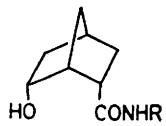
Intramolecular Nucleophilic and General Acid Catalysis in the Hydrolysis of an Amide

By JEFFREY J. MORRIS and MICHAEL I. PAGE*
(*Department of Chemical Sciences, The Polytechnic, Huddersfield* HD1 3DH)

Summary The relative effectiveness of intramolecular nucleophilic and general acid catalysis are compared in the hydroxide-ion catalysed hydrolysis of *N*-(2-aminoethyl)-2-*endo*-hydroxy-6-*endo*-norbornamide in its amino protonated form; the rate enhancement of *ca.* 10^9 is due mainly to nucleophilic catalysis by the neighbouring hydroxy group with only a relatively minor contribution from the protonated amino group acting as a general acid.

IT is often suggested that the transfer of a proton between electronegative atoms of substrates and those of acidic or basic groups in enzymes contributes to the large rate enhancement observed in enzyme-catalysed reactions.[1] The contribution of this effect may be divided into two separate problems. (i) What is the importance of catalysis by acidic or basic species compared with that of proton transfer to or from water? (ii) What is the importance of proton transfer in the enzyme–substrate complex being of lower kinetic order than the analogous intermolecular reaction involving acidic or basic species in solution? The first problem has been examined extensively,[2] but the second is less well understood.

α-Chymotrypsin is a proteolytic enzyme which catalyses the hydrolysis of amides. The mechanism of the reaction involves the formation of an acyl–enzyme intermediate by the transfer of the acyl group from the substrate to a serine hydroxy group in the enzyme.[3] For some substrates this process is thought to be facilitated by general acid catalysis, namely proton donation to the departing amino group from a protonated histidine residue in the enzyme.[4]



(I)

As a model for this enzyme-catalysed reaction we have studied the hydrolysis of some 2-*endo*-hydroxy-6-*endo*-norbornamides (I). The rate constants for the hydroxide-ion catalysed hydrolysis of these amides are *ca.* 10^7 to 10^8 fold greater than those for the corresponding amides lacking the neighbouring hydroxy group.[5] This rate acceleration is attributed to intramolecular catalysis in which the hydroxy group displaces the amine to form the lactone as an intermediate which is then hydrolysed to 2-*endo*-hydroxynorbornane-6-*endo*-carboxylic acid.

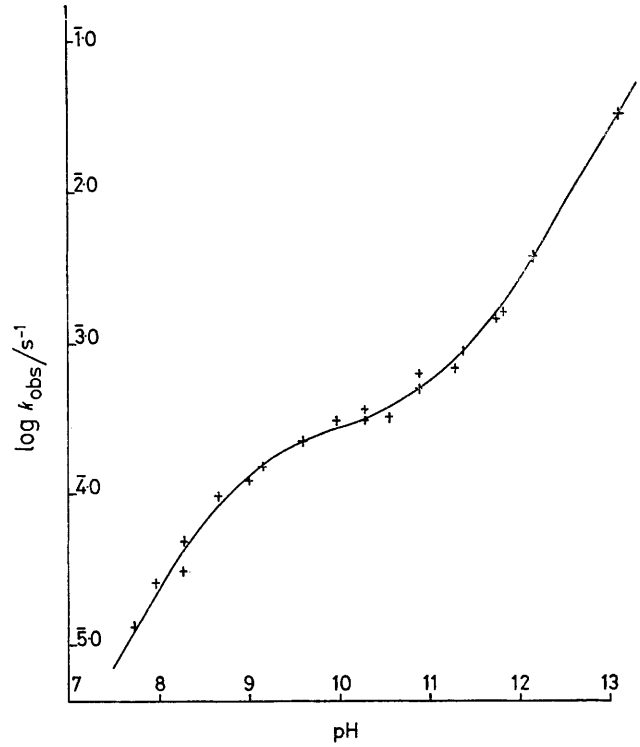


FIGURE. Plot of log k_{obs}/s^{-1} against pH for the hydrolysis of (I, R = $CH_2CH_2NH_2$). 30·0 °C, I = 0·2 M (KCl), in water. The solid line is calculated using the constants given in the text.

In order to examine the importance of general acid catalysis in this reaction, we have studied the hydrolysis of the amide derived from ethylenediamine (I, R = $CH_2CH_2NH_2$).† The pH–rate profile for the hydrolysis of this compound (Figure) is sigmoid, and is interpreted in terms of the hydroxide-ion catalysed hydrolysis of the amide with the amine nitrogen protonated (RNH_3^+) and non-protonated (RNH_2) [equation (1)]. The solid line of the Figure is calculated from equation (2) using K_W = $1{\cdot}48 \times 10^{-14}$, k_1 = 0·182 l mol^{-1} s^{-1} (s.d. 6·8%), k_2 =

$$\text{Rate} = k_1(RNH_2)(OH^-) + k_2(RNH_3^+)(OH^-) \quad (1)$$

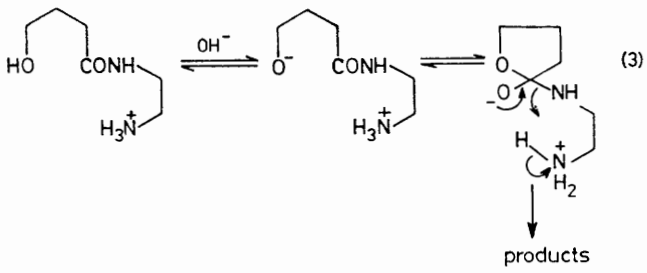
$$k_{obs} = (k_1K_WK_a/10^{-pH} + k_2K_W)/(10^{-pH} + K_a) \quad (2)$$

15·3 l mol^{-1} s^{-1} (s.d. 4·5%), and K_a = $9{\cdot}7 \times 10^{-10}$ mol l^{-1} (s.d. 7·3%). The value of k_2 is *ca.* 150 times greater than that predicted for the hydroxide-ion catalysed hydrolysis of an amide (I) where the pK_a of the conjugate acid of the departing amine is 7·5.‡ This rate enhancement is

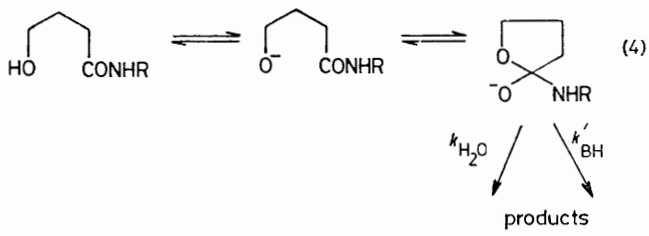
† M.p. 105—106 °C; i.r. (Nujol) 1640, 3100, 3260, 3290, and 3410 cm^{-1}; n.m.r. (CD_3OD) δ 4·20 (m, 1H), 3·30 (m, 3H), 2·90 (t, 2H), and 1·75 (m, 8H). Satisfactory elemental analyses were obtained.

‡ At pH >7 the rate law for the hydrolysis of *N*-substituted norbornamides is rate = k_{OH}[amide][OH$^-$] + k_B[amide][B] where B is a base catalyst. The dependence of log k_{OH} upon the pK_a of the conjugate acid of the leaving group amine is 0·3.

attributed to intramolecular general acid catalysis of hydrolysis by the protonated terminal amino group, facilitating breakdown of the tetrahedral intermediate [equation (3)]. The term k_2 is, of course, kinetically equivalent to a spontaneous hydrolysis of the unprotonated amide, but this is not observed for the other amides studied,[5] and ascription of this term to intramolecular general base catalysis by the terminal amino group removing a proton from the hydroxy group would involve the unlikely formation of a ten-membered cyclic transition state.



(3)



(4)

Intermolecular kinetic general base catalysis, by added amines and oxygen bases, is observed in the hydrolysis of (I).‡ However, this is attributed to the kinetically equivalent mechanism of general acid catalysed breakdown of the tetrahedral addition intermediate formed from hydroxide-ion and the amide [k'_{BH}, equation (4)]. The evidence for this pathway is that the dependence of the rate constants for the hydroxide-ion catalysed hydrolysis of a series of amides (I) upon the basicity of the leaving group amine gives a Brønsted β-value of 0·3.‡ This is consistent with rate-limiting breakdown of the tetrahedral intermediate with the departing amino group almost fully protonated by proton donation from water [k_{H_2O}, equation (4)]. An overall β-value of *ca.* 0·4 is expected for the conversion of an amide into a tetrahedral intermediate in which the nitrogen is protonated.[6]

Although the absolute rate increase caused by intramolecular general acid catalysis [equation (3)] relative to the 'water' reaction [k_{H_2O}, equation (4)] is considerable the contribution of intramolecularity itself is small. The second-order rate constant, k_2, for the hydroxide-ion catalysed hydrolysis of (I, R = $CH_2CH_2NH_3^+$) may be divided by the third-order rate constant§ k_{BH}, for the intermolecular general acid catalysed reaction of hydroxide-ion with (I, R = CH_2CH_2Me) *i.e.*, for the pathway proceeding *via* k'_{BH} [equation (4)]. This ratio of rate constants gives the effective molarity[7] of the protonated amino group in the intramolecular reaction [equation (3)] compared with a protonated amine of similar acidity in the intermolecular reaction [k'_{BH}, equation (4)]. The value obtained, *ca.* 1 l mol^{-1}, is small presumably because the entropy associated with the low-frequency vibrations in the loose transition-state involved offsets the large loss of translational and rotational entropy that normally occurs in intermolecular reactions.[7]

In summary, in our model system, nucleophilic catalysis makes a large contribution (a factor of *ca.* 10^8) to the rate enhancement whereas general acid catalysis makes a much smaller contribution (*ca.* 1). This is in agreement with the assessment of the importance of these contributions to enzyme-catalysed reactions.[8]

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§ This is kinetically equivalent to the rate constant for general base catalysis $k_{BH} = k_B K_a / K_W$.

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