A Metal-ion Catalysed Glucoside Hydrolysis

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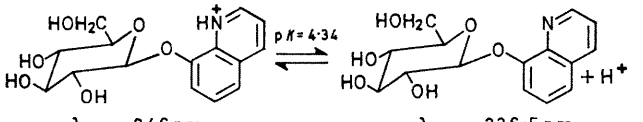
Summary The hydrolysis of 8-hydroxyquinoline β-D-glucoside is significantly catalysed by copper(II) ions at 70·1° and pH 5·5—6·0.

THE hydrolysis of glycosides is subject to acid catalysis which may be general or specific.[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 glycosides are susceptible to acid catalysis it might be expected that the glycosidic bond would also be subject to metal-ion catalysis, we have therefore studied the effect of copper(II)



(I)

ions on the hydrolysis of 8-hydroxyquinoline β-D-glucoside (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 hydrolysis of 8-quinolyl phosphate[6] and 8-quinolyl sulphate[7] have been shown to be catalysed by metal ions). The hydrolysis of the glucoside can readily be monitored by u.v. spectrophotometry. Table 1 lists values of the rate constants obtained for the acid-catalysed hydrolysis at 70·1°. A plot of log k_{obs} *versus* $-H_0$ is linear (slope = 0·74) and the reaction apparently proceeds by the expected A-1 pathway. However, in view of the dubious validity of the Hammett–Zucker hypothesis, these results do not in themselves provide sufficient evidence for an A-1 mechanism. The slope is rather low, the average value for glycoside hydrolysis lying in the range 0·89—1·04.[2]

Studies of the effect of copper(II) were also carried out at 70·1° to allow a direct comparison with the hydrogen-ion catalysis. No buffers were employed, but the pH of the solutions was always in the range 5·5—6·0, since the metal ion itself, has a pK_a of *ca.* 4·5, [$Cu(H_2O)_4 \rightleftharpoons Cu(H_2O)_3(OH)^+ + H^+$]. The p$K_a$ for the pyridine nitrogen ionisation (I) was determined spectrophotometrically to be 4·34 ± 0·03 at 25° and $I \rightarrow 0$. Good pseudo-first-order plots were obtained in every case, values of the pseudo-first-order rate constants (k_{obs}) are listed in Table 2. It is apparent that very substantial copper(II) catalysis occurs in this reaction, the value of k_{obs} with 1·25 × 10⁻³M-copper(II) is not markedly different from the value obtained with 4·18M-HCl. A plot of k_{obs} *versus* [Cu^{2+}] is linear and the rate expression takes the form $k_{obs} = k_u + k_{cat}$ [Cu^{2+}] where k_u is the rate constant for the uncatalysed reaction in the pH range 5·5—6·0 (which is neglible), and k_{cat} is the catalytic rate constant. The catalytic rate constant has the value 5·9 M^{-1} min^{-1} at 70·1°.

TABLE 1

Acid-catalysed hydrolysis of 8-hydroxyquinoline β-D-glucoside at 70·1°†

[HCl]	$-H_0$	$10^3 k_{obs}$ (min^{-1})
1·79	0·59	1·65
2·42	0·84	2·79
2·98	1·04	3·91
3·62	1·27	5·49
4·18	1·46	7·56

† The release of 8-hydroxyquinoline was monitored at 255 nm, rate constants were obtained from plots of log ($OD_\infty - OD_t$) *versus* time. Glucoside concentration 1·5 × 10⁻⁵ M.

TABLE 2

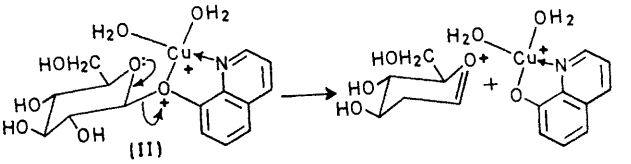
Copper(II)-catalysed hydrolysis of 8-hydroxyquinoline β-D-glucoside at 70·1°†

10^4 [Cu^{2+}]	$10^3 k_{obs}$ (min^{-1})
2·5	1·52
5·0	3·00
7·5	4·34
10·0	5·76
12·5	7·33

† Analytical wavelength 236 nm (λ_{max} for the glycoside). identical rate constants were obtained at 255·5 nm [λ_{max} for the copper(II) complex of 8-OH quinoline]. The pH was in the range 5·5—6·0. Glucoside concentration 1·5 × 10⁻⁵M.

It appears probable that the catalytic effect of copper(II) is due to the formation of the complex (II); binding of the glycosidic oxygen in a 5-membered chelate ring polarises the glucosidic bond in a manner analogous to that of the proton-catalysed reaction. The reaction should occur with glucosyl–oxygen fission as in the acid-catalysed reaction.[2]

It may be significant that possibly all α-amylases are metalloenzymes requiring calcium for activity. Human saliva amylase requires 1 g atom of calcium for full activity and the bacterial enzyme at least 4 g atoms.[2] The calcium may be removed by electrodialysis and the resulting apoenzymes have only 5—10% of the activity of the metalloenzymes. It is important to note that 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[9] and carbonic anhydrase[10] 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 bonding sites for the metal ion.



One of us (C.R.C.) thanks the Chemicals Manufacturing Company of New Zealand for financial support.

(*Received, May 5th*, 1970; *Com.* 677.)

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