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NEIGHBOURING ACETAMIDO-GROUP PARTICIPATION IN REACTIONS OF DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE*

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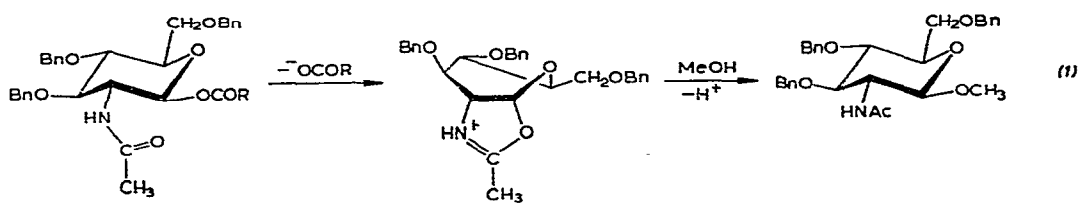
ABSTRACT

Kinetic measurements suggest that neighbouring acetamido-group participation occurs in the spontaneous hydrolysis and methanolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and in the spontaneous hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride. The methanolyses of these compounds proceed with predominant retention of configuration which is also consistent with neighbouring acetamido-group participation. The oxazoline intermediate which would arise from such a process was detected during methanolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride in the presence of bases by n.m.r., i.r., and u.v. spectroscopy. Attempts to isolate the oxazoline were unsuccessful.

INTRODUCTION

Inch and Fletcher[1] showed that 2-acetamido-1-*O*-acyl-2,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoses and β-D-galactopyranoses react with refluxing methanol to give the corresponding methyl β-D-glycosides with retention of configuration. They explained these results by invoking neighbouring-group participation by the amido group and formation of an intermediate oxazoline which underwent nucleophilic attack by methanol to give β-glycosides (eq. *1*). Hydrolysis in aqueous *p*-dioxane at 50° was thought to involve a similar process.



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*Dedicated to the memory of Professor Edward J. Bourne.

Piszkiewicz and Bruice studied the hydrolysis of *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside at 78.2°. The spontaneous hydrolyses of these compounds in the pH range 1.5 to 10.5 occurred 218 and 343 times faster, respectively, than those of the corresponding β-D-glucopyranosides. These results also suggest that neighbouring-group participation is occurring in the reaction of the 2-acetamido-2-deoxy-β-D-glucopyranosides with an oxazoline as intermediate. The acid-catalysed hydrolyses of *o*-nitrophenyl[2], *p*-nitrophenyl[2], phenyl[3], and methyl[3] 2-acetamido-2-deoxy-β-D-glucopyranosides occur 2.24, 2.14, 2.69, and 2.09 times faster, respectively, than those of the corresponding β-D-glucopyranosides, and hence, if there is any neighbouring-group participation in these reactions, the anchimeric assistance is not large. The spontaneous hydrolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside, which occurs with intramolecular, general acid catalysis, proceeds 7.09 times faster than that of *o*-carboxyphenyl β-D-glucopyranoside, a result which suggests that there is weak anchimeric assistance[3]. These results indicate that, for good leaving groups, *e.g.*, protonated alkoxy ($H\overset{+}{O}Me$) or protonated aryloxy ($H\overset{+}{O}C_6H_4NO_2$, $H\overset{+}{O}C_6H_5$), there is little anchimeric assistance, but for partly protonated aryloxy (hydrolysis of 2-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside), it is larger, and for unprotonated aryloxy, it is larger still. Thus, the poorer the leaving group, the greater is the anchimeric assistance by the acetamido group. In this work, we report further on the effect of the leaving group on the anchimeric assistance and describe the detection of the oxazoline intermediate.

RESULTS AND DISCUSSION

Hydrolysis of o-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. — This reaction was studied in buffers of ionic strength 1M at 65°. The rate constants showed a small dependence on buffer concentration (for example, see Table I). It is not clear if this dependence arose from catalysis by the components of the buffer or from a specific salt effect. Piszkiewicz and Bruice reported[3] that no buffer catalysis was detectable at ionic strength 0.3M and 78.2°. In our work, a similar dependence of the rate constant on buffer concentration was found whether the ionic strength was maintained constant with NaCl, KCl, KBr, or $NaClO_4$ (Table II), or if an ionic strength of 0.3M was used (Table III). In contrast, the rate constants for the hydrolysis of *o*-carboxyphenyl β-D-glucopyranoside decreased slightly with increasing concentration of buffer in an acetate buffer of pH 3.67 (*cf.* Table IV with Table I). Although these results suggest that the buffer catalysis in the hydrolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside is genuine, no attempt was made to evaluate the catalytic constants because of the large number of possible terms in the rate expression and the possibility that they are an artifact of the high ionic strength used. In the following discussion, use will be made only of the rate constants extrapolated to zero buffer concentration, k_0, and the pH–rate profile constructed from them (Table V).

TABLE I

HYDROLYSIS OF *o*-CARBOXYPHENYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE AT 65.0° IN ACETIC ACID–SODIUM ACETATE BUFFERS
(pH 3.67; IONIC STRENGTH MAINTAINED CONSTANT AT 1.00M WITH KCl)

[*AcOH*]	[*AcONa*]	*No. of runs*	*Av.* $10^4 k_{obs}$	$10^4 k_{calc}$
8.92	1.00	3	7.949	7.984
6.69	0.75	4	7.494	7.444
4.46	0.50	6	6.946	6.903
2.23	0.25	5	6.329	6.363

Intercept ($k_0 = 5.822 \times 10^{-4}$ sec^{-1} (s.d. 0.116×10^{-4} sec^{-1}). Slope of plot of k_{obs} versus [AcOH] = 0.242×10^{-4} l.mol^{-1}.sec^{-1} (s.d. = 0.021×10^{-4} l.mol^{-1}.sec^{-1}).

TABLE II

THE EFFECT OF VARYING THE SALT USED TO MAINTAIN THE IONIC STRENGTH CONSTANT AT 1.00M ON THE RATE CONSTANT (10^4 *k*/sec^{-1}) FOR THE HYDROLYSIS OF *o*-CARBOXYPHENYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE AT 65.0° AND pH 3.87

[*AcOH*][a]	[*AcONa*][a]	*Salt used*			
		NaCl	*KCl*	*KBr*	$NaClO_4$
		10^4 k/sec^{-1}			
5.64	1.00	6.57	6.51	6.60	6.51
4.23	0.75	6.44[b]	5.86	6.27	—
2.82	0.50	6.21[c]	—	5.88	6.14[d]
1.41	0.25	—	5.41	5.55	5.97[e]

[a]M. [b]pH 3.83. [c]pH 3.79. [d]pH 3.84. [e]pH 3.80. [f]Each rate constant is the average of 5 determinations.

TABLE III

HYDROLYSIS OF *o*-CARBOXYPHENYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE AT 65.0° IN ACETIC ACID–SODIUM ACETATE BUFFERS
(pH 3.87; IONIC STRENGTH MAINTAINED CONSTANT AT 0.30M WITH KCl)

[*AcOH*]	[*AcONa*]	*No. of runs*	*Av.* 10^4 k_{obs}	10^4 k_{calc}
1.69	0.30	5	5.873	5.850
1.13	0.20	5	5.574	5.658
0.56	0.10	6	5.445	5.466
0.17	0.03	5	5.369	5.332

Intercept ($k_0 = 5.280 \times 10^{-4}$ sec^{-1} (s.d. = 0.015×10^{-4} sec^{-1}). Slope of plot of k_{obs} versus [AcOH] = 0.312×10^{-4} l.mol^{-1}.sec^{-1} (s.d. = 0.009×10^{-4} l.mol^{-1}.sec^{-1}).

TABLE IV

HYDROLYSIS OF *o*-CARBOXYPHENYL β-D-GLUCOPYRANOSIDE AT 65.0° IN ACETIC ACID–SODIUM ACETATE BUFFERS
(pH 3.67; IONIC STRENGTH MAINTAINED CONSTANT AT 1.00M WITH KCl)

[*AcOH*]	[*AcONa*]	*No. of runs*	*Av.* 10^4 k_{obs}
8.92	1.00	5	0.2657
6.64	0.75	2	0.2906
4.46	0.50	3	0.3179
2.23	0.25	7	0.3316

TABLE V

THE DEPENDENCE OF k_0 FOR THE HYDROLYSIS OF *o*-CARBOXYPHENYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE ON pH AT 65.0° (I = 1.00M)

pH	*Buffer*	10^4 k_0	10^4 k_{calc}[a]
0.75	Cl_3CCO_2H	10.0	9.68
1.15	Cl_3CCO_2H	9.28	9.50
2.14	$ClCH_2CO_2H$	9.21	9.25
2.88	$ClCH_2CO_2H$	8.73	8.54
3.15	HCO_2H	7.51	7.90
3.55	HCO_2H	6.33	6.36
3.67	CH_3CO_2H	5.82	5.77
3.87	CH_3CO_2H	4.70	4.70
4.00	HCO_2H	3.51	4.01
4.12	CH_3CO_2H	3.64	3.39
4.60	CH_3CO_2H	1.56	1.48
5.12	CH_3CO_2H	0.532	0.501

[a]Calculated from the expression $k_{calc} = (k_1 + k_2 \times 10^{-pH})/(1 + K_a/10^{-pH})$, with $k_1 = 9.41$ sec^{-1}, $k_2 = 1.58 \times 10^{-4}$ $l.mol^{-1}.sec^{-1}$, and $K_a = 1.35 \times 10^{-4}$ $mol.l^{-1}$. The value of k_2 is not defined very accurately; K_a was determined independently under the conditions of the kinetic experiments to be 1.2×10^{-4} $mol.l^{-1}$.

This follows an equation similar to that of k_{obs} for the hydrolysis of *o*-carboxyphenyl β-D-glucopyranoside[4,5], *viz.* k_0[total substrate] = k_1[un-ionised form] + k_2[ionised form] $\times 10^{-pH}$, or $k_0 = (k_1 + k_2 \times 10^{-pH})/(1 + K_a/10^{-pH})$; where K_a, the dissociation constant for the carboxy group, is 1.35×10^{-4}; $k_1 = 9.41 \times 10^{-4}$ sec^{-1} and $k_2 = 1.58 \times 10^{-4}$ $l.mol^{-1}.sec^{-1}$ at 65°. The value of k_1 for the hydrolysis of *o*-carboxyphenyl β-D-glucopyranoside at 65°, extrapolated from the results of Piszkiewicz and Bruice[3], is 6.84×10^{-5} sec^{-1}, *i.e.*, about 13 times less, whereas the value for k_2, extrapolated from the results of Piszkiewicz and Bruice[3] and Capon *et al.*[5], is 1.35×10^{-4} $l.mol^{-1}.sec^{-1}$, which is almost the same as the above value. There therefore appears to be a small enhancement in the k_1 term, which can be ascribed to neighbouring-group participation by the 2-acetamido group, but not in the k_2 term.

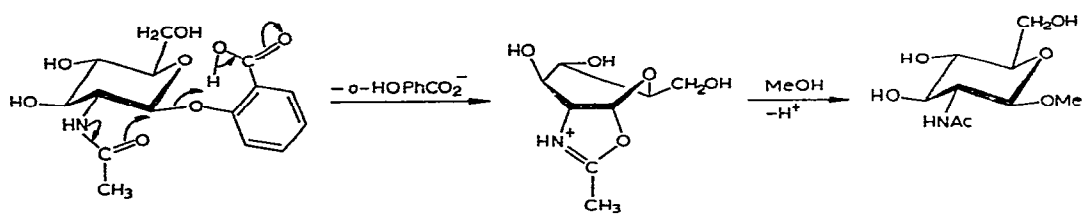
TABLE VI

PRODUCTS OF METHANOLYSIS OF *o*-CARBOXYPHENYL GLYCOPYRANOSIDES CALCULATED FROM OPTICAL ROTATIONS AT 365 nm AFTER 10 HALF-LIVES, AND THE CORRESPONDING RATE CONSTANTS

Glycoside	*Temperature (degrees)*	10^5 k/sec^{-1}	*Products*	
			α-D-Glycoside (%)	*β-D-Glycoside* (%)
α-D-Glucoside[a]	94	4.5	11.5	88.5
β-D-Glucoside[b]	94	14.0	84.0	16.0
2-Acetamido-2-deoxy-β-D-glucoside	94	555	7.6	92.4[c]
2-Acetamido-2-deoxy-β-D-glucoside	60	42.5	5.9	94.1[c]

[a]The methanolysis of phenyl α-D-glucopyranoside yielded 10% of methyl α-D-glucopyranoside and 90% of the β anomer (Ref. 6). [b]The methanolysis of phenyl β-D-glucopyranoside yielded 72% of methyl α-D-glucopyranoside and 28% of the β anomer (Ref. 6). [c]The n.m.r. spectrum of the product was identical with that of the β-glycoside and no signal for the anomeric proton of the α-glycoside could be detected.

In order to test further if the 2-acetamido group was participating, the methanolysis was studied, as the products are stable and hence the steric course of the reaction could be determined. Methanolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside occurs 39.6 times faster than that of *o*-carboxyphenyl β-D-glucopyranoside at 94°, and the product is greater than 90% methyl β-D-glucoside formed with retention of configuration (Table VI). In contrast, *o*-carboxyphenyl α- and β-D-glucopyranoside give mainly products of inversion of configuration. These results are best explained by neighbouring-group participation by the amido group in the methanolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (eq. 2).



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Hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. — The rate of hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside is almost independent of pH in the range 1.18–10.41 but increases strongly with pH at values greater than 11 (Table VII). The rate constant at 25° for the spontaneous hydrolysis is $k_1 = \sim 9.0 \times 10^{-5}$ sec^{-1}, and for the hydroxide-ion catalysed hydrolysis is $k_2 = \sim 3.9 \times 10^{-3}$ l.mol^{-1} sec^{-1}. The rate constant[7] for the spontaneous hydrolysis

TABLE VII

THE DEPENDENCE OF THE RATE CONSTANT FOR THE HYDROLYSIS OF 2,4-DINITROPHENYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE ON pH AT 25.0° (I = 0.1M)

pH	*Buffer*	10^5 k/sec^{-1}	*pH*	*Buffer*	10^5 k/sec^{-1}
1.18	HCl	9.37	7.27	Tris	7.77
1.80	$ClCH_2CO_2H$	9.56	9.12	Tris	8.23
3.73	CH_3CO_2H	8.92	10.41	Phosphate	7.61
4.83	CH_3CO_2H	9.46	11.29	Phosphate	9.99
5.71	CH_3CO_2H	9.41	13.0	NaOH	37.9

of 2,4-dinitrophenyl-β-D-galactopyranoside is 1×10^{-5} sec^{-1} at 25°; it would be expected that the corresponding glucoside would react more slowly, as reactions that involve formation of 1-galactopyranosyl cations normally occur faster than the corresponding reactions involving formation of glucopyranosyl cations[8]. The spontaneous hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside is therefore 10–20 times faster than that of the corresponding glucoside. The methanolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside ($k = 2.48 \times 10^{-4}$ sec^{-1} at 25°) yielded a product whose p.m.r. spectrum was identical to that of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, and no signal that could be attributed to the α anomer was observable. It is therefore concluded that this reaction occurs with at least 95% retention of configuration, and that the methanolysis and hydrolysis of this compound involve neighbouring acetamido-group participation. However, the anchimeric assistance appears to be less than that found[2] for the spontaneous hydrolysis of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside where the leaving group is poorer; the rate constant for this reaction is 1.72×10^{-5} sec^{-1} at 78.2°.

Hydrolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride. — This hydrolysis was followed in a pH stat[9]. When water was used as the solvent, the rate decreased with increasing pH in the range 8–11 (Table VIII). It was thought that this arose from variations in the ionic strength, and when 0.10M sodium perchlorate was

TABLE VIII

THE DEPENDENCE OF THE RATE CONSTANT FOR THE HYDROLYSIS OF 2-ACETAMIDO-2-DEOXY-β-D-GLUCOSYL FLUORIDE AT 25°

pH	10^3 k (*water*)	10^3 k (*0.1M NaClO$_4$*)	*pH*	10^3 k (*water*)	10^3 k (*0.1M NaClO$_4$*)
4.00	7.22	7.19	8.80	5.75	—
4.50	—	7.81	9.00	—	6.59
5.00	7.64	7.56	9.50	—	6.74
6.00	7.24	6.85	10.00	5.43	6.74
7.00	7.05	6.84	11.00	2.94	
8.00	7.30	6.64			

TABLE IX

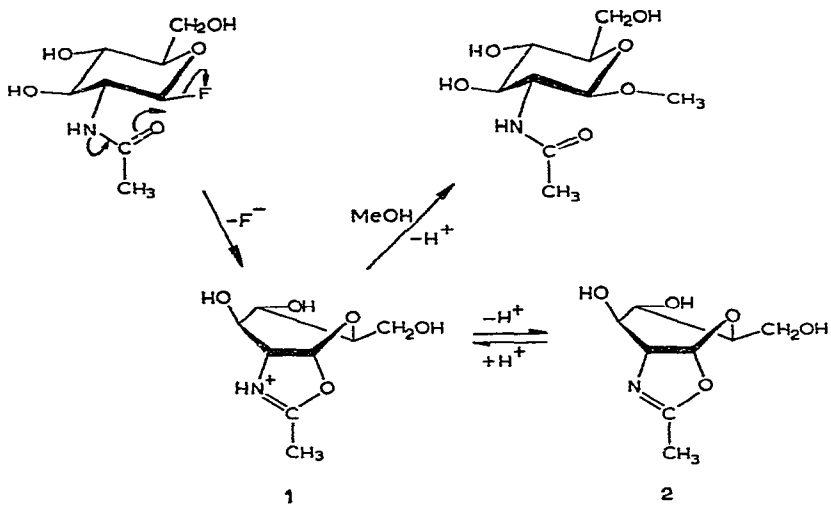
RATE CONSTANTS FOR THE HYDROLYSIS OF DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE

Leaving group	p-$O_2N \cdot C_6H_4 \cdot O^{-a}$	o-$O_2N \cdot C_6H_4 \cdot O^{-a}$	o-$HO_2C \cdot C_6H_4 \cdot O^{-b,c}$		2,4-$(NO_2)_2C_6H_3 \cdot O^{-b}$	F^{-b}
k/sec^{-1}	1.72×10^{-5}	1.45×10^{-4}	9.41×10^{-4}	2.96×10^{-3}	9×10^{-5}	7.1×10^{-3}
Temperature (degrees)	78.2	78.2	65	78.2	25	25

[a]Ref. 2. [b]This work. [c]Ref. 3.

used as solvent, the rate was almost independent of pH in the range 4–10. The average rate-constant was 7.1×10^{-3} sec^{-1}, *i.e.*, 79 times greater than for the spontaneous hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. Barnett[9] did not detect any spontaneous hydrolysis of β-D-glucopyranosyl fluoride after 10 min at pH 6.0 and 20°. These data put an upper limit of 8.5×10^{-5} sec^{-1} for the spontaneous hydrolysis of this compound, if it is assumed that 5% hydrolysis would have been detected. 2-Acetamido-2-deoxy-β-D-glucopyranosyl fluoride must react at least ~50 times faster than β-D-glucopyranosyl fluoride. It therefore seems that neighbouring-group participation is particularly effective with the fluoride leaving-group, and 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride is the fastest-reacting derivative of 2-acetamido-2-deoxy-β-D-glucose for which neighbouring amido-group participation has been detected (see Table IX). The second step in the hydrolysis of all the compounds listed in Table IX should be the same, namely, hydrolysis of the oxazoline, and detection of this should be easiest in the reaction of the fastest-reacting compound, *i.e.*, the fluoride. We therefore attempted to detect the intermediate oxazoline in the methanolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride.

Methanolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride. — The methanolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride in methanol-d_4 was followed by n.m.r. spectroscopy. The initial spectrum showed a singlet for NAc at δ 2.02 and a doublet of doublets for H-1 at δ 5.18 ($J_{H,H}$ 7.8 and $J_{H,F}$ 54 Hz). This spectrum changed to that of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside with a half life of ~800 sec at 35°. The NAc singlet now appeared at δ 2.00, and the H-1 signal was a doublet at δ 4.32 ($J_{H,H}$ 8.0 Hz). No signal attributable to an intermediate oxazoline could be detected. The reaction was formulated as proceeding through the protonated oxazoline **1**, and it was thought that there would be a better chance of detecting the deprotonated form. 2-Methyloxazoline has a pK_a of 5.5 in water[10], and



1 **2**

the stronger base collidine (pK_a 7.5; 1.5 mol. equiv.) was therefore added. The methanolysis was now much slower, so presumably the acid-catalysed methanolysis had been occurring in the first experiment. In addition, two new signals were detected in the n.m.r. spectrum, namely a doublet at δ 6.04 ($J_{H,H}$ 7.0 Hz) and a singlet at δ 2.03; the region of the spectrum between δ 4 and 6.1 is shown in Fig. 1. The signal at δ 6.04 reached a maximum value after ~100 min at 35° and disappeared after

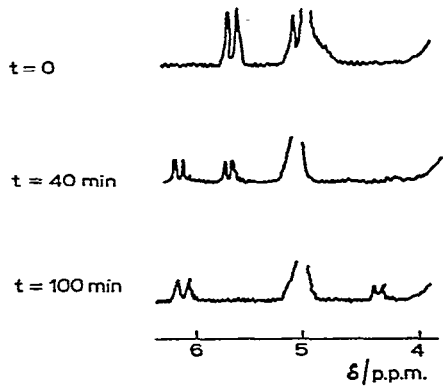


Fig. 1. The n.m.r. spectrum at different times of a reacting solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride in methanol-d_4 containing 1.5 molar equivalents of collidine. The spectrum amplitude is different for the three spectra. The lower-field half of the signal of the anomeric proton of the glucosyl fluoride occurs at $\delta = 5.44$ p.p.m. and part of the high-field half is just discernable in the spectrum at $t = 0$ as the shoulder on the CD_3OH signal at $\delta = \sim 4.9$ p.p.m. The doublet at $\delta = 6.04$ p.p.m. is that of the intermediate, and the doublet at $\delta = 4.32$ p.p.m. is that of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.

18 h; the spectrum was then identical to that of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (+ collidine). When the stronger base 1,8-bis(dimethylamino)-naphthalene[11] (pK_a in water, 12.34) was used, the only anomeric-proton signal visible in the n.m.r. spectrum after 90 min at 35° was that (δ 6.04) of the intermediate. After 24 h, this had been converted into ~30% of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. It was suspected that these new signals arose from the oxazoline **2**, and evidence which supports this view was obtained by following the changes in the i.r. and u.v. spectra. The i.r. spectra of a solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride in methanol-d_4 containing 1.5 molar equivalents of 1,8-bis(dimethylamino)naphthalene were measured in 0.04-mm barium fluoride cells. The initial solution showed the amide-I band at 1647 cm^{-1}, and this decreased with concurrent increase of a band at 1664 cm^{-1} which was attributed to the C=N stretching frequency of the oxazoline. The u.v. spectra were measured for a solution (10^{-2}M) which contained 1.5 molar equivalents of tributylamine. The change in absorbance at the tail of the $n \rightarrow \pi^*$ transition of the amide group at 240 nm decreased to a minimum after 2 h at 25° and then increased slowly to less than the original value after 26 h.

All these results suggest that the reaction of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride in methanol-d_4 containing base yields the oxazoline **2** which is slowly converted into methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. The coupling constant (7 Hz) for the anomeric proton is similar to that (6.4 Hz) reported[12] for the *O*-acetylated oxazoline derived from 2-acetamido-2-deoxy-D-galactose, and to that (7.0 Hz) found in this work for the *O*-acetylated oxazoline from 2-acetamido-2-deoxy-D-glucose (see Experimental). This value suggests that the dihedral angle for H-1/H-2 in the oxazoline is close to 0°, which is reasonable, as the fused, unsaturated, five-membered ring should be nearly planar.

An attempt was made to increase the ratio of the rate constants for formation and decomposition of the oxazoline still further by catalysing breaking of the C–F bond with zirconium nitrate[13]. In the absence of added base, however, a solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride containing 1.5 equivalents of zirconium nitrate yielded methyl 2-acetamido-2-deoxy-β-D-glucopyranoside immediately. In the presence of collidine, there was immediate precipitation of the zirconium.

Attempted isolation of the oxazoline. — Khorlin and his co-workers have reported[14] the preparation of the oxazoline derived from 2-acetamido-2-deoxy-D-glucose tetra-acetate by *O*-deacetylation with triethylamine in methanol. In our hands, however, this method always yielded a product whose i.r. spectrum showed the characteristic amide I and amide II bands at 1650 and 1540 cm^{-1}. An attempt to isolate the oxazoline by precipitation from a solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride and 1,8-bis(dimethylamino)naphthalene in methanol, kept for 90 min at 35°, was also unsuccessful. An attempt was also made to prepare the oxazoline by heating *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside in a sublimation apparatus at 120°/0.001 mmHg. After 12 h, a quantitative amount of salicylic acid had collected on the cold finger, but the residue was highly charred and its i.r. spectrum showed no band at ~1670 cm^{-1} characteristic of the oxazoline ring.

EXPERIMENTAL

General methods. — Melting points were determined by using a Kofler–Reichert hot-stage apparatus and are uncorrected.

P.m.r. spectra were measured on Varian T-60 or HA-100 spectrometers. Chemical shifts are recorded as δ values (p.p.m.) downfield from internal tetramethylsilane. ^{19}F-N.m.r. spectra were measured on Varian HA-100 or Jeol 60-MHz spectrometers.

Infrared spectra were obtained by using Unicam SP1000 and SP200 or Perkin–Elmer 257 spectrophotometers.

Optical rotations were measured at the sodium D line with a Perkin–Elmer 141 polarimeter and a 10-cm cell.

The pH values of the buffers were measured at the temperature of the kinetic experiments by using a Radiometer TTT1 titrator equipped with scale expander. At 25°, a Radiometer G202B glass electrode and Calomel type K-401 calomel electrode

were used, and at 65° a Radiometer G202BH glass electrode with the same calomel electrode.

o-Carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. — Methyl salicylate (2.2 g) was condensed with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (3.8 g) in the presence of potassium carbonate (7.0 g) in acetone (100 ml) overnight at room temperature. The salts were then filtered-off and the acetone was removed under reduced pressure. The residue was recrystallised twice from hot ethanol to give *o*-methoxycarbonylphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3.5 g, 58%) as white crystals, m.p. 201–202°, $[\alpha]_D^{30}$ −20.0° (*c* 1.0, chloroform); lit.[15] m.p. 202–203°, $[\alpha]_D^{22}$ −19.6°. This product was *O*-deacetylated by the method[16] of Zemplén to yield a white solid which was recrystallised from aqueous ethanol to give *o*-methoxycarbonylphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (2.4 g, 70%), m.p. 206–208°, $[\alpha]_D^{30}$ −51.5° (*c* 2, water); lit.[15] m.p. 207–208°, $[\alpha]_D^{22}$ −45° (water–methanol, 1:1).

The methyl ester was hydrolysed with standard barium hydroxide for 16 h. A stoichiometric quantity of standard sulphuric acid was then added, the barium sulphate was removed by centrifugation, and the supernatant solution was freeze-dried. The resulting powder was recrystallised from aqueous ethanol to give the title compound as the monohydrate (2.0 g, 80%), m.p. 151–152°, $[\alpha]_D^{30}$ −51.5° (*c* 1.0, water); lit.[17] m.p. 151–152°, $[\alpha]_D^{20}$ −54°.

2-Acetamido-2-deoxy-β-D-glucopyranosyl fluoride. — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (4 g) was allowed to react with sulphuric acid-dried silver fluoride (8 g) in dry acetonitrile (15 ml), following the method described by Helferich[18] for the preparation of β-D-glucopyranosyl fluoride. The flask was sealed and protected from the light, and then shaken for 48 h at room temperature. The reaction was followed by t.l.c. (methanol–chloroform, 8:92); chloride, R_F 0.7; product, R_F 0.55. The reaction mixture was then filtered through Celite 535, and the acetonitrile was removed on a rotary evaporator. The solid residue was dissolved in 15% methanol–chloroform and applied to a column (3 × 40 cm) of silica gel (chromatography grade) in order to adsorb dissolved silver salts. The column was eluted with this solvent system, and the fractions containing the fluoride were evaporated to ~15 ml. Ether (10 ml) was added and crystallisation then commenced. The 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl fluoride was recrystallised from methanol–chloroform to give white prisms (3.6 g, 95%), m.p. 156.5°. P.m.r. data ($CDCl_3$–CD_3OD): δ 1.94–2.90 (12 H, acetate methyls), 3–4 (m, 7 H, ring protons), 5.39 (doublet of doublets, 1 H, $J_{1,2}$ 6.8, $J_{F,1}$ 53 Hz), 6.15 (s, 1 H, N–H, disappears on D_2O addition). The identity of the H–F coupling was confirmed by ^{19}F decoupling. Irradiation of the low-field half of the ^{19}F quartet, centred at 56.444554 MHz, caused the lower half of the ^1H-spectrum, at δ 5.87, to collapse to a singlet. Irradiation of the high-field half of the ^{19}F quartet, centred at 56.444546 MHz, caused the high-field signal of the ^1H-spectrum, at δ 4.95, to collapse to a singlet at δ 5.39. ^{19}F-N.m.r. data: +5644.5 Hz (CF_3CO_2H), doublet of doublets, $J_{F,1}$ 58, $J_{F,2}$ 13 Hz.

Anal. Calc. for $C_{14}H_{26}FNO_8$: C, 48.1; H, 4.8; N, 4.0; F, 5.4. Found: C, 48.6; H, 5.9; N, 3.5; F, 5.0.

Deacetylation was difficult to carry out without loss of fluoride; the following method was satisfactory. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl fluoride (0.4 g) was dissolved in a mixture of dry methanol (4 ml) and dry chloroform (4 ml). Methanolic sodium methoxide (M, 0.3 ml) was added with shaking. After 1 min at room temperature, the solution was evaporated on a rotary evaporator to 2 ml, not using a water bath and causing cooling of the solution. Trituration and the addition of a few drops of dry ether effected crystallisation of the product, which was filtered-off and washed with ether; from addition of methoxide to crystallisation of the product took no more than 2 min. Yield, 0.2 g (65%); m.p. 199–201°; $[\alpha]_D^{25}$ 0° (*c* 0.4, methanol). I.r. spectrum: ν_{max} 3600–3200 (OH and N–H), 1660 (Amide I), and 1565 cm^{-1} (Amide II); there were no signals which could be ascribed to *O*-acetyl groups. P.m.r. data (CD_3OD spectrum taken immediately after dissolution): δ 2.02 (s, 3 H, NAc), 3–4 (m, 7 H, ring protons), 5.18 (doublet of doublets, 1 H, $J_{F,1}$ 54, $J_{1,2}$ 7.8 Hz). ^{19}F-N.m.r. data: +5780 Hz (with respect to CF_3CO_2H); $J_{F,1}$ 52, $J_{F,2}$ 11 Hz.

Anal. Calc. for $C_8H_{14}FNO_5$: C, 43.05; H, 6.3; N, 6.3; F, 8.5. Found: C, 42.5; H, 6.2; N, 6.2; F, 8.9.

*2-Methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyrano)-Δ²-oxazoline.* — This compound was prepared as a glass from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride by the method of Khorlin *et al.*[14], with the modification that collidine was removed from the reaction mixture by washing with aqueous cadmium chloride. I.r. spectrum (thin film); ν_{max} 3020(m), 2960(m), 1740(m), 1675(s), 1432(m), 1370(s), 1315(m), 1220(s), 1125(m), 1030(s), 938(s), and 750(s) cm^{-1}. N.m.r. data ($CDCl_3$): δ 5.93 (d, 1 H, $J_{1,2}$ 7.0 Hz), 5.26 (t, 1 H), 4.93 (d, 1 H), 4.20 (d, 3 H), 3.2–3.8 (m, 1 H), 2.03 (bs, 12 H). Identical material was obtained by the method of Pravdíc *et al.*[12]. *O*-Deacetylation of this compound using triethylamine in methanol, according to the method of Khorlin *et al.*[14], was unsuccessful. An oil was obtained which had the characteristic amide I and II bands at 1660 and 1540 cm^{-1}, and hence the oxazoline ring had been opened.

Other compounds. — *o*-Carboxyphenyl β-D-glucopyranoside[5], *o*-carboxyphenyl α-D-glucopyranoside[5], methyl α-D-glucopyranoside[19], methyl β-D-glucopyranoside[19], and 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[20] were samples prepared previously. Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was prepared by the method of Leaback and Walker[21]; m.p. 204–204°, $[\alpha]_D^{30}$ −45.6° (*c* 1.0, water); lit.[21] m.p. 204°, $[\alpha]_D^{19}$ −47.1° (water). Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (Koch–Light), recrystallised from aqueous ethanol, had m.p. 187–188°, $[\alpha]_D^{30}$ +128.4° (*c* 1.1, water).

Kinetic measurements. — The hydrolysis of *o*-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was followed by measuring the release of salicylic acid at 310 nm in a Cary 14 spectrophotometer. Reactions were followed for at least 3 half-lives, and rate constants were calculated from 20–30 values of the absorbance

read from the strip chart at various times using a generalised least-squares procedure[22]. Calculations were carried out on Glasgow University's KDF-9 computer. The hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was studied similarly, except that a Solatron Compact Data Logger was used to collect the absorbance values at convenient time-intervals. The pen-drive of the Cary 14 spectrometer drove the sweep arm of a potentiometer, across which was fed a voltage from a Mallory battery. This provided a voltage proportional to the absorbance which was fed directly to the logger which punched the value on to 5-hole paper tape. Rate constants were calculated using a generalised least-squares method[22] on the KDF-9 computer.

The kinetics of the methanolyses of the *o*-carboxyphenyl glucosides were measured by using the method of sealed bulbs. A standard solution ($\sim 10^{-2}$M) in methanol was prepared, and samples (2 ml) were sealed in Pyrex test-tubes. These were heated in a thermostat for a convenient time-interval and then quenched in iced. water. The optical rotation of each sample was measured at the sodium D-line, and the absorbance at 310 nm determined after dilution. Rate constants, calculated from the rotations and absorbances by the generalised least-squares method, were almost identical.

The hydrolysis of 2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride was followed by titrating the released acid with 0.10M carbonate-free sodium hydroxide in a pH stat, which consisted of a Radiometer titrator type TTTlc, Radiometer titrigraph type SBR2c, and Radiometer Autoburette type ABU12. The sample was weighed out to give 80% full-scale uptake of alkali at infinity (0.02 mmol). Thermostatted, boiled-out, distilled water (10 ml) or 0.1M sodium perchlorate (10 ml) was brought to the correct pH by addition of NaOH from the autoburette after standardising the meter, the sample was dissolved, and the reaction vessel replaced in the thermostat after shaking. About 20 values were read from the chart, and the first-order rate constant was calculated by the generalised least-squares method[22].

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