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# Determination of the Anomeric Configuration of Sugar Residues in Acetylated Oligo- and Polysaccharides by Oxidation with Chromium Trioxide in Acetic Acid

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A number of fully acetylated glycosides, oligosaccharide alditols, and polysaccharides have been treated with chromium trioxide in acetic acid. Pyranosidic pentose and hexose residues, in which the aglycone occupies an equatorial position in the most stable chair form, are readily oxidised, while corresponding residues, in which the aglycone occupies an axial position, are more resistant to oxidation. Similar results were obtained for carbohydrates containing pyranosidic 6-deoxy- and 2-acetamidodeoxy-sugar residues. The anomeric nature of sugar residues in oligosaccharides and polysaccharides can consequently be determined by this reaction, which may possibly also be used for selective degradation.

Determination of the anomeric nature of sugar residues in oligo- and polysaccharides is a recurrent problem in polysaccharide chemistry. For small oligosaccharides, this can be solved by polarimetry or NMR. Enzymic hydrolysis and reactions with specific antibodies or lectins may also provide valuable information.

Angyal and James [1,2] have recently demonstrated that chromium trioxide in acetic acid rapidly oxidises peracetylated hexopyranosides, in which the aglycone occupies an equatorial postition, giving 5-hexulosonates in high yields. The corresponding anomers in which the aglycone occupies an axial position are oxidised only slowly and give 1-*O*-acyl derivatives (*e.g.* the 1-*O*-formyl derivative from a methyl glycoside). Acetylated α- and β-furanosides are readily oxidised, yielding 4-hexulosonates.

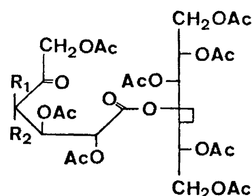
From these results, treatment with chromium trioxide in acetic acid appeared to offer a general method for distinguishing α- and β-pyranosidic linkages in acetylated glycosides, oligosaccharides, and polysaccharides. This possibility has now been investigated and the results are reported in the present communication.

## RESULT

Some acetylated methyl and ethyl glycosides of pentoses, hexoses, deoxyhexoses, and 2-acetamido-2-deoxy-hexoses were oxidized with chromium trioxide in acetic acid (see Table 1). The oxidations were followed by GLC using *myo*-inositol hexaacetate as an internal standard. For all sugars investigated, the anomer in which the aglycone occupies an equatorial position in the most stable chair form (generally the β-glycoside) disappeared considerably faster than the other anomer. It seems reasonable to assume that the former are oxidised to ketoesters and the latter to 1-formates or 1-acetates, in agreement with the results of Angyal and James. Both the methyl α- and the β-3,6-dideoxy-D-*xylo*-hexopyranosides (abequosides) were oxidised, the latter at a somewhat faster rate. Both glycosides were oxidised to 5-hexulosonates, as demonstrated by reduction of the reaction product with sodium borohydride, followed by identification of the expected products, 3,5-dideoxy-D-*xylo*-hexitol (abequitol) and 3,6-dideoxy-L-*arabino*-hexitol (ascarylitol) by GLC–MS. This result is not unexpected, as the difference in energy between the two chair forms of methyl α-abequoside should be comparatively small. It demonstrates, however, that the abequosidic linkage, which is rather labile to acid hydrolysis, is not hydrolysed under these reaction conditions.

Angyal and James [2] observed that the β-octaacetate of maltose was oxidised more readily than the α-octaacetate and also found similar differences between α- and β-pentaacetates of hexopyranosides. For this reason the disaccharides investigated were transformed into their alditol acetates before oxidation. The oxidations were carried out in the presence of *myo*-inositol hexaacetate and were followed by GLC of the alditol acetates of the sugars, obtained on acid hydrolysis of the reaction product.

Investigations with the nonaacetates of cellobiitol and maltitol demonstrated that the former could be quantitatively converted into the ketoester 1 under conditions which left the latter essentially intact. Compound 1 was isolated by TLC and its NMR spectrum displayed the typical two-proton singlet at $\tau$ 4.87 (the hydrogens at C-6 on the oxidised residue). Hydrolysis of 1 yielded one mole of D-glucitol. Borohydride reduction followed by hydrolysis afforded D-glucitol and L-iditol in the relative proportions 1.5 : 0.5. Analogously, the ketoester 2 was obtained by oxidation of lactitol nonaacetate, as




Compound 1 $R_1$=H $R_2$=OAc

Compound 2 $R_1$=OAc $R_2$=H

*Table 1.* Oxidation of oligosaccharide alditol acetates and peracetylated glycosides (1 h reaction time).

| Compound | % Recovery | Reaction temperature, °C |
|---|---|---|
| Et-α-Ara | 30 | 20 |
| Me-β-Ara | 90 | 20 |
| Me-α-G | 72 | 20 |
| Me-β-G | 13 | 20 |
| Me-α-Man | 100 | 20 |
| Me-β-Man | 32 | 20 |
| Me-α-Gal | 76 | 20 |
| Me-β-Gal | 4 | 20 |
| Me-α-GNAc [a] | 84 | 50 |
| Me-β-GNAc | 12 | 50 |
| $G \xrightarrow[\alpha]{1 \quad 4} G$ | 100 | 50 |
| $G \xrightarrow[\beta]{1 \quad 4} G$ | 0 | 50 |
| $G \xrightarrow[\beta]{1 \quad 4} Man$ | 19 | 50 |
| $G \xrightarrow[\beta]{1 \quad 6} G$ | 0 | 50 |
| $Gal \xrightarrow[\alpha]{1 \quad 6} G$ | 100 | 50 |
| $Gal \xrightarrow[\beta]{1 \quad 6} Gal$ | 11 | 50 |
| $Gal \xrightarrow[\beta]{1 \quad 4} G$ | 9 | 50 |
| $Man \xrightarrow[\alpha]{1 \quad 3} Gal \xrightarrow[\alpha]{1 \quad 2} Glyc$ | 100 | 50 |
| $Xyl \xrightarrow[\beta]{1 \quad 4} Xyl$ | 9 | 50 |

[a] Contains 10 % of Me-β-GNAc.

demonstrated by similar analyses. Reduction and hydrolysis in this case yielded a mixture of D-glucitol, D-galactitol, and L-altritol.

A number of fully acetylated oligosaccharide alditols were oxidised under the same conditions as used for the cellobiitol and maltitol nonaacetates (see Table 1). The α-pyranosidic sugar residues in these oligosaccharides were not attacked under conditions which oxidized 80 – 100 % of the β-pyranosidic residues.

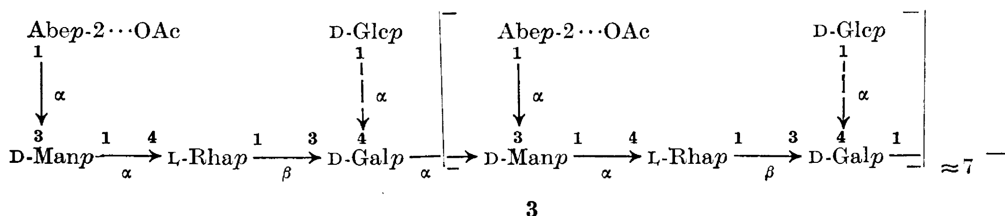
The oxidation of some fully acetylated polysaccharides was finally investigated (see Table 2). Guaran [3] consists of chains of (1 → 4)-β-linked D-mannose residues, about 60 % of which carry an α-D-galactopyranose residue in the 6-position. After oxidation for 2 h, the ratio of D-mannose to D-galactose had decreased to 20 % of its original value, thus showing the expected differ-

*Table 2.* Oxidation of peracetylated polysaccharides. Reaction temperature 50°C.

| Polysaccharide | Time of oxidation | Sugar analysis | | | | |
|---|---|---|---|---|---|---|
| | | Abe | L-Rha | D-Man | D-Gal | D-Glc |
| Guaran | 0 | – | – | 1.75 | 1.00 | – |
| | 1 | – | – | 1.03 | 1.00 | – |
| | 2 | – | – | 0.55 | 1.00 | – |
| *Salmonella typhimurium* | 0 | 0.45 | 0.95 | 1.00 | 1.24 | 0.67 |
| LT2 polysaccharide | 1 | 0.40 | 0.73 | 1.00 | 1.13 | 0.78 |
| | 2 | 0.45 | 0.38 | 1.00 | 1.12 | 0.70 |

ence of reactivity between the $\beta$-D-mannopyranosidic and the $\alpha$-D-galactopyranosidic residues.

The O-specific side-chains of the lipopolysaccharide (LPS) from *Salmonella typhimurium* LT2 may be represented by structure 3.[4,5]




**3**

In this structure, a dotted line indicates a substituent that is not present in all repeating units. All the sugar residues except the L-rhamnopyranose residues and the D-galactopyranose residue which links the O-specific side-chain to the core, have the $\alpha$-configuration. Naturally occurring L-rhamnose residues generally have the $\alpha$-configuration and the presence of $\beta$-L-rhamnopyranosidic residues in this LPS is an unusual feature. Oxidation of the fully acetylated LPS resulted in destruction of the L-rhamnose residues, in agreement with the proposed structure. The small decrease in D-galactose, in agreement with the low percentage of $\beta$-linked D-galactose residues, is probably fortuitous. The values for abequose, both in the original and the oxidised samples, are low, since a considerable part of this sugar is destroyed during the acid hydrolysis.

It proved difficult to obtain strictly reproducible results with the polysaccharides, probably because of the heterogeneous nature of the reagent. Thus the reaction rates seemed to depend upon the particle size of the chromium trioxide and of the agitation of the reaction mixture. Qualitatively, however, the results were always consistent.

## DISCUSSION

The anomeric nature of sugar residues in different carbohydrates can be determined by studying the reaction of their fully acetylated derivatives with

chromium trioxide in acetic acid. This method, which can be performed on a mg scale, will be useful for studying the oligosaccharides, obtained by graded hydrolysis of polysaccharides and which are often available only in small amounts. It may also be applied directly to polysaccharides. It is believed that it will become a valuable complement to other methods for determination of the anomeric nature of sugar residues, based upon polarimetry, NMR, enzymatic hydrolysis, or reactions with antibodies.

The pyranosidic sugar residues in which the aglycone occupies an equatorial position are oxidised to 5-hexulosonate residues, and the ester linkage formed on oxidation could be cleaved by subsequent alkaline hydrolysis. This would offer a new method for the controlled degradation of polysaccharides, which could be useful in structural polysaccharide chemistry. Ester linkages are cleaved during methylation with methylsulphinyl sodium – methyl iodide in methyl sulphoxide, according to Hakomori.[6] Methylation analysis of the original polysaccharide containing both oxidisable and resistant sugar residues and of the acetylated and oxidised sample could thus provide information on the sequences of some of these residues. Such possibilities are under investigation.

## EXPERIMENTAL

*General methods.* Concentrations were performed at reduced pressure. Gas-liquid chromatography was carried out on a Perkin-Elmer model 900 instrument fitted with a 3 % nitrile silicone-polyester copolymer (ECNSS-M) column. The alditol acetates were separated at 190°.

Sugar analyses were performed as described by Sawardeker *et al.*[7] TLC was performed on Silica gel G (E. Merck AG, Darmstadt) and the compounds were detected with 8 % aqueous sulphuric acid at 100°C.

*Oxidation of cellobiitol nonaacetate.* Powdered chromium trioxide (300 mg) was added to a solution of cellobiitol nonaacetate (100 mg) in glacial acetic acid (3 ml). The resulting suspension was agitated at 50°C in an ultrasonic bath. The reaction was followed by TLC using ethyl acetate – light petroleum (1 : 2, v/v) as irrigant. After 1 h, when no starting material remained, TLC showed only one new product. The reaction mixture was diluted with water (20 ml) and extracted with chloroform (3 × 20 ml). The combined chloroform extracts were dried over sodium sulphate and concentrated to dryness. The crude material (100 mg) was purified by preparative TLC to give 90 mg of pure oxidation product as a syrup. NMR of this product showed *inter alia* a singlet at $T = 4.87$;[2] sugar analysis gave 1.0 mol of D-glucitol. Part of the oxidation product (10 mg) was reduced with sodium borohydride (10 mg) in ethanol (5 ml). Sugar analysis of the reduced material revealed D-glucitol and L-iditol in the relative proportions 3 : 1.

*Oxidation of lactitol nonaacetate.* Lactitol nonaacetate was oxidised as described above for cellobiitol nonaacetate. The oxidation product was isolated in 90 % yield as a syrup. NMR of the oxidation product showed *inter alia* a singlet at $\tau = 4.91$; sugar analysis gave 1.0 mol of D-glucitol. After reduction with sodium borohydride in ethanol sugar analysis revealed L-altritol : D-galactitol : D-glucitol in the relative proportions 0.25 : 0.50 : 1.00.

*Oxidation of oligosaccharide alditol acetates and peracetylated glycosides.* The oligosaccharide alditol acetate or peracetylated glycoside (2 mg) was mixed with *myo*-inositol hexaacetate (2 mg) (as internal standard). Part of this mixture was subjected to a sugar analysis to determine the exact proportions of reactant and *myo*-inositol hexaacetate. Another part was oxidised with chromium trioxide in glacial acetic acid as described for cellobiitol nonaacetate. The reaction product was then analysed for nonoxidised sugars (oligosaccharide alditol acetates were first subjected to hydrolysis). The results are summarised in Table 1.

*Oxidation of acetylated guaran.* Acetylated guaran [8] (10 mg) was oxidised together with *myo*-inositol hexaacetate (5 mg) (internal standard) as described for cellobiitol nonaace-

tate. Samples were taken from the reaction mixture at 0, 1, and 2 h, hydrolysed and analysed for sugars. The results are presented in Table 2.

*Oxidation of the acetylated polysaccharide from* Salmonella typhimurium *LT2*. Lipid-free polysaccharide [8] from *S. typhimurium* LT2 (50 mg) was dissolved in formamide (10 ml). To this solution was added pyridine (5 ml) and acetic anhydride (4 ml) and the resulting mixture was allowed to stand at room temperature for 20 h. The reaction mixture was then poured into ice-water (100 ml) and dialysed. The resulting suspension of acetylated polysaccharide acetate was purified by precipitation from acetone with light petroleum to yield 60 mg of polysaccharide acetate.

The polysaccharide acetate (10 mg) was then oxidised and the oxidation products analysed as described for acetylated guaran. The results are shown in Table 2.

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