was filtered and recrystallized from ethyl acetate; m. p. 188°; $[\alpha]^{25}D$ −1.2° (*c*, 0.1 g. in 5 cc. ethanol).

Anal. Calcd. for $C_{14}H_{18}O_{10}$: C, 48.54; H, 5.24. Found: C, 48.56; H, 5.35.

Summary

Racemic α-hydroxy-β,β-dimethyl-γ-butyrolactone was readily resolved by the use of brucine and of diacetyl-*d*-tartaric anhydride. With brucine two different complexes have been prepared, both yielding the biologically active levorotatory lactone.

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Isolation and Structure of an Enzymatically Synthesized Crystalline Disaccharide D-Glucosido-D-ketoxyloside

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

In a preliminary report[1] evidence was presented indicating that preparations from the bacterium *Pseudomonas saccharophila*, capable of synthesizing sucrose[2] from glucose-1-phosphate and fructose, can also combine glucose-1-phosphate with L-sorbose or D-ketoxylose to form the corresponding disaccharides. One of these disaccharides has already been isolated in crystalline form and its structure appears to be α-D-glucopyranosido-α-L-sorbofuranoside.[3] The present work deals with the preparation and the molecular constitution of the other crystalline disaccharide, D-glucosido-D-ketoxyloside, formed from α-D-glucose-1-phosphate and D-ketoxylose by the phosphorylase from *Pseudomonas saccharophila*.

This disaccharide does not reduce Fehling solution or alkaline ferricyanide. Its empirical formula obtained by elementary analysis is $C_{11}H_{20}O_{10}$. The compound is practically unaffected by invertase, but is easily hydrolyzed with acid. When the disaccharide is hydrolyzed with acid and the glucose fermented out, an osazone is obtained which is identical with that of xylose. The specific rotation of the disaccharide is $[\alpha]D$ + 43°. Hydrolysis with 1 *N* hydrochloric acid changes the rotation to +16.2°. Taking Schmidt and Treiber's[4] value for the specific rotation of ketoxylose as −33.2°, the calculated rotation of an equimolar mixture of glucose and D-ketoxylose in water is +14.3°. The melting point of the disaccharide is 156–157°. Its rate of hydrolysis with acid is approximately 30% greater than that of sucrose. The acetylated derivative has a rotation in chloroform, $[\alpha]D$ +22° and a melting point of 180–181°.

Since the disaccharide is non-reducing, the glucose and D-ketoxylose units are obviously linked through the carbonyl groups. Inasmuch as the carbonyl group in ketoxylose occurs on the second carbon atom, the largest possible semi-acetal ring for the ketose component is the 2,5-furanose ring and the possibility of a pyranose ring is definitely excluded. Smaller rings such as the 2,3 or 2,4 ring are sterically improbable. The furanose structure of the ketoxylose was definitely confirmed experimentally by oxidation of the disaccharide with sodium periodate. A disaccharide consisting of glucopyranose and ketoxylofuranose glycosidically united through positions 1 and 2 of the aldose and ketose monosaccharides, would possess three adjacent free hydroxyls on carbon atoms 2, 3 and 4 in the glucose residue and two free hydroxyls on carbon atoms 3 and 4 in the ketoxylose residue. When subjected to oxidation, a disaccharide of this structure should consume two moles of periodate and form one mole of formic acid due to the glucose residue and consume one mole of periodate due to the ketoxylose residue. A total of 3 moles of periodate would thus be consumed and one mole of formic acid should be formed per mole of disaccharide. Actually, on oxidation of the carbohydrate with periodate, 2.96 moles of periodate are consumed and 0.95 mole of formic acid is formed.

Like the previously synthesized D-glucosido-L-sorboside,[3] this disaccharide gives a blue-green color with diazouracil, a reaction shown by Raybin[5] to be specific for compound sugars containing the same type of glycosidic glucosefructose linkage that exists in sucrose.[6] The fact that phosphorylase from *Pseudomonas saccharophila*, capable of synthesizing sucrose from glucose-1-phosphate and fructose,[2] can also effect the synthesis of a disaccharide from glucose-1-phosphate and D-ketoxylose, indicates that the linkage joining the two monosaccharide units in the glucosido-ketoxyloside is probably the same as that existing in sucrose. The formation of the disaccharide is a product of "de-phosphorolytic" condensation involving α-D-glucose-1-phosphate. This is good evidence that glucose exists in the disaccharide as the α-form.

(1) M. Doudoroff, W. Z. Hassid and H. A. Barker, *Science*, **100**, 315 (1944).

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

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

(4) O. T. Schmidt and R. Treiber, *Ber.*, **66**, 1765 (1933).

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

(6) C. B. Purves and C. S. Hudson, *ibid.*, **59**, 1170 (1937).

The above evidence and analogy with sucrose indicate that the structure of the disaccharide is α-D-glucopyranosido-β-D-ketoxylofuranoside as shown in Fig. 1. This compound and sucrose appear to be structurally identical, except that sucrose has an additional carbon atom with a primary alcohol group attached to the ring.

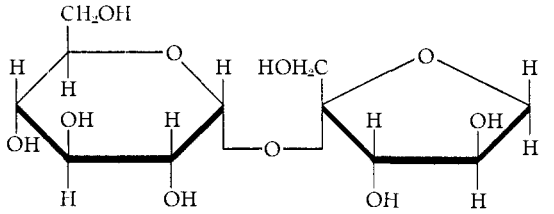

Fig. 1.—α-D-Glucopyranosido-β-D-ketoxylofuranoside.

It was previously noted[3] that the X-ray diffraction pattern of glucosido-sorboside showed some similarity with that of sucrose. It has now been found that glucosido-ketoxyloside likewise yields a diffraction pattern somewhat resembling that of sucrose in that six of the observed interplanar spacings in the two patterns have almost identical numerical values (Table I). However, the relative intensities of the spacings that are common to the two sugars are not the same. As in the case of glucosido-sorboside, the data do not support an interpretation that the pattern for the new sugar indicates contamination by sucrose. Of the three strongest lines in the sucrose pattern, only one, and that not the strongest, appears in the pattern for glucosido-ketoxyloside. The data at present available do not permit structural deductions for the two new disaccharides, but it appears reasonable to suggest that these sugars have crystal structures related to that of sucrose. Because of their similar configurations, the three disaccharides might be expected to attain crystal formation through similar types of molecular packing, thus giving rise to three crystal structures that are different from each other but that may have, nevertheless, some of their cell dimensions in close agreement.

Experimental

Enzymatic Synthesis and Isolation of Glucosido-ketoxyloside.—Partially purified phosphorylase was prepared from 7.5 g. of dry bacteria (*Pseudomonas saccharophila*) by the usual method.[2] The preparation was added to a solution containing 43 g. of potassium salt of glucose-1-phosphate and 14 g. of D-ketoxylose[7] and adjusted to *p*H 7.4. Barium acetate was then added to make the final concentration 0.1 *M*, the mixture was diluted to 680 ml. and kept at 37° with frequent shaking for twenty-four hours. The unchanged glucose-1-phosphate and the inorganic phosphate were removed from the reaction mixture by precipitation with alcohol followed by passage through ion exchange columns of Amberlite 1R-100 and Amberlite 1R-4.[4,8] The unreacted ketoxylose was eliminated by conversion into xylosazone and extraction of the osazone with ethyl acetate as previously described.[3] The solution, free of reducing sugar and other impurities, was evaporated *in vacuo* to a thick sirup at 40°, treated with hot absolute alcohol and stirred with a glass rod. After adding a small quantity of petroleum ether and allowing the mixture to remain *in vacuo* for several hours, the sirup was converted into crystals. The crystals were filtered, washed with a few ml. of absolute alcohol and dried *in vacuo* at 50°. The yield was 0.33 g.

Properties of the Disaccharide.—The disaccharide does not reduce Fehling or alkaline ferricyanide solution and is very soluble in water. Since ketopentose is part of the disaccharide molecule and produces furfural when heated with hydrochloric acid, no satisfactory Seliwanoff test for ketose could be obtained because the dark color due to furfural interferes with the red color given by ketose sugars in the presence of resorcinol. However, the characteristic color reaction for ketose sugars was obtained on a portion of the hydrolyzed disaccharide when it was heated with 1% cobaltous chloride solution on the steam-bath for a few minutes, then cooled and a few drops of ammonium hydroxide added.

The carbohydrate is practically unaffected by yeast invertase (Wallerstein), but is rapidly hydrolyzed with acid and with crude extracts of dried yeast, which possess α-glucosidase as well as invertase activity. It gives the blue-green color reaction with diazouracil[5] a test known to be specific for sucrose or other sugars containing the same type of glycosidic glucose-fructose linkage existing in sucrose.

Anal. Calcd. for $C_{11}H_{20}O_{10}$: C, 42.31; H, 6.41. Found: C, 42.17; H, 6.28; specific rotation, $[\alpha]_D$ +43° (in water, *c*, 2); melting point, 156–157°.

Hydrolysis of the Disaccharide and Identification of Products.—On hydrolysis of the disaccharide with 0.1 *N* hydrochloric acid and analysis of the reducing sugars by oxidation with ferricyanide, a reducing value of 94% was obtained, calculated on the basis of an equimolar mixture of glucose and xylose. Determination of pentose on the hydrolyzed solution gave a value of 79.4%, of the theoretical calculated as xylose.[9] No data are available regarding either the relative reducing power of ketoxylose or its furfural production. From these data it may be assumed that the ketoxylose has a lower reducing value and produces less furfural than xylose. After removing glucose in the hydrolyzate by fermentation with *Torula monosa*, an osazone was obtained which was identified as xylosazone.[10] A 1% solution of the disaccharide in 1 *N* hydrochloric acid hydrolyzed at room temperature gave a final specific rotation $[\alpha]_D$ +16.2°. The calculated rotation of an equimolar mixture of glucose and ketoxylose in water, assuming the specific rotation of ketoxylose to be −33.2°,[4] is 14.3°. The observed and calculated values are in fair agreement, considering that the solvent of the disaccharide was 1 *N* hydrochloric acid.

Rate of Hydrolysis of the Disaccharide.—A 2% solution of the disaccharide was hydrolyzed in 1 *N* hydrochloric acid at 21° and its rotation was followed until it became constant. When the course of hydrolysis was plotted against time, a logarithmic curve was obtained, indicating a first order reaction. The velocity constant *K* of the reaction is 0.0124. The velocity constant *K* for a 2% sucrose solution under the same conditions is 0.0095.

Acetylation.—A 0.08-g. sample of the disaccharide was treated with 0.6 ml. of pyridine and 0.4 ml. of acetic anhydride at 0° and the mixture kept at 3° for eighteen hours with occasional shaking until the sugar dissolved. The solution was poured into 2 ml. of ice water, the precipitated acetate filtered, washed with water until neutral and dried at 50° *in vacuo*. The dry material was dissolved in chloroform, evaporated at room temperature to a small volume, reprecipitated by the addition of petroleum ether

(7) The D-ketoxylose was prepared by autoclaving a 10% solution of D-xylose for forty-five minutes in the presence of 0.2 *M* phosphate buffer, *p*H 6.8. A considerable quantity of unreacted D-xylose was separated by crystallization, leaving a sirup consisting chiefly of D-ketoxylose.

(8) R. M. McCready and W. Z. Hassid, THIS JOURNAL, **66**, 560 (1944).

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

(10) C. A. Browne and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 685.

and dried. The yield was 0.095 g. The acetylated disaccharide was insoluble in water, but soluble in chloroform, acetone and to a lesser extent in ethanol.

Anal. Calcd. for $C_{11}H_{13}O_{10}(CH_3CO)_7$: C, 49.50; H, 5.61; CH_3CO, 49.67. Found: C, 49.29; H, 5.41; CH_3CO, 49.70; specific rotation, $[\alpha]_D$ +22° (in chloroform, *c*, 1); melting point 180–181°.

Oxidation of the Disaccharide with Sodium Periodate.—A 0.0312-g. sample of the disaccharide was oxidized at room temperature with 0.5 *M* sodium periodate.[11] The amount of periodate consumed in the reaction and the amount of formic acid liberated was estimated as previously described.[3] The results showed that 2.96 moles of periodate were consumed, giving rise to 0.95 mole of formic acid in the oxidation of one mole of disaccharide. These data are in agreement with a disaccharide consisting of a glucopyranose and a ketofuranose monosaccharide.

X-Ray Examination of α-D-Glucopyranosido-β-D-ketoxylofuranoside.—An X-ray diffraction pattern of the sugar was made by the powder method, using molybdenum radiation which had been rendered virtually monochromatic by filtration through a zirconium oxide screen. Sixteen lines were found, the three strongest, having approximately equal visual intensities, correspond to 6.0, 4.71 and 3.79 Å. spacings. The spacings are listed in Table I in comparison with the spacings for sucrose.

TABLE I

INTERPLANAR SPACINGS FOR GLUCOSIDO-KETOXYLOSIDE AND SUCROSE

Glucosido-ketoxyloside		Sucrose[a]	
Spacing in Å.	Intensity[b]	Spacing in Å.	Intensity
		7.6	s.
7.3	w.		
		6.9	s.
6.6	m.		
6.0	s.	6.0	v.v.w.
		5.7	m.
5.3	w.	5.4	v.w.
4.71	s.	4.71	v.s.
		4.50	m.s.
4.31	m.	4.30	m.
4.00	w.	4.00	s.
3.79	s.	3.79	w.
		3.59	v.v.s.
		3.37	v.v.w.
		3.22	v.w.
3.00	w.		
		3.10	v.w.
		2.88	m.s.
2.80	w.	2.80	v.w.
		2.07	v.v.w.
2.61	v.w.		
		2.58	v.w.
		2.49	v.w.
2.46	v.w.		
		2.41	v.w.
2.36	v.w.		
		2.34	m.
2.30	v.v.w.		
2.22	v.w.	2.24	m.
		2.18	v.w.
2.09	v.w.	2.06	w.m.

[a] The spacings for sucrose are those given by Hanawalt, *et al.*,[12] which are in good agreement with sucrose spacings found by the present authors. The six narrowest spacings in Hanawalt's original list are omitted since they fall outside of the range observed for the new sugar and have, therefore, no significance for comparison. The intensity designations have been changed to approximate qualitative estimates. [b] Intensity indicators: v. v. s. = very very strong, v. s. = very strong, s = strong, m. s. = medium strong, m = medium, w = weak, v. w. = very weak, v. v. w. = very very weak, w. m. = weak to medium.

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Summary

A non-reducing crystalline disaccharide consisting of D-glucose and D-ketoxylose has been synthesized from glucose-1-phosphate and D-ketoxylose through the action of a phosphorylase from *Pseudomonas saccharophila*.

On the basis of the data obtained from periodate oxidation of the disaccharide, it is concluded that the glucose residue possesses a pyranose and the ketoxylose residue possesses a furanose configuration.

The structure of the disaccharide appears to be similar to that of sucrose and the glucosido-sorboside previously described.

The new sugar may be designated as α-D-glucopyranosido-β-D-ketoxylofuranoside.

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(11) E. L. Jackson, "Organic Reactions," Vol. II, edited by R. Adams, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 341–375.

(12) J. D. Hanawalt, H. W. Rinne and L. K. Frevel, *Ind. Eng. Chem., Anal. Ed.*, **10**, 457 (1938); see also card index file of X-ray spacings issued by American Society for Testing Materials, 260 S. Broad Street, Philadelphia, Pa.