The Uncatalysed and Copper(II) Promoted Hydrolysis of 4-Nitrophenyl Glycinate

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The uncatalysed hydrolysis of 4-nitrophenyl glycinate has been studied over a range of pH at I = 0.1 mol dm^{-3} (KNO_3) and 35 °C. The ionization equilibrium of 4-nitrophenyl glycinate can be represented as in (i) where the pK is *ca.* 7.1 at 25 °C. Rate constants have been obtained for both

$$\underset{(HL^+)}{\overset{+}{N}H_3CH_2CO_2C_6H_4NO_2\text{-}4} \xrightleftharpoons{K} \underset{(L)}{NH_2CH_2CO_2C_6H_4NO_2\text{-}4} + H^+ \qquad \text{(i)}$$

water and base hydrolysis of the species L and HL$^+$. For the species L, k_{OH} = 2.3 × 10^2 dm^3 mol^{-1} s^{-1} and k_{H_2O} = 6.1 × 10^{-5} dm^3 mol^{-1} s^{-1} while for HL$^+$, k_{OH} = 3.8 × 10^5 dm^3 mol^{-1} s^{-1} and k_{H_2O} = 2.3 × 10^{-6} dm^3 mol^{-1} s^{-1} at 35 °C. The hydrolysis of the ester is strongly promoted by copper(II), and under the experimental conditions employed, it has been shown that the metal-ion promotion involves the steps (ii) and (iii) with K_M = 2.7 × 10^2 dm^3 mol^{-1} and k_{OH} = 6.6 × 10^6

$$Cu^{2+} + L \xrightleftharpoons{K_M} [CuL]^{2+} \qquad \text{(ii)}$$

$$[CuL]^{2-} + OH^- \xrightarrow{k_{OH}} \text{Products} \qquad \text{(iii)}$$

dm^3 mol^{-1} s^{-1} at 35 °C. Base hydrolysis of $[CuL]^{2+}$ is *ca.* 3 × 10^4 times faster than for L and some 18 times faster than for HL$^+$ at 35 °C. Possible mechanisms for these reactions are considered.

Since the initial discovery by Kroll[1] that transition-metal ions promote the hydrolysis of α-amino-acid esters, such reactions have been the subject of extensive kinetic and thermodynamic investigations.[2] These studies have almost exclusively involved the use of simple alkyl esters (methyl, ethyl, and isopropyl esters) which provide poor leaving groups. It is normally necessary to monitor these reactions by pH-stat techniques, and it is often not possible to vary greatly the metal to ligand ratios. The use of 4-nitrophenyl esters which provide a good leaving group presents a number of advantages. For example, (*a*) the reactions can be monitored spectrophotometrically, (*b*) it is possible to obtain precise kinetic data for the hydrolysis of the *N*-protonated ester (HL$^+$) and, (*c*) it is possible to study the hydrolysis over a wide range of metal to ligand ratios which allows the determination of both equilibrium and rate constants.

The present paper discusses studies of the uncatalysed and copper(II) promoted hydrolysis of 4-nitrophenyl glycinate (L). Copper(II) was chosen as the promoting metal ion as it is normally the most effective Lewis-acid catalyst of the M^{2+} cations of the first transition series. Currently the only reported investigation dealing with the interaction of metal ions with 4-nitrophenyl esters of amino-acids has been a study of the hydrolysis of 4-nitrophenyl carboalkoxyglycinates by hydroxo-complexes of mercury(II) chelates.[3]

Experimental

The ester 4-nitrophenyl glycinate hydrobromide was prepared by treatment of the *N*-carbobenzyloxy ester $PhCH_2OCONH$-$CH_2CO_2C_6H_4NO_2$-4 (B.D.H.) with HBr–$MeCO_2H$ essentially as described by Ben Ishai and Berger,[4] m.p. 213 °C (decomp.) [lit.,[5,6] 213 °C (decomp.)] (Found: C, 34.65; H, 3.25; N, 10.15. Calc. for $C_8H_9BrN_2O_4$: C, 34.65; H, 3.25; N, 10.15%).

Solutions of copper(II) were prepared from A.R. $Cu(NO_3)_2 \cdot 6H_2O$ and were standardised by normal methods.[7] Acetate buffer solutions (0.01 mol dm^{-3}) were prepared by literature procedures.[8] The ionic strength of the reactant solutions was adjusted with KNO_3, which was standardised using a cation-exchange resin.

Kinetic Measurements.—The hydrolysis of 4-nitrophenyl glycinate at low pH and the copper(II) promoted reactions were studied spectrophotometrically by monitoring the release of 4-nitrophenol. The kinetics of hydrolysis of the unprotonated ester (L) were monitored by pH-stat using the general experimental technique described elsewhere.[9] A high alkalinity glass electrode type G202B (Radiometer) was used as indicator electrode and a calomel K401 (Radiometer) as reference electrode. The electrode system was standardised with National Bureau of Standards phosphate buffer and borate buffer.[7]

Spectrophotometric monitoring was carried out using a Gilford 2400S spectrophotometer. A concentrated methanolic solution of the ester (0.05 cm^3) was added to the appropriate acetate buffer and the increase in absorbance due to the release of 4-nitrophenol monitored at 320 nm. Reactions were followed for 5—6 half lives and the A_∞ values recorded after 12—14 half lives. The observed first-order rate constants ($k_{obs.}$) were evaluated from the absorbance data using a desk top computer. The quoted rate constants, $k_{obs.}$, are the mean values of at least three kinetic runs, the experimental spread was normally less than ±5%. Derived rate constants were obtained from the least-squares slope and intercept of the linear plots as described in the Results and Discussion section. In all cases the initial ester concentration was 3.67 × 10^{-4} mol dm^{-3}. Values of the hydroxide ion concentration were determined from the pH using a molar activity coefficient calculated from the Davies' equation[10] (0.768 at 35 °C) and a value[11] of pK_w = 13.680 at 35 °C. Carbon dioxide is known to catalyse the hydrolysis of aryl esters of α-amino acids[6] and precautions were taken to prevent any CO_2 contamination of solutions.

Table 1. Hydrolysis of HL^+ in acetate buffer at $I = 0.1$ mol dm^{-3} (KNO_3) and 35 °C*

pH	$10^{10}[OH^-]$/ mol dm^{-3}	$10^4k_{obs.}/s^{-1}$
4.25	4.84	3.05
4.37	6.38	3.81
4.56	9.88	5.04
4.69	13.32	6.30
4.78	16.39	7.58

* Reaction monitored spectrophotometrically at 320 nm. Least-squares analysis gives $k_0 = 1.26 \times 10^{-4}$ s^{-1} ($k_{H_2O} = 2.27 \times 10^{-6}$ dm^3 mol^{-1} s^{-1}) and $k_{OH} = 3.83 \times 10^5$ dm^3 mol^{-1} s^{-1}.

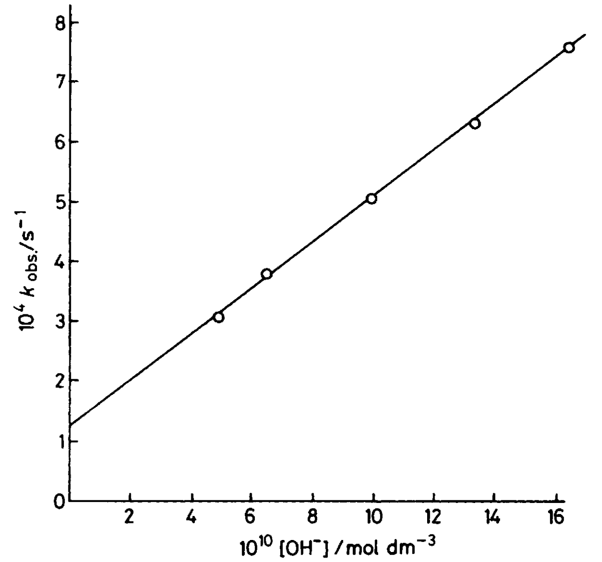


Figure 1. Hydrolysis of $\overset{+}{N}H_3CH_2CO_2C_6H_4NO_2$-4 ($HL^+$) at 35 °C and $I = 0.1$ mol dm^{-3} over the pH range 4.25—4.78

Results and Discussion

The ionization equilibrium of 4-nitrophenyl glycinate can be represented by equation (1). Rapid potentiometric titration of

$$\underset{(HL^+)}{\overset{+}{N}H_3CH_2CO_2C_6H_4NO_2\text{-}4} \overset{K}{\rightleftharpoons} \underset{(L)}{NH_2CH_2CO_2C_6H_4NO_2\text{-}4} + H^+ \quad (1)$$

the ester hydrobromide at 25 °C and $I = 0.1$ mol dm^{-3} (KNO_3) indicated a pK of *ca.* 7.1. Base hydrolysis of the ligand is quite rapid, and there is considerable pH drift at higher pH values.

The hydrolysis of the ester was studied in the pH range 4.25—4.78 at 35 °C, Table 1. In this pH range all the ester will be essentially present as HL^+. A plot of $k_{obs.}$ (the observed first-order rate constant) *versus* the hydroxide ion concentration is linear with a positive intercept, Figure 1, indicating that $k_{obs.} = k_0 + k_{OH}[OH^-]$. The k_0 term can be assigned to water attack on the protonated ester HL^+ and the k_{OH} term to base hydrolysis of HL^+. At 35 °C least-squares analysis gives $k_0 = 1.26 \times 10^{-4}$ s^{-1} and $k_{OH} = 3.83 \times 10^5$ dm^3 mol^{-1} s^{-1}. The k_0 rate constant can be converted to a second-order rate constant (k_{H_2O}) using the expression $k_{H_2O} = k_0/55.5$, where 55.5 mol dm^{-3} is the molar concentration of water. At 35 °C the value of k_{H_2O} is 2.27×10^{-6} dm^3 mol^{-1} s^{-1}. The nucleophilicity ratio $k_{OH}/k_{H_2O} = 1.7 \times 10^{11}$ at 35 °C is of the expected magnitude for the relative nucleophilicity of water and hydroxide ion in ester hydrolysis.[12,13]

Table 2. Hydrolysis of L at 35 °C and $I = 0.1$ mol dm^{-3} (KNO_3)*

pH	$10^5[OH^-]$/ mol dm^{-3}	$10^3k_{obs.}/s^{-1}$
8.15	0.38	3.99
8.50	0.86	5.60
8.60	1.08	6.13
8.70	1.36	6.44
8.74	1.50	6.82
8.89	2.11	8.18

* Least-squares analysis gives $k_0 = 3.37 \times 10^{-3}$ s^{-1} ($k_{H_2O} = 6.07 \times 10^{-5}$ dm^3 mol^{-1} s^{-1}) and $k_{OH} = 2.32 \times 10^2$ dm^3 mol^{-1} s^{-1}.

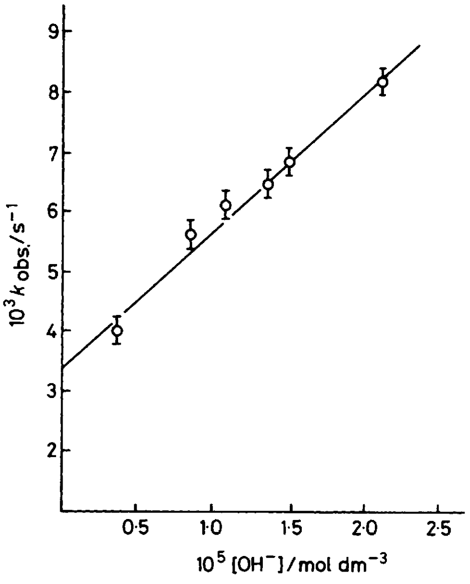


Figure 2. Hydrolysis of $NH_2CH_2CO_2C_6H_4NO_2$-4 (L) at 35 °C and $I = 0.1$ mol dm^{-3} over the pH range 8.15—8.89

The hydrolysis of the unprotonated ester L was studied by pH-stat at $I = 0.1$ mol dm^{-3} (KNO_3) in the pH range 8.1—8.9. The rate constants obtained at 35 °C are summarised in Table 2. In this case plots of $k_{obs.}$ *versus* the hydroxide ion concentration are also linear with a positive intercept indicating that $k_{obs.} = k_0 + k_{OH}[OH^-]$, Figure 2. Values of k_0 and k_{OH} were estimated from the least-squares intercept and slope of the plot. At 35 °C, $k_0 = 3.37 \times 10^{-3}$ s^{-1} and $k_{OH} = 2.32 \times 10^2$ dm^3 mol^{-1} s^{-1}. The k_0 term is considered to be due to water attack on the ester L with $k_{H_2O} = k_0/55.5 = 6.07 \times 10^{-5}$ dm^3 mol^{-1} s^{-1}. In this case the nucleophilicity ratio k_{OH}/k_{H_2O} is 3.8×10^7. Base hydrolysis of HL^+ is some 1.65×10^3 times faster than that of L at 35 °C.

The Copper(II) *Promoted Reaction.*—The reactions in the presence of copper(II) were all studied at a constant ester concentration of 3.67×10^{-4} mol dm^{-3}. Preliminary measurements at pH 4.56 and a total copper(II) concentration of 1.05×10^{-3} mol dm^{-3} established that the half life of the ester was *ca.* 5.1 min, indicating a significant rate enhancement. The reaction is first order in the ester concentration, and at low copper(II) concentrations, also shows a first-order dependence on the copper(II) concentration. At high metal to ligand ratios,

Table 3. Values of $k_{obs.}$ for the copper(II) promoted hydrolyses of 4-nitrophenyl glycinate at I = 0.1 mol dm^{-3} (KNO_3) at various pH values at 35 °C

$10^3[Cu^{2+}]$/ mol dm^{-3}	$10^3k_{obs.}$/ s^{-1}	$10^3[Cu^{2+}]$/ mol dm^{-3}	$10^3k_{obs.}$/ s^{-1}
(*a*) pH 4.25 ([OH$^-$] = 4.86 × 10^{-10} mol dm^{-3})			
0.0	0.31	9.8	2.41
2.8	1.48	14.0	2.64
7.0	2.14	22.4	3.14
(*b*) pH 4.37 ([OH$^-$] = 6.44 × 10^{-10} mol dm^{-3})			
0.0	0.38	10.5	4.01
2.1	1.77	16.8	4.20
5.6	2.86		
(*c*) pH 4.56 ([OH$^-$] = 9.79 × 10^{-10} mol dm^{-3})			
0.0	0.50	10.50	6.36
1.05	2.28	14.00	6.22
2.10	4.14	16.80	6.39
3.50	4.81	19.60	6.58
7.00	5.76		
(*d*) pH 4.69 ([OH$^-$] = 13.23 × 10^{-10} mol dm^{-3})			
0.0	0.63	5.6	7.49
1.4	3.60	11.2	8.63
2.8	5.22	17.5	8.65
(*e*) pH 4.78 ([OH$^-$] = 16.2 × 10^{-10} mol dm^{-3})			
0.0	0.76	4.20	7.80
0.52	2.90	7.14	10.08
1.05	4.94	10.50	10.78
2.10	6.58	16.80	10.82

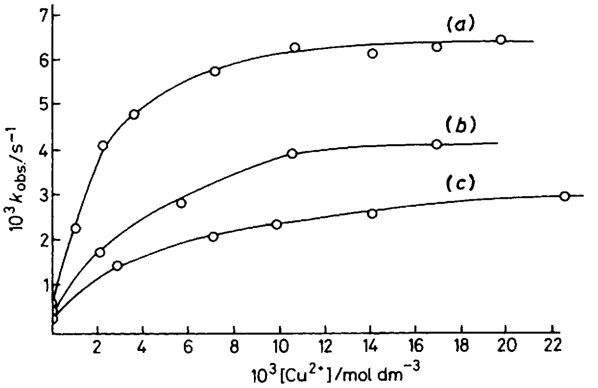


Figure 3. Plots of $k_{obs.}$ *versus* the total concentration of copper(II) at a total ester concentration of 3.67 × 10^{-4} mol dm^{-3} and pH (*a*) 4.56, (*b*) 4.37, (*c*) 4.25; temperature 35 °C and I = 0.1 mol dm^{-3} (KNO_3)

the reaction rate is independent of the copper(II) concentration, Table 3 and Figure 3.

The copper(II) promoted reaction can be rationalised in terms of equations (2) and (3). There is a rapid pre-equilibrium

$$Cu^{2+} + L \overset{K_M}{\rightleftharpoons} [CuL]^{2+} \qquad (2)$$

$$[CuL]^{2+} + OH^- \xrightarrow{k_{OH}} \text{Products} \qquad (3)$$

formation of $[CuL]^{2+}$ followed by a slow rate-determining base hydrolysis step. It can be readily shown that equation (4) applies to this system, where $k_{obs.}$ is the observed first-order rate constant at constant pH, k_0 is the rate constant due to the background solvolytic reaction in the absence of copper(II), and $k_{OH} = k/[OH^-]$. Rearranging equation (4) gives equation (5).

$$k_{obs.} = k_0 + \frac{kK_M[Cu^{2+}]}{(1 + K_M[Cu^{2+}])} \qquad (4)$$

$$1/(k_{obs.} - k_0) = 1/kK_M[Cu^{2+}] + 1/k \qquad (5)$$

A plot of $1/(k_{obs.} - k_0)$ *versus* $1/[Cu^{2+}]$ should be linear of slope $1/kK_M$ and intercept $1/k$. Such double reciprocal plots are indeed linear, Figure 4. Values of k and K_M obtained from such plots are summarised in Table 4. The average value of K_M is 270 dm^3 mol^{-1} (log K_M = 2.43) at 35 °C which may be compared with log K_M = 3.83 for the chelated copper(II) complex of ethyl glycinate at 25 °C.[14] A plot of k *versus* [OH$^-$] is linear, passing through the origin with a least-squares slope of 7.86 × 10^6 dm^3 mol^{-1} s^{-1} which is the value of k_{OH} at 35 °C and I = 0.1 mol dm^{-3}. The individual values of k_{OH} listed in Table 4 indicate the 'clean' first-order dependence on the hydroxide ion concentration and the lack of any solvolytic reaction.

Table 4. Values of k, k_{OH}, and log K_M obtained from plots of $(k_{obs.} - k_0)^{-1}$ *versus* $[Cu^{2+}]^{-1}$ at 35 °C and I = 0.1 mol dm^{-3} (KNO_3)

pH	$10^{10}[OH^-]$/ mol dm^{-3}	10^3k/s^{-1}	logK_M	$10^{-6}k_{OH}$/dm^3 mol^{-1} s^{-1}
4.25	4.86	3.23	2.30	6.7
4.37	6.44	4.76	2.30	7.4
4.56	9.79	7.69	2.48	7.9
4.69	13.23	10.53	2.45	7.9
4.78	16.20	12.82	2.61	7.9

Table 5. Plateau values of $k_{obs.}$ as a function of the hydroxide ion concentration at 35 °C*

pH	$10^{10}[OH^-]$/ mol dm^{-3}	$10^3k_{obs.}$/ s^{-1}	$10^{-6}k_{OH}$/dm^3 mol^{-1} s^{-1}
4.25	4.84	3.14	6.5
4.37	6.38	4.20	6.6
4.56	9.88	6.58	6.7
4.69	13.32	8.65	6.5
4.78	16.39	10.82	6.6

* Plateau values of $k_{obs.}$ taken from Table 3.

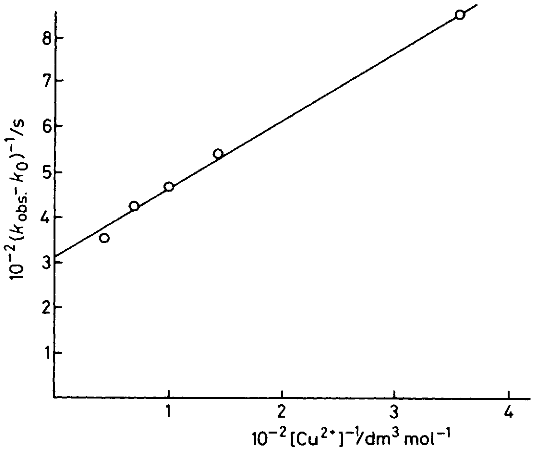
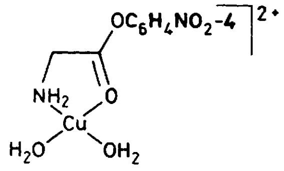


Figure 4. Double reciprocal plot for the copper(II) promoted hydrolysis of 4-nitrophenyl glycinate at pH 4.25 (35 °C and I = 0.1 mol dm^{-3}). Least-squares analysis gives an intercept of 3.1 × 10^2 s and a slope of 1.55 dm^3 mol^{-1} s with a correlation coefficient of 0.996

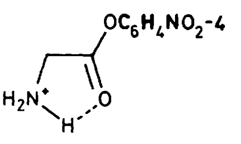
Table 6. Summary of the kinetic data at 35 °C

Reaction	k/dm^3 mol^{-1} s^{-1}
$HL^+ + H_2O$	2.3×10^{-6}
$HL^+ + OH^-$	3.8×10^5
$L + H_2O$	6.1×10^{-5}
$L + OH^-$	2.3×10^2
$[CuL]^{2+} + OH^-$	6.6×10^6



$[CuL]^{2+}$

(I)



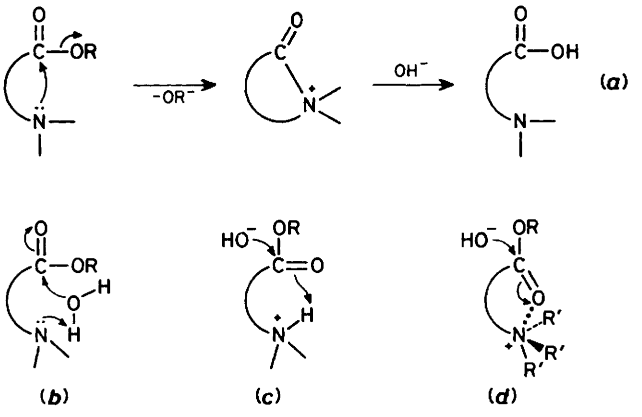
HL^+

(II)

The rate constant k_{OH} can also be obtained directly from the experimentally determined plateau values of $k_{obs.}$ (as all the substrate is present as $[CuL]^{2+}$). Values of k_{OH} determined by this method are listed in Table 5, giving a least-squares value of 6.6×10^6 dm^3 mol^{-1} s^{-1} with a correlation coefficient 0.9997.

The final kinetic data obtained are summarised in Table 6. A direct comparison of values of k_{OH} for $[CuL]^{2+}$, L, and HL^+ gives: $k_{OH}^{CuL^{2+}}/k_{OH}^{L} = 3 \times 10^4$, $k_{OH}^{CuL^{2+}}/k_{OH}^{HL^+} = 18$, and $k_{OH}^{HL}/k_{OH}^{L} = 1.65 \times 10^3$. The rate acceleration of 3.4×10^4 fold observed with $[CuL]^{2+}$ at 35 °C is consistent with the formation of the chelate (**I**) in which the aryloxycarbonyl group also acts as a donor, leading to significant polarisation of the carbonyl group.

The species HL^+ is also quite reactive towards base hydrolysis. A variety of mechanisms are available for neighbouring amino group facilitation of ester hydrolysis.[15] These mechanisms (Scheme) include (*a*) intramolecular nucleophilic catalysis, (*b*) intramolecular general base catalysis, (*c*) intramolecular general-acid specific-base catalysis, and (*d*) electrostatic facilitation due to the formal positive charge on the conjugate acid species. Mechanisms (*a*), (*b*), and (*c*) are kinetically indistinguishable. Base hydrolysis of $Et_3\overset{+}{N}CH_2CO_2Et$ is 200 times faster than that of CH_3CO_2Et due to electrostatic facilitation of the reaction.[16] In the present system intramolecular nucleophilic catalysis (which is favoured where a good leaving group is involved) is unlikely as the formation of a three-membered ring lactam is required. It is probable that a combination of electrostatic facilitation and 'hydrogen bond' catalysis[17,18] of the type shown in (**II**) accounts for the relatively high activity of the HL^+ species in base hydrolysis.



Scheme.

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References

1 H. Kroll, *J. Am. Chem. Soc.*, 1952, **74**, 2036.
2 R. W. Hay, 'Metal Ions in Biological Systems,' ed. H. Sigel, Marcel Dekker, New York, 1976, vol. 5, p. 173.
3 M. M. Werber and Y. Shalitin, *Bioinorg. Chem.*, 1973, **2**, 275.
4 D. Ben Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564.
5 M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, 1959, **81**, 3982.
6 R. W. Hay and L. Main, *Aust. J. Chem.*, 1968, **21**, 155.
7 J. Bassett, R. C. Denney, G. H. Jeffery, and J. Mendham, 'Vogels Textbook of Quantitative Inorganic Analysis,' Longman, London, 1978.
8 D. D. Perrin and B. Dempsey, 'Buffers for pH and Metal Ion Control,' Chapman Hall, London, 1974.
9 R. W. Hay, L. J. Porter, and P. J. Morris, *Aust. J. Chem.*, 1966, **19**, 1197.
10 C. W. Davies, *J. Chem. Soc.*, 1936, 2093.
11 R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions,' 2nd edn., Butterworths, London, 1979.
12 R. W. Hay and K. B. Nolan, *J. Chem. Soc., Dalton Trans.*, 1975, 1348.
13 R. W. Hay and A. K. Basak, *J. Chem. Soc., Dalton Trans.*, 1982, 1819.
14 C. Regardh, *Acta Pharm. Suec.*, 1966, **3**, 101.
15 T. C. Bruice and S. Benkovic, 'Bioorganic Mechanisms,' W. A. Benjamin, New York, 1966, vol. 1, p. 134 *et seq.*
16 R. P. Bell and F. J. Lindars, *J. Chem. Soc.*, 1954, 4601.
17 B. Hansen and A. Flormark, *Acta Chem. Scand.*, 1963, **17**, 1481.
18 B. Hansen, *Sven. Kem. Tidskr.*, 1963, **75**, 10.

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