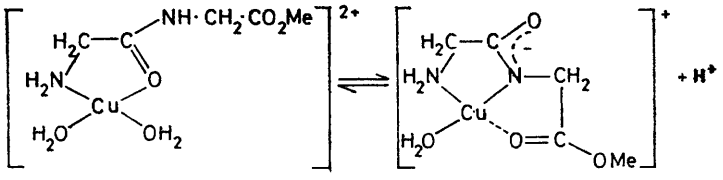
Copper(II)-promoted Hydrolysis of Ethyl Glycylglycinate, Ethyl Glycyl-β-alaninate, and Ethyl Glycyl-L-leucinate

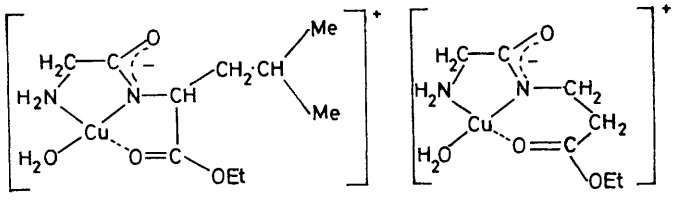
By **Robert W. Hay** * and **Kevin B. Nolan**, Chemistry Department, The University, Stirling

Copper(II)-promoted hydrolysis of the ethyl esters of glycylglycine, glycyl-β-alanine, and glycyl-L-leucine has been studied at 25 °C, I = 0·01 M, and pH values >7·6. Under these conditions, and at a 1 : 1 metal to ligand ratio, the peptide esters act as tridentate ligands, donation occurring from the terminal amino-group and the deprotonated amide nitrogen atom, with a weak interaction between the metal ion and the carbonyl group of the ester. An aqua ⇌ hydroxo equilibrium occurs in these complexes, and rate constants are reported for base hydrolysis of the aqua- and hydroxo-complexes. Rate accelerations of the order of 10^3 (compared with the unprotonated ligands) have been observed.

NAKON and ANGELICI [1] recently studied copper(II) complexes of the methyl esters of glycylglycine and glycylsarcosine. At low pH, the ligands chelate to the metal *via* the terminal amino-group and the amide carbonyl oxygen atom, (I). At higher pH, deprotonation of the amide linkage occurs in the glycylglycine derivative and donation occurs from the terminal amino-group, the deprotonated amide nitrogen atom, and possibly the ester group, as in (II). The formation of complexes such as (II), in which there is probably a weak interaction between the carbonyl group of the ester and the metal ion, suggests that hydrolysis of dipeptide esters should be susceptible to metal-ion catalysis. The amide–carbonyl-bonded complex (I) leads to metal-ion-promoted hydrolysis of the peptide linkage.[2] However, the deprotonated complex is not susceptible to hydrolysis at the peptide bond; indeed this bond is greatly stabilised by co-ordination of the nitrogen atom to copper as in



(I) (II)



(III) (IV)

[1] R. Nakon and R. J. Angelici, *Inorg. Chem.*, 1973, **12**, 1269.
[2] I. J. Grant and R. W. Hay, *Austral. J. Chem.*, 1965, **19**, 1189.

(III).[3] The only hydrolytic reaction expected to occur with (II) is therefore that of the ester group.

Three dipeptide ester ligands were chosen for the present study. In the glycyl-L-leucine ester complex, (III), there could be some steric hindrance to base hydrolysis by the bulky Bu^i group, while the glycyl-β-alanine ester system, (IV), allows a comparison to be made of the reactivity of five- and six-membered chelate rings.

EXPERIMENTAL

Ethyl glycylglycinate hydrochloride was used as received (Sigma) (Found: C, 36·4; H, 6·6; N, 14·3. Calc. for $C_6H_{13}ClN_2O_3$: C, 36·6; H, 6·7; N, 14·3%). Ethyl glycyl-β-alaninate hydrobromide and ethyl glycyl-L-leucinate hydrobromide monohydrate were prepared by coupling *N*-benzyloxycarbonylglycine and the appropriate amino-acid ethyl ester using dicyclohexylcarbodi-imide and cleaving the *N*-protecting group with HBr [4] (Found: C, 32·7; H, 5·9; N, 10·8. Calc. for $C_7H_{15}BrN_2O_3$: C, 32·9; H, 5·9; N, 11·0. Found: C, 38·3; H, 7·4; N, 9·0. Calc. for $C_{10}H_{23}BrN_2O_4$: C, 38·1; H, 7·4; N, 8·9%).

Kinetics and Measurements.—All kinetic measurements were carried out with a Radiometer TTT2 automatic titrator used as a pH-stat. A high-alkalinity glass electrode type G202B was used as indicator electrode and a saturated calomel electrode with a diffusion filter, type K401, as reference electrode. The electrode system was standardised at 25 ± 0·1 °C using 0·05M-potassium hydrogenphthalate (pH 4·008) and 0·01M-disodium tetraborate (pH 9·185).* The general technique employed in the kinetic measurements has been outlined.[5] All pH-stat studies were carried out at $I = 0{\cdot}01$M ($NaClO_4$) and 25 ± 0·1 °C. Low ionic strengths were necessary as at higher ionic strength 'salting out' of the complexes occurred. Values of the hydroxide-ion concentration were obtained from the pH using a molar activity coefficient of 0·905 [6] and a value of $pK_w = 14{\cdot}00$ at 25 °C.[7] The solutions used in the kinetic investigations were 5×10^{-4}M in both the ligand and copper(II). Reactions were followed for at least three half-lives. Values of k_{obs} were obtained from plots of log $(V_\infty - V_t)$ against time, where V_∞ is the final volume of base consumed and V_t that consumed at time t.

RESULTS AND DISCUSSION

The various equilibria occurring in solutions containing copper(II) and methyl glycylglycinate have been studied by potentiometric and i.r. techniques by Nakon and Angelici.[1] These authors estimated a pK value of 5·23 for the ionisation of the amide proton in the 1 : 1 copper(II) complex with methyl glycylglycinate. This value is *ca.* 1 pK_a unit higher than in the corresponding complex with glycylglycine (pK 4·31).[8] At pH > 5·8 in the ester system, precipitation occurred at 1 : 1 metal to ligand ratios at $I = 0{\cdot}10$M, but in solutions containing metal to ligand ratios of 1 : 2 precipitation did not occur and deprotonation of the amide linkage was followed by ester hydrolysis. The hydrolysis of ethyl glycylglycinate was studied by Meresaar and Ågren.[9] The various hydrolytic reactions are summarised in Scheme 1, where E is the ester, A is glycylglycine, and B is the ring closed

$$EH^+ + OH^- \xrightarrow{k_1} HA + EtOH \quad (1)$$

$$A^- + EtOH \quad (2)$$

$$E + OH^- \xrightarrow{k_2} \text{(2)}, \quad E + OH^- \xrightarrow{k_3} B + EtOH \quad (3)$$

$$E \xrightarrow{k_4} B + EtOH \quad (4)$$

SCHEME 1

product piperazine-2,5-dione; EH^+ and HA represent $H_3\overset{+}{N}\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et$ and $H_3\overset{+}{N}\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2^-$, respectively, and A^- is $H_2N\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2^-$. At 25 °C and $I = 1{\cdot}0$M the rate constants are $k_1 = 5{\cdot}2$ l mol^{-1} s^{-1} (approximate only), $k_2 = 0{\cdot}625$ l mol^{-1} s^{-1}, and $k_4 = 6{\cdot}86 \times 10^{-5}$ s^{-1}. Formation of the piperazinedione in basic solutions of glycylglycine esters is well established. Thus Nakon and Angelici [1] reported the appearance of a band at 1 625 cm^{-1} in basic solutions of methyl glycylglycinate which can be assigned to the amide carbonyl-stretching vibration of piperazine-2,5-dione. Significantly such a band was not observed at 2 : 1 ligand to copper ratios. Studies of the complexing behaviour of piperazine-2,5-dione have showed that bis(glycylglycinato)copper(II) is formed in basic solution.[10a]

The kinetics of base hydrolysis of ethyl glycylglycinate, ethyl glycyl-β-alaninate, and ethyl glycyl-L-leucinate were studied in the pH range 7·4—9·2. Using solutions which were 5×10^{-3}M in both the ligand and metal at $I = 0{\cdot}1$M ($NaClO_4$) led to precipitation at pH > 7. The addition of excess of ligand resulted in solubilisation of the precipitate, but the rate constants (k_{obs}) obtained were very dependent on the ligand to metal ratio used. This result is not surprising in view of the possible equilibria between 1 : 1, 2 : 1, and mixed-ligand complexes in such systems. The use of metal and ligand concentrations of 5×10^{-4}M at $I = 0{\cdot}01$M was successful. Precipitation did not occur at pH > 7 and plots of log $(V_\infty - V_t)$ against time were linear for at least three half-lives. In addition, complex formation was essentially complete as the rate constants did not vary when a slight excess of metal ion was used at pH *ca.* 7. The kinetic data obtained are summarised in Table 1. Values of $k_{obs}/[OH^-]$ were not constant and decreased as the pH increased.

* 1M = 1 mol dm^{-3}.

[3] M. M. Jones, T. J. Cook, and S. Brammer, *J. Inorg. Nuclear Chem.*, 1966, **28**, 1265.

[4] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids,' vol. 2, Wiley, 1961.

[5] R. W. Hay, L. J. Porter, and P. J. Morris, *Austral. J. Chem.*, 1966, **19**, 1197.

[6] C. W. Davies, *J. Chem. Soc.*, 1938, 2093.

[7] R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions,' 2nd edn., Butterworths, London, 1965.

[8] M. K. Kim and A. E. Martell, *Biochemistry*, 1964, **3**, 1169.

[9] U. Meresaar and A. Ågren, *Acta Pharm. Suecica*, 1968, **5**, 85.

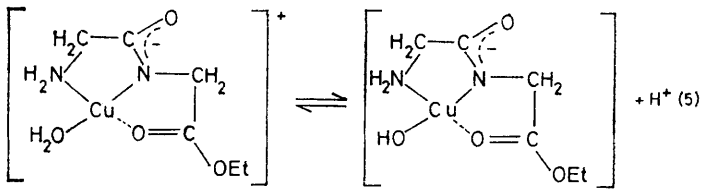
[10] (*a*) A. Nakahara, K. Sakurai, and Y. Nakao, *Bull. Chem. Soc. Japan*, 1965, **38**, 1051; 1966, **39**, 1608; (*b*) A. Albert and E. P. Sergeant, 'Ionisation Constants of Acids and Bases,' Methuen, London, 1962.

TABLE 1

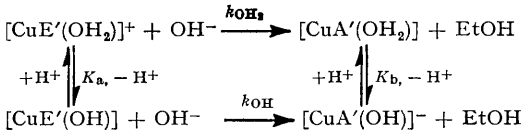
Base hydrolysis of 1 : 1 complexes of dipeptide esters with copper(II) at 25 °C and I = 0·01M

pH	k_{obs}/s^{-1}	$k_{obs}[OH^-]^{-1}$/ l mol^{-1} s^{-1}
(*a*) Ethyl glycylglycinate		
8·225	2·27 × 10^{-3}	1·22 × 10^3
8·095	1·95 × 10^{-3}	1·42 × 10^3
7·845	1·39 × 10^{-3}	1·80 × 10^3
7·69	1·11 × 10^{-3}	2·06 × 10^3
7·585	9·10 × 10^{-4}	2·14 × 10^3
(*b*) Ethyl glycyl-β-alaninate		
9·255	1·19 × 10^{-3}	6·00 × 10^1
9·030	9·20 × 10^{-4}	7·78 × 10^1
8·78	6·35 × 10^{-4}	9·54 × 10^1
8·647	5·11 × 10^{-4}	10·43 × 10^1
8·507	4·02 × 10^{-4}	11·33 × 10^1
(*c*) Ethyl glycyl-L-leucinate		
8·90	1·47 × 10^{-3}	1·68 × 10^2
8·71	9·52 × 10^{-4}	1·68 × 10^2
8·60	7·61 × 10^{-4}	1·73 × 10^2
8·49	6·32 × 10^{-4}	1·85 × 10^2

This behaviour is consistent with equilibrium (5) in which ionisation of a co-ordinated water molecule occurs.



The mechanism then takes the form shown in Scheme 2 where primes indicate deprotonation of the amide group.



SCHEME 2

It can readily be shown that for a scheme of this type equation (7) applies, so that plots of the left-hand side of

$$(k_{obs}/[OH^-])(K_a^{\ominus} + [H^+]) = k_{OH_2}[H^+] + k_{OH}K_a^{\ominus} \quad (7)$$

(7) against [H$^+$] should be linear of gradient k_{OH_2}, and intercept $k_{OH}K_a^{\ominus}$. Plots for the ethyl glycylglycinate and ethyl glycyl-β-alaninate systems are shown in the Figure. Best-fit values for $K_a^{\ominus}$ were computed using a Letagrop program [11] and the results are listed in Table 2.

TABLE 2

Second-order rate constants and p$K_a^{\ominus}$ values at 25 °C and I = 0·01M for the copper complexes

Ligand	p$K_a^{\ominus}$	k_{OH_2}/l mol^{-1} s^{-1}	k_{OH}/l mol^{-1} s^{-1}
Ethyl glycylglycinate	8·0	2·87 × 10^3	1·98 × 10^2
Ethyl glycyl-β-alaninate	9·1	1·36 × 10^2	1·51 × 10^1
Ethyl glycyl-L-leucinate			1·68 × 10^2

For the copper complex of glycylglycine, pK_b = 9·52 at I = 1·0M (KCl) and 25 °C. The use of literature values [7] for the activity coefficients gives pK_b = 9·87 at I = 0·01M and 25 °C. Thus, within the pH range of the kinetic measurements, essentially all the products are in the aqua-form, [CuA'(OH$_2$)]. Since the base hydrolysis [CuE'(OH)] ⟶ [CuA'(OH$_2$)] requires no net consumption of alkali, the volume of alkali consumed during the hydrolysis divided by the theoretical titre, *i.e.* the volume required if 1 mol reactant consumed 1 mol base, should equal the ratio [CuE'(OH$_2$)]$^+$: [CuE'(OH)]. The pK_a values of [CuE'(OH)$_2$]$^+$ calculated in this manner were in reasonable agreement with those given in Table 2. In the case of ethyl glycyl-L-leucinate, the pK_a value for the aqua ⇌ hydroxo equilibrium was lower than in the other systems. At pH values where significant amounts of the aqua-complex was present, complex formation was incomplete. Rate constants are therefore not reported for the aqua-species.

Catalytic Effects.—The rate constant for base hydrolysis of unprotonated ethyl glycylglycinate is *ca.* 0·63 l mol^{-1} s^{-1} at 25 °C and I = 1·0M; this value should not be greatly dependent on ionic strength. Base hydrolysis of the 1 : 1 aqua-complex with copper(II) had k = 2·87 × 10^3 l mol^{-1} s^{-1} at 25 °C and I = 0·01M, so that the rate acceleration is *ca.* 4·6 × 10^3. Such a rate acceleration is consistent with a degree of metal–ester carbonyl bonding. The rate acceleration for the hydroxo-complex was considerably lower, 3·2 × 10^2. Nakon *et al.*[12] argued that the reduced Lewis acidity of the metal ion when bonded to strong donor ligands can account for the trends observed in the rates of hydrolysis of mixed-ligand complexes, and that the charge carried by the complex is relatively unimportant. Such a conclusion is obviously not completely valid. Hydroxide ion is lower than water in the spectrochemical series and on the basis of Angelici's proposals the hydroxo-complex would be expected to undergo more rapid hydrolysis. It is now clear that rate accelerations for metal-promoted hydrolysis of carboxylic ester derivatives arise primarily from a much more positive entropy of activation in the promoted reactions. Thus Δ(Δ$S^{\ddagger}$) values of as much as +30 cal K^{-1} mol^{-1} have been reported,[13] corresponding to a rate acceleration of 3·2 × 10^6 at a constant enthalpy of activation.† Bimolecular reactions have Δ$S^{\ddagger}$ values in the range −5 to −15 cal K^{-1} mol^{-1}.[14] The entropy of

* The superscript ⊖ denotes a practical ionisation constant (ref. 10*b*).

† 1 cal = 4·184 J.

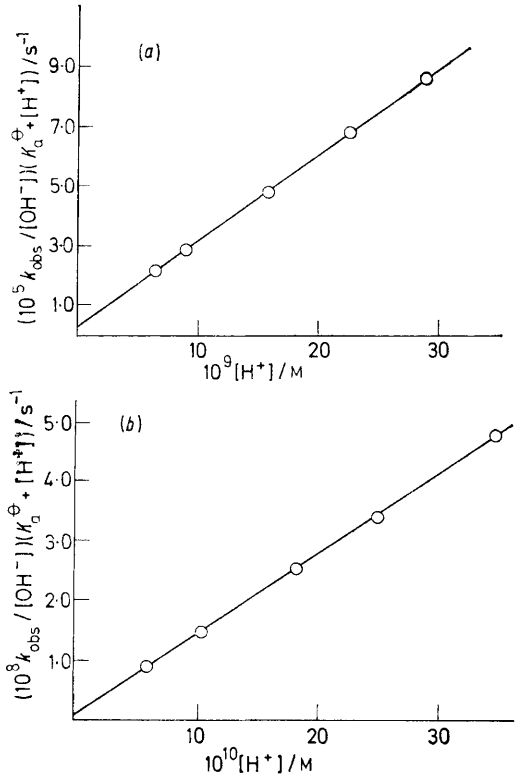
[11] N. Ingri and L. G. Sillén, *Arkiv. Kemi*, 1964, **23**, 97; L. G. Sillén, *Acta Chem. Scand.*, 1962, **16**, 159; 1964, **18**, 1085.

[12] R. Nakon, P. R. Rechani, and R. J. Angelici, *J. Amer. Chem. Soc.*, 1974, **96**, 2117.

[13] R. W. Hay and C. R. Clark, *J.C.S. Dalton*, submitted for publication.

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

activation is extremely sensitive to solvent effects. The orientation of solvent molecules around charges, or developing charges, results in a negative entropy change and the effect may be as large, or larger, than that resulting from the molecularity of the reaction. Addition of hydroxide ion to the free substrate results in a



Plots of $(k_{obs}/[OH^-])(K_a^{\ominus} + [H^+])$ against $[H^+]$ for the copper(II) complexes of (*a*) ethyl glycylglycinate, and (*b*) ethyl glycyl-β-alaninate

transition state of considerable polarity or hydrogen-bonding ability, requiring increased solvent structuring in its vicinity and a corresponding negative contribution to $\Delta S^{\ddagger}$. In the charged metal substrate complex, solvent structuring in the ground state would be expected to be extensive and the approach of the nucleophile to form the transition state will probably lead to minimal solvation requirements.

Base hydrolysis of the copper(II) complexes (aqua and hydroxo) of ethyl glycylglycinate proceeds much more rapidly than their ethyl glycyl-β-alaninate analogues. The relative rates for the aqua-complexes is *ca.* 21 and for the hydroxo-complexes, *ca.* 13. There are no kinetic data available for base hydrolysis of ethyl glycyl-β-alaninate; however, it is possible to calculate a reasonable approximate value. The rate constant for base hydrolysis of the unprotonated species of ethyl glycylglycinate (0·625 l mol^{-1} s^{-1} at 25 °C) is almost identical to that for ethyl glycinate (0·633 l mol^{-1} s^{-1}),[15] while methyl glycinate hydrolyses *ca.* twice as rapidly (1·28 l mol^{-1} s^{-1}).[15] Since the rate constant for methyl β-alaninate is 0·136 l mol^{-1} s^{-1},[16] a reasonable value for ethyl β-alaninate and ethyl glycyl-β-alaninate would be *ca.* 0·068 l mol^{-1} s^{-1}. Base hydrolysis of the 1 : 1 aqua-copper complex has a rate constant of 1·36 × 10^2 l mol^{-1} s^{-1} so that the rate acceleration is 2 × 10^3, a figure not greatly different from the value observed (4·6 × 10^3) for the corresponding complex of ethyl glycylglycinate. The kinetic measurements are therefore consistent with the view that a degree of metal–ester carbonyl bonding is also involved, requiring formation of a six-membered chelate ring. The rate constant for base hydrolysis of ethyl L-leucinate is 0·183 l mol^{-1} s^{-1} [15] at 25 °C and a comparable value would be expected for ethyl glycyl-L-leucinate. The rate acceleration for 1 : 1 hydroxo-copper(II) complex is *ca.* 9·2 × 10^2, a value somewhat higher than observed in the analogous glycylglycine system.

It is clear that copper(II) exerts a significant catalytic effect on the hydrolysis of dipeptide esters, the rate accelerations being of the order of 10^3. In cobalt(III) systems in which the carbonyl group of the ester is known to be bonded directly to the metal ion, rate accelerations of the order of 10^6 have been observed. The present results are therefore consistent with a rather weak interaction between the ester carbonyl group and the metal ion.

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[15] R. W. Hay and L. J. Porter, *J. Chem. Soc.* (*B*), 1967, 1261.
[16] R. W. Hay and P. J. Morris, *J. Chem. Soc.* (*B*), 1970, 1577.