Hydrolysis and Binding of Zinc to L-Aspartyl-L-phenylalanine Methyl Ester

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100-MHz N.m.r. studies are reported for the complexes formed between protons, zinc ions, and L-aspartyl-L-phenylalaninemethyl ester, aspartate, and L-phenylalanine methyl ester. The sites of metal-ion binding are identified and the relative rates of ester hydrolysis are discussed from a mechanistic viewpoint.

L-ASPARTYL-L-PHENYLALANINE methyl ester (Aspartame, G. D. Searle and Co. Trademark = ' Equa '), (1), is the latest addition to the list of artificial sweeteners. It is *ca.* 180 times as sweet as sugar and a daily consumption of 0.8 g will provide 5% of a person's normal daily intake of aspartic acid and *ca.* 10% of that of phenylalanine.[1] The sensitivity of taste buds in humans is known to be dependent on metal concentration and they are especially sensitive to the zinc concentration. Our formation-constant studies [2] have established that Aspartame does indeed form complexes with zinc ions and this paper reports some n.m.r. studies designed to identify the Aspartame donor groups that are complexed to the Zn^{II} and also presents our explanations of the mechanism of alkaline hydrolysis of Aspartame. The situation has been clarified by an examination of the hydrolysis characteristics and n.m.r. spectra of two fragments of Aspartame, aspartate (2) and phenylalanine methyl ester (3).

RESULTS

The protonation pK values and log formation constants are taken from ref. 2. During the potentiometric study it was noted (from the rates of electrode e.m.f. drift) that Aspartame was hydrolysed at pH >9 and that the presence of zinc seemed to stabilise the system against hydrolysis (although precipitation of $Zn[OH]_2$ did eventually occur). Paradoxically, phenylalanine methyl ester underwent a relatively slow hydrolysis but this process was catalysed

[1] *Chem. Eng. News*, 1974, August 5, 5.
[2] G. K. R. Makar, M. L. D. Touche, and D. R. Williams, *J.C.S. Dalton*, 1976, 1016.

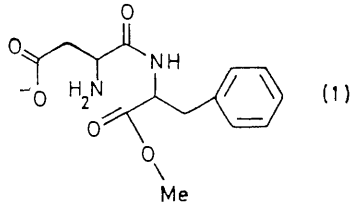
N.m.r. peaks and assignments

Ligand	10^3 Concentration / mol dm^{-3}	$10^3[Zn^{II}]$ / mol dm^{-3}	pD [a]	N.m.r. spectra: Peak (Hz)	No. of lines	Assignment
Aspartate	60	0	7.0	316	4	CH_2
				430	4	CH
	60	0	10.4	280	8	CH_2
				388	4	CH
	60	30	8.4	291	8	CH_2
				391	6	CH
Aspartame	50	0	7.0	288	*b*	CH_2 Asp
				346	*b*	CH_2 Phe
				366	1	$HOCH_3$
				406	1	OCH_3
				427	4	CH Asp
				493 [c]	?	CH Phe
				766	3	Ph
	50	50	7.0	308	*b*	CH_2 Asp
				346	*b*	CH_2 Phe
				368	1	$HOCH_3$
				406	1	OCH_3
				450	4	CH Asp
				495 [c]	?	CH Phe
				770	3	Ph

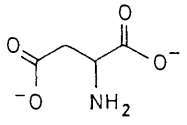
[a] pD = pH + 0.40 (P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188). [b] Multiple. [c] At the edge of the OH signal which obscured the C–H peaks.

by the presence of zinc ions to such an extent that formation constants for the zinc system could not be determined.

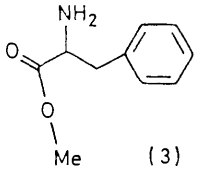
The Table lists the results of our 100-MHz n.m.r. studies which suggest the binding site of zinc in zinc–Aspartame, and this information leads on to an explanation of these



(1)



(2)



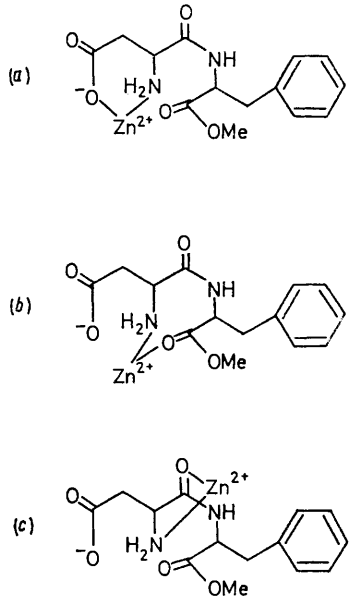
(3)

hydrolysis phenomena: the Figure shows three possible modes of zinc–Aspartame binding and the results in the Table show that there is no significant shift in the phenylalanine protons on adding zinc, whereas there is a substantial influence on the aspartyl protons. The former observation definitely excludes complexes (*b*) and (*c*) since both the ester and peptide involvement should have influenced the phenylalanine protons; this suggests that complex (*a*) is the main species present.

DISCUSSION

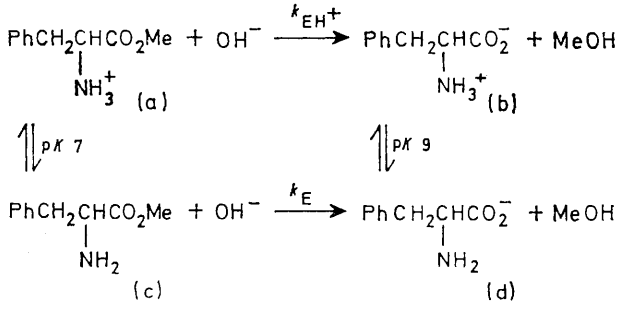
Table 2 of ref. 2 shows that the zinc–Aspartame system forms protonated complexes having pK values of 5.53 and 6.34. These protonated complexes considerably complicate the n.m.r. spectra and so we investigated the system at pH 7 where their concentrations were not noticeable in the spectra.

Scheme 1 suggests the modes of base hydrolysis for phenylalanine methyl ester [3] and the pH dependence of the reaction is understandable from the following observations. At pH > 9 only the lower reaction occurs and at pH < 7 only the upper reaction. However, at pH 7—9



FIGURE

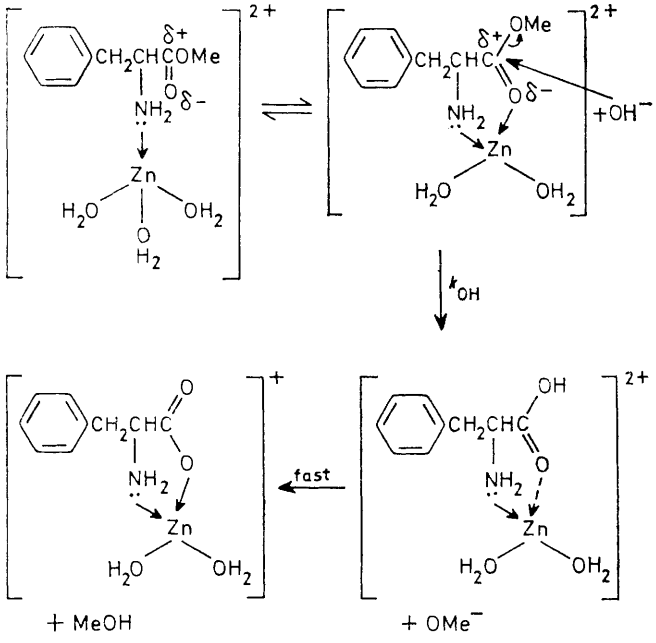
both reactions can occur together, but most of the ester will be in the unprotonated form (c) while the acid is in



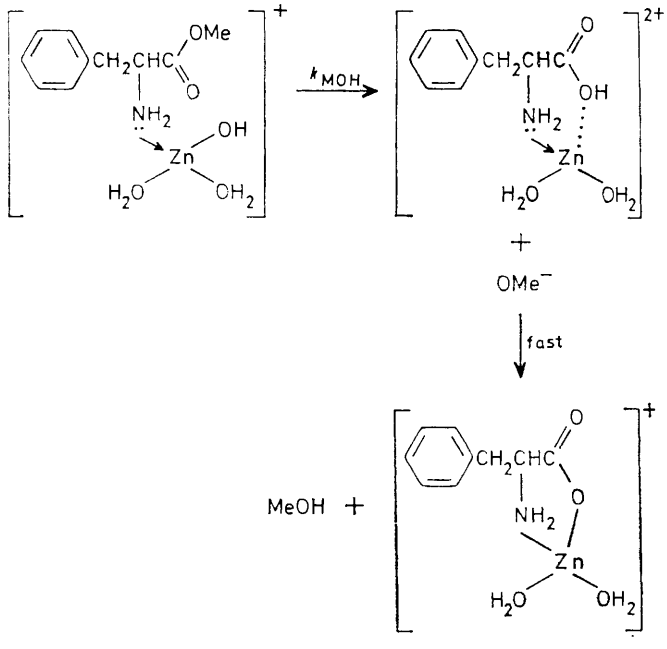
SCHEME 1

[3] R. W. Hay and P. J. Morris, *J. Chem. Soc.* (*B*), 1970, 1577.

the zwitterion form (b). Hence the main reaction is dependent on k_E with only a small contribution from k_{EH^+}.



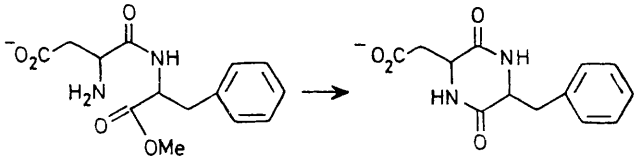
SCHEME 2



SCHEME 3

However, when the amine group of the ester is complexed to zinc ions there is probably some ester carbonyl–zinc bonding also (Scheme 2) [4] which increases the polarisation of the carbonyl bond and weakens the ester linkage, encouraging more rapid hydrolysis. An alternative mechanism (Scheme 3) involves internal hydroxide attack causing hydrolysis. The literature [4,5] favours Scheme 2 and suggests that the increase in the rate constant in the presence of Cu^{II} is one thousandfold.

Aspartame, on the other hand, when complexed as in Figure (*a*) uses donor groups remote from the ester linkage and so catalysis is not feasible. Further, if the electrode e.m.f. drift noted in the absence of metal ions is caused by the formation of a disubstituted dioxopiperazine (Scheme 4) through primary-amine influence



SCHEME 4

on the ester, complexing of the amine with zinc precludes Scheme 4 and explains the e.m.f. stabilisation noted in the presence of zinc ions.

EXPERIMENTAL

Aspartame was supplied by G. D. Searle and Co. and was used without further purification (Found: C, 57.2; H, 6.25; N, 8.9. Calc. for $C_{14}H_{18}N_2O_5$; C, 57.1; H, 6.15; N, 9.5%), m.p. 232 °C (decomp.). Aspartic acid was B.D.H. biochemical grade (Found: C, 35.9; H, 5.35; N, 10.5. Calc. for $C_4H_7NO_4$: C, 36.1; H, 5.30; N, 10.5%), m.p. >217 °C (decomp.). L-Phenylalanine methyl ester hydrochloride was obtained from Koch–Light Ltd (Found: C, 55.9; H, 6.35; N, 6.5. Calc. for $C_{10}H_{14}ClNO_2$: C, 55.7; H, 6.55; N, 6.5%), m.p. 155—156 °C (lit. 158—160 °C).

Solutions were prepared in D_2O and the pD was adjusted by the use of either sodium hydroxide in D_2O or 60% perchloric acid. The spectra were recorded on a Varian Associates HA 100-MHz spectrometer. Zinc carbonate was then added and the spectra were rerun. The reference probe used was tetramethylsilane.

Both aspartic acid and Aspartame have low solubilities (*ca.* 60 and 50 × 10^{-3} mol dm^{-3} at pH 7) and so the spectra were weaker than ideal and sometimes had a low signal to noise ratio. In order to observe the effect of adding zinc to a completely deprotonated ligand, spectra were recorded at pH *ca.* 10 for aspartate but, of course, the zinc aspartate signals were weak. COMPLOT [6,7] models suggested that this complex was present at a higher concentration at pD 8.4. These spectra were also recorded. Our results are listed in the Table.

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[4] R. J. Angelici and B. E. Leach, *J. Amer. Chem. Soc.*, 1968, **90**, 2499.

[5] R. W. Hay and K. B. Nolan, *J.C.S. Dalton*, 1974, 2542.

[6] A. C. Baxter and D. R. Williams, *J.C.S. Dalton*, 1974, 1117.

[7] D. D. Perrin and I. G. Sayce, *Talanta*, 1967, **14**, 833.