Partial Methylation Studies on Pustulan, Methyl α- and β-D-Glucopyranoside and Some Derivatives

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The syntheses of methyl 6-O-(2'-tetrahydropyranyl)-D-glucopyranoside and methyl 6-O-benzyl-D-glucopyranoside are described. The partial methylation of these substances, methyl α- and β-D-glucopyranoside, 1,6-anhydro-glucopyranose, and pustulan has been studied. The results are discussed with reference to the similar partial methylation of dextran.

The difference in reactivity observed between the three hydroxyl groups at C(2), C(3) and C(4) of an anhydroglucose unit in dextran could depend on many factors, for example, hydrogen bonding, electronic and steric effects. In order to evaluate the relative importance of these various factors in the partial methylation of dextran, methylation studies of the following model compounds have been performed; pustulan [β-D-(1→6)-polyglucose obtained from the lichen Umbilicaria pustulata], methyl α- and β-D-glucopyranoside, methyl 6-O-(2'-tetrahydropyranyl)-D-glucopyranoside, methyl 6-O-benzyl-D-glucopyranoside, and 1,6-anhydro-D-glucopyranose.

Methyl 2,3,4-tri-O-acetyl-6-O-(2'-tetrahydropyranyl)-D-glucopyranoside was prepared by reaction of methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside with dihydropyran in the presence of catalytic amounts of hydrochloric acid. The reaction mixture was purified by chromatography on a silicic acid-dimethyl sulphoxide column by which means the triacetate was obtained in a yield of 52%.

The acetate was deacetylated by stirring over-night with a strong anion exchange resin in 95% ethanol. In spite of the fact that the two tetrahydrofuran derivatives were obtained in a chromatographically pure form, none of them could be induced to crystallise.

Acid hydrolysis of 6-O-benzyl-3,5-O-benzylidene-1,2-O-isopropylidene-D-glucose yielded 6-O-benzyl-D-glucose, m.p. 119—122° Ohle and Tessman, who prepared this substance by another route, report m.p. 92—93.5°. It is evident from analytical and other experimental data that the substance, m.p. 119—122°, has the assumed structure. The material prepared by Ohle

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and Tessman may be a lower melting modification. Treatment of 6-O-benzyl-D-glucose with methanolic hydrogen chloride, followed by cellulose column chromatography, yielded the anomic mixture of methyl 6-O-benzyl-D-glucopyranosides.

The substituent in the 6-position of methyl 6-O-benzyl-D-glucopyranoside and the corresponding tetrahydropyranyl derivative is easily removed under mild conditions by catalytic hydrogenation and acid hydrolysis respectively. After partial methylation, removal of the 6-O-substituent and acid hydrolysis, these model substances yield the same O-methyl glucose as are present in an O-methyl dextran hydrolysate.1

Methylations were performed with dimethyl sulphate in 19% aqueous sodium hydroxide solution. The amount of dimethyl sulphate was adjusted to give degrees of substitution ranging from 0.1 to 0.2. At this low degree of substitution the relative initial rate constants \( k_2, k_3, k_4 \), and in some cases \( k_5 \), could be determined with comparatively good precision.1 The results from this investigation are summarized in Table 1.

As observed for dextran, all model substances have a lower reactivity at position 3 than at 2 and 4. The low reactivity of the C(3)-hydroxy group in dextran does not therefore depend on any special effect arising from the polymer, for example, steric hindrance.

Partial cyanoethylation of methyl \( \beta \)-D-glucopyranoside in sodium hydroxide solution also suggests that \( k_4 \geq k_5 ^5 \). Previous results published on the partial methylation of methyl \( \beta \)-D-glucopyranoside,6 however, indicates that \( k_3 \geq k_4 \). These results might according to one of the authors of that paper 7 be due to an incomplete electrophoretic separation of the mono-O-methyl glucose.

The ratio of \( k_4:k_3 \) is markedly higher for methyl 6-O-benzyl-D-glucopyranoside and methyl 6-O-(2'-tetrahydropyranyl)-\( \alpha \)-D-glucopyranoside than for dextran, postulan, and methyl \( \alpha \)-and \( \beta \)-D-glucopyranoside. In the latter group, the ratio of the rate constants for each compound is very similar.

Since the rates given in Table 1 are relative, no direct conclusions could be drawn from a comparison of the rate constants of, for example, dextran and methyl 6-O-(2'-tetrahydropyranyl)-\( \alpha \)-D-glucopyranoside. By studying the

<table>
<thead>
<tr>
<th>Substance</th>
<th>( k_2 )</th>
<th>( k_3 )</th>
<th>( k_4 )</th>
<th>( k_5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>8</td>
<td>1</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Postulan</td>
<td>9.3</td>
<td>1</td>
<td>3.1</td>
<td>13</td>
</tr>
<tr>
<td>Methyl ( \alpha )-D-glucopyranoside</td>
<td>7.8</td>
<td>1</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Methyl ( \beta )-D-glucopyranoside</td>
<td>7.3</td>
<td>1</td>
<td>3.1</td>
<td>12</td>
</tr>
<tr>
<td>Methyl 6-O-benzyl-D-glucopyranoside</td>
<td>12.1</td>
<td>1</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Methyl 6-O-(2'-tetrahydropyranyl)-( \alpha )-D-glucopyranoside</td>
<td>8</td>
<td>1</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>1,6-Anhydro-D-glucopyranose</td>
<td>2.5</td>
<td>1</td>
<td>1.8</td>
<td></td>
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</tbody>
</table>

*Table 1. The relative rate constants for the three hydroxyl groups in dextran, postulan and some model compounds during methylation with dimethyl sulphate in 19% sodium hydroxide solution.*

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simultaneous methylation of equimolar amounts of these substances with a small amount of dimethyl sulphate, however, it was possible to form a clear conception of the main differences in reactivity between the hydroxyl groups in the polymeric and monomeric compound. The partially methylated compounds were quantitatively separated by gel filtration, hydrolysed and fractionated on thick paper. The mono-O-methyl fractions were weighed and analyzed by gas chromatography and it was found that the reactivity of the C(4)-hydroxyl group increases and that the reactivities of the C(2)- and C(3)-hydroxyl groups decrease when passing from dextran to methyl 6-O-(2'\text{-tetrahydropyranyl})-α-D-glucopyranoside (Table 2). Furthermore, as the total degree of methylation is the same in both samples, their overall reactivity is approximately equal.

Table 2. The composition of the mono-O-methyl glucose fractions obtained from the simultaneous partial methylation of equimolar amounts of dextran and methyl 6-O-(2'\text{-tetrahydropyranyl})-α-D-glucopyranoside with dimethyl sulphate in 10 % sodium hydroxide solution.

<table>
<thead>
<tr>
<th>Partially methylated compound</th>
<th>mono-O-methyl glucose $^a$</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-O-Me</td>
<td>3-O-Me</td>
</tr>
<tr>
<td>Dextran</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Methyl 6-O-(2'\text{-tetrahydropyranyl})-α-D-glucopyranoside</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$ All figures are given in milligrams.

Methylations in the presence of a strong base certainly proceed via alkoxide ions.$^6$ The lower reactivity of the C(4)-hydroxyl group observed in dextran, pstulan, and methyl α- and β-D-glucopyranoside compared with the 6-substituted glucosides might therefore be due to the formation of a strong hydrogen bond between the alkoxide ion of this hydroxyl group and either a hydroxyl on the adjacent glucose unit (dextran, pstulan) or the C(6)-hydroxyl group on the same glucose unit (methyl α- and β-D-glucopyranoside). When a substituent unable to form such a hydrogen bond occupies the 6-position this hydrogen bond effect will disappear and induce an increased reactivity of the C(4)-hydroxyl group (cf. methyl 6-O-(2'\text{-tetrahydropyranyl})-α-D-glucopyranoside and methyl 6-O-benzyl-α-D-glucopyranoside).

The conformation of 1,6-anhydro-O-glucopyranose (1C) suggests that the low reactivity of the C(3)-hydroxyl group in this compound could depend on steric hindrance from the 1,6-anhydro-linkage.

It may be concluded that the general pattern in the reactivity of glucose polymers towards methylation in strong base follows that shown by the glucose derivatives and that no special effects are needed to account for the low reactivity of the C(3) hydroxyl group. The reactivity of the C(4)-hydroxyl

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group does seem, however, to depend on the nature of the substituent at C(6) and this may be due to hydrogen bonding effects. It must, however, be borne in mind that these changes in reactivity correspond to quite small differences in energy of activation, which might equally well have other explanations.

**EXPERIMENTAL**

Evaporations were performed under reduced pressure at a bath temperature of 40°C.


*Gas chromatography.* Apparatus: F & M 810. Column: 8 feet steel column packed with 5% butanediol succinate polyester (BDS) on Chromosorb W.

_Methyl 2,3,4-tri-O-acetyl-6-O-(2'-tetrahydropryranyl)-α-D-glucopyranoside._ Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside (2 g), dihydropryran (5 ml) and 37% hydrochloric acid (2 drops) were shaken together overnight. The reaction mixture was diluted with 20 ml dry acetone, neutralised with silver oxide, filtered and concentrated to a sirup. The sirup was dissolved in 20 ml of an isopropyl ether-DMSO mixture, (3 parts isopropyl ether + 7 parts isopropylether which had previously been equilibrated with an excess of DMSO) charged on a column (91 x 4 cm) packed with silica gel (Merck < 0.08 mm) to a height of 69 cm and eluted with the fore-mentioned solvent. The fractions were examined by thin layer chromatography (solvent A) and the fractions containing the desired compound were combined and concentrated. The residue was dissolved in chloroform and the chloroform solution washed with water (pH 8 with ammonia) to remove DMSO, dried over calcium sulphate and concentrated to a sirup (1.2 g, 52%) [α]D₂₀ +116° (chloroform, c 1.8) (Found: C 53.5; H 6.88. Calc. for C₁₉H₂₀O₁₀: C 53.5; H 6.97).

_Methyl 6-O-(2'-tetrahydropryranyl)-α-D-glucopyranoside._ Methyl 2,3,4-tri-O-acetyl-6-O-(2'-tetrahydropryranyl)-α-D-glucopyranoside (1 g) was dissolved in 95% ethanol (30 ml) and stirred with dry Dowex resin 1—X8 OH⁻ (10 g) over-night. The solution was then filtered and the resin washed thoroughly with ethanol and water (made slightly alkaline with ammonia). The filtrate and the washings were combined and evaporated to a sirup (0.65 g 92%) [α]D₂₀ + 91.4° (methanol, c 0.62) (Found: C 51.4; H 8.01. Calc. for C₁₉H₂₁O₁₁: C 51.8; H 7.97).

_B6-Benzyl-D-glucopyranose._ 6-O-Benzyl-3,5,6-tri-O-benzylidene-1,2-O-isopropylidene-D-glucose (2 g) was dissolved in 95% ethanol (30 ml), and 1% sulphuric acid (70 ml) was slowly added with warming on a waterbath. The solution was refluxed for 1 h after which the ethanol was distilled off and refluxing continued for a further 3 h. Neutralisation with anion exchanger IR-4B and concentration gave a slightly yellow sirup (1.1 g). Crystallisation from ethanol-ethyl ether yielded a hygroscopic substance, m.p. 119—122°. [α]D₂₀ + 46.4° (water, c 1.05) [lit. 4 m.p. 92—93.5°, [α]D₂₀ + 39.3° (water, c 1.7)]. (Found: C 57.1; H 6.71. Calc. for C₁₉H₁₉O₆: C 57.6; H 6.68). According to paper chromatography (Rₚ 0.77), paper electrophoresis (M₉ 0.80) and thin layer chromatography (solvent B) the product was contaminated with small amounts of glucose (about 1%).

_Methyl 6-O-benzyl-D-glucopyranoside._ 6-O-Benzyl-D-glucose (3.6 g) was dissolved in 2% anhydrous methanolic hydrogen chloride (50 ml) and refluxed on a waterbath for 2.5 h. The solution was neutralised with silver oxide, filtered, diluted with 50 ml methanol and charcoaled. Filtration and concentration gave a sirup which was fractionated on a cellulose column using water-saturated butanol as eluent yielding the product (3 g) [α]D₂₀ + 60.8° (water, c 1.1), Rₚ 0.81. (Found: C 58.5; H 7.13. Calc. for C₁₉H₂₁O₁₁: C 59.1; H 7.09).

_Pustulan._ Extracted from the lichen Umbilicaria pustulata, was kindly donated by Professor Bengt Lindberg, University of Stockholm, Sweden.

_Partial methylations._ A sample of a monosaccharide (1—2 g) was dissolved in 19% sodium hydroxide solution (50 ml). Dimethyl sulphate (1 ml) was added over 30 min with stirring under an atmosphere of nitrogen. The stirring was continued for 4 h. The reaction mixture was then neutralized with 2.5 M sulphuric acid and the sodium sulphate

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was precipitated by addition of 400 ml ethanol. The remaining solution was filtered and evaporated to dryness. (D.S. 0.1—0.2).

Debenzylation and hydrolysis. The partially methylated methyl 6-O-benzyl-D-glucoside was dissolved in ethanol (100 ml) and refluxed with stirring overnight with Raney-Nickel (5 g). After filtration and concentration the product was treated as below.

The partially methylated glucoside was dissolved in 1 M sulphuric acid (50 ml) and kept at 95°C for 6 h. The solution was deionised with ion exchangers IR-4B and IR-120, filtered and evaporated to dryness (0.8—0.9 g).

Analytical procedure. The hydrolysis products were fractionated by chromatography on thick paper. The mono-O-methyl ethers were then analysed by GLC as their trimethylsilyl derivatives. (90—170°C, 2°/min) and the ratio of 2-0-, 3-0-, 4-0-, and in some cases 6-0-methyl-glucose was determined.

Pustulan was methylated, hydrolysed and analysed according to the method described by Norrman.†

Simultaneous methylation of dextran and methyl 6-O-(2'-tetrahydropranyl)-α-D-glucopyranoside. Methyl 6-O-(2'-tetrahydropranyl)-α-D-glucopyranoside (2.05 g) and dextran (1.18 g) (equimolar amounts) were dissolved in 19% sodium hydroxide solution (150 ml). Dimethyl sulphate (2 ml) was added over 30 min with stirring under an atmosphere of nitrogen. The stirring was continued for 4 h. The reaction mixture was deionised with the ion exchangers IR-4B and IR-120, concentrated to a volume of 20 ml and charged on a Sephadex G-25-column (41 x 3 cm). The column gave a good separation of the two partially methylated compounds. These compounds were then hydrolysed and analysed as described above.

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