The Uncatalysed and the Copper(II) Promoted Hydrolysis of 4-Nitrophenyl L-leucinate

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Abstract

The uncatalysed hydrolysis of 4-nitrophenyl L-leucinate has been studied in detail over a range of pH and temperature at $I = 0.1$ M (KNO_3). Base hydrolysis of the ester is strongly promoted by copper(II) ions. Rate constants have been obtained for the following reactions (where EH^+ is the N-protonated ester and E is the free base form)

$EH^+ + OH^- \longrightarrow$ products

$E + OH^- \longrightarrow$ products

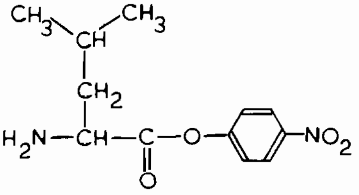
$E + H_2O \longrightarrow$ products

$CuE^{2+} + OH^- \longrightarrow$ products

Base hydrolysis of the copper(II) complex CuE^{2+} is 3.8×10^5 times faster than that of E and 75 times faster than that of EH^+ at 25 °C and $I = 0.1$ M. Activation parameters for these reactions have been determined and possible mechanisms are considered.

Introduction

The occurrence of a variety of metalloenzymes (e.g. carboxypeptidase A and carbonic anhydrase) which catalyse the hydrolysis of simple ester substrates, has stimulated much interest in the area of metal-ion promoted reactions of esters [1]. Since the initial discovery by Kroll [2] that transition metal ions promote the hydrolysis of α-amino-acid esters, such reactions have been the subject of extensive kinetic and thermodynamic investigations [1]. These studies have almost exclusively involved the use of simple alkyl esters (particularly methyl and ethyl 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 greatly vary the metal to ligand ratios. The use of 4-nitrophenyl esters which provide a good leaving group presents a number of advantages. For example, the reactions can be monitored spectrophotometrically, and since low ester concentrations are required it is possible to study the hydrolyses using a wide range of metal to ligand ratios. Currently the only published investigation using 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]. The present paper deals with the uncatalysed and copper(II) promoted hydrolysis of 4-nitrophenyl L-leucinate (I) and is one of a series dealing with 4-nitrophenyl esters of α-amino-acids*.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Experimental

The ester 4-nitrophenyl L-leucinate hydrobromide was prepared by treatment of the *N*-carbobenzyloxy ester (B.D.H) with HBr/HOAc essentially as described by Ben-Ishai and Berger [5]. About 0.1 mol of the *N*-carbobenzyloxy ester was treated with a saturated solution of HBr in glacial acetic acid. Carbon dioxide was evolved and the reaction was complete within a few minutes. After complete evolution of CO_2, dry ether (*ca.* 100 cm^3) was added to precipitate the ester hydrobromide. The mixture was placed in a refrigerator overnight, the product filtered off, washed thoroughly with ether and dried over sodium hydroxide pellets. The ester was recrystallised from dried ethanol by addition of dry ether, melting point 197 °C (decomposition) (literature [6] 199 °C (decomposition)). *Anal.* Calc. for $C_{12}H_{16}N_2O_4 \cdot HBr$: C, 43.27; H, 5.10; N, 8.41. Found: C, 43.10; H, 5.16; N, 8.30%.

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*For a discussion of the uncatalysed and copper(II) promoted hydrolysis of 4-nitrophenyl glycinate see ref. 4.

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Solutions of copper(II) prepared from A.R. $Cu(NO_3)_2 \cdot 6H_2O$ were standardised by normal methods [7]. Acetate buffer solutions were prepared by literature procedures [8]. The ionic strength was maintained at 0.1 M using standard KNO_3 solution, which was standardised by ion-exchange methods.

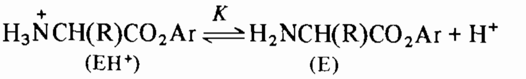
Kinetic Measurements

The hydrolysis of 4-nitrophenyl L-leucinate at low pH, and the copper(II) promoted reactions were studied spectrophotometrically by monitoring the release of 4-nitrophenol. The kinetics of base hydrolysis of the ester were monitored by pH-stat using methods described elsewhere [9]. Spectrophotometric monitoring was carried out with a Gilford 2400S spectrophotometer. A concentrated methanolic solution of the ester ($0.05\ cm^3$) was added to the appropriate thermostatted solution and the increase in absorbance due to the release of 4-nitrophenol monitored at 320 nm. In all cases the initial ester concentration was 1.70×10^{-4} M. For the copper(II) promoted reaction the copper concentrations were in large excess. Reactions were followed for 6–8 half lives and the A_∞ values were 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. Values of the hydroxide ion concentration were determined from the pH using the appropriate molar activity coefficients (calculated from the Davies' equation [10]*) and values of pK_w** obtained from the literature [11].

Carbon dioxide is known to catalyse the hydrolysis of aryl esters of α-amino-acids [12] and all kinetic measurements were carried out with freshly prepared buffer solutions and care taken to prevent any CO_2 contamination of solutions.

Results and Discussion

The ionisation equilibrium of 4-nitrophenyl L-leucinate ($R = CH_2CH(CH_3)_2$) can be represented by eqn. (1)



$$\underset{(EH^+)}{H_3\overset{+}{N}CH(R)CO_2Ar} \overset{K}{\rightleftharpoons} \underset{(E)}{H_2NCH(R)CO_2Ar} + H^+ \qquad (1)$$

Rapid potentiometric titration of the ester hydrobromide in 0.1 M KNO_3 indicated a p*K* of *ca.* 7.0. Base hydrolysis of the ester is quite rapid and there is considerable pH drift at higher pH values.

TABLE I. Base Hydrolysis of E at various Temperatures and $I = 0.1$ M $(KNO_3)^a$

Temperature (°C)	pH	10^5 [OH⁻] (M)	$10^3\ k_{obs}{}^b$ (s^{-1})
20	9.80	5.55	2.64(3)
	9.90	6.99	3.06(5)
	9.97	8.21	3.28(4)
	10.08	10.58	3.76(3)
	10.23	14.94	4.85(3)
	10.46	25.37	7.37(3)
25	9.20	2.07	3.00(3)
	9.43	3.51	3.31(4)
	9.77	7.68	4.50(5)
	9.98	12.46	6.03(3)
30	8.50	0.60	3.68(3)
	8.76	1.10	3.68(4)
	8.83	1.29	3.77(5)
	9.09	2.35	4.09(5)
	9.37	4.47	4.84(3)
	9.65	8.52	6.17(3)

[a] Reaction monitored by pH-stat. [b] Values in parenthesis are the number of kinetic runs.

Base hydrolysis of the unprotonated species E was studied by pH-stat in the pH range 9.2–10.0 (at 25 °C) at $I = 0.1$ M (KNO_3) and three temperatures. Values of the observed first order rate constants (k_{obs}) as a function of pH and temperature are listed in Table I.

A plot of k_{obs} *vs.* [OH⁻] is linear with a positive intercept, Fig. 1, so that $k_{obs} = k_o + k_{OH}[OH^-]$. The k_o term can be assigned to water attack on E as shown in eqn. (2).

$$E + H_2O \longrightarrow \text{products} \qquad (2)$$

$$E + OH^- \longrightarrow \text{products} \qquad (3)$$

This reaction is kinetically indistinguishable from the reaction of hydroxide ion with EH^+. Values of k_o were determined from the intercept of the plots and k_{OH} from the slope, Table II. The k_o rate constants were converted to second order constants using the expression $k_{H_2O} = k_o/55.5$, where 55.5 M is the molar concentration of water. For reaction (2), $\Delta H^{\ddagger} = 15.7$ kcal mol^{-1} and $\Delta S^{\ddagger}_{298} = -26$ cal K^{-1} mol^{-1}. While for base hydrolysis (reaction (3)), k_{OH} is 28.7 M^{-1} s^{-1} at 25 °C and $I = 0.1$ M with $\Delta H^{\ddagger} = 6.6$ kcal mol^{-1} and $\Delta S^{\ddagger}_{298} = -30$ cal K^{-1} mol^{-1}. Substantial negative entropies of activation are expected for bimolecular reactions of this type. Thus for the base hydrolysis of unprotonated ethyl leucinate $k_{OH} = 0.18$ M^{-1} s^{-1} at 25 °C and $I = 0.1$ M with $\Delta H^{\ddagger} = 7.5$ kcal mol^{-1} and $\Delta S^{\ddagger}_{298} = -39$ cal K^{-1} mol^{-1} [13]. At 25 °C the 4-nitrophenyl ester undergoes base hydrolysis some 160 times faster than the ethyl ester.

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*The molar activity coefficients at $I = 0.1$ M are 0.774 (20 °C); 0.772 (25 °C) and 0.770 (30 °C).

**The following values of pK_w were employed: 14.166_6 (20 °C); 13.996_5 (25 °C) and 13.833_0 (30 °C). Intermediate values of pK_w were determined from plots of $\ln K_w$ *vs.* 1/T.

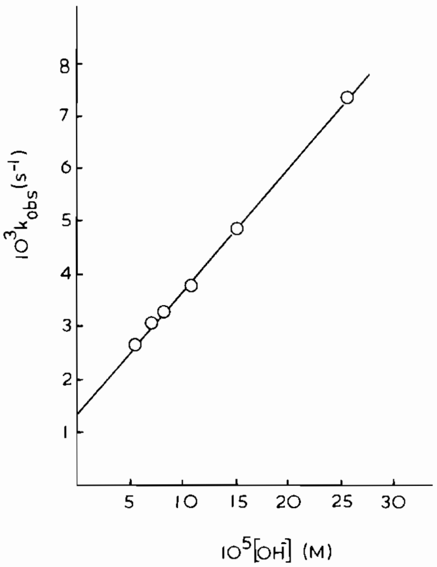


Fig. 1. Plot of k_{obs} *vs.* $[OH^-]$ for the hydrolysis of E at 20 °C and $I = 0.1$ M (KNO_3).

Base hydrolysis of the protonated species EH^+ was studied in the pH range 5–6 at a series of temperatures and $I = 0.1$ M (KNO_3). The rate constants k_{obs} obtained are summarised in Table III. Values of $k_{obs}/[OH^-] = k_{OH}$ are constant throughout the pH range indicating a first-order dependence on the hydroxide ion concentration and the lack of any solvolytic reaction. At 25 °C, the value of k_{OH} is 1.45×10^5 M^{-1} s^{-1}. Activation parameters obtained from the temperature dependence of k_{OH} are $\Delta H^\ddagger = 11.8$ kcal mol^{-1} and $\Delta S^\ddagger_{298} = +4.3$ cal K^{-1} mol^{-1}. A more positive entropy of activation is expected in this case as charge neutralisation occurs in the transition state leading to desolvation of the reactants.

TABLE II. The Temperature Dependence of the Rate Constants k_o and k_{OH} for the Hydrolysis of E at $I = 0.1$ M (KNO_3)

Temperature (°C)	$10^3 k_o$ (s^{-1})	$10^5 k_{H_2O}$[a] (M^{-1} s^{-1})	$10^{-1} k_{OH}$ (M^{-1} s^{-1})
20	1.36	2.45	2.33
25	2.29	4.13	2.87
30	3.40	6.13	3.49

[a] For k_{H_2O}, $\Delta H^\ddagger = 15.7$ kcal mol^{-1}; $\Delta S^\ddagger_{298} = -26$ cal K^{-1} mol^{-1}. For k_{OH}, $\Delta H^\ddagger = 6.6$ kcal mol^{-1}; $\Delta S^\ddagger_{298} = -30$ cal K^{-1} mol^{-1}. The rate constants k_{H_2O} were derived from the expression $k_{H_2O} = k_o/55.5$, where 55.5 M is the molar concentration of water.

TABLE III. Base Hydrolysis of EH^+ at various Temperatures and $I = 0.1$ M (KNO_3)

Temperature (°C)	pH	10^9 $[OH^-]$ (M)	$10^3 k_{obs}$ (s^{-1})	10^{-5} k_{OH}[a] (M^{-1} s^{-1})
22	5.19	1.85	0.21	1.14
	5.48	3.57	0.41	1.15
	5.86	8.68	0.92	1.06
	5.96	10.82	1.18	1.09
	6.14	16.31	1.85	1.13
$k_{OH} = 1.10 \times 10^5$ M^{-1} s^{-1}				
25	5.19	2.02	0.29	1.44
	5.48	3.94	0.58	1.47
	5.69	6.39	0.90	1.41
	5.84	9.02	1.31	1.45
	5.95	11.63	1.72	1.48
$k_{OH} = 1.44 \times 10^5$ M^{-1} s^{-1}				
30	5.19	2.96	0.49	1.66
	5.48	5.71	1.16	2.00
	5.67	8.86	1.68	1.90
	5.81	12.23	2.40	1.96
	5.92	15.72	3.05	1.94
$k_{OH} = 1.93 \times 10^5$ M^{-1} s^{-1}				

[a] From the temperature dependence of k_{OH}, $\Delta H^\ddagger = 11.8$ kcal mol^{-1} and $\Delta S^\ddagger_{298} = +4.3$ cal K^{-1} mol^{-1}.

Copper(II) Promoted Reaction

The copper(II) promoted reactions were studied at a total ester concentration of 1.70×10^{-4} M. The reaction was studied spectrophotometrically at 320 nm by monitoring the release of 4-nitrophenol. Table IV lists values of k_{obs} as a function of the copper(II) concentration at pH 4.48 and 30 °C. At copper(II) concentrations greater than 1×10^{-2} M, the reaction becomes independent of the copper(II) concentration with a limiting value of k_{obs} of *ca.* 6.9×10^{-3} s^{-1}, Fig. 2. The data in Table III establish that at 30 °C and pH 4.48 the value of k_{obs} in the absence of copper(II) would be *ca.* 0.12×10^{-3} s^{-1} at 30 °C. The kinetic behaviour is consistent with the scheme,

$$Cu^{2+} + E \overset{K}{\rightleftharpoons} CuE^{2+} \qquad (4)$$

$$CuE^{2+} + OH^- \xrightarrow{k_{OH}} CuA^+ + ArOH \qquad (5)$$

where A^- is the anion of L-leucine and ArOH is 4-nitrophenol. The first order dependence on $[OH^-]$ implied in eqn. (5) was confirmed by studies of the pH-dependence of the plateau value of k_{obs}, Table V. Values of $k_{OH} = k_{obs}/[OH^-]$ are constant with $k_{OH} = 1.28 \times 10^7$ M^{-1} s^{-1} at 30 °C. The temperature dependence of the k_{OH} values gives $\Delta H^\ddagger = 11.9$ kcal mol^{-1} and $\Delta S^\ddagger_{298} = +13$ cal K^{-1} mol^{-1}. The data reported in Table V were all determined using a copper(II) concentration of 10.5×10^{-3} M.

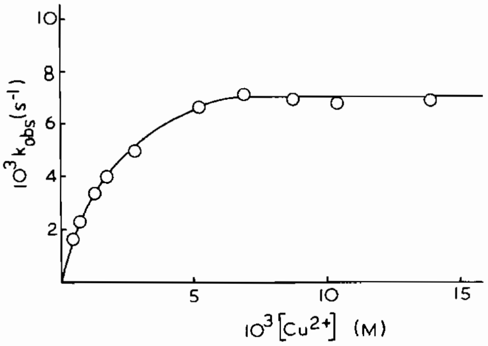


Fig. 2. Copper(II) dependence of the hydrolysis of 4-nitrophenyl L-leucinate at pH 4.48, $I = 0.1$ M (KNO_3) and 30 °C.

TABLE IV. Copper(II) Dependence of the Hydrolysis of 4-Nitrophenyl L-Leucinate at $I = 0.1$ M (KNO_3), pH 4.48 and 30 °C[a]

10^3 [Cu^{2+}] (M)	10^3 k_{obs} (s^{-1})	10^3 [Cu^{2+}] (M)	10^3 k_{obs} (s^{-1})
0.525	1.69	7.00	7.15
0.70	2.39	8.75	7.00
1.225	3.40	10.50	6.82
1.75	3.98	14.00	6.87
2.80	4.99		
5.25	6.69		

[a] Reactions monitored spectrophotometrically at 320 nm. Total ester concentration 1.70 × 10^{-4} M.

Rate constants at 25 °C for the various reactions, with the appropriate activation parameters are summarised in Table VI. Base hydrolysis of the copper(II) complex CuE^{2+} is 3.8 × 10^5 times faster than that of E and 75 times faster than that of EH^+ at 25 °C and $I = 0.1$ M. For base hydrolysis of EH^+ and CuE^{2+}, the values of $\Delta H^{\ddagger}$ are identical within experimental error and the 75 fold rate increase for CuE^{2+} compared with EH^+ arises solely due to a more positive value of $\Delta S^{\ddagger}_{298}$ for the metal complex. The base hydrolysis of an ester involves a bimolecular reaction with an associated substantial negative entropy of activation, as is clearly seen in the base hydrolysis of E where $\Delta S^{\ddagger}_{298} = -30$ cal K^{-1} mol^{-1}. For the base hydrolysis of positively charged ester species there is considerable charge neutralisation in the transition state with concomitant release of solvating water leading to more positive values of $\Delta S^{\ddagger}_{298}$.

A number of specific mechanisms can be considered for the substantial rate acceleration observed in the base hydrolysis of CuE^{2+}. The reaction could involve attack of 'external' hydroxide on the chelate ester species **II**, or there could be intramolecular attack by coordinated hydroxide on a monodentate ester species **III**.

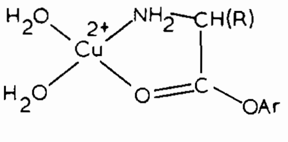
TABLE V. pH-Dependence of the Copper(II) Promoted Hydrolysis of 4-Nitrophenyl L-Leucinate at various Temperatures and $I = 0.1$ M (KNO_3)[a]

Temperature (°C)	pH	10^{10} [OH^-] (M)	10^3 k_{obs}[b] (s^{-1})	10^{-7} k_{OH} (M^{-1} s^{-1})
30	4.18	2.86	3.65(6)	1.28
	4.35	4.31	5.75(6)	1.33
	4.48	5.71	7.04(1)	1.23
	4.57	7.04	8.42(6)	1.20
	4.69	9.39	12.26(3)	1.31
	4.73	10.34	13.68(3)	1.32
$k_{OH} = 1.29 \times 10^7$ M^{-1} s^{-1}				
25	4.18	1.97	2.22(4)	1.13
	4.46	3.76	3.95(5)	1.05
	4.66	5.96	6.65(6)	1.12
	4.69	6.39	6.73(6)	1.05
	4.80	8.23	9.05(6)	1.10
$k_{OH} = 1.02 \times 10^7$ M^{-1} s^{-1}				
22	4.27	2.24	1.45(5)	0.65
	4.46	3.42	2.87(6)	0.84
	4.66	5.41	4.14(3)	0.77
	4.86	8.68	6.23(6)	0.72
$k_{OH} = 0.75 \times 10^7$ M^{-1} s^{-1}				

[a] Using a copper(II) concentration of 10.5 × 10^{-3} M. [b] Values in parenthesis are the number of kinetic runs carried out. From the temperature dependence of k_{OH}, $\Delta H^{\ddagger} = 11.9$ kcal mol^{-1} and $\Delta S^{\ddagger}_{298} = +13$ cal K^{-1} mol^{-1}.

TABLE VI. Summary of Rate Constants at 25 °C and Activation Parameters for the Hydrolytic Reactions of 4-Nitrophenyl L-Leucinate

Reaction	k_{25} (M^{-1} s^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}_{298}$ (cal K^{-1} mol^{-1})
E + H_2O	4.13 × 10^{-5}	15.7	−26
E + OH^-	2.87 × 10^1	6.6	−30
EH^+ + OH^-	1.45 × 10^5	11.8	+4.3
CuE^{2+} + OH^-	1.09 × 10^7	11.9	+13



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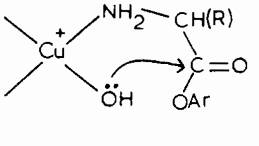
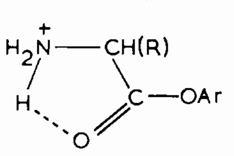


III

The copper(II) promoted reaction displays a first order dependence on the hydroxide ion concentration up to pH 4.80. The pK_a for the ionisation of the coordinated water molecule in the *N*-coordinated ester species, $Cu{-}OH_2 \rightarrow Cu{-}OH + H^+$ must be at least 6 for such a dependence to be observed. The p*K* values in copper(II) complexes normally fall in the range 5–7 so that a mechanism involving **III** cannot be immediately discarded. However, studies of the base hydrolysis of chelate ester species in kinetically inert cobalt(III) complexes have shown [16] that the rate accelerations of 10^4–10^6 normally observed, arise solely due to $\Delta S^{\ddagger}$. In the intramolecular reactions there is also a substantial contribution from $\Delta H^{\ddagger}$. Using such criteria a mechanism involving the chelate ester species **II** seems most appropriate to the present system, as the rate enhancement of 3.8×10^5 times at 25 °C arises solely due to a more positive value of $\Delta S^{\ddagger}$. The value of $\Delta H^{\ddagger}$ for the base hydrolysis of CuE^{2+} (11.9 kcal mol^{-1}) is considerably higher than that for E where $\Delta H^{\ddagger}$ is 6.6 kcal mol^{-1}, Table VI. Exactly analogous observations have been made in the palladium(II) promoted hydrolysis of α-amino acid esters where chelate ester species are involved [14]. Similar behaviour occurs in the copper(II) promoted hydrolysis of the methyl ester of ethylenediamine-monoacetate [15] which also involves a chelate ester species.

One interesting aspect of the system under study is the very high reactivity of EH^+ in base hydrolysis, where the ratio k_{EH^+}/k_E is 5.1×10^3 at 25 °C. For methyl glycinate this ratio is only 22.1 [9, 13] while for ethyl glycinate it is 36.1 at 25 °C [9], and for alkyl esters generally the ratio rarely exceeds 100. It is possible that with the aryl esters there is some additional facilitation of the reaction by H-bonding catalysis (**IV**), which has been recognised to lead to substantial rate accelerations in ester hydrolysis [17, 18]. Such catalysis has close similarities to the metal activated system **II**, a view supported by the close similarities of the activation parameters for the base hydrolysis of CuE^{2+} and EH^+.



IV

Acknowledgement

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