Studies on Clavulanic Acid. Part 1. Stability of Clavulanic Acid in Aqueous Solutions of Amines Containing Hydroxy Groups

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The kinetics of the decomposition of clavulanate ion, (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate, in aqueous solutions of 2-amino-2-methylpropane-1,3-diol, 2-amino-2-methylpropan-1-ol, 2-methoxyethylamine, 2,2,2-trifluorethylamine, and aminoacetonitrile have been studied. All reactions were conducted at 35.0 °C and 0.5M-ionic strength. In the pH range 8.05—9.80 the reaction is pseudo-first-order with respect to the concentration of clavulanate ion. The aminolysis rate constants can be expressed as a sum of the terms representing the uncatalysed or water-catalysed amine reaction, the self-assisted nucleophilic reaction, and the hydroxide-ion-catalysed nucleophilic attack of amine on the β-lactam moiety.

It has often been proposed [1,2] that the high catalytic activity of certain hydrolytic enzymes may result in part from concerted polyfunctional catalysis by both nucleophilic and electrophilic groups at the active site of the enzyme.

A number of systems have been proposed as models for these enzymes.[3] An important example is the mixture of Zn^{II} ions with tris(hydroxymethyl)aminomethane (Tris). In a previous paper,[4] we demonstrated the rapid decomposition undergone by clavulanic acid, (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1) under the following conditions: pH 7.50, 35.0 °C, 0.2M-Tris, and 10^{-3}M-
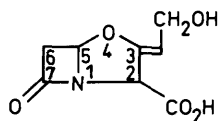
(1)

Zn^{II}. In the present work, we determine the catalytic effect exerted by certain amino alcohols similar in structure to Tris, one of them with the hydroxy group blocked. The study is aimed at ascertaining the nature of the functional groups involved in this catalytic effect.

Experimental

Materials.—Lithium clavulanate (98.8%) was supplied by Antibióticos S. A. (León, Spain). Other chemicals were of reagent grade. 2-Amino-2-methylpropane-1,3-diol was recrystallized from ethanol. 2-Methoxyethanolamine and 2-amino-2-methylpropan-1-ol were redistilled prior to use. Distilled and deionized water (18 MΩ resistance) was used for the preparation of buffer solutions.

Kinetic Procedure.—The amines were used both as buffers and as nucleophiles. A constant pH was maintained throughout the reaction with a pH-stat (a titrimeter assembly consisting of an E-614 Impulsomat, an E-655 Dosimat, and an E-632 pH-meter from Metrohm, Herisau, Switzerland). All reactions were conducted at 35.0 ± 0.1 °C and the ionic strength was adjusted to 0.5 mol l^{-1} with sodium perchlorate. A 1 × 10^{-4}M concentration of EDTA was used in order to avoid the catalytic

effect induced by small amounts of metal ions that may be present as impurities.

The rates of reaction were determined by monitoring the development of the absorbance at 312 nm of the product resulting from the reaction of clavulanate ion and imidazole,[5] on a Spectronic 2000 spectrophotometer (Bausch–Lomb). Under the same working conditions, this reaction is some three orders of magnitude faster than the decomposition of clavulanic acid.

The analyses of the experimental reproducibility, and of the various time plots, indicate the relative uncertainty on the observed rate constant, k_{obs}, to be 10% at the 95% confidence level.

Ionization Constants.—The apparent pK_a of each of the amines was determined at 35.0 °C and an ionic strength of 0.5 mol l^{-1} by potentiometric titration.

Results and Discussion

Over the pH range 8.05—9.80, the decomposition of lithium clavulanate in aqueous solution, in the presence of an excess of amines and at constant pH, follows pseudo-first-order kinetics. The pseudo-first-order rate constant, k_{obs}, obtained from the slope of the semilogarithmic plots of the residual concentration *versus* time by a least-squares treatment, is the sum of a first-order hydrolysis rate, k_{pH}, and the first-order rate constant depending on the concentration of amines, k_{amine}. The equation

$$k_{obs} = k_{pH} + k_{amine} \qquad (1)$$

allows the determination of k_{amine} once the value of k_{pH} is known for each pH.

In order to study the influence of the chemical species present in the aqueous solution of each amine on the stability of lithium clavulanate, and to determine its catalytic constants, experiments were carried out in which k_{obs} was experimentally determined as a function of the concentration of the amine, keeping constant the pH, ionic strength, temperature, and concentration of EDTA. The representation of k_{obs} and k_{amine} *versus* the total amine concentration, $[amine]_T$, for each pH, is linear for 2-amino-2-methylpropane-1,3-diol and 2-amino-2-methylpropan-1-ol. In the case of 2-methoxyethylamine (Figure 1), 2,2,2-trifluorethylamine, and aminoacetonitrile, the plots showed an upward curvature at any pH value.

In Tables 1, 2, and 3, the results obtained for 2-amino-2-
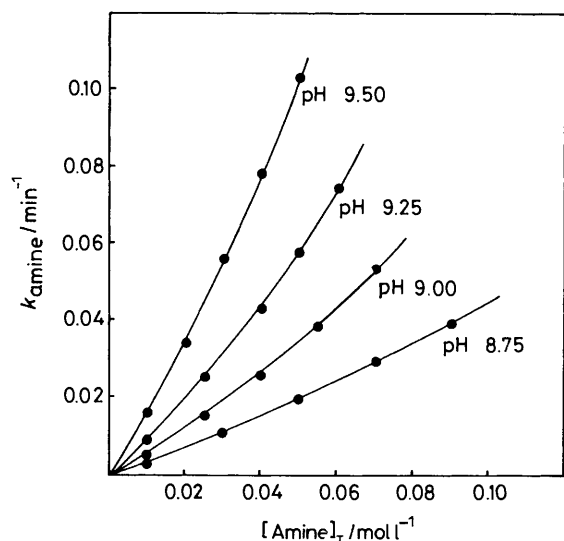
Figure 1. Pseudo-first-order rate constants, k_{amine}, versus total amine concentration for the reaction of lithium clavulanate with 2-methoxyethylamine at 35.0 °C, 2×10^{-4}M-EDTA, and 0.5M-ionic strength. Curves were calculated from equation (2) and rate constants in Table 3

Table 1. Pseudo-first-order rate constants, k_{obs}, for lithium clavulanate hydrolysis and aminolysis in 2-amino-2-methylpropane-1,3-diol buffers (pK_a 8.62) at 0.5M ionic strength, 1×10^{-4}M-EDTA, and 35.0 °C. Initial clavulanic acid concentration 5×10^{-4} mol l^{-1}

pH	$[Amine]_T/mol\ l^{-1}$	k_{obs}/min^{-1}
9.25	0.030	0.0120
9.25	0.060	0.0169
9.25	0.100	0.0236
9.25	0.150	0.0339
9.25	0.200	0.0412
8.85	0.030	0.0070
8.85	0.060	0.0111
8.85	0.100	0.0160
8.85	0.150	0.0225
8.85	0.200	0.0294
8.45	0.050	0.0061
8.45	0.100	0.0102
8.45	0.150	0.0145
8.45	0.200	0.0191
8.45	0.250	0.0243
8.05	0.050	0.0030
8.05	0.100	0.0052
8.05	0.180	0.0091
8.05	0.240	0.0118
8.05	0.300	0.0152

Table 2. Pseudo-first-order rate constants, k_{obs}, for lithium clavulanate hydrolysis and aminolysis in 2-amino-2-methylpropan-1-ol buffers (pK_a 9.52) at 0.5M ionic strength, 1×10^{-4}M-EDTA, and 35.0 °C. Initial clavulanic acid concentration 5×10^{-4} mol l^{-1}

pH	$[Amine]_T/mol\ l^{-1}$	k_{obs}/min^{-1}
9.80	0.020	0.0260
9.80	0.040	0.0350
9.80	0.060	0.0440
9.80	0.080	0.0521
9.80	0.100	0.0603
9.45	0.030	0.0157
9.45	0.060	0.0210
9.45	0.100	0.0306
9.45	0.150	0.0401
9.45	0.200	0.0501
9.15	0.050	0.0107
9.15	0.100	0.0168
9.15	0.150	0.0223
9.15	0.200	0.0282
9.15	0.250	0.0337
8.75	0.050	0.0050
8.75	0.100	0.0075
8.75	0.180	0.0120
8.75	0.240	0.0151
8.75	0.300	0.0180

Table 3. Pseudo-first-order rate constants, k_{obs}, for lithium clavulanate hydrolysis and aminolysis in 2-methoxyethylamine buffers (pK_a 9.35) at 0.5M ionic strength, 1×10^{-4}M-EDTA, and 35.0 °C. Initial clavulanic acid concentration 5×10^{-4} mol l^{-1}

pH	$[Amine]_T/mol\ l^{-1}$	k_{obs}/min^{-1}
9.50	0.0100	0.0258
9.50	0.0200	0.0443
9.50	0.0300	0.0658
9.50	0.040	0.088
9.50	0.050	0.113
9.25	0.0100	0.0157
9.25	0.0250	0.0315
9.25	0.040	0.0499
9.25	0.050	0.0644
9.25	0.060	0.081
9.00	0.0100	0.0093
9.00	0.0250	0.0188
9.00	0.040	0.0299
9.00	0.055	0.0423
9.00	0.070	0.0573
8.75	0.0100	0.0055
8.75	0.0300	0.0132
8.75	0.050	0.0218
8.75	0.070	0.0315
8.75	0.090	0.0414

methylpropane-1,3-diol, 2-amino-2-methylpropan-1-ol, and 2-methoxyethylamine are summarized.

These pseudo-first-order rate constants apparently obey the general relationship previously reported for penicillins[6-9] and cephalosporins:[8]

$$k_{amine} = k_1[amine] + k_2[amine]^2 + k_3[amine][OH^-] \quad (2)$$

Equation (2) can be re-written as:

$$\frac{k_{amine}}{[amine]} = k_1 + k_3[OH^-] + k_2[amine] \quad (3)$$

where k_1 represents the unassisted or water-catalysed reaction of free amine on the β-lactam moiety, k_2 represents the general base-catalysed and nucleophilic reaction assisted by a second molecule of amine, and k_3 is the hydroxide-ion-catalysed third-order rate constant of nucleophilic displacement by the amine. Equation (3) predicts that plots of $k_{amine}/[amine]$ versus [amine] will provide k_2 as the slope and $k_1 + k_3[OH^-]$ as the intercept. The plots of these intercepts versus hydroxide-ion concentration provide k_3 as the slope and k_1 as the intercept. It was noticed that the experimental uncertainties on k_1, k_2, and k_3 for 2,2,2-trifluoroethylamine and aminoacetonitrile are appreciably larger than for the more basic amino alcohols.

Representations of equation (3) for 2-amino-2-methyl-propane-1,3-diol, 2-amino-2-methylpropan-1-ol, and 2-methoxyethylamine from the data from Tables 1—3 are shown in
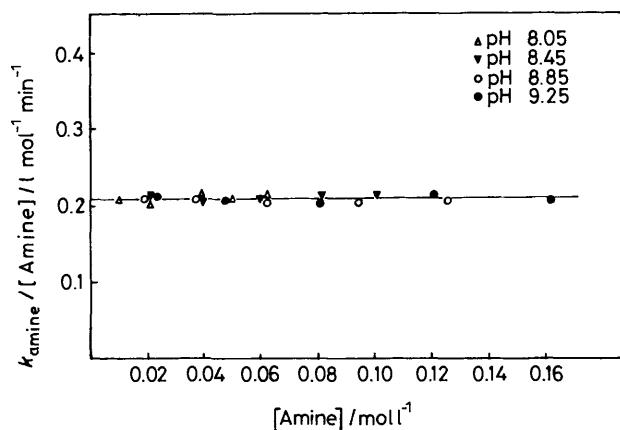
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Figure 2. Apparent second-order rate constants *versus* free amine concentration for the reaction of lithium clavulanate with 2-amino-2-methylpropane-1,3-diol at 35.0 °C, 1 × 10^{-4}M-EDTA, and 0.5M ionic strength
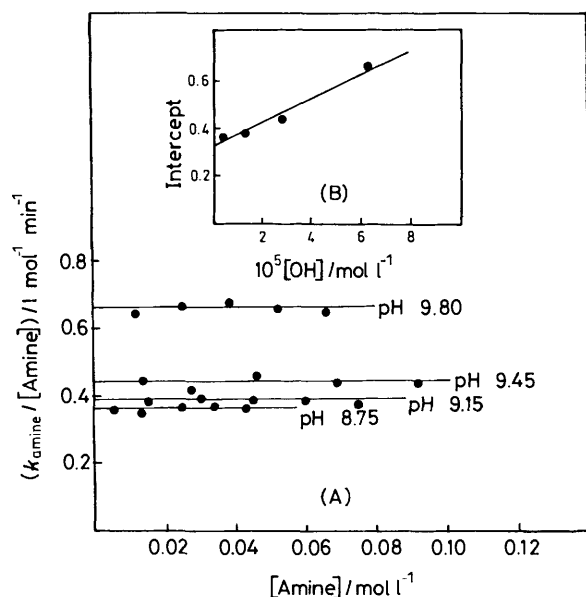

Figure 3. Apparent second-order rate constants *versus* free amine concentration (A) and the intercepts of Figure 3 (A) *versus* hydroxide-ion concentration (B) the reaction of lithium clavulanate with 2-amino-2-methylpropan-1-ol at 35.0 °C, 1 × 10^{-4}M-EDTA, and 0.5M ionic strength

Figure 4. Apparent second-order rate constants *versus* free amine concentration (A) and the intercepts of Figure 4 (A) *versus* hydroxide-ion concentration (B) the reaction of lithium clavulanate with 2-methoxyethylamine at 35.0 °C, 1 × 10^{-4}M-EDTA, and 0.5M ionic strength

Figure 5. Plot of the rate constant, k_1, for the reaction of clavulanate ion with amines as a function of pK_a at 35 °C and ionic strength 0.5 mol l^{-1}. The numbers refer to the amines listed in Table 4

Figures 2—4. As can be seen, for the 2-amino-2-methylpropane-1,3-diol, the constant k_{amine} is practically independent of the k_2[amine] and k_3[amine][OH$^-$] terms (Figure 2). In the case of 2-amino-2-methylpropan-1-ol, this constant is independent of the k_2[amine] term. In order to determine the values of k_1 and k_3, the intercepts are plotted with respect to the concentration of OH$^-$ (Figure 3). The k_{amine} constant for 2-methoxyethylamine depends on all the terms which appear in equation (3). In this case, the lines corresponding to each pH have practically the same slopes, which are finite, and allow for the determination of k_2 for this amine (Figure 4). The values k_1 and k_3 were determined as in the previous case. For 2,2,2-trifluoroethylamine and aminoacetonitrile k_{amine} also depends on all the terms which appear in equation (3).

The values of the constants for these amines are summarized in Table 4. It can be seen that the values of k_1 increase with increased pK_a values thus generating a Brønsted plot (Figure 5)
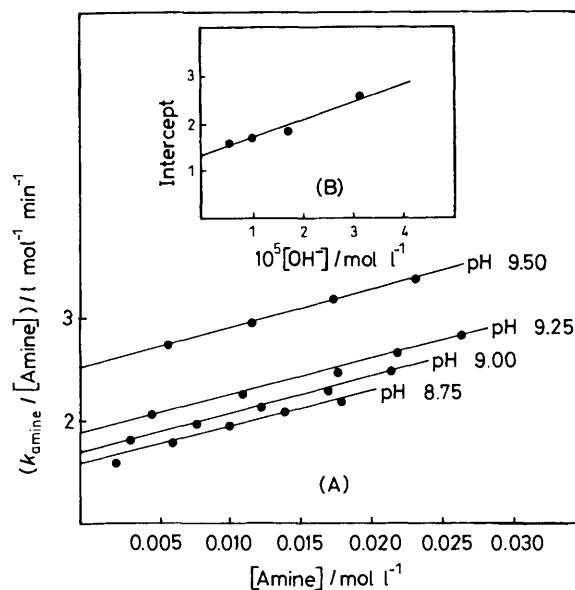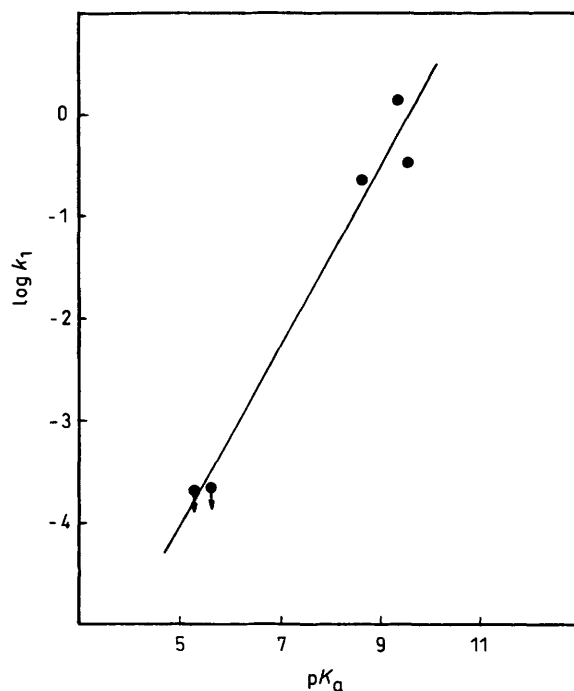
with a slope of 0.90. This value is quite close to that found for the aminolysis of benzylpenicillin and strongly suggests that the decomposition mechanism proposed by Morris *et al.*[10] is also operative in the present case.

For 2-amino-2-methylpropane-1,3-diol, the constant k_2 is practically zero, probably owing to steric hindrance in the simultaneous nucleophilic attack of two molecules of this amine. In 2-amino-2-methylpropan-1-ol, this hindrance may be

Table 4. Aminolysis rate constants for lithium clavulanate in the presence of the amines discussed at 35.0 °C, 1 × 10^{-4}M-EDTA, and 0.5M ionic strength

Amine	pK_a	k_1/l mol^{-1} min^{-1}	k_2/l^2 mol^{-2} min^{-1}	10^{-3} k_3/l^2 mol^{-2} min^{-1}
Aminoacetonitrile	5.34	$<2.1 \times 10^{-4}$	$\leqslant 8.9 \times 10^{-3}$	<0.015
2,2,2-Trifluoroethylamine	5.74	$<2.2 \times 10^{-4}$	$\leqslant 1.0 \times 10^{-2}$	<0.015
2-Amino-2-methylpropane-1,3-diol	8.62	0.22	a	0.19
2-Amino-2-methylpropan-1-ol	9.52	0.32	a	3.57
2-Methoxyethylamine	9.35	1.36	33.5	35.5

a Values too small for measurement.

R = H or CH$_3$
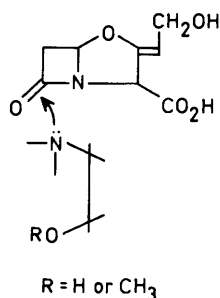
Scheme 1.

smaller because it has one less hydroxy group. These findings are in close agreement with those of Yamana et al.[11] on the reaction of benzylpenicillin with amines containing hydroxy groups.

For 2-methoxyethylamine, these steric effects are smaller than for the amino alcohols. This would ensure that none of the constants in the proposed kinetic model is zero.

These results strongly suggest that the fundamental process leading to the opening of the β-lactam moiety is the nucleophilic attack of the carbonyl group by the amino nitrogen (Scheme 1). We also noted that field effects induced by the hydroxy groups also reduce the nucleophilicity of the nitrogen. Thus both steric and electronic effects account for the fact that amino alcohols

and 2-methoxyethylamine are less efficient than aliphatic primary amines[12] at promoting the decomposition of clavulanate ion.

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