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Synthesis and Alkaline Degradation of Methyl 2,4,6-Tri-*O*-methyl-α- and -β-D-*ribo*-hexosid-3-ulose

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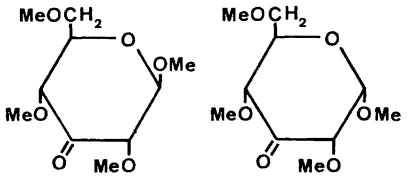
Methyl 2,4,6-tri-*O*-methyl-α- and -β-D-*ribo*-hexosid-3-ulose have been synthesized and their reactions in alkaline solution studied. The potential use of similar reactions in the controlled degradation of methylated polysaccharides is discussed.

There is a need in structural polysaccharide chemistry for specific degradation methods. The glycosidic linkage in a hexopyranoside, containing a 6-deoxy-6-*C*-*p*-toluenesulphonyl group, is cleaved in alkaline solution.[1] This reaction was recently used for the sequential degradation of the side chains in a methylated dextran in which the original hydroxymethyl groups had been replaced by *C*-*p*-toluene sulphonylmethyl groups.[2] After two degradation sequences, a methylated dextran was obtained, in which free hydroxyls at *C*-3 in some D-glucose residues marked the sites of the original branches. If the hydroxyl groups at *C*-3 could be oxidized to carbonyl groups and the product treated with alkali, the dextran chain could be cleaved at the hexos-3-ulose residues. Theander[3] has demonstrated that hexosid-3-uloses are rapidly degraded in alkaline solution. Characterisation of the fragments should provide information on the distribution of the branches in the original dextran. As a first step in this direction, some simple model substances have been prepared and their degradation in alkaline solution has been investigated.

A mixture of methyl 3-*O*-benzyl-2,4,6-tri-*O*-methyl-α- and -β-D-glucoside was prepared essentially as described by Freudenberg *et al.*[4] and separated into the pure anomers by column chromatography on silicic acid. Catalytic debenzylation followed by ruthenium tetroxide oxidation[5] yielded methyl 2,4,6-tri-*O*-methyl-α- and -β-D-*ribo*-hexosid-3-ulose (*6* and *5*).

The corresponding 2,3,4-tri-*O*-trideuteriomethyl analogues of *5* and *6* were also prepared. NMR spectra of *5* and *6*, of their deuterated analogues and of the intermediates were in agreement with the postulated structures. CD spectra of *5* and *6* showed a negative Cotton effect at 300 nm, $\theta_5 = 3009°$, $\theta_6 = 1319°$.

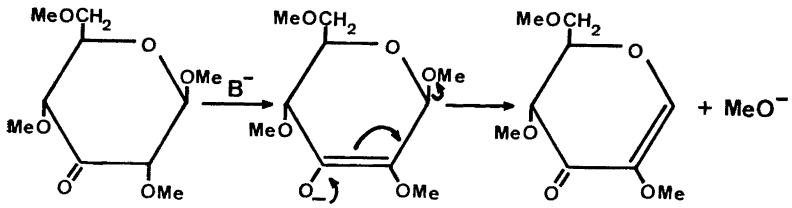


5 6

On treatment of methyl α- or -β-D-*ribo*-hexosid-3-ulose with alkali Theander[3] obtained, predominantly, 1,5-anhydro-D-*erythro*-2,3-hexo-diulose. This labile substance was formed by β-elimination of the aglycone. When methyl 2,4,6-tri-*O*-methyl-α- or -β-D-*ribo*-hexosid-3-ulose was treated with 0.1 M sodium ethoxide in ethanol no starting material remained after 30 min at 20°. The reaction product was, however, complex, and TLC revealed the presence of at least six components in comparable amounts. The same complex mixture was obtained when *5* and *6* were subjected to various alkaline conditions, using different bases, concentrations of base, and solvents.

For the proposed degradation of methylated polysaccharides, it was essential to establish the extent to which the glycosidic linkage was broken during the alkaline treatment. The 2,4,6-tri-*O*-trideuteriomethyl-analogues of *5* and *6* were therefore treated with sodium ethoxide in ethanol as above. The methanol liberated during this reaction was determined by GLC and the proportion of labelled to unlabelled methanol by GLC–MS. Comparable amounts of methanol (2.12 and 1.98 mol, respectively) were released from *5* and *6*. Of this, 1.01 and 0.98 mol, respectively, were unlabelled as determined by the relative intensities of the M−1 ions, *m/e* 33 ($CD_2 \overset{\oplus}{\cdots} OH$) and *m/e* 31 ($CH_2 \overset{\oplus}{\cdots} OH$). The results therefore demonstrate a quantitative cleavage of the glycosidic linkage in *5* and *6*.

In both cases, the first step in the degradation is probably the same as observed by Theander[3] for the corresponding non-methylated derivatives, as illustrated below for *5*.



The aglycone is eliminated from the enolate, but the product is labile and undergoes further reactions. The experiment indicates, however, that the degradation procedure for polysaccharides discussed above may be feasible.

EXPERIMENTAL

Concentrations were performed at reduced pressure at bath temperatures not exceeding 40°. Melting points are corrected. TLC was performed on Silica Gel F_{254} (Merck). Optical rotations were determined with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded with a Varian A60 A spectrometer, using tetramethylsilane as internal reference. Chemical shifts (τ) are given as ppm downfield from tetramethylsilane. CD spectra were determined on a Cary 60 instrument and GLC was performed on a Perkin-Elmer model 990 instrument fitted with a Poropac column. The separations were run at 100°. For GLC–MS the reaction mixtures were injected on a Poropac column fitted in a Perkin-Elmer 270 gas chromatograph–mass spectrometer. The spectra were recorded at a manifold temperature of 200°, an ionisation potential of 60 eV, an ionisation current of 80 μA, and an ion source temperature of 80°.

Methyl 3-O-benzyl-2,4,6-tri-O-methyl-β-D-glucoside (1). A mixture of 3-*O*-benzyl-2,4,6-tri-*O*-methyl-α- and -β-D-glucosides [5] (50 g) was added to the top of a silica gel column (80 × 7 cm) which was irrigated with light petroleum–ethyl acetate (1.8 : 1, v/v). The separation was followed by polarimetry. The first fraction to be eluted (methyl 3-*O*-benzyl-2,4,6-tri-*O*-methyl-β-D-glucoside) was obtained as a syrup which crystallised from hexane, (20.5 g) m.p. 34–38°, $[\alpha]_{578}$ −17° (*c* 1.2, chloroform). (Found: C 62.43; H 7.76. $C_{17}H_{26}O_6$ requires: C 62.55; H 8.04.) The NMR spectrum ($CDCl_3$) showed, *inter alia*, a doublet (τ 5.78, $J_{1,2}$ 7.5 Hz), identified as the signal from the anomeric proton.

Methyl 3-O-benzyl-2,4,6-tri-O-methyl-α-D-glucoside (2). Further elution of the column above yielded methyl 3-*O*-benzyl-2.4,6-tri-*O*-methyl-α-D-glucoside (24.8 g), chromatographically pure by TLC, $[\alpha]_{578}$ +99° (*c* 1.8, chloroform). The NMR spectrum ($CDCl_3$) showed, *inter alia*, a doublet (τ 5.08, $J_{1,2}$ 3.0 Hz), identified as the signal from the anomeric proton. The product was not further purified.

Methyl 2,4,6-tri-O-methyl-β-D-glucoside (3). Compound *1* (20 g) was dissolved in methanol (400 ml) and was hydrogenated at room temperature and atmospheric pressure using a catalyst of 10 % palladium on charcoal (0.3 g). When hydrogen consumption had ceased, the catalyst was filtered off. Concentration yielded a syrup which crystallised from light petroleum–ethanol (9 : 1, v/v) (14.2 g), m.p. 70–71.5°, $[\alpha]_{578}$ −26° (*c* 1.0, chloroform).[6] The NMR spectrum (CCl_4) showed, *inter alia*, a doublet (τ 5.92, $J_{1,2}$ 8.5 Hz), identified as the signal from the anomeric proton.

Methyl 2,4,6-tri-O-methyl-α-D-glucoside (2). Compound *2* (20 g) was hydrogenated as described above for the β-anomer. After processing a syrup was obtained (14.0 g), which was chromatographically pure on TLC, $[\alpha]_{578}$ +133° (*c* 0.47, chloroform). The NMR spectrum (CCl_4) showed, *inter alia*, a doublet (τ 5.27, $J_{1,2}$ 3.5 Hz), identified as the signal from the anomeric proton.

Methyl 2,4,6-tri-O-methyl-β-D-ribo-hexosid-3-ulose (5). Compound *3* (0.9 g) was dissolved in carbon tetrachloride (5 ml) and oxidized at room temperature by portionwise addition of 0.15 M ruthenium tetroxide in carbon tetrachloride. When no more ruthenium dioxide was formed the reaction mixture was filtered and the filtrate was concentrated to dryness. The resulting syrup crystallised from light petroleum–ethanol (9 : 1, v/v) (0.85 g), m.p. 117.5–119.5°, $[\alpha]_{578}$ −24° (*c* 1.0, chloroform). (Found: C 51.52; H 7.79. $C_{10}H_{18}O_6$ requires: C 51.26; H 7.76.) The NMR spectrum ($CDCl_3$) showed, *inter alia*, a doublet at τ 5.72, $J_{1,2}$ 7.5 Hz, identified as the signal from the anomeric proton. IR ($CHCl_3$) showed a single absorption in the carbonyl region at 1750 cm^{-1}. The CD spectrum gave a minimum at 300 nm with the amplitude $(\theta) = 3009°$.

Methyl 2,4,6-tri-O-methyl-α-D-ribo-hexosid-3-ulose (6). Compound *4* (1 g) was oxidized with ruthenium tetroxide, as described above, to yield a syrup which crystallised from light petroleum–ethanol (9 : 1, v/v) (0.9 g), m.p. 82.5–83.5°, $[\alpha]_{578}$ +164° (*c* 0.9, chloroform). (Found: C 51.38; H 7.75. $C_{10}H_{18}O_6$ requires: C 51.26; H 7.76.) The NMR spectrum ($CDCl_3$) showed, *inter alia*, a doublet (τ 4.81, $J_{1,2}$ 4 Hz), identified as the signal from the anomeric proton. IR showed a single absorption in the carbonyl region at 1750 cm^{-1}. The CD spectrum gave a minimum at 300 nm with the amplitude $(\theta) = 1319°$.

Methyl 2,4,6-tri-O-trideuteriomethyl-α- and -β-D-ribo-hexosid-3-ulose. These compounds were synthesized, using the same procedures as for the corresponding unlabelled derivatives.

Alkaline degradation. The 3-keto-derivative (20 mg) was dissolved in 0.10 M sodium ethoxide in ethanol (1.0 ml) and kept at 20°. The reaction was monitored by TLC, and

when no starting material remained (30 min) the reaction mixture was neutralised with acetic acid. TLC revealed the presence of a minimum of six new components in similar proportions and by GLC it was found that two moles of methanol had been liberated.

The keto-derivatives were also treated with sodium methoxide in methanol, sodium ethoxide in ethanol, and sodium *t*-butoxide in *t*-butanol with different concentrations of the base (0.1 M, 0.05 M, and 0.01 M). All these experiments, which were performed at room temperature, gave the same complex mixture from which no intermediate compound could be isolated.

Alkaline degradation of the labelled derivatives was performed as described above and in order to determine the ratio of labelled to non-labelled methanol the liberated methanol was analysed by GLC–MS.

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