

Anal. Calcd. for $C_9H_{18}O_6N_2$: C, 43.6; H, 6.5; OCH_3 , 42.7. Found: C, 43.7; H, 6.4; OCH_3 , 39.6.

(d) **2,3,4-Tri-O-methyl-D-glucose.**—A portion (87 mg.) of the methylated sugar lactone formed by bromine oxidation of the hydrolyzate of methylated *ti* glucofructosan (see above) was distilled in high vacuum to give a liquid (64 mg.), $[\alpha]^{20}_D +20^\circ$ in methanol (*c* 0.4). Paper chromatographic analysis followed by the hydroxamic acid test spray⁴⁴ showed that with three separate solvent systems, phenol saturated with water ($R_f = 0.9$), 1-butanol:ethanol:water (4:1:5) ($R_f = 0.86$) and methyl ethyl ketone:water azeotrope ($R_f = 0.84$) the lactone corresponded to 2,3,4-tri-O-methyl-D-glucono- δ -lactone.

Anal. Calcd. for $C_9H_{18}O_6$: equiv. wt., 220. Found: equiv. wt., 232.

An attempt to characterize the unknown lactone as crystalline 2,3,4-tri-O-methyl-D-glucosaccharo-1,5-lactone-6-methyl ester by oxidation with nitric acid followed by methanolysis was unsuccessful and treatment of the lactone ester with methanolic methylamine failed to yield the characteristic crystalline bis-methylamide of 2,3,4-tri-O-methyl-D-glucosaccharic acid.

Preparation of 3,4-Di-O-methyl-D-fructose and its Conversion to the Diamide of Methyl 3,4-Di-O-methylfructofuranosido-1,6-dicarboxylic Acid.—Fructose (1.0 g.) was dissolved in dry pyridine (40 ml.) and trityl chloride (6.2 g.) was added. After 2 days at room temperature, acetic anhydride (25 ml.) was added. The next day the mixture was poured with stirring into ice-water (300 ml.) and the resulting flocculent precipitate was separated and triturated with water until the washings were no longer acid to congo red paper. The product, after air-drying, was a powdery solid (10.3 g.) from which attempts to obtain crystalline 1,6-di-O-trityl-2,3,4-tri-O-acetyl-D-fructose were unsuccessful.

The crude material (9.27 g.) was dissolved in acetone (150 ml.) and methylated, at room temperature, with methyl sulfate and sodium hydroxide. After three successive methylations, the product being extracted after each methylation with chloroform, the crude methylated product was isolated and treated, at room temperature, with methanol (52 ml.) containing 0.5% hydrogen chloride. The gummy material went into solution when the mixture was shaken and soon thereafter trityl methyl ether separated as a copious, crystalline precipitate, m.p. 76° . The solvent was removed from the filtrate under reduced pressure after neutralization with silver carbonate, and impurities were removed by extraction with ether from aqueous solution. Water was removed under reduced pressure and the sirupy residue was

extracted with acetone. Removal of the acetone yielded sirupy methyl 3,4-di-O-methyl-D-fructoside (0.8 g.).

Anal. Calcd. for $C_9H_{18}O_6$: OCH_3 , 41.9. Found: OCH_3 , 39.0.

The methyl glycoside (106 mg.) was converted to the free sugar by treatment with 2.25% aqueous oxalic acid for 2 hours in a water-bath at 85° . Neutralization ($CaCO_3$), followed by filtration and removal of the solvent under reduced pressure, yielded a sirupy product (97 mg.) having $[\alpha]^{20}_D -23^\circ$ in methanol (*c* 3.2). The 3,4-di-O-methyl-D-fructose was converted, by the method described above for the preparation of 3,4,6-tri-O-methyl-1,2-O-isopropylidene-D-fructose, to 3,4-di-O-methyl-1,2-O-isopropylidene-D-fructose (117 mg.) which was a sirup having $[\alpha]^{20}_D -10^\circ$ in methanol (*c* 4).^{9,10} The fact that the rotation in this solvent is negative indicates that the substance is probably in the pyranose form, and hence is 1,2-O-isopropylidene-3,4-di-O-methyl-D-fructopyranose. Paper chromatograms of this derivative showed that it would afford a means of separating 3,4-dimethylfructose, with methyl ethyl ketone as the irrigating solvent, from dimethylhexoses not forming isopropylidene derivatives. The R_f value found for the isopropylidene derivative was 0.87 as compared with the value 0.29 for the free di-O-methyl sugar.

The slow moving component disappeared when the mixture of methylated sugars from methylated *ti* fructosan hydrolyzate was treated with acetone and sulfuric acid, indicating that 3,4-di-O-methyl-D-fructose was the sole dimethyl hexose component.

Conversion of the sirupy synthetic methyl 3,4-di-O-methyl-D-fructofuranoside (159 mg.) to crystalline methyl 3,4-di-O-methyl-D-fructofuranoside-1,6-dicarboxylic acid diamide by oxidation with nitric acid, methylation with methyl iodide and silver oxide, and treatment of the resulting sirupy dimethyl ester in methanolic solution with gaseous ammonia, as previously described, yielded a crude product (55 mg.) which, upon recrystallization from ethanol, had m.p. $192-193^\circ$ and $[\alpha]^{20}_D -68^\circ$ in water (*c* 0.6). There was no depression of the m.p. when mixed with the compound prepared in the same manner from the di-O-methyl-D-fructose component from the methylated *ti* glucofructan.

Anal. Calcd. for $C_9H_{18}O_6N_2$: C, 43.55; H, 6.45; N, 11.3. Found: C, 43.3; H, 6.4; N, 11.5.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF NEBRASKA]

The Preparation of 3-O- α -D-Glucopyranosyl-D-glucose¹

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3-O- α -D-glucopyranosyl-D-glucose has been isolated from a reversion mixture obtained by heating glucose and maltose in acid solution. The specific rotation of the oligosaccharide is $+87^\circ$. The crystalline phenylosazone of the compound yields an X-ray diffraction pattern identical with that of the phenylosazone of turanose, establishing the presence of an α -1,3-glucosidic bond in the new oligosaccharide.

Studies on the substrate specificity and on the mode of action of the carbohydrases have been greatly facilitated with the increased availability of pure oligosaccharides. These oligosaccharides have, for the most part, been obtained from either enzymatic digests of appropriate substrates with transglycosidases² or from partial acid hydrolysates

of polysaccharides.³ A number of glucosyl oligosaccharides^{4,5} also have been isolated from the reversion mixture obtained by heating glucose in acid solution.⁶ In the present paper, we describe methods for the isolation and characterization of a new oligosaccharide from a reversion mixture of

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(3) R. L. Whistler and D. I. McGilvray, *ibid.*, **23**, 79 (1954).

(4) A. Thompson, K. Anno, M. L. Wolfrom and M. Inatome, *THIS JOURNAL*, **75**, 1309 (1954).

(5) E. E. Bacon and J. S. D. Bacon, *Biochem. J.*, **58**, 396 (1954).

(6) E. Fischer, *Ber.*, **23**, 3687 (1890).

glucose and maltose. Structural evidence shows that this oligosaccharide is 3-O- α -D-glucopyranosyl-D-glucose. This compound is evidently identical with nigerose, a disaccharide isolated as a hydrolytic fragment of the polysaccharide, nigeran.⁷

In the present study, the oligosaccharide was isolated from the reversion mixture by a paper chromatographic procedure and was obtained as an amorphous yellow powder. The phenylosazone derivative of the compound was prepared in crystalline form and was characterized by its X-ray diffraction pattern. The specific rotation of the pure oligosaccharide is $+87^\circ$. It gives a positive Benedict test and a negative Seliwanoff test indicating the presence of a free aldehydic group. The reducing power of the oligosaccharide as measured by the method of Shaffer and Somogyi⁸ with copper sulfate reagent 60 was equivalent to that of turanose and only approximately 80% of the value for maltose. Since the oligosaccharide and turanose were oxidized to the same extent by alkaline copper sulfate, a similarity in structure of the two compounds might be expected.

Prolonged acid hydrolysis converted the new oligosaccharide quantitatively to glucose while partial acid hydrolysis yielded only glucose and unhydrolyzed oligosaccharide. These observations together with the R_f value (Table I) of the oligosaccharide point to a disaccharide structure for the compound. It is noted from the R_f values of the carbohydrates listed in Table I that the new compound moved on the paper chromatogram at a rate intermediate between turanose and maltose. According to French and Wild⁹ this type of chromatographic behavior would be characteristic of an oligosaccharide of two glucose units linked by an α -1,3-glucosidic bond. Since the compound was not fermented by bakers' yeast, the glucosidic bond is not susceptible to hydrolysis by yeast glucosidase.

TABLE I

APPARENT R_f VALUES OF GLUCOSYL COMPOUNDS AND OF THE REVERSION PRODUCTS FROM GLUCOSE AND MALTOSE

Compound	Apparent R_f value ^a Glucosyl compounds	Reversion products
Glucose	0.81	0.80
Turanose	.67	..
New oligosaccharide	.61	.60
Maltose	.54	.53
Cellobiose	.52	.50
Isomaltose	.39	.39
Lactose	.36	..

^a Since multiple ascent chromatography was used, apparent R_f values are recorded. These values are obtained by dividing the height to which the individual compounds have moved by the total height of the paper.

The phenylosazone derivatives of the compound and of turanose were prepared in crystalline form. Under the conditions used for qualitative identification of carbohydrate phenylosazones, the osazone of the compound crystallized from solution in a 20-minute heating period while the osazone of turanose

did not crystallize until cooled. Melting points and X-ray diffraction patterns for the crystalline phenylosazones of the oligosaccharide and of turanose were identical. Since the new oligosaccharide yields the same phenylosazone as turanose, the difference in the structure of turanose and the new compound must be at carbon atoms 1 and 2. In turanose, the glucosyl portion of the disaccharide is joined by an α -glucosidic bond to the 3-position of fructose and the reducing group is, therefore, located at carbon atom 2. On the basis of the evidence at hand, the glucosyl portion of the oligosaccharide is apparently joined by an α -glucosidic bond to the 3-position of a second glucose unit and the reducing group is located at carbon atom 1. The proposed structure for the oligosaccharide is 3-O- α -D-glucopyranosyl-D-glucose.

Experimental

Reversion Products of Glucose.—A sample of 6 g. of glucose was dissolved in 15 ml. of 0.1 *N* hydrochloric acid and heated in a boiling water-bath for five hours. At the end of this time the solution was neutralized with solid sodium carbonate. Qualitative chromatograms of 0.01-ml. samples of the neutralized solution were prepared and compared with similar chromatograms prepared from a reaction mixture of glucose and maltose. Aliquots of the neutralized solution were also subjected to fermentation treatment described in a subsequent section and were used to determine the yield of the new oligosaccharide.

Reversion Products of Glucose and Maltose.—Samples of 2 g. of glucose and 4 g. of maltose were dissolved in 15 ml. of 0.1 *N* hydrochloric acid, heated in a boiling water-bath for 5 hours, cooled to room temperature and neutralized with solid sodium carbonate. Comparison of the qualitative paper chromatogram of this solution with that obtained for the mixture described in the preceding section and with reference oligosaccharides showed that a number of disaccharides and trisaccharides had been produced by the acid treatment of either glucose and maltose or of glucose alone. Typical apparent R_f values for a number of reference compounds and for the reaction products are recorded in Table I. The sprayed chromatograms appeared very similar to that obtained by Bacon and Bacon⁵ for the reversion products of glucose.

Duplicate samples of 5 ml. of the neutralized reaction mixtures of glucose and maltose and of glucose were mixed with 5 ml. of bakers' yeast suspension (20%) and allowed to stand at room temperature for 48 hours. Examination of the clear supernatant solution by paper chromatography showed that the glucose and maltose had been completely removed by this treatment. Samples of 0.2 ml. of the supernatant solution were placed on paper strips and developed by methods previously described.¹⁰

The fast moving reducing compound was eluted from the paper and determined quantitatively with diphenylamine reagent.¹⁰ The yield of the compound from a mixture of glucose and maltose was 0.16 g. and the yield of the compound from glucose alone was 0.11 g. Since the yield of the oligosaccharide was higher from a mixture of glucose and maltose than from glucose alone, the mixture is to be preferred for preparing the oligosaccharide.

Preparation of 3-O- α -D-glucopyranosyl-D-glucose.—A solution of 12 g. of glucose and 24 g. of maltose in 90 ml. of 0.1 *N* hydrochloric acid was heated for 5 hours in a boiling water-bath, neutralized with sodium carbonate and stirred with 5 g. of bakers' yeast for 48 hours. Aliquots of 0.2 ml. of the supernatant solution were placed on paper chromatograms and developed by three ascents of the solvent system of 6 parts *n*-butyl alcohol, 4 parts of pyridine and 3 parts of water. Two strips (1 cm. wide) were cut from each chromatogram, sprayed with copper sulfate reagent¹¹ and used for marking the unsprayed portions of the chromatogram. The oligosaccharide was then extracted from the paper and further purified by repetition of the chromatographic separation.

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(8) P. A. Shaffer and M. Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

(9) D. French and G. M. Wild, *THIS JOURNAL*, **75**, 2612 (1953).

(10) J. H. Pazur, *J. Biol. Chem.*, **205**, 75 (1953).

(11) Reagent 60 described in reference 8 but without potassium iodide

ration. After final extraction and concentration the compound was precipitated with acetone, filtered and dried in a vacuum oven at 60°. The yield was 0.4 g. and the specific rotation of the compound was found to be +87° (*c* 1, water).

Reducing Power.—Samples of 1.6 mg. of the compound, of maltose and of turanose were used for the determination of reducing values with Reagent 60 of Shaffer and Somogyi.⁸ The values expressed in ml. of 0.005 *N* sodium thiosulfate were as follows: 4.46 for the new compound, 5.45 for maltose and 4.35 for turanose. The oligosaccharide and turanose possess approximately 80% of the reducing value of maltose.

Paper Chromatography.—Droplets of 0.01 ml. of the solutions of the new compound, glucose, maltose, turanose, lactose, isomaltose, cellobiose and the original reaction mixtures were placed near the bottom of a square (20 cm.) of filter paper (Eaton and Dikeman No. 613). The filter paper was rolled in a cylinder and placed in a solvent of *n*-butyl alcohol-pyridine-water (6:4:3 by volume). Four ascents of the solvent were used for developing the chromatogram. The finished strips were sprayed with copper sulfate reagent¹¹ and heated in an oven at 100° for 10 minutes in which time the areas at which carbohydrates were present turned yellow. The apparent *R_f* values obtained by dividing the distance to which the compounds had moved by the total height of the paper are recorded in Table I.

Fermentation Tests.—Samples of 0.2 ml. of 2% solutions of the new oligosaccharide, glucose, maltose and isomaltose were mixed with 0.2 ml. of 20% suspension of bakers' yeast. Aliquots of the digest were analyzed for reducing sugars at 0, 6, 24 and 48 hour reaction periods by paper chromatography. The analyses showed that under these conditions the glucose and maltose had completely disappeared from the reaction mixture in 24 and 48 hours, respectively, while the new oligosaccharide and isomaltose remained unchanged.

Acid Hydrolysis of the Oligosaccharide.—One ml. of a solution containing 4 mg. of the oligosaccharide was mixed with 1 ml. of 0.1 *N* hydrochloric acid. The test-tube containing the mixture was stoppered tightly and heated in an oven at 100° for 2 hours. Samples removed during heating at 0, 0.5, 1 and 2 hour intervals were placed on paper chromatograms and analyzed for reducing sugars. Examination of the sprayed chromatograms showed that only glucose and the unhydrolyzed compound were present during the course

of the hydrolysis. Further, in the 2-hour period the compound appeared to be completely hydrolyzed to glucose. Samples of 0.4 ml. of the 2-hour hydrolysate (equivalent to 0.8 mg. of the compound) and 0.8 mg. of glucose were used for determination of reducing values. The reducing values expressed in ml. of 0.005 *N* sodium thiosulfate were 5.46 for the acid hydrolysate of the compound and 5.42 for 0.8 mg. of glucose. The acid treatment had, therefore, converted the new compound quantitatively to glucose.

Preparation of Phenylsazone Derivative.—A solution of 0.1 g. of the oligosaccharide, 0.2 g. of phenylhydrazine hydrochloride and 0.3 g. of sodium acetate in 2 ml. of water was heated in a boiling water-bath for 30 minutes. The compound that precipitated from solution was collected on a filter, air-dried, and recrystallized from 3 ml. of ethyl alcohol; m.p. 203–205° dec., mixed m.p. with turanose phenylsazone 203–206° dec., literature value 204–206°.⁷ The X-ray diffraction pattern¹² for this compound gave the following data: 5.42¹³ – 50¹⁴; 5.13 – 40; 4.68 – 20 (double); 4.27 – 80; 4.05 – 80; 3.51 – 10; 3.32 – 100; 3.17 – 5; 2.69 – 50; 2.21 – 20; 2.10 – 10.

Preparation of Turanose Phenylsazone.—A solution of 0.2 g. of turanose, 0.4 g. of phenylhydrazine hydrochloride and 0.6 g. of sodium acetate in 5 ml. of water was heated in a boiling water-bath for 30 minutes. The phenylsazone which formed on cooling was collected on a filter and air-dried. It was recrystallized from 10 ml. of ethyl alcohol; m.p. 205–206° dec., literature values 200–205°¹⁵ dec., and 204–206°.⁷ The X-ray diffraction data for the turanose phenylsazone was: 5.42¹³ – 40¹⁴; 5.13 – 40; 4.68 – 20 (double); 4.25 – 70; 4.05 – 70; 3.50 – 10; 3.32 – 100; 3.16 – 10; 2.69 – 50; 2.21 – 10; 2.10 – 5. Comparison of these values with those in the preceding section shows that the phenylsazone of turanose and of the oligosaccharide are identical and, therefore, the glucose units of the oligosaccharide must be joined by the same type of linkage as is present in turanose, namely, the α -1,3-linkage.

(12) We wish to thank Mr. W. C. Robison, Electrical Engineering Department and the University Instrument Laboratory, University of Nebraska, for the X-ray patterns.

(13) Interplanar spacings, Å., CuK α radiation.

(14) Relative intensities on basis of 100 for the strongest line.

(15) C. S. Hudson, *J. Org. Chem.*, **9**, 470 (1944).

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[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

Synthesis of Maltose-1-C¹⁴, Maltobiono- δ -lactone-1-C¹⁴, and Lithium Maltobionate-1-C¹⁴ from 3-(α -D-Glucopyranosyl)-D-arabinose^{1,2}

BY HORACE S. ISBELL AND ROBERT SCHAFER

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A method is presented for the production of maltose-1-C¹⁴ from sodium cyanide-C¹⁴ and 3-(α -D-glucopyranosyl)-D-arabinose in 42% yield. The process includes the intermediate production of lithium maltobionate-1-C¹⁴ trihydrate and maltobiono- δ -lactone-1-C¹⁴. Lithium maltobionate trihydrate is the first metallic salt of maltobionic acid to be crystallized. It was prepared not only by the cyanohydrin synthesis but also by the electrolytic oxidation of maltose in the presence of lithium bromide and lithium bicarbonate. Crystalline maltobiono- δ -lactone is likewise a new substance. Concentration of Methyl Cellosolve (ethylene glycol monomethyl ether) solutions of maltobionic acid in the presence of seed crystals leads to a slow conversion of the acid to the crystalline lactone.

Numerous applications of C¹⁴-labeled maltose can be envisioned in the fields of enzymology, biochemistry and nutrition. Hence the development of a method for the synthesis of maltose-1-C¹⁴, similar to the methods previously described for the

production of other sugars^{3–6} was undertaken. The C¹⁴-labeled products were prepared by the following steps:

(3) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952).

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(5) H. S. Isbell and J. V. Karabinos, *ibid.*, **48**, 438 (1952); H. L. Frush and H. S. Isbell, *ibid.*, **51**, 167, 307 (1953); **54**, 267 (1955).

(6) H. S. Isbell, H. L. Frush and N. B. Holt, *ibid.*, **53**, 217, 325 (1954); H. S. Isbell, H. L. Frush and R. Schaffer, *ibid.*, **54**, 201 (1955).

(1) Part of a project on the development of methods for synthesis of radioactive carbohydrates, sponsored by the Atomic Energy Commission.

(2) Presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.