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## Note

# Determination of anomeric configurations of per-*O*-methylated sugars with chromium trioxide

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Determination of the positions of glycosidic linkages and of their anomeric configurations constitutes one of the main focuses of polysaccharide chemistry. The employment of g.l.c.–m.s. serves as a powerful tool for ascertaining the positions of glycosidic linkages, especially for complex mixtures of oligosaccharides available in only small amount[1–4]. In this case, per-*O*-methylated oligosaccharides are usually employed, both for their high volatility and the simplicity of their mass-spectral patterns.

It has been reported[5] that chromium trioxide preferentially oxidizes glycoside peracetates having equatorially oriented aglycons, and is useful for the determination of anomeric configurations, but g.l.c. of peracetates of oligosaccharides is difficult, and their mass-spectral patterns are generally complex. Consequently, a combination of g.l.c.–m.s. and chromium trioxide oxidation of per-*O*-methylated oligosaccharides provides a means of readily determining the positions and anomeric configurations of oligosaccharides. We now describe differences in the resistance of some per-*O*-methylated D-glucose derivatives to chromium trioxide oxidation.

The $\alpha$ and $\beta$ anomers of per-*O*-acetylated and per-*O*-methylated methyl D-glucopyranosides were oxidized with various concentrations of chromium trioxide (see Table I). In the case of the peracetates, as reported by Hoffman *et al.*[5], $\alpha$ anomers are not oxidized at 50° by chromium trioxide in all concentrations tested. $\beta$ Anomers are oxidized to the extent of over 90% after 1 h at 50°, at concentrations of chromium trioxide of above 0.24 mmol/mL.

Both anomers of per-*O*-methylated D-glucoside were more oxidizable, and were completely oxidized within 10 min at 50°, even at a chromium trioxide concentration of 0.12 mmol/mL, at which both anomers of peracetates were completely resistant. Under milder conditions (0.06 mmol/mL at 50°, or 0.12 mmol/mL at 25°), the

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TABLE I

THE RESULTS OF OXIDATION WITH CHROMIUM TRIOXIDE

| *Concentration of $CrO_3$ (mmol/mL)* | *Reaction temp. (degrees)* | *Compound*[a] | *Reaction time (min)* | | | | |
|---|---|---|---|---|---|---|---|
| | | | *10* | *20* | *30* | *45* | *60* |
| | | | *Amount remaining (%)* | | | | |
| 0.48 | 50 | Me-β-MG | 2.0 | 0 | 0 | 0 | 0 |
| | | Me-α-MG | 4.6 | 0 | 0 | 0 | 0 |
| | | Ac-α-MG | 97.3 | 96.2 | 90.8 | 86.2 | 82.9 |
| | | Ac-β-MG | 26.3 | 5.3 | 1.3 | 0 | 0 |
| 0.24 | 50 | Me-β-MG | 1.0 | 0 | 0 | 0 | 0 |
| | | Me-α-MG | 3.4 | 0 | 0 | 0 | 0 |
| | | Ac-α-MG | 94.9 | 95.6 | 95.4 | 92.1 | 82.9 |
| | | Ac-β-MG | 34.1 | 25.7 | 20.1 | 17.4 | 14.6 |
| 0.12 | 50 | Me-β-MG | 2.0 | 0.3 | 0 | 0 | 0 |
| | | Me-α-MG | 7.3 | 1.2 | 0 | 0 | 0 |
| | | Ac-α-MG | 102.3 | 103.9 | 102.7 | 102.7 | 101.7 |
| | | Ac-β-MG | 84.1 | 78.3 | 67.1 | 62.7 | 59.4 |
| 0.06 | 50 | Me-β-MG | 37.8 | 30.8 | 21.8 | 20.8 | 19.9 |
| | | Me-α-MG | 55.7 | 44.8 | 44.7 | 41.2 | 39.7 |
| | | Ac-α-MG | 101.2 | 101.5 | 99.8 | 101.0 | 102.2 |
| | | Ac-β-MG | 100.2 | 98.7 | 100.8 | 99.4 | 102.9 |
| 0.12 | 25 | Me-β-MG | 59.0 | 25.7 | 22.4 | 18.0 | 17.3 |
| | | Me-α-MG | 79.9 | 49.3 | 45.2 | 44.2 | 42.7 |
| | | Ac-α-MG | 102.3 | 99.3 | 99.2 | 102.4 | 101.8 |
| | | Ac-β-MG | 99.8 | 100.5 | 98.9 | 99.6 | 100.2 |
| 0.12 | 50 | Me-Mal | 61.4 | 52.4 | 48.3 | 42.4 | 38.9 |
| | | Me-Cel | 8.0 | 3.1 | 1.0 | 0.8 | 0.3 |
| 0.12 | 25 | Me-Mal | 77.8 | 66.4 | 64.1 | 66.4 | 60.2 |
| | | Me-Cel | 61.6 | 43.2 | 36.5 | 32.1 | 30.3 |

[a] Me-MG, per-*O*-methylated methyl D-glucoside; Ac-MG, per-*O*-acetylated methyl D-glucoside; Me-Mal, permethylated maltitol; Me-Cel, permethylated cellobiitol.

rates of reaction were low, and ~20% of the β anomer and ~40% of the α anomer was resistant to the oxidation; but, it is not possible to determine the anomeric configuration by this difference, because the remaining 20% was too large in relation to the difference in the amount of the two anomers remaining.

In the oxidation experiments on per-*O*-methylated disaccharide-alditols (maltitol and cellobiitol), it was possible to distinguish the α from the β linkage. Per-*O*-methylated cellobiitol was completely oxidized, but per-*O*-methylated maltitol was oxidized at 50° to the extent of only ~50% after 30 min, and 60% after 1 h, at a concentration of chromium trioxide of 0.12 mmol/mL. In an experiment at 25°, ~30% of per-*O*-methylcellobiitol remained intact. These results showed that the conditions of 0.12 mmol/mL during 30 min at 50° are suitable for determination of the anomeric configuration of the glycosidic linkage of per-*O*-methylated disaccharides.

## EXPERIMENTAL

Methyl per-*O*-acetyl- α- and β-D-glucopyranoside and methyl per-*O*-methyl-α- and β-D-glucopyranoside (55 μmol of each) were combined. To the mixture were added chromium trioxide and glacial acetic acid (5 mL), and the solution was kept at 25°, or 50°, with stirring. Periodically, an aliquot (0.3 mL) was poured into water (1 mL), the solution extracted with chloroform, and the extract analyzed by g.l.c. In the experiments on disaccharide-alditols, per-*O*-methylcellobiitol (55 μmol) and per-*O*-methylmaltitol (55 μmol) were separately oxidized. In all experiments, L-arabinitol peracetate was used as the internal standard.

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