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Acid Hydrolysis of Mono-hydroxyethyl Ethers of Methyl α- and β-D-Glucopyranoside

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The eight mono-hydroxyethyl ethers of methyl α- and β-D-glucopyranoside have been prepared and their acid hydrolysis investigated. They were all hydrolysed at a lower rate than the unsubstituted glucosides. The results are discussed in relation to similar studies of other ethers of these glucosides and to the acid hydrolysis of polysaccharides.

The rate of hydrolysis of a glycosidic linkage in a polysaccharide depends upon three factors; the nature of the sugar residue, the nature of the agluconic groups, and the substitution of the sugar residue.

Several sugar residues, which may be furanosidic or pyranosidic, α- or β-linked, occur in polysaccharides. The acid hydrolysis of simple glucosides, containing these residues, has been studied extensively and the relation between structure and rate of acid hydrolysis is reasonably well understood.

For polysaccharides the effect of the aglucone upon acid hydrolysis of a glycosidic linkage is essentially reduced to the difference between primary and secondary positions in a sugar residue. A linkage to a primary position (generally a 6-position) is more resistant to acid hydrolysis than a linkage to a secondary (2-, 3-, and 4-) position. On acetolysis, the linkages to primary positions are more readily cleaved than those to secondary positions, which is of practical importance.

A sugar residue in a polysaccharide could either be unsubstituted (terminal residue), monosubstituted (chain residue), or disubstituted (residue in a branching point). The results of Freudenberg and Blomqvist,[1] on hydrolysis of the cellodextrins, indicate that a terminal linkage is hydrolysed more readily than a central. Recent results by Feather and Harris [2] have confirmed this. They showed that, in cellotriose, the glucosidic linkage to the central glucose residue is hydrolysed 1.5 times faster than the linkage to the reducing glucose residue. Also here the relative rates are reversed on acetolysis.

On acid hydrolysis of branched polysaccharides, trisaccharides representing the branching point are formed only in low yields. It could well be that

the glycosidic linkage of disubstituted sugar residues are more resistant to acid hydrolysis than those of un- and monosubstituted residues. The formation of a branched trisaccharide should then require that a resistant linkage should be cleaved but two less resistant linkages survive, which could explain the low yields observed. McKee and Dickey,[3] who studied the acid hydrolysis of a 4-*O*-methyl glucuronoxylan also found that the xylosidic linkage of a disubstituted xylose residue (branching point) was more resistant to hydrolysis than that of a monosubstituted (chain) residue, and attributed this to the steric effects of the substituents.

The effect of substitution of a sugar residue upon the rate of acid hydrolysis has not been studied extensively. In a previous paper[4] we reported such studies on the mono-isopropyl ethers of methyl α- and β-D-glucopyranoside. Timell[5] recently reported similar studies on mono-methyl ethers of methyl β-D-glucoside and gentiobiose. The present paper reports hydrolysis studies on the monohydroxyethyl ethers of methyl α- and β-D-glucopyranoside.

The synthesis of the glucosides followed conventional lines. The 2- and 3-hydroxyethyl ethers were prepared by partial etherification of methyl 4,6-*O*-benzylidene α- or β-D-glucoside with methyl bromoacetate, separation of the mono-ethers by silicic acid chromatography, lithium aluminium hydride reduction, and removal of the benzylidene groups by mild acid hydrolysis. Similar etherification and reduction of methyl 2,3,6-tri-*O*-acetyl-β-D-glucoside yielded methyl 4-*O*-hydroxyethyl-β-D-glucoside. Treatment of the latter with methanol-acid yielded a mixture of the α- and β-forms, which were separated by chromatography on a strongly basic ion exchange resin.[6] 6-*O*-Hydroxyethyl-D-glucose was prepared as described by Timell[7] and transferred, by Fischer synthesis, into the methyl glucoside mixture, which was fractionated as above. The free sugars released on acid hydrolysis of the glucosides were investigated by paper electrophoresis in germanate buffer,[8] and showed the mobilities typical for 2-, 3-, 4-, and 6-substituted glucoses, respectively.

The hydrolysis studies on 0.05 M solutions of glucoside in 0.50 M aqueous sulphuric acid, were carried out at three different temperatures (70°, 80°, and 93°) and were followed polarimetrically as previously described.[4] For all substances studied a first order kinetics was observed and the plots of log

Table 1. Acid hydrolysis of mono-hydroxyethyl ethers of methyl α-D-glucopyranoside.

Methyl α-D-glucoside with hydroxyethyl group in position	$k \times 10^6$ sec^{-1}			k_{rel} [a] 80°	E kcal/mole	$\Delta S^{\neq}$ 80° [b]
	70.0°	80.0°	93.0°			
None	2.82	13.8	76.1	1	33.8	13.2
2	2.62	10.9	60.2	0.79	34.1	12.0
3	2.66	11.4	65.8	0.82	35.2	12.9
4	2.78	11.5	66.1	0.83	34.9	12.7
6	2.08	9.45	52.0	0.68	34.7	14.7

[a] k_{rel} refers to the parent unsubstituted glucoside.
[b] cal/degr. mole.

Table 2. Acid hydrolysis of mono-hydroxyethyl ethers of methyl β-D-glucopyranoside.

Methyl β-D-glucoside with hydroxyethyl group in position	$k \times 10^6$ sec^{-1}			k_{rel} [a] 80°	E kcal/mole	$\Delta S^{\neq}$ 80° [b]
	70.0°	80.0°	93.0°			
None	6.01	25.4	141.0	1	33.9	14.0
2	5.24	20.7	100.0	0.81	32.0	11.2
3	5.23	21.8	119.5	0.86	33.3	11.1
4	5.60	22.6	125.3	0.89	33.8	13.2
6	4.35	18.2	98.0	0.71	33.8	13.8

[a] k_{rel} refers to the parent unsubstituted glucoside.
[b] cal/degr. mole.

k versus $1/T$ gave straight lines. The results are summarised in Tables 1 and 2. The $\Delta S^{\neq}$ values are all of the same order of magnitude, indicating that all the substances studied are hydrolysed by the same or a similar mechanism. The differences between the E and $\Delta S^{\neq}$ values are rather small and often within the experimental errors. A discussion of the results in terms of these parameters is therefore hardly justified. It seems to be significant, however, that the $\Delta S^{\neq}$ values for the isopropyl ethers [4] are generally higher, and those of the hydroxyethyl ethers generally lower than those of the parent glucosides.

In Table 3, the rates of hydrolysis at 80°, relative to those of methyl α- and β-D-glucopyranoside, are summarised. The values for the isopropyl ethers and the methyl ethers in the β-series, determined by Timell,[5] are also included in the table.

If the effect of a substituent could be divided into a sterical and an inductive effect, one should assume that the introduction of a bulky substituent would render the glucoside more stable to acid hydrolysis. This effect should increase in the order $CH_3- < HOCH_2CH_2- < (CH_3)_2CH-$. An electron releasing group would render the glucoside more labile and this effect should increase in the order $HOCH_2CH_2- < CH_3- < (CH_3)_2CH-$. The effect should of course also depend upon the position of the substituent.

Table 3. Relative rates of hydrolysis, at 80° of mono etherified methyl α- and β-D-glucopyranosides.

Substituent in position	α		β		
	$-OCH(CH_3)_2$	$-OCH_2CH_2OH$	$-OCH_3$	$-OCH(CH_3)_2$	$-OCH_2CH_2OH$
None	1	1	1	1	1
2	1.16	0.79	0.86	1.99	0.81
3	1.33	0.82	0.99	1.84	0.86
4	1.02	0.83	0.88	1.11	0.89
6	0.78	0.68	0.67	0.84	0.71

A common feature is that substitution in the 6-position with either methyl, isopropyl, or hydroxyethyl, reduces the rate of hydrolysis. The introduction of the bulky isopropyl group in all other positions enhances the rate of acid hydrolysis, indicating that steric effects are less important than inductive effects. In agreement with this all hydroxyethyl ethers are hydrolysed at lower rates than the corresponding isopropyl ethers. Another observation is that the effect of isopropylation is higher in the β- than in the α-series but no such tendency is observed for the hydroxyethyl ethers.

This simple analysis of the results is completely upset when the methyl ethers are considered. Steric and inductive effects should contribute to render the hydroxyethyl ethers more resistant to acid hydrolysis than the methyl ethers. The differences are, however, small and for two pairs the methyl ethers are more resistant. It is therefore evident that the analysis of the results in terms of steric and inductive effects involves a considerable oversimplification and that other effects, due to solvation and dipole-dipole interactions, must also be taken into account. Considering that the ratio between the highest and the lowest rate for the 22 substances in Table 3 is only 3, it does not seem feasible to give a rational explanation for the differences in their rates of acid hydrolysis.

A sugar residue in the chain or in a branching point of a polysaccharide is substituted by other sugar residues. Of the different ether groups; methyl, isopropyl, and hydroxyethyl, the latter is probably the best model for such a sugar residue, as far as bulk and inductive effects are concerned. The inductive effect of a glycosidically linked sugar residue or a hydroxyethyl ether group should render the glycosidic linkage more resistant to acid hydrolysis. When, as in a glucose residue, the crowding of substituents is increased in the transition state, bulky substituents should also stabilise the glycosidic linkage. The results of the model experiments indicate that inductive effects are more important than steric effects but also show that it is not possible to rationalise the results only in terms of inductive and steric effects.

The investigations have thrown some light upon the way in which substitution may effect the acid hydrolysis of linkages in polysaccharides but it must be addmitted that the effects involved are not fully understood.

EXPERIMENTAL

All melting points are corrected. Evaporations were done under reduced pressure at a bath temperature below 40°.

Paper electrophoresis was performed on Whatman No. 3MM filter paper, using 0.05 M germanate buffer at pH 10.7.[8]

Thin layer chromatography was performed on silica gel G (E. Merck, AG) and column chromatography on silicic acid (100 mesh, Mallinckrodt).

Methyl 2-*O*- and 3-*O*-hydroxyethyl α- and β-D-glucopyranoside

Carboxymethylation of methyl 4,6-O-benzylidene-α-D-glucoside. A mixture of benzylidene compound (10 g), dimethyl formamide (100 ml), methyl bromoacetate (150 ml), and Drierite (5 g) was stirred at room temperature for 1 h. Silver oxide (15 g) was then added

over a period of 6 h and stirring continued overnight. Silver salts were removed by filtration and washed with dimethyl formamide (20 ml). Chloroform was added to the combined filtrates, which were then washed with 6 % aqueous potassium cyanide (300 ml). The aqueous phase was washed with chloroform (2 × 100 ml) and the chloroform extracts combined, washed with water (2 × 100 ml), dried over calcium chloride and concentrated. The remaining syrup was fractionated on a silicic acid column (6.5 × 70 cm) using ethyl acetate-light petroleum (2:1) as irrigant. The fractionation was followed by thin layer chromatography (same solvent system) and fractions containing pure components were combined and concentrated. The mono-ethers, R_F 0.70 and 0.54, respectively, were eluted in the following order:

Methyl 4,6-O-benzylidene-2-O-carboxymethyl-α-D-glucoside methyl ester. (0.8 g), amorphous, $[\alpha]_{578}^{21}$ +129° (*c* 1.0, chloroform).

Methyl 4,6-O-benzylidene-3-O-carboxymethyl-α-D-glucoside methyl ester. (1.2 g), m.p. 100—102° (crystallised from ethyl acetate-light petroleum), $[\alpha]_{578}^{21}$ +140° (*c* 1.0, chloroform). (Found: C 58.1; H 6.12; O 35.8. $C_{17}H_{22}O_8$ requires: C 57.7; H 6.22; O 36.1).

Carboxymethylation of 4,6-O-benzylidene-β-D-glucoside (11 g) as well as fractionation of the products was performed in the same way as reported above for the α-isomers. The mono-ethers, having on thin layer chromatography the R_F values 0.77 and 0.54, respectively, were eluted in the following order:

Methyl 4,6-O-benzylidene-2-O-carboxymethyl-β-D-glucoside methyl ester (1.8 g), m.p. 169—171° (from ethyl acetate-light petroleum) $[\alpha]_{578}^{21}$ −54° (*c* 1.0, chloroform). (Found: C 57.8; H 6.19; O 36.2. $C_{17}H_{22}O_8$ requires: C 57.7; H 6.22; O 36.1).

Methyl 4,6-O-benzylidene-3-O-carboxymethyl-β-D-glucoside methyl ester (1.6 g), m.p. 118—120° (from ethyl acetate-light petroleum). $[\alpha]_{578}^{21}$ −13° (*c* 1.0, chloroform). (Found: C 57.9; H 6.20; O 36.2. $C_{17}H_{22}O_8$ requires: C 57.7; H 6.22; O 36.1).

Reduction of a carboxymethyl derivative was performed by dissolving it (1 part) in dry tetrahydrofuran (15 parts). To the stirred solution, which was kept under nitrogen, was added in portions an excess of lithium aluminium hydride, after which the mixture was refluxed for 3 h. Excess of hydride was decomposed by careful addition of ethyl acetate, followed by a few drops of water. The mixture was then dried over sodium sulphate, filtered and concentrated. The following substances were prepared by this method in yields between 60—70 % of the theoretical:

Methyl 4,6-O-benzylidene-2-O-hydroxyethyl-α-D-glucoside m.p. 140—142° (from ethanol) $[\alpha]_{578}^{21}$ +111° (*c* 1.0, chloroform). (Found: C 58.9; H 6.51; O 34.5. $C_{16}H_{22}O_7$ requires: C 58.9; H 6.72; O 34.4).

Methyl 4,6-O-benzylidene-3-O-hydroxyethyl-α-D-glucoside, amorphous, $[\alpha]_{578}^{21}$ +129° (*c* 1.5, chloroform).

Methyl 4,6-O-benzylidene-2-O-hydroxyethyl-β-D-glucoside, m.p. 177—178° (from ethyl acetate-light petroleum), $[\alpha]_{578}^{21}$ −65° (*c* 2.0, chloroform). (Found: C 59.3; H 6.48; O 34.5. $C_{16}H_{22}O_7$ requires: C 58.9; H 6.72; O 34.4).

Methyl 4,6-O-benzylidene-3-O-hydroxyethyl-β-D-glucoside, amorphous, $[\alpha]_{578}^{21}$ −53° (*c* 1.5, chloroform).

Hydrolytic removal of benzylidene groups was performed by dissolving the derivative (1 part) in acetone-water, 3:1 (10 parts) and M aqueous hydrochloric acid (0.13 parts), followed by refluxing for 4 h. The cooled solution was neutralised with Dowex 3 (free base) and concentrated. The substances listed below were prepared by this method in almost quantitative yields. The purity of the substances were checked by thin layer chromatography (chloroform-ethanol 3:1).

Methyl 2-O-hydroxyethyl-α-D-glucopyranoside, amorphous, $[\alpha]_{578}^{21}$ +121° (*c* 1.5, water). M_G of the free sugar 0.1.

Methyl 3-O-hydroxyethyl-α-D-glucopyranoside, amorphous, $[\alpha]_{578}^{21}$ +126° (*c* 1.0, water). M_G of the free sugar 1.45. A sample of 3-*O*-hydroxyethyl-D-glucose prepared according to Shyluk and Timell,[7] showed the same mobility.

Methyl 2-O-hydroxyethyl-β-D-glucopyranoside, m.p. 135—136° (from ethanol). $[\alpha]_{578}^{21}$ −23° (*c* 1.0, water). (Found: C 46.0; H 7.53; O 47.3. $C_9H_{18}O_7$ requires: C 45.4; H 7.58; O 47.1). M_G of the free sugar 0.1.

Methyl 3-O-hydroxyethyl-β-D-glucopyranoside, amorphous, $[\alpha]_{578}^{21}$ −21° (*c* 2. water). M_G of the free sugar 1.45.

Methyl 4-*O*-hydroxyethyl α- and β-D-glucopyranoside

Carboxymethylation of methyl 2,3,6-tri-O-acetyl-β-D-glucopyranoside. The title compound [9] (11 g) was dissolved in methyl bromoacetate (150 ml) and silver oxide (15 g) was added over a period of 8 h at room temperature. The stirring was continued for 24 h, after which the reaction product was isolated by extraction with chloroform, washed with potassium cyanide and concentrated to a syrup (4.96 g).

Reduction of the carboxymethyl group and reductive removal of the acetyl groups was performed as described above for the benzylidene derivatives. The resulting syrup was fractionated on a silicic acid column (6.5 × 70 cm) using chloroform-ethanol (3:1) as irrigant. Paper electrophoresis of a hydrolysate of the first fraction revealed one main compound (M_G 0.3) together with a small amount of glucose.

The anomeric methyl 4-O-hydroxyethyl-D-glucosides. Syrupy methyl 4-*O*-hydroxyethyl-β-D-glucoside (3.0 g) was dissolved in anhydrous methanol (100 ml) and Dowex 50 (H^+), (10 g), previously washed with anhydrous methanol, was added. The mixture was stirred under reflux for 16 h, filtered and concentrated. The resulting syrup was fractionated on a Dowex 1 (OH^-) column [6] (6.5 × 90 cm) irrigated with water. The fractionation was followed polarimetrically and the products first eluted, the methyl 4-*O*-hydroxyethyl α- and β-D-glucoside, were well separated from each other and from other components.

Methyl 4-O-hydroxyethyl-α-D-glucopyranoside, (1.4 g), amorphous, $[\alpha]_{578}^{21}$ +129° (*c* 1.0, water).

Methyl 4-O-hydroxyethyl-β-D-glucopyranoside (1.1 g) m.p. 158.5—159° (from ethanol). $[\alpha]_{578}^{21}$ −18° (*c* 1.5, water). (Found: C 45.5; H 7.60; O 47.1. $C_9H_{18}O_7$ requires: C 45.4; H 7.58; O 47.1).

Methyl 6-*O*-hydroxyethyl α- and β-D-glucopyranoside

6-*O*-hydroxyethyl-D-glucose (1.2 g), prepared according to Shyluk and Timell,[7] was treated with methanol-Dowex 50 (H^+) and the isomers separated as described above.

Methyl 6-O-hydroxyethyl-α-D-glucopyranoside (0.6 g), amorphous, $[\alpha]_{578}^{21}$ +113° (*c* 2.0, water).

Methyl 6-O-hydroxyethyl-β-D-glucopyranoside (0.3 g), amorphous. $[\alpha]_{578}^{21}$ −22° (*c* 1.5, water).

Kinetic determinations

The hydrolyses were followed polarimetrically and the experimental conditions were the same as in the previous study [4] on the acid hydrolysis of the analogous isopropyl ethers.

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