[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, AND THE DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF CALIFORNIA]

The Structure of an Enzymatically Synthesized Reducing Disaccharide D-Glucosido-L-arabinose

BY W. Z. HASSID, M. DOUDOROFF, A. L. POTTER AND H. A. BARKER

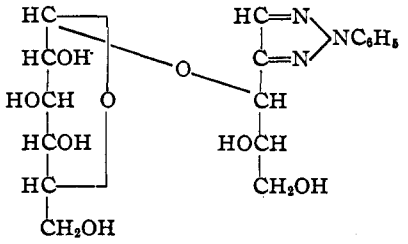
It has been shown that sucrose phosphorylase preparations from *Pseudomonas saccharophila* catalyze the reversible reaction between glucose-1-phosphate and certain ketose sugars forming disaccharides with the liberation of inorganic phosphate. Thus, sucrose and several other non-reducing disaccharides, namely, D-glucosido-L-sorboside, D-glucosido-D-xyloketoside, and D-glucosido-L-araboketoside[1,2,3,4] were prepared.

It appeared at first as though this enzyme were only capable of catalyzing the reaction between ketose monosaccharides and glucose-1-phosphate. Since these disaccharides are non-reducing and their ketose constituents exist in the furanose form, they can be considered as analogs of sucrose. However, it was later discovered that the same enzyme will catalyze a reaction between glucose-1-phosphate and L-arabinose to form a reducing disaccharide having no obvious structural relation to sucrose or to any of the previously prepared sucrose analogs.[4]

The present investigation is concerned with the structural configuration of the crystalline reducing disaccharide formed from α-D-glucose-1-phosphate and L-arabinose under the influence of the enzyme from *Pseudomonas saccharophila*.

This disaccharide reduces Fehling and alkaline ferricyanide solutions. It contains two molecules of water of crystallization and has a specific rotation $[\alpha]_D$ in water of $+156°$. On hydrolysis with acid it produces one mole of D-glucose and one mole of L-arabinose. The phenylosotriazole derivative of the disaccharide prepared according to Hudson, *et al.*,[5] is readily hydrolyzed with acid to D-glucose and L-arabinose phenylosotriazole, showing that the L-arabinose constitutes the free reducing unit in the disaccharide. Like the previously isolated disaccharides, it is formed by the agency of phosphorylase from *Pseudomonas saccharophila* as a result of "de-phosphorolytic" condensation involving α-D-glucose-1-phosphate, indicating that glucose exists in the disaccharide as the α-form.

On oxidation of the phenylosotriazole derivative of the disaccharide with sodium periodate, three moles of periodate are consumed with the formation of one mole each of formic acid and formaldehyde per mole of phenylosotriazole derivative. The structure of this compound is, therefore, 3-[α-D-glucopyranosido]-L-arabinose phenylosotriazole in which D-glucose is attached through carbon atom 1 to carbon atom 3 of L-arabinose as shown by Formula (I).



1. 3-[α-D-Glucopyranosido]-L-arabinose phenylosotriazole

If the D-glucose in the D-glucopyranosido-L-arabinose phenylosotriazole were attached to carbon atom 4 of the L-arabinose derivative, oxidation of this compound with sodium periodate would require two moles of periodate and would liberate one mole of formic acid with no formaldehyde production. Junction of D-glucose to carbon atom 5 of the L-arabinose phenylosotriazole would require three moles of periodate whereby one mole of formic acid would be produced, and no formaldehyde formed.

On methylation of the disaccharide with dimethyl sulfate and sodium hydroxide a hexamethylmethyl derivative of the carbohydrate was obtained. When this fully methylated derivative (II) was hydrolyzed with acid, 2,3,4,6-tetramethyl-D-glucose (III) and dimethyl-L-arabinose (IV) were produced. Since position 3 in the L-arabinose component (I) was shown to be occupied in glycosidic linkage with D-glucose, the dimethyl-L-arabinose could be either the 2,5- or 2,4-dimethyl derivative (IV), depending on whether the L-arabinose unit originally exists in the disaccharide in the furanose or pyranose form. The ring type of the L-arabinose was ascertained by subjecting the dimethyl-L-arabinose to oxidation with sodium periodate, after it had been oxidized with hypoiodite to the corresponding lactone (V) and subsequently hydrolyzed to the straight chain, dimethyl-L-arabonic acid (VI).

If the dimethyl derivative were the 2,5-dimethyl-L-arabonic acid, it would possess a pair of adjacent hydroxyls, on positions 3 and 4, which on oxidation with sodium periodate would consume one mole of periodate in the reaction. On the other hand, the 2,4-dimethyl-L-arabonic acid (VI), lacking a pair of adjacent hydroxyls, cannot

(1) W. Z. Hassid, M. Doudoroff and H. A. Barker, THIS JOURNAL, **66**, 1416 (1944).

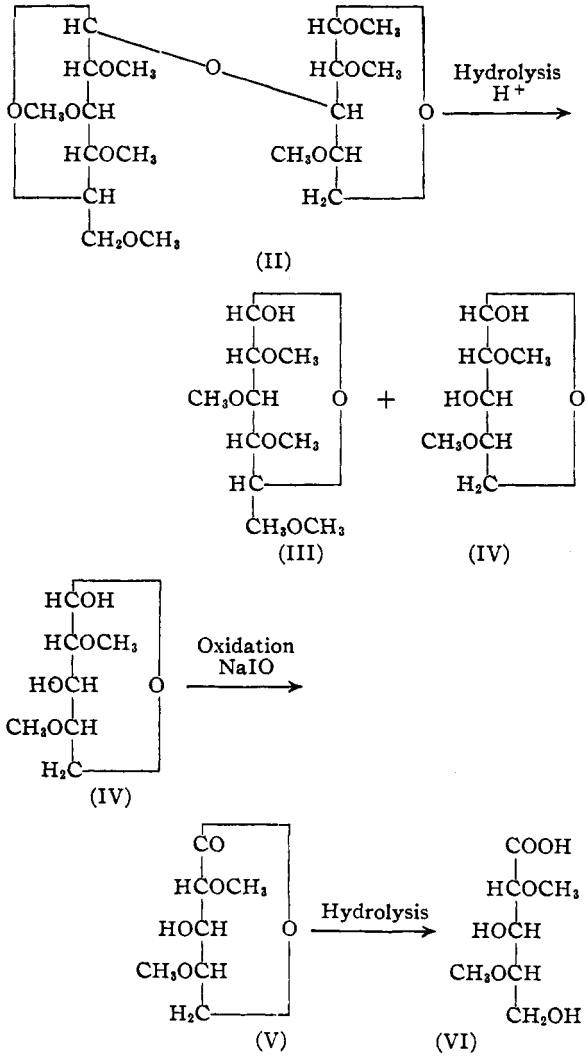
(2) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, *ibid.*, **67**, 1394 (1945).

(3) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, *ibid.*, **68**, 146 (1946).

(4) M. Doudoroff, W. Z. Hassid and H. A. Barker, *J. Biol. Chem.*, **168**, 733 (1947); M. Doudoroff, H. A. Barker and W. Z. Hassid, *ibid.*, **168**, 725 (1947).

(5) R. M. Hann and C. S. Hudson, THIS JOURNAL, **66**, 735 (1944); W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **67**, 939 (1945).

be oxidized. Actually, no periodate was consumed when the dimethyl-L-arabonic acid was treated with this reagent. This shows that the dimethyl derivative is 2,4-dimethyl-L-arabonic acid. The free hydroxyl in position 3 is obviously restored in the dimethyl-L-arabinose when the methylated disaccharide is hydrolyzed; the hydroxyl in position 5 is formed when its internal ring is broken in the process of hydrolysis of the lactone to dimethyl-L-arabonic acid, which is a straight chain compound.



A more direct confirmation that the dimethyl-L-arabinose possesses a pyranose configuration was obtained from the study of the rate with which its lactone derivative is hydrolyzed to the open chain acid. Haworth, *et al.*,[6] found that the six-membered ring sugar lactones hydrolyze in water to their corresponding straight chain acids at a distinctly greater rate than the five-membered ring sugar lactones. The gamma lactones require approximately two weeks for complete opening of the ring, while the delta lactones are converted completely into the open chain acids within a few hours. It is thus possible to determine whether a given lactone has a furanose or pyranose configuration.

When the dimethyl-L-arabono lactone was dissolved in water, it was found to be almost completely hydrolyzed within four hours. This was indicated by a change of its rotation from $[\alpha]_D$ + 60 to 24° (Fig. 1). A constant value of $[\alpha]_D$ +17° was reached within less than twenty-four hours. Since the rate of change in rotation of this methylated lactone due to hydrolysis is high, it strongly indicates that the lactone possesses a pyranose configuration. This observation confirms the periodate oxidation data, showing that the dimethyl derivative is 2,4-dimethyl-L-arabonic acid.

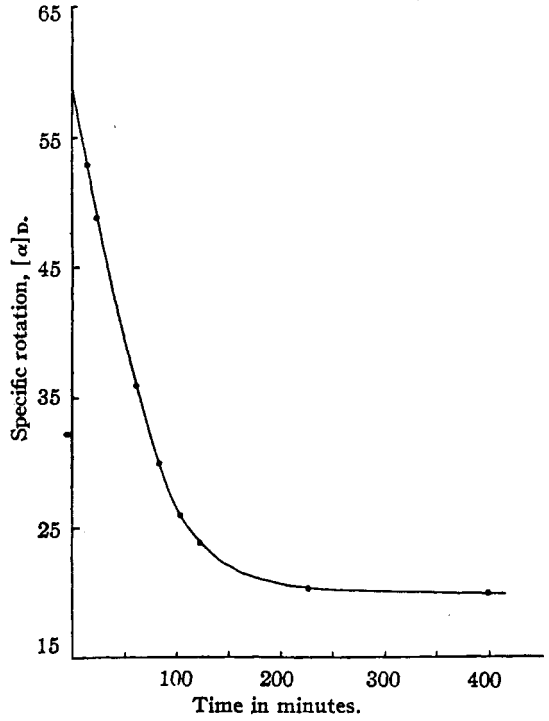


Fig. 1.—Hydrolysis of dimethyl-L-arabonolactone.

On the basis of these results the structural formula for this reducing disaccharide may be written as in Fig. 2.

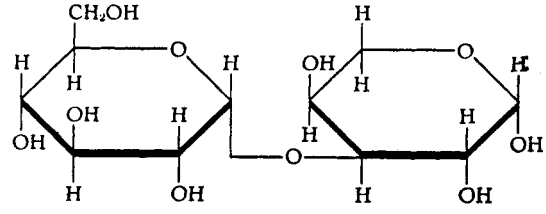


Fig. 2.

(6) W. N. Haworth, "The Constitution of Sugars," Edward Arnold and Company, London, 1929, p. 24; S. Baker and W. N. Haworth, *J. Chem. Soc.*, **127**, 365 (1925).

Experimental

Enzymatic Synthesis of D-Glucosido-L-arabinose.—A mixture of 30 g. of glucose-1-phosphate, 16 g. of L-arabinose and 400 ml. of 0.1 *M* barium acetate was incubated for eighteen hours at *p*H 7.0 and 35° in the presence of enzyme extracted from the bacteria *Pseudomonas saccharophila* by the method previously described.[1,2] The unused glucose-1-phosphate was removed by precipitation with alcohol and the unused L-arabinose by fermentation with a selected strain of *Escherichia coli*.[7] After passage through ion exchange columns and concentration of the solution *in vacuo*, the sugar was crystallized from absolute alcohol in the usual manner.[2,3] The yield was 5.4 g.

Properties of the Disaccharide.—The disaccharide is hygroscopic, very soluble in water and has a sweet taste. It reduces Fehling or alkaline ferricyanide solution and after hydrolysis with acid and subsequent neutralization gives the orcinol-hydrochloric acid reaction (Bial), characteristic for pentose sugars. This reducing disaccharide does not give the diazouracil reaction[8] specific for sucrose and the other synthetic disaccharides[2,3] which apparently contain the type of glycosidic glucose–fructose linkage existing in sucrose.

The carbohydrate is practically unaffected by yeast invertase and has a much greater resistance to acid hydrolysis than the previously synthesized non-reducing disaccharides, containing ketose units. Its rate of hydrolysis with acid is about one-third as great as that of maltose.

Fig. 3.—Phenylosazone of D-glucosido-L-arabinose (× 93).

(7) The removal by extraction of its osazone derivative was precluded by the reducing nature of the synthesized carbohydrate.

(8) H. W. Raybin, THIS JOURNAL, **55**, 2603 (1933); **59**, 1402 (1937).

Anal. Calcd. for $C_{11}H_{20}O_{10}\cdot 2H_2O$: C, 37.93; H, 6.90. Found: C, 38.26; H, 6.89; specific rotation $[\alpha]_D$ +156°, (in water, *c*, 2).

Hydrolysis of the Disaccharide and Identification of Products.—A 0.02-g. sample of the disaccharide was hydrolyzed with 0.5 *N* hydrochloric acid at 100° for six hours. Analysis of the hydrolyzed solution for reducing sugars by oxidation with ferricyanide,[9] showed a reducing value of 96% calculated on the basis of an equimolar mixture of glucose and L-arabinose and taking into consideration that the disaccharide contains two molecules of water of crystallization. After removal of the glucose from the hydrolyzate by fermentation with *Tortula monosa*, an osazone was prepared which was identified as arabosazone. Determination of pentose on an original sample of the disaccharide gave the theoretical value of L-arabinose.[10] A 2% solution of the disaccharide in 0.5 *N* hydrochloric acid hydrolyzed at 100° gave a final specific rotation $[\alpha]_D$ +71.0°. The calculated rotation of a hydrolyzed disaccharide consisting of D-glucose and L-arabinose and containing two molecules of water of crystallization is $[\alpha]_D$ +72.3°.

Rate of Hydrolysis of the Disaccharide.—The hydrolysis of a 2% solution of the D-glucosido-L-arabinose in 6 *N* hydrochloric acid was followed by observing the change of rotation at 50°. The rate of hydrolysis was compared with that of a similar solution of maltose under identical conditions. The course of hydrolysis of this disaccharide was represented by a logarithmic curve, indicating a first order reaction. The velocity constant *K* of the reaction is 4.37×10^{-3}. The velocity constant *K* of a 2% maltose solution, determined under the same conditions, is 1.28×10^{-2}, showing that the rate of hydrolysis of the D-glucosido-L-arabinose with acid is approximately one-third as great as that of maltose.

Phenylosazone.—One gram of the D-glucosido-L-arabinose was mixed in a test-tube with 2 g. of phenylhydrazine hydrochloride and 3 g. of sodium acetate. The mixture was dissolved in 14 ml. of water and heated on a steam-bath for thirty minutes. Upon cooling to room temperature crystals of the phenylosazone separated out, which appeared under the microscope as yellowish-green needles (Fig. 3). After placing the test-tube with the contents in the refrigerator for several hours at 4° the osazone was filtered, recrystallized from 50% alcohol and dried *in vacuo* at 30°. The yield was 0.735 g.

D-Glucosido-L-arabinose Phenylosotriazole.—A suspension of 0.50 g. of phenylosazone derivative of the new disaccharide was dissolved in 50 ml. of water, heated to the boiling point and a solution of 0.265 g. of copper sulfate pentahydrate (1.1 molecular equivalents) in 5 ml. of water was added. The mixture was heated under a reflux condenser for thirty minutes from the time of addition of the copper sulfate solution, then cooled and filtered. The filtrate, after neutralization with 0.20 g. of calcium carbonate, was extracted with ether to remove aniline. The solution was then run through ion exchange columns (Duolite C-3 and A-3, obtained from the Chemical Process Co., San Francisco) to remove inorganic impurities and evaporated to dryness *in vacuo* at 50°. The residue was extracted with hot alcohol, filtered, and the extract evaporated *in vacuo* to a small volume. Upon the addition of petroleum ether and upon stirring, crystals of the phenylosotriazole derivative were obtained. After cooling to 0°, the crystals were filtered on a sintered glass filter, washed with petroleum ether three times and dried *in vacuo* at 40°. The product was then recrystallized from 3 ml. of hot alcohol. A yield of 0.276 g. was obtained.

Anal. Calcd. for $C_{17}H_{23}N_3O_8$: C, 51.37; H, 5.83; N, 10.58. Found: C, 50.89; H, 5.82; N, 10.38; specific rotation, $[\alpha]_D$ +80° (in water, *c*, 2); melting point, 126.5°.

Oxidation of the D-Glucosido-L-arabinose Phenylosotriazole with Sodium Periodate.—A 0.0397-g. sample

(9) W. Z. Hassid, *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).

(10) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).

(0.1 millimole) of the derivative was oxidized at room temperature with 0.5 *M* sodium periodate. The amount of periodate consumed in the reaction and the amount of formic acid liberated was estimated by the usual procedure.[11] The formaldehyde produced in the reaction was determined photocolorimetrically with 1,8-dihydroxynaphthalene-3,6-disulfonic acid by the method of MacFayden.[12]

The results showed that three moles of periodate were consumed, giving rise to one mole of formic acid and one mole of formaldehyde in the oxidation of one mole of the phenylosotriazole derivative.

Acetylation.—A 0.1-g. sample of the disaccharide was acetylated with 0.46 ml. of acetic anhydride in the presence of pyridine at 0° as previously described.[1] The acetylated derivative was insoluble in water but soluble in chloroform and acetone. The yield was 0.11 g.

Anal. Calcd. for $C_{11}H_{13}O_{10}(CH_3CO)_7$: CH_3CO, 49.67. Found: CH_3CO, 48.88; specific rotation, $[\alpha]_D$ +111° (in chloroform, *c*, 2).

Methylation of D-Glucosido-L-arabinose.—The disaccharide was methylated by the method of Haworth and Leitch[13] as follows: One gram of the sugar was dissolved in a minimum of hot water (approximately 1 ml.) and the solution cooled to 30°. A total of 3.8 ml. of methyl sulfate and 8 ml. of 30% sodium hydroxide were added in eight equal portions with vigorous stirring. The first portion (0.5 ml.) of the methyl sulfate and that of the sodium hydroxide (1 ml.) were added at 30°. It is important that the sodium hydroxide be added very slowly so as not to allow the solution to become alkaline at any time during the methylation procedure. The temperature was then raised to 40° and the treatment was repeated with similar portions of methyl sulfate and sodium hydroxide solution. The mixture was then kept overnight at room temperature and on the following day tested for its reducing value. It was found to have no reducing property. Thereafter the temperature was raised to 60° and a third portion corresponding to one-eighth of the original volume of methyl sulfate was introduced and a similar proportion of sodium hydroxide was added drop by drop, with vigorous stirring. The remainder of the reagents was added at 70° in the same order and in similar fractions, stirring being continued throughout the addition. Finally the temperature was raised to 100° for a period of half an hour and the solution allowed to cool to room temperature. The solution was dried with anhydrous sodium sulfate, filtered and the chloroform distilled off. The yield was 1.03 g.

The incompletely methylated product was further methylated by the method of Pacsu and Trister[14] by dissolving in ether and treating with sodium and then methyl iodide. The yield obtained of the hexamethylmethyl D-glucosido-L-arabinose was 0.58 g.

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OCH_3, 52.91. Found: 51.70; specific rotation, $[\alpha]_D$ +122° (in water, *c*, 2.34).

Hydrolysis of the Methylated Disaccharide and Separation of the 2,3,4,6-Tetramethyl-D-glucose and Dimethyl-L-arabinose.—The methylated D-glucosido-L-arabinose (0.56 g.) was dissolved in 20 ml. of 2 *N* hydrochloric acid and the solution was heated on a steam-bath for three hours. The hydrolyzate was cooled and extracted six times with equal volumes of chloroform. The solution was evaporated to a small volume on the steam-bath and dried by contact with a mixture of anhydrous sodium sulfate and anhydrous magnesium sulfate for forty minutes. A small amount of charcoal was then added and after fifteen more minutes the mixture was filtered. After evaporating this solution to dryness *in vacuo*, a yield of 0.31 g. (Fraction I) of crude 2,3,4,6-tetramethyl-D-glucose was obtained. The specific rotation of this product (in water, *c*, 1.67) was $[\alpha]_D$ +87°.

The tetramethylglucose was purified as follows: The crude product (Fraction I) was dissolved in 20 ml. of water, extracted six times with equal volumes of chloroform and the chloroform dried with a mixture of anhydrous sodium sulfate and anhydrous magnesium sulfate. A small amount of charcoal was then added and after a few minutes the solution was filtered. Upon evaporation of the chloroform solution to dryness, a yield of 0.27 g. of 2,3,4,6-tetramethyl-D-glucose was obtained.

Anal. Calcd. for $C_6H_8O_2(OCH_3)_4$: OCH_3, 52.6. Found: OCH_3, 51.6; specific rotation, $[\alpha]_D$ +81° (in water, *c*, 2).

The aqueous solution left after Fraction I was separated was again extracted six times with equal volumes of chloroform and the chloroform was evaporated, dried and clarified as before. Upon evaporation to dryness, 0.02 g. (Fraction II) of sirup was obtained. This small amount of sirup, probably dimethylarabinose, was discarded.

The aqueous solution contained practically all the dimethyl-L-arabinose, inasmuch as the dimethyl arabinose derivative is greatly in favor of the aqueous phase. The aqueous solution was passed through a 140-ml. capacity Duolite A-3 column to adsorb the hydrochloric acid. The column was washed with six volumes of water and the combined solutions evaporated to dryness *in vacuo*. The residue was then extracted with chloroform, clarified with charcoal, filtered and evaporated to dryness again. The yield of dimethyl-L-arabinose sirup was 0.180 g. (Fraction III).

Anal. Calcd. for $C_5H_8O_3(OCH_3)_2$: OCH_3, 34.8. Found: OCH_3, 34.2; specific rotation, $[\alpha]_D$ +61° (in alcohol, *c*, 1.56).

Oxidation of Dimethyl-L-arabinose to its Lactone.—The oxidation of the dimethyl-L-arabinose was carried out by a modified method of Goebel.[15] The sirup (0.165 g.) was dissolved in 33 ml. of 0.3 *N* barium iodide–iodine solution (7.5 g. of barium iodide and 3.81 g. of iodine dissolved in 100 ml. of water) to which 50 ml. of 0.4 *N* barium hydroxide was added in three minutes at constant rate of flow while stirring. The mixture was allowed to stand for fifteen minutes and was then acidified with 0.925 ml. of concentrated sulfuric acid which was diluted with 7.5 ml. of water. An excess of lead carbonate (7.5 g.) was immediately added and the mixture was rapidly stirred until it became neutral to congo red. The precipitate was permitted to settle and the supernatant liquid decanted. The precipitate was washed several times by decantation. The supernatant liquid and the washings, containing the methylated arabonate, was evaporated *in vacuo*, thus removing the iodine, and the concentrated solution filtered and the residue washed with water. The filtrate was then treated with 0.1 g. of silver sulfate to precipitate the iodide. The precipitate was filtered and the filtrate was treated with hydrogen sulfide to remove the excess silver and lead and filtered again. The solution was neutralized with barium hydroxide, allowed to stand for a few hours and filtered. It was then passed through a 25-ml. capacity Duolite C-3 column and washed with several volumes of water. The solution was concentrated *in vacuo* to dryness, adding water several times during the course of distillation, in order to remove any volatile acids that might be present. The dry residue was extracted with hot alcohol and the alcohol evaporated to dryness *in vacuo*. The yield of dimethyl-L-arabonic lactone was 0.14 g. When a sample of 0.0295 g. of the lactone was neutralized with 0.1 *N* sodium hydroxide, 1.50 ml. of this reagent was consumed. The theoretical requirement is 1.68 ml. of 0.1 *N* sodium hydroxide.

Anal. Calcd. for $C_5H_6O_3(OCH_3)_2$: OCH_3, 35.2. Found: OCH_3, 35.2.

Hydrolysis of Dimethyl-L-arabonolactone.—A sample of 0.0492 g. of the lactone was dissolved in 5 ml. of water and its rate of hydrolysis was followed by observing the

(11) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937); **62**, 958 (1940); R. M. Hann, W. D. Maclay and C. S. Hudson, *ibid.*, **61**, 2432 (1939).

(12) D. A. MacFayden, *J. Biol. Chem.*, **158**, 107 (1945).

(13) W. N. Haworth and G. C. Leitch, *J. Chem. Soc.*, **113**, 195 (1918).

(14) E. Pacsu and S. M. Trister, THIS JOURNAL, **61**, 2442 (1939).

(15) W. F. Goebel, *J. Biol. Chem.*, **72**, 809 (1927).

change of rotation in a polariscope. It was found that the lactone was almost completely hydrolyzed to the acid within four hours. A change from the initial rotation of $[\alpha]_D$ +60° to +24° occurred within that time. After twenty-four hours the rotation had reached $[\alpha]_D$ +17°, which remained constant thereafter (Fig. 1). Since the rate of change in the rotation of this lactone due to hydrolysis is high, it is concluded that this methylated lactone, and therefore also the original pentose unit, possesses the pyranose configuration.

Oxidation of the Dimethyl-D-arabonic Acid.—A 0.017-g. sample of the lactone was oxidized by allowing it to remain at room temperature with 0.5 *M* sodium periodate[11] for thirty hours. In aqueous solution the lactone is converted to the straight chain acid in a few hours. Analysis of the solution showed that no periodate was used up, indicating that the substance was not oxidized, apparently, due to the fact that it did not contain any adjacent free hydroxyl groups. When a similar sample of 2,3,6-trimethylgluconic acid was oxidized with periodate under the same conditions, one mole of periodate was consumed in the reaction.

Acknowledgment.—The work reported in this paper was supported in part by a grant from the Corn Industries Research Foundation.

Summary

A reducing crystalline disaccharide consisting of D-glucose and L-arabinose has been synthesized from glucose-1-phosphate and L-arabinose by the agency of a phosphorylase from the organism *Pseudomonas saccharophila*.

On oxidation of the phenylosotriazole derivative of this disaccharide with sodium periodate, three moles of periodate are consumed with the formation of one mole each of formic acid and formaldehyde per mole of phenylosotriazole derivative. These data indicate that in the disaccharide D-glucose is linked through carbon atom 1 to carbon atom 3 of L-arabinose.

Methylation of the disaccharide produced a hexamethylmethyl derivative, which on hydrolysis with acid gave rise to 2,3,4,6-tetramethyl-D-glucose and 2,4-dimethyl-L-arabinose.

Evidence that the dimethyl-L-arabinose possesses the pyranose configuration was obtained from the rate of hydrolysis of the dimethyl-L-arabonolactone derivative to its acid and also from periodate oxidation of this lactone.

On the basis of these data, the new reducing disaccharide may be designated as 3-[α-D-glucopyranosido]-L-arabopyranose.

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1,5-Anhydro-4-(β-D-glucopyranosyl)-D-glucitol,[1] 1,5-Anhydro-6-(β-D-glucopyranosyl)-D-glucitol and 1,5-Anhydro-D-galactitol[2]

BY HEWITT G. FLETCHER, JR., AND C. S. HUDSON

The palladium-catalyzed addition of hydrogen to the double bond of an acetylated 2-hydroxyglycal may, because of the new asymmetry introduced at carbon two, give rise to either or both of the predicted diastereomeric sugar alcohol anhydrides. Thus Zervas[3] reduced 2,3,4,6-tetraacetyl-2-hydroxy-D-glucal in a 61% yield to an anhydrohexitol which later was demonstrated[4] to be 1,5-anhydro-D-mannitol (styracitol), while Richtmyer, Carr and Hudson[4c] have more recently succeeded in obtaining a 4% yield of 1,5-anhydro-D-glucitol (polygalitol) by the same process.

The catalytic reduction of 2,3,4-triacetyl-2-hydroxy-D-xylal, which might lead to either 1,5-anhydro-xylitol or 1,5-anhydro-D-arabitol or to both, has recently been investigated[5]; only 1,5-anhydro-xylitol, isolated as its triacetate in a yield of 83%, was obtained.

In addition to the two cases above, the reduction of three other acetylated 2-hydroxy-glycals has been reported in the literature. Maurer and Plötner[6] reduced both heptaacetyl-2-hydroxy-cellobial (I) and heptaacetyl-2-hydroxy-gentiobial (V) in the presence of palladium to corresponding heptaacetyl-1,5-anhydro-(β-D-glucopyranosyl)-hexitols in yields, respectively, of 62 and 53%. The free anhydrides were termed "1.4-glucosido-styracitol" and "1.6-glucosido-styracitol," that is, as derivatives of an anhydride (styracitol) which is now known to have the mannitol configuration. Apparently these names were chosen solely on the assumption that the course of the reduction of the acetates of the two substituted 2-hydroxy-glycals had been similar to that of 2,3,4,6-tetraacetyl-2-hydroxy-D-glucal, which, as mentioned above, appears to give predominantly 1,5-anhydro-D-mannitol. Definitive proof, such as might have been obtained by the hydrolysis of the 1,5-anhydro-(β-D-glucopyranosyl)-hexitols, was apparently not adduced.

A third acetylated 2-hydroxy-glycal, 2,3,4,6-tetraacetyl-2-hydroxy-D-galactal (IX), was reduced in the presence of palladium by Freuden-

(1) By D-glucitol we denote the hexitol corresponding in configuration to D-glucose and commonly termed sorbitol.

(2) This communication represents a portion of a paper presented before the Division of Sugar Chemistry and Technology at the Atlantic City meeting of the American Chemical Society, April 15, 1947.

(3) L. Zervas, *Ber.*, **63**, 1689 (1930).

(4) (a) L. Zervas and I. Papadimitriou, *Ber.*, **73**, 174 (1940); (b) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 64 (1943); (c) N. K. Richtmyer, C. J. Carr and C. S. Hudson, *ibid.*, **65**, 1477 (1943); (d) R. C. Hockett and Maryalice Conley, *ibid.*, **66**, 464 (1944).

(5) H. G. Fletcher and C. S. Hudson, *ibid.*, **69**, 921 (1947).

(6) K. Maurer and K. Plötner, *Ber.*, **64**, 281 (1931).