Most Efficient Intramolecular General Acid Catalysis of Acetal Hydrolysis by the Carboxy Group

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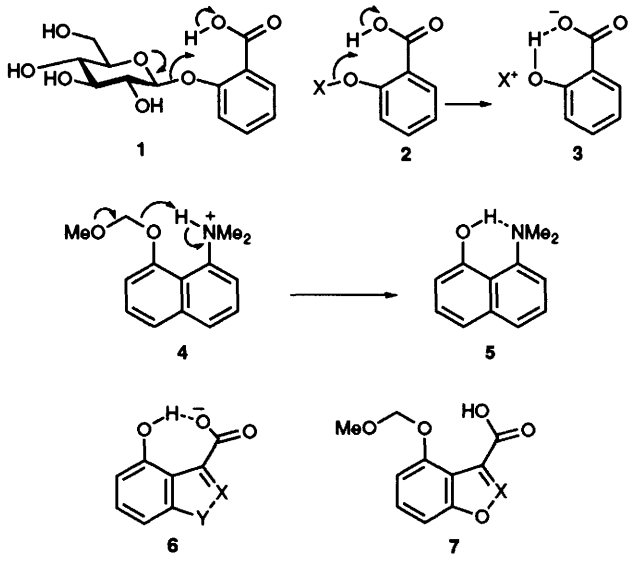
The hydrolysis of 4-methoxymethoxybenzisoxazole-3-carboxylic acid (halflife 31 s at 39 °C) is the fastest measured for a methoxymethyl acetal: catalysis by the neighbouring CO_2H group is facilitated by a strong intramolecular hydrogen bond.

Glycosidases such as lysozyme use CO_2H, the strongest general acid available on amino acid side-chains, to protonate the oxygen atoms of the group leaving the anomeric centre.[1] The classic model for this part of the enzyme mechanism is the hydrolysis of salicyl-β-D-glucoside **1**.[2]

The salicylic acid system turns out to be uniquely reactive in reactions of this sort **2**.[3,4] We believe that the key to this reactivity lies in the strong intramolecular hydrogen bond known to stabilise the product **3**, and also, presumably, the transition state leading to it.[5] Evidence is the efficient intramolecular general acid catalysis observed in the hydrolysis of the methoxymethyl naphthol **4**, which also gives a product **5** known to be stabilised by a strong intramolecular hydrogen bond.[5]



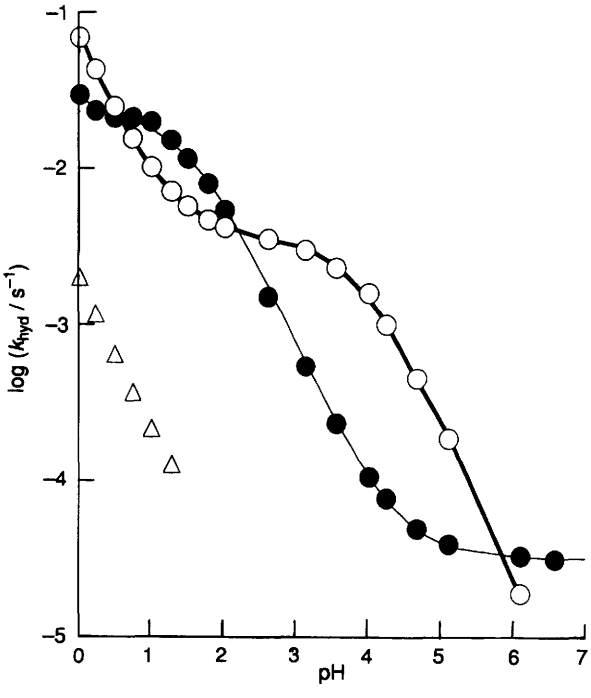


Fig. 1 pH-rate profiles for the hydrolysis of acetal acids **7** (X = N), closed circles [and for comparison (triangles) its methyl ester], and **7** (X = CH, open circles), at 39 °C and ionic strength 1.0 mol dm^{-3}. The points are experimental, the curves calculated using the rate constants and pK_as given in Table 1.

We report two new systems, based on the planar 6,5-fused system **6**, designed to form intramolecular hydrogen bonds with geometries more favourable than that of the salicylate monoanion.

The methoxymethyl acetal (**7**, X = N) of 3-carboxybenzisoxazole-4-ol[6] has a halflife at 39 °C and ionic strength 1 mol dm^{-3} of 31 s, and is 16 times more reactive than the methoxymethyl acetal of salicyclic acid[7] under the same conditions. Closer comparison with the salicyclic acid derivative shows that the $MeOCH_2O$ group of **7** (X = N) is intrinsically less reactive (the second-order rate constant k_H for hydrolysis of the CO_2H form by H_3O^+ is 2.8 times slower) but that the CO_2H group is a stronger acid (pK_a 1.55 compared with 3.77[7]). The efficiency of catalysis does not depend on the pK_a of the CO_2H group in the salicyclic acid system,[7] but could do so in the case of the new system **6**. So, we prepared for comparison the methoxymethyl acetal **7** (X = CH) of 3-carboxybenzofuran-4-ol.[8] This compound has a pK_a of 3.84, close to that of the salicyclic acid derivative. The acetal† is hydrolysed six times more slowly than **7** (X = N), but still 2.4 times faster than the salicyclic acid derivative; suggesting that the strength of the general acid is a significant factor determining reactivity in these 6,5-fused systems. The rate constants and pK_as appear in Table 1.

In the absence of detectable intermolecular catalysis it is not possible to measure effective molarities,[4] so we use relative reactivity under standard conditions as a simple guide to catalytic efficiency. By this criterion **7** (X = N) shows the most efficient intramolecular general acid catalysis yet measured for acetal hydrolysis. This further strengthens the evidence that proton transfer is facilitated by a strong intramolecular hydrogen bond. We have suggested that the efficiency of this part of the enzyme mechanism for glycoside hydrolysis may be enhanced by assistance from a nucleophile in the C–O cleavage process,[1] though any such synergy must involve a very subtle dependence on the extent and degree of coupling of bond making and bond breaking. In the hydrolysis of the methoxymethyl acetals discussed here there is expected to be weak nucleophilic participation by solvent water.[9] We have demonstrated stronger nucleophilic catalysis by CO_2^- in the

Table 1 Kinetic data for the hydrolysis of acetal acids **7**, X = N and CH, and their methyl esters, at 39 °C and ionic strength 1.0 mol dm^{-3}

X in **7**	k_H/dm^3 mol^{-1} s^{-1}	k_0/s^{-1}[a]	pK_a
N	$6.8 \pm 3.7 \times 10^{-3}$	$2.24 \pm 0.23 \times 10^{-2}$	1.55 ± 0.10
N, ester	$2.11 \pm 0.02 \times 10^{-2}$		
CH	$7.03 \pm 0.16 \times 10^{-2}$	$3.55 \pm 0.15 \times 10^{-3}$	3.84 ± 0.02
CH, ester	$3.13 \pm 0.02 \times 10^{-2}$		

[a] k_0 is the pH-independent rate constant calculated for the hydrolysis of the acetal acid. The pH-independent reaction of the anion of **7** (X = N) observed at pH > 7 is decarboxylation.[11]

related system **4**,[5] and have new evidence of intramolecular nucleophilic catalysis of the hydrolysis of a related glucoside.[10] We are now in a position to construct glycosides with a range of neighbouring catalytic groups capable of reproducing the main features of the generally accepted mechanism of action of many glycosidases and thus to address directly the question of synergy.

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Footnote

† Only the acetal group reacts: the benzofuran ring is intact in the hydrolysis product at all pHs studied. Acetal acids **7** were prepared and characterised as their methyl esters, which were converted to the sodium salts by treatment with sodium trimethylsilanolate in diethyl ether.

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