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The Oxidation of Glycosides

V. Oxidation of Methyl 6-*O*-Trityl-α-D-glucopyranoside and Methyl 6-*O*-Trityl-β-D-glucopyranoside with Chromium Trioxide *

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Methyl 6-*O*-trityl-α-D-glucopyranoside and methyl 6-*O*-trityl-β-D-glucopyranoside have been oxidised with chromium trioxide in acetone. After detritylation of the resulting mixture, the 2-oxo-, 3-oxo and 4-oxo-methylglucosides in both the α- and the β-series were isolated. The position of the oxo-groups were shown by characterising the sugars formed after hydrolysis of the reduced oxoglucosides.

In part IV [1] the isolation of methyl β-D-3-oxoglucopyranoside and methyl β-D-6-oxoglucopyranoside from the reaction mixture after oxidation of methyl β-D-glucopyranoside with dichromate in the presence of oxalic acid was described. These two oxocompounds were isolated only in low yield with the aldehyde predominating. In order to improve the yields of the 3-oxo-glucoside and the other potential oxidation products, the primary hydroxyl at $C_{(6)}$ was now protected by tritylation prior to oxidation (V). The oxidation of methyl 6-*O*-trityl-α-D-glucopyranoside (I) was also investigated.

Chromium trioxide-pyridine [2] was tried as oxidant, but chromium trioxide in acetone was found to give better yields of oxoglucosides. After oxidation (see Experimental) the brown precipitate of chromium oxides was filtered off and the trityl groups were removed by mild hydrolysis. The chromium in the solution was reduced by ethanol at the same time. The water soluble, neutral part of the oxidation mixture, obtained after separation of triphenylcarbinol and other water insoluble products followed by deionizing, was investigated by paper chromatography and was found to contain at least a dozen different components. The mixture was separated by chromatography on a carbon column and by chromatography and electrophoresis on thick filter paper. The yields are given in Table 1 of the methyl β-3-oxoglucoside (VII) and the

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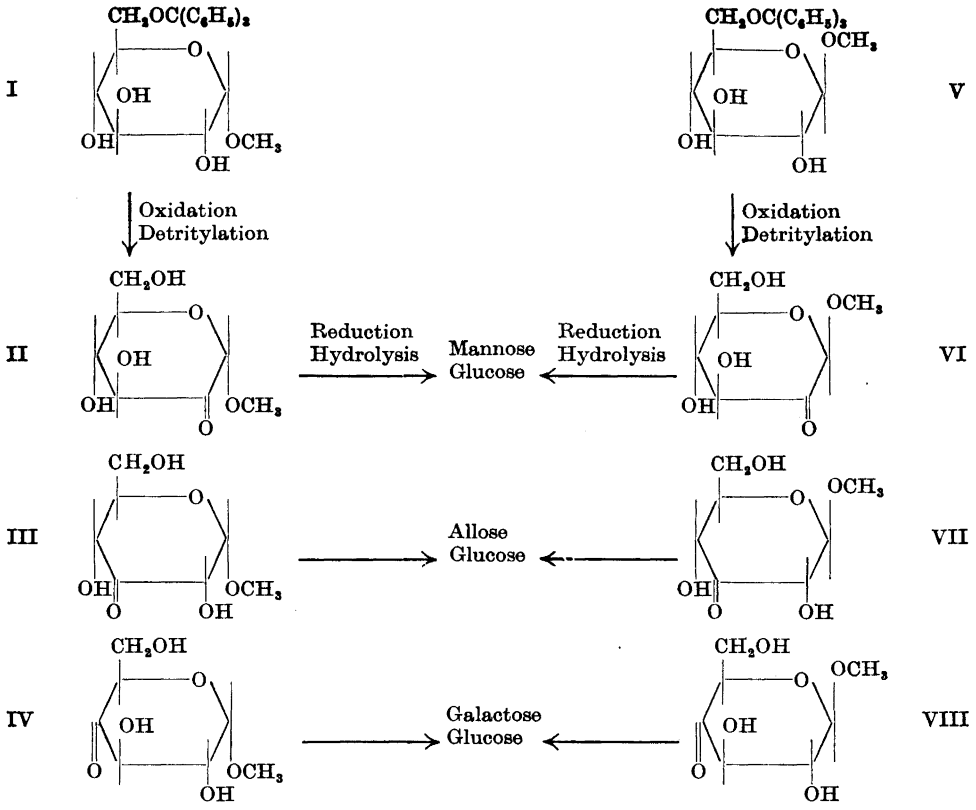

unoxidised methyl β-glucoside, obtained after carbon column chromatography of the reaction product from the oxidation of V with varying amounts of chromium trioxide.

The yield of VII has a flat maximum of about 4.5 % at 1—2 moles oxidant per mole of sugar; at the same time there is still much unchanged methyl glucoside left. The amount of oxoglucosides is obviously limited by further oxidation of these compounds and perhaps also by side reactions, *e.g.* oxidation of glucose at $C_{(1)}$. In addition to VII which was crystalline and identical to the substance m.p. 129.5-131°, $[\alpha]_D$-62° in water, previously isolated, there were also isolated two other ketoglucosides in the β-series (VI and VIII), and the corresponding compounds in the α-series (II, III and IV). VI was crystalline, m.p. 120-121°, and $[\alpha]_D$-47° in water. VIII and the last three were amorphous, but chromatographically and electrophoretically pure. The specific rotation of III was $[\alpha]_D$ +84° in water. The structure of the oxoglucosides was proved by characterising the sugars obtained after reduction with Raney nickel[3] and hydrolysis of the glucoside formed (compare the scheme above). Each sugar gave glucose and one of the sugars mannose, allose or galactose. Constants and details of the separation are given in Table 2.

Table 1. Oxidation of methyl 6-*O*-trityl-β-D-glucopyranoside with chromium trioxide in acetone (The yields are given as a percentage of amount of starting material calculated as methyl β-glucoside

Moles chromium trioxide per mole of trityl sugar	Water soluble, neutral products	Methyl glucoside	Methyl 3-oxo-glucoside
1.0	64	46	4.0
1.5	53	31	4.4
1.5	57	33	4.9
4.0	18	9	3.2

The amounts of 3-oxoglucosides formed were much higher than those of the 2- and 4-oxoglucosides. This is obvious also from the direct paper chromatogram of the hydrolysed oxidation mixture, before the separation procedure. On the other hand it was found in preliminary experiments that the 2-oxo and 4-oxoglucosides are more labile than the corresponding 3-oxo-compound at a pH near the neutral point and give epimerisation and decomposition products. As they emerge from the carbon column together with unchanged methyl glucoside their separation is more complicated than that of 3-oxoglucoside, which is obtained directly from the carbon column almost pure, crystallises readily (in the β-series) and is less labile.

The differences in the R_F values and the relative rates of the electrophoretic migration in hydrogen sulphite buffer between the various oxoglucosides is remarkable and much more marked than for, *e.g.*, hexoses. All oxoglucosides gave strong reducing reactions with the silver nitrate-sodium ethoxide reagent and a particularly sensitive and characteristic colour reaction was given by the resorcinol-hydrochloric acid reagent. In addition to unchanged methyl glucoside and mono-ketoglucosides there were many other compounds present in small amounts, amongst which were: (in the order of emergence from the carbon column) salts, traces of arabinose, glucose (about 0.1 %), methyl 6-oxoglucoside (isolated in the β-series only), a group of substances probably condensation products, with low R_F values and finally products with high R_F values, which might be dioxo compounds, emerging from the column at about 10 % ethanol. One or more of the latter group gave a strong red-violet colour reaction with the resorcinol-hydrochloric acid reagent and one gave a strong yellow colour with anisidine hydrogen chloride. A small amount (0.3 %) of the last mentioned compound was isolated both in the α-series and β-series and after reduction and subsequent hydrolysis gave spots corresponding to glucose, galactose and also arabinose in smaller amount on paper chromatograms. They gave positive Schiff reactions, consumed hypoiodite [5] (corresponding to a purity of about 70 %), and the electrophoretic migration in hydrogen sulphite had the magnitude expected for a dioxo compound. On the paper chromatograms they gave one dominating spot with a high R_F value and a minor one with low R_F value with trailing between, probably explained by a slow equilibrium between different forms (behaving similarly to methyl-β-6-

Table 2. Oxo-glucosides; preparation and characteristics.
Oxidant per mole = 1.5. The yields are calculated as percentage of the amount of glucoside before oxidation. Data of the carbon column chromatography and the solvents and buffers used by the paper chromatography and electrophoresis are given in Experimental. f. = fluorescence; a. = absorption.

	Methyl α-D-oxoglucopyranosides			Methyl α-D-glucopyranoside
	2-oxo	3-oxo	4-oxo	
Yield (%)	0.3	2.0	0.4	31
Percentage of ethanol in the eluant, when the substance emerged from the carbon column	3.8	6.9	4.9	4.0
Paper chromatography $R_{Glucose}$ values				
Solvent A	1.78	3.38	2.47	2.63
Solvent B	1.42	2.05	1.61	1.64
Paper electrophoresis M_V values, buffer D (50°)	0.93	0.07	0.75	0
Colour reactions on paper chromatograms (the data in brackets refer to the colour in UV-light):				
Anisidine hydrogen chloride	yellow (brownish yellow a.)	yellow (brownish yellow a.)	yellow (brownish yellow a.)	
Resorcinol-hydrochloric acid	brick red (red-brown a.)	orange (orange f.)	brick red (red-brown a.)	

oxoglucoside [1]). These facts might indicate the presence of methyl-4.6-dioxoglucosides in the α- and β-series, respectively.

Only 0.5 % of the material was soluble in water directly after the oxidation and this gave approximately the same chromatographic picture as the main mixture obtained after hydrolysis, (*i.e.* methyl glucoside and methyl 3-oxoglucoside predominated) showing that the protecting trityl group is hydrolysed to a very small extent during the oxidation. In spite of that the 6-oxoglucoside and a possible dioxo compound containing an aldehyde-group were formed (about 0.7 % isolated altogether), which might indicate tritylation on secondary hydroxyls to some extent leaving the primary hydroxyl group free.

An interesting observation was made on the rate of detritylation in a case where the strength of the acid for the hydrolysis following the oxidation of V was unintentionally lower than usual. Methyl β-glucoside and VII were isolated by carbon column chromatography from this "weak" hydrolysate and from the hydrolysate obtained after further hydrolysis of the water insoluble material with the usual strength of acid. The total amount of methyl

Table 2. (continued)

	Methyl β-D-oxoglucopyranosides				Methyl β-D-glucopyranoside
	2-oxo	3-oxo	4-oxo	6-oxo	
Yield (%)	0.5	4.7	0.4	0.4	32
Percentage of ethanol in the eluant, when the substance emerged from the carbon column	3.0	6.6	4.6	5.3	3.4
Paper chromatography $R_{Glucose}$ values					
Solvent A	1.54	3.69	2.21	1.10	2.44
Solvent B	1.19	1.87	1.41	0.87 2.20	1.55
Paper electrophoresis M_V values, buffer D (50°)	0.79	0.56	0.81	1.25	0
Colour reactions on paper chromatograms (the data in brackets refer to the colour in UV-light):					
Anisidine hydrogen chloride	yellow (brownish yellow a.)	yellow (brownish) yellow a.)	yellow (brownish yellow a.)	reddish brown (grey yellow a.)	
Resorcinol-hydrochloric acid	brick red (red brown a.)	orange (orange f.)	brick red (red. brown a.)	weak pink (reddish a.)	

β-glucoside was distributed 65 to 35 % between the "weak" and "normal" hydrolysates, respectively, but the corresponding values for the oxoglucoside were 10 to 90 %, indicating that removal of the trityl group from the latter is more difficult.

EXPERIMENTAL

Melting points corrected. All evaporations were made under reduced pressure and at a bath temperature under 40°, unless freeze-drying was used. Whatman 1 filter papers were used for paper chromatography and electrophoresis except for the preparative separations, which were made on Whatman 3 MM filter papers, previously washed thoroughly with water.

Solvents and buffers used:

A. Ethyl acetate-acetic acid-water, 3 : 1 : 3 (the upper phase).
B. Butanol-ethanol-water, 10 : 3 : 5.
C. Butanol-pyridine-water, 3 : 1 : 1.5.
D. Hydrogen sulphite buffer [4] pH 4.7, 0.1 M. (The electrophoresis was run at 50° if not otherwise stated.)
E. Borate buffer [6] pH 10.0, 0.1 M.

Oxidation of methyl 6-*O*-trityl-β-D-glucopyranoside and fractionation of the product

Methyl 6-*O*-trityl-β-D-glucopyranoside (V)[7], dried in a vacuum over P_2O_5 at 70° (65.0 g) was dissolved in acetone (800 ml), which had been purified by refluxing in the presence of potassium permanganate, drying over potassium carbonate and distilling. A solution of chromium trioxide (22.5 g), corresponding to 1.5 moles of oxidant per mole of sugar, in purified acetone (800 ml), at about 5°, was poured in a slow stream with agitation into the sugar solution which was kept in an ice water bath. This procedure took about 20 min and the temperature was kept below 15°. As has been previously observed[8], it is important to add the chromium trioxide to the solvent in small portions with agitation; it then dissolves readily to give a reddish brown solution. If the two chemicals are mixed in the reverse order, *i.e.* acetone to chromium trioxide it usually inflames!

The oxidation was carried out by refluxing the mixture for 30 min (leaving the mixture at room temperature for a day gave almost the same result). During the oxidation a dark brown precipitate formed. The solution was cooled and filtered and the precipitate was washed with acetone, refluxed with fresh acetone (400 ml) for 10 min and again filtered and washed. This procedure was repeated three times. After the last treatment the amount of extractable material was negligible. The brown chromium oxides, probably hydrated, were heated to about 300—400°, giving green Cr_2O_3 (77 % of the theoretical amount). The combined filtrates and washings were evaporated to a dark brown clear solution (adjusted to 540 ml with acetone), which was mixed with 6 N ethanolic hydrogen chloride (60 ml). The acid treatment was performed at 25° for 30 min. The colour turned to green and there was a typical odour of acetaldehyde, indicating a reduction of chromium in the solution. Silver carbonate was added with agitation and cooling (the temperature kept below +10°) until a drop of the solution showed pH 5 when put on a moist pH paper. The silver precipitate was filtered off and washed first with acetone and then with water. The aqueous acetone solution was evaporated to a small volume and the crops of triphenyl carbinol separating during this process were removed (82 % of the theoretical amount, m.p. 159—161°). The concentrated aqueous solution was extracted with chloroform several times and then passed through columns of the ion exchange resins Amberlite IR-120 (H^+) and IR-4B (free base). The solution, which had a pH about 4.5 was evaporated to a thick, light green-yellow syrup (15.9 g). To make sure that the detritylation was complete, the concentrated chloroform extract (3.3 g), was given a further treatment with hydrogen chloride as described above using hydrogen chloride at twice the concentration but yielded negligible amounts of water soluble material (0.1 g), which gave the same paper chromatographic picture as the main water extract. The possibility of detritylation during the oxidation procedure was checked by taking an aliquot of the acetone solution directly after oxidation, shaking it with water and chloroform several times and evaporating the water phase, which had a pH of about 5. The water soluble material (0.15 g) gave approximately the same paper chromatographic picture as the main water extract.

The water soluble, deionised material (15.9 g) was fractionated on a charcoal-Celite column (48 × 6 cm) using linear gradient elution (16 l 0—20 % aqueous ethanol) in the usual way. The substances present emerged in the following order: salts, arabinose (traces only), glucose, unknown substances (small amounts), methyl-β-glucoside together with 2-, 4- and 6-oxoglucoside, 3-oxoglucoside and unknown substances, probably condensation or decomposition products of oxocompounds, and also dioxo compounds. Methyl β-glucoside crystallised directly on evaporation of the fractions containing this substance (m.p. 110—111° after recrystallisation from ethanol). Further amounts of methyl β-glucoside and the 2-, 4- and 6-oxoglucosides were isolated from the mother liquors by repeated separations on Whatman 3 MM papers run in solvent A. The final separation of the 4-oxoglucoside was carried out by electrophoresis in buffer D at room temperature and subsequent ion exchange of eluants from the paper strips. The 3-oxoglucoside was obtained quite pure from the column, in a fraction with only small amounts of methyl β-glucoside and other substances. It crystallised directly on evaporation, and was recrystallised from *n*-propanol. The yield was increased by working up the mother liquors and higher fractions from the column by separations on thick papers. The amounts isolated and the characteristics of the oxoglucosides and methyl β-glucoside are given in Table 2.

The figures are averages of two similar batches. Glucose (0.03 g) and the 6-oxoglucoside (0.12 g) were isolated only in one case. By fractionation on thick papers a compound which might be a dioxoglucoside was isolated (0.09 g) from the late fractions from the carbon column.

As the oxo compounds were quite labile, particularly the 2- and 4-oxoglucosides, which were epimerised to 3-oxoglucoside and further transformed to other products even at pH 4—7, the eluants from the final thick paper separations were evaporated by freeze-drying in silicone treated flasks.

Oxidations were also carried out with 1.0 and 4.0 moles of oxidant, respectively, per mole of sugar and the product formed worked up as described above. but only methyl β-glucoside and the 3-oxoglucoside were isolated (Table 1).

In one case, with 1.5 moles oxidant per mole of sugar, the strength of the acid used for the hydrolysis was unintentionally lower than usual. The water soluble material obtained from this "weak" hydrolysis and from a further hydrolysis of the chloroform extract using the normal conditions were both worked up separately on a carbon column in the usual way. The total amount of methyl β-glucoside was distributed as 64 and35% in the "weak" and "normal" hydrolysate, respectively, and the corresponding figures for methyl β-3-oxoglucoside were 10 and 90 %.

Characterisation of methyl β-oxoglucosides

Methyl-β-D-3-oxoglucopyranoside (VII) was identical with that previously isolated [1], but the physical data, m.p. 129.5—131°, $[\alpha]_D^{22}$ −62° (c = 2, water), recorded now are more accurate since more substance was available. After reduction of VII (1.00 g) with Raney nickel [3], hydrolysis with 0.5 N sulphuric acid for 16 h at 100° and neutralisation with the ion exchange resin Amberlite IR-4B, the sugars formed were separated on thick papers using solvent A (2 × 16 hours). After elution, evaporation and recrystallisation from ethanol, D-allose (0.32 g, m.p. and mixed m.p. 127°) was isolated.

A small amount of *methyl β-D-2-oxoglucopyranoside* (VI) was obtained in crystalline form from ethanol but the values given here, m.p. 120—121° and $[\alpha]_D^{22}$ −47° (c = 2, water) (Found: C 44.1; 6.43; OCH_3 16.9. Calc. for $C_7H_{12}O_6$ (192.2): C 43.7; H 6.26; OCH_3 16.1) were obtained when more substance became available from a procedure which will be described in a later publication.

Small amounts (about 40 mg) of VI and *methyl β-D-4-oxoglucopyranoside* (VIII), respectively, were reduced, hydrolysed and deionised as above and the sugars formed fractionated on thick filter paper in solvent C for two days. It was shown by paper chromatography and electrophoresis in solvents A—C and buffers D and E, that the only sugars formed were glucose and mannose (from VI) and glucose and galactose (from VIII). Glucose was characterised as the phenylosazone, m.p. 206—208° from VI and VIII, undepressed on admixture with authentic material, mannose as the phenylhydrazone, m.p. and mixed 195—196°, and galactose as the methylphenylhydrazone, m.p. and mixed m.p. 188—189°, in the usual way.

The Methyl β-D-6-oxoglucopyranoside was shown to be identical with that previously isolated [1] by paper chromatography and electrophoresis; it gave a positive reaction with Schiff's reagent and gave only glucose on reduction and subsequent hydrolysis as above.

The possible *methyl β-D-4,6-dioxoglucopyranoside* had the $R_{Glucose}$ values 2.92, 0.65 (B) and M_V value 2.25 (D, at 50°), gave a strong lemon yellow reaction with anisidine hydrogen chloride, a positive Schiff's reaction and consumed hypoiodite [5] (corresponding to a purity of 70 %). After reduction and subsequent hydrolysis, glucose, galactose and smaller amounts of arabinose were shown by paper chromatography.

Oxidation of methyl 6-*O*-trityl-α-D-glucopyranoside

Methyl 6-*O*-trityl-α-D-glucopyranoside (I) [7] was oxidised and the product worked up using the same amounts and conditions as described above for the corresponding β-compound. Data for the isolation and characteristics of the mono-oxoglucosides isolated are given in Table 2. The methyl α-glucoside recovered had m.p. 165—165.5° after recrystallisation from ethanol. A possible 4,6-dioxoglucoside corresponding to that in the β-series was also isolated (0.07 g).

Characterisation of methyl α-oxoglucosides

The same technique of reduction, hydrolysis, neutralisation and preparative isolation and characterisation of the sugars formed was used as in the β-series.

Methyl α-D-*2-oxoglucopyranoside* (II) gave glucose phenylosazone, m.p. and mixed m.p. 207—208°, and mannose phenylhydrazone, m.p. and mixed m.p. 196—198°.

Methyl α-D-*3-oxoglucopyranoside* (III) gave very little glucose, sufficient only for characterisation by paper chromatography and electrophoresis. The predominant D-allose was isolated, m.p. and mixed m.p. 126—127°. Amorphous III showed $[\alpha]_D^{22}$ +84° (c = 2, water).

Methyl α-D-*4-oxoglucopyranoside* (IV) gave glucose phenylosazone, m.p. and mixed m.p. 206—208°, and galactose methylphenylhydrazone, m.p. and mixed m.p. 186—187°.

The possible *methyl* α-D-*4,6-dioxoglucopyranoside* had the $R_{Glucose}$ values 2.64, 0.62 (B) and M_V values 2.06 (D, at 50°), gave a strong lemon yellow reaction with anisidine hydrogen chloride, a positive Schiff's reaction, consumed hypoiodite [5] (corresponding to a purity of 69%) and gave glucose, galactose and small amounts of arabinose on reduction and subsequent hydrolysis.

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