The Hydrolysis of Alkyl-3'Uridyl Phosphate or Acetate Esters Can be Usedto Estimate the pK_a of Neopentyl Alcohol

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Abstract

The research project aim is to determine the pK_a of neopentanol alcohol because it has been used in many biological models as a leaving group. The pK_a of neopentanol alcohol can be obtained by measuring the observed rate for compound **1** under identical conditions to that reported for a similar series of alkyl uridyl phosphate diesters. The pK_a of neopentyl alcohol was derived from the reported Brønsted plot for the hydroxide-catalyzed hydrolysis of alkyl uridyl phosphate diesters and the observed rate constant for uridine 3′-neopentyl phosphate **1**. As a result, the best estimate for the pK_a of neopentanol alcohol can established from this work. The corresponding pK_a of neopentyl alcohol from the observed rate of the hydroxide-catalyzed hydrolysis of **1** is 17.3, which is significantly greater than the value of 15.5 used in earlier reports. This new value can be used now to estimate a better half-life for pH-independent DNA cleavage. This allows us to use correctly the neopentyl group as a mimicked model for biological systems such as that in DNA cleavage.

Keywords: Phosphate and Acetate Hydrolysis, Neopentanol

1. Introduction

The pK_a of leaving groups is obviously important value because many biological and organic reactions are catalyzed by acids or bases in different solutions, but what is pK_a and what does mean? pK_a gives us a thought about how much is the acidity of a given hydrogen atom in a substrate. The p*K*a value is very useful parameter because, it is necessary to guess where the compound or substrate will be protonated or deprotonated and what is the pH reaction would be needed. It would be ineffective to use a very low pH to deprotonate a compound has a high pK_a but, using a very high pH where a low one

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will be good this would be like trying to break a walnut using a heavy thing-you would succeed but your nut would be totally damaged in the process. Once you understand the trends involved, you should have a good feel for the pK_a values of commonly encountered compounds and also be able to predict the values for unfamiliar compounds. However, at the end of the nineteenth century it was understood that such compounds are acidic only if they produce hydrogen ions H^+ in aqueous solution-the more acidic the compound, the more hydrogen ions it produces[1, 3].

In this work, our thinking leads us to use the Brønsted plot (LFER) for uridine 3´-alkyl-phosphate diesters (RNA models) to find out the best estimate for the pK_a of neopentyl alcohol. Somebody may ask why we select this brønsted plot to estimate the pK_a of the neopentyl alcohol (poor leaving group). The answer will be that the much more negative of β_{lg} -1.28 [4] for the reaction used in this work (sharp gradient of relationship) means that errors in the rate of reaction are reduced in the pK_a estimate. Furthermore, the reaction is a better reference to correlate the data obtained here as it also involves a phosphate diester, rather than a carboxylic ester.

The mechanistic evidence for phosphoryl transfer reactions in RNA bonds has been well characterized to achieve a clear understanding concerning the biological process. In the fact, RNA has a very efficient internal nucleophile (2´–hydroxyl), which leads to rapid RNA transesterification, whereas DNA has no similar internal nucleophile to cleave its bonds [5, 6].

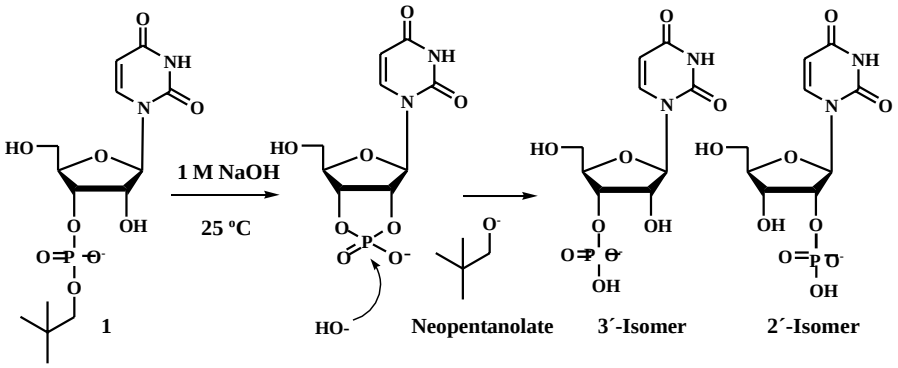


Figure 1. Proposed mechanism for the hydroxide catalyzed hydrolysis of **1** via formation of uridine 2´,3´-cyclic monophosphate.

2. Results and Discussion

In an effort to determine the pK_a of neopentyl alcohol, substrates **1** and **2** with neopentyl group as a leaving group have been prepared for this purpose. Fig 1 shows chosen substrate **1** to mimic the phosphate diester cleavage of RNA. activated models for the key structure of RNA have successfully been established by replacing real linkage group of alkyl group with the pK_a of the 3′ or 5′ OH as a leaving group ≈ 14.3 [7, 8] in RNA by a range of leaving groups with pK_a from12.24 to 17.1 and in this work the leaving group replaced by neopentyl group as shown in Fig 1. Designed good model to quantify the pK_a of neopentyl alcohol as a leaving group can be facilitated by inserting this group with an activated substrate as here in compound **1**. This thought can give us a good estimate for the pK_a of neopentyl alcohol from the observed rate of RNA model hydrolysis at low temperature [9, 10]. Compound **1** contains the neopentyl group as a leaving group to prevent attack at carbon without hindering attack at the phosphorus [11]. Indeed, the hydrolysis of substrate **1** is observable at ambient temperature when the neopentyl group is a leaving group [4]. Under the reaction conditions, the elution system in table I and retention time in table II for the starting diester and the products alcohol with the intermediate monoester and the internal reference were monitored at 224 nm where all materials can be detected using the HPLC-UV.

Table 1.The elution system used in hplc for phosphate diester kinetics on a column [a]

Substrate	Time / min	% Acetonitrile	0.1 % TFA [b]
1	0	0.1	99.9
	9	1	99
	25	15	85
	35	0.1	99.9

a Column 4 × 150 mm, particle size 5 mm, flow rate 1 ml min^{-1}. ***b*** Acetonitrile content (%, v/v) in 0.025 M acetic acid–sodium acetate buffer, containing 0.1 M ammonium chloride [4, 8].

Table 2.Retention times of uridine 3´-neopentyl phosphate, its 2´,3´-isomers and internal reference on a column

Retention time (min)			
3´-Isomer	2´-Isomer	Internal Reference	Uridine-3´neopentyl Phosphate
4.22	4.41	14.53	25.33

The hydrolysis of uridine 3´-neopentyl phosphate diester in 1 M NaOH was initiated by 0.5 mM of substrate **1** with internal reference. The progress of the reaction then analyzed by HPLC on a column (4 x 150 mm, 5 µm) in a run of 35 min for, using an appropriate solvent system to isolate peaks. This was loaded onto the column and eluted with solvent system as detailed in table I, at a flow rate of 1 ml min^{-1}.

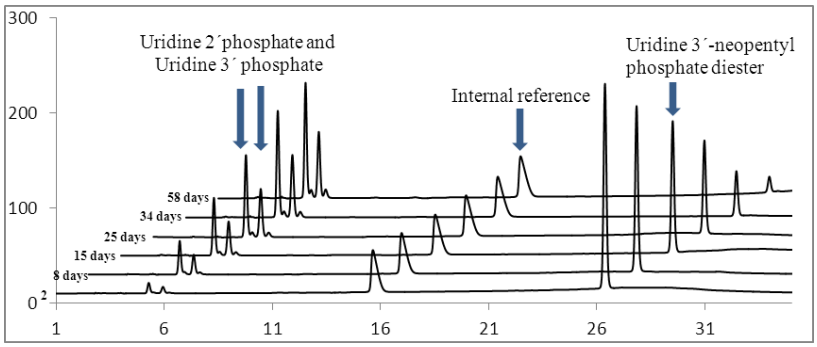


Figure 2. HPLC traces for the hydroxide catalyzed hydrolysis of compound **1** in 1 M NaOH, monitored at 25 °C to observe the disappearance of the uridine 3´-neopentyl phosphate diester and corresponding appearance of uridine 2´- and 3´-phosphate, with concomitant release of neopentyl alcohol

At 25 °C, we could observe the disappearance of uridine 3´-neopentyl phosphate diester and corresponding appearance of uridine- 2´- and 3´-monophosphates, which are formed from the subsequent hydrolysis of 2´, 3´–cUMP product by HPLC analysis of aliquots removed at various time intervals during two months (Figure 2).

The pseudo-first-order rate constant was measured at 1 M NaOH by plotting the relative integration peak area against time. This gives an observed rate of 4.81×10^{-7} s^{-1}; the reaction is catalyzed by hydroxide (Figure 3).

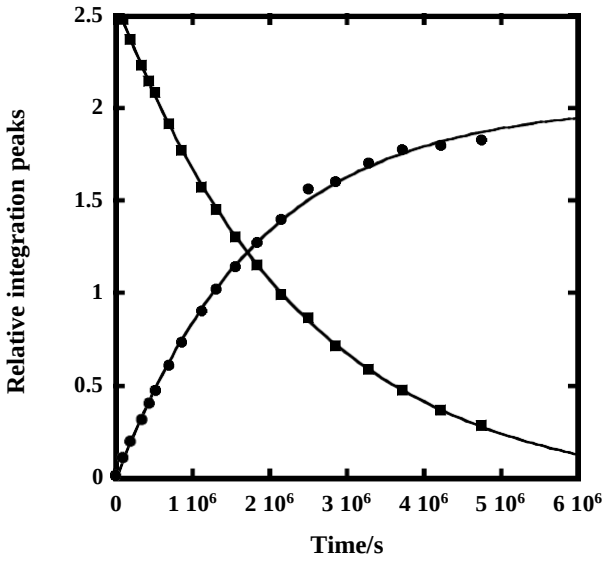


Figure 3. Hydroxide ion-catalyzed hydrolysis of substrate 54 in alkaline solution of 1 M NaOH. (●), appearance of the (U2´-P and U3´-P) and (■), disappearance of the substrate54. The reaction was monitored by HPLC at 260 nm and 25 °C, R^2> 0.99.

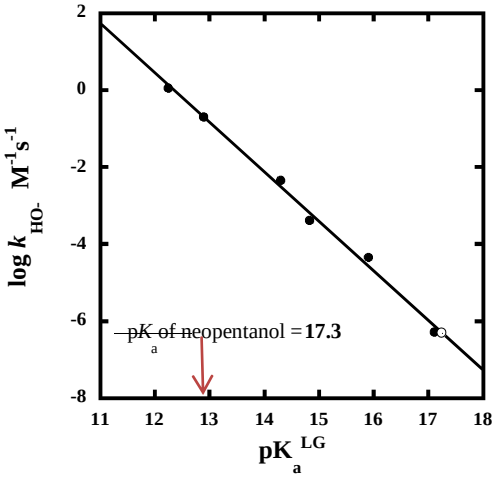


Figure 4. The Brønsted plot for the second order rate constants of the hydroxide-catalyzed hydrolysis of uridine 3´-alkyl-phosphate diesters [4] used to estimate the pK_a for neopentanol alcohol (17.3). The solid line has a gradient β_{lg} = – 1.28 ± 0.05; intercept 15.9 ± 0.7. The data was obtained at 25 °C and I = 1 M.[34]

Using the reported Brønsted plot for uridine 3´-alkyl-phosphate diesters (Figure 4), the pK_a of neopentyl alcohol was derived from the plot by reading the corresponding pK_a for the observed rate constant for **1**. The pK_a of neopentyl alcohol from the observed rate is 17.3.

This value of 17.3 is in good agreement with the value of 17.9 estimated for 2,2-dimethyl-3-(4-sulfonylphenyl) propyl, derived from the hydroxide catalyzed hydrolysis of acetate esters at 25 °C [2, 12]. This reaction has a small β_{lg} of – 0.25, which means that the error in the rate measurements will be amplified in the estimated pK_a. The much more negative of β_{lg} -1.28 for the reaction used in this work means that errors in the rate of reaction are reduced in the pK_a estimate. Furthermore, the reaction is a better reference to correlate the data obtained here as it also involves a phosphate diester, rather than a carboxylic ester This new value 17.3 for the pK_a of neopentanol is about two units greater than that used in earlier papers (15.5), where it was assumed that the pK_a would be the same as for methanol [13, 15] This new value is used to estimate a better half-life for pH-independent DNA cleavage in this work.

The pK_a of 2, 2-dimethyl-3-phenyl propanol alcohol is very similar compound to neopentyl alcohol just attached a ring of sodium sulfonyl phenyl to facilitate the solubility and monitoring by HPLC. The pK_a of this alcohol was measured by studying the hydrolysis of the acetate ester at 25 °C.

Figure 5 gives an observed rate k_{obs} 4.35×10^{-3} s^{-1} in 0.1 M KOH and ionic strength 1 M at 25 °C and therefore $k_{OH} = 4.35 \times 10^{-2}$ M^{-1} s^{-1}. The reported equation for the base hydrolysis of similar compounds can be used to estimate the pK_a of the alcohol. The pK_a of the alcohol was estimated to be 17.9 by solving reported equation, which derived by Bruice *et al* [15] for a series of acetate esters under the same conditions. log (k_{OH} /M^{-1} s^{-1} at 25 °C) = (3.12 ± 0.16) – (0.25 ± 0.01) pK_{LG}

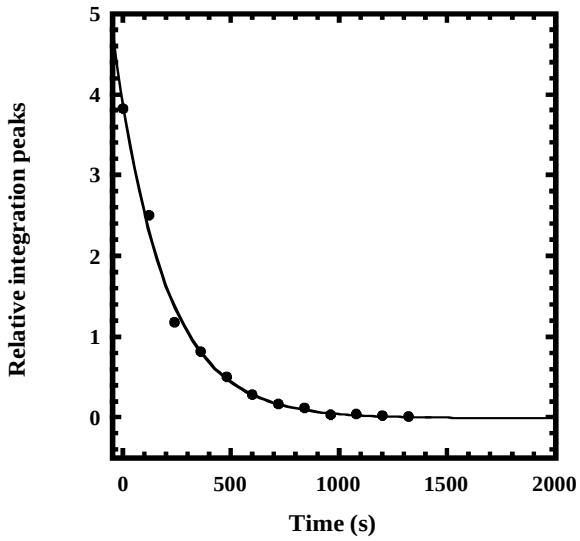
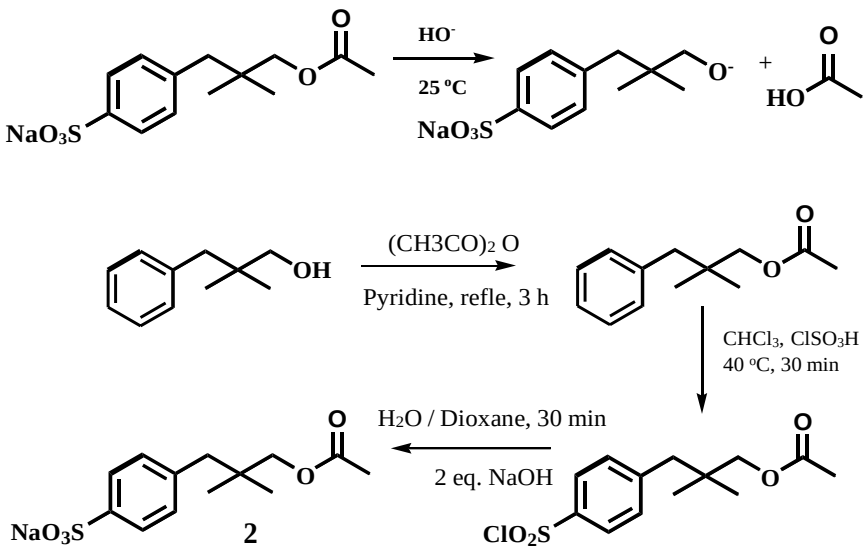


Figure 5. Hydrolysis of acetate ester 25 in 0.1 M KOH at 25 °C.

3. Materials and Methods

Uridine 3´-neopentyl phosphate diester was provided by Prof. Williams, University of Sheffield and Heidi korhonen, from Turku University – Finland. 1H-NMR (500 MHz, D2O ; 7.80 (2d, J=8.0Hz, H-C(6)) ; 5.88 (d, J=5 Hz, H-C(1´)) ; 5.81 (d, J=8 Hz, H-C(5)) ; 4.50 – 4.46 (m, H-C(3´)) ; 4.37 – 4.35 (t, H-C(2´)) ; 4.24 – 4.23 (q , J=3 Hz, H-C(4´)) ; 3.84 – 3.81 (dd, J_1=12.5 Hz, J_2=2.5 Hz, H-C (5´)); 3.76 – 3.73 (dd, J_1=12.75 Hz, J_2=4.5 Hz, H-C(5´´)) ; 3.51 – 3.46 (m, CH2-O) ; 0.83 (s, 9H, 3 Me). HR-ESI-MS calculated for $C_{14}H_{22}N_2O_9P^-$ 393.11, found 393.1146.

2,2-dimethyl-3-phenyl propyl acetate was prepared by reaction of 2,2-dimethyl-3-phenylpropan-1-ol with acetic anhydride in dichloromethane in the presence of pyridine. The sulfonation was carried out with chlorosulfonic acid followed by treatment with sodium hydroxide to yield the target 2,2-dimethyl-3-phenyl propyl acetate (Scheme 1) δ_H (250 MHz, $CDCl_3$) 0.9 (6 H, s, CH_3), 2.1 (3 H, s, CH_3 –C = O), 2.7 (2 H, s, CH_2 - Ar), 3.8 (2 H, s, CH_2 - O), 7.4 (2 H, d, $^3J_{HH}$ 8.5, Ar - H), 7.9 (2 H, d, $^3J_{HH}$ 8.8, Ar - H).



Scheme 1. Preparation of sodium sulfonyl 2, 2-dimethyl-3-phenyl propyl acetate.

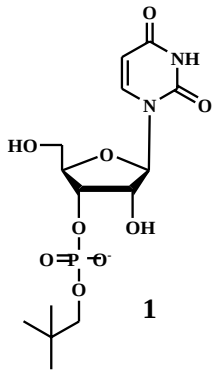
Kinetic experiments were investigated in plastic tube with closed lid immersed in an oil bath at 25 ± 0.1°C. The initial concentration of substrate **1** was 0.5 mM with 4-sodium sulfonyl toluene as an internal reference. The progress of the cleavage of **1** in 1 M NaOH was monitored at 260 nm and 25 °C. The aliquots were taken from the running reaction at required time intervals and quenched with hydrochloric acid to pH 7, then analyzed by HPLC on a column (4 x 150 mm, 5 µm) in 35 min for each run, using an appropriate solvent system to isolate peaks, which is a mixture of acetic acid, sodium acetate buffer (40 mM, pH= 4.4, [NH4Cl] =0.1M) and acetonitrile content spanning 0.1 to 15 as an eluent. 99.9 % buffer was eluted as a solvent system in a 9 min then followed by a gradient (25 min) up to 15% of acetonitrile. This was loaded onto the column and eluted with solvent system as detailed in **table** I, at a flow rate of 1 ml min^{-1}. The pseudo-first-order rate constant was obtained by plotting the relative peak area against time.

Kinetic studies of **2** involved a 5 mM solution of **2** in 0.1 M KOH with an internal standard of sodium p-toluene sulfonate (2.5 mM). This was sealed in a plastic tube and kept at a constant temperature (25 ± 0.1) °C by immersion in a circulating oil bath. Samples were removed at various time intervals and quenched with hydrochloric acid to

pH 7 for analysis by HPLC. The peak areas of reactants and products were normalized against the internal standard and the relative peak areas were plotted against time.

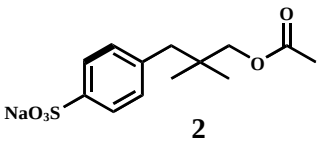
4. Conclusion

The hydrolysis of **1** and **2** as modeled substrates (Fig. 6) for neopentyl as a leaving group under identical conditions to that reported for a series of alkyl uridyl phosphate diesters or acetates have been used to provide a better estimate for the pK_a of neopentyl alcohol derived from Brønsted plots and the observed rate constants for **1** and **2**. accordingly the pK_a of neopentyl alcohol from the observed rate of the hydroxide-catalysed hydrolysis of **1** is **17.3** and for **2** is **17.9** ,which is significantly greater than the value of 15.5 published and used in earlier reports. Thus, the average value of the pK_a of neopentyl alcohol is **17.6**. This new value can be used to estimate a better half-life for pH-independent DNA cleavage.



1

Sodium Uridine 3´-neopentyl phosphate diester



2

Sodium sulfonyl- 2, 2-dimethyl -3-phenyl propyl acetate

Figure 6. two substrates attached with neopentyl alcohol as a leaving group to estimate the pK_a of neopentyl alcohol.

Acknowledgment

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5. References

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