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A profound solvent effect on the diketopiperazine formation of the new dipeptide angiotensin-converting enzyme inhibitor, Moexipril

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Summary

The effect of organic solvents on the degradation kinetics, products and mechanisms of Moexipril (**1**) was investigated at 25 °C. Like those observed in aqueous solution, the degradation kinetics leading to the diketopiperazine (**2**) and hydrolysis product (**3**) in mixed solvents were found to be pseudo-first-order. Added organic solvent, however, produced a dramatic effect on the rate and product ratio of the degradation. Thus, the hydrolysis process (k_3) was suppressed in solvents with high organic content. The two water or neutral catalyzed cyclization processes (k_1 and k_2) responsible for the diketopiperazine formation, on the other hand, were enhanced by the addition of organic solvent; a maximum increase of 5.5 and 29-fold for k_1 and k_2, respectively, was observed in 75–90% ethanol solutions. Attempts were made to rationalize such rate enhancement through changes in solvation energy, pK_a values of **1** and equilibrium constants of the intermediates with added organics. The application of the results to some of the formulation problems is discussed.

Introduction

Diketopiperazine (DKP) formation has become a major stability problem for the formulation of *N*-carboxyalkyl dipeptide analogs that have recently been discovered to be potent angiotensin-converting enzyme (ACE) inhibitors (Wyvratt and Patchett, 1985; Dzau, 1986; Webber and Zusman, 1986). In the solid state, this degradation has led to the strict requirement of maintaining low relative humidity manufacturing conditions and the use of dessicant in packaged bottles (Shiromant and Bavitz, 1986). In water, the DKP formation was found to be strongly pH dependent (Bouvette and Digenis, 1987; Gu and Strickley, 1987). For example, we have shown that Moexipril (RS-10085, **1**) degraded almost entirely to its DKP analog (**2**) at pH values equal to or below the carboxylic acid pKa_1 (≈ 3.05). At pH values above 3.1 the rate of DKP formation decreased and eventually was replaced by the formation of the hydrolysis product, RS-10029 (**3**), at pH values above the amine pKa_2 ($= 5.4$) (Gu and Strickley, 1987) (Scheme 1).

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In this paper we report a study of an unusual solvent effect on the various degradation pathways of **1**. This information is generated in order to facilitate the overall formulation developmental program of **1** where various organic and mixed aqueous solvent systems are used.

Materials and Methods

Chemicals

Moexipril (**1**) hydrochloride, Moexipril diketopiperazine (**2**) and RS-10029 (**3**) were obtained form the Institute of Organic Chemistry (Syntex Research). High-performance liquid chromatographic (HPLC)-grade acetonitrile, tetrahydrofuran and water (purified using Nanopure system) were used to prepare the mobile phase. Alcohol (both U.S.P. and absolute) was used as received. All other chemicals were analytical grade and used as supplied.

Dissociation constants (pK_a)

The measurements of the dissociation constants in mixed aqueous organic solvents followed the procedures reported elsewhere (Gu and Strickley, 1987) except that the standard KOH solutions were also prepared in mixed aqueous organic solvents. The standard deviation of the pK_a values is typically 0.1 unit.

Kinetic methods

Acetate (prepared from sodium acetate and acetic acid) and phosphate buffer (prepared from NaH_2PO_4 and Na_2HPO_4) solutions containing 0.010 M total buffer concentrations were freshly prepared. The buffer solution was mixed with organic solvents (v/v) to obtain the desired ratio. The apparent pH of the drug solution was measured using a Radiometer model PHM 64 Research pH meter equipped with a Radiometer model GK 2410C combination electrode. The pH meter was calibrated using standard aqueous buffer solutions. All kinetics were studied under controlled conditions of room temperature (25 °C) and the pH value for a given solution remained essentially unchanged throughout the investigation.

In a typical experiment, a drug solution of the desired concentration was stored in a 10 ml volumetric flask until predetermined time periods had elapsed, when 1.0 ml of the solution was removed, diluted with mobile phase and assayed by HPLC immediately. Details of the stability-specific reverse-phase HPLC method and instrumentation have been described elsewhere (Gu and Strickley, 1987). The method employed an Altex Ultrasphere-ODS 5 μm column and a mobile phase of ammonium phosphate buffer (0.05 M, pH 2.0)/acetonitrile/tetrahydrofuran (55 : 35 : 10). The flow rate was maintained at 1.0 ml/min and the wavelength of detection was 220 nm. Excellent linearity was obtained via area integration for compounds **1**–**3** and their relative response factors were used to calculate the % of product formation.

Results and Discussion

Kinetics

The stability of the hydrochloride salt of **1**, the selected raw material form, was first studied in mixed aqueous-ethanol solutions at room temperature in order to evaluate the effect of organic solvents. Similarly to those found in the aqueous solution, the kinetics of the degradation in mixed aqueous-ethanol solutions were strictly pseudo-first-order (Fig. 1) when followed by a stability-specific HPLC method (see Materials and Methods). The t_{90} (time to reach 90% drug remaining) values calculated from the observed rate constants as well as the apparent pH values measured for each mixed aqueous-ethanol solution are summarized in Table 1. At a drug concentration of 0.10 mg/ml, the t_{90} value in water was 51 days and the pH of the solution was 3.9. This t_{90} value in water is therefore consistent with that predicted from results obtained at elevated temperature and pH 3.9 (Gu and Strickley, 1987). The addition of ethanol had a significant effect on the rate of degradation as the t_{90} values initially displayed a gradual decrease as the % ethanol in solution increased showing a 23-fold rate increase in 75%–90% ethanol solutions ($t_{90} = 2.2$ days). However, at even higher ethanol content, the degradation

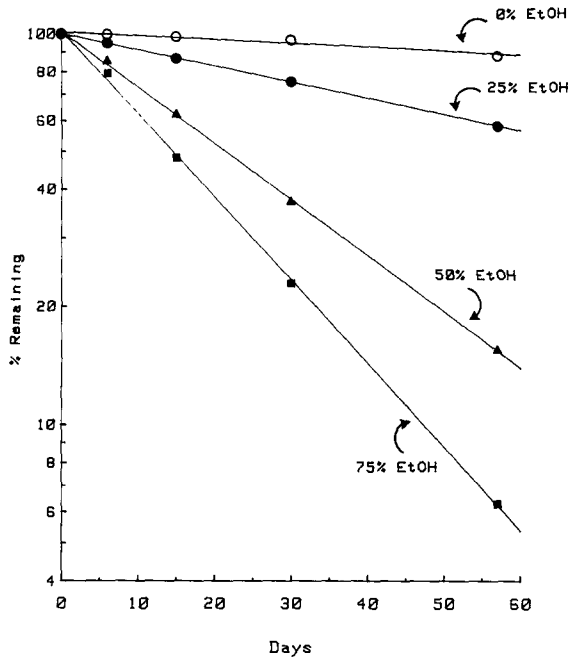


Fig. 1. Pseudo-first-order kinetics of the degradation of 0.10 mg/ml of 1 in aqueous-ethanol solutions at 25 °C. % ethanol: (○) 0, (●) 25, (▲) 50, (■) 75.

rate slowed down considerably as the t_{90} value in 95% ethanol increased to 25 days.

The effect of pH on the degradation kinetics in mixed aqueous-ethanol solutions was also evaluated. The results are listed in Table 1. A significant (5-fold) increase in rate was also observed when 0.01 N HCl or acetate buffer (pH 4.3) was used as the aqueous diluent. However, a reversal in this trend occurred when the pH of the aqueous solution was increased to pH = 6.8. Thus, the t_{90} values increased from 38 days in phosphate buffer (0% ethanol) to 155 days in 50% ethanol and to greater than 1 year in 90% ethanol (Table 1).

In order to determine whether the profound solvent effect is peculiar to ethanol, the stability of drug was also investigated in dimethylacetamide (DMA), dimethylformamide (DMF), dimethylsulfoxide (DMSO) and propylene glycol (PG) mixed aqueous solutions at a drug concentration of 0.10 mg/ml. It is clear from the results summarized in Table 2 that up to 3–7-fold increase in rate was observed in these mixed solvents. Again,

TABLE 2

Stability of 0.10 mg/ml Moexipril (1) hydrochloride salt in mixed-aqueous solutions

Solvents	t_{90} (days) [a,b]			
Ratio [c]:	10/90	50/50	90/10	100/0
DMA/H_2O	17.0	2.3	4.6	20.4
DMF/H_2O	19.2	5.9	TS	TS
DMSO/H_2O	20.4	4.3	16.0	TS
PG/H_2O	28.2	5.2	6.9	TS

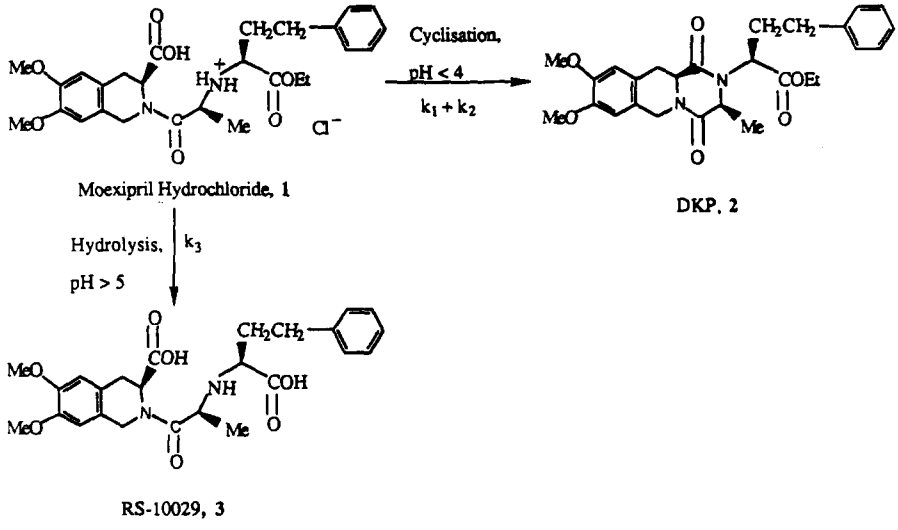
[a] All samples were assayed in duplication and standard deviations on the rate data are typically <10%.
[b] TS denotes too slow to be followed at 2 month end point.
[c] Ratio: proportion of organics vs water.

TABLE 1

Stability of 0.10 mg/ml Moexipril (1) hydrochloride salt in aqueous-ethanol solutions

Aqueous solution	t_{90} [a,b,c] (days)						
% EtOH:	0	10	25	50	75	90	95
H_2O	51	27	11	3.2	2.2	2.2	25
	(3.9)	(4.1)	(4.4)	(4.9)	(5.2)	(4.2)	
0.01 N	5.5	5.2	3.2	1.5	1.4	1.9	18
HCl	(2.0)	(2.4)	(2.8)	(3.3)	(3.4)	(3.1)	
Acetate	60	–	20	17	15	12	25
buffer	(4.3)		(4.6)	(5.5)	(6.0)	(5.8)	
Phosphate	38	41	100	155	210	TS	
buffer	(6.8)	(6.9)	(7.0)	(7.6)	(8.3)	(7.9)	TS

[a] All samples were assayed in duplicate and standard deviations on the rate data are typically < 10%.
[b] Values in parentheses are measured apparent pH values.
[c] TS denotes too slow to be measured at 2 month end point.



Scheme 1.

when water was absent, the degradation was suppressed (Table 2).

Product distribution

Intramolecular aminolysis yielding DKP (**2**) and ester hydrolysis yielding RS-10029 (**3**) (Scheme 1) are by far the two most important pathways for degradation of **1** in mixed aqueous-organic solvents. In all cases studied, these two products accounted for at least 98% of reacted **1**. In addition to the strong pH dependence, the % organic solvent in the solution also affects the product distribution significantly. Fig. 2 shows the dramatic effect of % ethanol on the product ratio (expressed as % cyclization) in various mixed aqueous buffer-ethanol solutions. Thus, in 0.010 N HCl-ethanol solutions, DKP (**2**) was the sole product regardless of the % ethanol in the solution. In aqueous acetate buffer with a pH value of 4.2, hydrolysis product **3** became important and accounted for 25% of the total products. However, with ethanol in acetate buffer solutions, hydrolysis product **3** was suppressed and DKP (**2**) essentially accounted for 99% of the total products in 90% or higher ethanol solutions. That the formation of DKP (**2**) was enhanced in higher % organic solvents can best be seen in phosphate buffer (pH = 6.8)-ethanol solutions. Thus, DKP (**2**) accounted for only 2% of the total products in aqueous phosphate buffer but rose to 52% in 50% ethanol solution and reached 98% of the total products in 90% or higher ethanol solutions.

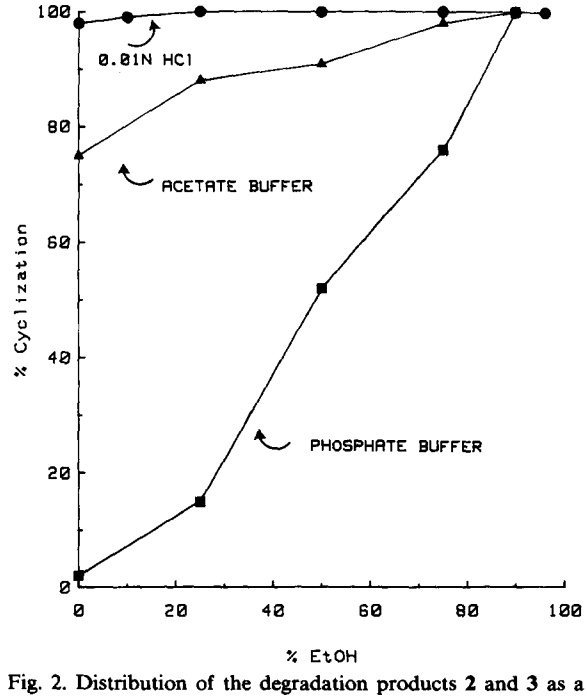


Fig. 2. Distribution of the degradation products **2** and **3** as a function of % ethanol with aqueous portion of 0.01 N HCl (●), acetate buffer (▲) and phosphate buffer (■). Standard deviations for all data points are typically ≤10%.

pKa of 1 in aqueous-ethanol solutions

In aqueous solution, the two pKa values of **1** were found to control the relative importance of the degradation pathways (Gu and Strickley, 1987). For this reason, the two apparent pKa values of **1** in mixed aqueous-ethanol solutions were determined, using a titration method (see materials and methods). The true pKa values in the mixed solvent system can be calculated according to the following:

$$\mathrm{p}K\mathrm{a} = \mathrm{p}K\mathrm{a}' - \delta \qquad (1)$$

where δ is the correction constant which takes into account the variability in glass electrode response for mixed solvent systems (Bates, 1973; Perrin and Dempsey, 1974). Both pKa′ and pKa values are shown in Fig. 3. Although the goal of this study is not to estimate the actual aqueous pKa values from those obtained in the mixed solvents, it is interesting to examine the effect of solvent on the carboxylic acid p$K\mathrm{a}_1$ and the amine p$K\mathrm{a}_2$ in a peptide structure like **1**. A plot of either the apparent pKa′ or true pKa values vs % ethanol

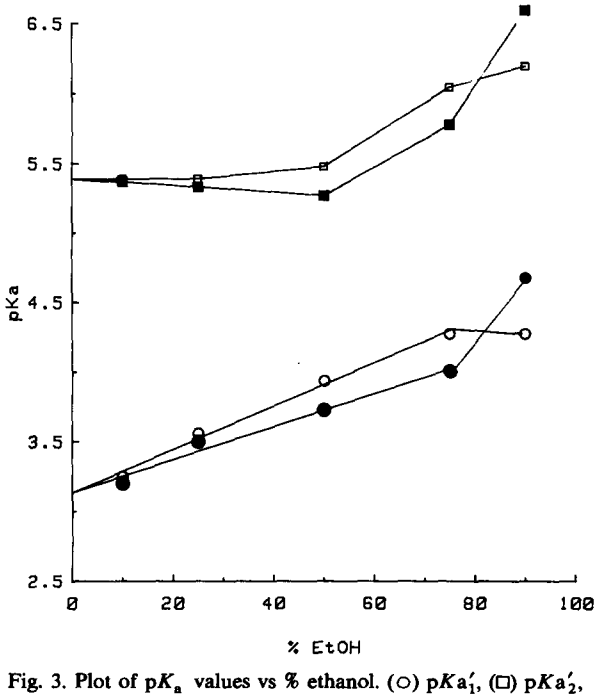


Fig. 3. Plot of pK_a values vs % ethanol. (○) p$K\mathrm{a}_1'$, (□) p$K\mathrm{a}_2'$, (●) p$K\mathrm{a}_1$, (■) p$K\mathrm{a}_2$.

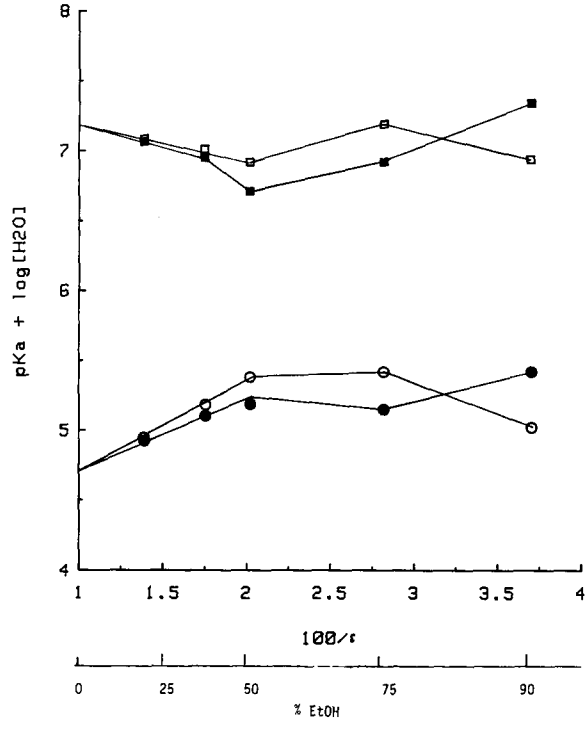


Fig. 4. Plot of $pK_a + \log[H_2O]$ vs $100/\epsilon$. (○) p$K\mathrm{a}_1$, (□) p$K\mathrm{a}_2'$, (●) p$K\mathrm{a}_1$, (■) p$K\mathrm{a}_2$.

using the data points obtained in $\leq 75\%$ ethanol solutions yields a straight line with an extrapolated p$K\mathrm{a}_1$ value of 3.1 at 0% ethanol. Both the true p$K\mathrm{a}_1$ and the apparent p$K\mathrm{a}_1'$ values in 90% ethanol solution however deviate significantly from the regression line (Fig. 3).

A more theoretical treatment of the solvent effect on the pKa is defined by the following expression (Newton et al., 1982):

$$\mathrm{p}K\mathrm{a} + \log[\mathrm{H_2O}] = \frac{1}{\epsilon}\left(e^2/2.303rkT\right) + c \qquad (2)$$

where ϵ is the dielectric constant of the solvent, e is the ionic charge, r is the mean ionic diameter, k is the Boltzman constant, T is the absolute temperature and c is a constant independent of the solvent system. Because of the diminution of ionic dissociation with concomitant increase in ion-pair association (Cookson, 1974) Eqn 2 usually applies only to those solvents with $\epsilon > 50$. Plots of the p$K\mathrm{a}_1'$ and p$K\mathrm{a}_1$ values + $\log[\mathrm{H_2O}]$) of **1** vs $1/\epsilon$

are shown in Fig. 4. Linear relationships can be identified at $\lesssim 50\%$ ethanol ($\epsilon \simeq 50$) but not in $\gtrsim 50\%$ ethanol solutions, consistent with the limitation of Eqn 2. Extrapolation to 0% ethanol yields an identical value ($pKa + \log[H_2O]$) of 4.8. When a value of 1.74 ($= \log[H_2O]$ in 0% ethanol) is subtracted from 4.8, a pKa_1 value of 3.1 is obtained.

Both the apparent pKa'_2 and actual pKa_2 values in aqueous-ethanol solvents did not vary significantly except in solutions with $> 50\%$ ethanol content. Linear relationships were observed only at $\lesssim 50\%$ ethanol for which plots were constructed of the apparent pKa'_2 and actual pKa_2 values vs % ethanol (Fig. 3) and apparent pKa'_2 values + $\log[H_2O]$ vs $1/\epsilon$ (Fig. 4). The extrapolated pKa'_2 value from all three plots is 5.4 at 0% ethanol. No apparent linearity can be identified when the actual pKa_2 values are plotted according to Eqn 2. However, extrapolation from the two actual pKa_2 values obtained in 10 and 25% ethanol solutions also yields the value of 5.4 for pKa_2 at 0% ethanol (Fig. 4), indicating that the application of Eqn 2 in this case is even more limited. The extrapolated pKa values for **1** in 0% organic solvent ($pKa_1 = 3.1$; $pKa_2 = 5.4$) are therefore consistent with those measured directly ($pKa_1 = 3.05$; $pKa_2 = 5.4$) within experimental error. Thus, it appears that simple treatment of the pKa values (from plots of % organic) is equally effective to the more theoretical procedure (i.e., Eqn 2), if not better, for **1**.

Possible reasons for the unusual solvent effect

Because different mixed solvents have differing pH scales, direct comparison of the rate in mixed solvents can sometimes be misleading (Bates, 1973). It is therefore useful to analyze the rate data by different kinetic processes. In aqueous solution the rate of degradation of **1** in the acidic to neutral pH region is defined by (Gu and Strickley, 1987):

$$\text{Rate} = k_{obs}[\mathbf{1}]_t \tag{3}$$

$$k_{obs} = k_1 f_{1c} + k_2 f_{1z} + k_3 f_{1a} \tag{4}$$

where $[\mathbf{1}]_t$ is the total concentration of **1** in solution and f_{1c}, f_{1z} and f_{1a} are the fractions of the cation, zwitterion and anion of **1**, respectively (see Scheme 2 for structure assignment). It was found from the detailed product analysis in aqueous solution that k_1 and k_2 processes led primarily to the DKP **2** formation, and the k_3 process gave hydrolysis product **3** as the major species.

The effect of solvent on the water- or neutral-catalyzed ester hydrolysis (Eqn 5) has been studied extensively (Patai, 1969).

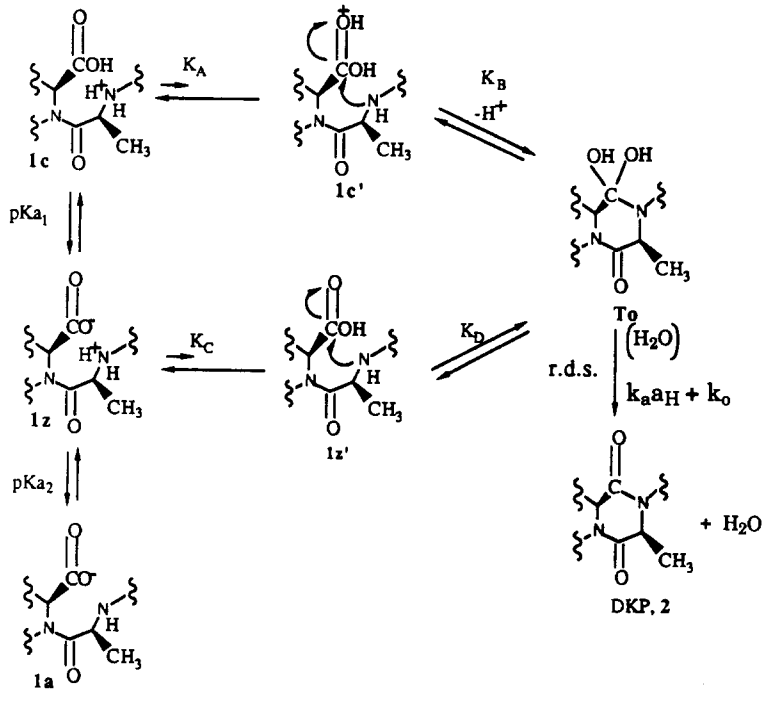
$$RC(O)OR' + H_2O \rightarrow RC(O)OH + R'OH \tag{5}$$

In general, the hydrolysis is suppressed with added organic solvent due to the decrease in water concentration. Thus, the increased stability (t_{90} from 38 days in water to > 1 year in 90% ethanol) and the change in product distribution (from 95% of **3** in water to $\sim 0\%$ of **3** in 90% ethanol) observed in phosphate buffer-ethanol solutions can be attributed to the suppression of the water- or neutral-catalyzed hydrolysis process (k_3).

On the other hand, the DKP **2** formation was found to be accelerated by addition of organic solvent with the greatest rate enhancement observed in $\sim 75\%$ ethanol (Table 1). Using the true pH and the pKa values observed in 0–75% ethanol, the rate data for cyclization were substituted into Eqn 6 and the results are summarized in Table 3.

$$k_{obs} \text{ for cyclization} = k_1 f_{1c} + k_2 f_{1z} \tag{6}$$

Thus, the maximum rate enhancement in mixed aqueous-ethanol solutions is 5.5- and 29-fold for the k_1 and k_2 processes, respectively. Because both the cation **1c** and zwitterion **1z**, are non-nucleophilic, the mechanism of the DKP **2** formation must be more complex than that indicated by Eqn 6. In fact, due to its resemblance to enzyme-catalyzed amide bond formation, intramolecular cyclization of amino esters and amino acids is of particular interest and has been investigated extensively employing a few model compounds (Camilleri et al., 1979; Abdallah and Moodie, 1983; Fife and Duddy, 1983). If the cyclization of **1** follows a similar mechanism, then the rate-determining step would be the dehydration of tetrahedral intermediate To (Scheme 2). The simplest



Scheme 2.

rate law according to Scheme 2 is:

$$\text{Rate} = (k_a a_H + k_0) f_{To} [\mathbf{1}]_t \quad (7)$$

or

$$k_{obs} = k_a K_A K_B f_{1c} + k_0 K_C K_D f_{1z} \quad (8)$$

Thus, any change in the rate constant and/or equilibrium constants in Eqn 8 resulting from added organic solvent would affect k_1 and k_2 in Eqn 6. Organic solvents such as ethanol are known to stabilize cations and to destabilize anions (Bates, 1973). Qualitatively, this can be partially explained by the inductive effects of the ethyl groups as shown in Scheme 3.

Scheme 3.

The increase in electronic density from added ethanol would strengthen the interaction between the cationic charge and the solvent molecule in the primary layer, but weaken the interaction with anions (Bennetto et al., 1966; Andrews et al., 1968). If we assume that the stabilization provided by ethanol is similar for the cations, **1c** and **1c'** (no change in K_A'), then the stabilization for the neutral To by ethanol must be greater (increase in K_B) to account for the up to 5.5-fold increase in k_1 (Eqn 8 and Table 3).

TABLE 3

Effect of ethanol on the kinetics of DKP 2 formation at 25 °C

% EtOH	k_1 ($M^{-1} s^{-1}$) [a]	k_2 ($M^{-1} s^{-1}$) [a]
0	2.0×10^{-7}	1.1×10^{-8}
25	4.5×10^{-7}	4.2×10^{-8}
50	9.3×10^{-7}	1.6×10^{-7}
75–90	1.1×10^{-6}	3.2×10^{-7}

[a] Standard deviation is typically 30 ± 40% of the reported values.

On the other hand, solvation energy provided by the organic solvent for the neutral species but not for zwitterions would indicate an increase in K_C but no effect on K_D with an overall effect of an increase in f_{To}. According to Eqns 6 and 8, this could contribute to the up to 29-fold increase in k_2 observed in 75–90% ethanol solutions (Table 3) even though an increase in k_0 is also possible. One of the findings providing evidence showing that K_C increases with added organic solvent is that of the decrease in the difference between the amine and the carboxylic pKa values as the % of ethanol increased (Fig. 3). Finally, when the ethanol content in mixed solvents increased to 95%, the rate of DKP formation decreased sharply (Table 1). Although water is a product of the reaction, the effect of its formation on the % organic in a given cosolvent is negligible, due to the small amount of drug used (1 mg/ml) in the study. The dehydration of the tetrahedral intermediate, To, on the other hand, requires water as a catalyst (Scheme 2) (Adballah and Moodie, 1983). Thus, when $[H_2O]$ is reduced significantly in high organic content solvents, the rate of the dehydration process is also reduced, resulting in suppression of the overall rate of cyclization.

Implication to Formulation Development

The predicted 25°C shelf-life (t_{90}) of **1** in aqueous solution was reported previously to be approx. 2 months at the pH of maximum stability (pH = 4.5). The use of cosolvent is often an effective means of circumventing such undesirable shelf-life (Irwin et al., 1984; Mojaverian and Repta, 1984). However, it was surprising to find that the addition of 50% ethanol or PG enhanced the instability of **1** in aqueous solution (with no buffer control) (Tables 1 and 2). Attempts at converting the hydrochloride salt of **1** to other cation salts where cosolvents were used also led to severe drug degradation (Dvorack, 1988). These observations have led to an extensive investigation of the effect of ethanol as well as other commonly used organic solvents on drug stability. From the detailed kinetic and product distribution data presented in this study, it is clear that the rate enhancement of intramolecular cyclization to DKP **2** formation in cosolvents is responsible for the unexpected reactivity of **1**. Storage of the hydrochloride salt of **1** in cosolvents without pH adjustment is therefore not recommended.

The hydrolysis reaction of **1**, on the other hand, was suppressed efficiently with added organic solvents. When the pH values of the aqueous ethanol solutions of **1** were adjusted to above 6, where the hydrolysis reaction dominated, the stability of the drug solution improved dramatically (Table 1). Thus, at pH 6, a parenteral formulation of **1** with a shelf-life of greater than 2 years appears to be achievable in $\geq 90\%$ aqueous ethanol solutions.

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