k_2) = k_1/(1 + k_{-1}/k_2) = (k'_1K_w/K_a)[OH]/(1 + k_{-1}/k_2) \quad (3)$$

The parameters k_1, k_{-1} and k_2 are defined as in eqn. (1) and k'_1 is the second order rate constant for reaction of hydroxide ion with neutral ester; K_w is the autoprotolysis constant and K_a is the dissociation constant of the 2′-hydroxyl group. Each rate constant possesses a discrete Brønsted equation ($\log k_x = \beta_x pK_a^{Lg} + c_x$) and equation (4)[4] can be derived which governs the overall non-linear Brønsted type dependence.

$$\log k_{OH} = \log k_1 - \log (1 + k_{-1}/k_2) = \beta_1 pK_a^{Lg} + c_1 - \log \{1 + 10^{((\beta_{-1} - \beta_2)pK_a^{Lg} + c_{-1} - c_2)}\} = \beta_1 pK_a^{Lg} + c_1 - \log \{1 + 10^{(\Delta\beta pK_a^{Lg} + c_o)}\}^{10} \quad (4)$$

The fit of the data to eqn. (4) possesses an excellent correlation coefficient and yields parameters which are given in the legend to Fig. 1.

The theoretical Brønsted plot for eqn. (2) would exhibit no breakpoint. The breakpoint for eqn. (1) occurs at the pK_a^{Lg} where $k_{-1} = k_2(\beta_{-1}pK_a^o + c_{-1} = \beta_2 pK_a^o + c_2)$ *i.e.* at $pK_a^o = -c_o/\Delta\beta$. The parameter pK_a^o is the value of pK_a^{Lg} at the intersection point of the two asymptotic limbs of the Brønsted plot. It is unlikely that the non-linear plot is due to structural differences between phenoxide and alkoxide ions because in other displacement reactions, where the transition state remains constant over the pK_a range of both hydroxyl types, the Brønsted plots are linear.[11] There is no reason to suppose that in the present case the difference in transition state structures, arising from the pK_a change, is due to different structural types; indeed, the bond forming step is one bond removed from the substituent change [X in eqns. (1) and (2)]. The break in the free energy relationship is caused by a change in rate limiting step in

† Electronic supplementary information (ESI) available: Tables of rate parameters and Brønsted plots for the alkaline cyclisation of R_P uridine-3′-phosphorothioate esters and 2-hydroxypropylphosphate esters ($HOCH(CH_3)CH_2OPO_2OX$). See http://www.rsc.org/suppdata/ob/b4/b406926a/

‡ While this article was being prepared we learnt of a paper in press which indicated similar conclusions and also obtained *prima facie* evidence for an intermediate in the metal ion complex-catalysed formation of methyl aryl phosphate esters.[31]

DOI: 10.1039/b406926a

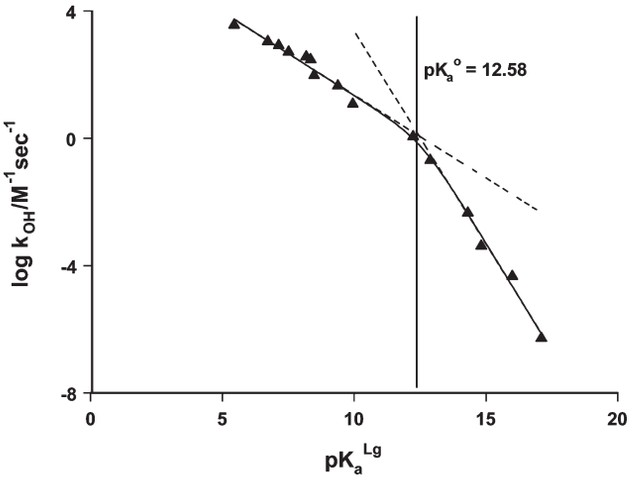
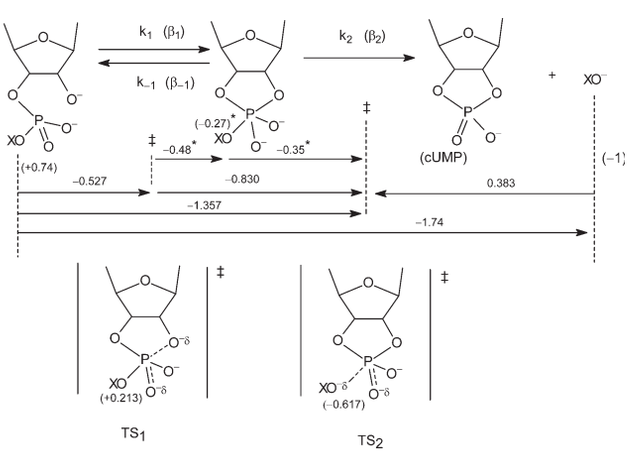


Fig. 1 Brønsted dependence of k_{OH} for the cyclisation of uridine 3′-phosphate esters at 25 °C. Data and conditions are from Table 1 (supplementary data†)[6,7,15] and the curved line (solid) is theoretical from eqn. (4) using the following parameters: $\beta_1 = -0.52 \pm 0.05$; $\Delta\beta = 0.82 \pm 0.08$; $c_1 = 6.57 \pm 0.41$; $c_o = -10.17 \pm 1.12$; $pK_a^o = 12.40 \pm 0.16$ ($r = 0.9980$). See the text for the intersecting straight lines (dashed) which have the equations: $\log k_{OH} = -0.521pK_a + 6.57$ and $\log k_{OH} = -1.34pK_a + 16.74$.

a mechanism which has at least two steps. The slope of each line of the non-linear Brønsted plot refers to the effective charge in the transition structure of the corresponding rate limiting step ($\beta_1 - \Delta\beta$ refers to k_2 and β_1 to k_1).

This system provides the first classical type[4] demonstration of a transient intermediate[12] in the cyclisation of ribose 3′-phosphate esters and to our knowledge such non-linear free energy relationships, diagnostic of intermediates, have never previously been reported in displacement reactions in either cyclic or acyclic phosphate derivatives.

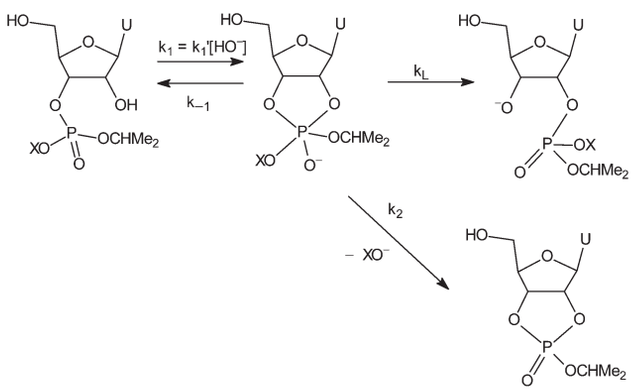
The present observation and deduction of the phosphorane structure enables the effective charge map[13,14] of the reaction to be assigned confidently (Scheme 1).



Scheme 1 Effective charge map for the cyclisation of uridine 3′-phosphate esters. Figures refer to the changes in effective charge; data in brackets refer to the effective charge on particular groups. Starred figures are calculated assuming $\alpha_{-1} = \alpha_2$.[18]

The experimental pK_a for the ionisation of the 2′ hydroxyl of the uridine 3′-phosphate ethyl ester is 12.85[15] close to that (13.03) calculated from the Taft equation ($pK_a = 15.9 - 1.42\Sigma\sigma^*$).[16] A true quasi-symmetrical reaction should exhibit a break in the Brønsted dependence at the pK_a corresponding to that of the attacking nucleophile. The quasi-symmetrical criteria do not hold in the cyclisation case but the observed pK_a^o (12.58) is within error limits close to that of the entering 2′-hydroxyl ion (12.85). The similarity between pK_a^o and the pK_a of the 2′-hydroxyl group is probably fortuitous because entropic and angle strain factors respectively increase and decrease its value. A decrease in pK_a^o would be due to angle strain imparted by the ^{c}O–P–O^a endocyclic angle of ~109° (where the ring bridges equatorial and axial positions) compared with 99°[17] for such an angle in an acyclic phosphorane. An increase in pK_a^{Lg} would derive from an entropic effect because the k_2 step should be faster for a given pK_a^{Lg} since it involves the production of two species whereas the k_{-1} step generates only a single reactant. It is possible that each of these factors would alter the pK_a^o by only a small extent. Further information regarding the effective charge on the leaving oxygen in the transient intermediate can be deduced if it can be assumed that Leffler's α is the same for breakdown of intermediate forward to products (α_2) and back to reactants (α_{-1}).[18]

The hydroxide ion catalysed transesterification of uridine 3′-alkyl, isopropyl phosphate esters (Scheme 2)[19] has values of k_{-1} and k_L which are predicted to be almost the same for all the esters in the series so that the value of k_{OH} is $k'_1k_L/(k_{-1} + k_L) = 0.5k'_1$ and the β_{rg}[19] value of −0.57 refers to the addition (k'_1) of the 2′-hydroxyl anion to the phosphorus to form the phosphorane; this Brønsted selectivity agrees with the β_1 for the diester reaction.



Scheme 2 Cyclisation and transesterification of uridine 3′-phosphotriesters.

In the case of the above triesters the mechanism of isomerisation is likely to involve a pentacoordinate intermediate which has to undergo pseudorotation to an isomer before collapsing to the 2′-phosphate ester. It is unlikely that the cyclisation traverses a concerted mechanism because the common pentacoordinate structure must be stable enough to exist for the period of a pseudorotation to allow isomerisation to occur. The slopes $\beta_{rg} = -0.57$ and $\beta_{Lg} = -1.38$[19] refer to k_1 and k_1k_2/k_{-1} respectively. The value of β_{Lg} is similar to that observed for the present phosphodiester which is considered to have the same rate limiting step.

Displacements at an acyclic phosphorus with good leaving groups involve concerted mechanisms[5] in contrast to the stepwise mechanism in the cyclisation process. The formation of an oxyphosphorane ring is an important factor as the ring structure confers extra stability and under certain conditions phosphoranes, analogous to the putative transient intermediates, can be synthesised and isolated.[20] In the alkaline diester reaction isomerisation of 3′- to 2′-phosphate esters does not occur probably because the dianionic phosphorane is too short lived for pseudorotation to occur. The conclusion that a stepwise process is involved complements that from the absence of a ^{15}k-isotope effect[21] on the cyclisation of the 4-nitrophenyl uridine 3′-phosphate ester. Rate limiting addition of oxyanion to the phosphorus bearing good leaving groups is also consistent with the observation that the Leffler α_{Lg} values for the cyclisation reactions are relatively small for hydroxide ion or imidazole catalysis (~0.3)[6] and for the ribonuclease reaction (~0.1);[22] this indicates only weak coupling between proton transfer and the fission of the bond to the leaving group consistent with a stepwise process. The absence of a ^{15}k isotope effect for the base catalysed cyclisation of the 4-nitrophenyl ester implies zero charge development on the leaving oxygen.[23] The observation of a change in effective charge (−0.527) is not inconsistent with this result as the isotope effect refers to the oxygen atom specifically whereas the substituent effect (which yields the effective charge) is derived from global charge including the bonding and solvation changes.[24]

The observation of an intermediate in the intramolecular displacement reaction at the monoanionic phosphorus (phosphodiester) level does not affect the previous conclusions of concerted displacements at acyclic phosphorus at the neutral (triester) or dianion (monophosphate) levels.[5] At the phosphodiester level the concerted displacement mechanism holds for the acyclic case.[25]

More data are necessary if an intermediate is to be assigned to the cyclisation of esters of the corresponding uridine 3′-phosphorothioic acids ($S_pU_{P(S)}OAr$).[26] A single point is available for an alkyl ester ($S_pU_{P(S)}O$Adenosine)[27] and this lies 2 orders of magnitude below the subtended Brønsted plot of the aryl esters even though it is itself an upper limit for 25 °C. This provides only *prima facie* evidence for a change in rate limiting step (indicating a transient intermediate) and more data is required before a definitive answer can be made.[28]

Further corroborating evidence for an intermediate can be obtained from the rate constants for the alkaline hydrolysis of 2-hydroxypropyl phosphate esters ($HOCH(CH_3)CH_2OPO_2OX$); these rate constants were originally reported by Brown and Usher to possess a linear Brønsted plot against the pK_a's of the leaving hydroxyl groups.[29] However, a value of 17.1 is more reasonable the pK_a's of the isopropanol and cyclohexanol were originally assigned to be 19.[7] Using the new pK_a values leads to the observation that the Brønsted plot is non-linear[30] providing support for the present conclusions of a pentacoordinate phosphorane intermediate in this class of reaction.§ The data can be fitted to eqn. (4) and the derived parameters are broadly in line with the values of those for the uridine-3′-phosphate ester case (see supplementary material†).

Notes and references

§ We are grateful to a referee for reminding us of the earlier work carried out by Brown and Usher.

1 (*a*) J. Emsley and C. D. Hall, *The Chemistry of Phosphorus*, Harper & Row, New York, 1976, pp. 318–338; (*b*) D. M. Perreault and E. V. Anslyn, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 432.

2 (*a*) F. H. Westheimer, *Acc. Chem. Res.*, 1968, **1**, 70; (*b*) P. C. Haake, *J. Am. Chem. Soc.*, 1961, **83**, 1102.

3 R. D. Cook, P. C. Turley, C. E. Diebert, A. H. Fierman and P. C. Haake, *J. Am. Chem. Soc.*, 1972, **94**, 9260; R. D. Cook, C. E. Diebert, W. Schwarz, P. C. Turley and P. C. Haake, *J. Am. Chem. Soc.*, 1973, **95**, 8088.

4 (*a*) A. Williams, *Concerted Organic and Bio-Organic Mechanisms*, CRC Press, Boca Raton, USA, 2000, pp. 26–29; (*b*) A. Williams, *Free Energy Relationships in Organic and Bio-Organic Chemistry*, Royal Society of Chemistry, Cambridge, 2003, pp. 163–166; (*c*) B. M. Anderson and W. P. Jencks, *J. Am. Chem. Soc.*, 1960, **82**, 1773.

5 A. Williams, *Concerted Organic and Bio-Organic Mechanisms*, CRC Press, Boca Raton, USA, 2000, pp. 168–181.

6 A. M. Davis, A. D. Hall and A. Williams, *J. Am. Chem. Soc.*, 1988, **110**, 5105.

7 M. Kosonen, E. Youseti-Salakdeh, R. Strömberg and H. Lönnberg, *J. Chem. Soc., Perkin Trans. 2*, 1997, 2661.

8 E. P. Serjeant and B. Dempsey, *Ionization Constants of Organic Acids in Aqueous Solution, IUPAC Chemical Data Series 23*, Pergamon Press, Oxford, 1979.

9 $k_1 = (k'_1K_w/K_a)[OH^-]$ where K_a is the ionisation constant of the 2′-hydroxyl group; k_{OH} is measured from the alkaline hydrolysis of ester with unionised 2′-hydroxyl group ($k_{obs} = k_{OH}[OH^-]$).

10 $c_o = c_{-1} - c_2$ and $\Delta\beta = \beta_{-1} - \beta_2$.

11 (*a*) A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1244; (*b*) T. C. Bruice, T. H. Fife, J. J. Bruno and N. E. Brandon, *Biochemistry*, 1962, **1**, 7; (*c*) H. Al-Rawi and A. Williams, *J. Am. Chem. Soc.*, 1977, **99**, 2671.

12 Haake's observation[3] refers to an accumulating intermediate.

13 A. Williams, *Free Energy Relationships in Organic and Bio-Organic Chemistry*, Royal Society of Chemistry, Cambridge, 2003, Chapter 3.

14 N. Bourne, S. A. Ba-Saif and A. Williams, *J. Org. Chem.*, 1984, **49**, 1200.

15 S. Acharya, A. Földesi and J. Chattopadhyaya, *J. Org. Chem.*, 2003, **68**, 1906.

16 P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, 1960, **82**, 795.

17 T. A. Steitz and W. N. Lipscomb, *J. Am. Chem. Soc.*, 1965, **87**, 2488.

18 If it is assumed that k_{-1} and k_2 have the same Leffler α value then α can be calculated from the data in Scheme 1 to be 0.48; this value would place an effective charge of −0.27 on the leaving oxygen (XO^-) in the transient intermediate.

19 M. Kosonen, R. Seppänen, O. Wichmann and H. Lönnberg, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2533.

20 (*a*) Y. Segal and I. Granoth, *J. Am. Chem. Soc.*, 1978, **100**, 5130; Y. Segal and I. Granoth, *J. Am. Chem. Soc.*, 1979, **101**, 3687; (*b*) F. Ramirez, M. Nowakowski and J. F. Maracek, *J. Am. Chem. Soc.*, 1977, **99**, 4515; R. Sarma, F. Ramirez, B. McKeever, M. Nowakowski and J. F. Maracek, *J. Am. Chem. Soc.*, 1978, **100**, 5391.

21 A. C. Hengge, K. S. Bruzik, A. E. Tobin, W. W. Cleland and M.-D. Tsai, *Bioorg. Chem.*, 2000, **28**, 119.

22 A. M. Davis, A. C. Regan and A. Williams, *Biochemistry*, 1988, **27**, 9042.

23 (*a*) A. C. Hengge, *Acc. Chem. Res.*, 2002, **35**, 105; (*b*) A. C. Hengge, W. A. Edens and H. Elsing, *J. Am. Chem. Soc.*, 1994, **116**, 5045.

24 A. Williams, *Free Energy Relationships in Organic and Bio-Organic Chemistry*, Royal Society of Chemistry, Cambridge, 2003, pp. 55–74; and A. Williams, *Adv. Phys. Org. Chem*, 1991, **27**, 1.

25 S. A. Ba-Saif, A. M. Davis and A. Williams, *J. Org. Chem.*, 1989, **54**, 5483.

26 H. Almer and R. Strömberg, *J. Org. Chem.*, 1996, **118**, 7921.

27 P. M. J. Burgers and F. Eckstein, *Biochemistry*, 1979, **18**, 592.

28 The data obey eqn. (4) but the fit cannot be trusted as there is a large extrapolation required between the aryl esters and the adenosine ester; this results in an unrealistic pK_a^o of 8.42 (see supplementary data†).

29 D. M. Brown and D. A. Usher, *J. Chem. Soc.*, 1965, 6558.

30 The data fit eqn. (4) (see supplementary data†) but there are too few to obtain reliable Brønsted and pK_a^o parameters.

31 M. Padovani, N. H. Williams and P. Wyman, *J. Phys. Org. Chem.*, 2004, **17**, 472.