Demethylation Kinetics of Aspartame and L-Phenylalanine Methyl Ester in Aqueous Solution

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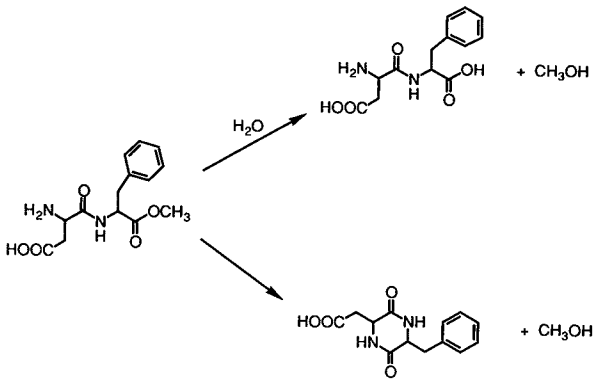
The kinetics of demethylation of aspartame and L-phenylalanine methyl ester were studied in aqueous solution at 25°C over the pH range 0.27–11.5. The pseudo-first-order rate constant for aspartame was resolved into individual contributions from methyl ester hydrolysis and diketopiperazine formation. pH–rate profiles were quantitatively described by chemically reasonable kinetic schemes. Aspartame is maximally stable at pH 4 (t_{90} = 53 days at 25°C); phenylalanine methyl ester, at pH 3. The potentiometrically measured pK_a values were pK_{a1} 3.19 and pK_{a2} 7.87 for aspartame and pK_a 7.11 for phenylalanine methyl ester.

KEY WORDS: aspartame; stability; pH–rate profile; peptide ester; hydrolysis.

INTRODUCTION

Our laboratory is engaged in systematic studies of solvent effects on chemical phenomena. We have developed a theoretical framework for the interpretation of such studies and have made applications to solvent effects on solubility (1,2), surface tension (3), and molecular complex formation (4,5) in binary solvent mixtures. As the next major step in these investigations, we plan to extend them to solvent effects on chemical reaction rates. Among the systems we have selected for study is the decomposition of aspartame, in part because of its practical importance, but also because aspartame is a representative of the peptide class of compounds. Aspartame, L-α-aspartyl-L-phenylalanine methyl ester, is a noncarbohydrate sweetener that is widely used in foods, beverages, and pharmaceuticals. The stability of aspartame is of considerable importance to the formulator because its degradates are not sweet. Under typically mild conditions of storage (solution phase at room or refrigerator temperature) aspartame (A) can undergo demethylation via classical intermolecular hydrolysis to give the dipeptide L-α-aspartyl-L-phenylalanine (AP) or via intramolecular aminolysis to form 3-carboxymethyl-6-benzyl-2,5-diketopiperazine (DKP); these reactions are shown in Scheme I.



Scheme I

We required an understanding of the demethylation kinetics of aspartame in aqueous solution before experiments in binary aqueous–organic mixtures, which use the aqueous system as a reference, could be rationally designed. We therefore examined aspartame decomposition over a wide pH range. Several studies of aspartame stability have been published (6–11), but these studies had other aims, and a comprehensive pH–rate profile was not available. We also report similar data for the demethylation of L-phenylalanine methyl ester (P), which degrades only via intermolecular hydrolysis. In later solvent effect studies, we used P as a model for aspartame hydrolysis.

MATERIALS AND METHODS

Aspartame and L-α-aspartyl-L-phenylalanine were obtained from Sigma Chemical (St. Louis, MO) and Bachem (Philadelphia, PA), respectively. L-Phenylalanine, L-phenylalanine methyl ester, and sodium hexanesulfonate were obtained from Aldrich Chemical (Milwaukee, WI). Acetonitrile (HPLC grade) was obtained from EM Science (Gibbstown, NJ). Buffer components were of reagent grade. Aqueous solutions and buffers were prepared with distilled deionized water (Sybron-Barnstead PCS system; Boston, MA). All pH measurements were made at 25°C with an Orion Research Model 701A pH meter (Cambridge, MA) equipped with a Corning semimicro pH combination electrode (Corning, NY). The pK_a values for aspartame and phenylalanine methyl ester were determined by potentiometric titration in 0.100 *M* NaCl (12).

HPLC Assay. The chromatographic work station consisted of a Waters 501 isocratic solvent delivery module (Bedford, MA), a Waters 484 tunable absorbance detector operated at 254 nm, a Rheodyne (Cotati, CA) model 7125 injection valve equipped with a 100-μL loop, a Kipp and Zonen Model BD 40 chart recorder (Delft, Holland), and a 4.6 × 250-mm Econosphere octylsilane cartridge column (5-μm average particle size) from Alltech (Deerfield, IL). Peak areas were integrated using a Rainin Dynamax HPLC Method Manager, Version 1.1, (Woburn, MA), interfaced to an Apple Macintosh SE computer (Cupertino, CA). The mobile phase consisted of 80 parts 0.04 *M* phosphate buffer (pH 2.23)/0.01 *M* 1-sodium hexanesulfonate, and 20 parts aceto-

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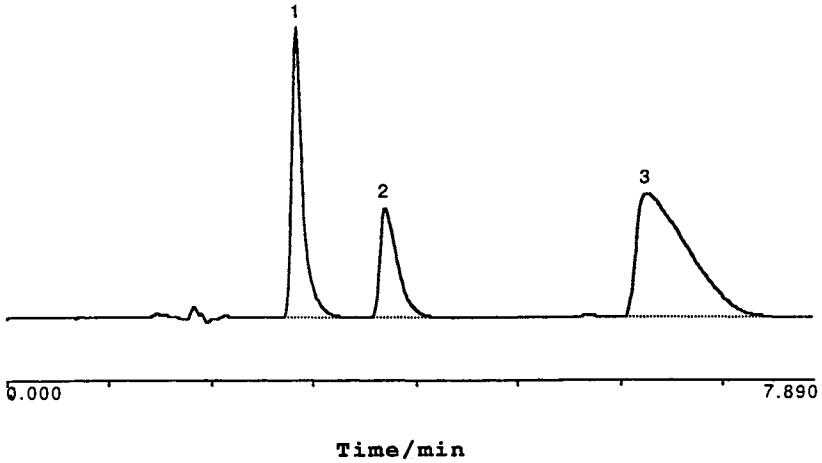

Fig. 1. Chromatogram of aspartame and its decomposition products after 20.0 min at pH 10.08 and 25.0°C in water. Peak 1, DKP; peak 2, AP; peak 3, aspartame.

nitrile. The flow rate was 2.0 mL/min. A typical chromatogram of aspartame and its degradates is shown in Fig. 1; details of the assay development, with chromatograms, have been reported elsewhere (13).

Kinetics Studies. The degradation of aspartame and phenylalanine methyl ester was studied over the pH range 0.27 to about 11.5 at 25.00 ± 0.05°C. The buffer concentrations used are listed in Table I. For pH values higher than 1.0, the ionic strength was adjusted to 0.100 *M* with sodium chloride. Samples were stored in a water bath and aliquots were removed at appropriate intervals for HPLC analysis. The pH was monitored during the reaction. If necessary, the pH was adjusted with dropwise addition of 1 *N* sodium hydroxide. Reactions having half-lives of less than 80 days were followed for at least one half-life.

The kinetics samples having pH values less than 2.23 had an initial analyte concentration of 25 m*M* and were diluted 1:5 in water prior to analysis; all other samples had an initial analyte concentration of 5 m*M* and required no sample preparation.

RESULTS AND DISCUSSION

pK_a Values. The apparent potentiometric pK_a values, measured at 0.100 *M* ionic strength and 25°C, were pK_{a1} 3.19 ± 0.01 and pK_{a2} 7.87 ± 0.02 for aspartame and pK_a 7.11 ± 0.02 for phenylalanine methyl ester. Presumably pK_{a1} describes the carboxylic acid ionization and pK_{a2} the dissociation of the amine group in aspartame.

Aspartame Kinetics. The kinetics of aspartame demethylation were studied in aqueous solution at 25°C over the pH range 0.27 to 11.43. Pseudo-first-order kinetics were observed, and the pseudo-first-order rate constants for loss of aspartame, k_{obs}, obtained by linear least-squares regression of semilogarithmic first-order plots, are listed in Table I. The reproducibility of k_{obs} is 2–4%. Since most of the buffer concentrations are low, no corrections for possible buffer catalysis were applied. Acetate buffers in the pH range 4.2–5.7 showed some growth of microorganisms during the duration of the reactions, so these studies were rejected.

The degradation kinetics and mechanisms of aspartame demethylation are affected by the ionization of the carboxylic acid and the terminal amine moieties on the aspartic acid residue. Below pH 3, aspartame exists mainly in the cationic form, designated A_{0+}, the first subscripted symbol denoting the charge state of the COOH group and the second of the NH_2 group; between pH 3 and 8, the predominant form is the zwitterion, A_{-+}; and above pH 8, A_{-0} predominates. Scheme II incorporates the likely, and kinetically distinct, reactions.

$$A_{0+} + H^+ \xrightarrow{k_1} \text{products}$$

$$A_{0+} \xrightarrow{k_2} \text{products}$$

$$A_{-+} \xrightarrow{k_3} \text{products}$$

$$A_{-0} \xrightarrow{k_4} \text{products}$$

$$A_{-0} + OH^- \xrightarrow{k_5} \text{products}$$

Scheme II

The rate expression for this kinetic scheme is

$$\text{rate} = k_{obs}[A] = k_1[A_{0+}][H^+] + k_2[A_{0+}] + k_3[A_{-+}] + k_4[A_{-0}] + k_5[A_{-0}][OH^-] \quad (1)$$

where [A] represents the total aspartame concentration at any time; that is,

$$[A] = [A_{0+}] + [A_{-+}] + [A_{-0}] \quad (2)$$

Combination of Eqs. (1) and (2) with the appropriate expres-

Table I. Kinetics Data for the Demethylation of Aspartame and Phenylalanine Methyl Ester

	Aspartame				Phenylalanine methyl ester	
Buffer	pH	$10^6 k_{obs}/sec^{-1}$	$10^6 k_{DKP}/sec^{-1}$	$10^4 k_{AP}/sec^{-1}$	pH	$10^6 k_{obs}/sec^{-1}$
1.000 *N* HCl	0.28	11.0	—	—	0.27	1.37
0.500 *N* HCl	0.59	4.92	—	—	0.59	0.636
0.250 *N* HCl	0.91	2.12	—	—	0.89	0.300
0.100 *N* HCl	1.33	0.713	—	—	1.27	0.159
0.0500 *N* HCl	1.82	0.252	—	—	1.53	0.0532
0.0300 *N* HCl	2.25	0.100	—	—	1.74	0.0407
0.0100 *N* HCl	—	—	—	—	2.21	0.0246
0.0100 *M* H_2P[a]	—	—	—	—	2.74	0.0388
0.00800 *M* H_2P, 0.00200 *M* KHP	—	—	—	—	2.90	0.0302
0.00600 *M* H_2P, 0.00400 *M* KHP	2.82	0.0766	—	—	3.12	0.0432
0.00400 *M* H_2P, 0.00600 *M* KHP	3.09	0.0617	—	—	3.34	0.0414
0.00200 *M* H_2P, 0.00800 *M* KHP	3.39	0.0529	—	—	—	—
0.00900 *M* HOAc,[b] 0.00100 *M* NaOAc	3.75	0.0272	—	—	3.75	0.0330
0.00800 *M* HOAc, 0.00200 *M* NaOAc	4.02	0.0232	—	—	4.14	0.0555
0.00700 *M* HOAc, 0.00300 *M* NaOAc	—	—	—	—	4.27	0.0583
0.00500 *M* HOAc, 0.00500 *M* NaOAc	—	—	—	—	4.65	0.0850
0.00300 *M* HOAc, 0.00700 *M* NaOAc	—	—	—	—	5.01	0.147
0.00200 *M* HOAc, 0.00800 *M* NaOAc	—	—	—	—	5.22	0.203
0.00100 *M* HOAc 0.00900 *M* NaOAc	—	—	—	—	5.54	0.389
0.0321 *M* NaH_2PO_4, 0.00357 *M* Na_2HPO_4	5.82	1.09	1.09	—	5.81	1.08
0.0286 *M* NaH_2PO_4, 0.00714 *M* Na_2HPO_4	6.19	2.40	2.40	—	6.17	2.06
0.0250 *M* NaH_2PO_4, 0.0107 *M* Na_2HPO_4	—	—	—	—	6.41	2.94
0.0179 *M* NaH_2PO_4, 0.0179 *M* Na_2HPO_4	6.78	7.64	7.64	—	6.76	5.01
0.0107 *M* NaH_2PO_4, 0.0250 *M* Na_2HPO_4	7.16	18.3	18.3	—	7.09	7.27
0.00714 *M* NaH_2PO_4, 0.0286 *M* Na_2HPO_4	7.38	39.7	39.7	—	—	—
0.00357 *M* NaH_2PO_4, 0.0321 *M* Na_2HPO_4	7.66	53.0	53.0	—	7.46	9.48
0.100 *M* NaCl[c]	—	—	—	—	7.49	9.80
0.100 *M* NaCl[c]	—	—	—	—	7.86	11.5
0.100 *M* NaCl[c]	—	—	—	—	8.25	13.3
0.100 *M* NaCl[c]	—	—	—	—	8.67	16.5
0.0175 *M* $NaHCO_3$, 0.00750 M Na_2CO_3	8.92	224	202	0.217	9.03	17.8
0.0150 *M* $NaHCO_3$, 0.0100 *M* Na_2CO_3	—	—	—	—	9.33	26.1
0.0125 *M* $NaHCO_3$, 0.0125 *M* Na_2CO_3	9.58	298	232	0.653	9.62	41.3
0.00750 *M* $NaHCO_3$, 0.0175 *M* Na_2CO_3	10.00	402	259	1.43	10.04	77.5
0.00500 *M* $NaHCO_3$, 0.0200 *M* Na_2CO_3	—	—	—	—	10.23	112

Table I. Continued

Buffer	Aspartame pH	Aspartame $10^6 k_{obs}/sec^{-1}$	Aspartame $10^6 k_{DKP}/sec^{-1}$	Aspartame $10^4 k_{AP}/sec^{-1}$	Phenylalanine methyl ester pH	Phenylalanine methyl ester $10^6 k_{obs}/sec^{-1}$
0.00250 *M* $NaHCO_3$, 0.0225 *M* Na_2CO_3	—	—	—	—	10.44	172
0.0123 *M* Na_2HPO_4, 0.00528 *M* Na_3PO_4	10.41	621	199	4.21	10.83	496
0.00880 *M* Na_2HPO_4, 0.00880 *M* Na_3PO_4	10.87	1240	224	10.1	11.08	970
0.00528 *M* Na_2HPO_4, 0.0123 *M* Na_3PO_4	11.21	2830	267	25.6	11.38	1770
0.00176 *M* Na_2HPO_4, 0.0158 *M* Na_3PO_4	11.43	4200	226	40.0	11.60	2600

[a] H_2P represents phthalic acid; KHP is potassium acid phthalate.
[b] HOAc represents acetic acid; NaOAc is sodium acetate.
[c] Sodium hydroxide was added to adjust pH.

sions (14) for the fractions $[A_{0+}]/[A]$, $[A_{-+}]/[A]$, and $[A_{-0}]/[A]$ gives

$$k_{obs} = \frac{k_1[H^+]^3 + k_2[H^+]^2 + k_3K_{a1}[H^+] + k_4K_{a1}K_{a2} + k_5K_{a1}K_{a2}K_w/[H^+]}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (3)$$

The constants k_1, k_2, k_3, k_4, and k_5 in Scheme II are found by fitting k_{obs} from Table I to Eq. (3). In the pH range 0.28–1.5, Eq. (3) simplifies to

$$k_{obs} = k_1[H^+] \quad (4)$$

allowing k_1 to be estimated. The slope of the plot of log k_{obs} versus pH is -1.0 at low pH, consistent with the assumption of a reaction that is first order in hydrogen ion. At very high pH, Eq. (3) simplifies to

$$k_{obs} = k_5[OH^-] \quad (5)$$

and k_5 is obtained from the data at high pH. Figure 2 is a plot of log k_{obs} against pH, showing these extreme sections of the curve controlled by k_1 and k_5. The values estimated for k_1 and k_5 are 2.05×10^{-5} and 1.50 $sec^{-1}\ M^{-1}$, respectively.

In the pH range 5.82–11.43,

$$k_{obs} = k_{DKP} + k_{AP} \quad (6)$$

where k_{DKP} and k_{AP} are the *base-promoted* formation rate constants of DKP and AP, respectively. Since the ratio of the product concentrations is equal to the ratio of the formation rate constants at any time during the reaction (15), Eq. (7),

$$\frac{[AP]}{[DKP]} = \frac{k_{AP}}{k_{DKP}} \quad (7)$$

k_{DKP} and k_{AP} can be calculated; these values are listed in Table I. In our work, the concentration ratio was measured at "infinite" time. [Gaines and Bada (6) claim that AP and DKP are in equilibrium at 100°C, but we did not observe this at 25°C.]

In the pH range 5.8–9.6, where the free amino group of A_{-0} is available for cyclization but the solution hydroxide concentration is not high, diketopiperazine formation is the dominant degradation route. Figure 3 is a plot of log k_{DKP} and log k_{AP} against pH. As expected, the rate of DKP formation increases with pH until essentially all of the aspartame is in the A_{-0} form, when the rate becomes independent of pH. The rate of AP formation, on the other hand, being

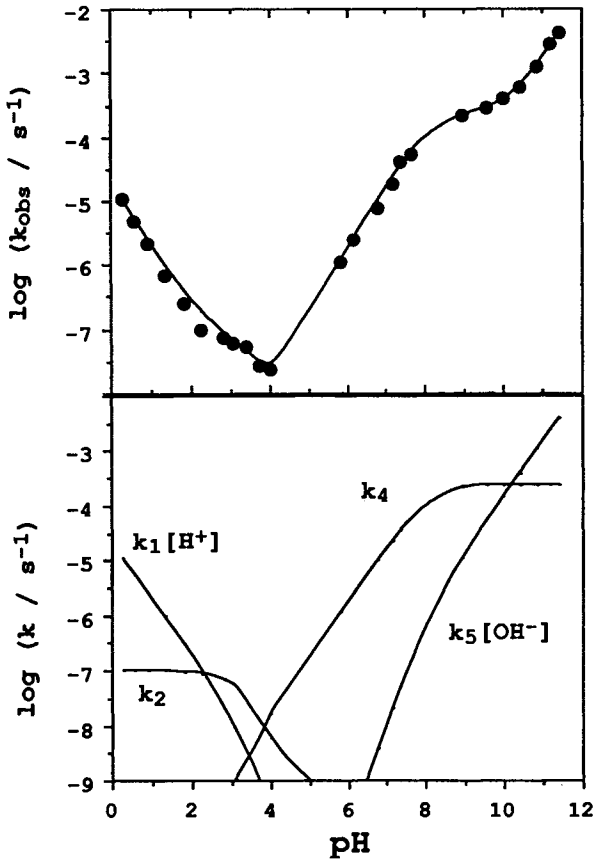

Fig. 2. Top: pH–rate profile for the decomposition of aspartame at 25°C in aqueous solution. The solid line is drawn with Eq. (3) and the parameter values are listed in the text. Bottom: Contributions of the k_1, k_2, k_4, and k_5 steps in Scheme II to k_{obs}. The sum of the contributions gives the solid line in the top panel.

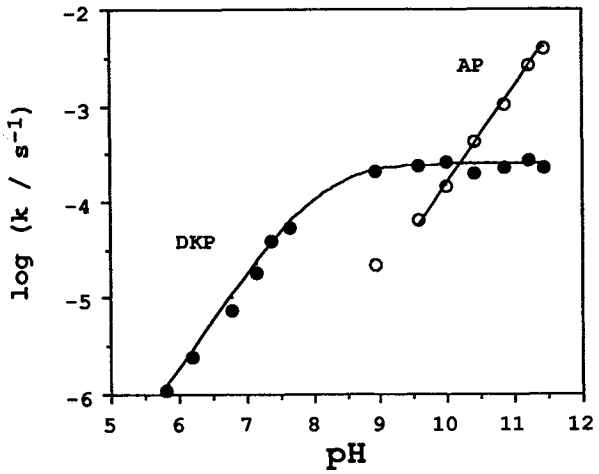

Fig. 3. Plot of k_{AP} and k_{DKP}, the pH-dependent rate constants for dipeptide and diketopiperazine formation, respectively, against pH, for the demethylation of aspartame.

catalyzed by the hydroxide ion, increases with pH until this rate finally dominates at high pH. The rates of formation of AP and DKP are equal at pH 10.2.

From the plateau value of k_{DKP} the intrinsic constant k_{DKP} is estimated to be 2.43×10^{-4} sec^{-1}; this quantity can be identified as k_4 in Scheme II. From the relationship $k_{DKP} = F_{-0}\, k_{DKP}$, we get Eq. (8):

$$k_{DKP} = k_{DKP}\, K_{a2}/([H^+] + K_{a2}) \qquad (8)$$

which, in rearranged form, shows that a plot of k_{DKP}/k_{DKP} versus $[H^+]$ should yield a straight line with a slope equal to $1/K_{a2}$. From this plot a kinetically determined value of 8.14 was found for pK_{a2}, which is in fair agreement with the potentiometric estimate of 7.87. The kinetic estimate for K_{a2} was used in fitting the pH–rate profile because the fit was less satisfactory when the potentiometric estimate was used.

With the estimates of k_1, k_4, k_5, K_{a1}, and K_{a2} available, only k_2 and k_3 remain as unknowns in Eq. (3). Moreover, the shape of the pH–rate curve in the pH region 2–4 suggests that k_2 is greater than k_3. Therefore k_3 was provisionally set to zero, and the data were fitted to Eq. (3) with k_2 as the sole unknown. A value of 1×10^{-7} sec^{-1} was determined for k_2. The resultant curve-fit is satisfactory, and the solid line in Fig. 2 is drawn with Eq. (3) and these parameter values: 2.05×10^{-5} sec^{-1} M^{-1} for k_1, 1×10^{-7} sec^{-1} for k_2, 0 for k_3, 2.43×10^{-4} sec^{-1} for k_4, 1.50 sec^{-1} M^{-1} for k_5, 6.46×10^{-4} M for K_{a1}, and 7.24×10^{-9} M for K_{a2}. Thus at pH values below 4, acid-catalyzed hydrolysis (k_1) to give the dipeptide is the major degradation pathway, with a small contribution from an uncatalyzed hydrolysis term (k_2). In the pH region 4–9, diketopiperazine formation dominates, through the k_4 term. Above pH 9, aspartame degrades via parallel reaction pathways to form the diketopiperazine and the dipetide. The base-catalyzed hydrolysis dominates above pH 11, through the k_5 term. Figure 2 also shows the separate contributions made to k_{obs} by the several reactions in Scheme II. Aspartame is maximally stable at pH 4, having a half-life of 346 days and a shelf life (t_{90}) of 53 days at 25°C.

Phenylalanine Methyl Ester Kinetics. The kinetics of hydrolysis of phenylalanine methyl ester were studied at 25°C over the pH range 0.27 to 11.60. Pseudo-first-order kinetics were observed, and the rate constants are given in Table I. Figure 4 shows the pH–rate profile. The kinetic results are described in Scheme III, where P_+ represents the protonated reactant and P_0 the neutral form.

$$P_+ + H^+ \xrightarrow{k_1} \text{products}$$

$$P_+ \xrightarrow{k_+} \text{products}$$

$$P_0 \xrightarrow{k_0} \text{products}$$

$$P_0 + OH^- \xrightarrow{k_{OH}} \text{products}$$

Scheme III

The rate expression for Scheme III is

$$\text{rate} = k_{obs}[P] = k_H[P_+]\,[H+] + k_+[P_+] + k_0[P_0] + k_{OH}[P_0][OH^-] \qquad (9)$$

where [P] represents the total reactant concentration, so $[P] = [P_+] + [P_0]$. Equation (9) yields Eq. (10) as the relationship between k_{obs} and the hydrogen ion concentration.

$$k_{obs} = \frac{k_H[H^+]^2 + k_+[H^+] + k_0K_a + k_{OH}K_aK_w/[H^+]}{[H^+] + K_a} \qquad (10)$$

Using $K_a = 7.82 \times 10^{-8}$ (from the potentiometrically determined pK_a value), the rate parameters of Eq. (10) were evaluated by fitting the k_{obs} values of Table I to Eq. (10) by nonlinear regression (16). These values were obtained:

$$k_H = 2.7 \times 10^{-6} \text{ sec}^{-1}\, M^{-1}$$
$$k_+ = 3 \times 10^{-8} \text{ sec}^{-1}$$
$$k_0 = 1.5 \times 10^{-5} \text{ sec}^{-1}$$
$$k_{OH} = 0.74 \text{ sec}^{-1}\, M^{-1}$$

The smooth curve in Fig. 4 was calculated with Eq. (10) and these values. Figure 4 also shows the contributions of the separate terms to the observed rate constant.

Comparison of Rate Constants. Table II lists the rate constants for analogous rate terms in the reactions of aspartame (A) and phenylalanine methyl ester (P) as found in this work. The descriptions of these reactions in Table II relate to Schemes II and III and require amplification.

The specific acid-catalyzed reaction is a bimolecular process, probably a nucleophilic attack by water on the protonated ester group. The catalytic protonation occurs (for both A and P) on a molecule that is already carrying a pos-

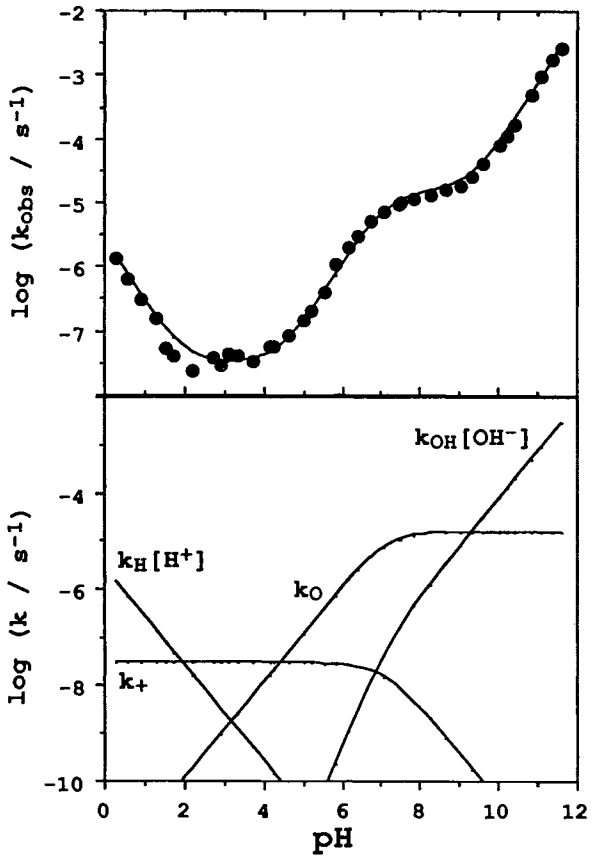

Fig. 4. Top: pH–rate profile for the decomposition of L-phenylalanine methyl ester at 25°C in aqueous solution. The solid line is drawn with Eq. (10) and the parameter values are listed in the text. Bottom: Contributions of the k_H, k_+, k_0, and k_{OH} steps in Scheme III to k_{obs}. The sum of the contributions gives the solid line in the top panel.

itive charge. The greater reactivity of A relative to P is then explicable in terms of the greater distance between the protonated amine and the ester group in A.

The reaction described as the uncatalyzed hydrolysis of the RNH_3^+ species has a rate term of the form $[RNH_3^+][H_2O]$, which is kinetically equivalent to $[RNH_2][H_3O^+]$, this second form being a specific acid-catalyzed reaction of the neutral reactant. The latter mechanism is probably more likely; however, a firm basis for preferring one of these forms to the other does not exist. A reacts slightly more rapidly than does P by this route.

The third entry in Table II is the most disparate one in this comparison. For A, this route constitutes diketopiperazine formation, which is a result of intramolecular nucleophilic attack by the amino group on the ester function. For P, however, this route constitutes ester hydrolysis, and the rate term $[RNH_2][H_2O]$ is kinetically equivalent to $[RNH_3^+][OH^-]$, which seems a more probable form on electrostatic grounds. Obviously, P does not constitute a model for A via this route, since the two reactants undergo different reactions in this region.

A reacts slightly faster than does P via specific base-catalyzed ester hydrolysis, despite the negative charge on the A molecule in this pH region. For comparison, the corresponding rate constant for the specific base-catalyzed ester hydrolysis of methyl acetate is 0.23 $sec^{-1} M^{-1}$ (17). The significantly greater susceptibility to alkaline hydrolysis of the two amino acid esters is of practical interest and suggests further studies on related compounds.

Table II. Rate Constants for Comparable Terms in Reactions of Aspartame (A) and Phenylalanine Methyl Ester (P) at 25°C

Reaction	Rate constant[a] A	Rate constant[a] P	k_A/k_P
H^+ catalysis of ester hydrolysis	2.05×10^{-5}	2.7×10^{-6}	7.6
Uncatalyzed hydrolysis of RNH_3^+ species	1×10^{-7}	3×10^{-8}	3.3
Uncatalyzed reaction of RNH_2 species	2.43×10^{-4}	1.5×10^{-5}	16.2
OH^- catalysis of ester hydrolysis	1.50	0.74	2.0

[a] See text for units.

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