Acta Chemica Scandinavica B 30 (1976) 631—634

Oxidation—Degradation of Methyl 2,3-Di-*O*-ethyl-4-*O*-propyl-α-D-glucopyranoside and Methyl 2,3-Di-*O*-ethyl-6-*O*-propyl-α-D-glucopyranoside

PER-ERIK JANSSON, LENNART KENNE, BENGT LINDBERG and SIGFRID SVENSSON *

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm, Sweden

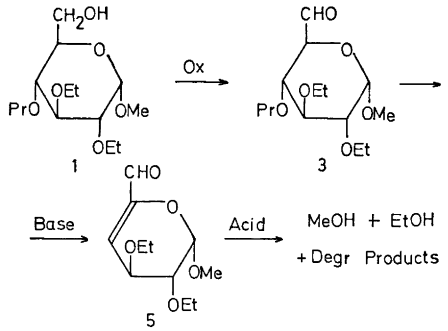
The degradation of methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-*gluco*-hexo-dialdo-1,5-pyranoside and methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-D-*xylo*-hexopyranosid-4-ulose by treatment with base followed by acid hydrolysis under mild conditions and with acid only have been investigated. These model experiments provide an understanding of the degradation of methylated hexodialdo-1,5-pyranosyl and hexopyranosyl-4-ulose residues in a polysaccharide on similar treatment.

A method for the specific degradation of polysaccharides starting from methylated polysaccharides with a limited number of free hydroxyl groups in defined positions has recently been described.[1,2] The hydroxyl groups are oxidized to carbonyl functions and subsequent treatment with base leads to β-elimination with the formation of unsaturated sugar residues. The latter are finally degraded by acid hydrolysis under mild conditions. Glycosyl groups (aglycones) and glycose residues (reducing) are released from the oxidized residue during these treatments. The course of the degradation may be followed by borohydride reduction, re-etherification using trideuteriomethyl or ethyl iodide, hydrolysis and characterization of the products. The results provide information on the sequence of sugar residues in the original polysaccharide. Other degradation methods, based upon β-elimination of sulfone derivatives of polysaccharides and of polysaccharides containing uronic acid residues, have also been described.[2]

The course of the degradation preceded by oxidation has been studied using partially etherified glycosides with free hydroxyl groups at C-2,[3] C-3[4,5] or C-4 and C-6[6] as model substances. We now report similar studies on glycosides having a free hydroxyl at C-4 or C-6.

RESULTS AND DISCUSSION

Methyl 2,3-di-*O*-ethyl-α-D-glucopyranoside was partially propylated using propyl iodide and silver oxide. The main reaction products, methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-glucopyranoside (*1*) and the corresponding 6-*O*-propyl derivative (*2*) were obtained in fair yields. They were identified by MS of the derived alditol acetates. The corresponding carbonyl derivatives, *3* and *4*, were prepared by oxidation with chlorine–dimethyl sulfoxide–triethylamine.[7] The identities of the carbonyl com-



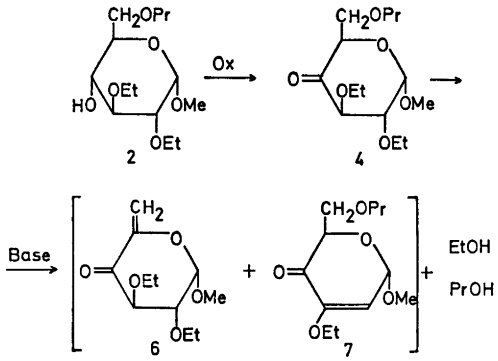
* Present address: Department of Clinical Chemistry, University Hospital, S-221 85 Lund, Sweden

pounds were confirmed by reduction with sodium borodeuteride, whereby *1* gave a product monodeuterated at C-6, and *2* afforded a mixture of *2* and the corresponding D-galacto-pyranoside (1:7), deuterated at C-4.

Treatment of methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-*gluco*-hexodialdo-1,5-pyranoside (*3*) with 0.25 M sodium ethoxide in dichloro-methane-ethanol (2:1) yielded methyl 4-deoxy-2,3-di-*O*-ethyl-α-L-*threo*-hex-4-enodialdo-1,5-pyranoside (*5*), identified by its NMR spectrum. The formation of *5* is consistent with results of Beving and Theander,[8] who investigated the alkaline degradation of a related substance.

The unsaturated aldehyde (*5*) was treated with 50 % aqueous acetic acid at 100 °C, and the release of methanol and ethanol was followed by GLC. After 14 h, all the methanol (from C-1) and approximately 90 % of the ethanol (from C-2 and C-3) had been released. The other reaction products were not investigated. The related methyl 4-deoxy-β-L-*threo*-hex-4-enodi-aldo-1,5-pyranoside on treatment with acid yields 2,5-furandicarboxaldehyde and 4*H*-pyran-2-carboxaldehyde-4-one.[8] and these products were probably also formed in the present experiment. This type of degradation should be well suited for the specific degradation of polysaccharides, as is the chemically analogous uronic acid degradation.[8]

The degradation of *2* was expected to be more complicated as the β-elimination of its oxidized product, methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-D-*xylo*-hexopyranosid-4-ulose (*4*), could take two alternative courses, giving *6* or *7*. The carbonyl compound *4* was treated with 0.2 M sodium butoxide in butanol at 25 °C for 20 min, and the released alcohols were analysed by GLC. The molar proportions of



methanol, ethanol and propanol were 0.73, 1.45 and 0.35, respectively. We established that these alcohols were not formed by the action of heat on labile reaction products during GLC by removing the alcohols from the reaction mixture and injecting a sample into the gas chromatograph. On subsequent treatment with 50 % aqueous acetic acid at 100 °C for 12 h, the figures for the released alcohols increased to 0.95, 1.89 and 0.59. The amounts formed during the treatment with acid for 12 h as above were determined by removing the alcohols formed during the base treatment before treatment with acid. The figures, 0.24, 0.45 and 0.30, for methanol, ethanol and propanol, respectively, agree well with the other values.

These results demonstrate that on degradation of *4*, a high percentage of substituents in all positions is eliminated during the alkaline step and that the elimination of substituents at C-1, C-2 and C-3 is essentially complete after the mild acid hydrolysis. Elimination of the substituent at C-6, however, is incomplete. The elimination of substituents other than those at C-1 during the alkaline step may be a disadvantage when the method is applied to polysaccharides. If such a substituent is a sugar residue, it will be released as a reducing sugar residue, which will then be degraded further. This, and the incomplete elimination at C-6, make the results less clearcut than those obtained with the carbonyl group in other positions. This was apparent during studies of a bacterial polysaccharide.[9] Elimination of the substituent at C-1 during the alkaline step was also observed for a hexodialdo-1,5-pyranosid-4-ulose.[6]

Some glycosides containing carbonyl groups are considerably more sensitive to acid hydrolysis than their parent glycosides, for example, hexodialdopyranosides,[10] and the hexodialdo-1,5-pyranosid-4-ulose referred to above. When methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-*gluco*-hexodialdo-1,5-pyranoside (*3*) was treated with 50 % aqueous acetic acid at 100 °C for 12 h, the release of methanol, ethanol and propanol, per mol of starting material, were 0.38, 0.80 and 0.30 mol, respectively. Similar treatment of methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-D-*xylo*-hexo-pyranosid-4-ulose (*4*) released 0.75, 0.96 and 0.45 mol, respectively. These model ex-

periments indicate that the degradation of a methylated polysaccharide containing hexodialdo-1,5-pyranosyl or hexopyranosyl-4-ulose residues by treatment with acid under mild conditions may give useful structural information.

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 of all substances were recorded with a Varian A 60 A or a Varian XL-100 spectrometer, using tetramethylsilane as internal reference and were in agreement with the postulated structures. GLC separations were performed on a Perkin-Elmer F-30 instrument using a glass column containing 5 % UCON 50HB 2000 on Porapak Q (alcohols) and on a Hewlett-Packard 5830A instrument using a glass capillary column (25 m × 0.25 cm) wall-coated with SP-1000 (partially alkylated alditol acetates). For GLC-MS a Varian MAT 311-SS-100-gas chromatograph-mass spectrometer fitted with a glass column containing 3 % OV-225 on Gas Chrom Q was used. Hydrolyses were performed with 0.25 M sulfuric acid for 16 h at 100 °C.

*Methyl 2,3-di-*O*-ethyl-α-*D*-glucopyranoside.* Methyl 4,6-*O*-benzylidene-2,3-di-*O*-ethyl-α-D-glucoside[11] (4.7 g) in ethanol (20 ml) was hydrogenated at room temperature and atmospheric pressure using a catalyst of 10 % palladium on carbon (0.6 g) to give a chromatographically pure syrup (3.5 g), $[\alpha]_{578}^{25}$ +122° (*c* 1.4, chloroform).

*Methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-*D*-glucopyranoside (2).* Silver oxide (4.0 g) was added to methyl 2,3-di-*O*-ethyl-α-D-glucopyranoside (3.3 g) in propyl iodide (50 ml) and the mixture stirred for 4 h at 70 °C. The silver salts were filtered off, washed with chloroform and the combined filtrates concentrated to dryness. The reaction products were separated on a Silica Gel column (5 × 70 cm) irrigated with ethyl acetate – light petroleum (1:1). The separation was followed by TLC using the same solvent. The components eluted were identified by NMR and by MS of the derived alditol acetates.[12] The first fraction to be eluted, methyl 2,3-di-*O*-ethyl-4,6-di-*O*-propyl-α-D-glucoside, was obtained as a syrup (0.2 g), $[\alpha]_{578}^{25}$ +92° (*c* 1.3, chloroform). The next compound eluted (methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-D-glucopyranoside (*2*)) was also obtained as a syrup (1.3 g), $[\alpha]_{578}^{25}$ +100° (*c* 1.0, chloroform).

*Methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-*D*-glucoside (1).* Further elution of the column above yielded the title compound (*1*) as a chromatographically pure syrup (1.0 g), $[\alpha]_{578}^{25}$ +127° (*c* 1.1, chloroform). Unchanged starting material (0.9 g) was finally eluted.

*Methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-*D*-glucohexodialdo-1,5-pyranoside (3).* The oxidation reagent was prepared by adding dimethyl sulfoxide (3.5 ml) to a 1 M solution of chlorine in anhydrous dichloromethane (10 ml) in a sealed flask under vigorous stirring at −45 °C. A white precipitate appeared during the addition. The glucoside 1 (0.45 g) in dichloromethane (2 ml) was added with the aid of a syringe, and the reaction mixture was stirred at −45 °C for 3.5 h. Triethylamine (2.8 ml) was added and the mixture kept at the same temperature for further 10 min, and was then raised to room temperature. It was confirmed by TLC (chloroform–ethanol, 19:1) that all starting material had reacted. The product was purified by chromatography on a silica gel column (15 × 4 cm) irrigated with acetone–ethyl acetate, 1:1, and the last traces of dimethyl sulfone, formed during the oxidation, were removed on a column (30 × 2.5 cm) of Sephadex LH-20, irrigated with chloroform. The title compound (0.32 g) crystallized and was recrystallized from hexane, m.p. 86.5–88 °C $[\alpha]_{578}^{25}$ +129° (*c* 1.0, chloroform). Elemental analysis could not be performed because of the lability of the compound. The NMR spectrum showed, *inter alia*, a signal at δ 9.78, $J_{5,6} < 1$ Hz, assigned to the aldehyde proton. After addition of deuterated water (1 drop), the NMR showed signals at δ 0.93 (t, *J* 7 Hz, 3 H), 1.23 (t, *J* 7 Hz, 6 H), 1.54 (m, *J* 7 Hz, 2 H), 3.3–4.2 (complex signals, 13 H), 4.80 (d, $J_{5,6}$ 3.5 Hz, 1 H), and 4.85 (d, $J_{1,2}$ 3 Hz, 1 H). The substance crystallized as the *gem*-diol, as no carbonyl band but a strong hydroxyl band was present in the IR spectrum (KBr).

*Methyl 2,3-di-*O*-ethyl-6-*O*-propyl-α-*D*-xylohexopyranosid-4-ulose (4)* was prepared by oxidation of *2* (0.42 g), using the same procedure as above, except that ethyl acetate –light petroleum (1:1) was used for TLC and ethyl acetate–light petroleum (2:1) for column chromatography. The title compound was obtained as a chromatographically pure syrup (0.37 g), $[\alpha]_{578}^{25}$ +166° (*c* 1.9, chloroform). A strong carbonyl band was present in the IR spectrum at 1740 cm^{-1} and NMR showed signals at δ 0.92 (t, *J* 7 Hz, 3 H), 1.25 (t, *J* 7 Hz, 6 H), 1.54 (m, *J* 7 Hz, 2 H), 3.3–4.4 (complex signals 14 H), and 4.97 (d, $J_{1,2}$ 3.5 Hz).

Oxidation–reduction of 1 *and* 2. Oxidation of *1* (10 mg) in the presence of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (10 mg) was performed as described above. The product obtained on concentration was dissolved in ethanol (5 ml), and sodium borodeuteride (20 mg) was added. After 14 h at room temperature, the solution was neutralized with Dowex 50 (H^+) and the product analysed by GLC-MS of the derived alditol acetates. The yield of methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-glucopyranoside, monodeuterated at C-6, was estimated at 95 %. In an analogous experiment

2 was transformed to a mixture of D-glucose and D-galactose derivative, 1:7, deuterated at C-4, the total yield being 79 %.

Methyl 4-deoxy-2,3-di-O-ethyl-α-L-threo-hex-4-enodialdo-1,5-pyranoside (5). The aldehyde *3* (46 mg) was treated with 0.25 M sodium ethoxide in dichloromethane–ethanol (2:1, 4.5 ml) at room temperature for 50 min. Dowex 50 (H^+) was added to pH 7, filtered off, and the solution concentrated to dryness yielding *5* as a chromatographically pure syrup (31 mg), $[\alpha]_{578}^{25}$ +220° (*c* 1.0, chloroform). The NMR spectrum showed, *inter alia*, signals at: δ 1.24 (t, *J* 7 Hz, 6 H), 3.50 (s, 3 H), 4.27 (dd, $J_{3,4}$ 2.5 Hz, $J_{2,3}$ 8 Hz, 1 H), 5.06 (d, $J_{1,2}$ 2.5 Hz, 1 H), 5.86 (d, $J_{3,4}$ 2.5 Hz, 1 H) and 9.19 (s, 1 H).

Treatment of 5 with acid. A solution of *5* (10 mg) and 2-propanol (1 mg) in 50 % aqueous acetic acid (0.5 ml) was kept in a sealed tube at 100 °C. Analyses of alcohols by GLC showed that methanol (1 mol) and ethanol (1.8 mol) had been released after 14 h. In the analyses, molar responses of the alcohols and partial formation of acetate esters were accounted for.

Treatment of 4 with acid and base. A solution of *4* (10 mg) and 2-propanol (1 mg) in 0.2 M sodium butoxide in butanol (0.15 ml) was kept at room temperature for 20 min. The release of methanol (0.73 mol), ethanol (1.45 mol) and propanol (0.35 mol) was determined by GLC. On addition of 50 % aqueous acetic acid and heating for 12 h at 100 °C the values for the amounts of alcohols released increased to 0.95, 1.89 and 0.59 mol, respectively.

In a separate experiment, *4* (10 mg) was treated with base as described above, after which the reaction mixture was neutralized with acetic acid and concentrated to dryness. Treatment of the product with 50 % aqueous acetic acid containing 2-propanol as an internal standard at 100 °C for 12 h and analysis by GLC showed that methanol (0.24 mol), ethanol (0.45 mol) and propanol (0.30 mol) had been released.

Acknowledgements. The authors are indebted to Statens Naturvetenskapliga Forskningsråd for financial support.

REFERENCES

1. Kenne, L., Lönngren, J. and Svensson, S. *Acta Chem. Scand. 27* (1973) 3692.
2. Lindberg, B., Lönngren, J. and Svensson, S. *Adv. Carbohydr. Chem. 31* (1975) 185.
3. Kenne, L. and Svensson, S. *Acta Chem. Scand. 26* (1972) 2144.
4. Kenne, L., Larm, O. and Svensson, S. *Acta Chem. Scand. 26* (1972) 2473.
5. Kenne, L., Larm, O. and Svensson, S. *Acta Chem. Scand. 27* (1973) 2797.
6. Jansson, P.-E., Kenne, L. and Svensson, S. *Acta Chem. Scand. B 30* (1976) 61.
7. Corey, E. J. and Kim, C. U. *Tetrahedron Lett. 12* (1973) 919.
8. Beving, H. F. G. and Theander, O. *Acta Chem. Scand. B 29* (1975) 577.
9. Lindberg, B., Lönngren, J., Rudén, U. and Nimmich, W. *Carbohydr. Res. 42* (1975) 83.
10. Beving, H. F. G. and Theander, O. *Acta Chem. Scand. B 29* (1975) 641.
11. Marvel, J. T., Sen, S. K., Uenaka, F. T., Berry, J. W. and Deutschman, Jr. A. J. *Carbohydr. Res. 6* (1968) 18.
12. Björndal, H., Hellerquist, C. G., Lindberg, B. and Svensson, S. *Angew. Chem. Int. Ed. 9* (1970) 610.

Received December 21, 1975.