The Palladium(II) Promoted Hydrolysis of the Methyl Esters of Glycyl-L-Leucine, Glycyl-L-Alanine and L-Alanylglycine

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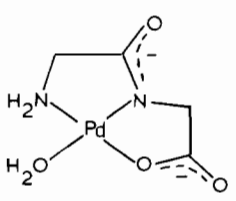
Abstract

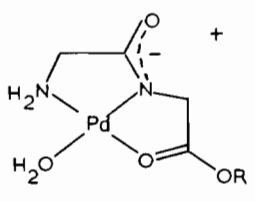
The palladium(II)-promoted hydrolysis of the methyl esters of glycyl-L-leucine, glycyl-L-alanine and L-alanylglycine have been studied at 25 °C and $I = 0.1$ M in the pH range 4–5. At a 1:1 metal to ligand ratio the peptide esters act as tridentate ligands, donation occurring via the terminal amino group, the deprotonated amide nitrogen, and the carbonyl group of the ester. Due to the high Lewis acidity of Pd(II) rapid hydrolysis of the ester function by water and hydroxide ion occurs. Rate constants k_{OH} and k_{H_2O} have been obtained for base hydrolysis and water hydrolysis of the coordinated peptide esters at 25 °C. The rate constants for base hydrolysis are 3.4×10^6 M^{-1} s^{-1} (L-alaglyOMe), 6.4×10^6 M^{-1} s^{-1} (gly-L-alaOMe) and 2.3×10^7 M^{-1} s^{-1} (gly-L-leuOMe). Base hydrolysis of the coordinated peptide esters is at least 10^6 times that of the free unprotonated ligand. Activation parameters have been obtained for both water and base hydrolysis of the Pd(II) complex of methyl L-alanylglycinate and possible mechanisms for the hydrolyses are considered.

Introduction

Metal-induced ionisation of a proton from amide (peptide) nitrogens with subsequent metal binding to the deprotonated nitrogen is a characteristic feature in the complexation of palladium(II) by small peptides [1–10]. Thus, at pH 4 dipeptide ligands interact with Pd(II) to form planar complexes in which the amino, deprotonated amide and carboxylate oxygen act as donors. A representative structure involving glycylglycine is shown in I. Since Pd(II) does not form hydroxo-complexes until pH > 8 in solutions of dipeptides [5] the fourth site on Pd(II) is occupied by a water molecule*. Dipeptide esters would be expected to form a complex of type II in which the alkoxycarbonyl group of the ester also acts as a donor. This view is supported by the observation [7] that base hydrolysis of coordinated methyl glycylglycinate is accelerated by a factor of *ca.* 10^5 fold when compared with the free ester ligand. Palladium(II) has many attractive features as a Lewis acid catalyst due to (a) its fixed coordination number of four, (b) its high Lewis acidity (the $[Pd(H_2O)_4]^{2+}$ ion being square planar is much more acidic ($pK_a = -2.3$) than octahedrally coordinated divalent ions [11]); (c) its high formation constants with N,O donor ligands and (d) its intermediate kinetic lability. For this reason we have studied the palladium(II) promoted hydrolysis of a series of dipeptide methyl esters. The deprotonated peptide is not susceptible to hydrolysis at the peptide bond, indeed this bond is greatly stabilised by coordination of the deprotonated peptide nitrogen to metal ions such as copper(II) [12, 13] and cobalt(III) [14]. As a result ester hydrolysis can be studied in isolation without any competing effects due to peptide bond hydrolysis.



I



II R = Me

Experimental

Glycyl-L-leucine, glycyl-L-alanine and L-alanylglycine were purchased from Fluka. Methyl glycyl-

*It should be noted that the nature of the fourth ligand remains uncertain in those solutions to which Cl^- has been added either internally as $[PdCl_4]^{2-}$, or externally as an ionic strength control. Even in supposedly Cl^- free solutions, the Cl^- flow from pH electrodes may be of significance. Kim and Martin [26] have recently determined the formation constants for Cl^- binding to $[Pd(gly-gly)H_2O]$ and $[Pd(gly-phe)H_2O]$ using intensity changes in the ultraviolet spectrum. For both peptide complexes $\log K_{Cl} = 1.90$. As a result it must be assumed that there is some Cl^- binding at the fourth site in the present system.

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L-leucinate hydrochloride was prepared as follows. To ice-cold methanol (150 cm^3) was added dropwise with constant stirring $SOCl_2$ (2 g, slight excess). The addition was maintained at such a rate that the temperature was always less than 5 °C. On completion of the addition, glycyl-L-leucine (1 g) was added and the solution refluxed for 4 h. The solvent was then removed on a rotary evaporator and the ester crystallised by the addition of diethyl ether. The dipeptide ester hydrochloride was recrystallised from methanol/ether and air dried (yield 0.9 g). *Anal.* Calc. for $C_9H_{19}N_2O_3Cl$: C, 48.3; H, 8.0; N, 11.7. Found: C, 48.5; H, 7.9; N, 11.9%.

The hydrochlorides of methyl glycyl-L-alaninate and methyl L-alanylglycinate were prepared similarly. The glycyl-L-alanine derivative gave an oil on removal of the solvent methanol and was crystallised by addition of a large excess of diethyl ether (*ca.* 500 cm^3) followed by vigorous shaking. On standing overnight in a refrigerator the pure ester hydrochloride crystallised (0.3 g). *Anal.* Calc. for $C_6H_{13}N_2O_3Cl$: C, 36.65; H, 6.65; N, 14.25. Found: C, 36.3; H, 6.4; N, 14.5%. The L-alanylglycine derivative also gave an oil. Addition of ice-cold diethyl ether (200 cm^3) followed by vigorous shaking and scratching gave the crystalline ester hydrochloride. *Anal.* Calc. for $C_6H_{13}N_2O_3Cl$: C, 36.65; H, 6.65; N, 14.25. Found: C, 36.8; H, 6.5; N, 14.2%.

Kinetics and Measurements

All kinetic measurements were carried out using 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 (s.c.e.) with diffusion filter, type K401, as reference electrode. The electrode system was standardised at 25 ± 0.1 °C using 0.05 M potassium hydrogen phthalate (pH 4.008) and 0.01 M disodium tetraborate (pH 9.185). At the other temperatures the recommended pH standards were employed [15]. The general technique employed in the kinetic measurements has been outlined previously [16]. All pH-stat studies were carried out at $I = 0.1$ M ($NaClO_4$) with the dipeptide ester hydrochlorides 2.25×10^{-4} M and $K_2[PdCl_4]$ of the same concentration. One mol of base was consumed per mol of the ester in the pH-stat measurements. Values of the hydroxide ion concentration were obtained from the pH using a molar activity coefficient γ_1 of 0.772 [17] and a value of $pK_w = 13.997$ at 25 °C [18]. At the other temperatures the appropriate values are $\gamma_1 = 0.770$, $pK_w = 13.833$ (30 °C); $\gamma_1 = 0.768$, $pK_w = 13.680$ (35 °C); and $\gamma_1 = 0.766$, $pK_w = 13.535$ (40 °C).

For the potentiometric titrations the solutions used were 1×10^{-3} M in both $K_2[PdCl_4]$ and the ligand (dipeptide or dipeptide ester hydrochloride); $I = 0.1$ M ($NaClO_4$) at 25 °C. In this case pH measurements were made with a Radiometer PHM 64 Research pH-meter.

Results and Discussion

Potentiometric titration of a 1:1 molar mixture of $K_2[PdCl_4]$ with glycylglycine indicates the release of two protons from the protonated amino-group and the peptide bond to give the complex **I** [7]. Formation of the 1:1 complex is essentially complete by pH 4. Similar behaviour is also observed with methyl glycylglycinate hydrochloride and $K_2[PdCl_4]$ at 1:1 molar ratios [7]. Quite analogous observations were made with the three dipeptides and dipeptide ester hydrochlorides used in the present study, Figs. 1 and 2.

Hydrolysis of the ester ligand occurs in the pH range 4–5. One mol of base is consumed per mol of the peptide ester on hydrolysis in the presence of one mol equivalent of $K_2[PdCl_4]$ in this pH range. Values of k_{obs} (the observed first-order rate constant at constant pH) for the hydrolysis of methyl L-alanylglycinate at various temperatures are summarised in Table I.

Plots of k_{obs} *vs.* $[OH^-]$ are linear with a positive intercept, Fig. 3. The kinetic data conform to the equation $k_{obs} = k_o + k_{OH}[OH^-]$, where k_o can be assigned to a solvolytic pathway (water attack on the complex) and k_{OH} to base hydrolysis. These two processes can be represented by eqns. (1) and (2).

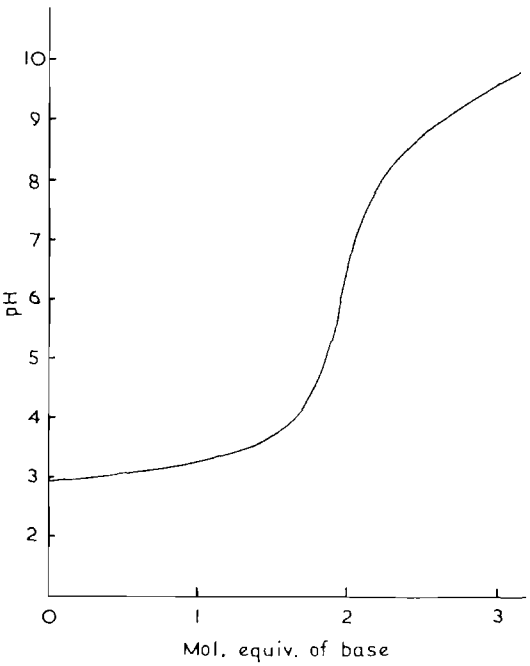


Fig. 1. Potentiometric titration of a 1:1 mixture (1×10^{-3} M) of $K_2[PdCl_4]$ and L-alanylglycine at 25 °C and $I = 0.1$ M ($NaClO_4$).

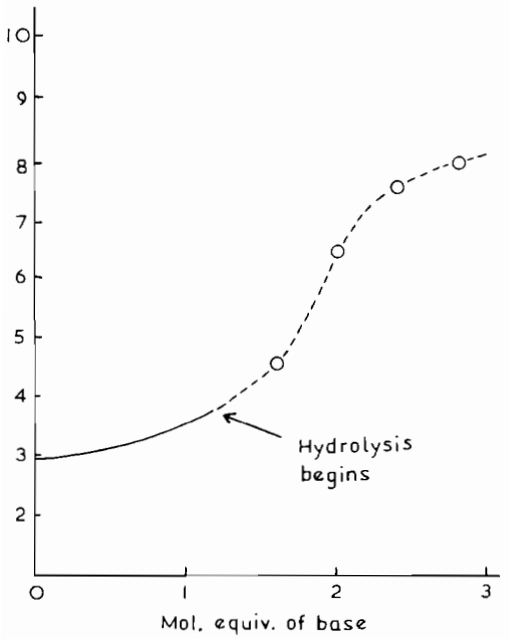


Fig. 2. Potentiometric titration of a 1:1 mixture (1×10^{-3} M) of $K_2[PdCl_4]$ and methyl L-alanylglycinate hydrochloride at 25 °C and $I = 0.1$ M ($NaClO_4$). Hydrolysis begins at the point shown, and it was necessary to titrate rapidly to obtain the experimental points at higher pH.

TABLE I. Hydrolysis of the 1:1 Complex of Methyl L-Alanylglycinate with Palladium(II) at Various Temperatures and $I = 0.1$ M ($NaClO_4$)

Temperature (°C)	pH	10^{10} [OH^-] (M)	10^3 k_{obs} (s^{-1})
25	4.12	1.72	1.97
	4.23	2.22	2.15
	4.34	2.86	2.39
	4.67	6.11	3.46
	4.77	7.69	3.98
30	4.03	2.04	3.79
	4.11	2.46	4.07
	4.30	3.81	4.86
	4.46	5.50	5.83
	4.52	6.32	6.25
35	3.75	1.53	6.30
	3.89	2.11	6.92
	3.92	2.26	7.12
	4.04	2.98	7.89
	4.16	3.93	8.82
40	3.61	1.55	11.63
	3.69	1.87	11.98
	3.75	2.14	12.54
	3.80	2.40	13.04
	3.85	2.69	13.69

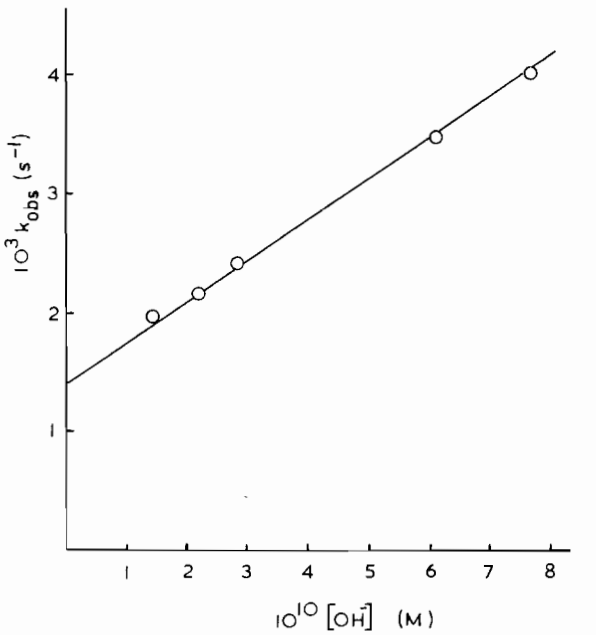


Fig. 3. Plot of k_{obs} *vs.* the hydroxide in concentration for the hydrolysis of the 1:1 complex of Pd(II) with methyl L-alanylglycinate at 25 °C and $I = 0.1$ M.

$$[Pd(peptideOMe)(H_2O)]^+ + H_2O \xrightarrow{k_{H_2O}} [Pd(peptide)(H_2O)]^0 + MeOH + H^+ \quad (1)$$

$$[Pd(peptideOMe)(H_2O)]^+ + OH^- \xrightarrow{k_{OH}} [Pd(peptide)(H_2O)]^0 + MeOH \quad (2)$$

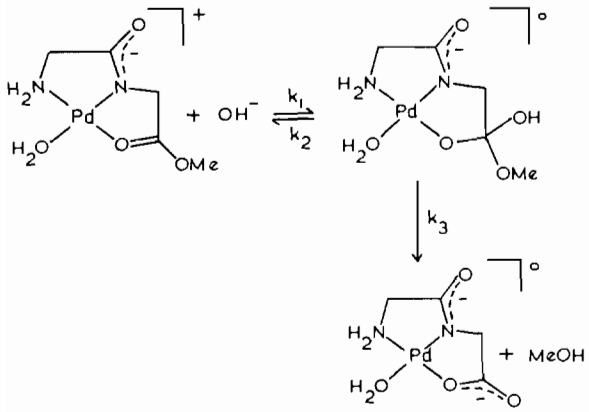
As both reactions consume base they are monitored by the pH-stat technique. Least-squares analysis of the data at 25 °C gives $k_o = 1.41 \times 10^{-3}$ s^{-1} and $k_{OH} = 3.35 \times 10^6$ M^{-1} s^{-1} with a correlation coefficient of 0.9998. Values of k_o were converted to k_{H_2O} rate constants using the expression $k_{H_2O} = k_o/55.5$, where 55.5 M is the molar concentration of water, giving $k_{H_2O} = 2.54 \times 10^{-5}$ M^{-1} s^{-1}. The ratio $k_{OH}/k_{H_2O} = 1.3 \times 10^{11}$ which is of the expected magnitude (10^{10}–10^{12}) for the relative nucleophilicities of hydroxide ion and water in reactions of this type. The rate constants k_{OH} and k_{H_2O} at various temperatures are summarised in Table II for the hydrolysis of methyl L-alanylglycinate. The requisite activation parameters are $\Delta H^{\ddagger} = 91$ kJ mol^{-1} and $\Delta S^{\ddagger}_{298} = -28$ JK^{-1} mol^{-1} (for k_{H_2O}) and $\Delta H^{\ddagger} = 86$ kJ mol^{-1} and $\Delta S^{\ddagger}_{298} = 168$ JK^{-1} mol^{-1}. These values are very comparable with those previously obtained [7] for the hydrolysis of methyl glycylglycinate, Table III. The most significant feature of these reactions is the large positive entropy of activation in the base hydrolysis reactions. A large negative entropy of activation would be expected in a bimolecular reaction of the type shown in eqn. (2) with $\Delta S^{\ddagger}$

TABLE II. Values of k_{OH} and K_{H_2O} for the Hydrolysis of the 1:1 Complex of Methyl L-Alanylglycinate with Palladium-(II) at various Temperatures and I = 0.1 M ($NaClO_4$)[a]

Temperature (°C)	$10^5\ k_{H_2O}$ ($M^{-1}\ s^{-1}$)	$10^{-6}\ k_{OH}$ ($M^{-1}\ s^{-1}$)
25	2.54 ± 0.03	3.35 ± 0.03
30	4.75 ± 0.07	5.75 ± 0.08
35	8.48 ± 0.09	10.52 ± 0.2
40	15.6 ± 0.5	18.4 ± 1.3

[a]For k_{H_2O}, $\Delta H^{\ddagger}$ = 91 ± 1 kJ mol^{-1} and $\Delta S^{\ddagger}_{298}$ = −28 ± 3 JK^{-1} mol^{-1} (correlation coefficient = 0.999). For k_{OH}, $\Delta H^{\ddagger}$ = 86 ± 2 kJ mol^{-1} and $\Delta S^{\ddagger}_{298}$ = 168 ± 6 JK^{-1} mol^{-1} (correlation coefficient = 0.992).

in the range −20 to −60 JK^{-1} mol^{-1} [19], which is in fact observed in the water reaction. The base hydrolysis reaction can be considered in terms of Scheme 1. Attack by hydroxide ion on the positively charge metal complex will lead to an overall charge



Scheme 1. Base hydrolysis of the peptide ester complexes.

neutral tetrahedral intermediate and considerable desolvation. The metal ion effectively 'solvates' the developing negative charge on the carbonyl oxygen when nucleophilic attack occurs, thus reducing the solvation requirement which occurs in the absence of the metal ion and which leads to a substantial negative entropy of activation. The results are in

TABLE IV. Hydrolysis of the 1:1 Complex of Methyl Glycyl-L-Alaninate with Palladium(II) at 25 °C and I = 0.1 M ($NaClO_4$)[a]

pH	10^{10} [OH^-] (M)	$10^3\ k_{obs}$ (s^{-1})
3.06	1.50	1.76
3.36	2.99	1.87
3.45	3.68	1.96
3.64	5.70	2.04
3.76	7.51	2.16
3.81	8.43	2.22

[a]k_{H_2O} = (1.68 ± 0.04) × 10^{-5} M^{-1} s^{-1}; k_{OH} = (6.39 ± 0.4) × 10^6 M^{-1} s^{-1} (correlation coefficient 0.9927).

TABLE V. Hydrolysis of the 1:1 Complex of Methyl Glycyl-L-Leucinate with Palladium(II) at 25 °C and I = 0.1 M ($NaClO_4$)[a]

pH	10^{10} [OH^-] (M)	$10^3\ k_{obs}$ (s^{-1})
3.59	5.08	1.49
3.60	5.19	1.62
3.72	6.85	1.84
3.82	8.63	2.20
3.86	9.46	2.35
3.91	10.61	2.62
4.11	16.82	4.26

[a]k_{H_2O} = (5.07 ± 1.9) × 10^{-6} M^{-1} s^{-1}; k_{OH} = (2.29 ± 0.1) × 10^7 M^{-1} s^{-1} (correlation coefficient = 0.9941).

general agreement with the observations made with analogous cobalt(III) complexes [20–23]. Reactions involving the attack of an 'external' nucleophile such as water and hydroxide ion on a 'coordinated' carbonyl function leads to rate accelerations of 10^5–10^6 for all substrates independent of the leaving group. In addition, the rate enhancement is due entirely to entropy factors with no contribution from the $\Delta H^{\ddagger}$ term [20].

The hydrolyses of the 1:1 complexes of methyl glycyl-L-alaninate and methyl glycyl-L-leucinate with Pd(II) were also studied at 25 °C, Tables IV and V.

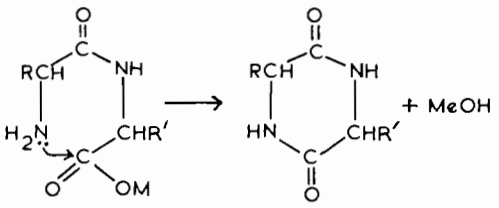
TABLE III. Activation Parameters for Base Hydrolysis (k_{OH}) and Water Hydrolysis (k_{H_2O}) of the 1:1 Complexes of Palladium-(II) with Methyl L-Alanylglycinate and Methyl Glycylglycinate at I = 0.1 M ($NaClO_4$)[a]

Ester	$k^{25}_{H_2O}$ ($M^{-1}\ s^{-1}$)	k^{25}_{OH} ($M^{-1}\ s^{-1}$)	$\Delta H^{\ddagger}(H_2O)$ (kJ mol^{-1})	$\Delta S^{\ddagger}_{298}(H_2O)$ (JK^{-1} mol^{-1})	$\Delta H^{\ddagger}(OH^-)$ (kJ mol^{-1})	$\Delta S^{\ddagger}_{298}(OH^-)$ (JK^{-1} mol^{-1})
glyglyOMe	6.9 × 10^{-6}	1.55 × 10^5	90	−42	79	118
L-alaglyOMe	3.4 × 10^{-6}	2.5 × 10^5	91	−28	86	168

[a]Values for glyglyOMe from ref. 7.

Values of k_{OH} are 6.4×10^6 M^{-1} s^{-1} (gly-L-alaOMe) and 2.3×10^7 M^{-1} s^{-1} (gly-L-leuOMe) at $I = 0.1$ M.

It is difficult to determine precise rate constants for the base hydrolysis of the free dipeptide esters ($NH_2CH(R)CONHCH(R')CO_2Me$) due to the competing formation of the piperazine-2,5-dione (**III**). Maresaar and Agren [24] have estimated that k_{OH} for the hydrolysis of $NH_2CH_2CONHCH_2CO_2Et$ is *ca.* 0.63 M^{-1} s^{-1} at 25 °C and $I = 1.0$ M.



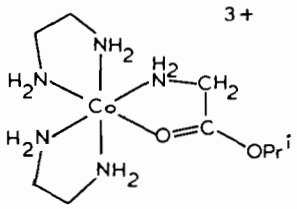
III

Since methyl esters normally undergo base hydrolysis at *ca.* twice the rate of ethyl esters, a reasonable estimate for the base hydrolysis of the methyl ester of the dipeptide is $k_{OH} = 1.25$ M^{-1} s^{-1} at 25 °C. On the basis of known rate constants for the hydrolysis of methyl glycinate ($k_{OH} = 1.28$ M^{-1} s^{-1}), methyl α-alaninate ($k_{OH} = 1.11$ M^{-1} s^{-1}) and methyl leucinate ($k_{OH} = 0.46$ M^{-1} s^{-1}) [25] it appears that the rate constants for the dipeptide esters will be very similar to those observed for the methyl ester of the C-terminal amino acid of the dipeptide, *i.e.* for L-alaglyOMe $k_{OH} = 1.3$ M^{-1} s^{-1}, for gly-L-alaOMe, $k_{OH} = 1.1$ M^{-1} s^{-1} and for gly-L-leuOMe, $k_{OH} = 0.5$ M^{-1} s^{-1} at 25 °C. On this basis the rate accelerations observed in base hydrolysis of the Pd(II)–peptide ester complexes compared with the free ligands are in the range 2.6×10^6 to 4.6×10^7 fold, Table VI. These rate accelerations are of a similar magnitude to that observed with the chelate glycine ester complex $[Co(en)_2(glyOPr^i)]^{3+}$ (**IV**) where $k_{OH} = 1.5 \times 10^6$ M^{-1} s^{-1} at 25 °C and $I = 1.0$ M [14] leading to a rate acceleration of *ca.* 10^6 fold.

TABLE VI. Rate Constants k_{H_2O}, k_{OH} and Reactivity Ratios for the Hydrolysis of the 1:1 Palladium(II) Complexes of the Dipeptide Esters at 25 °C and $I = 0.1$ M ($NaClO_4$)[a]

Peptide ester	10^5 k_{H_2O} (M^{-1} s^{-1})	10^{-6} k_{OH} (M^{-1} s^{-1})	k_{OH}/k_{H_2O}
L-alaglyOMe	2.5	3.4	1.3×10^{11}
gly-L-alaOMe	1.7	6.4	3.8×10^{11}
gly-L-leuOMe	0.5	22.9	4.6×10^{12}

[a]For the free unprotonated dipeptide esters the estimated (see text) values of k_{OH} are 1.3 M^{-1} s^{-1} (L-alaglyOMe), 1.1 M^{-1} s^{-1} (gly-L-alaOMe) and 0.5 M^{-1} s^{-1} (gly-L-leuOMe) at 25 °C.



IV

References

1 E. W. Wilson and R. B. Martin, *Inorg. Chem., 9,* 528 (1970).
2 H. Sigel and R. B. Martin, *Chem. Rev., 82,* 385 (1982).
3 T. P. Pitner, E. W. Wilson, Jr. and R. B. Martin, *Inorg. Chem., 11,* 738 (1972).
4 R. B. Martin, in H. Sigel (ed.), 'Metal Ions in Biological Systems', Vol. 1, Marcel Dekker, New York, 1974.
5 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc., 102,* 7906 (1980).
6 D. L. Rabenstein, A. A. Isab and M. M. Shoukry, *Inorg. Chem., 21,* 3234 (1982).
7 R. W. Hay and M. P. Pujari, *J. Chem. Soc., Dalton Trans.,* 1083 (1984).
8 N. Ueyama, M. Nakata and A. Nakamura, *Inorg. Chim. Acta, 55,* L61 (1981).
9 N. Ueyama, K. Sasaki, M. Nakata and A. Nakamura, *Bull. Chem. Soc. Jpn., 55,* 2364 (1982).
10 B. D. Reverend, C. Loncheux, T. Kowalik and H. Kozlowski, *Inorg. Chim. Acta, 66,* 205 (1982).
11 D. W. Barnum, *Inorg. Chem., 22,* 2297 (1983).
12 I. J. Grant and R. W. Hay, *Aust. J. Chem., 19,* 1189 (1965).
13 M. M. Jones, T. J. Cook and S. Brammer, *J. Inorg. Nucl. Chem., 28,* 1265 (1966).
14 D. A. Buckingham, C. E. Davis, D. M. Foster and A. M. Sargeson, *J. Am. Chem. Soc., 92,* 5571 (1970); N. E. Dixon and A. M. Sargeson, in T. G. Spiro (ed.), 'Zinc Enzymes', Wiley, New York, 1983.
15 'Vogel's Textbook of Quantitative Inorganic Analysis', 4th edn., Longmans, London, 1978, p. 578.
16 R. W. Hay, L. J. Porter and P. J. Morris, *Aust. J. Chem., 19,* 1197 (1966).
17 C. W. Davies, *J. Chem. Soc.,* 2093 (1938).
18 R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions', 2nd edn., Butterworths, London, 1965.
19 L. L. Schaleger and F. A. Long, *Adv. Phys. Org. Chem., 1,* 1 (1963).
20 C. J. Boreham, D. A. Buckingham and F. R. Keene, *Inorg. Chem., 18,* 28 (1979).
21 D. A. Buckingham, D. M. Foster and A. M. Sargeson, *J. Am. Chem. Soc., 90,* 6032 (1968).
22 D. A. Buckingham, D. M. Foster, L. G. Marzilli and A. M. Sargeson, *Inorg. Chem., 9,* 11 (1970).
23 D. Buckingham, J. M. Harrowfield and A. M. Sargeson, *J. Am. Chem. Soc., 96,* 1726 (1974).
24 U. Meresaar and A. Agren, *Acta Pharm. Suec., 5,* 85 (1968).
25 R. W. Hay and P. J. Morris, in H. Sigel (ed.), 'Metal Ions in Biological Systems', Vol. 5, Marcel Dekker, New York, 1976.
26 S.-H. Kim and R. B. Martin, *J. Am. Chem. Soc., 106,* 1707 (1984).