Solvent Effects on Chemical Processes. 8. Demethylation Kinetics of Aspartame in Binary Aqueous–Organic Solvents

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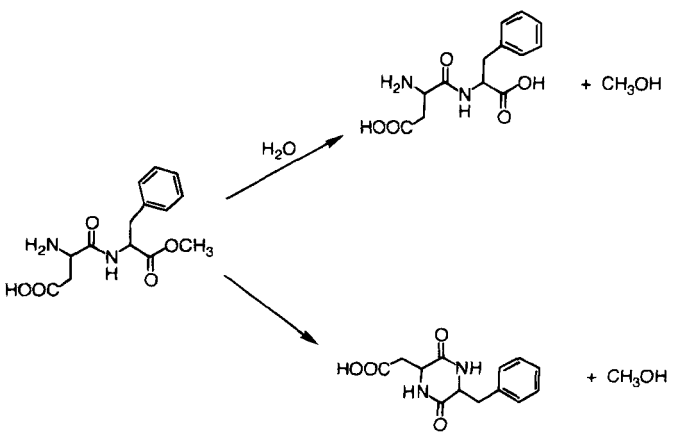
Abstract □ The kinetics of demethylation of aspartame were studied in binary aqueous–organic solvent mixtures at 25 °C under two solution conditions, namely 1.0 M HCl (pH 0.28 in water) and carbonate buffer (pH 10.1 in water). Under these conditions solvent effects on the acid dissociation constants of aspartame do not complicate the interpretation of the kinetics. The organic cosolvents were acetone, acetonitrile, dimethyl sulfoxide, dioxane, tetrahydrofuran, and methanol. The observed kinetic solvent effects were modest in magnitude, not exceeding a factor of 3 in rate constant, relative to the fully aqueous solution. The rate changes included both increases and decreases, and in some solvent mixtures extrema were observed. It is concluded that at least two contributory factors, identified as an electrostatic (dielectric constant) effect and a solvation effect, must be operating to produce the observed kinetic solvent effects.

Introduction

Earlier papers in this series described our phenomenological theory of solvent effects and its application to solubility,[1–3] surface tension,[4] molecular complex formation,[5,6] and absorption spectra.[7] The extension to reaction rates constitutes a more difficult task, which we begin in the present paper by describing our experimental findings on solvent effects in the kinetics of demethylation of the artificial sweetener aspartame. A secondary purpose of this work is to clarify some results and interpretations recently reported by other workers, in this journal, on the effects of organic solvents on rates of aspartame decomposition.[8,9]

We chose to study aspartame degradation because this important compound is a peptide, because of the practical matter that its maximum shelf-life (t_{90}) at 25 °C in aqueous solution is only 53 days,[10] and because one of its decomposition reactions, diketopiperazine formation, is also an important degradation pathway for proteins and peptides,[11,12] some cephalosporins,[13–16] cycloserine,[17–19] and some ACE inhibitors.[20,21] As a preliminary to the present work we carried out a study of the kinetics of aspartame demethylation in fully aqueous medium.[10] Aspartame can undergo intermolecular acid-catalyzed and base-catalyzed ester hydrolysis and intramolecular aminolysis, this last reaction yielding a diketopiperazine product; the overall reactions are shown in Scheme 1. The aqueous pK_a values of aspartame[10] at 25 °C are pK_1 = 3.2 (COOH) and pK_2 = 8.0 (NH_2), so below pH 3 aspartame exists mainly as the cation A_{0+}, the first subscript denoting the charge on the COOH group and the second symbol the charge on the NH_2 group. Between pH 3 and 8 the predominant form is the zwitterion A_{-+}, and above pH 8 the anion A_{-0} predominates.

The kinetics of aspartame demethylation were described by rate equation 1 and the rate constant values $k_1 = 2.05 \times 10^{-5}$ M^{-1} s^{-1}, $k_2 = 1 \times 10^{-7}$ s^{-1}, $k_3 = 0$, $k_4 = 2.43 \times 10^{-4}$ s^{-1}, $k_5 =$ 1.50 M^{-1} s^{-1}. Figure 1 shows the contribution of the individual rate terms as a function of pH. These results provide a sound basis for the design of experiments into the effect of solvent composition on the kinetics of aspartame demethylation. Clearly it is desirable to be able to assign the observed effects to the individual rate constants of eq 1 and Figure 1. Although practical considerations might motivate a study in the pH region 2–5, where aspartame is maximally stable, Figure 1 shows that the results of such a study would be difficult to interpret, with the three quantities k_1, k_2, and k_4 making contributions to the observed rate in this portion of the pH scale. For example, Yalkowski et al.[9] reported that the rate of degradation in a binary aqueous–organic medium decreases with respect to that in a fully aqueous solution at pH 2; whereas Sanyude et al.[8] describe the opposite behavior at pH 4.5. Both of these groups accounted for their observations on the basis of classical electrostatic arguments. We have chosen to study the effect of binary aqueous–organic solvents on the aspartame kinetics at pH 0.28, where the k_1 term demonates the kinetics, and at pH 10.1, where the k_4 and k_5 terms both contribute to the rate.



Scheme 1—Demethylation of aspartame by ester hydrolysis (upper route) and diketopiperazine formation.

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

Experimental Section

Materials and Methods—Aspartame was from Sigma Chemical (St. Louis, MO), and L-α-aspartylphenylalanine was purchased from Bachem Feinchemikalien (Bubendorf, Switzerland). The organic solvents, which were obtained from EM Science (Gibbstown, NJ), were of HPLC grade.

Kinetic Studies—Binary aqueous–organic solvents were prepared both gravimetrically and volumetrically, so their compositions were known on both a weight basis (weight percent, mole fraction) and a volume basis (postmixing volume percent, molarity). All kinetics were

® Abstract published in *Advance ACS Abstracts*, November 1, 1994.

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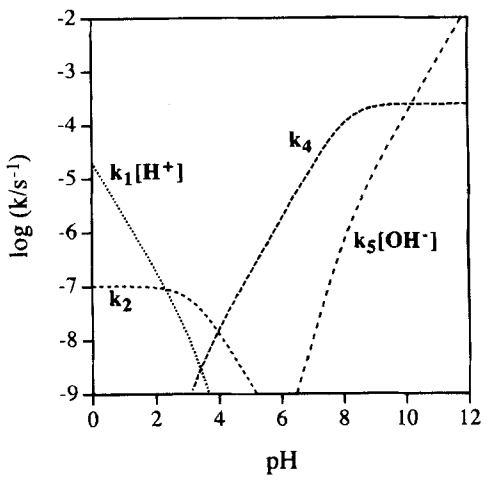


Figure 1—Contributions of the individual rate terms of eq 1 to the observed rate constant for aspartame demethylation.[10]

carried out at 25.0 °C and were followed by HPLC analysis. The assay development and analytical details have been described.[8,11]

In the acid-catalyzed studies the medium was 1.0 M HCl; the measured pH in the fully aqueous medium was 0.28. The initial aspartame concentration was 25 mM. The kinetic samples were diluted 1:5 with water prior to HPLC analysis. After determining that first-order kinetics are obeyed for four half-lives, reactions were followed for one half-life. For the studies in the basic region, the buffer consisted of 0.0025 M sodium bicarbonate, 0.0225 M sodium carbonate, and 0.0300 M sodium chloride; the ionic strength was 0.100 M and the pH in the fully aqueous medium was 10.1. The apparent pH was monitored during reaction, and if it changed, it was brought back to the initial value with the dropwise addition of 1 M sodium hydroxide; the resulting changes in volume and ionic strength were negligible. In these basic solutions the initial reactant concentration was 5 mM. Reaction samples were quenched prior to analysis by 1:2 dilution in 0.1 M HCl. These reactions were followed for one to two half-lives.

Data Analysis—The observed rate constant k_{obs} was extracted from the analytical data by nonlinear regression (using the SYSTAT program) of eq 2,

$$\text{Percent reactant remaining} = \alpha e^{-k_{obs}t} \qquad (2)$$

where the pre-exponential factor α is expected to be 100.0. The uncertainty in k_{obs} is the standard deviation within a single run.

Results and Discussion

Solvent Effects on pK_a and pH—The present paper is not the place for an extended review of this important subject, but a few remarks are appropriate. General surveys have been given by Bates[22] and Perrin and Dempsey.[23]

The ionization of a neutral acid involves charge separation, so the solvent effect on the ionization constant is expected to be dominated by the electrostatic contribution. Indeed, it is observed that the pK_as of carboxylic acids undergo very substantial increases (corresponding to acid weakening) when organic solvents are incorporated into aqueous solutions.[24-27] In the present work on the acid-catalyzed hydrolysis of aspartame, the pH (aqueous) was 0.28, about 3 units to the acid side of the pK_1 (COOH) of aspartame. Since pK_1 will increase as organic solvents are added, we can be confident that in all of these solutions the aspartame remains in substantially the A_{o+} form.

Dissociation of a cationic acid such as a protonated amine does not constitute charge separation, so the electrostatic influence should be minor, and specific solvation effects may dominate the solvent effect. Experimentally it is found[24] that pK_a values for some amines undergo modest decreases as

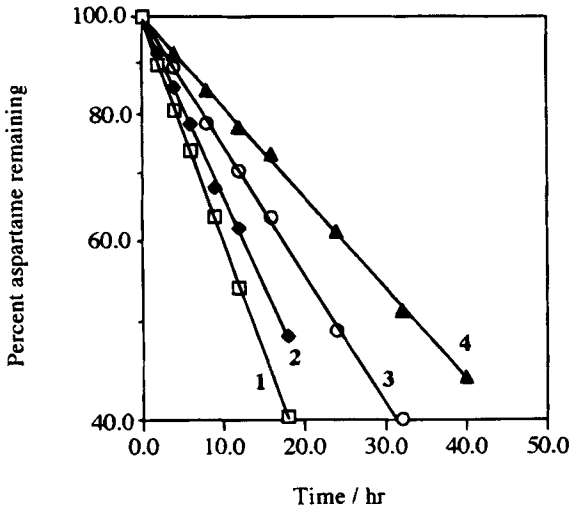


Figure 2—Representative first-order plots for the acid-catalyzed hydrolysis of aspartame in acetone–water solvents. Key: 1, $x_2 = 0.000$; 2, $x_2 = 0.0558$; 3, $x_2 = 0.1327$; 4, $x_2 = 0.2466$.

organic solvents are added. For example, the pK_a of aniline changes from 4.62 in water to 4.33 in 40 vol % methanol/water.

Of course, it is not only pK_a that may experience a solvent effect—the pH itself may change, both through the role of the autoprotolysis constant, which is solvent dependent,[28-30] and the role of the pK_a of the buffer acid. The effect is apparently that pH always increases with increasing organic solvent concentration.[28,31-33] The position of an acid–base equilibrium is controlled both by pK_a and pH, and the shifts that have been described above indicate strongly that, in the basic solutions used in the present work, the amino group was substantially in the conjugate base form in all solutions. That is, in the fully aqueous system pH is 2 units higher than the amine pK_a, and this difference is expected to increase with organic solvent composition.

Solvent Effects in Acid Solution—The analytical data in all instances satisfied the first-order rate equation (eq 2); the pre-exponential factor α was 100.0 within the limits of experimental uncertainty. Figure 2 shows some typical first-oder plots. The precision of k_{obs} in a single run was 1–2%. Table 1 lists rate constants and solvent compositions for the acidic conditions.

The solvent effect on a reaction rate, $\delta_M\Delta G^{\ddagger}$, is defined[34] by eq 3,where k_B is Boltzmann's constant, T is the absolute

$$\delta_M\Delta G^{\ddagger} = -k_B T \ln \frac{k(x_2)}{k(x_2=0)} \qquad (3)$$

temperature, and x_2 is the mole fraction of organic cosolvent in the reaction medium. That is, the solvent effect at any cosolvent concentration is referred to the fully aqueous system ($x_2 = 0$). In subsequent figures the term "solvent effect" signifies the dimensionless quantity $\delta_M\Delta G^{\ddagger}/k_BT$. A change of 0.7 in this quantity corresponds to a factor of 2 change in the rate constant.

Figure 3 is a plot of the solvent effect on k_{obs} in the acidic solutions, the organic cosolvents being acetone, acetonitrile, dimethyl sulfoxide (DMSO), 1,4-dioxane, and tetrahydrofuran (THF). In this figure the solvent effect is plotted as a function of the volume fraction of cosolvent; a plot against mole fraction is similar in appearance. Two features of Figure 3 are obvious: (1) the magnitudes of the solvent effects are modest; no rate constant ratio exceeds a factor of 3; (2) the solvents exhibit a considerable dispersion in their solvent effect values, and they even differ in the functional forms of the dependence

Table 1—Kinetic Data for the Acid-Catalyzed Hydrolysis of Aspartame at 25 °C in 1.0 M HCl[a]

Φ_2[b]	x_2	$10^2k_{obs}/h^{-1}$	$10^4k_H/M^{-1}\,h^{-1}$	Φ_2[b]	x_2	$10^2k_{obs}/h^{-1}$	$10^4k_H/M^{-1}\,h^{-1}$
			Acetone				
0.00	0.0	5.05(0.05)	9.09	0.35	0.1102	3.29(0.06)	8.62
0.05	0.0124	5.07(0.06)	9.47	0.40	0.1327	2.92(0.03)	8.29
0.10	0.0258	4.73(0.04)	9.29	0.45	0.1557	2.50(0.07)	7.58
0.15	0.0404	4.20(0.06)	8.70	0.50	0.1823	2.33(0.04)	7.69
0.20	0.0558	4.07(0.07)	8.90	0.55	0.2126	2.27(0.02)	8.24
0.25	0.0726	3.30(0.03)	7.64	0.60	0.2466	2.05(0.02)	8.30
0.30	0.0902	3.43(0.05)	8.40				
			Acetonitrile				
0.00	0	4.83(0.05)	8.69	0.35	0.1480	2.01(0.02)	5.39
0.05	0.0173	4.38(0.01)	8.26	0.40	0.1715	1.89(0.02)	5.43
0.10	0.0357	3.86(0.04)	7.66	0.45	0.2069	1.83(0.03)	5.75
0.15	0.0553	3.49(0.04)	7.30	0.50	0.2400	1.81(0.05)	6.21
0.20	0.0763	2.91(0.02)	6.45	0.55	0.2775	1.84(0.03)	7.00
0.25	0.0986	2.55(0.02)	5.98	0.60	0.3174	1.85(0.03)	7.78
0.30	0.1221	2.28(0.02)	5.71				
			Dimethyl Sulfoxide				
0	0	4.83(0.03)	8.69	0.35	0.1165	4.07(0.03)	11.16
0.05	0.0129	4.79(0.03)	9.05	0.40	0.1397	3.85(0.01)	11.41
0.10	0.0267	4.85(0.03)	9.63	0.45	0.1655	3.54(0.02)	11.37
0.15	0.0419	4.75(0.06)	9.98	0.50	0.1944	3.08(0.03)	10.85
0.20	0.0577	4.60(0.02)	10.21	0.55	0.2275	2.65(0.03)	10.36
0.25	0.0754	4.45(0.04)	10.51	0.60	0.2641	2.22(0.01)	9.75
0.30	0.0942	4.30(0.02)	10.90				
			Dioxane				
0	0	4.92(0.04)	8.85	0.35	0.0970	3.12(0.03)	8.45
0.05	0.0107	4.66(0.04)	8.80	0.40	0.1182	2.82(0.01)	8.27
0.10	0.0223	4.51(0.04)	8.94	0.45	0.1405	2.56(0.03)	8.14
0.15	0.0350	4.24(0.03)	8.90	0.50	0.1660	2.38(0.02)	8.32
0.20	0.0485	4.07(0.05)	9.03	0.55	0.1943	2.24(0.01)	8.61
0.25	0.0635	3.66(0.03)	8.65	0.60	0.2273	2.15(0.02)	9.28
0.30	0.0799	3.33(0.02)	8.40				
			Tetrahydrofuran				
0	0	4.85(0.07)	8.73	0.35	0.1012	2.36(0.04)	6.28
0.05	0.0112	4.55(0.06)	8.60	0.40	0.1216	2.14(0.03)	6.16
0.10	0.0235	4.27(0.02)	8.48	0.45	0.1444	1.96(0.01)	6.10
0.15	0.0367	3.83(0.05)	8.03	0.50	0.1706	1.82(0.01)	6.22
0.20	0.0509	3.45(0.14)	7.67	0.55	0.2033	1.71(0.01)	6.61
0.25	0.0664	3.09(0.03)	7.28	0.60	0.2327	1.82(0.01)	7.68
0.30	0.0828	2.68(0.01)	6.67				

[a] Quantities in parentheses are standard deviations. [b] Postmixing volume fraction of organic cosolvent.

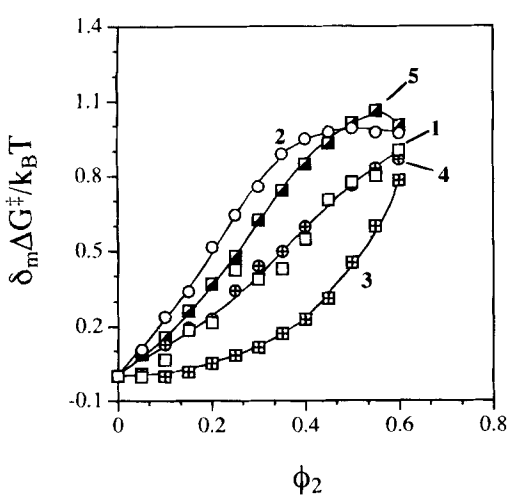


Figure 3—Solvent effect on the first-order rate constant for acid-catalyzed hydrolysis of aspartame as a function of volume fraction of organic cosolvent. Key: 1, acetone; 2, acetonitrile; 3, DMSO; 4, dioxane; 5, THF.

of solvent effect on solvent composition. (In this and later figures, the lines have no theoretical basis but are drawn to indicate experimental trends.)

The rate constant k_{obs}, on which Figure 3 is based, includes the concentration of the reactant water, which of course varies in these studies. We account for this change by converting the apparent first-order rate constant k_{obs} to a second-order rate constant k_H by eq 4,where c_1 is the molar concentration

$$k_H = \frac{k_{obs}}{c_1} \tag{4}$$

of water. The solvent effect on k_H, the second-order rate constant calculated with eq 4, is shown in Figure 4. The comments concerning Figure 3 apply also to Figure 4, with even greater dispersion being seen; moreover, several of the curves exhibit extrema. (It is appropriate to mention here that the operation described by eq 4 is supported by experimental work,[35,36] though such studies are complicated by the possible concurrent presence of a medium effect.)

The conventional interpretation of kinetic solvent effects makes use of electrostatic arguments, with the dielectric constant of the medium being the controlling variable; thorough treatments of this approach are available.[37,38] The reaction taking place in the present work, in 1.0 N HCl, is the acid-catalyzed hydrolysis of protonated aspartame, species A_{o+} in the earlier symbolism. A rapid pre-equilibrium consisting of the protonation of A_{o+} occurs, followed by water attack

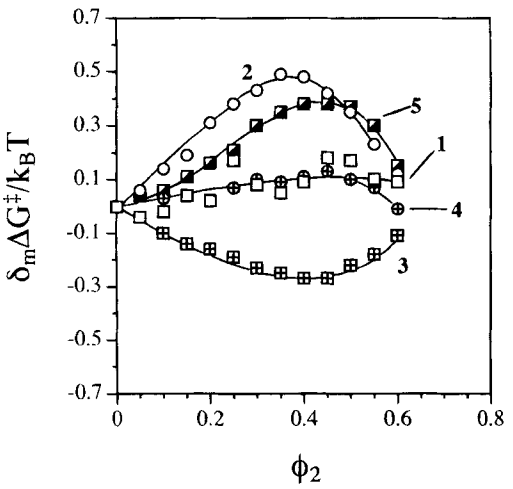


Figure 4—Solvent effect on the second-order rate constant for acid-catalyzed hydrolysis of aspartame; for key to solvents see Figure 3.

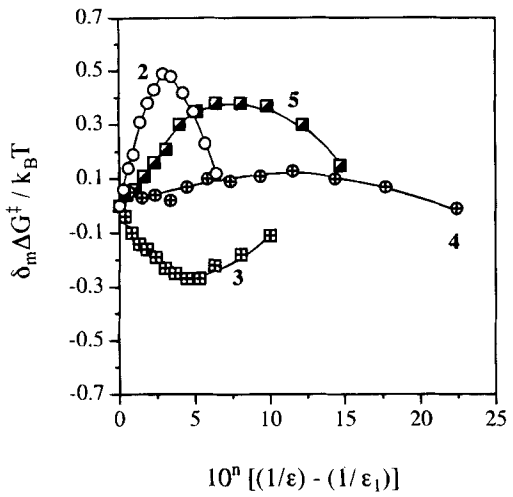


Figure 5—Solvent effect on the second-order rate constant for acid-catalyzed hydrolysis of aspartame as a function of the reciprocal of the dielectric constant, ϵ, of the medium; for key to solvents, see Figure 3; ϵ_1 is the dielectric constant of water. The parameter n is 3 except for DMSO, for which n is 4.

to form a tetrahedral intermediate.[39] With the decrease in dielectric constant produced as the concentration of organic cosolvent increases, the pre-equilibrium will be shifted to favor the species A_{o+}, thus having the indirect effect of slowing the reaction; the rate-determining process, however, is not expected to be very sensitive to the dielectric constant, because the initial state and transition state have similar charge distributions.

Now, the dielectric constant is a decreasing monotonic function of cosolvent concentration in all of these solvent mixtures. Electrostatic theories predict a linear dependence of solvent effect with the reciprocal of dielectric constant, the details of the case depending upon the dipole moments of initial and transition states. Such theories lead to the expectation that, for a given reaction, all organic solvents will yield the same rate effects when the dielectric constant is taken as the independent variable. Figure 5 is a plot of solvent effect on k_H against the reciprocal of the dielectric constant. (Dielectric constants were taken from literature sources.[40–45]) It is clear that the dielectric constant cannot be the sole, or even the dominant, property controlling the solvent effect, because of the disparate behavior and extrema seen in this plot. We are therefore led to postulate a second effect, one that is dependent upon the identity of the cosolvent; a specific solvation effect can reasonably be suggested as the cause of the considerable dispersion seen in Figures 3–5.

Solvent Effects in Basic Solution—The observed rate constant in the basic region can be decomposed into rate constants k_{AP} and k_{DKP}, which respectively describe the intermolecular base-promoted ester hydrolysis, to give the dipeptide L-α-aspartyl-L-phenylalanine (AP), and the intramolecular cyclization, which yields 3-(carboxymethyl)-6-benzyl-2,5-diketopiperazine (DKP). These rate constants are obtained from k_{obs} by solving eqs 5 and 6, which describe this

$$k_{obs} = k_{DKP} + k_{AP} \tag{5}$$

$$\frac{k_{AP}}{k_{DKP}} = \frac{[AP]_\infty}{[DKP]_\infty} \tag{6}$$

set of parallel reactions.[46] The concentration of AP at "infinity" time was obtained from the HPLC analysis, and $[DKP]_\infty$ was then calculated from the mass balance relationship $[aspartame]_o = [AP]_\infty + [DKP]_\infty$. Table 2 lists values of k_{obs}, k_{AP}, and k_{DKP} for each of the solvent systems. Figure 6 displays the solvent effect on the observed first-order rate constant in the carbonate buffer solutions. (The organic cosolvent concentration was extended nearly to the point at which the carbonate buffer precipitated in these studies.) Although the observed solvent effects are small, as in the acid region, the key features of the results are very clearcut: the several cosolvents generate widely different solvent effect profiles.

In Table 2, values of k_{DKP} and k_{AP}, derived from k_{obs} by means of eqs 5 and 6, are tabulated. We now make the connection between k_{DKP} and k_{AP} and the quantities in eq 1. From eq 1, in the basic region where A_{-o} is essentially the only aspartame species present, we can write eq 7,where [A]

$$k_{obs}[A] = k_4[A_{-o}] + k_5[A_{-o}][OH^-] \tag{7}$$

$$k_{obs} = k_4F_{-o} + k_5F_{-o}[OH^-] \tag{8}$$

represents total aspartame concentration. Eq. (8) is an equivalent form, where $F_{-o} = [A_{-o}]/[A]$ is the fraction of aspartame in the A_{-o} form. It is easy to show the relationship $F_{-o} = K_2/(K_2 + [H^+])$. Now, as we have seen, in these basic solutions pH $\gg pK_2$, so $[H^+] \ll K_2$, and $F_{-o} = 1$, a condition we used in writing eq 7. Thus eq 8 becomes

$$k_{obs} = k_4 + k_5[OH^-] \tag{9}$$

Comparing eq 9 with eq 5 and recalling that AP formation is a hydroxide-promoted reaction allow these identities to be written:

$$k_{DKP} = k_4 \tag{10}$$

$$k_{AP} = k_5[OH^-] \tag{11}$$

Over a wider pH region where A_{-o} is not the sole form of aspartame, this description must be generalized,[10] but in the present circumstances eqs 10 and 11 serve to relate the experimental constants k_{DKP} and k_{AP} to the parameters of the rate equation. The term $k_4[A_{-o}]$ may also be written in the kinetically equivalent form $k_4'[A_{-+}][OH^-]$, implying ester hydrolysis via this route. However, our earlier study[10] shows that only DKP formation occurs in the pH region dominated by the k_4 term.

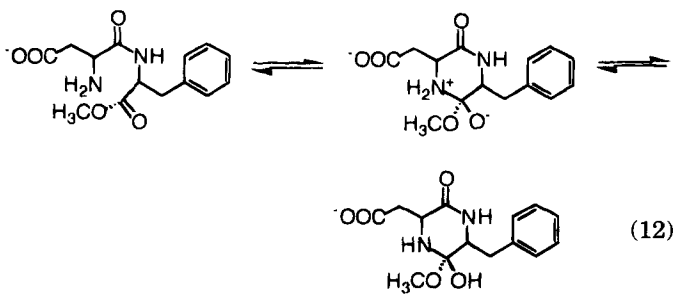
Diketopiperazine formation occurs by intramolecular nucleophilic attack of the amino group on the ester carboxyl

Table 2—Kinetic Data for Aspartame Demethylation at 25 °C in Carbonate Buffer[a,b]

Φ_2[c]	x_2	10^2k_{obs}/min^{-1}	10^2k_{AP}/min^{-1}	10^2k_{DKP}/min^{-1}	Φ_2[c]	x_2	10^2k_{obs}/min^{-1}	10^2k_{AP}/min^{-1}	10^2k_{DKP}/min^{-1}
				Acetone					
0	0	2.43(0.07)	0.85	1.58	0.30	0.0910	3.14(0.02)	0.65	2.49
0.05	0.0125	2.63(0.05)	0.76	1.87	0.35	0.1110	3.29(0.07)	0.64	2.64
0.10	0.0260	2.89(0.05)	0.75	2.14	0.40	0.1328	3.23(0.02)	0.63	2.61
0.15	0.0406	3.04(0.08)	0.64	2.40	0.45	0.1565	3.45(0.02)	0.57	2.88
0.20	0.0543	3.00(0.04)	0.65	2.35	0.55	0.2124	3.36(0.02)	0.48	2.89
0.25	0.0729	3.31(0.06)	0.75	2.56					
				Acetonitrile					
0	0	3.09(0.05)	1.05	2.04	0.35	0.1496	1.76(0.01)	0.55	1.22
0.05	0.0175	2.83(0.06)	0.98	1.85	0.40	0.1796	1.73(0.02)	0.54	1.19
0.10	0.0359	2.87(0.03)	1.07	1.80	0.45	0.2116	1.47(0.01)	0.42	1.05
0.15	0.0558	2.47(0.01)	0.89	1.58	0.50	0.2462	1.68(0.01)	0.40	1.28
0.20	0.0762	2.13(0.03)	0.75	1.38	0.55	0.2845	1.57(0.02)	0.34	1.24
0.25	0.0998	2.07(0.03)	0.73	1.35	0.60	0.3264	1.85(0.05)	0.34	1.51
0.30	0.1240	1.88(0.02)	0.62	1.27	0.65	0.3764	1.98(0.01)	0.32	1.65
				Dimethyl Sulfoxide					
0	0	2.68(0.03)	0.91	1.77	0.30	0.0953	4.49(0.70)	1.90	2.60
0.05	0.0131	2.88(0.10)	1.02	1.86	0.35	0.1158	3.67(0.03)	1.91	1.76
0.10	0.0270	3.43(0.09)	1.32	2.12	0.40	0.1389	3.29(0.05)	1.71	1.58
0.15	0.0422	3.63(0.07)	1.59	2.04	0.45	0.1637	4.71(0.16)	2.63	2.08
0.20	0.0581	3.64(0.04)	1.74	1.90	0.50	0.1923	4.39(0.05)	2.90	1.49
0.25	0.0758	4.04(0.15)	1.83	2.21					
				Dioxane					
0	0	2.65(0.03)	1.00	1.66	0.30	0.0803	2.13(0.03)	0.76	1.37
0.05	0.0109	2.58(0.14)	0.97	1.61	0.35	0.0982	2.48(0.02)	0.85	1.63
0.10	0.0226	2.37(0.04)	0.87	1.50	0.40	0.1190	1.88(0.02)	0.62	1.26
0.15	0.0352	2.46(0.03)	0.97	1.49	0.45	0.1414	1.76(0.03)	0.49	1.27
0.20	0.0488	2.06(0.03)	0.77	1.29	0.50	0.1663	1.85(0.02)	0.52	1.33
0.25	0.0637	2.22(0.05)	0.88	1.35	0.55	0.1953	1.87(0.01)	0.46	1.42
				Methanol					
0	0	2.82(0.03)	0.95	1.87	0.40	0.2180	2.73(0.01)	1.39	1.34
0.05	0.0226	2.86(0.03)	1.05	1.82	0.45	0.2555	2.52(0.03)	1.25	1.28
0.10	0.0462	2.93(0.07)	1.14	1.78	0.50	0.2923	2.57(0.04)	1.29	1.29
0.15	0.0712	2.87(0.03)	1.14	1.73	0.55	0.3331	2.56(0.06)	1.42	1.14
0.20	0.0978	3.06(0.02)	1.29	1.77	0.60	0.3766	2.56(0.06)	1.41	1.15
0.25	0.1256	2.18(0.03)	0.80	1.38	0.65	0.4262	2.38(0.03)	1.24	1.14
0.30	0.1547	2.33(0.04)	0.96	1.37	0.70	0.4784	2.39(0.04)	1.20	1.19
0.35	0.1857	2.61(0.03)	1.27	1.34	0.75	0.5370	2.32(0.03)	1.13	1.19
				Tetrahydrofuran					
0	0	2.22(0.04)	0.72	1.50	0.25	0.0664	1.70(0.02)	0.66	1.03
0.05	0.0113	2.34(0.04)	0.91	1.43	0.30	0.0837	1.59(0.01)	0.60	0.99
0.10	0.0236	2.17(0.02)	0.91	1.26	0.35	0.1024	1.37(0.11)	0.53	0.86
0.15	0.0369	1.93(0.03)	0.82	1.11	0.40	0.1227	1.42(0.03)	0.47	0.94
0.20	0.0509	1.98(0.02)	0.86	1.11	0.45	0.1461	1.32(0.04)	0.41	0.91

[a] Quantities in parentheses are standard deviations. [b] pH = 10.1 in the $x_2 = 0$ systems. [c] Postmixing volume fraction of organic cosolvent.

function, with the intermediate formation of a tetrahedral species, as shown schematically in eq 12. Charge separation



(12)

in the transition state leading to the intermediate suggests that a decrease in medium dielectric constant will act to decrease k_{DKP}. Figure 7 shows that the solvent effect on diketopiperazine formation is small and variable in direction.

Formation of the dipeptide AP occurs by classical intermolecular hydroxide attack on the methyl ester function. Little charge separation takes place, and the electrostatic contribution to k_5 should be small. However, k_{AP} also includes the hydroxide ion concentration, according to eq 11, and this is subject to alteration as the solvent composition changes. It is true that the apparent pH was maintained substantially constant throughout these studies, but this does not ensure that [OH^-] remains constant, because the autoprotolysis constant is solvent-dependent; moreover, there is no thermodynamic basis for relating a pH reading in one solvent to that in another solvent. Thus we may expect solvent effects on k_{AP} through this influence. We find (Figure 8) that methanol has very little effect on k_{AP}, four of the cosolvents produce rate decreases, and DMSO acts to produce an appreciable rate increase. This last effect may be a consequence of the unusual capability of DMSO for increasing the alkalinity of aqueous–DMSO solutions.[47]

The disparity of solvent effects generated by the different organic cosolvents shows, as it did above for the acid-catalyzed kinetics, that the electrostatic dielectric constant effect cannot be the dominant factor controlling these solvent effects, although it probably makes a contribution. We therefore

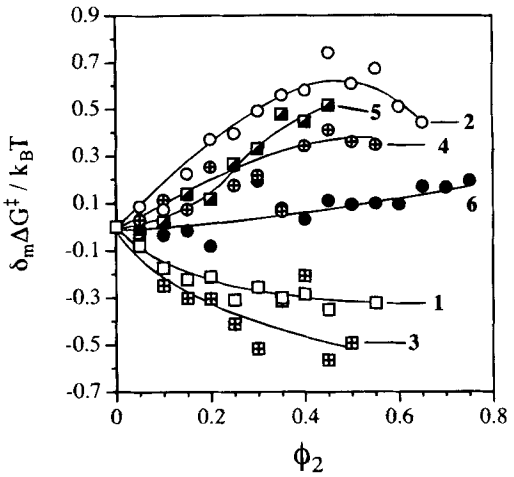


Figure 6—Solvent effect on the first-order rate constant for aspartame demethylation in basic solution. Key: 1, acetone; 2, acetonitrile; 3, DMSO; 4, dioxane; 5, THF; 6, methanol.

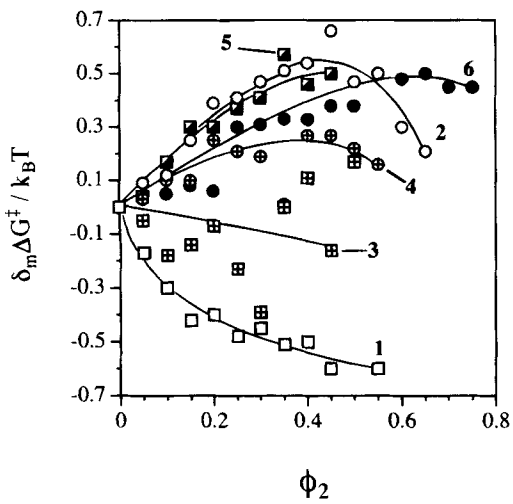


Figure 7—Solvent effects on k_{DKP}; for key to solvents, see Figure 6.

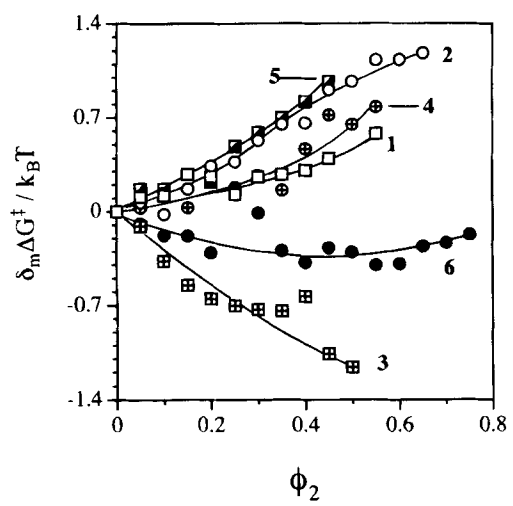


Figure 8—Solvent effects on k_{AP}; for key to solvents, see Figure 6.

conclude that a second effect, postulated to be a solvation effect generated through solute–solvent interactions, is responsible for the variability in responses to solvent identity and composition seen in our experimental results.

Interpretation of Literature Results—Labile compounds are sometimes formulated in mixed solvent systems to enhance their stability. Two recent studies examined the effect of cosolvents on the rate of demethylation of aspartame, reaching different conclusions. As we will see, however, there is no discrepancy, though there certainly are complications.

Sanyude et al.[8] reported that cosolvents increase the reaction rate relative to the fully aqueous system. Their work was performed at pH 4.5, where aspartame is maximally stable. Figure 1 shows that both the k_2 and the k_4 terms are important at this pH. Addition of a cosolvent will increase pK_1 and will decrease pK_2, as we have noted above. Both of these shifts will act to increase the observed demethylation rate, even if k_2 and k_4 are unchanged. Thus a full interpretation of the solvent effect at pH 4.5 requires consideration of the individual solvent effects on pK_1, pK_2, k_2, and k_4, a complicated situation. Nevertheless, the result of Sanyude et al.[8] is consistent with known behavior.

Yalkowsky et al.,[9] working at pH 2.0, found that cosolvents decrease the demethylation rate. At pH 2.0, in water, about 94% of aspartame is in the A_{o+} form, and this percentage will increase as cosolvent is incorporated. Thus only the k_1 and k_2 routes are important, with k_1 dominating. Our studies in the acid region show (Figure 3) that cosolvents decrease the decomposition rate, in agreement with the results of Yalkowsky et al.[9]

The essential point is that the interpretation of solvent effects in such a system requires a detailed understanding of the several rate and equilibrium processes that contribute to the observed effect.

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Acknowledgments

This work was supported by a grant from Glaxo, Inc. R.D.S. was a Fellow of the American Foundation for Pharmaceutical Education (1989–1992) and a Bristol-Myers Squibb Fellow (1991–1992).