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Intramolecular General Base Catalysed Alcoholysis of Amides

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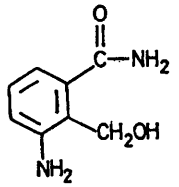
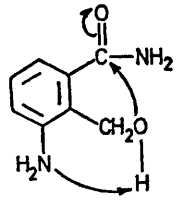
Summary The pH-independent cyclization of 3-amino-2-hydroxymethylbenzamide proceeds with intramolecular general base catalysis at a rate 10^3 times greater than that for the 4-, 5-, and 6-amino, or unsubstituted 2-hydroxymethylbenzamide.

The α chymotrypsin catalysed hydrolysis of ester and amide substrates involves formation and decomposition of an acyl enzyme intermediate which is an ester of serine-195.[1]



(1)



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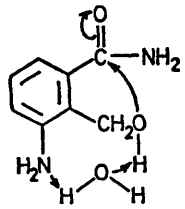

(4)

In the generally accepted mechanism histidine-57 assists acylation of serine by classical general base catalysis, partially abstracting a proton from the hydroxyl group as it attacks the carbonyl of the substrate.[1]

Neighbouring hydroxyl groups have been studied as intramolecular nucleophiles in the decomposition of amides[2] and carbamate esters.[3] The latter reactions[3] proceed through attack of the oxygen anions, with effective molarities for the neighbouring group of 10^5—10^8 M in comparison with analogous bimolecular reactions ($s^{-1}/M^{-1}\ s^{-1}$). Apparent hydroxide ion catalysis of the cyclization of 2-hydroxymethylbenzamide[4] and ethyl 2-hydroxymethylbenzoate[5] to phthalide is also 10^5 times more favourable than hydroxide ion catalysed hydrolysis of benzamide or ethyl benzoate. The cyclization reactions of the 2-hydroxymethylbenzoic acid derivatives provide models for acylation of α-chymotrypsin by amides and esters since catalysis by general bases, including imidazole, is observed.[4,5] There have been no previous studies of intramolecular alcoholysis reactions where catalysis by a general base is also intramolecular.

We have measured rate constants for the cyclization of 3-amino-2-hydroxymethylbenzamide (**1**) to 4-aminophthalide at 30° in 50% dioxan–H_2O (v/v) and for comparison purposes the corresponding rate constants of the 4-, 5-, and 6-amino derivatives. The amino-2-hydroxymethylbenzamides were synthesized by reduction of the corresponding nitro derivatives. The rate of cyclization of (**1**) was measured by following the appearance of aminophthalide at 290 nm. The Figure shows log k_{ex} *vs.* pH at 30° where k_{ex} is the rate constant obtained by extrapolation to zero buffer concentration. The inflection point in the profile at pH 3·5 corresponds closely with the spectrophotometrically determined amino group pK_a of 3·8. The pH-independent reaction from pH 3·5—10 ($k_0 = 10^{-3}\ s^{-1}$) is approximately 10^3 times faster than with 2-hydroxymethyl-

benzamide[4] and its 5- and 6-amino substituted derivatives. Due to a relatively rapid hydroxide ion catalysed reaction, there is no plateau in the profile for cyclization of 4-amino-2-hydroxymethylbenzamide (**2**). Log k_{ex} *vs.* pH for (**2**) in

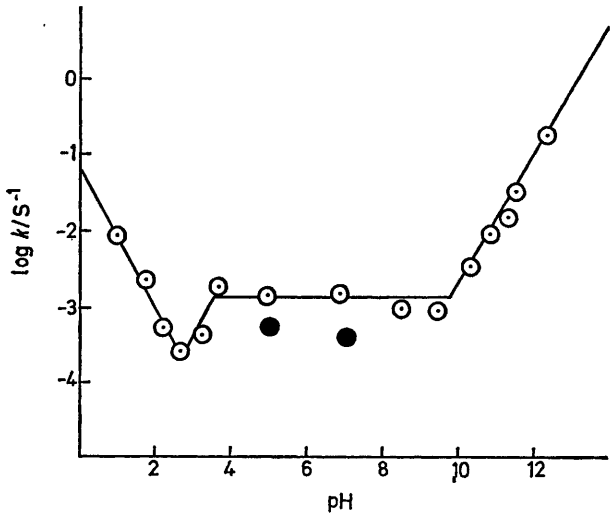

FIGURE. Plot of log k_{ex} for cyclization of (**1**) *vs.* pH at 30° and $\mu = 0{\cdot}5$ in 50% dioxan–H_2O (v/v) ⊙, and 50% dioxan–D_2O ●. The rate constants are for appearance of product (4-amino phthalide) and were obtained by extrapolation to zero buffer concentration.

50% dioxan–H_2O intersects at 10^{-6} s^{-1} at 30° which represents a maximum value for a pH-independent reaction. Thus, the rate constant for pH-independent cyclization of (**1**) must be at least 10^3 times greater than that for cyclization of the 4-amino derivative. The amino group of the 4- and 5-derivatives cannot interact with either the hydroxymethyl group or a tetrahedral intermediate.

The pH-independent cyclization of (**1**) proceeds 2·82 times more slowly in 50% dioxan–D_2O than 50% dioxan–H_2O indicating that proton transfer takes place in the critical transition state. Bimolecular general base catalysis is not statistically significant in hydrolysis of (**1**) in contrast with the pronounced general base catalysis observed with the other 2-hydroxymethylbenzamides in the series. It would not be expected that bimolecular buffer catalysis would compete with an intramolecular reaction. The rate enhancement of 10^3 in conjunction with the solvent isotope effect is therefore most likely due to intramolecular general base catalysis by the neighbouring amino group. A Stuart Briegleb model reveals that the steric situation is not favourable for the concerted process in (**3**), and this is substantiated by the ΔS^* of -23 J K^{-1} mol^{-1} for the pH-independent reaction. General base catalysis through one or more solvent molecules (**4**) would be in accord with the highly negative ΔS^* and would partially avoid the steric complications of mechanism (**3**).

Employing the pK_{app} of (**1**) of 3·5, an effective molarity of 15M can be calculated for the 3-amino group in comparison with bimolecular catalysis by an amine of the same pK_a in cyclization of the 4-amino derivative. In view of the rate enhancement of 10^3 due to the neighbouring amino group of (**1**), it is clear that if the appropriate functional groups were properly aligned, as is presumably the case in the active sites of enzymes, much larger rate enhancements and effective molarities would be possible.

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