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Pseudocellobiouronic Acid, Synthesis and Acid Hydrolysis

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4-*O*-β-D-Glucopyranosyl-D-glucuronic acid, pseudocellobiouronic acid, has been synthesised. The rates of acid hydrolysis of this substance, cellobiouronic acid and cellobiose have been determined.

By following the change in viscosity of cellulose, dissolved in 81 % phosphoric acid, Marchessault and Rånby [1] obtained results which they stated were due to the presence of a low percentage of linkages in the cellulose, which were more sensitive than the ordinary linkages. They attributed these weak linkages to the presence of oxidised residues in the cellulose, *e.g.* glucuronic acid residues or residues containing carbonyl groups. Starting from the known fact that aldobiouronic acids are considerably more stable towards acid hydrolysis than the corresponding neutral disaccharides, they advanced a theory to account for the presence of weak linkages in molecules containing these oxidised residues. If it is assumed that the rate-controlling step in the acid hydrolysis of a glycoside is the heterolysis of the conjugate acid formed on addition of a proton to the glycosidic oxygen atom, electron withdrawing groups in the sugar residue, as carboxyl and carbonyl groups, should stabilise the linkage. On the other hand, when such groups are present in the aglucone, the same inductive effect should render the glycosidic linkage more labile towards acid hydrolysis. If, for example, a glucuronic acid residue were present in the cellulose chain, the uronide linkage should be more stable but the glucosidic linkage to the uronic acid residue should be more labile than the ordinary glucosidic linkages in the molecule.

This hypothesis seems to involve considerable oversimplifications, and ignores steric effects. Recent results by Easty [2] and by Nakano and Rånby [3] also show that the differences in rates of acid hydrolysis between methyl glucopyranosides and methyl glucopyranosiduronic acids are comparatively small, the former being hydrolysed only 1.5—2.5 times more rapidly in aqueous solutions. The appararent activation energy is even higher for the glucosides than for the uronides. Nakano and Rånby [3] have further shown that the results obtained in 81 % phosphoric acid and in dilute aqueous solutions are not strictly comparable, and that it is questionable whether the degradation of

the uronides in the former solvent is due to acid hydrolysis. Theander [4] has demonstrated that oxidation products of methyl β-D-glucopyranoside, carrying a carbonyl in the 2- or 3-position, are somewhat more labile in acid solutions than methyl β-D-glucopyranoside itself. This is the opposite to what is expected from the above theory. Here also it is questionable whether the hydrolysis of the glycosidic linkage is the primary reaction.

A comparison of the rates of hydrolysis of cellobiose, 4-*O*-(β-D-glucopyranosiduronic acid)-D-glucose (cellobiouronic acid) and 4-*O*-β-D-glucopyranosyl-D-glucuronic acid (pseudocellobiouronic acid), representatives of the three types of linkages in cellulose containing some glucuronic acid residues, discussed above, would throw some light upon these questions. The synthesis of cellobiouronic acid was reported in an earlier communication [5] from this laboratory and simultaneously by Jayme *et al.*[6] No chemical synthesis of a pseudoaldobiouronic acid seems to have been reported but Barker *et al.*[7] have described the enzymatic synthesis of 2-*O*-α-D-glucopyranosyl-D-glucuronic acid.

1,6-Anhydro-cellobiose hexaacetate (I) was converted to 2,3,2′,3′,4′,6′-hexa-*O*-acetyl-α-cellobiosyl chloride (II) by treatment with titanium tetrachloride in chloroform. The reaction is analogous to the ring opening of 1,6-anhydro-D-glucose triacetate, studied by Zemplén and Csürös.[8] Acetic acid was added to the system as suggested by Csürös *et al.*[9] Treatment of II with mercuric acetate in acetic acid yielded the heptaacetate (III) which was oxidised to the pseudocellobiouronic acid heptaacetate (IV) by potassium permanganate in acetic acid, as devised by Stacey [10] for a similar reaction. The oxidation was sluggish, compared with the analogous oxidation of 2,3,2′,3′,4′-penta-*O*-acetyl-1,6-anhydrocellobiose.[5] The primary hydroxyl group in the reducing glucose residue of cellobiose therefore seems to be considerably sterically hindered compared with the corresponding group in the non-reducing glucose residue. This was also indicated by the catalytic oxidation of benzyl-β-cellobioside,[6] in which neither of these groups is protected, to the cellobiouronic acid derivative in good yield. Attempts to oxidise III with oxygen and a platinum catalyst in butanone were also unsuccessful. Part of IV was converted to the crystalline methyl ester heptaacetate, m.p. 143—145°, $[\alpha]_D^{20}$ −22°, and the remainder was deacetylated to give the free pseudocellobiouronic acid (V). The crystalline product, $[\alpha]_D^{20}$ + 22° → + 16° melted with decomposition at 177—180°.

The structure of the product, which is evident from its mode of synthesis, was substantiated by hydrolysis to D-glucose and D-glucuronic acid. Borohydride reduction followed by acid hydrolysis gave D-glucose and L-gulonic acid.

The acid hydrolysis of cellobiose, pseudocellobiouronic acid and cellobiouronic acid was made in 1 M sulphuric acid. For the first two substances, the reaction was followed polarimetrically, at 70°, 80°, and 90°. As expected, the theoretical final reading was never reached, due to some decomposition of the products, this being small for cellobiose but greater for pseudocellobiouronic acid. The values for the rate constants, calculated from the observed rotations and the theoretical end value, therefore decreased continuously but the values for the first third of the reaction were reasonably constant. The hydro-

lysis of cellobiouronic acid, which was more resistant, was studied at 80°, 90°, 100°, and 110°. The solutions rapidly turned dark, thus preventing the reaction being followed polarimetrically. The reaction was therefore followed by paper chromatographic determination of the glucose formed, using D-xylose as an internal standard. The values for the rate constants obtained by this method were not very accurate compared with those obtained by the polarimetric method. The values are summarised in Table 1. Moelwyn-Hughes [11] reports an activation energy of 30 710 cal/mole for the acid hydrolysis of cellobiose.

It is seen that pseudocellobiouronic acid is hydrolysed at about the same rate as cellobiose and there is little difference in their apparent activation energies. This is obviously contrary to the predictions of Marchessault and Rånby,[1] discussed above. The rate constant for cellobiouronic acid, at 90° is about 35 times lower than that for cellobiose, in agreement with the known stabilities of aldobiouronic acids and their corresponding neutral disaccharides. A rational interpretation of this difference, and the much smaller differences observed between the rates of hydrolysis of the methyl glucopyranosides and the methyl glycopyranosiduronic acids must await an investigation of several model substances and a theoretical treatment of the results in which inductive and steric effects as well as different possible reaction mechanisms are considered.

EXPERIMENTAL

Melting points are corrected. Concentrations were performed under reduced pressure at a bath temperature of 40°.

2,3,2',3',4',6'-Hexa-O-acetyl-α-cellobiosyl chloride (II). 1,6-Anhydro-cellobiose hexaacetate (10 g), titanium tetrachloride (4 ml) and acetic acid (0.3 ml) were added to anhydrous, ethanol-free chloroform (200 ml). A yellow precipitate formed immediately. The mixture was refluxed (bath temperature 65°) for 10 h, cooled and washed successively with ice-water, saturated aqueous sodium hydrogen carbonate and water, dried over calcium chloride, decolourised with a small amount of activated carbon and concentrated. The residue was crystallised from chloroform-ethyl ether yielding 7.2 g of

Table 1. Rate constants for the hydrolysis of cellobiose, pseudocellobiouronic acid and cellobiouronic acid in 1 M sulphuric acid. (The values are obtained by interpolation from the observed values, determined at slightly different temperatures).

	Rate constants × 10^6 sec^{-1}		
Temperature °C	Cellobiose	Pseudocellobiouronic acid	Cellobiouronic acid
70	24.7	29.4	
80	97.1	106	2.6
90	353	353	10
100			36
110			130
Apparent activation energy cal/mole	33 000	31 000	35 000

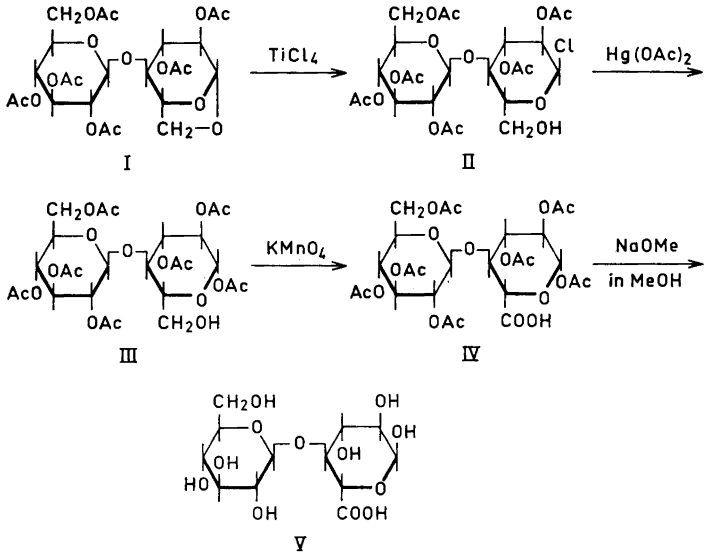
material, m.p. 220–224°. Crystallisation from acetone-isopropyl ether yielded the pure substance, m.p. 231–232°, $[\alpha]_D^{20}$ + 79° (*c* 2.0 in chloroform). [Found: Cl 5.80. $C_{24}H_{33}Cl$ requires: Cl 5.78.]

1,2,3,2',3',4',6'-Hepta*-O-*acetyl-β-cellobiose (III). *II* (20 g) and mercuric acetate (22 g) were dissolved in acetic acid (400 ml) and kept at room temperature overnight. The solution was then poured into ice-water (1000 ml) and extracted with chloroform (5 × 200 ml). The chloroform solution was washed with aqueous sodium hydrogen carbonate and water, dried over calcium chloride and concentrated to a syrup. This was crystallised from ethanol, yielding the almost pure substance (15.2 g), m.p. 198–200°. Further crystallisations from ethanol raised the m.p. to 202–203°, $[\alpha]_D^{20}$ –22° (*c* 8.0 in chloroform). [Found: C 48.9; H 5.65; O 45.2. $C_{26}H_{36}O_{18}$ requires: C 49.1; H 5.70; O 45.2.]

Hepta-O-*acetyl-pseudocellobiouronic acid (IV).* The cellobiose heptaacetate (10 g) was dissolved in analytically pure acetic acid (100 ml). Finely powdered potassium permanganate (6 g) was added in portions with stirring, over 8 days. Preliminary oxidations, followed by thin layer chromatography on silica gel (ethanol-butanol-water, 3:10:5) indicated that these were the optimal conditions for obtaining a good yield of the acid. Excess permanganate was destroyed by sodium oxalate and the reaction mixture poured into water, filtered and extracted with chloroform. The chloroform solution was washed with water, dried over calcium chloride and concentrated. The resulting syrup was fractionated on a silica gel column (5 × 50 cm), using chloroform as irrigant. Unchanged starting material was eluted before the acid, which was obtained as a non-crystalline syrup (6.0 g), containing some impurities.

Methyl ester heptaacetate of pseudocellobiouronic acid. Part of the above product was dissolved in methanol and methylated with diazomethane in ethyl ether. The reaction product crystallised from ethanol and after repeated crystallisations from methanol had m.p. 143–145° and $[\alpha]_D^{20}$ –22° (*c* 0.5 in chloroform). [Found: C 49.2; H 5.48; O 45.9. $C_{27}H_{36}C_{19}$ requires: C 48.8; H 5.46; O 45.7.]

Pseudocellobiouronic acid (V). The heptaacetate (IV, 3.8 g) was dissolved in anhydrous methanol (10 ml), to which sodium methoxide (from 0.2 g sodium) in methanol (50 ml) was added. After about 5 min a pale yellow precipitate was formed. The mixture was kept for 30 min at room temperature, neutralised with 0.1 M acetic acid, concentrated to a small volume and filtered through a column of Dowex 50 (H^+). The solution, which in addition to pseudocellobiouronic acid contained smaller amounts of glucose, glucuronic acid and unidentified products, was concentrated and the residue fractionated on a



I II III IV V

cellulose column (ethyl acetate-acetic acid-water, 3:1:1). The chromatographically pure pseudocellobiouronic acid thus obtained (1.9 g) crystallised from ethanol, m.p. 177–180° (decomp.), $[\alpha]_D^{20}$ + 22° → + 16° (*c* 0.5 in water). [Found: C 40.0; H 6.13; O 53.8. $C_{12}H_{20}O_{12}$ requires: C 40.5; H 5.66; O 53.9].

Characterisation of pseudocellobiouronic acid. Paper chromatography of an acid hydrolysate of pseudocellobiouronic acid revealed the presence of glucose, glucuronic acid and glucurono-3,6-lactone. When the hydrolysis was preceded by borohydride reduction, spots corresponding to glucose, gulonic acid and gulono-1,4-lactone were observed. D-Glucose (30 mg), m.p. 143–145° and L-gulono-1,4-lactone (30 mg), m.p. 179–181°, identical with authentic specimens, were obtained from the reduced acid (100 mg) by fractionation of the hydrolysate on thick filter paper (ethyl acetate-pyridine-water, 8:2:1).

Kinetic experiments

Hydrolysis of cellobiose and pseudocellobiouronic acid. The substance (100 mg) was dissolved in 1 M sulphuric acid (6 ml) and transferred to a 10 cm polarimeter tube, through the jacket of which water from the thermostat (70°, 80° or 90°) was circulated. The temperature of the solution in the tube was determined. The optical rotation was recorded by a Perkin Elmer 141 photoelectrical polarimeter, connected to a potentiometer recorder. The expected final rotation was determined by measuring the optical rotations of the reaction products, D-glucose and D-glucuronic acid, in the same solvent at the same temperatures. The rate constants were calculated in the ordinary manner, assuming first order kinetics. Two typical runs are given in Table 2.

Hydrolysis of cellobiouronic acid. The previously prepared cellobiouronic acid [5] was obtained crystalline, m.p. 190° (decomp.), after seeding with material,[6] kindly supplied by Professor Jayme. Samples of cellobiouronic acid (20 mg) in 1 M sulphuric acid (1.5 ml) were heated, in sealed tubes, at 80°, 90°, 100° or 110° for various times (6–8 different times for each temperature). The tubes were then cooled down, opened and to 1 ml of the solution was added the same volume of 0.1 M D-xylose solution. This mixture was deionised and the ratio of glucose to xylose determined by quantitative paper chromatography according to Saeman *et al.*[12] The values for the rate constants, calculated from these figures, are given in Table 1. A typical run is given in Table 3. To check the method,

Table 2. Hydrolysis of cellobiose and pseudocellobiouronic acid in 1 M sulphuric acid at 90°C. (The table gives only some of the observed values).

Cellobiose at 88.4°C			Pseudocellobiouronic acid at 88.5°C		
Time sec	$a°$	$k \times 10^6$ sec^{-1}	Time sec	$a°$	$k \times 10^6$ sec^{-1}
0	0.565	—	0	0.364	—
240	0.589	292.4	240	0.403	291.7
360	0.600	289.2	420	0.430	289.3
480	0.611	290.1	480	0.440	294.3
600	0.622	292.6	540	0.451	302.8
720	0.631	286.6	600	0.458	296.4
900	0.645	284.7	900	0.502	303.8
1200	0.667	283.3	1200	0.535	292.9
1500	0.688	284.6	1500	0.563	282.0
∞ *	0.919	—	∞ *	0.941	—
	Mean value	287.9		Mean value	294.2

* Obtained from the optical rotation(s) of the pure product(s) of hydrolysis.

Table 3. Hydrolysis of cellobiouronic acid in 1 M sulphuric acid at 100°C.

Time min	conc. of cellobiouronic acid mg/ml	$k \times 10^6$ sec^{-1}
0	10.86	—
30	10.19	34.89
60	9.44	38.95
120	8.02	42.11
185	7.34	35.19
270	6.33	33.34
375	5.57	29.64
	Mean value	35.7

the rate of hydrolysis of cellobiose was also determined by this technique. The rate constants obtained, 21, 79, and 260 at 70°, 80°, and 90° respectively, are of the same order of magnitude although consistently lower than those determined by the more accurate polarimetric method.

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