

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Structure of Corn Hull Hemicellulose. Part V. Partial Hydrolysis and Identification of 4-*O*- β -D-Xylopyranosyl-D-xylose and 5-*O*- β -D-Galactopyranosyl-L-arabofuranose^{1,2}

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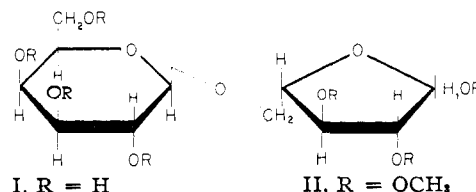
Partial hydrolysis of corn hull hemicellulose gives in addition to arabinose, xylose, galactose and 2-*O*-[α -D-glucopyranosiduronic acid]-D-xylose, a number of neutral oligosaccharides. Two of these oligosaccharides have been shown in a previous communication of this series, to be 3-*O*- α -D-xylopyranosyl-L-arabinose and 4-*O*- β -D-galactopyranosyl-D-xylose. Of the remaining oligosaccharides, two are shown herein to be 4-*O*- β -D-xylopyranosyl-D-xylose and 5-*O*- β -D-galactopyranosyl-L-arabofuranose.

Hydrolysis of corn hull hemicellulose³ with 0.01 *N* hydrochloric acid has been shown previously⁴ to give xylose, arabinose, galactose, 2-*O*-[α -D-glucopyranosiduronic acid]-D-xylose^{5,6} together with a number of neutral oligosaccharides. Two of the neutral oligosaccharides were shown to be 3-*O*- α -D-xylopyranosyl-L-arabinose and 4-*O*- β -D-galactopyranosyl- β -D-xylose.⁴ This paper is concerned with the isolation and identification of two more disaccharides, namely, 4-*O*- β -D-xylopyranosyl-D-xylose (xylobiose) and 5-*O*- β -D-galactopyranosyl-L-arabofuranose (I, R = H).

The identification of 4-*O*- β -D-xylopyranosyl-D-xylose is based upon the following experimental evidence. Upon acid hydrolysis, it gave only xylose. It had m.p. 184–185° and showed $[\alpha]_D -30^\circ$ changing to -23° (water), constants which are in agreement with the values reported⁷ for a specimen of 4-*O*- β -D-xylopyranosyl-D-xylose obtained from corncob holocellulose. The xylobiose was also recognized as its phenylosazone which had m.p. 205° and $[\alpha]_D -50^\circ$ (pyridine:ethanol).

The structure of the hitherto unknown disaccharide, 5-*O*- β -D-galactopyranosyl-L-arabofuranose (I, R = H) rests on the following facts. The disaccharide showed $[\alpha]_D -13^\circ$ (equilibrium value in water) and gave upon acid hydrolysis galactose and arabinose as revealed by paper chromatography. Treatment of I (R = H) with phenylhydrazine afforded a phenylosazone which upon hydrolysis gave a reducing sugar which was chromatographically identical with galactose. This established that the arabinose residue constituted the reducing end of the disaccharide and also that C₂ of the arabinose moiety was not involved in the biose linkage. Methylation of I (R = H) first with methyl sulfate and alkali and then with methyl iodide and silver oxide afforded the fully methylated derivative (II, R = OCH₃), $[\alpha]_D -36^\circ$ in methanol which gave upon hydrolysis a mixture ($[\alpha]_D +38^\circ$ in methanol) of approximately equal

amounts of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose. The former was identified as *N*-phenyl-D-galactopyranosylamine 2,3,4,6-tetramethyl ether⁸ by melting point, specific rotation and by comparison with an authentic specimen while the latter was converted to the characteristic 2,3-di-*O*-methyl-L-arabinose 1,4-bis-*p*-nitrobenzoate.



From the above results it may be concluded that the disaccharide is either 5-*O*-D-galactopyranosyl-L-arabofuranose (I, R = H) or 4-*O*-D-galactopyranosyl-L-arabopyranose. A decision between these two structures may be reached by applying the rules of isorotation.⁹

The contribution of the L-arabopyranose residue will be highly positive and approximately equal to the equilibrium value (+104°) observed for L-arabinose, whereas that of the L-arabofuranose residue will be about -40° on the assumption that it will be approximately the same as that of 2,3,5-tri-*O*-methyl-L-arabofuranose.¹⁰ Inspection of the glycosides of D-galactopyranose shows that the contribution of those of the α -type would be about $+180^\circ$ while those of the β -type would be approximately 0° . Providing that intermolecular interaction has little effect on the contribution of each residue of a disaccharide, it is possible to make the following approximations. A combination of the L-arabopyranose residue with an equilibrium rotation of $+104^\circ$ with the α -D-galactopyranosyl residue having $[\alpha]_D +180^\circ$ will result in a disaccharide with a high positive rotation ($+120^\circ$ to $+150^\circ$). The combination of the L-arabopyranose residue ($+104^\circ$) with a β -D-galactopyranosyl residue ($+0^\circ$) would produce a value for the disaccharide of about $+50^\circ$. The third possible combination consisting of the L-arabofuranose residue (-40°) with the α -D-galactopyranosyl residue ($+180^\circ$) would result in a value of about $+70^\circ$ while the fourth and last possible combination, consisting of the L-arabofuranose residue (-40°)

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(2) This research was done under contract with the United States Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service.

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and the β -D-galactopyranosyl residue ($+0^\circ$), would produce a disaccharide with a rotation of about -20° . This last value is approximately the same as that (-13°) observed for the new D-galactosyl-L-arabinose disaccharide (I, R = H), and it is tentatively concluded that I (R = H) is a β -D-galactopyranosyl-L-arabofuranose. The isolation of 2,3-di-*O*-methyl-L-arabinose from the fully methylated disaccharide proves that the biose linkage must engage either C₄ or C₅ of the arabinose residue, but only a 1,5-linkage would permit a disaccharide structure with an arabofuranose residue. The disaccharide (I, R = H) is therefore designated 5-*O*- β -D-galactopyranosyl-L-arabofuranose.

The previous isolation of 4-*O*- β -D-galactopyranosyl-D-xylose,⁴ *O*-L-galactopyranosyl-(1 \rightarrow 4)-*O*-D-xylopyranosyl-(1 \rightarrow 2)-L-arabinose¹¹ and 3-*O*- α -D-xylopyranosyl-L-arabinose^{4,11} together with 4-*O*- β -D-xylopyranosyl-D-xylose and 5-*O*- β -D-galactopyranosyl-L-arabinose of the present paper, brings to five the number of neutral oligosaccharides thus far isolated and characterized from the hydrolysis of corn hull hemicellulose.

Since the available information from methylation studies on the corn hull hemicellulose reveals that the galactose residues give rise only to 2,3,4,6-tetra-*O*-methyl-D- and -L-galactose,¹²⁻¹⁴ it appears that all the galactose residues occupy terminal positions in the highly branched chain complex. The results from the study of the structure of the oligosaccharides containing galactose, produced by graded hydrolysis of the hemicellulose, show that the terminal non-reducing units of galactose are linked to the complex in at least three different ways.

Experimental

The following solvents were used for the partition chromatography of sugars and their derivatives: (A) pyridine:ethyl acetate:water (1:2.5:3.5—upper layer)¹⁵; (B) 1-propanol:water azeotrope¹⁶; (C) 2-butanone:water azeotrope¹⁷; and (D) benzene:ethanol:water (200:47:15—upper layer).¹⁸ *p*-Anisidine trichloroacetate¹⁹ was used as a spray reagent for the detection of sugars and their derivatives. R_x and R_G values represent the rate of movement on the paper relative to xylose and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, respectively. All evaporations were carried out *in vacuo* at 30–40°.

Isolation and Identification of 4-*O*- β -D-Xylopyranosyl-D-xylose.—It was observed that when the mixture of disaccharides, obtained by the fractionation of the corn hull hemicellulose hydrolyzate on a charcoal: Celite column²⁰ as previously described,⁴ was resolved on a cellulose column²¹ using solvent B, 3-*O*- α -D-xylopyranosyl-L-arabinose was

followed by a mixture of the same disaccharide and 5-*O*- β -D-galactopyranosyl-L-arabinose (I) and then by a third component having R_x 0.47, a value that is very close to that of 3-*O*- α -D-xylopyranosyl-L-arabinose (R_x 0.49). It was noted, however, that the rotation ($[\alpha]_D -7^\circ$ in water) of this crude component (R_x 0.47) was different from that of 3-*O*- α -D-xylopyranosyl-L-arabinose ($+175^\circ$). This new component shown herein to be 4-*O*- β -D-xylopyranosyl-D-xylose, was purified by rechromatography on a cellulose column using solvent B followed by recrystallization from ethanol. This crystalline disaccharide had m.p. 184–185° and $[\alpha]_D^{25} -30^\circ$ changing in 5 hr. to -23° (equil. value in water (*c* 2)); lit. values: m.p. 185–186°, $[\alpha]_D^{25} -32^\circ \rightarrow -25.5^\circ$. A solution of the xylobiose (10 mg.) in 0.1 *N* sulfuric acid (2 ml.) was heated (sealed tube) for 5 hr. in a boiling water-bath. The solution was neutralized (BaCO₃), evaporated to a sirup and found by paper chromatography (solvent A) to contain only xylose.

To a solution of xylobiose (62 mg.) in water (1.5 ml.) were added phenylhydrazine (0.175 ml.), 20% acetic acid (1.0 ml.) and sodium bisulfite (10 mg.). The solution was heated for 2 hr. at 100° (bath temp.). The crystals of xylobiose phenylosazone hemihydrate which separated were filtered, washed with water and recrystallized from 60% ethanol (yield 41 mg.), m.p. 205–207° dec. and $[\alpha]_D^{25} -6^\circ$ (5 min.) changing to -50° (26 hr., constant value) (*c* 0.7) in pyridine:ethanol (7:3); lit. value for the monohydrate,⁷ m.p. 195–196° dec., $[\alpha]_D^{25} -22.5^\circ \rightarrow -77^\circ$ (33 hr.). *Anal.* Calcd. for C₂₂H₃₈O₇N₄·1/2H₂O: C, 56.3; H, 6.2; N, 11.9. Found: C, 56.2; H, 6.8; N, 11.7.

Identification of 5-*O*- β -D-Galactopyranosyl-L-arabofuranose.—This disaccharide (I, R_x 0.33, 50 mg.), obtained in a pure state by repeated chromatography on a column of cellulose, had $[\alpha]_D^{25} -13^\circ$ in water (*c* 1); the material did not crystallize. A solution of I (10 mg.) in 0.5 *N* H₂SO₄ (2 ml.) was heated (sealed tube) for 5 hr. in a boiling water-bath. The hydrolyzate was neutralized (BaCO₃), concentrated to a sirup and chromatographed using solvent A; two components corresponding to galactose and arabinose were detected.

The sugar (5 mg.) was dissolved in water (0.15 ml.) and after the addition of phenylhydrazine (0.015 ml.), 20% acetic acid (0.1 ml.) and sodium bisulfite (5 mg.), the solution was heated for 2 hr. at 70–80° (bath temp.). The light yellow crystalline osazone which separated on cooling was centrifuged, washed once with water and directly subjected to hydrolysis in the following manner. The phenylosazone was dissolved in ethanol (0.5 ml.) and after the addition of 0.5 *N* sulfuric acid (2 ml.), the solution was heated for 3 hr. at 100° (bath temp.). The reaction mixture was neutralized (BaCO₃), filtered and the solution concentrated to a sirup. Paper chromatographic analysis of the hydrolyzate (solvent A) showed the presence of galactose but no arabinose.

Methylation of 5-*O*- β -D-Galactopyranosyl-L-arabofuranose.—The sugar (39 mg.) was dissolved in water (3 ml.) and the solution placed in a 250-ml. centrifuge bottle which was flushed with a current of nitrogen. The solution was cooled to 0° and then 40% potassium hydroxide (3 ml.) and methyl sulfate (1 ml.) were added dropwise in this order with vigorous stirring. In this manner, 21 ml. of potassium hydroxide and 7 ml. of methyl sulfate were added over a period of 2 hr. After the mixture had attained room temperature (25°), it was further treated with methyl sulfate (3 ml.) and potassium hydroxide (10 ml., 40%) as previously described. After stirring for another 1.5 hr., the reaction mixture was heated at 90–100° for 1 hr., cooled, diluted with water (10 ml.) and after stirring with chloroform (100 ml.), it was filtered and the residue washed with chloroform. The chloroform solution was washed twice with a saturated solution of sodium sulfate, dried (Na₂SO₄) and evaporated to give a colorless sirup (50 mg.).

The methylated disaccharide thus obtained was dissolved in methyl iodide (10 ml.), and after the addition of silver oxide (3 g.) the mixture was refluxed for 10 hr. The product, isolated in the usual manner, was remethylated as before to give methyl 5-*O*-(2,3,4,6-tetra-*O*-methyl- β -D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabofuranoside (II), a light yellow sirup (34 mg.), $[\alpha]_D^{25} -36^\circ$ in methanol (*c* 0.6).

Hydrolysis of Methyl 5-*O*-(2,3,4,6-Tetra-*O*-methyl-D-galactopyranosyl)-2,3-di-*O*-methyl-L-arabofuranoside (II).—A solution of the methylated disaccharide (II, 28 mg.) in 0.7 *N* sulfuric acid (10 ml.) was heated for 8 hr. at 100° (bath

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temp.). The hydrolyzate was neutralized (BaCO₃), filtered and evaporated to a sirup (24 mg.) which showed $[\alpha]^{25}_D +38^\circ$ in methanol (*c* 0.8) and $+81^\circ$ in water (*c* 0.8). Paper chromatographic analysis using solvents C and D revealed the presence of two main components, a and b, whose R_G values corresponded to those of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose (see Table I) and

TABLE I

Methylated sugar	R_G (solvent C)	R_G (solvent D)
Component a	0.49	0.22
Component b	.80	.86
2,4-Di- <i>O</i> -methyl-L-arabinose	.33	.16
2,5-Di- <i>O</i> -methyl-L-arabinose	.75	.30
2,3-Di- <i>O</i> -methyl-L-arabinose	.49	.21
2,3,4,6-Tetra- <i>O</i> -methyl-D-galactose	.80	.86

a small amount of a third component c which moved faster than 2,3,4,6-tetra-*O*-methyl-D-galactose. This component c was shown to be a reducing methylated disaccharide, since on rehydrolysis it afforded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose. The methylated fragments from the hydrolysis of II were isolated in the pure form by sheet paper chromatography using solvent D.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-galactose.—The 2,3,4,6-tetra-*O*-methyl-D-galactose component, obtained as a sirup (6 mg.), had $[\alpha]^{25}_D +54^\circ$ in ethanol (*c* 1). It was boiled for 5 hr. with ethanol (2 ml.) containing aniline (0.1 ml.). Removal of the excess of the aniline and the solvent afforded a crystalline residue. Recrystallization from ethyl acetate gave *N*-phenyl-D-galactopyranosylamine 2,3,4,6-tetramethyl ether, m.p. and mixed m.p. 196–197°, $[\alpha]^{20}_D -73^\circ$ in acetone (*c* 0.2); lit. values⁹ m.p. 192°, $[\alpha]_D -77^\circ$ (acetone).

Identification of 2,3-Di-*O*-methyl-L-arabinose.—The 2,3-di-*O*-methyl-L-arabinose fraction obtained as a sirup (5 mg.) showed $[\alpha]^{25}_D +84^\circ$ in water (*c* 1.5). The sirup (3 mg.) was dissolved in dry pyridine (2 ml.) and after addition of *p*-nitrobenzoyl chloride (50 mg.) the solution was heated at 100–110° (bath temp.) for 2 hr. After keeping the reaction mixture overnight at room temperature, the excess of *p*-nitrobenzoyl chloride was destroyed by addition of a solution of sodium bicarbonate and the resulting solution diluted with 50 ml. of water. The *p*-nitrobenzoate, which separated as a pale yellow precipitate, was extracted with chloroform. The chloroform extract was dried (Na₂SO₄), and evaporated to give a sirup which crystallized on trituration with methanol. Recrystallization from ethanol gave 1,4-bis-*O*-*p*-nitrobenzoyl-2,3-di-*O*-methyl-L-arabinose, m.p. and mixed m.p. 150–153°.

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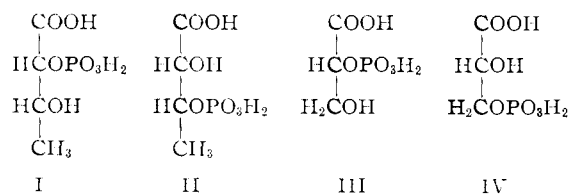
The D-erythro-2,3-Dihydroxybutyric Acid Monophosphates

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The synthesis of D-erythro-2,3-dihydroxybutyric acid 2- and 3-phosphates is described. These compounds are analogs of the D-glyceric acid monophosphates and have been used in studies of the enzyme specificity of enolase and glyceric acid phosphate mutase.

This paper describes the synthesis of the 2-phosphate (I) and 3-phosphate (II) of D-erythro-2,3-dihydroxybutyric acid. These substances are analogs of the glycolytic intermediates D-glyceric acid 2-phosphate (III) and D-glyceric acid 3-phosphate (IV). As such they are useful for a study of the substrate specificity of the enzymes enolase and glyceric acid phosphate mutase.



The key intermediate for the synthesis of these butyric acid derivatives is 2-*O*-benzyl D-erythro-2,3-dihydroxybutyric acid. This was prepared by benzylation in the 4-position of an appropriately blocked D-rhamnoside (V–VII); followed by hydrolysis of the acid labile blocking groups, and oxidative cleavage to remove C1 and C2 of the D-rhamnose portion of the molecule (VIII–X). The remaining steps are similar to those used in this Laboratory for syntheses of D-glyceric acid phosphates.¹ X was esterified to XI, which was phos-

phorylated and the product XII then unblocked by hydrogenation and saponification to give II. Benzoylation of XI, followed by debenzoylation, gave XIII which was phosphorylated to XIV. Unblocking of XIV by hydrogenation and saponification gave I.

These two new organic phosphates parallel in chemical properties the glyceric acid phosphates; they show approximately the same optical rotations; and the 3-isomer gives the same exaltation of the rotation in the presence of molybdate ions. Acid-catalyzed phosphate migration occurs with the same ease, and approaches about the same equilibrium mixture of 2- and 3-phosphates obtained by similar treatment of the glyceric acid phosphates.

Biochemical studies carried out with these new analogs have shown that, (1) they are substrates for glyceric acid phosphate mutase,² being interconverted by the mutase, however, at rates several thousandths less rapidly than the D-glyceric acid phosphates; and (2) the D-erythro-2,3-dihydroxybutyric acid 2-phosphate does not act as a substrate for enolase, but is a very effective competitive inhibitor of this enzyme.³ Additional experiments are under way designed for the more complete delineation of the substrate specificity of the latter enzyme.

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