Carboxy-group Participation in Acetal Hydrolysis. The Hydrolysis of Benzaldehyde Disalicyl Acetal

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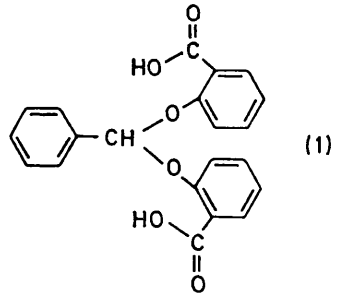
Summary The pH–rate constant profile for hydrolysis of benzaldehyde disalicyl acetal in either 50% dioxan–H_2O (v/v) or in H_2O at 25° is bell-shaped and reveals a maximum enhancement of k_{obs} in comparison with the dimethyl ester of $2{\cdot}7 \times 10^9$

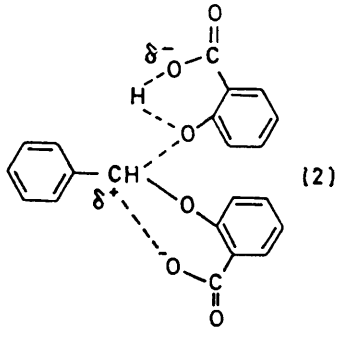
THE three-dimensional structure of the glycosidic enzyme lysozyme has been elucidated by *X*-ray crystallographic analysis,[1] and the complete amino-acid sequence of the enzyme has been determined [2] Carboxyl groups from glutamic acid-35 and aspartic acid-52 presumably form part of the active site The pH–rate profile for the enzyme is bell-shaped, indicating that two groups are possibly involved [3] Several mechanisms have been suggested[4,5] which differ in detail, but all involve glutamic acid-35 acting as a general acid A mechanism receiving recent support involves general acid catalysis by glutamic acid-35 and electrostatic stabilization of a developing carbonium ion by the aspartate anion [5]

In view of the detailed information concerning the structure of lysozyme, a number of physical organic studies have been carried out to determine the structural features in an acetal that will lead to general acid catalysed hydrolysis reactions [6] Intramolecular facilitation of acetal hydrolysis by one carboxyl group has been observed in several cases,[7–9] but bifunctional catalysis of acetal hydrolysis by two carboxyl groups has not been observed [8]

In an attempt to obtain rate enhancements in simple chemical systems of the magnitude seen in enzymatic reactions and to determine whether bifunctional catalysis is a chemically reasonable mechanism for lysozyme, we have studied various dicarboxyl substituted acetals In the Figure is shown a plot of k_{obs} for appearance of salicylic acid, *vs* pH for the hydrolysis of benzaldehyde disalicyl acetal (**1**) in 50% dioxan–H_2O (v/v) at 25°



(1)



(2)

A similar profile resulted with H_2O as solvent A bell-shaped plot is obtained Thus, the observed kinetics for hydrolysis of (**1**) are similar to those observed in lysozyme-catalysed reactions In comparison with the corresponding dimethyl ester (k_H $4{\cdot}37 \times 10^{-3}$ M^{-1} s^{-1} in 50% dioxan–H_2O) the maximum difference in k_{obs} is $2\ 7 \times 10^9$, showing that intramolecular carboxyl group participation is occurring This rate enhancement is considerably greater than

Rate constants for hydrolysis of benzaldehyde disalicyl acetal at 25°

Solvent	$k_1 \times 10^{-4}$ (M^{-1} s^{-1})	$k_2 \times 10^{-4}$ (M^{-1} s^{-1})	k_{H_2A} (s^{-1})	k_{H_1A} (s^{-1})	pK_a	pK_a'
50% Dioxan-H_2O	1 83	1170	0 042	2 74	5 64	6 63
H_2O	0 0219	9 75	0 105	3 23	3 32	4 48

found previously with monocarboxyl substituted acetals where rate enhancements compared to the corresponding methyl esters or *para* carboxyl substituted isomers are 600 for 2 methoxymethoxybenzoic acid,[7] 10^4 for *o*-carboxyphenyl β D glucoside,[7] and 10^5—10^6 with 2-(*o*-carboxyphenoxy)tetrahydropyran and benzaldehyde methyl *o*-carboxyphenyl acetal [9]

The curve in the Figure was calculated from equation (1) and the rate constants reported in the Table The constants, k_{H_2A} and k_{H_1A} are rate constants for intramolecular general

$$k_{obs} = \frac{k_{H_2A}a_H^2 + k_{H_1A}a_H K_a}{a_H^2 + K_a a_H + K_a K_a'} \quad (1)$$

acid catalysis of the hydrolysis of the un-ionized and monoanionic species, and K_a and K_a' are the first and second acid dissociation constants The kinetic equivalencies shown in equations (2) hold, where k_1 and k_2 are the rate

$$\begin{aligned} k_{H_2A} &= k_1 K_a \\ k_{H_1A} &= k_2 K_a' \end{aligned} \quad (2)$$

constants for hydronium ion catalysed hydrolysis of the monoanion and dianion species, respectively Buffer acid catalysis by dichloroacetic acid is observed with the dimethyl ester of (**1**) Therefore, it is likely that mechanistically similar intramolecular general acid catalysis is occurring with (**1**) rather than a kinetic equivalent possibility

While the monoanionic species is the most active, its associated rate constant for general acid catalysis is only 65 times that for the un-ionized compound On the basis of inductive effects it would be expected that the monoanion would hydrolyse fastest (σ for CO_2^- is 0 while that for CO_2H is 0 45), but a difference of 65 times is perhaps too large to be ascribed entirely to a substituent effect This is especially so when it is considered that the carboxyl group of the monoanion is a weaker acid by 1 pK_a unit Thus, it is possible that some electrostatic carbonium ion stabilization is occurring, see (**2**), but any participation by the carboxylate anion can be giving only a relatively small increase in the rate constant The product of the reaction is the cyclic acylal (1 equivalent of salicylic acid is released) This

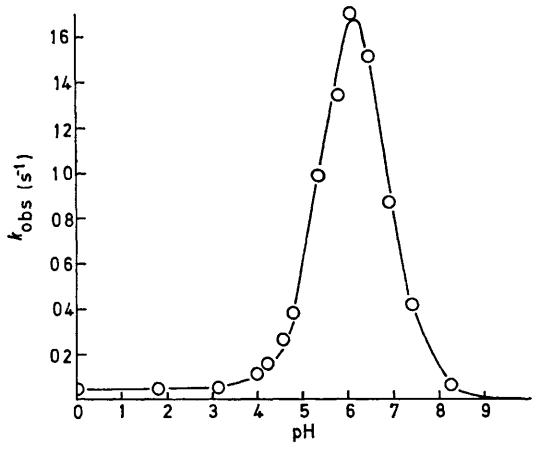


FIGURE *Plot of* k_{obs} *for appearance of product* vs pH *for hydrolysis of benzaldehyde disalicyl acetal in* 50% *dioxan*–H_2O *at* 25° (μ = 0 05)

could result from carbonium ion capture by the carboxylate anion and does not prove direct involvement in the critical transition state Most of the rate enhancement of $2{\cdot}7 \times 10^9$ in comparison with the dimethyl ester must result from participation by one group If the ionized species reaction involves intramolecular general acid catalysis, as is most likely, then it is clear that this mechanism is capable of giving rise to rate enhancements of the magnitude seen in enzymatic reactions

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