Acid-catalysed and Metal-ion-catalysed Hydrolysis of 8-Quinolyl β-D-Glucopyranoside

By **Charles R. Clark** and **Robert W. Hay,*** Chemistry Department, University of Stirling, Stirling, Scotland

The hydrolysis of 8-quinolyl β-D-glucopyranoside has been studied over a wide range of acidity. In the pH region 1·0—5·2, hydrolysis of the free base form and of the *N*-protonated glucoside occurs. There is little evidence for intramolecular general-acid catalysis in the hydrolysis of the *N*-protonated glucoside. In more strongly acidic media (1·79—5·02M-HCl) at 70 °C, only the specific-acid-catalysed hydrolysis of the *N*-protonated glucoside occurs. Application of various mechanistic criteria [ϕ = +0·49; $k(D_2O)/k(H_2O)$ = 1·1; $\Delta S_{333}^{\ddagger}$ = +3·0 cal K^{-1} mol^{-1}] suggest that the reaction proceeds by a predominantly *A*-2, rather than the expected *A*-1 pathway. The hydrolysis of the glucoside is susceptible to metal ion catalysis in the pH range 5·5—6·2. Copper(II), nickel(II), and cobalt(II) catalysis has been studied. Copper(II) is a particularly effective catalyst and it is estimated that the Cu^{II} complex is hydrolysed *ca.* 10^5—10^6 times faster than the uncomplexed glycoside in the pH range 5·5—6·2.

THE hydrolysis of glycosides is susceptible to both general- and specific-acid catalysis.[1,2] In most theories of glycosidase action it is postulated that the glycoside undergoes hydrolysis in the enzyme–substrate complex with an acidic group of the enzyme providing general-acid catalysis and sometimes a basic group also providing nucleophilic catalysis.[3,4] Intramolecular general-acid catalysis has recently been suggested to account for the strong rate enhancing effect of the *ortho*-carboxy-group in the hydrolysis of 2-carboxyphenyl β-D-glucoside.[5] Since hydrolysis of glycosides is susceptible to acid catalysis it might be expected that cleavage of the glycosidic bond would also be subject to metal ion catalysis. We have therefore studied the effect of metal ions on the hydrolysis of 8-quinolyl β-D-glucopyranoside (I). 8-Hydroxyquinoline was chosen as the aglycone in order to provide a binding site for the metal ion close to the glucosidic bond (the hydrolyses of 8-quinolyl phosphate[6] and 8-quinolyl sulphate[7] have been shown to be catalysed by metal ions) and the presence of the 8-quinolyl system allows ready monitoring of the reaction by u.v. spectrophotometry.

EXPERIMENTAL

Deuterium chloride (Merck; isotopic purity 99%) was used as received without dilution. All other reagents were B.D.H. AnalaR grade.

8-Quinolyl β-D-Glucopyranoside.—Acetobromoglucose (3·0 g, 7·9 mmol), 8-hydroxyquinoline (4·5 g, 31 mmol), and potassium hydroxide (1·7 g) were dissolved in 50% acetone–water and set aside for 24 h. The acetone was evaporated off and the residue, in benzene, was washed with M-sodium hydroxide, then water, dried ($MgSO_4$), and evaporated. The 8-quinolyl tetra-*O*-acetylglucoside (1·78 g, 46%) had m.p. 159—160° (from ethanol), $[\alpha]_D^{25}$ −53·4°, (lit.,[8] $[\alpha]_D^{25}$ −57·1°, m.p. 162—163°). Deacetylation with a catalytic amount of sodium methoxide in methanol gave 8-quinolyl β-D-glucopyranoside (90%). Recrystallisation from water to which a trace of dioxan had been added and drying *in vacuo* at 25° gave a monohydrate (Found: C, 55·4; H, 6·2; N, 4·1. Calc. for $C_{15}H_{17}NO_6,H_2O$: C, 55·5; H, 5·9; N, 4·3%), as previously reported.[8] The m.p. (184—185°) which is observed upon slow heating is characteristic of the anhydrous compound; the hydrate may melt at a considerably lower temperature.

Kinetic Measurements.—With the exception of studies with deuterium chloride, all kinetic measurements were carried out by the ' sealed tube ' technique. A few crystals of the glycoside were added to the reaction medium (100 ml). Samples (6—7 ml) of this solution were then placed in each of twelve Pyrex tubes, which were then sealed and simultaneously immersed in a thermostatted bath containing ethylene glycol. The mixtures were allowed to equilibrate for 30 min before one tube was removed and its contents rapidly quenched in ice–water. Additional tubes were then removed at suitable intervals, and their contents quenched and analysed spectrophotometrically (Hilger–Watts Uvispek spectrophotometer). Measurements of the deuterium solvent isotope effect were carried out by using stoppered 1 cm silica cells in the thermostatted cell holder of the instrument. The acid-catalysed hydrolyses were monitored at 255 nm ($\lambda_{max.}$ for the conjugate acid of 8-hydroxyquinoline). Metal-ion-catalysed hydrolyses were followed both at 236·5 nm ($\lambda_{max.}$ for the free base form of the glycoside) and at 255 nm. In the latter case identical rate constants, within experimental error, were obtained at both wavelengths.

In kinetic investigations of glycoside hydrolysis in the pH region 1·0—5·2, spectrophotometric analyses were also carried out at 255 nm after acidification of the reaction mixtures with an equivalent volume of 3M-HCl. These reactions were normally followed to about 60% completion; however for the very slow runs in the pH range 4·5—5·2, rate constants were estimated from the percentage hydrolysis after 450 h. The pH values of the solutions were measured on initiation and completion of the reactions. Mixtures exhibiting a pH drift greater than ±0·03 units were discarded. In all kinetic runs glycoside concentrations were in the range 1·5—3·0 × 10^{-5}M.

[1] J. N. BeMillar, *Adv. Carbohydrate Chem.*, 1967, **22**, 25.
[2] B. Capon, *Chem. Rev.*, 1969, **69**, 407.
[3] D. E. Koshland in ' The Mechanism of Enzyme Action,' eds. W. D. McElroy and B. Glass, John Hopkins Press, Baltimore, 1954, p. 608; E. H. Fischer and E. A. Stein, *Enzymes*, 1960, **4**, 313; M. L. Bender and R. Breslow, in ' Comprehensive Biochemistry,' vol. 2, eds. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1962, p. 38; I. Wallenfels and P. O. Malhotra, *Adv. Carbohydrate Chem.*, 1961, **16**, 239; F. C. Mayer and J. Larner, *J. Amer. Chem. Soc.*, 1959, **81**, 188.
[4] G. Lowe, *Proc. Roy. Soc.* (*B*), 1967, **167**, 431.
[5] B. Capon, M. C. Smith, E. Anderson, R. H. Dahm, and G. H. Sankey, *J. Chem. Soc.* (*B*), 1969, 1038.
[6] Y. Murakami, J. Sunamoto, and H. Sadamori, *Chem. Comm.*, 1969, 983; Y. Murakami and J. Sunamoto, *Bull. Chem. Soc. Japan*, 1971, **44**, 1939.
[7] R. W. Hay and J. A. G. Edmonds, *Chem. Comm.*, 1967, 969; R. W. Hay, C. R. Clark, and J. A. G. Edmonds, *J.C.S. Dalton*, in the press.
[8] E. M. Montgomery, N. K. Richtmeyer, and C. S. Hudson *J. Org. Chem.*, 1945, 194.

Rate constants were obtained from the slopes of plots of log ($OD_\infty - OD_t$) *versus* time (t), where OD = optical density. Linear plots over at least two half lives were obtained for both the acid- and metal-ion-catalysed reactions. Values of OD_∞ in the cobalt(II) catalysed reactions were obtained from solutions of cobalt(II) to which the requisite quantity of 8-hydroxyquinoline had been added. This technique was necessary because of degradation of the ligand after 3—4 half lives.

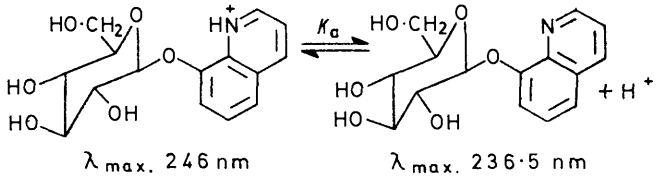
The activation energy for the acid-catalysed hydrolysis was obtained from the slope of a plot of log k_{obs} *versus* $1/T$, where k_{obs} is the observed pseudounimolecular rate constant. The entropy of activation was calculated from equation (i),

$$\Delta S^\ddagger/4{\cdot}576 = \log k_r - 10{\cdot}753 - \log T + E_a/4{\cdot}576T \quad \text{(i)}$$

where $k_r = k_{obs}/[H^+]$. As log k_{obs} usually varies linearly with $-H_0$ for glycoside hydrolysis, it has been customary,[9] for the purpose of calculating $\Delta S^\ddagger$, to define $k_r = k_{obs}/h_0$. In the present study the alternative approach was adopted, as k_{obs} followed $[H^+]$ more closely than h_0. This approach only slightly alters the resultant value of $\Delta S^\ddagger$, in this case by *ca.* 0·9 cal K^{-1} mol^{-1}.

RESULTS AND DISCUSSION

The pK_a for the equilibrium in Scheme 1 was determined spectrophotometrically to be 4·34 ± 0·03 at 25° and $I \longrightarrow 0$ as described by Albert and Serjeant.[10]



SCHEME 1

The pH–rate profile for the hydrolysis of 8-quinolyl β-D-glucopyranoside (Table 1) is shown in Figure 1.

TABLE 1

The pH–rate profile for the hydrolysis of 8-quinolyl β-D-glucopyranoside at 89·8° and I = 0·1M (KCl)

pH	$10^5 k_{obs}/min^{-1}$	pH	$10^5 k_{obs}/min^{-1}$
1·08 [a]	46·1	2·98 [b]	7·8
1·19 [a]	34·2	3·40 [b]	7·4
1·26 [a]	30·4	3·81 [c]	5·0
1·39 [a]	21·7	4·40 [d]	2·7
1·55 [a]	19·2	4·79 [d]	1·5
1·90 [a]	11·4	5·18 [d]	0·67
2·32 [a]	7·8		

[a] HCl–KCl buffer. [b] $ClCH_2{\cdot}CO_2H$–KOH buffer. [c] HCO_2-H–KOH buffer. [d] AcOH–KOH buffer.

A variety of buffers were employed (Table 1). General-acid catalysis was not observed; identical rate constants were obtained after two-fold dilution of buffers with 0·1M-potassium chloride.

[9] W. G. Overend, C. W. Rees, and J. S. Sequira, *J. Chem. Soc.*, 1962, 3429.

In the pH range 1·0—5·2 the rate law takes the form of equation (ii). Excellent agreement between the

$$\text{Rate} = k_{obs}[\text{Total Substrate}] = k_1[BH^+] + k_2[BH^+]a_{H^+} \quad \text{(ii)}$$

observed and calculated values of k_{obs} could be obtained by using values of $pK_a^p = 4{\cdot}1$, $k_1 = 7{\cdot}6 \times 10^{-5}$ min^{-1}, and $k_2 = 4{\cdot}1 \times 10^{-3}$ l mol^{-1} min^{-1} at 89·8°. The kinetic

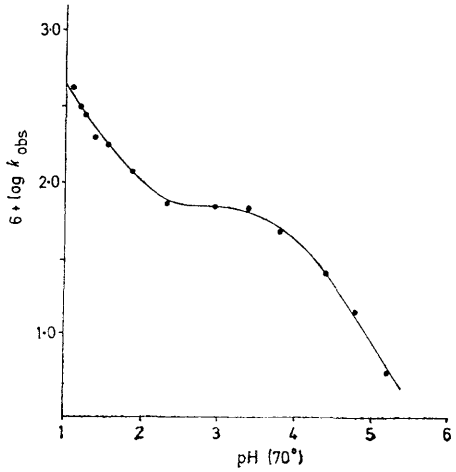


FIGURE 1 pH–Rate profile for the hydrolysis of 8-quinolyl β-D-glucopyranoside at 89·8° (I = 0·1M); the line is theoretical [based on equation (ii)] and the points are experimental

pK_a is a 'practical' constant and is thus not directly comparable with the thermodynamic constant at 25°. The second term on the right hand side of equation (ii) is attributable to a specific-acid-catalysed reaction of the protonated species BH^+. The first term involving k_1 may arise from one of two kinetically indistinguishable processes: (*a*) specific-acid-catalysed hydrolysis of B, or (*b*) 'spontaneous' hydrolysis of BH^+, which could involve intramolecular general-acid catalysis.

Normally, the specific-acid-catalysed hydrolysis of phenyl β-D-glucopyranosides is relatively insensitive to the nature of the substituents in the aglycone. Thus in the series of compounds studied by Nath and Rydon,[11] the most rapid hydrolysis was observed with 2-methoxyphenyl β-D-glucoside, which was hydrolysed only 27 times more rapidly than the most unreactive substrate, 4-nitrophenyl β-D-glucoside ($\rho = -0{\cdot}66$). If the reaction involved a specific-acid-catalysed hydrolysis of B, the second-order rate constant (k^*) would be related to k_1 by the equation $k^* = k_1/K_a$. The kinetically determined value of K_a gives $k^* = 0{\cdot}96$ l mol^{-1} min^{-1} at 89·8°, which is *ca.* 16 times greater than the second-order rate constant for the hydrolysis of phenyl β-D-glucoside at the same temperature. A comparison with Nath and Rydon's data shows that the relative value of k^* is certainly within the range expected for substituent effects alone, and there appears to be little evidence for an intramolecular general-acid-catalysed reaction.

[10] A. Albert and E. P. Serjeant, 'Ionisation Constants of Acids and Bases,' Methuen, London, 1962.

[11] R. L. Nath and H. N. Rydon, *Biochem. J.*, 1954, **57**, 1.

It has been suggested [12] that the ready acid-catalysed hydrolysis of 2-pyridyl glycosides involves intramolecular general-acid catalysis, but this view has recently been criticised.[2] Subsequent work has shown that 2-pyridyl β-D-glucoside is hydrolysed only 8 times more rapidly than 4-pyridyl β-D-glucoside. It has been suggested that the driving force for the reaction is attributable to the *N*-protonated 2-hydroxypyridine being a particularly good leaving group, since it isomerises to give the more thermodynamically stable 2-pyridone.

The absence of intramolecular general-acid catalysis in the hydrolysis of the 8-quinolyl glucoside is probably due to the protonated quinolyl nitrogen atom being too distant from the glucosyl oxygen atom for proton transfer to occur. In fact proton transfer to the ring oxygen atom would appear to be stereochemically more favourable; however, hydrolysis by such a pathway is unfavourable for aryl glucosides.[2] Intramolecular catalysis in the hydrolysis of 2-carboxyphenyl glucosides is dependent on both the orientation of the carboxy-group and its ability to approach closely the reaction centre.[5] At acidities greater than 0·5M-HCl, only the specific-acid-catalysed hydrolysis of BH^+ occurs. The reaction was studied at 70·1° over the acidity range 1·8—5·0M-HCl (Table 2). Plots of log k_{obs} *versus* both

TABLE 2

Acid-catalysed hydrolysis of 8-quinolyl β-D-gluco-pyranoside [a]

	[HCl]/M	$-H_0$ [b]	T/°C	10^3k_{obs}/min^{-1}
(a)	5·02	1·77	70·1	11·7
	4·18	1·46	70·1	7·56
	3·62	1·27	70·1	5·48
	2·98	1·04	70·1	3·91
	2·42	0·84	70·1	2·79
	1·79	0·59	70·1	1·65
	2·24		85·0	13·9
	2·24		70·0	2·36
	2·24		55·0	0·308

[a] The data in the latter part of the table give E_a = 29·4 kcal mol^{-1} ($\Delta H^{\ddagger}$ = 28·8 kcal mol^{-1}) and $\Delta S^{\ddagger}_{333}$ = 3·0 ± 2·0 cal K^{-1} mol^{-1}. [b] Interpolated from the data of M. A. Paul and F. A. Long (*Chem. Rev.*, 1957, **57**, 1).

(*b*) Solvent deuterium isotope effect at 58·3 °C

Medium	10^3k_{obs}/min^{-1}	$k(D_2O)/k(H_2O)$
6·54M-DCl	5·72	1·14
6·54M-HCl	5·07	

log [H^+] and $-H_0$ show considerable curvature (Figure 2). In view of the dubious validity of the Hammett–Zucker hypothesis, the Bunnett–Olsen plots are to be preferred. Plots of (log k_{obs} + H_0) *versus* (log [H^+] + H_0) are linear and the slope (ϕ parameter) is +0·49. This value lies within the range +0·22—0·56 associated with reactions in which water is involved as a nucleophile in the rate-determining step (*A*-2), and differs significantly from the values found (<0) for unimolecular processes (*A*-1).[13]

The deuterium solvent isotope effect [$k(D_2O)/k(H_2O)$ = 1·1; Table 2] is typical for transition states in which there is considerable involvement by water.[14] For *A*-1

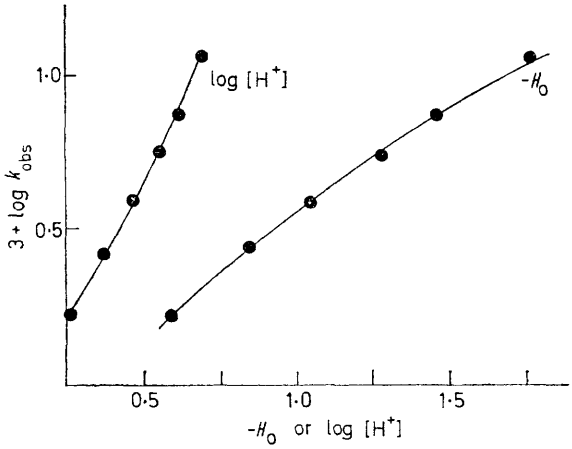
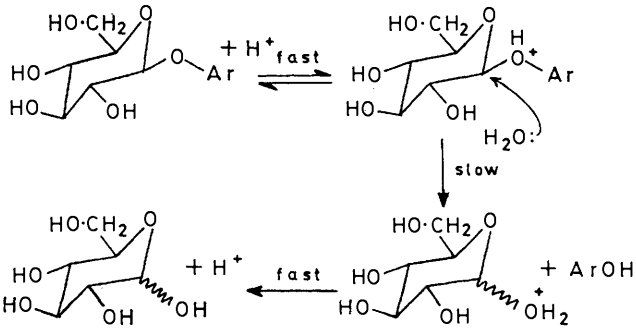


FIGURE 2 Plots of log k_{obs} *versus* $-H_0$ and log [H^+] for the acid-catalysed hydrolysis of 8-quinolyl β-D-glucopyranoside

reactions, $k(D_2O)/k(H_2O)$ ratios of 1·9—2·5 are commonly observed:[15] thus for the hydrolyses of methyl α-D-glucoside [9] and methyl 2-deoxy-α-D-glucopyranoside [16] the values are 1·9 and 2·5, respectively.

The entropy of activation ($\Delta S^{\ddagger}_{333}$ = +3·0 cal K^{-1} mol^{-1}; Table 2) is considerably lower than the mean value (+13·7 cal K^{-1} mol^{-1}) observed for a series of twenty-four glycopyranosides;[9] however, the reduction in $\Delta S^{\ddagger}$ by +10·7 cal K^{-1} mol^{-1} is less than that (*ca.* 20 cal K^{-1} mol^{-1}) which characteristically separates *A*-1 and *A*-2 processes within the same class of compounds.[17] The various mechanistic criteria are thus more consistent with an *A*-2 process (Scheme 2).



SCHEME 2

Recent studies of the mercury(II)-catalysed solvolysis of thioglucosides have shown that the reaction occurs with almost complete inversion of configuration at C-1.[18]

[12] G. Wagner and G. Valz, *Pharmazie*, 1967, **22**, 548; G. Wagner and H. Frenzel, *Z. Chem.*, 1965, **5**, 454; *Arch. Pharm.*, 1967, **300**, 591.

[13] J. F. Bunnett and F. P. Olsen, *Canad. J. Chem.*, 1966, **44**, 1917.

[14] C. A. Bunton and V. J. Shiner, *J. Amer. Chem. Soc.*, 1961, **83**, 3207.

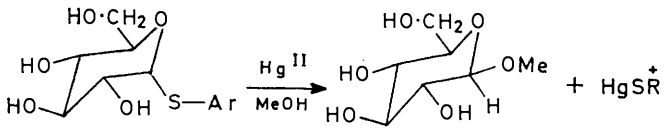
[15] J. G. Pritchard and F. A. Long, *J. Amer. Chem. Soc.*, 1958, **80**, 4162.

[16] C. Armour, C. A. Bunton, S. Patai, L. H. Selman, and C. A. Vernon, *J. Chem. Soc.*, 1961, 412.

[17] L. L. Schaleger and F. A. Long, *Adv. Phys. Org. Chem.*, 1963, **1**, 1.

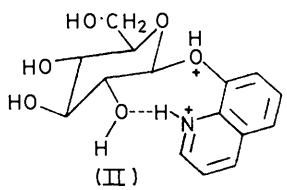
[18] R. J. Ferrier, N. Vethaviyasar, and R. W. Hay, *Carbohydrate Res.*, 1973, **27**, 55.

Thus for the mercury(II)-catalysed methanolysis of phenyl 1-thio-α-D-glucopyranoside, methyl β-D-glucopyranoside is produced with 100% stereospecificity (Scheme 3).

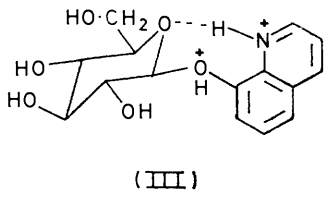


SCHEME 3

Bunnett plots for the hydrolysis of pyranosides have yielded rather conflicting results.[19] The w values suggest that some reactions proceed by an A-2 rather than the expected A-1 pathway. Interactions within the glycoside favouring an increase in the positive character of C-1 could lead to nucleophilic participation by water. An examination of stereo-models indicates that the protonated quinolyl group can interact with (*a*) the C-2



(II)



(III)

hydroxy-group (II) and (*b*) the ring oxygen atom (III). Hydrogen-bonding to the C-2 hydroxy-group (II) is clearly unfavourable, as the interaction involves an 8-membered chelate ring with a concomitant freezing of rotation about several bonds. In addition, electronic effects at C-2 cause profound effects on hydrolysis rates. Buncel and Bradley [20] have observed that replacement of the C-2 hydroxy-group by the more strongly electron-withdrawing chloro-group in methyl β-D-glucopyranoside resulted in a 35-fold rate decrease and a partial A-2 reaction. In the case of 8-quinolyl β-D-glucopyranoside the A-2 character of the reaction is more pronounced, but the hydrolysis is only 10 times slower than that of phenyl β-D-glucopyranoside. The hydrolysis rate is of the order expected for an aryl β-D-glucopyranoside containing a strongly electron-withdrawing group in the aglycone. The alternative structure (III) is thermodynamically more favourable since rotation is restricted about fewer bonds. In such a structure there is less ' lone pair availability ' on the oxygen atom to provide resonance stabilisation of a developing A-1 transition state.

Metal-ion-catalysed Hydrolysis.—Catalysis by copper(II), nickel(II), and cobalt(II) was studied at 70·1° to allow a direct comparison with the proton-catalysed reaction. Buffers were not employed in the study, but the pH of the reaction mixtures fell within the range 5·5—6·2 owing to the buffering effect of the equilibrium $M(H_2O)_6^{2+} \rightleftharpoons [M(H_2O)_5(OH)]^+ + H^+$. The reactions were carried out under pseudounimolecular conditions (metal-to-ligand ratios in excess of 20 : 1). Under these conditions the reactions were first order in the glycoside, and the values of k_{obs} at various metal ion concentrations are listed in Table 3. For a particular metal ion, k_{obs}

TABLE 3

Metal-ion-catalysed hydrolysis of 8-quinolyl β-D-glucopyranoside at 70·1° and I = 0·1M (KCl)

(a) Copper(II) catalysis

$10^4[M^{2+}]$/M	10^4k_{obs}/min^{-1}	$(k_{obs}/[M^{2+}])$/ l mol^{-1} min^{-1}
2·5	15·2	6·04
5·0	30·0	6·00
7·5	43·4	5·79
10·0	57·6	5·76
12·5	73·3	5·87

$k_{cat} = 5·9 \pm 0·2$ l mol^{-1} min^{-1}

(*b*) Nickel(II) catalysis

$10^3[M^{2+}]$/M	10^4k_{obs}/min^{-1}	$(10^2k_{obs}/[M^{2+}])$/ l mol^{-1} min^{-1}
2·00	0·429	2·15
3·00	0·620	2·10
4·01	0·833	2·08
5·01	1·04	2·04

$k_{cat} = (2·1 + 0·1) \times 10^{-2}$ l mol^{-1} min^{-1}

(*c*) Cobalt(II) catalysis

$10^2[M^{2+}]$/M	10^4k_{obs}/min	$(10^3k_{obs}/[M^{2+}])$/ l mol^{-1} min^{-1}
0·67	0·27	4·1
1·67	0·73	4·4
2·50	1·14	4·46
3·33	1·51	4·52

$k_{cat} = (4·3 \pm 0·2) \times 10^{-3}$ l mol^{-1} min^{-1}

is given by equation (iii), where k_u is the rate constant

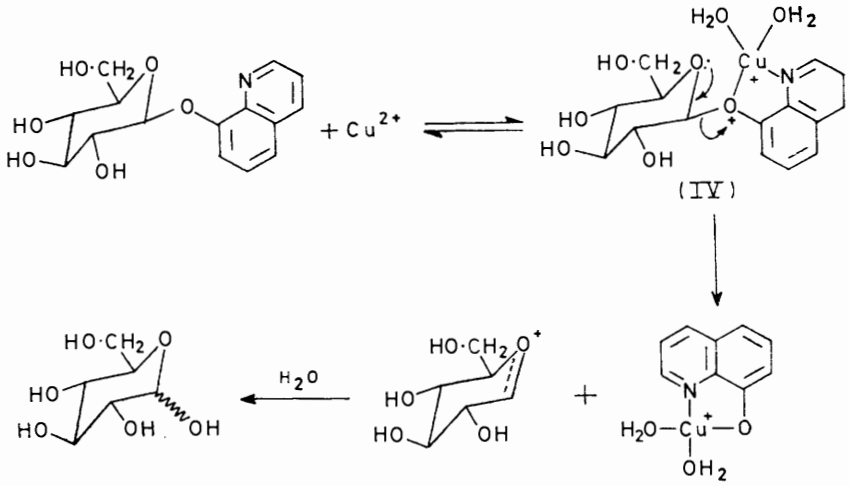
$$k_{obs} = k_u + k_{cat}[M^{2+}] \quad \text{(iii)}$$

for the uncatalysed reaction (which is negligible in the pH range 5·5—6·2), and k_{cat} is the catalytic rate constant. Even at the highest metal ion concentrations employed no evidence was obtained for a zero-order dependence on the metal ion concentration. At 70·1° the values of k_{cat} are 5·9 [copper(II)], $2·1 \times 10^{-2}$ [nickel(II)], and $4·3 \times 10^{-3}$ l mol^{-1} min^{-1} [cobalt(II)]. The catalytic rate constants are composite rate constants, *i.e.* $k_{cat} = kK$ where k is the rate constant for the decomposition of the complex and K is its formation constant ($M^{2+} + L \rightleftharpoons ML^{2+}$; $K = [ML^{2+}]/[M^{2+}][L]$). At the high metal ion concentrations employed it is probable the 1 : 1 complex ML^{2+} is the only species of kinetic significance. As it is unlikely that the actual reactivities of the complexes do not vary by a factor of greater than ten within the series studied, the relative values of k_{cat} of 1380 : 5 : 1 probably only reflect the formation constant differences.

It is clear that the metal ions exert a profound catalytic effect on the hydrolysis of the glycoside; thus the value of k_{obs} obtained with 10^{-3}M-copper(II) is similar to the value obtained in 3·62M-HCl. Under such conditions (metal : ligand = 660 : 1) the ligand is not fully complexed so that hydrolysis of the metal complex within

[19] J. F. Bunnett, *J. Amer. Chem. Soc.*, 1961, **83**, 4978.
[20] E. Buncel and P. R. Bradley, *Canad. J. Chem.*, 1967, **45**, 515.

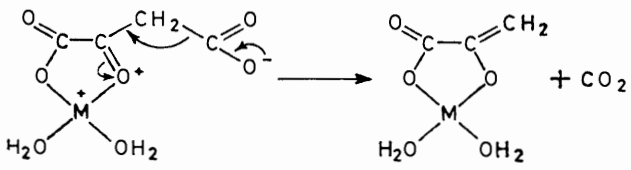
the pH range 5·5—6·2 is probably at least 10^5—10^6 times faster than the 'spontaneous' hydrolysis of the glycoside in this pH range. It seems probable that the metal-ion-catalysed hydrolysis of 8-quinolyl β-D-glucoside proceeds *via* the pathways shown in Scheme 4. Formation of the 1 : 1 complex (IV) polarises the glycosidic oxygen atom and leads to cleavage of the glucosyl–oxygen bond. This mechanism is completely analogous to the normal *A*-1 pathway for proton catalysis. Similar complexes have recently been proposed to account for the copper(II)-catalysed hydrolyses of 8-quinolyl sulphate [7] and 8-quinolyl phosphate.[6] A linear free energy relationship of the Brönsted type occurs between k_{cat} and the formation constants (K_f) for the 1 : 1 complexes of copper(II), nickel(II), and cobalt(II) with 8-hydroxyquinoline: $\log k_{cat} = 1{\cdot}01 \log K_f - 11{\cdot}5$ ($\log K_f = 12{\cdot}2$, 9·9, and 9·1 for the copper, nickel, and cobalt complexes, respectively).[21]



SCHEME 4 Metal-ion-catalysed hydrolysis of 8-quinolyl β-D-glucopyranoside

Similar correlations in metal-ion-catalysed reactions between reaction rates and formation constants have been observed previously. Such relationships appear to be most successful where the chosen complex closely approximates to the transition state of the reaction. Thus the rate constants for the metal-ion-catalysed decarboxylation of oxaloacetic acid (Scheme 5), do not correlate well with the formation constants of the metal oxaloacetates; however, a good correlation is observed [22] with the formation constants for the metal oxalates which closely approximate to the transition state for the decarboxylation.

The pronounced metal-ion catalysis in the hydrolysis of 8-quinolyl β-D-glucoside suggests that similar metal-catalysed processes may be of importance in the action of the glycosidases. Human saliva amylase, for example, requires 1 g atom of calcium for full activity and the amylase from *B. subtilis* at least 4 g atoms. The calcium may be removed by electrodialysis and the resulting apoenzymes have only 5—10% of the activity of the metalloenzymes.[23] However, present evidence suggests that the calcium is involved in maintaining the tertiary structure of the enzyme rather than taking a direct part in its catalytic action. It is important to note than in model studies it is necessary to provide binding sites for the metal ion on the substrate molecule. In the zinc metalloenzymes such as carboxypeptidase A [24] it appears that three of the ligand attachments are to the apoenzyme, while the fourth can be used to interact with the substrate. The hydrolysis of simple aryl glycosides is not catalysed by metal ions; however, this does not exclude the direct participation of a metal ion in the enzymic reaction since the apoenzyme can provide the requisite binding sites for the metal ion.



SCHEME 5

We thank the S.R.C. for a grant.

[3/1059 *Received*, 23*rd May*, 1973]

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