Hydrolysis of Nucleosides Related to Wyosine

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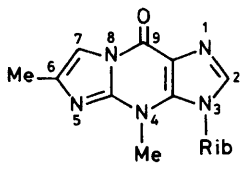
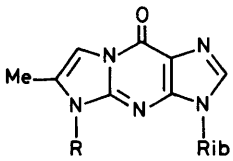
The glycosidic bond of 3-β-D-ribofuranosylwye (**1**) has been found to undergo not only hydrogen ion catalysed cleavage but also general base catalysed or nucleophilic cleavage; steric assistance has been shown to account for its unusual susceptibility to acid.

A characteristic of wyosine from *Torulopsis utilis* $tRNA^{Phe}$ is the unusual lability of the glycosidic bond under acidic conditions.[1] The present paper describes the results of kinetic studies on the hydrolysis of 3-β-D-ribofuranosylwye (**1**),[2] the most probable structure for wyosine, and nucleosides structurally related to (**1**).

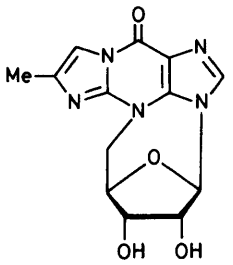
Pseudo-first-order rate constants ($k_{obs.}$) for the hydrolysis of the glycosidic bond of the demethyl analogue (**2**),[1b] the positional isomer (**3**),[1b] and the cyclonucleoside analogue (**4**)[1b,3] of (**1**) in 0.1 M aq. HCl at 85 °C (4.4×10^{-3}, 3.3×10^{-3}, and 6.3×10^{-3} min^{-1}, respectively) were found to be the same order of magnitude but rather smaller than $k_{obs.}$ (1.7×10^{-2} min^{-1}) for the hydrolysis of the glycosidic bond of guanosine under these conditions.† For comparison, the rates for (**2**) and (**3**) were measured at 75 and 95 °C in 0.1 M aq. HCl and the rate constants, 4.5×10^{-7} and 2.8×10^{-7} min^{-1}, at 25 °C were calculated from the Arrhenius equation. These values are smaller than those for the hydrolysis of (**1**) and 3-methylguanosine (**5a**) (4.4×10^{-1} and 9.8×10^{-1}, respectively)[2b] at 25 °C in 0.1 M aq. HCl by a factor of 10^{-6}. Thus, the 3-methylguanosine structure in (**1**) appears to be responsible for the unusual susceptibility of the glycosidic bond of (**1**) to acidic hydrolysis.



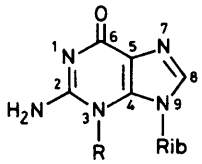
(**1**)



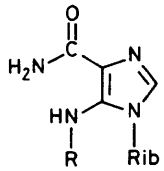
(**2**) R = H
(**3**) R = Me



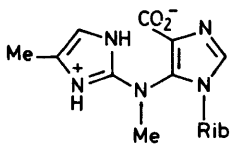
(**4**)



(**5**) **a**; R = Me
b; R = Et
c; R = $CHMe_2$



(**6**) **b**; R = Et
c; R = $CHMe_2$



(**7**)

Rib = β-D-ribofuranosyl

The rates of acidic hydrolysis of (**1**) are independent of buffer concentration and the rate–pH profile suggests that the hydrolysis of (**1**) is influenced by the hydrogen ion catalysed solvolysis of both the unprotonated, S, and protonated species, SH^+, as shown in equation (1), where $k^0_{obs.}$ is the

$$k^0_{obs.}[S]_T = k_1[S]a_H + k_2[SH^+]a_H \quad (1)$$

apparent first-order rate constant at zero buffer concentration, $[S]_T$ the total concentration of (**1**), and a_H the hydrogen

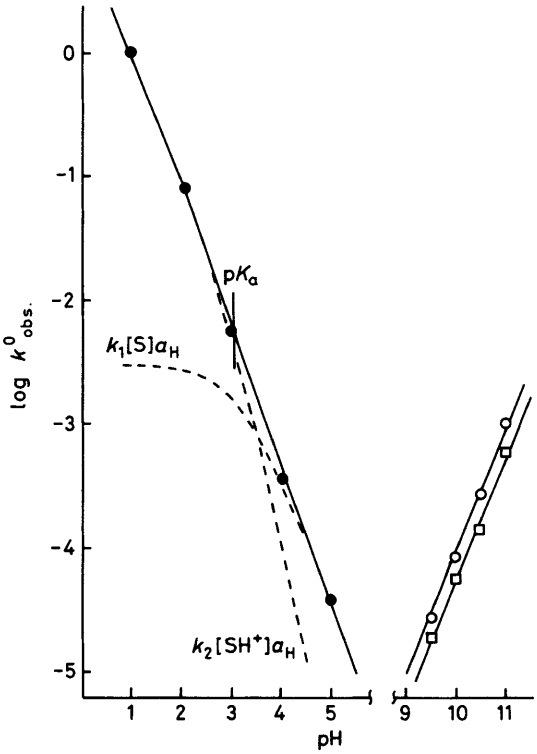


Figure 1. Rate–pH profiles for the hydrolysis of the glycosidic bond of (**1**) at 25 °C and ionic strength 1.0 (●) and for the disappearance (○) and glycosidic bond cleavage (□) of (**1**) at 40 °C and ionic strength 1.0.

Table 1. Acid dissociation constants and second-order rate constants for the hydrogen ion catalysed hydrolysis of the unprotonated and protonated species at 25 °C and ionic strength 1.0.

Compound	pK_a	k_1 /l mol^{-1} min^{-1}	k_2 /l mol^{-1} min^{-1}
(**1**)	3.06 ± 0.05	3.5	10
(**5a**)	3.99 ± 0.06	18	23
(**5b**)	3.86 ± 0.07	28	42
(**5c**)	3.83 ± 0.04	130	140

† Reese and Whittall have also reported that the glycosidic linkage of (**4**) is more stable than that of guanosine in 0.9 M aq. HCl at 37 °C (ref. 3).

ion activity. At 25 °C and ionic strength 1.0 a value of 3.06 for pK_a was determined for (**1**), and good agreement between the experimental (the closed circles in Figure 1) and calculated values (the solid line) of $k^0_{obs.}$ was obtained when the second-order rate constants, k_1 and k_2, were taken to be the values given in Table 1.

Acid catalysed hydrolyses of the glycosidic bond of (**5a**)[2b] and its higher alkyl homologues [(**5b**): m.p. 168 °C (decomp.); (**5c**): m.p. 65 °C (decomp.), prepared from (**6b,c**)[4] by cyanation[2b] followed by treatment with $NaOCHMe_2$ in 37 and 10% yield, respectively] also obey rate law (1). The results summarised in Table 1 show that the bulkier the substituent at the 3-position of (**5**) the more the rate of hydrogen ion catalysed glycosidic bond cleavage of both the unprotonated and protonated species is enhanced.

Under alkaline conditions, the glycosidic bond and the base moiety of (**1**) are cleaved competitively and the rate constants increase with increasing buffer concentration at constant pH. The ring-fission product was obtained as a glass and characterised as (**7**) by ^{1}H n.m.r. [δ $(CD_3)_2SO$ 1.96 (3H, CMe), 3.20 (3H, NMe), and 5.37 (1H, d, *J* 5 Hz, anomeric proton)] and field desorption mass spectroscopy (M^+ 353). The limiting rate constants for the disappearance of (**1**) at zero buffer concentration ($k^0_{obs.}$), 40 °C, and ionic strength 1.0 (the open circles in Figure 1) are proportional to hydroxide ion activity and the second-order rate constant was estimated to be 3.3×10^{-1} l mol^{-1} min^{-1}, when the ionic product of H_2O was taken as 2.9×10^{-14} mol^2.[5] For the hydroxide ion catalysed cleavage of the glycosidic bond of (**1**) (the open squares in Figure 1), a second-order rate constant of 1.9×10^{-1} l mol^{-1} min^{-1} was obtained. Since (**1**) remained unchanged at pH 7.0 and 25 °C for 40 days, hydrolysis of the unprotonated species catalysed by H_2O can be neglected. Thus, it can be seen from Figure 1 that (**1**) is practically stable between pH 6 and 9 at room temperature.

The present results suggest that a reduction in the steric repulsion between the 4-methyl and 3-ribofuranosyl groups is a major driving force in the rapid acidic hydrolysis of the glycosidic bond of (**1**) and this work should help in the isolation of wyosine and its congeners and in the modification of $tRNAs^{Phe}$.

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