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# Oxidation—Degradation of Methyl 4-*O*-(2,3-Di-*O*-methyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-methyl-β-D-glucoside

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Methyl 4-*O*-(2,3-di-*O*-methyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-methyl-β-D-glucoside has been synthesized. The product obtained after oxidation was subjected to degradation by base and/or mild acid treatment. The use of these degradation procedures for specific degradation of polysaccharides is discussed.

In previous publications [1–4] a specific degradation procedure, using model compounds, is reported. Methylated polysaccharides or glycoconjugates having a limited number of free hydroxyl groups at specific positions [5] are suitable for sequential degradation. The degradation method involves oxidation of the free hydroxyl groups to carbonyl functions, β-elimination with formation of α,β-unsaturated ketones or aldehydes and further degradation of these derivatives by mild acid hydrolysis. Employing this method the glycosidic linkage of a methylated sugar residue with a free hydroxyl group in the 2-, 3-, 4-, or 6-position can be specifically cleaved. The degradation method has been applied in structural studies of polysaccharides.[6–8]

Several bacterial polysaccharides contain hexopyranoside residues substituted with pyruvic acid, ketalically bound to O-4 and O-6. Formaldehyde acetalically bound to the same positions has also been observed.[9] It should be possible to use the oxidation-degradation procedure after selective removal of such groups from a methylated polysaccharide. The removal of the ketal group, when preceded by carboxyl reduction, may be performed by acid hydrolysis under mild conditions without simultaneous hydrolysis of glycopyranosidic linkages. The possibility of degrading polysaccharides by this route has now been investigated using the disaccharide methyl 4-*O*-(2,3-di-*O*-methyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-methyl-β-D-glucoside as a model substance.

## RESULTS AND DISCUSSION

Methyl β-maltoside was treated with benzylidene bromide in pyridine [10] to yield methyl 4-*O*-(4,6-*O*-benzylidene-α-D-glucosyl)-β-D-glucoside (I). Methylation of I with methyl iodide/sodium hydride in dioxane gave crystalline methyl 4-*O*-(4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucosyl)-2,3,6-tri-*O*-methyl-β-D-glucoside (II), which after hydrogenation afforded the desired model compound methyl 4-*O*-(2,3-di-*O*-methyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-methyl-β-D-glucoside (III). The structure of III was confirmed by NMR spectroscopy and sugar analysis of its hydrolysate.

The model compound (III) was oxidised with a chlorine–dimethyl sulfoxide complex,[11] but part of the reaction mixture was withdrawn before the addition of triethylamine. Sugar analysis of the latter product showed no oxidation of the hydroxyl groups.

The oxidised product was treated with base and acid under different conditions. The cleavage of the glycosidic linkage was evaluated by GLC from the amount of liberated methyl 2,3,6-tri-*O*-methyl-β-D-glucoside. Methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside was used as an internal standard. These analyses showed that

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about 25 % of the glucosidic bond was cleaved during the treatment with triethylamine, the work-up procedure and/or by thermal degradation in the gas chromatograph.

On treatment of oxidised III with sodium ethoxide in dichloromethane-ethanol followed by hydrolysis under mild conditions (50 % aqueous acetic acid, 100 °C, 3 h), a quantitative cleavage of the glycosidic linkage of the oxidised residue was obtained. It was, however, also possible to generate glycosidic cleavage by either base or mild acid hydrolysis alone. Selective degradation is therefore feasible in the presence of groups that are acid labile.

In a separate experiment the product from the oxidation of III was reduced with sodium borodeuteride, hydrolysed and the resulting mixture of sugars analysed, as alditol acetates, by GLC-MS.[12] The ratio of 2,3-di-*O*-methyl-D-glucose to 2,3,6-tri-*O*-methyl-D-glucose was 1:7, indicating that most of the oxidised residues were degraded during the alkaline conditions of the borohydride reduction. The 2,3-di-*O*-methyl-D-glucose was deuterated at C-6, showing that the small percentage of oxidised residues that not degraded had been oxidised at C-6 but not at C-4.

The results thus demonstrate that the degradation procedure can be applied to methylated polysaccharides with free hydroxyls at C-4 and C-6 of hexopyranoside residues. The glycosidic linkages of the oxidised residues may be cleaved either by treatment with base or with acid under mild conditions. The consecutive treatment with base and acid is, however, recommended, as complete degradation of only partially oxidised residues may otherwise not be obtained.

## EXPERIMENTAL

Concentrations were performed at reduced pressure at bath temperatures not exceeding 40 °C. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded with a Varian A60 A spectrometer, using tetramethylsilane as internal reference. NMR spectra were recorded for all substances and were in agreement with the postulated structures. GLC separations were performed on a Perkin-Elmer model 900 instrument using a glass capillary column (25 m × 0.25 mm) wall-coated with SP-1000 (LKB-Products, Sweden). Peak areas were measured with a Hewlett-Packard 3370B electronic integrator. For GLC-MS a Perkin-Elmer 270 gas chromatograph – mass spectrometer fitted with an OV-225 S.C.O.T. column was used. Mass spectra were recorded at an ionisation potential of 70 eV and with an ion source temperature of 120 °C. Hydrolyses were performed with 90 % formic acid for 1 h at 100 °C followed by treatment with 0.25 M sulfuric acid for 16 h at 100 °C.

*Methyl 4-O-(4,6-O-benzylidene-α-D-glucosyl)-β-D-glucoside (I).* Methyl β-maltoside[13] (1.1 g) and benzylidene bromide (1.0 g) in pyridine (18 ml) were refluxed for 2 h. The solution was then allowed to cool and concentrated to dryness. The reaction mixture was separated on a Silica gel column (3 × 50 cm) using ethyl acetate as irrigant. The separation was monitored using TLC and the fractions containing I were concentrated to dryness yielding chromatographically pure I (1.0 g) as a syrup, $[\alpha]_D^{25}$ + 35° (*c* 0.4, ethanol).

*Methyl 4-O-(4,6-O-benzylidene-2,3-di-O-methyl-α-D-glucosyl)-2,3,6-tri-O-methyl-β-D-glucoside (II).* Methyl iodide (10 ml) was added to a mixture of I (1.0 g) and sodium hydride (1.0 g) in dioxane (20 ml). The mixture was refluxed for 4 h, cooled, and excess sodium hydride was destroyed by the addition of ethanol (10 ml). The solution was concentrated to dryness and partitioned between chloroform and water. The chloroform phase was collected and evaporated, yielding crystalline II which was recrystallised from ethanol – hexane (1:3, v/v). The yield of II was 1.1 g, m.p. 161 – 162 °C, $[\alpha]_D^{25}$ + 52° (*c* 1.0, chloroform). (Found: C 58.78; H 7.38. $C_{25}H_{38}O_{11}$ requires: C 58.35; H 7.44).

*Methyl 4-O-(2,3-di-O-methyl-α-D-glucopyranosyl)-2,3,6-tri-O-methyl-β-D-glucoside (III).* A solution of II (1.1 g) in ethanol (50 ml) was hydrogenated over palladium on carbon (10 %, 0.1 g) at room temperature and atmospheric pressure. When the hydrogen consumption had ceased, the catalyst was filtered off and the solution concentrated to dryness, yielding III as a chromatographically (TLC) pure syrup (0.9 g), $[\alpha]_D^{25}$ + 74° (*c* 1.4, chloroform). Sugar analysis of the hydrolysate gave equimolar amounts of 2,3,6-tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose, analysed by GLC-MS as their alditol acetates.

*Oxidation of III.* The oxidation reagent was prepared by adding dimethyl sulfoxide (0.3 ml) to a 1 M solution of chlorine in anhydrous dichloromethane (1 ml) under vigorous stirring at − 45 °C. A white precipitate appeared during the addition. Compound III (24 mg) and methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside (13 mg) as an internal standard (relative molar proportion 1.08:1) in dichloromethane (2 ml) were added with the aid of a syringe, and the reaction mixture was stirred for 4 h at − 45 °C.

To show that III was quantitatively regenerated from the oxidation complex, part of the reaction mixture (1/10) was withdrawn, hydrolysed and the resulting sugars were analysed

as their alditol acetates. Triethylamine (0.25 ml) was added to the remaining part and the mixture was kept at −45 °C for another 10 minutes, and then allowed to rise to room temperature. TLC showed that no starting material was left. Part of the reaction mixture (1/10) was hydrolysed and the sugars analysed as their alditol acetates by GLC-MS. The ratio of 2,3-di-*O*-methyl-D-glucose to 2,3,6-tri-*O*-methyl-D-glucose was 0.03:1, demonstrating that most of the residues with free hydroxyl groups had been oxidised. Dimethyl sulfoxide and triethylamine hydrochloride were removed by chromatography on a Silica gel column (3 × 10 cm) using acetone−ethylacetate (1:1, v/v) as eluent and the eluate was concentrated to dryness.

*Degradation of oxidised III.* A. Part of the product (10 %) was analysed directly on GLC. The relative molar proportion of methyl 2,3,6-tri-*O*-methyl-β-D-glucoside and methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside (the internal standard) was 0.25:1.

B. Part of the product (30 %) was treated with 1 M sodium ethoxide in ethanol (0.2 ml) and dichloromethane (1.0 ml) for 1 h at room temperature, neutralized with glacial acetic acid and concentrated to dryness. The product was treated with 50 % aqueous acetic acid (1 ml) at 100 °C. Samples were withdrawn at intervals and analysed on GLC. The relative molar proportion of methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside to the internal standard was 1.05:1 after 3 h.

C. Part of the product (30 %) was treated with 1 M sodium ethoxide in ethanol (0.2 ml) and dichloromethane (1 ml) at room temperature. Samples were withdrawn at intervals, neutralised with Dowex 50 ($H^+$) and analysed on GLC. The relative molar proportion of methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside to the internal standard was 1.05:1 after 2 h.

D. Part of the product (30 %) was treated with 50 % aqueous acetic acid (1 ml) at 100 °C. Samples were withdrawn at intervals and analysed on GLC. The relative molar proportion of methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside to the internal standard was 1.05:1 after 4 h.

*Reduction of oxidised III.* Compound III (2 mg) was oxidised as above and the organic solvents removed upon evaporation. Sodium borodeuteride (20 mg) was added to oxidised III in ethanol−water (1:1, 2 ml). The reaction mixture was kept for 14 h at room temperature, neutralised with Dowex 50 ($H^+$), filtered, hydrolysed, and the sugars analysed by GLC-MS of their alditol acetates. 2,3,6-Tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose were obtained in the proportion 1:0.14. MS demonstrated that the latter substance was monodeuterated at C-6 but not at C-4.

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