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A New Method of Degrading Glucose to Xylose and Galactose to Arabinose

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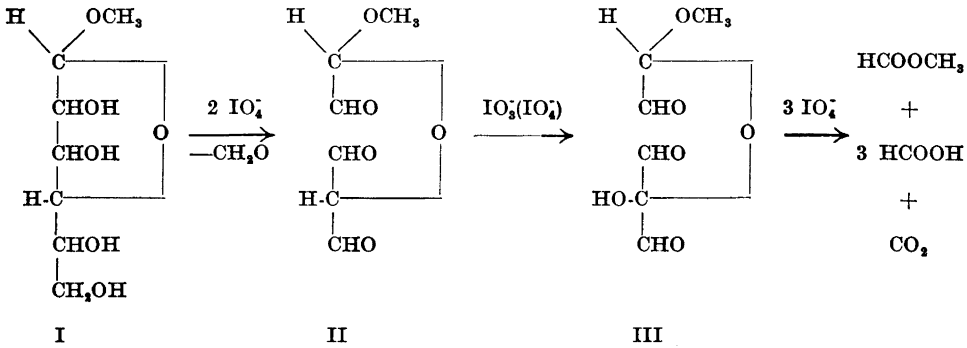
It has been found that by treatment with periodic acid of such aldohexofuranosides in which the hydroxyl groups at C_2 and C_3 are in *trans*-position, experimental conditions can be established so that the oxidation is restricted to the glycol grouping outside the ring. Formaldehyde is therefore formed, and besides this a monoaldehyde is obtained which by reduction gives an aldopentofuranoside. The method makes it possible to correlate the anomers of the aldohexofuranosides with those of the aldopentofuranosides.

Oxidation of methyl aldohexopyranosides and methyl aldopentofuranosides with periodic acid leads to structurally identical dialdehydes, the optical rotations of which make it possible to correlate the configuration at carbon 1 in the two types of methylosides [1,2]. A marked difference in the oxidation of the two types of methylosides is that the hexopyranosides give formic acid beside the dialdehyde.

On oxidizing a methyl aldohexofuranoside (I) with periodic acid under the same conditions, one would expect the formation of a trialdehyde (II) beside one mole of formaldehyde.

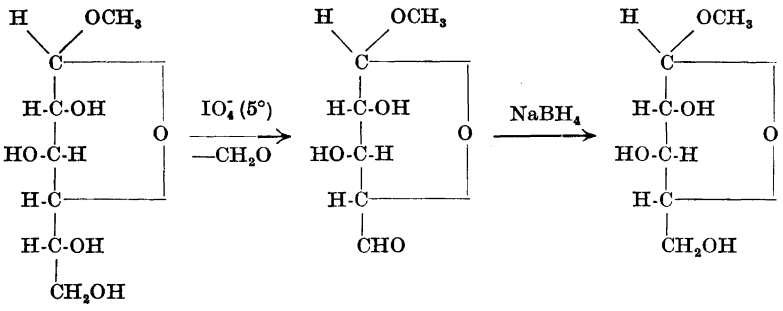


I II III

Experience has, however, shown that the oxidation goes further, and it has been suggested that this is due in the first place to the oxidation of the activated hydrogen atom at C_4 to a hydroxyl group (III). As vicinal hydroxyaldehydes also are easily oxidized by periodic acid [3], the result will be that the whole molecule is disintegrated. Accordingly Hirst *et al.*[4] found that α-methyl-D-mannofuranoside on oxidation gave formaldehyde, formic acid, methyl formate and carbon dioxide. It is therefore not possible to determine the configuration at carbon atom 1 in the aldohexofuranosides in the same way as with the other types of methylosides. On oxidation of α-methyl-D-mannofuranoside and β-ethyl-D-galactofuranoside with lead tetraacetate, Criegee [5] found that in the mannofuranoside the link between C_2 and C_3 was split up first, while in the galactofuranoside the link between C_5 and C_6 was cleaved more easily than that between C_2 and C_3. He meant that the reason for this was that with *cis*-configuration a ring-glycol is attacked more easily by lead tetraacetate than a glycol in which there is free rotation between the carbon atoms, and that the latter glycol is attacked easier than a ring-glycol with *trans*-configuration. Later Price and Knell [6] and Hudson [7] have found that the same is the case on oxidation with periodic acid.

In the present investigation these experiences have been verified. It was further found that starting with galacto- or glucofuranosides in which the configuration at C_2—C_3 is *trans*, the oxidation by lowering the temperature could be localized mainly to the C_5—C_6 position. The oxidation accordingly proceeded as shown in formulae IV—V and VII—VIII. When the temperature during the oxidation was kept at about + 5° each mole of furanoside on consuming one mole of periodic acid gave one mole formaldehyde. After removing iodate and periodate with lead dithionate as described by O'Dea and Gibbons [8], the formaldehyde was determined colorimetrically by means of chromotropic acid according to Eergiwe [9]. During the oxidation the rotation at first changed rapidly, but after one mole periodic acid had been consumed it became practically constant. The oxidation could therefore be followed in a polarimeter. At the appropriate time the oxidation was stopped by addition of barium hydroxide and the C_5-aldehyde isolated as a colourless syrup. Reduction of the aldehyde group would lead to methyl pentofuranoside as shown in the formulae VI and IX. This method therefore represents a degradation of glucose and galactose to xylose and arabinose, respectively.

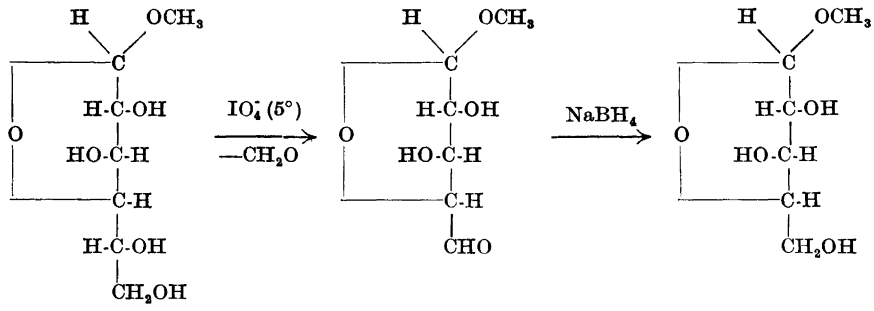
Sodium borohydride was used as a reducing agent. Experimentally it was found that the α- and β-methyl-D-glucofuranosides gave α- and β-methyl-D-xylofuranosides and that the α- and β-methyl-D-galactofuranosides gave β- and α-methyl-L-arabofuranosides, respectively. The four pentofuranosides were identified by means of paper chromatography. Xylose was also identified by preparing the dibenzylidene derivatives of the dimethyl acetal and comparing it with a sample prepared from authentic xylose. As the glucofuranosides used were slightly impure, the specific rotation of the xylofuranosides prepared from them would not give any proof of their identity and they were not subjected to a more detailed investigation. In the case of the galactofuranosides the specific rotation of the arabofuranosides obtained by degradation (Table 1) agree fairly well with those of the pure substances (—125° and + 118°). The β- and α-methyl-L-arabofuranosides were therefore oxidized



IV α-Methyl-D-glucofuranoside

V

VI α-Methyl-D-xylofuranoside



VII α-Methyl-D-galactofuranoside

VIII

IX β-Methyl-L-arabofuranoside

further with periodic acid, giving D'- and L'-methyl-L-hydroxymethyldiglycolaldehyde, respectively. After reduction of these the specific rotation of the enantiomeric diglycols formed were found (Table 1) to be in agreement with the values found before [2,10].

On hydrolyzing the diglycols with mineral acid, methanol, glycerol and glycolaldehyde were obtained. The glycolaldehyde was determined as 2,4-dinitrophenylhydrazone of the *O*-benzoate.

As will be seen this new method of degradation makes it possible to correlate the anomeric aldohexofuranosides to the anomers of the pentofuranosides.

EXPERIMENTAL

β-Methyl-D-galactofuranoside

To a solution of 0.7264 g (3.75 mmole) of the furanoside in water (20 ml) periodic acid (10 ml of 0.430 M solution) was added and the temperature kept at 5°. The optical rotation in a 10 cm tube which was −2.48° after 3 min changed to −2.10° after 25 min and then became constant. The concentration of the monoaldehyde was calculated as 2.00 g in 100 ml and the specific rotation accordingly −105.0°. After 30 min excess of

Table 1. Oxidation of methyl-D-galactofuranosides and methyl-D-glucofuranosides.

Substance	Meth.fur. $[\alpha]_D^{20}$	mole H_5IO_6 per mole furano-side	mole CH_2O per mole furano-side	mono-alde-hyde $[\alpha]_D^{5}$	pento-side $[\alpha]_D^{20}$	mole H_5IO_6 per mole furano-side	dialde-hyde $[\alpha]_D^{20}$	Diglycol $[\alpha]_D^{20}$
β-Meth.-D-galactofur.	−112°	1.06 1.06	1.05 1.02	−105.0° −107.8°	−116.1° −119.7°	1.01 1.01	−116.9° −118.3°	+10°
α-Meth.-D-galactofur.	+104°	1.08	0.99	+102.3°	+114.6°	1.05	+148.8°	−11.1°
β-Meth.-D-glucofur.	−60°	1.11	1.02	−78.5°	−80.3°	—	—	—
α-Meth.-D-glucofur.	+112.5°	1.04	—	+116.2°	+168.2°	—	—	—

periodic acid and the amount of formaldehyde were determined, and after 35 min further oxidation was prevented by adding a hot solution of barium hydroxide until pH 9.5.

Determination of periodic acid: The solution (2 ml) was diluted with water (10 ml) and sodium bicarbonate added until basic reaction. After adding 0.1 g potassium iodide and 5 ml 0.2016 N sodium arsenite the solution was left for 15 min and then titrated with 9.50 ml 0.1015 N KI_3. These data correspond to a consumption of 1.06 mole periodic acid per mole galactoside.

Determination of formaldehyde: The solution (0.3 ml) was neutralized with sodium bicarbonate and diluted with water to 100 ml. To 1 ml of this solution was added 1 ml lead dithionate (2 % in water). After centrifugating, 1 ml of the clear solution was diluted with 9 ml of a solution of 0.25 g chromotropic acid in 250 ml 60 % sulphuric acid. After 30 min the solution was centrifuged and then heated on steam-bath for 40 min. All operations were carried out in darkness. After cooling the absorption at 570 mμ, measured on a Beckmann DU spectrophotometer, was 0.345. As standards of 4.7, 7.1, and 11.8 μg formaldehyde in the same volum showed the absorptions 0.248, 0.413, and 0.680. The absorption 0.345 corresponds to 5.90 μg formaldehyde or 1.05 mole per mole galactofuranoside oxidized.

The isolation of the monoaldehyde was carried out in the same way as described for the dialdehyde by Jackson and Hudson [1]. The monoaldehyde was obtained as a syrup (0.424 g) which was dissolved in water (10 ml) and reduced in the course of one hour by adding 0.1 g sodium borohydride. Excess borohydride was destroyed by bubbling carbon dioxide through the solution until pH 7. After treatment with active carbon α_D in a 10 cm tube was −4.84° and as the concentration of the pentofuranoside was calculated as 4.17 g in 100 ml the specific rotation was −116.1°. In a parallel experiment 0.291 g of β-methyl-D-galactofuranoside gave a monoaldehyde and a pentofuranoside with the specific rotations −107.8° and −119.7°, respectively.

In order to isolate the reduction product, the solution was concentrated at reduced pressure (bath-temperature 35−40°) and the residue dried in a vacuum desiccator above calcium chloride. The residue was shaken with cold anhydrous ethanol (4 times with 10 ml and 3 times with 5 ml) and the ethanolic extract then concentrated *in vacuo*. The resulting syrup was dissolved in water (200 ml) and the solution passed successively through 100 g of Amberlite IR-45 (weak anion exchange type) and 100 g of Amberlite IR-120 (strong cation exchange type). On concentrating the eluate under reduced pressure a pale yellow syrup was obtained which was chromatographed on Whatman paper No. 1 using methyl ethyl ketone as moving phase and *m*-phenylenediamine hydrochloride

(0.6 g in 100 ml 70 % ethanol) as developer. The dominating spot had the same R_F-value as α-methyl-L-arabofuranoside while a smaller spot had the same R_F-value as arabinose.

Oxidation of the pentofuranoside: A solution of the syrupy pentofuranoside in water (10 ml) after treatment with active carbon had $\alpha_D^{20} = -3.34°$ in a 10 cm tube showing that it contained 0.270 g (1.65 mmole) α-methyl-L-arabofuranoside. The solution was mixed with 5 ml 0.430 M periodic acid (2.15 mmole). After 3 h at room temperature α_D^{20} became constant at $-2.12°$ and $[\alpha]_D^{20}$ accordingly $-116.9°$ ($c = 1.77$). In a second experiment was found $-118.3°$. An analysis carried out as described above showed that each mole furanoside had consumed 0.98 mole periodic acid. The dialdehyde was isolated as described for the monoaldehyde and was obtained as a slightly impure syrup (0.140 g) which after dissolving in water (10 ml) was reduced with 0.1 g sodium borohydride. The optical rotation in a 10 cm tube was now found to be $+0.15°$ and as the concentration of the diglycol was calculated as 1.41 g/100 ml the optical rotation was $+10.6°$.

The diglycol was benzoylated and after hydrolyzing the acetal group, the 2,4-dinitrophenylhydrazone of glycolaldehyde-*O*-benzoate was prepared. Recrystallized three times from chloroform-light petroleum, m. p. 187—188°; no depression on mixing with authentic sample. (Found: C 52.24; H 3.68; N 16.41. Calc. for $C_{15}H_{12}O_6N_4$: C 52.38; H 3.55; N 16.20.)

α-Methyl-D-galactofuranoside

The oxidation of this furanoside was performed in the same way as described for β-methyl-D-galactofuranoside. The optical rotation became practically constant in 30 min when $[\alpha]_D$ was $+102.3°$. Analysis showed that each mole furanoside consumed 1.08 mole periodic acid and led to the formation of 0.99 mole formaldehyde. Starting with 0.3188 g of α-methyl-D-galactofuranoside 0.143 g monoaldehyde was obtained which after dissolving in water was reduced by adding 50 mg sodium borohydride. Excess of borohydride was quenched with carbon dioxide and the solution treated with active carbon when it was found that the specific rotation of the pentofuranoside was $+114.6°$. The furanoside was isolated as described above. On a paper chromatogram the main spot had the same R_F-value as β-methyl-L-arabofuranoside. Also in this case a smaller spot with the same R_F-value as arabinose showed that some hydrolysis of the furanoside had taken place.

As a further control the furanoside (0.106 g) was oxidized with periodic acid. Per mole it consumed 1.05 mole periodic acid and the specific rotation of the dialdehyde formed was found to be $+148.8°$. Reduction of the dialdehyde with sodium borohydride led to a diglycol with the specific rotation $-11.1°$.

β-Methyl-D-glucofuranoside

To a solution of 0.3901 g (2.01 mmole) of the slightly impure furanoside (syrup) 10 ml 0.272 M periodic acid was added and the temperature kept at 5°. In 15 min the rotation became practically constant and the specific rotation of the monoaldehyde found to be $-78.5°$. Analysis showed that each mole of the glucofuranoside had consumed 1.11 mole of periodic acid and formed 1.03 mole formaldehyde.

The monoaldehyde (0.195 g) isolated in the usual way was reduced with 0.15 g sodium borohydride and the specific rotation of the reduction product determined to be $-80.3°$. The pentofuranoside was isolated as described for the arabofuranoside, but with the exception that in this case the solution was treated first with the cation exchanger and thereafter with the anion exchanger (Amberlite IR-4B). The result was that more of the furanoside was hydrolyzed and the paper chromatogram showed two spots of nearly equal strength, one with the same R_F-value as β-methyl-D-xylofuranoside and the other with that of xylose. A mixture of ethyl acetate-propanol-water (5:3:2) was used as moving phase.

The presence of xylose was verified in the following way. The pentofuranoside was hydrolyzed in 1 N hydrochloric acid by heating to 100° for 2 h. After removing the acid

by treatment with an ion exchange resin (IR-4B) the solution was concentrated *in vacuo* and the last trace of water removed in a vacuum desiccator above calcium chloride. The residue was dissolved in 4 ml anhydrous methanol containing 0.5 N hydrogen chloride, and 1 ml freshly distilled benzaldehyde was added. After 8 days the dibenzylidene derivative formed was filtered and washed thoroughly first with water and then with cold methanol. Recrystallized from ethyl acetate, m. p. 213.5—214°; no depression on mixing with the dibenzylidene dimethylacetal prepared from D-xylose. (Found: C 67.84; H 6.45; MeO 16.38. Calc. for $C_{21}H_{24}O_6$: C 67.73; H 6.50; MeO 16.65.)

α-Methyl-D-glucofuranoside

The crystalline furanoside, m. p. 61—62° (0.1576 g = 0.81 mmole) dissolved in water (5 ml) was oxidized by adding 3.5 ml 0.272 M periodic acid (0.95 mmole). At 5° the rotation became approximately constant in 20 min when the specific rotation of the monoaldehyde was found to be +116.2°. Analysis showed that each mole furanoside had consumed 1.04 mole periodic acid.

The monoaldehyde (87 mg isolated as above) was reduced with 50 mg sodium borohydride and the specific rotation of the reduction product was found to be +168.2°. Isolated in the usual way the reduction product gave on a paper chromatogram one main spot with the same R_F-value as α-methyl-D-xylofuranoside and a smaller spot showing the presence of xylose.

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