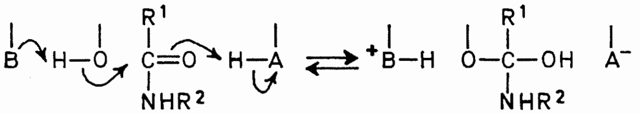
Models for Enzyme–Substrate Complexes: Kinetics of Hydrolysis of Bicyclic Immonium Ether Perchlorates

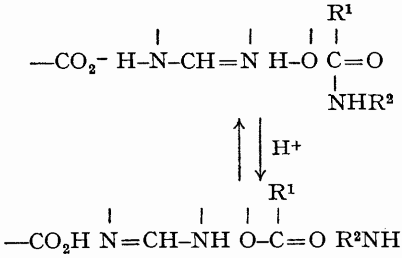
By B. A. W. COLLER, F. W. EASTWOOD,* L. Y. FOO, and N. C. G. H. Y. HO
(*Chemistry Department, Monash University, Wellington Road, Clayton, Victoria, 3168, Australia*)

Summary The rates of hydrolysis of bicyclic immonium ether perchlorates have been measured and the results have been interpreted in terms of unstable cyclol intermediates; on reaction with sodium methoxide the perchlorates yielded bicyclic lactam acetals.

THE changes in bonding postulated to occur in a number of mechanisms which have been put forward for enzymic hydrolysis can be represented by the following equation in which an initial tetrahedral intermediate, formed by the attack of a hydroxyl group on the amide carbonyl, collapses into an acylated enzyme and a free amine.[1]

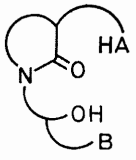


However, the published *X*-ray crystallographic data on α-chymotrypsin[2] do not give direct evidence of a specific group concerned with protonation of the carbonyl oxygen atom but show that the base which removes the seryl hydroxyl proton is made up of a tautomeric system which relays a negative charge:



$$\text{—CO}_2^-\ \text{H–N–CH=N H–O}\ \underset{\text{NHR}^2}{\overset{\text{R}^1}{\text{C}}}\text{=O} \quad \overset{\text{H}^+}{\rightleftarrows} \quad \text{—CO}_2\text{H N=CH–NH O–}\overset{\text{R}^1}{\text{C}}\text{=O R}^2\text{NH}_2$$

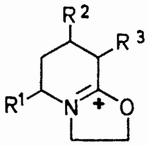
In order to test the validity of ideas of this kind, we are attempting the synthesis of model compounds which in solution should show reversible intramolecular addition of a hydroxy-group to an amide under conditions which do not involve any considerable change in the acidity of the solvent medium. Such models of the enzyme–substrate complex require an amide linkage, a hydroxy-group, an acidic function (HA) to protonate the amide carbonyl, and a basic function (B) to remove the proton from the hydroxy-group. In our laboratory the design of such molecules has centred on *N*-hydroxyalkyl-lactams having one or both of the groups HA and B attached as shown in the schematic formula (**1**).



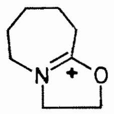
(1)

We report kinetic studies on the alkaline hydrolysis of the cations (**2**)—(**6**) which were investigated as a preliminary to the study of compounds of type (**1**). *N*-(2′-Hydroxyethyl) derivatives of a series of 2-piperidones and of 2-oxohexamethyleneimine and *N*-(3′-hydroxypropyl) derivatives of 2-pyrrolidone, a series of 2-piperidones and of 2-oxohexamethyleneimine were treated with perchloric acid[3] to form the corresponding crystalline perchlorate salts of the bicyclic immonium ether cations (**2**)—(**6**).†

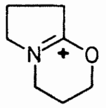
	R¹	R²	R³
a	H	H	H
b	Me	Me	H
c	Me	Me	Me
d	H	H	Me



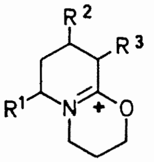
(2)



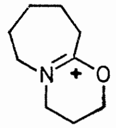
(3)



(4)



(5)



(6)

Treatment of each of the salts (**2**)—(**6**) with alkali under preparative conditions yielded the corresponding *N*-(ω-hydroxyalkyl)-lactams. The rates of hydrolysis of each compound were obtained from tracings of time against the volume of 0·05 M-sodium borate buffer added in maintaining constant pH at 25°.[4] The curves gave straight line Guggenheim plots from which pseudo-first-order rate constants $k_{(1)}$ were calculated. The results were analysed on the basis of the kinetic equation using the steady-state approximation for the intermediate B. With the exception of the

$$\text{A} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{B} \xrightarrow{k_2} \text{C}$$

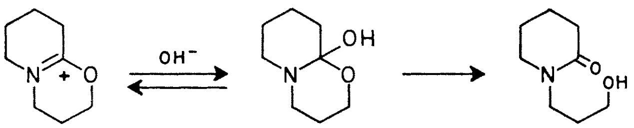
compound (**6**), first-order dependence on hydroxide ion concentration was confirmed when essentially constant values of the pseudo-second-order rate constant $k_{(2)}$ in the expression $k_{(2)} = k_1k_2/(k_{-1} + k_2)$ were obtained over a range of pH values. The determined values of $k_{(2)}$ for each compound are given in the Table.

The mechanism of hydrolysis of each of the compounds (**2a**)—(**5c**) has been interpreted in terms of the formation of

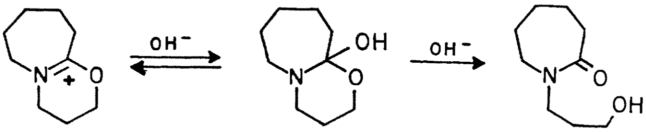
---

† Satisfactory analyses and spectroscopic data have been obtained for all compounds described in this communication.

a tetrahedral intermediate which rapidly collapses to yield the hydroxy-lactam, *e.g.*



For the perchlorate (**6**), the shape of the titration curves measured at constant pH under the same conditions differed significantly from those of the other salts, indicating that the reaction involved more complex kinetics. Analysis of the data confirmed a second-order dependence on hydroxide concentration at lower pH. It was assumed that this second-order dependence lay in the breakdown of the tetrahedral intermediate according to the equation,



and the rate equation was modified to have the steady state form $-\mathrm{d}A/\mathrm{d}t = k_{(1)}A = k_1k_2A[\mathrm{OH}^-]/(k_{-1} + k_2[\mathrm{OH}^-])$. From a plot of $k_{(1)}/[\mathrm{OH}^-]^2 = k_{-1}/k_1k_2 + [\mathrm{OH}^-]/k_1$, the determined value of k_1 at 25° was 571 ± 70 l mole^{-1}sec^{-1} and the ratio $k_{-1}/k_2 = 10^{-6} \times 1{\cdot}92 \pm 0{\cdot}02$ l mole^{-1}.

Evidence in support of the position of attack by the nucleophile in the reactions was adduced from the formation of the corresponding bicyclic lactam acetals N–C(OMe)O from (**2**a), (**3**), (**4**), (**5**a), and (**6**) when the respective perchlorate salts were treated with sodium methoxide in ether.

The second-order rate constants given in the Table reflect the increased resistance of the oxazinium salts (**4**), (**5**a), (**5**b), and (**5**c) to attack by hydroxide ion compared with the oxazolium salts (**2**a), (**2**b), (**2**c), (**2**d), and (**3**). Within each series, also, increased substitution can be seen to decrease the rate of hydrolysis both by a general decrease in polarity of the substrate and, particularly with substitution at the α-position to the immonium ether carbon atom, by direct steric hindrance. While the rate of hydrolysis of (**6**) is comparable with those of the remaining oxazinium salts, the evidence points to a marked increase in the stability of the cyclol intermediate since attack by a hydroxide ion is required for its breakdown into the amide system.

Pseudo-second-order rate constants for the hydrolysis of immonium ether perchlorates at 25°

Compound	pH range[a]	$k_{(2)}$ (l mole^{-1}sec^{-1})	95% confidence limits
(**2**a)	6·91—7·41	5270	±156
(**2**b)	7·12—7·72	4474	±264
(**2**c)	7·32—7·91	2444	±147
(**2**d)	7·22—7·82	3838	±188
(**3**)	7·71—8·22	3190	±148
(**4**)	8·31—8·81	301	± 12
(**5**a)	8·21—8·71	932	± 29
(**5**b)	8·40—8·71	242	± 11
(**5**c)	8·32—8·71	188	± 10

[a] Measurements were made at 6 or 7 different pH's over the range indicated for each compound.

Comparison of the chemical shift of the protons on the α-carbon atom in the n.m.r. spectra of the unsubstituted immonium ethers [δ 2·74—3·0 (D_2O)], the lactam acetals [δ 1·78—2·1 (CCl_4)], and the hydroxy-lactams [δ 2·3—2·55 ($CDCl_3$)] gave a direct method for detecting a tetrahedral intermediate. Hydrolysis of the perchlorate salt (**6**) into the corresponding hydroxy-lactam could be followed at ambient temperature in the n.m.r. spectrometer, but no evidence for the presence of the tetrahedral intermediate was obtained.

(*Received, May 7th*, 1970; *Com.* 698.)

---

[1] M. L. Bender and F. J. Kézdy, *J. Amer. Chem. Soc.*, 1964, **86**, 3704; T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, Benjamin, New York, 1966.

[2] D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, 1969, **221**, 337; M. R. Hollaway, *Ann. Reports*, 1968, **65**, *B*, 612.

[3] N. J. Leonard, K. Conrow, and R. R. Sauers, *J. Amer. Chem. Soc.*, 1958, **80**, 5185; W. S. Schneider and B. Muller, *Chem. Ber.*, 1960, **93**, 1579.

[4] R. P. Bell and B. A. W. Coller, *Trans. Faraday Soc.*, 1964, **60**, 1087.