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Synthesis of Cellobiouronic Acid

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4-*O*-(β-Glucopyranosyluronic acid)-D-glucose or cellobiouronic acid (I) was synthesised starting from 1,6-anhydrocellobiose (IV).

4-*O*-(β-D-Glucopyranosyluronic acid)-D-glucose or cellobiouronic acid has been isolated from hydrolysates of the specific polysaccharides of *Pneumococcus* Types III [1] and VIII [2,3] and its structure has been determined by Hotchkiss and Goebel.[4] The similar serological activity of these and some other bacterial polysaccharides and of oxidised cellulose is apparently related to the presence of cellobiouronic acid residues.

In a recent paper, Marchessault and Rånby [5] have discussed the nature of certain linkages in cellulose, which have an increased sensitivity to acid hydrolysis and have attributed them to the presence of glucuronic acid residues in the cellulose chain. It is known that a biouronic acid linkage, as in cellobiouronic acid (I), is considerably more resistant to acid hydrolysis than an ordinary glycosidic linkage, as in cellobiose (II). Rånby and Marchessault now suggest that the pseudo-biouronic acid linkage, as in pseudo-cellobiouronic acid (III), must be considerably more sensitive to acid hydrolysis than the other linkages.

They also suggest that inductive effects, due to the electrophilic carboxyl groups, are responsible for the known resistance of the biouronic linkage (I) and for the postulated weakness of the pseudo-biouronic linkage (III). A comparison of the rates of acid hydrolysis of the three model substances (I, II and III) is therefore a matter of some interest. The present paper describes the synthesis of cellobiouronic acid (I).

1,6-Anhydro-cellobiose (IV) was prepared by treatment of phenyl-β-cellobioside with alkali.[6] Tritylation and acetylation yielded the pentaacetate trityl ether (V) and detritylation of V gave the pentaacetate (VI), in which all the hydroxyls of cellobiose (II) except the primary hydroxyl in the non-reducing glucose residue are protected. Oxidation of VI with permanganate in acetic acid, as previously used in a similar syntheses of glucuronic acid by Stacey [7], yielded the pentaacetate of 1,6-anhydro-cellobiouronic acid, VIII. This substance was not isolated but was deacetylated and subjected to acid hydrolysis. The 1,6-anhydride linkage was preferentially hydrolysed and the cellobiouronic acid (I) formed was purified from small amounts of contaminating

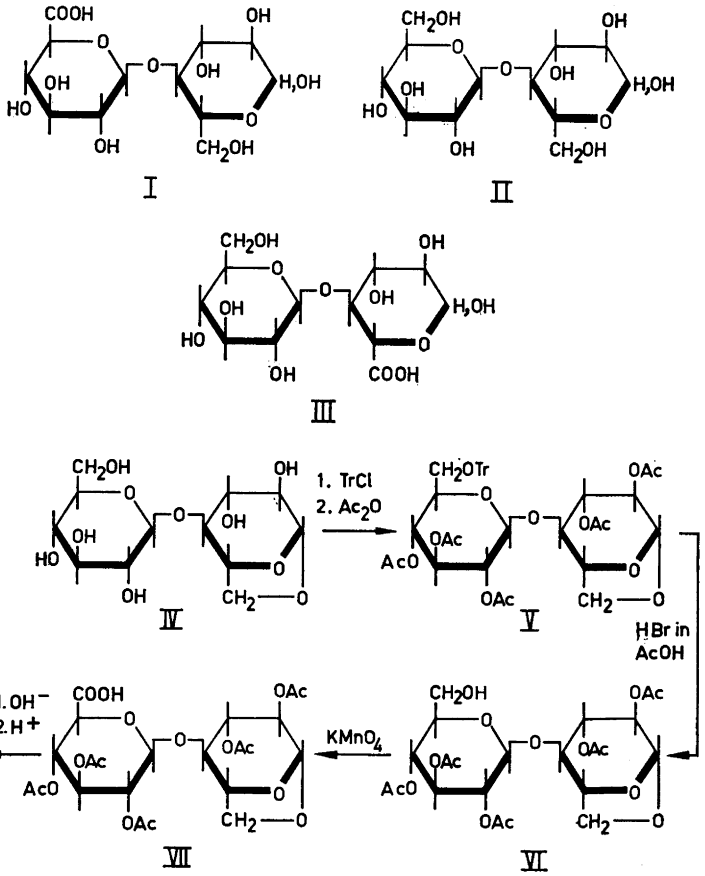
glucose and glucuronic acid by ion exchange chromatography. The amorphous product was chromatographically and electrophoretically pure and gave the α-heptaacetate methyl ester with m.p. 251—252 °, $[\alpha]_D^{20}$ + 44° (in chloroform), in good agreement with previously recorded values for this substance.[2,3] *

EXPERIMENTAL

Melting points are corrected. Concentrations were done under reduced pressure.

1,6-Anhydro-cellobiose (IV) was prepared according to Montgomery *et al.*[6] An attempt to prepare the intermediate phenyl-hepta-*O*-acetyl-β-cellobioside from octa-*O*-acetyl-α-cellobiose, phenol and *p*-toluene-sulphonic acid by the method of Helferich and Schmitz-Hillebrecht [8], failed, probably because of the difficulty of separating the mixture of α- and β-glycosides formed.

2,3,2',3',4'-Penta-O-*acetyl-6'*-O-*trityl-1,6-anhydrocellobiose.* (V) 1,6-Anhydrocellobiose (5 g) was dissolved in anhydrous pyridine (100 ml) and trityl chloride (6.6 g) was added.



* *Added in proof.* In a recent paper [*Chem. Ber.* **95** (1960) 356] Jayme and Werner report the synthesis of cellobiuronic acid by a different route.

The solution was kept overnight, acetic anhydride (20 ml) was then added and after two days the mixture was poured into ice-water (1 000 ml) containing acetic acid (50 ml). The precipitate was collected and crystallised from ethanol, yielding the pure substance (8.0 g), m. p. 119—120°, $[\alpha]_D^{20}$ —50° (chloroform *c*, 1.1). (Found: C 63.3, H 5.71. Calc. for $C_{41}H_{44}O_{15}$: C 63.5; H 5.58.)

*2,3,2',3',4'-Penta-*O*-acetyl-1,6-anhydro-cellobiose* (VI). The trityl derivative (V) (8.0 g) was dissolved in acetic acid (60 ml) and a saturated solution of hydrogen bromide in acetic acid (15 ml) was added. The mixture was shaken for 45 sec. and the trityl bromide was removed by filtration. The solution was immediately poured into ice-water (500 ml) and extracted with chloroform (3 × 100 ml). The chloroform solution was washed with ice-water (5 × 100 ml), dried over calcium chloride and concentrated to a sirup. The sirup crystallised on addition of ether and scratching and was recrystallised from chloroform-ether. The crystals (2.7 g) had m. p. 156—157° and $[\alpha]_D^{20}$ —133°, (chloroform, *c*, 2.0). (Found: 49.4; H 5.75. Calc. for $C_{22}H_{30}O_{15}$: C 49.4; H 5.66.)

*4-*O*-(β-*D*-Glucopyranosyluronic acid)-*D*-glucose (I).* The pentaacetate (VI) (1.0 g) was dissolved in analytically pure, homologue-free acetic acid (10 ml) and finely powdered potassium permanganate (500 mg, 20 % excess) was added. The mixture was stirred at room temperature, and the reaction was followed by chromatography on dimethyl sulphoxide-impregnated paper [9], using benzene as the mobile phase. The starting material and the reaction product had R_F-values of 0.9 and 0.65, resp. The spots were developed with silver nitrate-sodium ethoxide [10].

After two days, when the reaction was complete, the mixture was concentrated to dryness, dissolved in 0.1 N aqueous potassium hydroxide (50 ml) and kept overnight. The precipitate of manganese dioxide was filtered off and the clear, colourless solution obtained was filtered through a column of cation exchange resin, Amberlite IR 120 (H^+).

The solution was concentrated by freeze-drying, dissolved in 0.5 N sulphuric acid and then kept at 100°. The hydrolysis was followed by paper chromatography (ethyl acetate-acetic acid-water, 3:1:1) and paper electrophoresis (0.1 M borate buffer, pH 10). After 12 h the starting material had disappeared. The solution was neutralised with barium carbonate, barium sulphate was removed by filtration and the salts were converted to acids by filtering through a column of Amberlite IR 120 (H^+). The solution which in addition to cellobiouronic acid contained traces of glucose and glucuronic acid (chiefly as glucurone) was added to the top of a column (23 × 2 cm) of anion exchange resin, Dowex 2, in the acetate form. The column was eluted, first with water (3 000 ml) and then with 3 N aqueous acetic acid (500 ml). The latter eluate, which contained chromatographically and electrophoretically pure cellobiouronic acid was concentrated to dryness, dissolved in water and freeze-dried, yielding the compound as a white, amorphous powder (415 mg).

Methyl ester α-heptaacetate of cellobiouronic acid. Cellobiouronic acid (100 mg) was dissolved in methanol (7 ml) and an excess of diazomethane in ether was added. After a few minutes the solution was concentrated to a sirup, and this was then dissolved in acetic anhydride (10 ml) containing zinc chloride (0.5 g) and kept at 60° for 90 min.

The solution was concentrated and the residue was crystallised from ethanol, yielding the methyl ester acetate (60 mg), m. p. 251—252°, $[\alpha]_D^{20}$ +44° (chloroform, *c* 1.2).

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