

## A Contribution to the Study of the Configuration of Anomeric Sugars

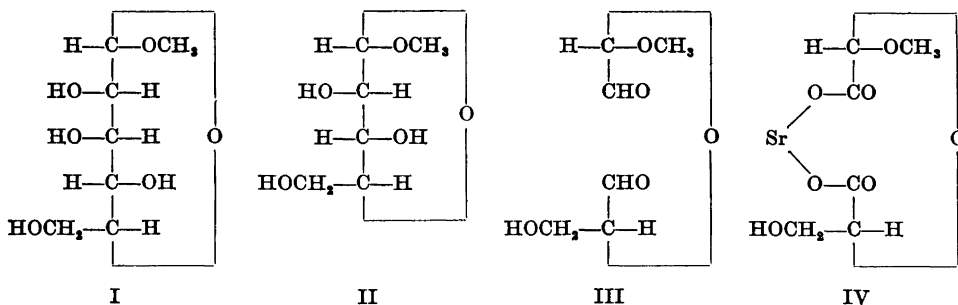
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The following methyl aldopentofuranosides have been oxidized by means of periodic acid:  $\alpha$ -D-arabofuranoside,  $\alpha$ - and  $\beta$ -L-arabofuranosides,  $\alpha$ -D-xylofuranoside, and  $\beta$ -D-ribofuranoside. The oxidation of an  $\alpha$ -D-pentofuranoside gave the same product as obtained by Jackson and Hudson on oxidation of an  $\alpha$ -D-hexopyranoside and corresponding results were arrived at when the  $\beta$ -D- as well as the  $\alpha$ -L- and the  $\beta$ -L-forms of the two ring-types of glycosides were oxidized. The correlation between the configuration at carbon atom 1 in the anomeric forms of these types of glycosides is thus established. Reduction of the primarily obtained oxidation products led to enantiomeric diglycols because the asymmetry at carbon atom 4 in this way was eliminated. On discussing the configuration the importance of the use of X-ray methods for determination of the absolute configuration of the anomeric forms is underlined. It is further emphasized that a rational nomenclature for anomeric sugars and their derivatives ought to be worked out and eventually accepted by international agreement.

Jackson and Hudson<sup>1</sup> introduced the method of periodate oxidation in determining the ring structure and alpha-beta configuration of methyl glycosides. By the oxidation of an  $\alpha$ -methyl-D-aldohexopyranoside (I) two moles of periodic acid were consumed resulting in the formation of one mole formic acid and a substance III designated D'-methoxy-D-hydroxymethyl-diglycolaldehyde and having the specific rotation  $+121^\circ$ . It was found that all the four  $\alpha$ -D-aldohexopyranosides which were investigated gave the same diglycolaldehyde with rotation  $+121^\circ$ . The dialdehyde could be oxidized to a dibasic acid with the rotation  $+26^\circ$  which was transformed into a strontium salt (IV) with rotation  $-53^\circ$ . As the configuration at carbon atoms 1 and 5 of the glycoside were retained during these processes, it could be inferred that the configuration also at carbon atom 1 must be the same in all  $\alpha$ -D-aldohexopyranosides.

As could be expected the  $\beta$ -methyl-D-aldohexopyranosides gave a dialdehyde different from that obtained from the anomeric  $\alpha$ -forms. On the other



hand Jackson and Hudson found that periodate oxidation of the anomeric forms of methyl aldopentopyranosides led to enantiomeric dialdehydes, a result which is in accordance with the fact that carbon atom 5 in this case is not asymmetric. The mentioned authors also showed that  $\alpha$ -methyl-D-arabofuranoside (II) gave an oxidation product identical with that obtained from the  $\alpha$ -methyl-D-aldohexopyranosides and that therefore the configuration at carbon atom 1 in this furanoside must be the same as in the  $\alpha$ -D-hexopyranosides. We have found that this holds also for other aldopentofuranosides as will be described below.

Smith and co-workers<sup>2</sup> showed that the dialdehydes could be reduced to diglycols either by hydrogenation in the presence of Raney nickel catalyst or by the action of sodium borohydride in aqueous solution. Because the asymmetry of carbon atom 5 in this way is eliminated, enantiomeric diglycols are obtained and consequently also in the case of the aldohexopyranosides the anomers of the D-series can be correlated to those of the L-series.

On the whole the application of the periodate oxidation method to the glycosides has demonstrated a definite correlation between anomers within the series of the aldohexopyranosides, the aldopentopyranosides, and the aldopentofuranosides, and also correlated the anomers of the aldopentofuranosides with those of the aldohexopyranosides. It has, however, not established a correlation between anomers of the aldohexopyranosides and those of the aldopentopyranosides and it does not, of course, give any proof of the absolute configuration at carbon atom 1.

The question of the configuration at carbon atom 1 is of considerable interest and has been the object of many papers. Especially interesting are those of Sørensen and Trumpy<sup>3</sup> and of Riiber and Sørensen<sup>4</sup>. The tentative configuration at C<sub>1</sub> which has in fact been generally accepted as correct is based mainly upon the application of Hudson's rules of isorotation and the displacement rules of Freudenberg and Kuhn, but partly also upon some chemical evidences such as the electric conductivity of glucose solutions to which boric acid has been added, the molecular refraction and the rate of hydrolysis of anomeric glycosides as compared with those of *cis-trans* isomeric diols investigated by Vavon, and further the predominant occurrence of certain glycosides by the addition of methanol to 1,2-oxides of sugars. For sugars belonging to the glucose and galactose series the application of these methods are mostly in agreement with the tentatively accepted configuration. On the other hand,

as especially shown by Riiber <sup>5</sup>, molecular refractions and rates of hydrolysis in the case of sugars belonging to the mannose series (mannose, rhamnose, and lyxose) lead to an opposite configuration of that obtained by means of the rules of isorotation and displacement. This anomaly in the mannose series has not yet found an explanation. It would, however, seem reasonable to believe that it has bearing on the various conformations in which the pyranoses and pyranosides can occur. It has been shown in this institute <sup>6</sup> that in the case of the furanosides in which the molecules are supposed to be more or less plane no anomaly is found in the rates of hydrolysis of those of mannose as compared with those of sugars belonging to the glucose and galactose series.

Even if the periodate oxidation method and the other physical and chemical methods mentioned above would indicate a certain configuration at carbon atom 1 in the anomeric sugars and their derivatives this would not represent a strict proof of the configuration. It is therefore of considerable importance that the application of X-ray methods during the later years seems to prove conclusively the correctness of the tentatively accepted configuration at carbon atom 1. Thus Furberg <sup>7</sup> in 1950 showed that the  $\beta$ -D-ribofuranoside which is built into the cytidine molecule has *trans*-position of the groups bound to carbon atoms 1 and 2. The same author <sup>8</sup> has later shown that  $\beta$ -D-arabopyranose contains the hydroxyl groups at C<sub>1</sub> and C<sub>2</sub> in *cis*-position. (It should be remarked that the nomenclature used is based upon the application of the rules of isorotation and displacement mentioned above.) McDonald and Beevers <sup>9</sup> established the *cis*-position of the hydroxyl groups at C<sub>1</sub> and C<sub>2</sub> in  $\alpha$ -D-glucopyranose and McGeachin and Beevers <sup>10</sup> the *trans*-position of the same groups in  $\alpha$ -L-rhamnopyranose monohydrate.

Although the absolute configuration of the anomeric forms of the sugars and their derivatives can be established by means of X-ray investigations the question of a rational nomenclature is still open. As already emphasized by Riiber and Sørensen <sup>4</sup> it must be hoped that this will be taken up and settled by international agreement.

By means of column chromatography several crystalline methyl aldofuranosides have been prepared in this institute <sup>6</sup>, amongst them the one used by Jackson and Hudson:  $\alpha$ -methyl-D-arabofuranoside. Our sample of the latter which had the specific rotation  $+128^\circ$  on oxidation with periodate gave a dialdehyde with rotation  $+120^\circ$  in good agreement with the values  $120-121^\circ$  obtained by the oxidation of the  $\alpha$ -methyl-D-aldohexopyranosides. Jackson and Hudson starting from a slightly impure arabofuranoside (rotation  $+123^\circ$ ) found the rotation of the dialdehyde to be  $+117.3^\circ$ . The other aldopentofuranosides which we have oxidized are the  $\alpha$ - and  $\beta$ -methyl-L-arabofuranosides, the  $\alpha$ -methyl-D-xylofuranoside, and the  $\beta$ -methyl-D-ribofuranoside. (The results of the oxidation of some aldohexofuranosides will be given in another paper.) The present results are collected in Table 1 from which it will be seen that the configuration at carbon atom 1 is the same in the  $\alpha$ -D- and  $\beta$ -D-forms, respectively, as in the corresponding forms of the D-aldohexopyranosides and that there accordingly is the same relationship in the case of the L-methylsides.

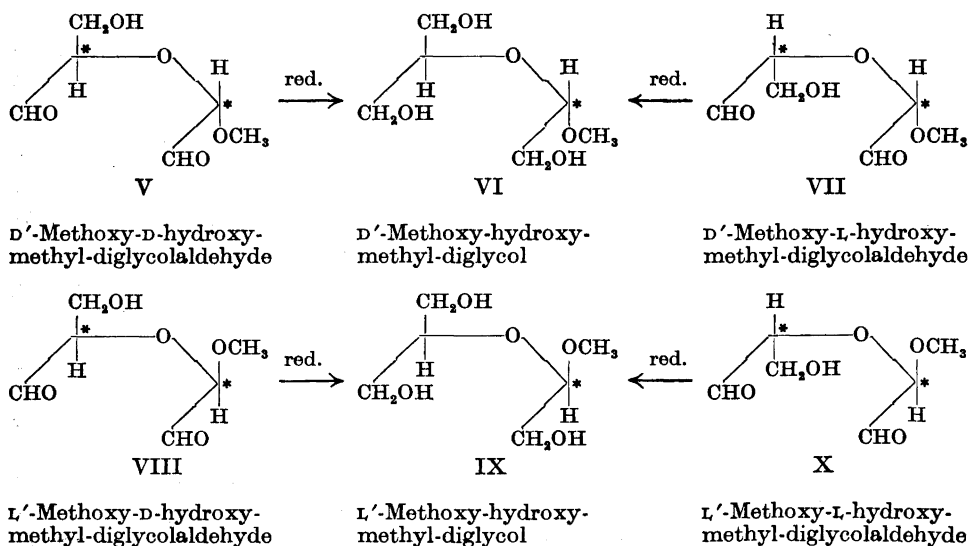
Owing to the small quantities of furanosides at our disposal only the dialdehyde from  $\alpha$ -methyl-D-xylofuranoside was oxidized further. This dialdehyde, according to Jackson and Hudson called D'-methoxy-D-hydroxymethyl-

Table 1. Oxidation of some methyl-aldopentofuranosides.

Substance	Methyl-furanoside [ $\alpha$ ] <sub>D</sub> <sup>20</sup>	Dialdehyde [ $\alpha$ ] <sub>D</sub> <sup>20</sup>	Sr-salt of dibasic acid [ $\alpha$ ] <sub>D</sub> <sup>20</sup>	Diglycol [ $\alpha$ ] <sub>D</sub> <sup>20</sup>
$\alpha$ -Methyl-D-arabofuranoside	+128°	+120.1°		-13.3°
$\alpha$ -Methyl-L- »	-128°	-121.2°		+12.5°
$\beta$ -Methyl-L- »	+118°	+150.6°		-11.1°
$\alpha$ -Methyl-D-xylofuranoside	+182°	+119.7°	-53.3°	-10.5°
$\beta$ -Methyl-D-ribofuranoside	-49.8°	-152.5°		+15.0°

diglycolaldehyde, on oxidation with strontium hypobromite gave the crystalline strontium salt of D'-methoxy-D-hydroxymethyl-diglycolic acid. The composition of the salt was controlled by analysis and it had a specific rotation of  $-53.3^\circ$  in good agreement with that of the strontium salt obtained from the  $\alpha$ -D-aldohexopyranosides.

As will be seen from the formulae below reduction of the dialdehydes should lead to enantiomeric diglycols because the asymmetry at carbon atom 4 is eliminated. The specific rotations of the diglycols given in Table 1 show that within the limit of the experimental error, unavoidable by the small quantities used, this is the case. Sodium borohydride was found to be most suitable for the reduction of small quantities of the dialdehydes. The process was carried out in aqueous solution at room temperature and took place very rapidly. The reduction product, a methoxy-hydroxymethyl diglycol, was easily hydrolyzed in mineral acid to glycerol, glycolaldehyde, and methanol. After removing the methanol by evaporation, glycerol was identified as the tris-O-p-nitrobenzoate, m.p. 197–98°. From the hydrolysate the glycolaldehyde could not be isolated as a hydrazone as it would directly give the osazone. We therefore proceeded in this way that the trihydric alcohols (VI, IX) were



transformed into the tris-O-benzoates in which the acetal linkings could be opened by treatment with N hydrochloric acid in acetic acid without removing the benzoyl groups. On addition of 2,4-dinitrophenylhydrazine the crystalline hydrazone of glycolaldehyde-O-benzoate separated which was identified by analysis and comparison with an authentic specimen. Keeping the hydrazone in contact with the hydrazine solution for some time resulted in the formation of the osazone of glyoxal.

## EXPERIMENTAL

### $\alpha$ -Methyl-D-xylofuranoside

*Oxidation with periodic acid.* To a solution of 0.1649 g (1.01 mmole) of the furanoside in 2.1 ml water 6.6 ml 0.3524 M  $H_5IO_6$  (2.32 mmole) were added. After 100 min the optical rotation became constant  $\alpha_D^{20} = +2.18^\circ$  in a 10 cm tube. The concentration of the dialdehyde was calculated to be 1.83 % and the specific rotation accordingly  $+119.7^\circ$ . The consumption of periodic acid was determined in samples of 1 ml solution. After dilution with water (10 ml) sodium bicarbonate was added to basic reaction and then 5 ml 0.2016 N sodium arsenite and 0.1 g potassium iodide. After 15 min the solution was retitrated with 7.30 ml 0.1011 N  $KI_3$ . This corresponds to 1.10 mole  $H_5IO_6$  per mole xyloside. The dialdehyde was isolated as described by Jackson and Hudson.

*Oxidation of the dialdehyde.* A quantity of 0.35 g dialdehyde collected from several experiments was oxidized with 1 ml bromine in the presence of 6 g strontium carbonate. The crystalline strontium salt obtained was recrystallized three times by dissolving in hot water and adding hot ethanol until precipitation began. Yield 0.3 g strontium D'-methoxy-D-hydroxymethyl-diglycolate dihydrate. After drying in a vacuum until constant weight the rotation was  $[\alpha]_D^{20} = -53.3^\circ$  (c 2.5 w). The dihydrate was used for analysis. (Found: C 22.54; H 3.67. Calc. for  $C_6H_5O_7Sr \cdot 2H_2O$ : C 22.81; H 3.80; Sr 27.76.) For determination of Sr 0.0836 g strontium diglycolate was dissolved in water till 50 ml. To parallels of 10 ml were added 2 ml conc. ammonia, 5 drops 0.1 % phthalein purpur, 10 ml 0.00955 M EDTA and 10 ml ethanol. Retitrated until light blue colour with 4.20 and 4.23 ml 0.00199 N strontium nitrate. Found 28.06, 27.90 % Sr.

*Reduction of the dialdehyde.* To 0.103 g of the dialdehyde dissolved in 10 ml water 0.1 g sodium borohydride was added in the course of one hour. After standing for another hour excess of borohydride was destroyed by bubbling carbon dioxide through the solution until pH 7. The solution was then treated with active carbon after which the rotation in a 10 cm tube was  $\alpha_D^{20} = -0.11^\circ$ . As the concentration was calculated to be 1.05 % diglycol the specific rotation was  $-10.5^\circ$ . In order to isolate the reduction product the solution was evaporated at reduced pressure (bath  $35^\circ$ ) and the last trace of water removed in a vacuum desiccator above calcium chloride. The residue was extracted three times with 20 and three times with 10 ml cold anhydrous ethanol. After treatment with active carbon the ethanolic solution was concentrated *in vacuo* (bath  $30^\circ$ ) giving about 0.07 g of a not quite pure syrup.

*Determination of the glycolaldehyde.* The syrup (0.07 g) was dissolved in 5 ml pyridine, benzoyl chloride (0.05 ml) added and the solution heated on the steam bath 40 min. After cooling the solution was poured on 15 ml ice-water saturated with sodium bicarbonate and then extracted three times with chloroform. The chloroform solution was washed thoroughly with water, dried with calcium chloride and the chloroform evaporated *in vacuo*. In order to remove the pyridine the temperature of the bath was in the end raised to  $50-60^\circ$ . The resulting syrup did not crystallize. Dissolved in 5.5 ml glacial acetic acid the rotation in a 10 cm tube was  $-0.16^\circ$ . On addition of 0.5 ml conc. hydrochloric acid the optical rotation disappeared in the course of 10 min. After 20 min 2,4-dinitrophenylhydrazine (0.05 g) was added and dissolved easily on stirring. Shortly afterwards a crystalline orange-coloured substance separated which after washing with ethanol was

recrystallized by adding light petroleum (60–70°) to a warm solution of the hydrazone in chloroform. M. p. 187–88° and no depression on mixing with 2,4-dinitrophenylhydrazone of glycolaldehyde-O-benzoate prepared from erythritol<sup>11</sup>. (Found: C 52.46; H 3.55; N 16.07. Calc. for  $C_{15}H_{13}O_6N_4$ : C 52.32; H 3.51; N 16.27.)

If the hydrazone were left in the mother liquor for some days (or heated with it) the colour changed to dark red indicating that the hydrazone had been transformed into the osazone. Recrystallized from nitrobenzene, m. p. 310–315° (d). (An osazone prepared from glyoxal had the same appearance and melting point.) (Found: C 40.06; H 2.50; N 26.46. Calc. for  $C_{14}H_{10}O_8N_4$ : C 40.20; H 2.41; N 26.79.)

### $\alpha$ -Methyl-D-arabofuranoside

To a solution of 0.2636 g (1.61 mmole) furanoside 5 ml 0.430 M  $H_5IO_6$  (2.15 mmole) was added. After 100 min the optical rotation became constant,  $\alpha_D^{20} = +1.93^\circ$  in a 10 cm tube. As the concentration of dialdehyde was calculated to be 1.61 % the specific rotation was  $+120.1^\circ$ . Analysis carried out as described for the xylofuranoside showed that 1.01 mole periodic acid had been consumed per mole arabofuranoside. The dialdehyde isolated (0.12 g) was dissolved in 10 ml water and reduced with 0.1 g sodium borohydride. After reduction the rotation of the solution was  $\alpha_D^{20} = -0.16^\circ$  and as the concentration of diglycol was calculated to be 1.2 % the specific rotation was  $-13.3^\circ$ . The 2,4-dinitrophenylhydrazone of glycolaldehyde-O-benzoate prepared in the same way as described above had m. p. 184–86°.

### $\alpha$ -Methyl-L-arabofuranoside

$\alpha$ -L-Arabofuranoside: 0.1607 g (0.98 mmole) in 7 ml water; added 3.0 ml 0.430 M  $H_5IO_6$  (1.29 mmole). After 90 min  $\alpha_D^{20} = -1.90^\circ$ . Concentration of dialdehyde calculated to 1.57 % and the specific rotation accordingly  $-121.2^\circ$ . Analysis showed that 1 mole furanoside had consumed 0.97 mole periodic acid. The dialdehyde (0.088 g) reduced as above gave a solution with  $\alpha_D^{20} = +0.11^\circ$ . As the concentration of diglycol was 0.88 % the specific rotation was  $+12.5^\circ$ . The 2,4-dinitrophenylhydrazone of glycolaldehyde-O-benzoate had m. p. 185–87°.

### $\beta$ -Methyl-L-arabofuranoside

$\beta$ -L-Arabofuranoside: 0.1219 g (0.74 mmole) in 4.8 ml water; added 2.2 ml 0.408 M  $H_5IO_6$  (0.898 mmole). After 180 min rotation constant  $\alpha_D^{20} = +2.56^\circ$ . Concentration of dialdehyde 1.70 % and specific rotation accordingly  $+150.6^\circ$ . Analysis showed that 1 mole furanoside had consumed 1.04 mole periodic acid. Two experiments gave in all 0.072 g dialdehyde which dissolved in 8 ml water was reduced with 0.05 g sodium borohydride in the course of 10 min. The solution which contained 0.90 % diglycol had  $\alpha_D^{20} = -0.10^\circ$  and the specific rotation was accordingly  $-11.1^\circ$ . The quantity of the hydrazone of glycolaldehyde-O-benzoate was in this case too small to be recrystallized. It had m. p. 179–181°.

### $\beta$ -Methyl-D-ribofuranoside

$\beta$ -D-Ribofuranoside: 0.1307 g (0.80 mmole) in 5 ml water; added 3 ml 0.430 M  $H_5IO_6$  (1.29 mmole). After 20 min the rotation became constant  $\alpha_D^{20} = -2.44^\circ$ . Concentration of dialdehyde 1.60 % and specific rotation  $-152.5^\circ$ . One mole of ribofuranoside consumed 1.02 mole periodic acid. The dialdehyde from two experiments (0.16 g) was dissolved in 10 ml water and reduced in 90 min with 0.1 g borohydride. The solution containing 1.60 % of diglycol had  $\alpha_D^{20} = +0.24^\circ$  and the specific rotation  $+15.0^\circ$ . The diglycol isolated (0.12 g) was transformed into a 2,4-dinitrophenylhydrazone with m. p. 187–88°.

## REFERENCES

1. Jackson, E. L. and Hudson, C. S. *J. Am. Chem. Soc.* **59** (1937) 994.
2. Abdel-Akher, M., Cadotte, J. E., Lewis, Bertha, Montgomery, R., Smith, F. and Van Cleve, J. W. *Nature* **171** (1953) 474.
3. Sørensen, N. A. and Trumpy, B. *Kgl. Norske Videnskab. Selskabs Skrifter* **1934** No 6.
4. Riiber, C. N. and Sørensen, N. A. *Kgl. Norske Videnskab. Selskabs Skrifter* **1938** No 1.
5. Riiber, C. N. and Minsaas, J. *Ber.* **60** (1927) 2402; Riiber, C. N. *Kgl. Norske Videnskab. Selskabs Forh. IV* (1931) 157.
6. Augestad, I. and Berner, E. *Acta Chem. Scand.* **8** (1954) 251; **10** (1956) 911.
7. Furberg, S. *Acta Cryst.* **3** (1950) 325.
8. Furberg, S. and Hordvik, A. *Acta Chem. Scand.* **11** (1957) 1594.
9. McDonald, T. R. R. and Beevers, C. A. *Acta Cryst.* **5** (1952) 654.
10. McGeachin, H. Mc. and Beevers, C. A. *Acta Cryst.* **10** (1957) 227.
11. Ohle, H. and Melkonian, G. A. *Ber.* **74** (1941) 291.

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