ACTA CHEMICA SCANDINAVICA 22 (1968) 949–952

Synthesis of Ketodextrans

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A ketodextran (0.39 C=O per hexose residue), with most of the carbonyl groups in the 3-position has been prepared by oxidation of dextran 2,4-phenylboronate with dimethyl sulphoxide—acetic anhydride. Another ketodextran, with a similar distribution of carbonyl groups, was obtained on oxidation of unprotected dextran with the same reagent. Reduction of either of the above ketodextrans yielded a modified dextran, in which about 25 % of the original D-glucose residues have been transferred into D-allose residues.

Selective modification of polysaccharides is of interest in several respects, *e.g.* in connection with enzyme specificity and immunochemistry. Such modifications have been achieved by different routes. In most of these, a tosyl ester, either in primary or secondary position, has been replaced by another group and in this manner amino groups have been introduced in 6-positions [1] and D-glucopyranose residues have been converted into D-altropyranose residues.[2] Oxidation of 6-*O*-trityl cellulose [3] with dimethylsulphoxide—acetic anhydride reagent [4] yielded a product essentially oxidised in the 2-position and reduction of this product yielded a polysaccharide with a high percentage of D-mannopyranose residues.

The present paper reports oxidation studies on dextran, an essentially α-(1→6)-linked glucan. It has previously been demonstrated [5,6] that D-xylosides, as their 2,4-phenylboronates, can be oxidised with dimethylsulphoxide—acetic anhydride to D-*erythro*-pentopyranosid-3-uloses in high yields. Dextran, in dimethylsulphoxide—benzene, reacted with phenylboronic acid to form a 2,4-phenylboronate, indicated by a low optical rotation of the reaction product ($[\alpha]_{578}$ + 60°, calculated on the original dextran). When the dextran 2,4-phenylboronate was treated with dimethylsulphoxide—acetic anhydride a ketodextran (ketodextran I) was obtained. It contained about 0.39 carbonyl groups per hexose residue, as determined by the hydroxylamine

Table 1. Relative proportions of sugars on acid hydrolysis of reduced ketodextran.

Ketodextran	D-Glucose	D-Allose	D-Mannose	D-Galactose
I	69	27	3.5	0.75
II	69	25	2.5	3.1
III	68	27	3.0	0.80
IV	67	26	2.5	3.5

method.[7] Reduction of ketodextran I with sodium borohydride yielded a polysaccharide which on acid hydrolysis gave a mixture of D-glucose, D-allose, D-mannose, and D-galactose in the relative proportions given in Table 1. Borohydride reduction of methyl α-D-*ribo*-hexapyranosid-3-ulose gives D-alloside and D-glucoside in the relative proportions 69:1.[6] The steric course on borohydride reduction of corresponding α-D-hexopyranosid-2- and -4-uloses, derived from glucose, are not known. If it is assumed that the two products; D-glucoside—D-mannoside and D-glucoside—D-galactoside, respectively, are formed in equal amounts, the sugars formed from the reduced ketodextran I account for about 0.36 carbonyl groups per hexose residue, in good agreement with the value determined by the hydroxylamine method. As expected, the oxidation has occurred essentially in the 3-position. The oxidation observed in the 2- and 4-positions is probably due to incomplete reaction with the phenylboronic acid. Further, the dextran is slightly branched and the terminal residues should probably give 4,6-phenylboronate esters, leaving the 2- and 3-positions unprotected.

Surprisingly, almost the same result was obtained when unprotected dextran was oxidised with the dimethyl sulphoxide—acetic anhydride reagent. The product, ketodextran II, also contained 0.39 carbonyl groups per hexose residue and an investigation of a hydrolysate of the reduced product (Table 1) revealed that oxidation had occurred essentially in the 3-position. The only significant difference was that the galactose content, indicating carbonyl groups in the 4-position, was higher in reduced ketodextran II than in reduced ketodextran I.

Theander [8] has shown that methyl β-D-*arabino*-hexopyranosid-2-ulose is readily rearranged into methyl β-D-*ribo*-hexopyranosid-3-ulose under mild alkaline conditions. On treatment with aqueous sodium borohydride, however, reduction is faster than rearrangement, and only methyl β-D-glucoside and methyl β-D-mannoside are formed. It may not be excluded, therefore, that the carbonyl distribution in ketodextran II was the result of an equilibrium established during the isolation of the product. In separate experiments, the oxidation of protected and unprotected dextran were repeated, the reaction mixtures poured into water, washed with chloroform and borohydride directly added to the solution. The carbohydrate compositions of hydrolysates

of the products (ketodextran III and IV, respectively, Table 1) were not significantly different from those obtained from reduced ketodextran I and II, respectively. It seems most probable, therefore, that the high percentage of carbonyl groups in 3-positions in ketodextran II is a result of preferential oxidation in this position. The difference between oxidation of dextran and 6-*O*-trityl-cellulose, which is selectively oxidised in the 2-positions, is striking.

The formation of different hexopyranosid-diulose residues may be expected on oxidation of unprotected glucose residues in dextran. Borohydride reduction of such residues should give rise also to D-gulose, D-altrose, and D-talose residues. In the analytical procedure used, the sugars are reduced to alditols, which are separated as their acetates, by GLC.[9] D-Gulose, which gives L-glucitol on reduction, would be overlooked but D-altrose and D-talose both yield talitol, which, although relatively difficult to separate from mannitol,[10] should be detected.

EXPERIMENTAL

Dextran 2,4-phenylboronate. Dextran NRRL B-512, a partially hydrolysed product of $\bar{M}_w$ 12 000, (6.4 g) and phenylboronic acid (4.2 g) were dissolved in dry dimethyl sulphoxide (100 ml) and benzene (100 ml) was gradually added while heating to reflux temperature. The water formed during the reaction, was collected in a Dean-Stark apparatus and measured. After 18 h no more water was formed, showing that the esterifiation had gone to completion. The optical rotation $[\alpha]_{578}^{20}$ + 60°, of the reaction mixture was measured and calculated for dextran. The reaction product was not isolated but the solution used for the oxidation was freed from benzene by distillation.

Oxidation of dextran 2,4-phenylboronate. Acetic anhydride (75 ml) was gradually added during 10 min to the solution (100 ml) of dextran 2,4-phenylboronate in dimethyl sulphoxide. The slightly turbid solution was kept at 40° for 2 h, filtered and the slightly coloured solution poured into a mixture of chloroform (300 ml) and water (300 ml). The aqueous layer was washed with chloroform (2 × 100 ml), dialysed for 2 days against tap water, concentrated and poured into ethanol. The light brown polysaccharide material (4.8 g), ketodextran I, showed $[\alpha]_{578}^{20}$ + 120° (*c* 0.2, water). The carbonyl content of the product was determined by the hydroxylamine method.[7] IR showed a single absorption in the carbonyl region at 1735 cm^{-1}.

Oxidation of dextran. Dextran (6.4 g) in dimethyl sulphoxide (100 ml) was oxidised and the mixture worked up as above, to yield ketodextran II (5.0 g). Elemental analysis showed the presence of a small percentage of sulphur (< 2 %) in the product.

Reduction of ketodextrans. Sodium borohydride (0.5 g) in water (10 ml) was gradually added to a solution of ketodextran (0.5 g) in water (10 ml). After 10 h at room temperature, excess Dowex 50 (H^+) was added; the mixture filtered, the aqueous solution concentrated and boric acid removed by codistillation with methanol. The slightly yellow polysaccharide (0.5 g) showed no absorbtion in the carbonyl region in IR. The product obtained from ketodextran I had $[\alpha]_{578}^{20}$ + 170° (*c* 0.1, water).

In separate experiments, the reaction mixtures obtained on oxidation of protected and unprotected dextran (ketodextran III and IV), were poured into chloroform-water and the aqueous phases washed with chloroform as above. They were then treated with excess of borohydride and the products worked up by dialysis, concentration and precipitation with ethanol.

Analysis of component sugars. Reduced ketodextran (100 mg) was treated with 0.5 M sulphuric acid (10 ml) at 100° for 10 h, cooled, neutralized with barium carbonate, filtered and concentrated. The product was reduced with sodium borohydride and subsequently acetylated with acetic anhydride-pyridine. The mixture of acetylated alditols was analysed by GLC using an ECNSS—M column.[9] The 1,6-anhydride of D-allose (14 % at equilibrium [11]) was formed during hydrolysis of reduced ketodextrans. The acetate of this anhydride has a lower retention time than those of the acetylated hexitols. The

hexitol acetate mixture was also analysed by GLC-mass spectrometry, and for each peak assumed to be a hexitol acetate, a mass spectrum typical for these compounds was obtained.[12]

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Received October 25, 1967.