# Concerted general acid and nucleophilic catalysis of acetal hydrolysis. A simple model for the lysozyme mechanism

**Kathryn E. S. Dean and Anthony J. Kirby ***

*University Chemical Laboratory, Cambridge, UK CB2 1EW*

***Received (in Cambridge, UK) 29th November 2001, Accepted 16th January 2002***
***First published as an Advance Article on the web 6th February 2002***

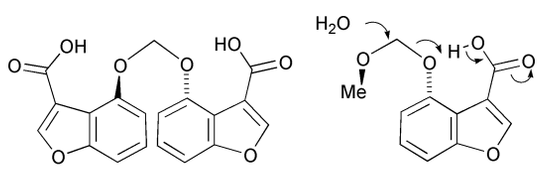
The monoanion is the most reactive ionic form of the symmetrical formaldehyde acetal of 4-hydroxybenzofuran-3-carboxylic acid **1**. The evidence is consistent with a mechanism in which the carboxylate anion acts as a nucleophile to assist the general acid catalysed cleavage of the C–O bond to the leaving group: the initial product is the cyclic acylal **5** and the extra acceleration derived from the participation of the neighbouring nucleophile (through an unpromising 7-membered ring) contributes some two orders of magnitude to a total rate enhancement of almost five orders of magnitude over the rate expected for specific acid catalysis.

Ever since the crystal structure of lysozyme revealed that a pair of carboxy groups—under the right conditions—could catalyse the rapid hydrolysis of intrinsically highly unreactive glycosides the mechanism by which they act has been of special interest.[1] It was immediately clear that new chemistry was involved. First, glutamic acid-35 apparently acted as a general acid. This conclusion was logical—acetal hydrolysis always needs acid—and it was not long before examples of intramolecular general acid catalysis of acetal hydrolysis appeared.[2] The role of aspartic acid-52, on the other hand, either as a nucleophile in a double displacement process, or in the electrostatic stabilisation of an intermediate cation, has been settled only very recently, pretty conclusively in favour of the nucleophilic mechanism.[3] Nucleophilic catalysis of acetal hydrolysis is by now a fairly well understood process in model systems, favoured when an unsymmetrical acetal bears a very good leaving group,[4] and enforced when the oxocarbocation intermediate of the $S_N1$-type mechanism is of too high energy to exist for a significant length of time under the hydrolysis conditions.[5]

Of particular interest is the question of the efficiency of the catalytic process. A simple alkyl glycoside has a half-life in water at pH 7 of the order of 100,000 years, but this is reduced to less than a second in the active site of a glycosidase. Yet general acid catalysis is typically inefficient in intramolecular models,[6] and highly efficient nucleophilic catalysis of *acetal* hydrolysis has never been observed. The intriguing and important possibility arises that in the cleavage of the glycosidic C–O bond there may be synergy between the proton transfer from the general acid and the formation of the new bond to the nucleophile. Both nucleophilic and general acid catalysis are normally observed only for the hydrolysis of acetals with good leaving groups: the idea is that very efficient nucleophilic catalysis may turn a poor leaving group into one good enough for general acid catalysis to become possible; and *vice versa*. The relevant property of the scissile C–O bond is the degree of polarisation in the sense $C^+$–$O^-$, and we know from crystal structure correlations[7] that C–O(R) bonds are longer, and more polarised in this sense, for better leaving groups $RO^-$: the same polarisation is involved in the nucleophilic displacement of $RO^-$. Very recent work shows this polarisation induced by intramolecular hydrogen bonding by a COOH group.[8]

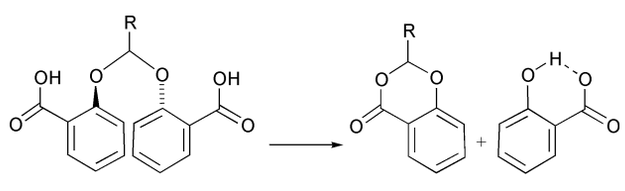
We are now looking for experimental evidence for such synergy in acetal (ideally glycoside) cleavage reactions which are subject to both efficient general acid and efficient nucleophilic catalysis. So far we have developed systems where intramolecular general acid catalysis by COOH approaches the levels of efficiency characterised by nucleophilic catalysis,[9] and are currently building them into glycosides with efficient neighbouring carboxylate nucleophiles. We report here results with a simpler model system **1**, based on the molecular geometry developed to support highly efficient intramolecular general acid catalysis, which should be capable of nucleophilic catalysis also. The evidence is good that the two mechanisms are concerted, and reinforce each other.

The molecular geometry which supports the most efficient intramolecular general acid catalysis in simple systems is the 6,5-fused bicyclic structure. One of our first systems of this sort was the benzofuran-3-carboxylic acid derivative **2**.[10] As it is a formaldehyde acetal, nucleophilic involvement of the solvent is expected to be enforced.[11] So a better neighbouring (carboxylate) nucleophile is expected to participate in the hydrolysis of the monoanion of the symmetrical acetal **1**, presumably in the rate determining C–O cleavage step and thus in concert with the general acid catalysis.




**1** **2**

The most directly relevant previous work on this problem is that of Anderson and Fife[12] who found a total rate enhancement of over $10^9$ for the hydrolysis of the monoanion of benzaldehyde disalicyl acetal **3** (R = Ph), compared with its dimethyl ester (Scheme 1). The product was the cyclic acylal **4**




**3** **4**

**Scheme 1**

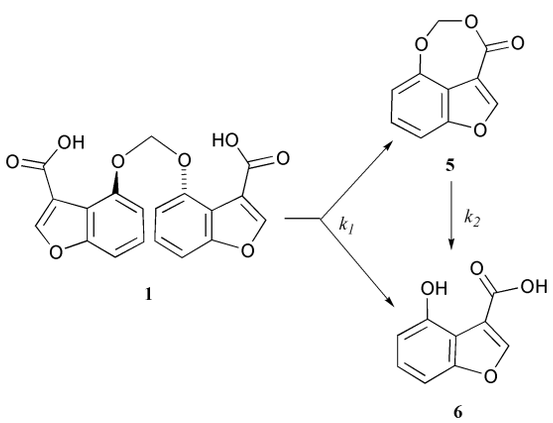
(R = Ph) and bifunctional catalysis appeared to be involved. However, its contribution was small and its origin not clearly identified: the salicylic acid system is complicated by the conjugation of the leaving group oxygen with the *ortho*-carboxy group, and the unsymmetrical compound with one *ortho*- and one *para*-carboxy group (for which bifunctional catalysis is

 DOI: 10.1039/b110948k

impossible) also showed a bell-shaped pH–rate profile. Furthermore, the cyclic acylal **4** (R = H) was not a product in the reaction of the corresponding (but far less reactive) formaldehyde acetal **3** (R = H), which also showed enhanced reactivity of the monoanion; so nucleophilic participation by carboxylate could be ruled out.

## Results and discussion

The hydrolysis of formaldehyde acetal **1** is relatively slow (formaldehyde acetals are typically hydrolysed some $10^5$ times more slowly than the corresponding benzaldehyde derivatives), and was followed conveniently at 60 °C. The absorbance change was clearly biphasic at most pH values, indicating a two-stage reaction, so the data were analysed for consecutive first order processes. The expected cyclic acylal intermediate **5** was prepared independently and its hydrolysis ($k_2$, Scheme 2, Table 1)




**Scheme 2**

followed under the same conditions. The raw data for the hydrolysis of the acetal **1** were analysed in two ways, treating the rate constant for the second step first as an independent variable, and alternatively setting it to the known value for the hydrolysis of **5**. The derived values for the rate constant $k_1$, for the first step, did not differ significantly (Table 2), but the fit was slightly better, as might be expected, for the former method.

The pH–rate profile (Fig. 1) for the first step ($k_1$) shows the bell-shaped maximum near pH 4 expected if the reaction of the monoanion is dominant near pH 4, superimposed on the region of unit slope expected for the specific acid catalysed reaction of an acetal. The pH–rate profile for the hydrolysis of the presumed cyclic acetal intermediate **5** (also plotted in Fig. 1) shows the expected acid and base catalysed regions, and a significant pH-independent region between pH 3–5. The evidence from the kinetic analysis and from the UV spectra is unambiguous: acetal **1** is hydrolysed effectively quantitatively to the hydroxy-acid **6** and the acylal **5**, which is itself hydrolysed in step 2

**Table 1** Kinetic data for the hydrolysis of cyclic acylal **5** at 60 °C in 10% acetonitrile–water, $I$ = 1 mol dm$^{-3}$

| $10^4\ k_H$/dm$^3$ mol$^{-1}$ s$^{-1}$ | $10^6\ k_0$/s$^{-1}$ | $k_{OH}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | $R$ |
|---|---|---|---|
| 4.6 ± 0.4 | 3.5 ± 0.4 | 250 ± 40 | 0.979 |

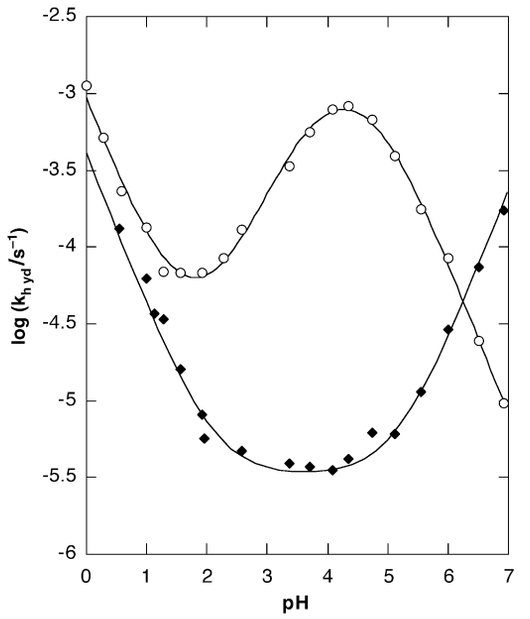


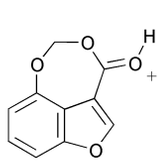

**Fig. 1** pH–rate profiles for the hydrolysis of diacid **1** (circles) and the cyclic acetal **5** (diamonds), in 10% acetonitrile–water at 60 °C. The points are experimental, the curves calculated, based on the rate and dissociation constants shown in Tables 1 and 2.

(Scheme 2) to formaldehyde (hydrate) and a second molecule of **6**.

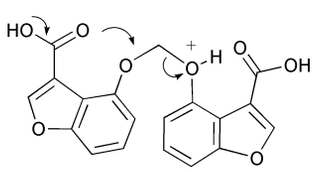
### Mechanism

*The hydrolysis of the acylal* **5** involves acid and base catalysed reactions and a pH-independent process which is buffer catalysed. The base catalysed reaction can only be $B_{AC}2$ hydrolysis at the ester carbonyl group. Buffer catalysis could also be $B_{AC}2$ hydrolysis at the ester carbonyl but *probably* involves nucleophilic attack at the acetal $CH_2$: it is insensitive to the p$K_a$ of the oxyanion (Brønsted $\beta$ around 0.2), as expected[4] for nucleophilic catalysis of acetal hydrolysis, and the rate of the pH-independent reaction, thought to involve water as a nucleophile, is closely similar to that of the 2,4-dinitrophenyl phenyl acetal of formaldehyde under similar conditions (60 °C, 20% dioxan).[13]

The mechanism of the acid catalysed hydrolysis of the acylal **5** most likely involves the attack of water on the conjugate acid **5H**$^+$. There is insufficient evidence to choose between acetal attack and addition to the ester C=O group, though the latter is the more likely because (i) "internal return" must compete with fragmentation of the hemiacetal leaving group, and (ii) the phenolic oxygen is stereoelectronically disadvantaged as an *n*-donor in the almost planar 7-membered ring.




**5H**$^+$



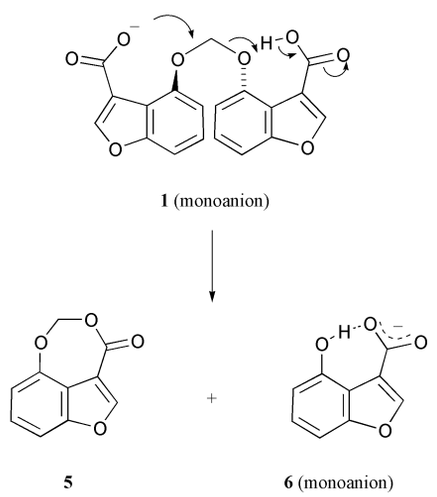

**1H**$^+$

**Table 2** Rate and kinetic dissociation constants for the hydrolysis of acetal **1** at 60 °C in 10% acetonitrile–water, $I$ = 1 mol dm$^{-3}$

| | Constants obtained varying both $k_1$ and $k_2$ ($R$ = 0.998) | | Constants obtained with $k_2$ fixed ($R$ = 0.995) | |
|---|---|---|---|---|
| $k_H$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 9.24 ± 0.40 × 10$^{-4}$ | | 7.61 ± 0.49 × 10$^{-4}$ | |
| $k_{0(1)}$/s$^{-1}$ | 3.69 ± 0.29 × 10$^{-5}$ | | 4.13 ± 0.41 × 10$^{-5}$ | |
| $k_{0(2)}$/s$^{-1}$ | 1.40 ± 0.15 × 10$^{-3}$ | | 1.31 ± 0.10 × 10$^{-3}$ | |
| $K_{a(1)}$ | 1.40 ± 0.15 × 10$^{-4}$ | p$K_{a1}$ = 3.8 | 1.46 ± 0.23 × 10$^{-4}$ | p$K_{a1}$ = 3.8 |
| $K_{a(2)}$ | 1.74 ± 0.11 × 10$^{-5}$ | p$K_{a2}$ = 4.8 | 1.60 ± 0.16 × 10$^{-5}$ | p$K_{a2}$ = 4.8 |

*The hydrolysis of the acetal dicarboxylic acid* **1** also involves three different mechanisms in the pH-range 0–7. The monoanion is the most reactive species (there is no significant reaction involving the dianion under the conditions) and the pH–rate profile (Fig. 1) indicates both spontaneous and acid-catalysed reactions of the neutral diacid. This last, acid-catalysed reaction may be a normal specific-acid catalysed acetal hydrolysis—the kinetic analysis fits a simple, one-step conversion to products below pH 1—but we cannot rule out a nucleophilic role for a neighbouring COOH group (as in $\mathbf{1H^+}$: we attempted to measure hydrolysis rates for the methyl ester **7** for control data, but the reaction was complicated by the competing hydrolysis of the ester groups). The spontaneous reaction of the neutral dicarboxylic acid between pH 1.5–2.5 presumably involves general acid catalysis by one COOH group: the ratio $k_{0(1)}/k_{H}K_{a(1)}$ (the acceleration over the $H_3O^+$-catalysed reaction[14]) is 285, similar to the value of 349 found for the methoxymethyl derivative **2**.[10]

The reaction of primary interest for this work is the hydrolysis of the monoanion of **1**, which is responsible for the maximum in the pH–rate profile at pH 4.3 (Scheme 3). The




**Scheme 3**

increase in reactivity over the spontaneous reaction of the neutral diacid is a modest 38-fold, coincidentally similar to the value of 26 observed for the disalicyl derivative **3** (R = H),[12] which was attributed to the differential substituent effect of carboxylate *vs.* COOH groups on the donor oxygen. However, the catalytic and leaving groups in **1** are in different rings and do not interact significantly, and the initial product is the cyclic acylal **5**, produced by nucleophilic attack of the neighbouring carboxylate group. The efficiency of a *7-exo-tet*[15] nucleophilic substitution is expected to be low,[6] typically being two orders of magnitude less than that observed for *6-exo-tet* reactions such as that looked for in the benzaldehyde disalicyl acetal **3**. Thus the rate enhancement observed in this system is $8.7 \times 10^4$ over the rate expected if only specific acid catalysis occurred, suggesting that a much larger rate enhancement could be observed in a system with a more favourable geometry for intramolecular nucleophilic attack.

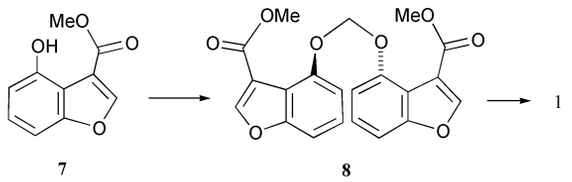
Thus the experimental evidence strongly indicates not only that concerted general acid and nucleophilic catalysis can occur in such systems, but also that the synergy between properly positioned nucleophilic and general acid and catalytic groups, as found in the active sites of lysozyme and many other retaining glycohydrolases, could itself account for very substantial rate enhancements. †

† A referee reminds us that—as for the lysozyme reaction—kinetic evidence does not distinguish between the nucleophilic mechanism we prefer and a "stabilizing electrostatic interaction between an oxocarbenium ion intermediate and a neighboring carboxylate anion". As discussed above, the nucleophilic mechanism is now firmly established for the lysozyme reaction, and an oxocarbenium ion is an unlikely intermediate in the hydrolysis of a formaldehyde acetal. More detailed arguments for our preference have been presented many times. (See for example, G.-A. Craze and A. J. Kirby, *J. Chem. Soc., Perkin Trans. 2*, 1974, 61–66.)

## Experimental

### Synthesis

The dimethyl ester **8** of **1** was prepared by the reaction of the sodium salt of **7** with chloroiodomethane, then converted to the diacid or its dipotassium salt. The acylal **5** was prepared under similar conditions from the parent hydroxyacid **6**.




**Methanal bis(3-methoxycarbonylbenzo[*b*]furan-4-yl) acetal 8.** A solution of 3-methoxycarbonyl-4-hydroxybenzo[*b*]furan[16] **7** (97 mg, 0.5 mmol) in methanol (8 mL) was added slowly to a stirred solution of sodium methoxide (30 mg, 0.56 mmol) in methanol (2 mL). The solution was stirred at room temperature for 20 h, the solvent removed under reduced pressure and the resultant white solid dried *in vacuo*. The colourless sodium salt was dissolved in DMSO (4 mL), chloroiodomethane (0.05 mL, 0.69 mmol) was added and the mixture heated at 90 °C for 18 h. The brown solution was allowed to cool to room temperature, poured into water (16 mL) and extracted with $CH_2Cl_2$ (4 × 15 mL). The combined organic extracts were washed with water (10 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure to give a brown solid which was chromatographed ($SiO_2$, $CH_2Cl_2$) to give the *diester* (65 mg, 66%) as a white solid; mp 128–129 °C; $R_f(CH_2Cl_2)$ 0.21; $\nu_{max}(CDCl_3)/cm^{-1}$ 2999 (C–H), 2953 (C–H), 2848 (C–H), 1736 ($COOCH_3$), 1599 (Ar), 1553 (Ar) and 1499 (Ar); $\delta_H$(400 MHz; $CDCl_3$) 8.16 (2 H, s, C(2)H), 7.32 (4 H, m, C(6)H and C(7)H), 7.23 (2 H, dd, *J* 6.5 and 2.6, C(5)H), 6.00 (2 H, s, $OCH_2O$) and 3.88 (6 H, s, $OCH_3$); $\delta_C$(100.6 MHz; $CDCl_3$) 162.9+ (*C*$OOCH_3$), 157.3+ (C(7a)), 151.4+ (C(4)), 150.8+ (C(2)), 125.4− (C(6)), 115.0+ (C(3a)), 114.5+ (C(3)), 110.8− (C(7)), 106.6− (C(5)), 92.6+ ($CH_2$) and 51.8+ ($OCH_3$); *m*/*z* (+FAB) 396; (Found: $M^+$, 396.0858. $C_{21}H_{16}O_8$ requires *M*, 396.0845).

**Methanal bis(3-carboxybenzo[*b*]furan-4-yl) acetal 1.** Sodium trimethylsilanolate (1 mol $dm^{-3}$ solution in $CH_2Cl_2$, 0.252 mL, 0.252 mmol) was added to a stirred solution of the diester **8** (50 mg, 0.126 mmol) in $CH_2Cl_2$ (2 mL) and the resultant solution was stirred at room temperature for 18 h. The fine white suspension was extracted with aqueous sodium hydroxide solution (1%, 4 × 20 mL). The combined aqueous extracts were acidified to approximately pH 2 by addition of aqueous hydrochloric acid (3 mol $dm^{-3}$, Universal Indicator Paper) to form a white precipitate which was collected by filtration and dried under reduced pressure to give the *diacid* (32 mg, 68%) as a white solid; mp 148–150 °C; $\nu_{max}$(solid)/$cm^{-1}$ 3200–2600 (O–H), 2924 (C–H), 1711 (COOH), 1597 (Ar), 1552 (Ar), 1499 (Ar) and 1090 (C–O); $\delta_H$(500 MHz; $(CD_3)_2SO$) 12.7 (2 H, br s, OH), 8.53 (2 H, s, C(2)H), 7.37 (2 H, dd, *J* 8.2 and 1.3, C(7)H)), 7.34 (2 H, t, *J* 7.9, C(6)H), 7.27 (2 H, dd, *J* 7.6 and 1.3, C(5)H) and 5.95 (2 H, s, $OCH_2O$); $\delta_C$(125.7 MHz; $(CD_3)_2SO$) 163.5+ (COOH), 157.0+ (C(7a)), 151.6+ (C(2)), 151.3+ (C(4)), 126.7− (C(6)), 115.3+ (C(3a)), 115.1+ (C(3)), 110.7− (C(5)), 106.8− (C(7)) and 92.4+ ($OCH_2O$); *m*/*z* (+ES) 391 (100%,

$MNa^+$) (Found: $MNa^+$, 391.0414. $C_{19}H_{12}O_8Na$ requires, 391.0430).

**Methanal bis(3-carboxybenzo[*b*]furan-4-yl) acetal (1), dipotassium salt.** The diester **8** (20 mg, 0.05 mmol), water (0.5 mL), methanol (0.5 mL) and aqueous potassium hydroxide (2 mol $dm^{-3}$, 0.005 mL, 0.10 mmol) were stirred at 80 °C for 18 h. The mixture was allowed to cool to room temperature and the solvents were removed under reduced pressure to give the *dipotassium salt* (22 mg, 100%) as an off-white solid; mp 240–250 °C, dec.; $\nu_{max}$(solid)/$cm^{-1}$ 2921 (C–H), 1606 ($COO^-$), 1587 (Ar), 1546 (Ar), 1494 (Ar) and 1395 ($COO^-$); $\delta_H$(500 MHz; $D_2O$) 7.86 (2 H, s, C(2)H), 7.30 (2 H, t, *J* 8.2, C(6)H), 7.23 (2 H, d, *J* 8.2, C(5)H or C(7)H), 7.18 (2 H, d, *J* 8.0, C(5)H or C(7)H) and 5.96 (2 H, s, $OCH_2O$); $\delta_C$(125.7 MHz; $D_2O$) 171.5+ ($COO^-$), 156.4 (C(7a)), 150.4 (C(4)), 145.7+ (C(2)), 125.8− (C(6)), 119.7+ (C(3)), 115.6 (C(3a)), 109.5− (C(5) or C(7)), 106.4 (C(5) or C(7)), 91.7+ ($OCH_2O$); *m*/*z* (+ES) 482 (100%, $MK^+$) (Found: $MK^+$, 482.9266. $C_{19}H_{10}O_8K_3$ requires, 482.9287). Some $^{13}C$ signals were not observed in the APT spectrum.

**6,7,8,9-Tetrahydro-2*H*-2,6,8-trioxa-9-oxobenz[*cd*]azulene 5.** 3-Carboxy-4-hydroxybenzo[*b*]furan[17] (70 mg, 0.39 mmol), potassium carbonate (227 mg, 1.64 mmol), chloroiodomethane (0.2 mL, 484 mg, 2.75 mmol) and DMSO (3 mL) were heated at 100 °C for 4.5 h. The dark brown reaction mixture was allowed to cool to room temperature, poured into water (50 mL) and extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic extracts were washed with water (15 mL), dried ($MgSO_4$) and the solvents were removed under reduced pressure to give a dark brown oil which was chromatographed twice ($CH_2Cl_2$–hexane, 1 : 1) to give the *acylal* **5** (24 mg, 32%) as a white solid, mp 112–113 °C; $R_f$($CH_2Cl_2$–hexane, 1 : 1) 0.09; $\nu_{max}$($CDCl_3$)/$cm^{-1}$ 3034 (C–H), 2952 (C–H), 1740 (C=O), 1608 (Ar), 1558 (Ar) and 1501 (Ar); $\delta_H$(500 MHz; $CDCl_3$) 8.35 (1 H, s, C(1)H), 7.32 (1 H, t, *J* 8.0, C(4)H), 7.28 (1 H, dd, *J* 8.4 and 0.9, C(3)H), 6.95 (1 H, dd, *J* 7.7 and 0.9, C(5)H) and 5.75 (2 H, s, C(7)H); $\delta_C$(125.7 MHz; $CDCl_3$) 163.6+ (C=O), 156.2+ (C(2a)), 152.6+ (C(5a)), 152.4+ (C(1)), 126.9− (C(4)), 113.8+ (C(5c)), 112.4+ (C(5b)), 111.1− (C(5)), 106.8− (C(3)) and 91.7+ (C(7)); *m*/*z* (+ESI) 213 (100%, $MNa^+$) (Found: $MNa^+$, 213.0159. $C_{10}H_6O_4Na$ requires, 213.0164).

### Kinetic methods

Inorganic buffer reagents were of AnalaR grade. Water was triply distilled through an all glass apparatus and degassed with argon. KOH and HCl stock solutions (2 mol $dm^{-3}$) were made by dilution of BDH Convol® concentrates. Buffer solutions were made by adding the appropriate amount of the 2.0 mol $dm^{-3}$ stock solutions of KOH or HCl to standard solutions of the acid or base forms respectively of the buffer compound in grade A volumetric flasks. The correct amount of 2.0 mol $dm^{-3}$ KCl solution was added to adjust the ionic strength, *I*, to 1.0 mol $dm^{-3}$. The correct amount of acetonitrile was added to make the final acetonitrile content 10% (v/v), and the final volume obtained by addition of water. Whenever practical the pH value of buffer solutions was measured under the conditions of the kinetic run using a Radiometer PHM82 pH meter fitted with a Russell CTWL electrode calibrated using standard buffer solutions. For HCl solutions too concentrated for the pH value to be measured by this method, the pH value was calculated using the equation: $pH = -log[HCl] + \Delta pH$, where ΔpH is the activity correction, measured as the difference between the calculated and measured pH values extrapolated from a plot for those HCl solutions for which it was possible to measure the pH accurately. ΔpH was zero at 60 °C for buffers containing 10% acetonitrile.

Spectroscopic data were acquired using a Varian Cary 3 UV/Vis. spectrophotometer fitted with a thermostatted cell-holder. Stock solutions of the cyclic acylal **5** were prepared in acetonitrile, and stock solutions of the diacid **1** in dilute aqueous KOH. In all cases the concentrations of stock solutions were approximately 0.01 mol $dm^{-3}$. Runs were started by injecting 0.020 mL of stock solution into 2.5 mL of preheated buffer solution in a quartz cuvette of 1.0 cm path length, giving a final substrate concentration of approximately $1 \times 10^{-5}$ mol $dm^{-3}$. The concentration of the buffer was always at least 100 times higher than that of the substrate to ensure pseudo first order conditions.

Repetitive absorbance *versus* wavelength scans from 200 to 400 nm were run at 39 or 60 °C. No isosbestic points were observed in the hydrolysis of the diacid **1**. An initial decrease in absorbance with maximum change at 254 nm was followed by an increase in absorbance with maximum change at 256 nm. For the hydrolysis of the cyclic acylal **5** an isosbestic point was observed at 273 nm and the maximum absorbance increase at 256 nm.

**Hydrolysis of the cyclic acylal 5.** Rate constants for the hydrolysis of the cyclic acylal **5** were measured over a range of pH values between 0 and 7, at 60 °C in aqueous buffers containing 10% acetonitrile, $I = 1$ mol $dm^{-3}$. Preliminary scans showed well-defined isosbestic points indicating a one-step reaction, which was studied by following the increase in absorbance at 256 nm. In all cases first order exponential curves were observed. Varying the buffer concentration showed catalysis by formate, acetate and phosphate buffers, so rate constants measured at four different buffer concentrations were extrapolated to zero buffer concentration to give solvolysis rates. These rate constant values at zero buffer concentration were plotted against the pH values of the buffer solutions to give the pH–rate profile (Fig. 1). The calculated curve was obtained by fitting the experimental data to eqn. (1) below.

$$k_{obs} = k_H a_H + k_0 + k_{OH} K_w / a_H \quad (1)$$

Here $k_{obs}$ is the observed rate constant for acylal hydrolysis; $k_H$ the second order rate constant for specific acid catalysed acylal hydrolysis; $k_0$ the first order rate constant for pH-independent acylal hydrolysis; $k_{OH}$ the second order rate constant for specific base catalysed acylal hydrolysis; and $K_w$ the ionisation constant of water = $1 \times 10^{-13.034}$ at 60 °C.[18] (The values of the rate constants obtained appear in Table 1).

**Hydrolysis of the diacid 1.** Hydrolysis of the diacid **1** to directly give two equivalents of the acid **6** would be expected to show a change in absorbance with time given by eqn. (2):

$$Abs = Abs_0 e^{-k_{obs}t} + \frac{\varepsilon_C}{\varepsilon_A} 2Abs_0 (1 - e^{-k_{obs}t}) \quad (2)$$

where: $k_{obs}$ = observed rate constant for the hydrolysis reaction; $\varepsilon_A$ and $\varepsilon_C$ = absorption coefficients for the diacid **1** and the product acid **6**, respectively. However, fitting of the time course of the absorbance change for the hydrolysis of the diacid **1** to this equation was satisfactory only for data obtained below pH 1, so the data were analysed in terms of two consecutive first order processes (see Scheme 2) using eqn. (3):

$$Abs = Abs_0 e^{-k_1 t} + \frac{\varepsilon_B}{\varepsilon_A} Abs_0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) + \frac{\varepsilon_C}{\varepsilon_A} Abs_0 \left\{ 2 - e^{-k_1 t} + \frac{1}{k_2 - k_1} (k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \right\} \quad (3)$$

Here $k_1$ and $k_2$ are the rate constants for the first and second steps of the hydrolysis reaction; $\varepsilon_A$, $\varepsilon_B$ and $\varepsilon_C$ are the absorption

coefficients for the diacid **1**, the intermediate and the acid **6** respectively.[19]

Eqn. (3) was used to fit the observed change in absorbance with time in two different ways: either directly, with no constraints on the value of $k_2$; or by substituting into the equation the measured values of the rate constant $k_2$ for the hydrolysis of the supposed intermediate **5**. The two methods gave closely similar values for the rate constant $k_1$ for the first step in the hydrolysis. Furthermore, these values were independent of buffer concentration: but when the equation was used to obtain rate constants for both steps catalysis by formate, acetate, phosphate and TRIS buffers was indicated for the second step of the reaction, as found for the hydrolysis of the acylal **5**.

The rate constants $k_1$ obtained for the first step of the hydrolysis of diacid **1** (Table 2) were plotted against pH to give the profile shown in Fig. 1. The calculated curve was obtained by fitting the data (in column 1) to eqn. (4).

$$k_{1(obs)} = [k_H(a_H)^3 + k_{0(1)}(a_H)^2 + k_{0(2)}K_{a(1)}(a_H)]/[(a_H)^2 + K_{a(1)}(a_H) + K_{a(1)}K_{a(2)}] \quad (4)$$

Here: $k_{1(obs)}$ is the observed rate constant for the first step; $k_H$ the second order rate constant for specific acid catalysed hydrolysis; $k_{0(1)}$ and $k_{0(2)}$ the first order rate constants for the spontaneous hydrolysis of the neutral diacid and the mono-anion, respectively; $K_{a(1)}$ and $K_{a(2)}$ are the first and second dissociation constants of the diacid.

## Acknowledgements

KESD is grateful for a Studentship awarded by the Biotechnology and Biological Sciences Research Council of Great Britain.

## References

1 T. Imoto *et al.*, in*The Enzymes*, P. D. Boyer, Ed., Academic Press, New York, 1970, vol. 7, p. 665; . For an up to date account see G. J. Davies, M. L. Sinnott and S. G. Withers, in *Comprehensive Biological Catalysis*, M. L. Sinnott, Ed., Academic Press, New York, 1998, vol. 1, pp. 119–207.
2 B. Capon, M. C. Smith, E. Anderson, R. H. Dahm and G. H. Sankey, *J. Chem. Soc., B*, 1969, 1038–1047.
3 D. J. Vocadlo, G. J. Davies, R. Laine and S. G. Withers, *Nature*, 2001, **412**, 835–838.
4 G.-A. Craze, A. J. Kirby and R. Osborne, *J. Chem. Soc., Perkin Trans. 2*, 1978, 357–368.
5 N. S. Banait and W. P. Jencks, *J. Am. Chem. Soc.*, 1991, **113**, 7951–7958; N. S. Banait and W. P. Jencks, *J. Am. Chem. Soc.*, 1991, **113**, 7958–7963.
6 A. J. Kirby, *Adv. Phys. Org. Chem.*, 1980, **17**, 183–278.
7 A. J. Kirby, *Adv. Phys. Org. Chem.*, 1994, **29**, 87–183.
8 A. D. Bond, A. J. Kirby and E. Rodriguez, *Chem. Commun.*, 2001, 2266–2267.
9 E. Hartwell, D. R. W. Hodgson and A. J. Kirby, *J. Am. Chem. Soc.*, 2000, **122**, 9326–9327.
10 A. J. Kirby and A. Parkinson, *J. Chem. Soc., Chem. Commun.*, 1994, 707–708.
11 P. R. Young and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 8238–8248.
12 E. Anderson and T. H. Fife, *J. Am. Chem. Soc.*, 1973, **95**, 6437–6441.
13 Unpublished work with O. E. Desvard.
14 A. J. Kirby and F. O'Carroll, *J. Chem. Soc., Perkin Trans. 2*, 1994, 649–656.
15 J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 1976, 734–736.
16 A. Parkinson, *PhD Thesis*, University of Cambridge, 1993.
17 G. Kneen and P. J. Maddocks, *Synth. Commun.*, 1986, **16**, 1635–1636.
18 D. R. Lide, *CRC Handbook of Chemistry and Physics*, 80th edn., 1999.
19 K. E. S. Dean, *PhD Thesis*, University of Cambridge, 2000.