The Carbohydrate-catalysed Hydrolysis of Cephalosporins

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The decomposition of cephaloridine, cephtazidime, cephaclor, cephalothin, cephaloglycin, cephalexin and cephradine catalysed by glucose, galactose, maltose, sucrose, mannitol and α-methylglucoside in aqueous solutions of pH 9–11 is reported. The rate of decomposition depends upon the structure of the cephalosporin, the more important feature being the electron withdrawing nature of the substituent attached to the exocyclic C-3 methylene carbon atom. At pH 9.5 a Hammett ρ_I value of 2.9 is obtained from a plot of log k_2, the apparent second-order rate constant, *versus* σ_I for the glucose catalysed reaction. At pH *ca.* 9 plots of k_o *versus* catalyst concentration appear linear, but above pH *ca.* 10 such plots are curved. The extent of catalysis increases at higher pH values. Carbohydrates with a hemiacetal OH group are significantly better catalysts than those that do not contain this functionality, though catalysis by non-hemiacetal groups is evident. The difference in reactivity between the two types of OH at pH 9.5 lies between 10 and 15-fold. The results are interpreted in terms of a mechanism that involves nucleophilic catalysis *via* the hemiacetal anion of the carbohydrate. Curvature of the k_o *versus* [catalyst] plots is explained by the formation of non-catalytic dimer between the anionic and neutral forms of the catalyst.

The hydrolysis of penicillins and cephalosporins catalysed by carbohydrates and polyhydric alcohols is relevant both to drug stability and to β-lactam allergy. For example, penicillins and cephalosporins are commonly dispensed in syrup preparations; the shelf life of such a preparation is thus linked to the kinetics and mechanism by which carbohydrates catalyse β-lactam hydrolysis. Moreover, the formation of penicilloate esters from sugars and penicillins is well documented;[1] and it has been shown that such esters are capable of eliciting penicilloyl specific allergic reactions in sensitized animals.[2]

The kinetics and mechanism of the sucrose accelerated degradation of penicillins has been studied by Bundgaard and co-workers.[3] A linear relationship between the degradation rate and the sucrose concentration up to 10% w/v was observed, and the rate accelerating effect of sucrose was found to be directly proportional to the hydroxide ion concentration in the pH range 6–10.2. The presence of a penicilloate ester of sucrose in the reaction pathway was demonstrated by penamaldate analysis, and kinetic analysis showed that the catalysed hydrolysis of penicillins to penicilloic acids proceeds entirely through a nucleophilic pathway with the intermediate formation of penicilloyl-sucrose esters. A similar process also was described for the degradation of cephalosporins in the presence of various carbohydrates and polyhydric alcohols.[4] At pH 6.5, the degradation rates increased linearly with the concentration of the hydroxy compound up to 15% w/v. This rate accelerating effect was found to be directly proportional to the hydroxide ion activity up to pH values of about 11. Above pH 11, the rate constants tended to level off slightly with increasing pH. It was proposed that this catalytic effect is due to a mechanism which involves nucleophilic opening of the cephalosporin β-lactam by an alkoxide ion derived from the carbohydrate of polyalcohol.

More recently, the alcohol-catalysed hydrolysis of benzylpenicillin was studied by Page and co-workers[5] who reported that alkoxide ion catalysis proceeds *via* a nucleophilic pathway, the intermediate ester being detected in some cases. The Brönsted β_{nuc} for the dependence upon the alkoxide ion was found to be 0.97, this value being compatible with rate-limiting ring opening of the β-lactam. In related work the ability of carbohydrates to act as nucleophilic catalysts in the hydrolysis of *p*-nitrophenyl esters has also been demonstrated.[6,7]

Interested in more clearly establishing the role of the carbohydrate catalyst in cephalosporin degradation, we have investigated the hydrolysis of a series of cephalosporins **1a–h**, in the presence of several carbohydrates (glucose, galactose, sucrose, maltose), an α-methylglucoside and one polyalcohol (D-mannitol). Our results are reported herein.

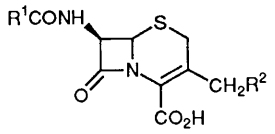
Experimental

Substrates and Reagents.—The cephalosporins and the carbohydrates studied were commercial products and were used as received. Sodium carbonate, hydrogen carbonate, perchlorate and hydroxide were Analar reagents.

Kinetics.—A small aliquot (50 μdm^3) of a solution of cephalosporin (3×10^{-3} mol dm^{-3}) in water was injected into a thermostatted curvette containing 3 cm^3 of the required buffer solution. Initial substrate concentrations were therefore *ca.* 5×10^{-5} mol dm^{-3}. Ionic strength was maintained at the appropriate value by the use of sodium perchlorate. The change in UV spectrum was monitored at 260 nm with respect to time, and pseudo-first-order rate constants k_o, were obtained from plots of $\ln(A_t - A_\infty)$ *versus* time, where A_t is the absorbance at time t and A_∞ is the absorbance after at least 10 half-lives. In the presence of carbohydrates A_∞ values are not constant, a slow reaction, due to further reaction of the products is observed. Therefore, a computer program was used to calculate pseudo-first-order rate constants using a non-linear least squares method which treated A_∞ as an adjustable parameter. The validity of the method was tested by comparing the k_o values so obtained with those derived from an initial rate method at known initial cephalosporin concentrations. The agreement between the two was ± 10%. Individual rate constants are reproducible to ± 5%.

Results and Discussion

A plot of the pseudo-first-order rate constants, k_o obtained for the hydrolysis of cephalosporins **1a–g** in carbonate buffer at



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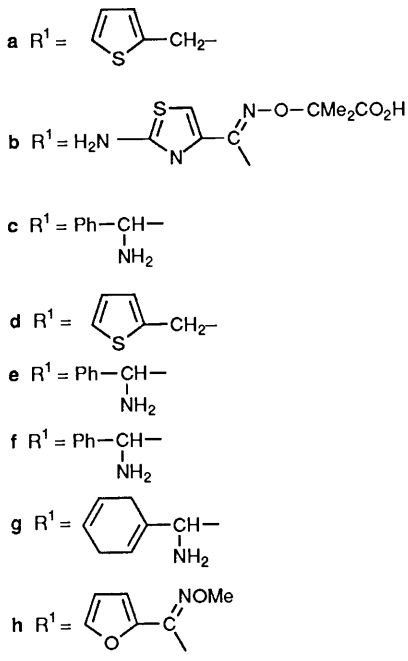

a R^1 = R^2 =  cephaloridine

b $R^1 = H_2N$ R^2 =  cephtazidine

c R^1 = Ph—CH(NH$_2$)— R^2 = Cl cephaclor

d R^1 = $R^2 = OCOCH_3$ cephalothin

e R^1 = Ph—CH(NH$_2$)— $R^2 = OCOCH_3$ cephaloglycine

f R^1 = Ph—CH(NH$_2$)— R^2 = H cefalexin

g R^1 = R^2 = H cephradine

h R^1 = $R^2 = OCONH_2$ cefuroxime

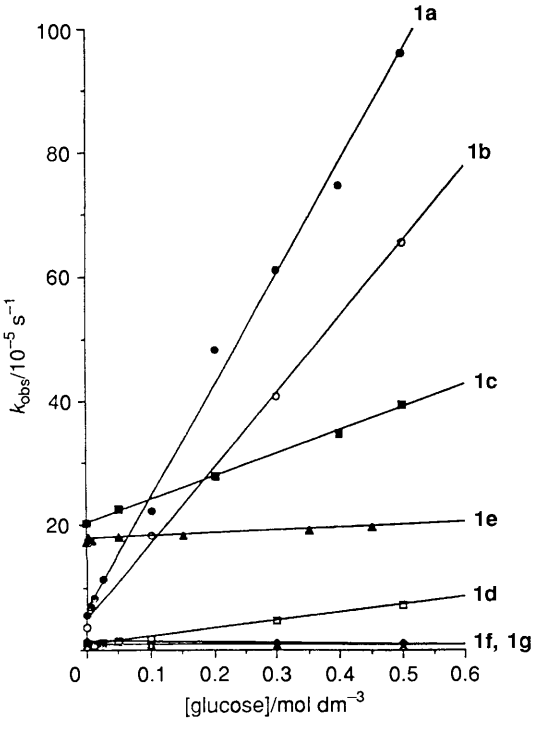

Fig. 1 Dependence of the pseudo-first-order rate constants upon [glucose] for the catalysed hydrolysis of cephalosporins **1a**–**h** at pH 9.5 and 35 °C

pH = 9.5 and ionic strength 0.14 mol dm^{-3}, in the presence of glucose is shown in Fig. 1. Similar plots obtained with other carbohydrates, demonstrating the presence of a carbohydrate catalysed as well as a spontaneous hydrolysis of the cephalosporins (as indicated by the intercepts). The spontaneous hydrolysis is dependent on both pH and buffer concentration, but since this is a well known reaction we did not study it further. At this pH k_0 values vary linearly with the catalyst concentration, and the values of the second-order rate constants, k_2, obtained from the slopes of such plots are presented in Table 1.

These data indicate that the extent of catalysis depends upon the structure of both cephalosporin and the carbohydrate. For the cephalosporins, both of the groups R^1 and R^2 appear to contribute to the magnitude of catalysis. For example, the relative reactivities of compounds **1c**,**d**,**f**, for which R^1 is constant, vary in the order **1c** $>^8$ **d** $>^3$ **f**. This is precisely the order expected if the difference in electron withdrawing ability of the group R^2 influences the rate of reaction as it does for both the OH^--catalysed hydrolysis and the aminolysis of cephalosporins.[8,9] Similarly, comparison of compounds **1a** and **d** reveals that the pyridinium substituent has a 15-fold greater accelerating affect on the rate of reaction than does the acetoxy group.

It is not so easy to discern a reason for the effect of the R^1 group. Comparison of compounds **1a** with **b** and **d** with **e** (the k_0 values determined for **1f** and **g** are too small to make reliable comparisons meaningful) reveal rate effects of *ca.* 1.5–3. The large differences between the structure of the R^1 groups in **1a** and **b** make it impossible to identify the reason for any rate differences between the two compounds. However, the electron withdrawing effects of the thiazolylmethyl group in **1d** and the aminobenzyl group in **1e** are roughly the same (calculated values of Taft σ^* are 0.52 and 0.54, respectively[10]). Even so, **1d** is three times more reactive than **1e**. The difference in reactivity remains unclear at this time.

It would appear from the data in Table 1 that the group R^2 has a much greater influence on the catalytic rate constants than R^1. Indeed, the k_2 values for the glucose catalysed hydrolysis of

Table 1 Second-order rate coefficients, k_2, for the carbohydrate catalysed hydrolysis of cephalosporins **1a–g**, in carbonate buffer at pH 9.50, 35 °C and μ = 0.14 mol dm^{-3}

	$10^3 k_2$/dm^3 mol^{-1} s^{-1}							
	1a	**b**	**c**	**d**	**e**	**f**	**g**	**h**
Glucose	1.83	1.22	0.372	0.128	0.044	0.013	0	0.106
Galactose	1.44							
Maltose	2.66	2.5						
Sucrose	0.944	0.611	0.142	0.030				
Mannitol	0.278		0.031	0.025	0			

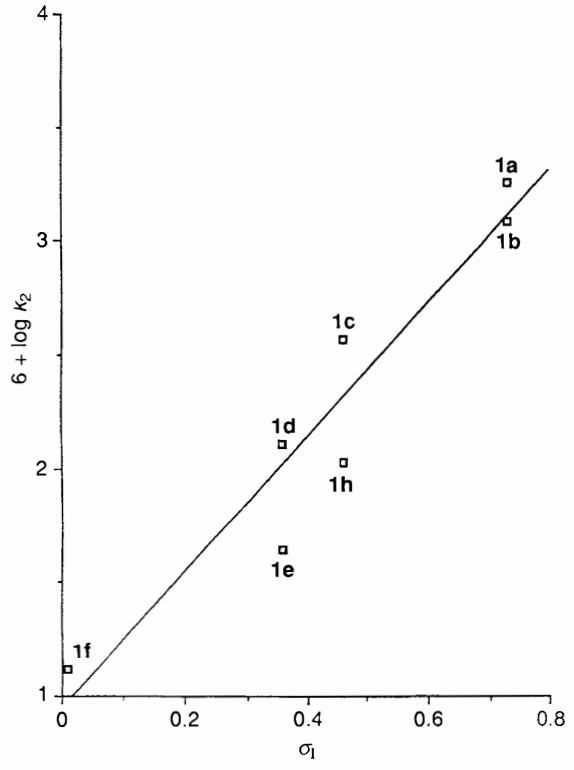

Fig. 2 Variation of the rate constants, k_2, *versus* σ_I for the glucose-catalysed decomposition of cephalosporins **1a–f**

compounds **1a–f** were found to correlate with the σ_I values of R^2 (Fig. 2) despite the fact that R^1 is not constant. Interestingly, the best line through the data is one that almost joins **1a** and **d** or **1c** and **f**, pairs of compounds for which R^1 is held constant. The value of ρ_I, 2.9, is of similar magnitude to that found for the OH$^-$-catalysed hydrolysis of cephalosporins, 2.5.[8] A similar correlation with ρ_I = 3.8 has been found for the aminolysis of cephalosporins.[9] Therefore, the difference in reactivity is the result of the inductive effect of the R^2 group. The most important structural characteristic of the sugars appears to be the hemiacetal OH group. The better catalysts are those in which this OH is free. When the hemiacetal OH is blocked (sucrose) or is not present (mannitol) the magnitude of catalysis is significantly smaller. This is confirmed by the results in Table 2 which show that catalysis by glucose is significantly greater than that by α-methylglucoside. Since the OH in the hemiacetal position is relatively acidic in both pentoses and hexoses,[11] we infer from these results that the more active catalytic species must be the anion resulting from ionization of the hemiacetal OH. Nevertheless, the observation that sucrose, mannitol and α-methylglucoside all exert some catalytic effect implies that the non-hemiacetal hydroxy groups are also able to catalyse the decomposition of the cephalosporins. Using the catalytic rate constant date, k_2, in Tables 2 and 3, together with the number of hemiacetal and non-hemiacetal hydroxyl groups in each of the catalysts, and making the assumption that each non-hemiacetal hydroxy group has identical reactivity, it is possible to calculate that, at these pH values, the hemiacetal OH has 10–15 times the catalytic activity as the other OH groups.

At higher pH values, plots of the pseudo-first-order rate constants for the hydrolysis of **1a** catalysed by glucose and maltose are clearly curved (Fig. 3). This curvature in the plots of k_o *versus* [polyalcohol] has not been reported previously. However, we have determined each rate constant three times, and have repeated these experiments with different batches of carbohydrate and substrate, obtaining similar results in all cases. We are therefore confident that the curved nature of the plots is not an artefact of the system, and is mechanistically significant. Two observations are worthy of note. First, at any one carbohydrate concentration the increasing rate with increasing pH is consistent with the anion of the carbohydrate being the catalytic species; at higher pH values the anion concentration is increased. Second, at any one pH value the downward curvature of each plot suggests the formation of a complex involving the catalytic species. We can envisage two possible types of complex: (i) equilibrium formation of a tetrahedral intermediate formed by attack of the carbohydrate anion at the β-lactam carbonyl group; and (ii) formation of a non-catalytic dimeric species between the anionic and neutral forms of the catalyst, *e.g.* RO$^-$ ··· HOR. Curvature of k_o *versus* [catalyst] plots would be observed in the former mechanism if breakdown of the tetrahedral intermediate becomes rate limiting; a saturation effect is then observed. Curvature of the plots in the second case arises if the carbohydrate anion is the only catalytic species; since dimer formation is a second-order process, the relative extent of its formation is greater at higher carbohydrate concentrations. The concentration of 'free' carbohydrate anion is therefore relatively smaller and catalysis becomes relatively less extensive.

The best computer fit (solid lines in Fig. 3) to the experimental data (individual points in Fig. 3) was achieved using the reactions outlined in Scheme 1, which includes a step involving dimer formation between the carbohydrate anion and the neutral carbohydrate.

$$\text{CarbOH} + \text{OH}^- \overset{K_b}{\rightleftharpoons} \text{CarbO}^- + \text{H}_2\text{O}$$

$$\text{CarbOH} + \text{CarbO}^- \overset{K_D}{\rightleftharpoons} (\text{CarbOH} \cdots {}^-\text{OCarb})$$

$$\text{CarbO}^- + \text{Cephalosporin} \xrightarrow{k_1} \text{Products}$$

$$\text{Cephalosporin} \xrightarrow{k_2} \text{Products}$$

Scheme 1 Component reactions of the carbohydrate catalysed decomposition of cephalosporins

It follows from Scheme 1 that the rate of decomposition of the β-lactam is given by eqn. (1): where the k_2[Cephalosporin] term

$$\text{Rate} = k_1[\text{Cephalosporin}][\text{CarbO}^-] + k_2[\text{Cephalosporin}] \quad (1)$$

refers to the combined hydroxide ion and buffer catalysed processes. Since the total carbohydrate concentration, [CarbOH]$_T$, is given by eqn. (2): then the concentration of the carbohydrate anion catalyst, [CarbO$^-$], is given in eqn. (3). As

$$[\text{CarbOH}]_T = [\text{CarbOH}] + [\text{CarbO}^-] + 2[(\text{CarbO}^- \cdots \text{HOCarb})] \quad (2)$$

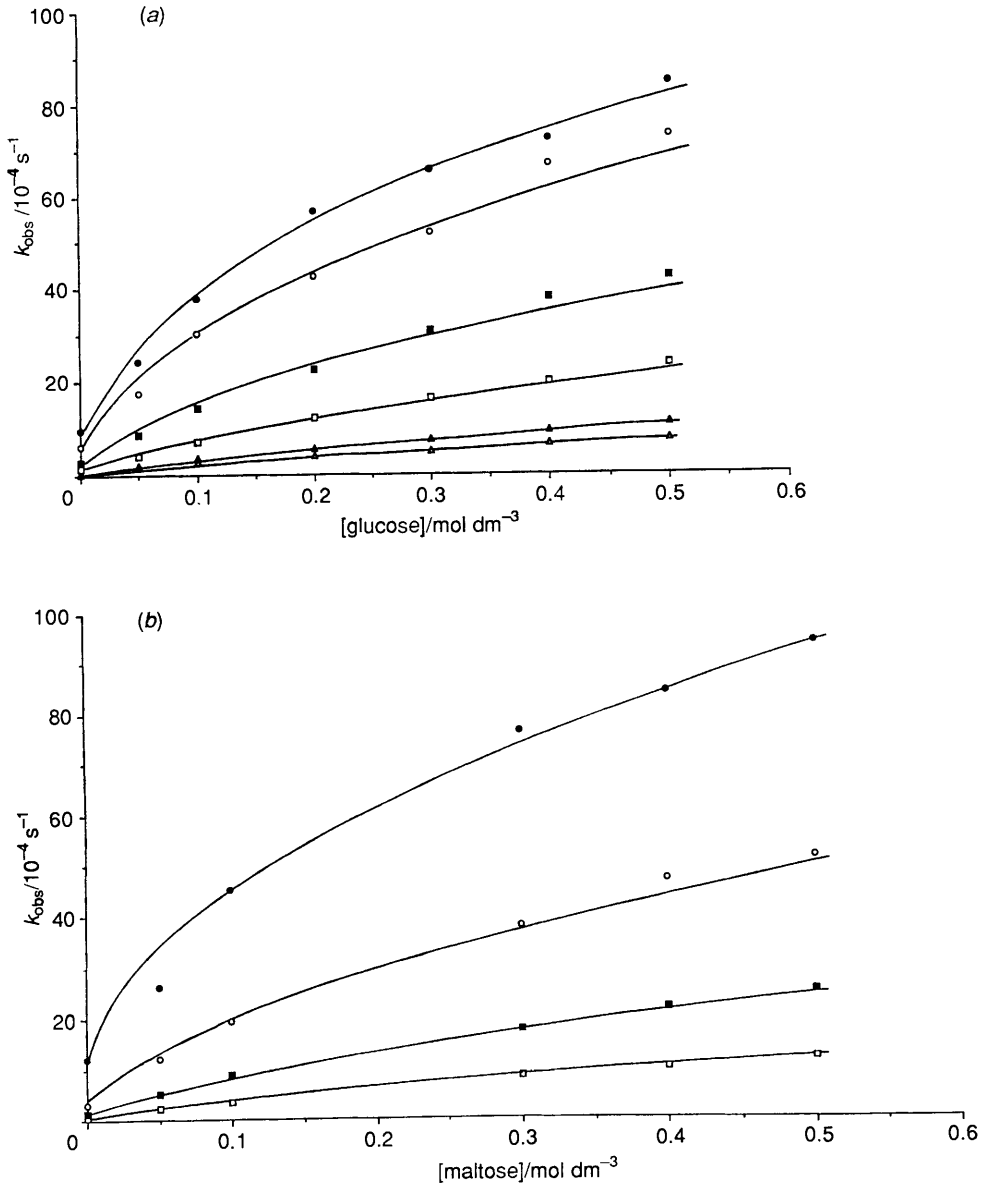

Fig. 3 pH Dependence of k_o for the carbohydrate-catalysed decomposition of **1a** by (*a*) glucose (pH = ●, 10.88; ○, 10.72; ■, 10.32; □, 9.88; ▲, 9.49; △, 9.27) and (*b*) maltose (pH = ●, 10.98; ○, 10.49; ■, 9.98; □, 9.57). Points are experimental data, lines are theoretical fit (see the text).

Table 2 Values of k_o and k_2 for the glucose and α-methylglucoside catalysed hydrolysis of **1a** and **c** in carbonate buffer at 35 °C and μ = 0.5 mol dm^{-3}

	pH	[Glucose]/mol dm^{-3}	$10^5 k_o$/s^{-1}	$10^3 k_2$/dm^3 mol^{-1} s^{-1}	[α-Methylglucoside]/mol dm^{-3}	$10^5 k_o$/s^{-1}	$10^3 k_2$/dm^3 mol^{-1} s^{-1}
1a	9.12	0	4.56	1.36	0	5.36	0.103
		0.1	22.6		0.1	6.44	
		0.3	44.5		0.3	7.86	
		0.5	75.3		0.5	10.7	
1c	9.23						
		0	21.9	0.444	0	21.0	0
		0.1	24.1		0.1	21.6	
		0.3	32.6		0.3	21.0	
		0.5	44.1		0.5	20.4	

$$[CarbO^-] = \{[1 + 8K_DK_b[OH^-][CarbOH]_T/(1 + K_b[OH^-])^2]^{\frac{1}{2}} - 1\} \times (1 + K_b[OH^-])/4K_D \quad (3)$$

the pseudo-first-order rate constant, k_o, is given by eqn. (4),

$$k_o = k_1[CarbO^-] + k_2 \quad (4)$$

eqns. (3) and (4) may be used to compare the theoretical data to those obtained experimentally. Fig. 3 shows that Scheme 1 does indeed give a very satisfactory fit. From these plots, the values of K_b and K_D and k_1 for the glucose- and maltose-catalysed decomposition of cephaloridine **1a** can be obtained and these are contained in Table 3. The value for K_b of 76 dm^3 mol^{-1} for glucose is very close to that, 75.8 dm^3 mol^{-1}, obtained from the

Table 3 Values of the constants k_1, K_b and K_D for the carbohydrate catalysed decomposition of cephaloridine at 35 °C

Catalyst	k_1/dm^3 mol^{-1} s^{-1}	k_b/dm^3 mol^{-1}	K_D/dm^3 mol^{-1}
Glucose	9.4×10^{-1}	76	405
Maltose	7.1×10^{-1}	76	230

reported values of pK_a and K_W at 35 °C.[4] Interestingly, the value obtained from our data for the glucose-catalysed decomposition of cephaloridine, 0.94 dm^3 mol^{-1} s^{-1}, is similar to the value of 1.25 dm^3 mol^{-1} s^{-1} obtained by Bundgaard and Larsen.[4] Moreover, the values of k_1 reveal that glucose is a slightly better catalyst than maltose, an order that is apparently the reverse of that implied from Table 1. This is attributable to the extent of non-catalytic dimer formation, which is greater for glucose than for maltose. The data in Table 1, when corrected for the differing extents of such dimer formation, reveal a similar trend to those in Table 3 for the reaction of the carbohydrate with the β-lactam.

Scheme 1 outlined here identifies the alkoxide anion of the carbohydrate as the catalytic species. We have not identified the product of the reaction, however. Since we monitor the loss of the absorption at 260 nm it is clear that opening of the β-lactam ring is occurring. Following the work of Bungaard[3,4] and Page,[8] we assume that the carbohydrate acts as a nucleophile (rather than general base) catalyst forming an intermediate ester which is unstable and undergoes further decomposition. This would account for the absence of a stable infinity absorbance reading, as has been observed elsewhere.[9] Indeed, Bungaard has identified the formation of such an ester in the reaction of sucrose with benzylpenicillin.[3]

In conclusion, we have shown that cephalosporins undergo catalysed degradation in the presence of carbohydrates and related compounds. Catalytic activity increases at higher pH values. The most important structural feature contributing to reactivity in the cephalosporins is the inductive electron withdrawing ability of the substituent attached to the carbon atom of exocyclic 3-methylene group, as would be expected from the related aminolysis reactions. For the carbohydrate catalyst, the most important structural requirement is the presence of a hemiacetal OH group; when this is blocked, for example in sucrose or α-methylglucoside, catalysis is significantly diminished.

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Paper 3/04888H
Received 12*th August* 1993
Accepted 8*th September* 1993