also be apparent that plastic or rubber devices most likely should not be repeatedly sterilized with ethylene oxide if there is any possibility that chlorides are present. Many hospitals apparently reuse disposable devices a number of times and feel that general washing of the item and sterilization with ethylene oxide ensure the safety of the item. This practice should be discouraged.

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Relative Substituent Effects on Alkaline Solvolysis of β-Lactams (2-Azetidinones) and Amides

RICHARD J. WASHKUHN* and JOSEPH R. ROBINSON†

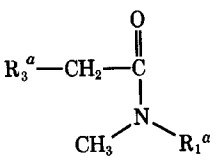
Abstract □ The effect of aliphatic and aromatic substituents, in the 1- and 3-positions of β-lactams, on the rate of solvolysis were examined and compared to analogous substituent effects in the model linear amides. It was found that substituent effects in the β-lactams could be quantitated using the Taft equation, and that a greater sensitivity to polar effects exists in the β-lactams as compared to the model linear amides. In addition, for single substituents in the 1- and 3-positions of the lactams, there is less steric influence on the rate of solvolysis as compared to linear amides. The mechanism of alkaline hydrolysis of these β-lactams appears to be the rate-limiting attack of hydroxide ion at the carbonyl carbon of the amide group and not the breakdown of an ionized tetrahedral intermediate as has been proposed for γ-lactams. Hydrolytic studies on β-lactams at 80° yield a different pathway of degradation, because deamination of the amino acid appears to be rate limiting; thus, elevated temperature studies on β-lactams should be carried out with caution. Finally, it is concluded that β-lactams are not unusually stable in their reactivity toward nucleophiles, such as hydroxide and methoxide ions, and their lability closely follows the parent linear amides.

Keyphrases □ 2-Azetidinones and linear amides—solvolysis mechanism, substituent effects □ β-Lactams, unfused, and linear amides—solvolysis mechanism, substituent effects □ Solvolysis (alkaline) mechanism, substituent effects—unfused β-lactams and linear amides

An enormous effort has been expended investigating various physicochemical and biological aspects of penicillins and cephalosporins (1–4), but comparatively little work has been done on the physicochemical properties of β-lactams. This is somewhat surprising, considering that present thinking (5–8) concerning the mode of action of the penicillins and cephalosporins centers around the acylating potential of the β-lactam moiety in these compounds. Recent work (9) demonstrated that fusion of the β-lactam ring to other rings can introduce considerable strain into the β-lactam ring which, when coupled with polar inductive effects arising from the C-3 side chain and the prevention of amide ground-state resonance, helps explain the unusual lability of these antibiotics to nucleophilic attack. However, the question still remains whether β-lactams are unusual in their stability toward nucleophiles, such as hydroxide and methoxide ions, as compared to corresponding linear amides. In addition, a mechanism of solvolysis of these agents has not been reported.

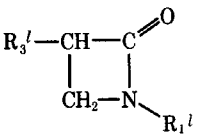
A summary of the effect of substituents on reactivity for a variety of β-lactams was reported (10), and these effects are all qualitative in nature. The only quantitative results of substituent effects on reactivity appears to be the work by Holley and Holley (11–13). These investigators examined the solvolysis of some substituted β-lactams, amides, and penicillins in 85% ethanolic solutions. They discussed, in qualitative terms, the effect of structural variation on reactivity. Recent work concerning the effect of ring size in fused ring β-lactams on the rate of hydrolysis was published by Earle *et al.* (9) and Moll (14).

The purposes of the present study were: (*a*) to investigate substituent effects on the rates of alkaline hydrolysis and solvolysis of unfused β-lactams and (*b*) to compare these effects to analogous substituent effects exhibited in normal amide solvolysis to determine if β-lactams are



amide structure

I



β-lactam structure

II

Table I—β-Amino Acids Prepared by the Blicke and Gould Method (19)

R[a]	Yield, %	Melting Point[b]
C_6H_5—	70	125–126°
$C_6H_5CH_2CH_2$—	95	194°
CH_2=CH—CH_2—	80	165°
$(CH_3)_3C$—	90	150–152°
$C_6H_5CH_2$—	97	195–196°
$(CH_3)_2CH$—	70	183°
Cyclo-C_6H_{11}—	90	193–194°
$CH_3CH_2CH_2$—	70	172°
CH_3CH_2—	50	191–192°
CH_3—	95	200–201°
H—	25	224–225°

[a] $RNHCH_2CH(C_6H_5)CO_2H$. [b] All temperatures are corrected.

substantially different from their corresponding linear amides. Structures I and II show the structural similarities between the amides and the β-lactams. In addition, since the mechanism for solvolysis of β-lactams has apparently not been reported, the possible pathways for solvolysis of these agents are explored here.

EXPERIMENTAL

Reagents—All chemicals and solvents were of analytical grade. Nevertheless, some had to be further purified prior to use since trace impurities and small moisture content can seriously affect the product yield in the various syntheses performed in this study. Methanol and ethanol were purified by distillation from metallic sodium and the corresponding formate ester, as described by Vogel (15). To ensure complete dryness in all solvents, an all-glass distillation apparatus was used and distillations were carried out immediately prior to use. Diethyl ether and benzene were purified by distillation from sodium wire as described by Vogel (15). Ethyl bromide and methyl iodide, used in the prepartion of Grignard reagents, were purified by distillation.

Analytical grade dimethylaniline was purified by distillation, using the acetic anhydride method (16) to remove any secondary amines present, and then dried with anhydrous potassium carbonate, filtered, and redistilled from sodium hydroxide. Pyridine was purified in an analogous manner as described by Vogel (16). Thionyl chloride was purified by procedures set forth by Friedman and Wetter (17); acetyl chloride was purified by fractional distillation, and propionyl and phenylacetylchloride were purified by methods described by Fieser and Fieser (18). Magnesium turnings were washed thoroughly with anhydrous diethyl ether, dried, and stored in a desiccator prior to use. Water was doubly distilled from alkaline permanganate in an all-glass distillation apparatus.

Synthesis—The various β-lactams were prepared by one of two methods: Blicke and Gould's method (19), involving an amine hydrochloride acid chloride intermediate, or Holley and Holley's method (11–13), involving a Grignard reagent with a β-amino ester. Tables I and II present the various β-amino acids and esters synthesized in this study, together with yields and melting or boiling points. The β-lactams prepared by both methods are presented in Table III, together with yields and melting or boiling points. Structures of β-amino acids, β-amino esters, and β-lactams were confirmed by IR and PMR spectra as well as elemental analysis.

Table II—β-Amino Esters Prepared by the Holley and Holley Method (11–13)

R_1[a]	R_2[a]	R_3[a]	Yield, %	Boiling Point[b] (°/mm. Hg)
H—	CH_3CH_2—	CH_3—	50	63–64°/13
H—	CH_3CH_2—	CH_3CH_2—	70	70–72°/14
H—	CH_3CH_2—	$(CH_3)_2CH$—	52	125°/0.3
H—	CH_3CH_2—	$C_6H_5CH_2$—	55	131°/2.7
H—	CH_3CH_2—	Cyclo-C_6H_{11}—	54	119°/0.5
CH_3—	CH_3—	CH_3—	65	68–70°/18
CH_3—	CH_3—	CH_3CH_2—	81	67–68°/13
CH_3—	CH_3—	$(CH_3)_2CH$—	50	63–65°/2
CH_3—	CH_3—	$C_6H_5CH_2$—	41	104–106°/0.4
CH_3—	CH_3—	Cyclo-C_6H_{11}—	65	83–85°/0.4

[a] R_3—$NHCH_2C(=R_1)H$—CO_2R_2. [b] All temperatures are corrected, and pressures were determined using a Todd Universal vacuum gauge.

Table III—β-Lactams Prepared in This Study[a]

R_3[l]	R_1[l]	Yield, %	Melting Point[b] (°) or Boiling Point[b] (°/mm. Hg)
H—	CH_3—	12	68–69°/18
H—	CH_3CH_2—	12	67–69°/14
H—	$C_6H_5CH_2$—	15	110°/2
CH_3—	CH_3—	20	65–66°/12
CH_3—	CH_3CH_2—	22	76–78°/14
CH_3—	$C_6H_5CH_2$—	50	100–102°/0.7
CH_3—	$(CH_3)_2CH$—	20	38–40°/1
CH_3—	Cyclo-C_6H_{11}—	40	72–75°/0.3
C_6H_5—	CH_3—	30	106–107°/0.4
C_6H_5—	CH_3CH_2—	75	140–142°/2
C_6H_5—	$CH_3CH_2CH_2$—	50	102–105°/0.1
C_6H_5—	$C_6H_5CH_2$—	70	72°
C_6H_5—	$(CH_3)_2CH$—	80	130–133°/0.2
C_6H_5—	Cyclo-C_6H_{11}—	50	60–61°

[a] The first eight compounds were prepared by the Holley and Holley method (11–13) and the remainder by the Blicke and Gould method (19). [b] All temperatures are corrected, and pressures were determined using the Todd Universal vacuum gauge.

Amides of acetic, propionic, and phenylacetic acids were prepared either by direct addition of an ethereal solution of an amine to the acid chloride solution or by the conventional method employing pyridine as a catalyst. The resulting amides were then purified by filtration, followed by repeated extraction with 5% hydrochloric acid and 5% sodium hydroxide solutions. The ethereal solution of amide was washed with water and dried with $MgSO_4$; finally the solvent was evaporated at reduced pressures. The residue was then either vacuum distilled or recrystallized to yield the pure amide. Melting and boiling points of these amides coincided with those available in the literature (Table IV). IR and PMR spectra, as well as elemental analysis, were used to confirm structures of these compounds.

Apparatus—IR spectra for all synthesized compounds were obtained using a Beckman IR-5A or IR-9. PMR spectra were taken with a Varian A-60 spectrometer. Measurements and adjustments of pH were made with a Corning model 12 pH meter equipped with an expanded scale, using a Beckman type E3 wide-range glass electrode. The pH meter and electrode system was standardized at 25° against a phosphate buffer as described by Bates (20). Water and oil bath temperatures were maintained to 0.1° with Sargent Thermonitor electronic relays. Reaction progress was monitored on a Cary 11, 14, or 15 recording spectrophotometer with thermostated cell compartments.

Procedures—The following procedure was used for reactions of convenient rapidity to warrant direct measurement of absorbance change as a function of time. A 2- or 5-cm. spectrophotometer cell, containing an alkaline solution at the desired pH, was temperature equilibrated in the cell compartment. After equilibration, 1 or

Table IV—Melting and Boiling Points of Some Amides

R_3[a]	R_1[a]	Melting Point[a] (°) or Boiling Point[a] (°/mm. Hg)	
H—	H—	203°/760	(27–28°)
H—	CH_3—	165°/760	
H—	$C_6H_5CH_2$—	90–91°/1.0	(25°)
H—	Cyclo-C_6H_{11}—	86–87°/1.0	
CH_3—	H—	205–207°/760	
CH_3—	CH_3—	175°/760	
CH_3—	$C_6H_5CH_2$—	111–112°/1.0	
CH_3—	Cyclo-C_6H_{11}—	90–91°/1.0	
C_6H_5—	H—	58°	
C_6H_5—	CH_3—	40–41°	
C_6H_5—	$C_6H_5CH_2$—	175–176°/1.0	(63–64°)
C_6H_5—	Cyclo-C_6H_{11}—	161–163°/1.0	

[a] All temperatures are corrected, and pressures were determined using the Todd Universal vacuum gauge.

Table V—Second-Order Rate Constants for Alkaline Hydrolysis of Some Disubstituted β-Lactams in Water at 25°

Compound	$k_{OH} \times 10^4 (M^{-1}$ min.$^{-1})^a$	*SD*	Number of Determinations
1,3-Dimethyl-2-azetidinone	10.3	0.4	3
1-Methyl-3-hydrogen-2-azetidinone	22.6	0.15	6
1-Methyl-3-phenyl-2-azetidinone	27.2	0.17	4
1-Ethyl-3-methyl-2-azetidinone	3.70	0.25	2
1-Ethyl-3-hydrogen-2-azetidinone	8.32	0.20	3
1-Ethyl-3-phenyl-2-azetidinone	9.51	0.11	3
1-Benzyl-3-methyl-2-azetidinone	12.9	0.8	3
1-Benzyl-3-hydrogen-2-azetidinone	32.0	0.2	6
1-Benzyl-3-phenyl-2-azetidinone	35.0	0.5	5

[a] Determined on the basis of hydroxide-ion activity.

2 μl. of pure liquid was injected into the cell and mixed thoroughly. With low melting solids, the compound was first melted and then the liquid was injected. With high melting solids, a given amount of solid was accurately weighed corresponding to a 10^{-3} *M* concentration and placed into 200 ml. of alkaline solution at the desired temperature, mixed rapidly, and equilibrated; absorbance changes were monitored as a function of time.

Wavelengths chosen to monitor absorbance changes were obtained by comparison of UV spectra of the β-lactam with that of the corresponding amino acid or, in the case of amides and the methoxide-catalyzed methanolysis studies, by comparison of the initial UV spectrum with that obtained 48 hr. later. All the wavelengths used varied between 225 and 230 nm., depending upon substituents, temperature, and nucleophiles involved in the reaction.

For reactions with exceedingly slow rates at 25, 35, and 45°, the procedure described earlier was followed except that absorbances were determined in an intermittent manner. For reactions at 80°, the following procedure was employed. A 2.0×10^{-3} *M* solution of the β-amino acid or β-lactam was prepared in an alkaline 25% v/v methanol–water solution, mixed well, and placed into 5-ml. ampuls. The ampuls were sealed and placed into an oil bath at 80°. At appropriate time intervals, samples were removed from the oil bath and quenched in an ice–brine mixture, and absorbances were measured.

Table VI—Second-Order Rate Constants for Alkaline Hydrolysis of Amides in Water at 25°

Compound	$k_{OH} \times 10^4 (M^{-1}$ min.$^{-1})^a$	*SD*	Number of Determinations
N,*N*-Dimethylacetamide	11.0	0.5	3
N,*N*-Dimethylpropionamide	11.3	0.4	3
N,*N*-Dimethylphenylacetamide	8.0	0.4	3
N-Methylacetamide	4.08	0.25	2
N-Methylpropionamide	3.50	0.31	2
N-Methylphenylacetamide	4.57	0.43	2
N-Methyl-*N*-benzylacetamide	2.35	0.16	2
N-Methyl-*N*-benzylpropionamide	1.18	0.20	2
N-Methyl-*N*-benzylphenylacetamide	3.0	0.4	2

[a] Determined on the basis of hydroxide activity.

Table VII—Pseudo-First-Order Rate Constants for Alkaline Hydrolysis of 1-Benzyl-2-azetidinone as a Function of pH at 25°

Molar Concentration of NaOH	$pH_{app.}$[a]	$pH_{corr.}$[b]	$k_{obs.} \times 10^3$ min.$^{-1}$
3 *M*	13.94	14.54	11.3
2 *M*	13.82	14.20	5.3
1.5 *M*	13.71	14.00	3.3
1.0 *M*	13.59	13.80	2.0
0.5 *M*	13.36	13.46	0.99
0.1 *M*	13.00	13.00	0.32
	12.55	12.55	0.13
	12.00	12.00	0.031

[a] Measured pH. [b] pH corrected for sodium-ion concentration.

Methoxide-catalyzed methanolysis of the β-lactams was carried out in a methanolic solution of sodium methoxide prepared by reacting freshly cut sodium with anhydrous methanol, followed by filtration through a fine-sintered-glass funnel, and diluting to the desired concentration of sodium methoxide with anhydrous methanol. The final concentration of sodium methoxide in methanol was determined by accurately pipeting a small volume of the basic solution into 50 ml. of water and titrating this solution with standardized 0.1 *N* hydrochloric acid aqueous solution to a mixed indicator end-point. The mixed indicator was composed of a neutralized 0.1% bromcresol green solution and a 0.1% solution of sodium alizarin sulfonate.

Pseudo-first-order conditions were maintained in all kinetic experiments, since a large excess of hydroxide or methoxide ion was present at all times. Pseudo-first-order rate constants were converted to second-order rate constants using hydroxide or methoxide concentration or activity. For methoxide-catalyzed methanolysis, the rate constants were calculated on the basis of concentration rather than activity; the aqueous solution studies were determined on the basis of either concentration or activity, depending upon temperature. At 25°, activity measurements were used; at elevated temperatures, concentration was employed. Many pH measurements required inclusion of a sodium-ion correction factor.

Identification of Reaction Products—*Alkaline Hydrolysis of β-Lactams at 25°*—Identification of the reaction products following alkaline hydrolysis of the β-lactams was accomplished in three ways: (*a*) comparison of molar absorptivities to the amino acids, (*b*) IR spectral data, and (*c*) TLC. The molar absorptivity of the reaction products was calculated and coincided with the theoretical value for the amino acid. Comparison of the IR spectrum of the reaction product with that of the amino acid indicated they were

Table VIII—Effect of Temperature on Second-Order Rate Constants for Alkaline Hydrolysis of β-Lactams and Linear Amides in 1.0 and 2.0 *M* NaOH

	$k_{OH} \times 10^4\ M^{-1}$ min.$^{-1}$[a]				
	45°		35°	25°	
Compound	2 *M* NaOH	1 *M* NaOH	2 *M* NaOH	2 *M* NaOH	1 *M* NaOH
1-Benzyl-2-azetidinone	105.0	102.0	57.1	25.2	20.2
1-Methyl-2-azetidinone	109.0	67.3	39.9	18.0	21.3
1-Methyl-3-methyl-2-azetidinone	32.3	44.2	18.0	8.39	6.13
1-Methyl-3-phenyl-2-acetidinone	89.7	73.5	42.5	19.6	18.0
N,*N*-Dimethylacetamide	39.8	37.4	20.4	9.8	6.1
N,*N*-Dimethylpropionamide	36.8	34.0	21.8	10.1	6.2
N,*N*-Dimethylphenylacetamide	28.5	21.2	15.2	7.61	3.67

[a] The second-order rate constants are based on concentration of hydroxide ion as determined by titration at 25°.

Table IX—Activation Parameters for Alkaline Hydrolysis of β-Lactams and Linear Amides

Compound	$\Delta H^{\neq}_{25°}$, kcal./mole	$\Delta G^{\neq}_{25°}$, kcal./mole	$\Delta S^{\neq}_{25°}$, e.u.
1-Benzyl-2-azetidinone	13.9	23.4	−32.2
1-Methyl-2-azetidinone	13.4	23.6	−34.3
1-Methyl-3-methyl-2-azetidinone	15.2	24.2	−30.1
1-Methyl-3-phenyl-2-azetidinone	13.3	23.6	−34.7
N,N-Dimethylacetamide	14.6	24.1	−31.9
N,N-Dimethylpropionamide	13.7	24.1	−34.9
N,N-Dimethylphenylacetamide	13.9	24.3	−35.2

identical compounds. TLC provided further verification that the amino acid was the sole product.

The chromatographic procedure was as follows. A 5×10^{-2} *M* solution of the β-lactam 1-benzyl-2-azetidinone, a 5×10^{-2} *M* solution of its corresponding amino acid, and a 1:1 mixture of the two solutions were prepared. Preliminary investigations with these solutions suggested that a 1:1 mixture of methanol and chloroform could efficiently separate the amino acid from the β-lactam on a 5 × 20-cm. silica gel TLC plate. The average R_f values associated with each are 0.18 and 0.73, respectively, based on six determinations. A 5×10^{-2} *M* solution of the β-lactam was then prepared in 2 *M* NaOH solution, reacted for 2 days, neutralized with concentrated HCl to pH = 7.0, and then placed on the TLC plate together with the three known solutions. Following elution, the R_f values were calculated, and the R_f value for the reaction product was identical to that of the amino acid in the two known solutions. Only one spot appeared from the reaction products.

Alkaline Hydrolysis of β-Lactams at 80°—At 80°, different reaction products are involved in the alkaline hydrolysis of β-lactams, since the final absorbance after complete reaction does not coincide with the theoretical based on the molar absorptivities of the corresponding amino acid. The final absorbances were significantly higher than predicted, suggesting that a conjugated system is present in the reaction product. Isolation of the reaction products was not carried out; however, some inference concerning their identity can be made. In the case of the β-lactam 1-propyl-3-phenyl-2-azetidinone, the reaction was allowed to proceed to completion at 80° and then the system was acidified with concentrated hydrochloric acid. The acidified reaction solution was extracted with chloroform and a UV spectrum was taken. Comparison of this spectrum with that of atropic acid in chloroform showed that the molar absorptivities and maximum wavelengths were the same, suggesting that the major reaction product is atropic acid. Further evidence supporting this was derived from investigation of the alkaline-catalyzed deamination of the corresponding amino acid under identical conditions. The rates of reaction for the amino acid were approximately the same as the lactam, under identical conditions, suggesting that this is indeed what is occurring. The fact that β-amino acids undergo a deamination process is well documented in the literature (21, 22).

RESULTS

Second-order rate constants for the alkaline hydrolysis of a variety of 1,3-disubstituted 2-azetidinones are presented in Table V, together with standard deviations and number of determinations associated with each 2-azetidinone. Alkaline hydrolytic second-order rate constants for some linear amides are presented in Table VI. Included in the table are standard deviations and number of determinations associated with each amide. For both the β-lactams and the amides, a pH range of 12.0–14.54 was used in the hydrolytic studies. Below pH 12.0, the reaction is inconveniently slow; above pH 14.54, the sodium-ion correction factor becomes exceedingly large. In addition, the uncorrected pH measurement is no longer on the scale of the pH meter.

Pseudo-first-order rate constants for the alkaline hydrolysis of 1-benzyl-2-azetidinone were observed as a function of hydroxide-ion activity over a moderate range of pH values (Table VII). Each pseudo-first-order rate constant as a function of pH was determined from at least two determinations at the specific pH. The standard deviations of these rate constants were all less than 5% of the average and generally in the range of 2–3% of the average. The lower limit of pseudo-first-order conditions is likely to be approached at pH's less than 13.0, so a buffer was added to ensure constant pH. A variety of buffer concentrations was used to establish that no buffer catalysis occurred in the system.

Table X—Methoxide-Catalyzed Methanolysis of β-Lactams and Linear Amides at 45°

Compound	$k_{OCH_3} \times 10^3$ M^{-1} min.$^{-1}$[a]
1-Benzyl-2-azetidinone	11.1
1-Methyl-2-azetidinone	12.6
1-Methyl-3-methyl-2-azetidinone	3.0
1-Methyl-3-phenyl-2-azetidinone	5.6
N,N-Dimethylacetamide	0.25
N,N-Dimethylpropionamide	0.10

[a] Second-order rate constants are based on concentration of methoxide ion as determined by titration at 25°.

Salt effects on the rate of alkaline hydrolysis were also investigated; small changes in the rate constant occurred over substantial salt concentrations. Since this study was carried out in 1.0 *M* sodium hydroxide solution, it was necessary to use salt concentrations in the range of 2–4 *M*.

The effect of temperature on the second-order rate constants for the alkaline hydrolysis of various 1,3-disubstituted 2-azetidinones and some of their linear amide analogs are shown in Table VIII. These second-order rate constants are based on concentration rather than activity, since accurate estimates of activity could not be readily obtained at temperatures other than 25°. The second-order rate constants were converted to units of M^{-1} sec.$^{-1}$, and the activation parameters were then evaluated for each compound at each concentration of hydroxide ion. The energies of activation were determined from Arrhenius plots and converted to enthalpies of activation at 25°; the entropies of activation were calculated using the equations involved in transition state theory. The values of $\Delta H^{\neq}$, $\Delta S^{\neq}$, and $\Delta G^{\neq}$ at each sodium hydroxide concentration were then averaged (Table IX). The values of $\Delta G^{\neq}$ in the two concentrations of sodium hydroxide were quite close to the average for each compound, suggesting that these averages are reasonably accurate. The values of $\Delta H^{\neq}$ and $\Delta S^{\neq}$, on the other hand, fluctuated to a much greater extent from their respective averages. These fluctuations were random in nature and a result of the large standard deviations associated with the rate constants at the specific temperature and concentration of sodium hydroxide.

Second-order rate constants at 45° for the methoxide-catalyzed methanolysis of a variety of 1,3-disubstituted 2-azetidinones and some corresponding linear amides are shown in Table X. The second-order rate constants were determined on the basis of concentration of methoxide ion.

Some β-lactams were subjected to hydrolysis at elevated temperatures (80°). All the β-lactams tested appeared to undergo a relatively

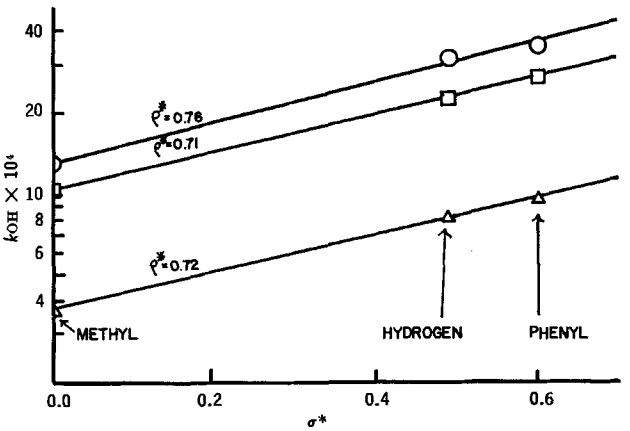


Figure 1—*Plot of second-order rate constants for alkaline hydrolysis at 25° of some 2-azetidinones, with substituent variations in the 3-position,* versus *Taft's substituent constant,* σ*. Key:* ○, *1-benzyl series;* □, *1-methyl series; and* △, *1-ethyl series.*

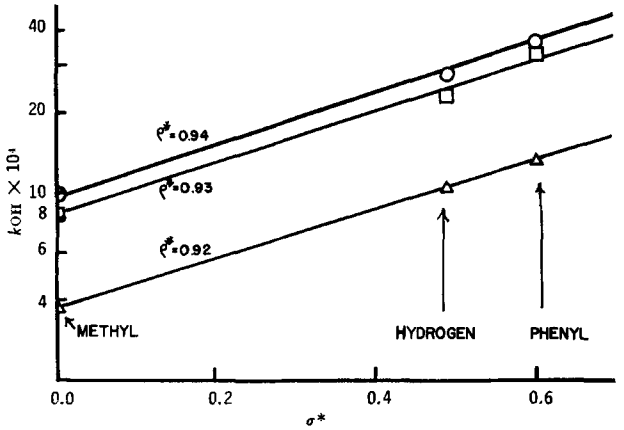


Figure 2—*Plot of second-order rate constants for alkaline hydrolysis at 25° of some 2-azetidinones, with substituent variations in the 1-position,* versus σ^*. *Key:* O, *3-phenyl series;* □, *3-hydrogen series; and* △, *3-methyl series.*

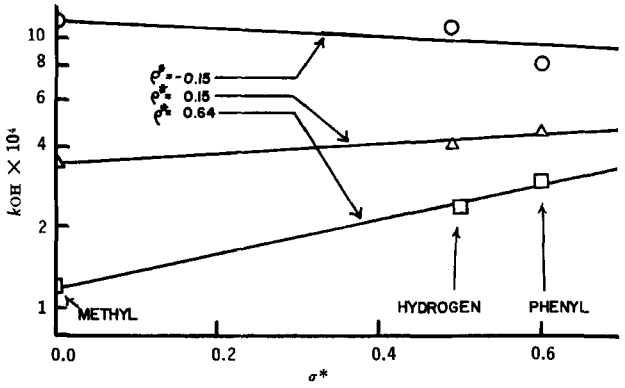


Figure 3—*Effect of substituent variations in the acyl position on the second-order rate constants for alkaline hydrolysis of some amides* versus σ^*. *Key:* O, N,N-*dimethylamides;* □, N-*methyl,* N-*benzylamides; and* △, N-*methylamides.*

rapid cleavage of the β-lactam ring followed by a slower deamination step. Earlier workers (11–13) used elevated temperatures in their studies of β-lactam solvolysis. Since their method of following the reaction could not detect this elimination, their results could be specious. This points out once again that elevated temperature studies to measure stability of a compound must be made with caution.

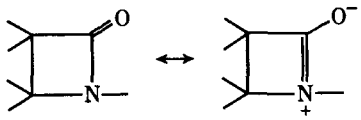
DISCUSSION

β-Lactams—*Substituent Effects in 3-Position*—A number of qualitative and semiquantitative comparisons of the rates of hydrolysis of a series of β-lactams with structures were made during the penicillin program and were summarized by Ballard and Melstrom (10). The general opinion was that substituent effects in the 3-position of 2-azetidinones governed the rates of hydrolysis primarily through a steric influence (21); this conclusion resulted from observations for introduction of two groups into the 3-position. For introduction of a single substituent, this is not the case since substituent effects in the 3-position may be correlated using Taft's polar substituent constant (22), as demonstrated in Fig. 1 for the data obtained in this study[1]. The slopes of these plots are not influenced by the substituent in the 1-position since each series of β-lactams, with variations in the 3-position, has very nearly parallel sensitivities to polar effects in the alkaline hydrolysis as judged by the similar ρ^* values. Insertion of an additional substituent in the 3-position introduces a considerable steric factor; a 25-fold decrease in rate is observed for the 3,3-dimethyl lactam relative to its monomethyl analog (23).

The effects of temperature variation on the ρ^* values obtained from the Taft plot for substituent variations in the 3-position of 1-methyl-2-azetidinones were negligible, suggesting that the isokinetic temperature for this reaction series might be significantly different from the temperature utilized in this study[2].

Substituent Effects in 1-Position—Holley and Holley (11, 12) observed a retardation of an order of magnitude in reactivity when comparing the 1-alkyl-substituted 2-azetidinones to the unsubstituted compound, and they suggested that this was primarily due to a steric effect. In the present studies, it was found that within a series of alkyl substituents in the 1-position, the substituent effects are dependent upon both polar and steric parameters since neither Taft's polar substituent constant, σ^*, nor the steric parameter, E_s, alone could effectively correlate substituent effects on the rates of hydrolysis. It was necessary to define the substituent as $-CH_2R$ to linearize the Taft plot (Fig. 2). Interpretation is not quite clear when a methylene group is inserted between the substituent and the nitrogen, because both steric and polar effects of the substituent are altered by insertion of the methylene group. Here again, the slopes of the Taft plot appear to be independent of the substituent in the 3-position, as suggested by the similar values exhibited for each series.

Comparison of the ρ^* values for the substituent effects in each position reveals that the 1-position is influenced by polar effects to a greater degree than the 3-position. The ρ^* values for variation in the 1-position could conceivably be even greater than the observed 0.93 for all series since insertion of a methylene group diminishes transmission of the polar effects of the substituent due to the greater distance involved between substituent and the reactive site. The enhanced sensitivity observed for the 1-alkyl substituents may be due to enhanced transmission of polar effects by the free pair of electrons present on the nitrogen since these are more fluid than the sigma electrons in the carbon backbone. Another possible explanation may involve amide resonance in the β-lactam molecule (Scheme I), which would be affected to a greater



Scheme I

degree by polar effects in a 1-position than in the 3-position.

Amides—*Substituent Effects in Acyl Portion*—Substituent effects on the alkaline hydrolysis of acyl substituted amides may also be correlated by the Taft relationship (Fig. 3). The substituent constants were defined on the basis of the substituent on the α-carbon rather than directly attached to the carbonyl group so that direct comparison of inductive effects may be made. In this instance, the slopes of the Taft plots for each series are independent of the substituent on the nitrogen and appear to be associated with the inductive effect of the substituent on the nitrogen rather than a steric effect. The negative slope for the alkaline hydrolysis of the *N,N*-dimethylamides is unexpected since alkaline hydrolysis of amides and esters is usually facilitated by lower electron density at the carbonyl carbon. Interpretation of this negative value must be made with caution because the magnitude is very small. An explanation for the negative value might be that the reaction temperature may be near the isokinetic temperature of the reaction for this series. A similar situation may exist for the *N*-methylamides since the magnitude of ρ^* is also very small.

The effect of temperature on the rates of hydrolysis of the *N,N*-dimethylamide series was investigated and does not appear to influence the values of ρ^* appreciably. However, the magnitudes of the

[1] The polar substituent constant (σ^*) expresses the purely polar effects of a substituent in an aliphatic reaction. Utilization of this substituent constant is generally through the following equation:

$$\log k/k_0 = \sigma^*\rho^*$$

where one plots the logarithm of the ratio of the rate constants of the particular reaction at hand against σ^*, presumably yielding a straight line with a slope equal to ρ^*. The sign and magnitude of the slope are interpreted as the electronic requirements for that particular reaction, *e.g.*, a negative slope indicates that the reaction is favored by high electron density.

[2] The isokinetic temperature is the temperature when $\delta\ \Delta G^{\neq} = 0$; that is, substituent effects on the free energy change disappear at this temperature. Thus, the $\Delta H^{\neq}$ and $T\ \Delta S^{\neq}$ terms exactly offset each other at the isokinetic temperature.

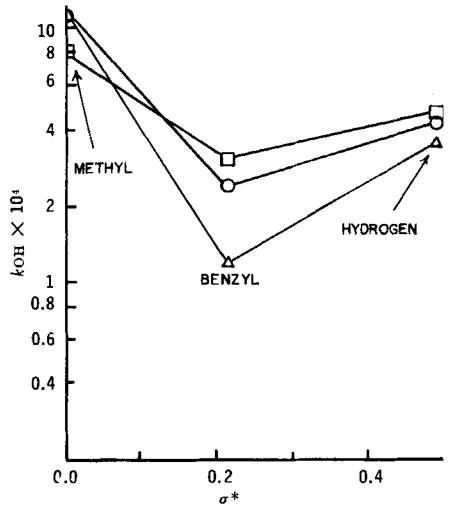


Figure 4—*Plot of second-order rate constants for alkaline hydrolysis at 25° of some amides, with substituent variations on the nitrogen,* versus σ^*. *Key:* ○, *acetamides;* □, *phenylacetamides; and* △, *propionamides.*

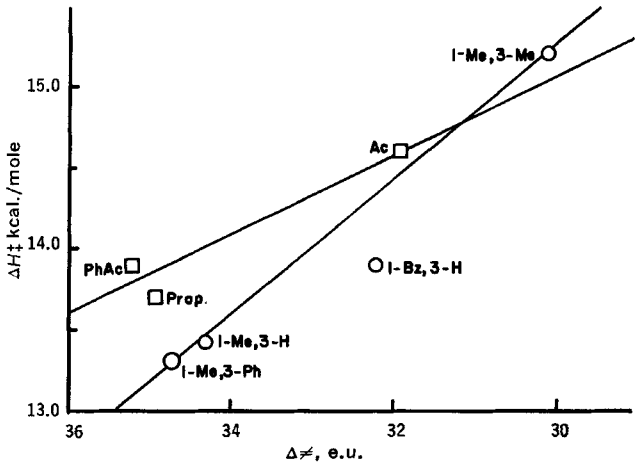


Figure 5—*Enthalpies of activation* versus *entropies of activation for the alkaline hydrolysis of amides and β-lactams. The lines drawn have a slope value of 300°K. for amides and 525°K. for β-lactams. Key:* ○, *β-lactams with variations in the 3-position; and* □, *acyl variations of amides.*

ρ^* values are difficult to estimate due to the significant standard deviations (10–20%) associated with the rate constants.

Substituent Effects on Amide Nitrogen—Variation of substituents on the amide nitrogen could be successfully correlated with Taft substituent constants, as illustrated in Fig. 4. This may conceivably be explained by a change in the rate-determining step; however, this interpretation must be made with caution since only three substituents are involved and no ^{18}O-exchange studies were done. Biechler and Taft (24) observed that alkaline hydrolysis of anilides included a term in the rate expression that was second order with respect to hydroxide ion and attributed this to a rate-determining base-catalyzed expulsion from an anionic tetrahedral intermediate. Others (25–28) reported that the hydrolysis of anilides is subject to general base catalysis, suggesting a similar mechanism. Bender and Thomas (29) observed that ^{18}O-exchange with the solvent occurs more rapidly than hydrolysis, thereby suggesting that breakdown of the tetrahedral intermediate is the rate-determining step. Other ^{18}O-exchange studies (29) suggest that the ratio of hydrolysis to exchange varies with the number of substituents on the nitrogen.

Comparison of β-Lactams with Their Acyclic Analogs—Comparison of the substituent effects on the 3-position of β-lactams with that in the acyl portion of linear amides reveals that both are correlatable with Taft's substituent constants. However, unlike the β-lactams, the ρ^* values are greatly influenced by substituents on the nitrogen in the case of linear amides. The sensitivities of the alkaline hydrolysis of β-lactams to substituent effects in the 3-position also appear to be greater than those observed in the alkaline hydrolysis of linear amides.

The effect of 1-position substituents on the alkaline hydrolysis of β-lactams could also be correlated with σ^*, but similar substituents on the nitrogen in linear amides could not, suggesting that the mechanisms of hydrolysis are different and that direct comparisons may not be readily made. The sensitivities of alkaline hydrolysis of β-lactams to polar substituents in the 1-position are not influenced by substituent variations in the 3-position, unlike those of linear amides where variations on the nitrogen are influenced by the acyl portion of the molecule.

The activation parameters for the alkaline hydrolysis of some β-lactams and their acyclic analogs are presented in Table IX. No significant differences in the values for β-lactams and the amides are apparent. The free energies of activation for the alkaline hydrolysis of β-lactams are slightly less than those of the corresponding amides. This may be due to ring strain in the β-lactams. A plot[3] of $\Delta H^{\neq}$ *versus* $\Delta S^{\neq}$ for the alkaline hydrolysis of both series is illustrated in Fig. 5. By considering only the variations in the 3-position of the β-lactams, a line with a slope of 525°K., corresponding to the isokinetic temperature for this series, may be drawn through the three points, whereas a much smaller value (275–300°K.) is observed for similar acyl variations in the amide series. The large difference in the isokinetic temperature for each series does coincide with the expected differences based on the ρ^* values in Figs. 1 and 3.

The methoxide-catalyzed methanolysis of β-lactams and amides was studied for two reasons: (*a*) to note the effect of a different nucleophile on the rates of reaction of β-lactams and amides, and (*b*) to establish steric requirements involved in both series since the methoxide ion is much larger than the hydroxide ion. The different reaction complexities involved in alkaline hydrolysis and methoxide solvolysis, such as different nucleophilicities and solvent composition, are recognized; this simple interpretation is only for comparison of β-lactams and amides. These studies on the relative alkaline hydrolysis to methoxide-catalyzed methanolysis of β-lactams and amides reveal that the β-lactams exhibit approximately the same reactivity to either nucleophile, while linear amides are significantly less reactive to the methoxide ion, suggesting that steric hindrance to nucleophilic attack is much less in the β-lactams.

Mechanism—Vinnik and Moiseyev (30) speculated on the hydrolytic mechanism for lactams and amides in aqueous solutions of potassium hydroxide and concluded that formation of a stable tetrahedral intermediate was the appropriate mechanism. This mechanism was based primarily on IR spectral evidence exhibited by a γ-lactam, γ-butyrolactam, in concentrated aqueous solutions of

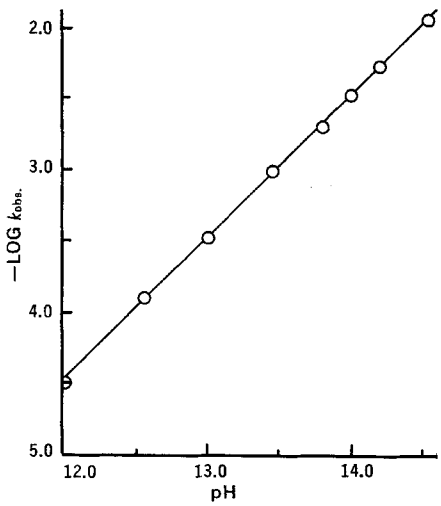


Figure 6—*Partial pH–rate profile for alkaline hydrolysis of 1-benzyl-2-azetidinone at 25°.*

[3] This treatment and interpretation are highly speculative considering the small number of points, the large error associated with each value, and the relatively small differences in $\Delta H^{\neq}$ and $\Delta S^{\neq}$.

Table XI—Some Physicochemical Constants Associated with Amides, β-Lactams, and Antibiotics

Compound	Bond[a] Lengths, Å C—N	Bond[a] Lengths, Å C=O	Distance of[a] Nitrogen from Plane of Ring, Å	C=O[a] Stretching Frequencies, cm.$^{-1}$	$k_{OH}(M^{-1}$ sec.$^{-1})$[b]	Antibiotic[a] Activity
Penicillins	1.34–1.37	1.17–1.20	0.40	1795–1775	10^{-1}–1	Yes
Δ^3-Cephalosporins	1.38–1.48	1.21–1.28	0.22–0.24	1790–1765	10^{-1}	Yes
Δ^2-Cephalosporins	1.34	1.22	0.065	1760–1755	10^{-3}–10^{-2}	No
Fused β-lactams[b,c]	—	—	—	1750	10^{-3}–10^{-2}	No
Unfused β-lactams	1.33	—	0	1760–1730	10^{-6}–10^{-5}	No
Amides[b]	1.32	—	0	1690–1670	10^{-6}–10^{-5}	No

[a] From *Reference 31.* [b] This work. [c] *References 11–13.*

potassium hydroxide. They noted the appearance of several bands with frequencies of 1555, 1740, and 1395 cm.$^{-1}$, the intensities of which increased as the alkali concentration became greater. The band with frequency at 1555 cm.$^{-1}$, appearing at low concentrations of potassium hydroxide, was ascribed to the monoionized intermediate; the frequencies of 1740 and 1395 cm.$^{-1}$, which appeared at approximately 30% potassium hydroxide, were ascribed to the doubly ionized species. IR spectral studies for 1-benzyl-2-azetidinone were performed in a similar fashion. The appearance of spectral bands was not evident as a function of potassium hydroxide concentrations up to 25% (w/v) KOH. In addition, kinetic studies performed by simultaneously measuring the disappearance of the carbonyl-stretching band for the β-lactam and the appearance of the carbonyl-stretching band for the amino acid suggested that the rates associated with each were quite similar. This indicates that a mechanism involving a stable charged intermediate would not be applicable in this case.

Further evidence inconsistent with a mechanism involving a charged intermediate was derived from the partial pH–rate profile and the salt effect on the rates of alkaline hydrolysis for 1-benzyl-2-azetidinone. The partial pH–rate profile is shown in Fig. 6. The slope of this plot indicates that the alkaline hydrolysis is first order with respect to hydroxide ion over a pH range of 12.0–14.54. Since linearity is observed, no charged intermediate is apparently participating in the reaction, a requirement implicit in the mechanism of Vinnik and Moiseyev (30), further discounting charged intermediate formation as a rate-determining factor. Therefore, it appears, for the β-lactams employed in this study, that the rate-determining step is attack of hydroxide ion rather than breakdown of the tetrahedral intermediate.

Comparison of β-Lactams and Amides with Penicillins and Cephalosporins—Sweet and Dahl (31) published data dealing with some structural parameters associated with various penicillins and cephalosporins and related them to biological activity. Table XI, a summary of present observations in conjunction with their results, indicates that chemical reactivity and biological activity parallel a variety of structural parameters associated with the β-lactam ring. The apparent differences in reactivity and biological activity from the penicillins to linear amides is reflected by the degree of planarity of the nitrogen in the β-lactam ring. As the nitrogen atom becomes more pyrimidal in nature, the chemical reactivity increases almost six orders of magnitude. Woodward (32) suggested that normal amide resonance occurs in β-lactams which are not fused to another ring and, consequently, the C—N and C=O bond lengths would be very similar to those of a linear amide provided the resonance involved in both instances occurs to the same extent. Decreasing the resonance capabilities of the β-lactam ring would then result in a decrease in the C=O bond length and an increase in the C—N bond length. In penicillins, changes in bond lengths coincide with the expected changes in reactivity, both being sensitive to the planar configuration of the nitrogen since less overlap between the π-electron orbitals of the carbonyl group and the lone pair of electrons on the nitrogen should exist.

In addition to the lack of planarity of the nitrogen in the β-lactam ring, the biologically active cephalosporins have another factor, enamine resonance, which may account for the unusual lability of the β-lactam amide bond. This, in addition to the nonplanarity of the nitrogen, would result in diminished amide resonance; consequently, the bond lengths would be lengthened to an even greater extent. On the other hand, Δ^2-cephalosporins have a nitrogen in the ring which is nearly planar and have no enamine resonance. This suggests that the bond lengths should be very similar to the unfused β-lactams and linear amides and this does coincide with the observed values (33).

CONCLUSIONS

The enhanced sensitivity to alkaline hydrolysis of penicillins and Δ^3-cephalosporins relative to unfused β-lactams is not primarily due to substituent effects, considering the relatively small changes in the rate constants for alkaline hydrolysis observed as the polarity of the substituent on the ring is varied. Similarly, strain induced into the β-lactam ring associated with fusion to another ring may not totally account for the unusual lability. However, fusion of another ring to the β-lactam ring in conjunction with inhibition of normal amide resonance does effectively account for the five or six orders of magnitude difference in reactivity. Unfused β-lactams are more closely associated with linear amides than penicillins or Δ^3-cephalosporins, as evidenced by the similarities exhibited in kinetic, structural, and spectral properties.

Even though unfused β-lactams are very similar in most respects to amides, some dissimilarities are apparent when comparisons are made. The sensitivities of alkaline hydrolysis to substituent effects are very different for β-lactams and amides, and this difference may be a result of the differences in isokinetic temperatures. For the temperature range employed in this study, alkaline hydrolysis of various unfused β-lactams has an isokinetic temperature which is far removed from the experimental temperature, unlike that observed for amide hydrolysis. Differences in steric hindrance to nucleophilic attack are also apparent, as suggested by reactivities observed in the methoxide-catalyzed methanolysis of amides and β-lactams relative to hydroxide-catalyzed hydrolysis. Differences in mechanism of alkaline hydrolysis may also be involved. In the case of alkaline hydrolysis of β-lactams, the rate-determining step is the attack of the nucleophile. For the alkaline hydrolysis of linear amides, the rate-determining step is dependent upon the number of substituents on the amide nitrogen and may involve either nucleophilic attack or decomposition of the tetrahedral intermediate. These differences, however, are not sufficient to warrant classification of the unfused β-lactams with penicillins and Δ^3-cephalosporins solely on the basis of ring strain.

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Significance of Vehicle Composition I: Relationship between Topical Vehicle Composition, Skin Penetrability, and Clinical Efficacy

JAMES OSTRENGA, CAROLYN STEINMETZ, and BOYD POULSEN

Abstract □ The penetration of two topical steroids, fluocinolone acetonide and fluocinonide, through human abdominal skin was investigated for various propylene glycol–water gels. Release, penetration, and *in vivo* data were compared as a function of vehicle composition. The similarity between the *in vivo* and *in vitro* composition profiles for both steroids suggested that clinical efficacy can be predicted from *in vitro* data and from the physical properties of the steroids. The correlations indicated that the *in vivo* results were directly dependent upon penetrability.

Keyphrases □ Fluocinolone acetonide, fluocinonide topical gels—skin penetration □ Topical gels, fluocinolone acetonide, fluocinonide—release, penetration, *in vivo*, *in vitro* data □ Vehicle composition profiles, *in vivo*, *in vitro*—fluocinolone acetonide, fluocinonide topical gels □ Pharmacokinetics, skin penetration—fluocinolone acetonide, fluocinonide topical gels, drug efficacy, vehicle and drug physical properties, effects

In the formulation of vehicles for topical drugs, the efficacy of such dosage forms is often dependent on the composition of the vehicle. The ability of a drug in a topical formulation to penetrate the skin and exert its effect is dependent on two consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface, and then it must penetrate this natural barrier en route to the site of action. Many so-called "vehicle effects" reported in the literature are consequences of these two diffusional processes. Depending on which process proceeds slower, either event could determine the overall effectiveness of the topical dosage form. These two processes are intimately related, and both are dependent upon the physical properties of the drug, vehicle, and barrier.

The physical picture is one in which a single molecular species, the drug, experiences a changing environment as it diffuses out of the vehicle and across the skin. Poulsen *et al.* (1) reported on vehicle effects regarding the relative release characteristics of gels with varying compositions. The main objectives of this study were to explore the second process of diffusion, penetration of the drug through human skin, and to show that it is rate controlling when a topical dosage form is applied to normal skin. A more general objective was to provide insight into the manner in which the physical–chemical properties of drug and vehicle can be utilized to increase a formulation's effectiveness. Such information hopefully should aid in the intelligent design of topical dosage forms.

THEORETICAL CONSIDERATIONS

The transport of drugs across the skin barrier may be considered a process of passive diffusion. The flux, J (moles cm.$^{-2}$ sec.$^{-1}$), for