[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Isolation of 5-O-β-D-Glucopyranosyl-D-glucose from Hydrol

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The presence of 5-O-β-D-glucopyranosyl-D-glucose in the disaccharide fraction of a representative sample of hydrol has been established through carbon column chromatography. The sugar was characterized as the known, crystalline *p*-toluenesulfonylhydrazone. Evidence is presented that the disaccharide arises in the mixture from the acid-catalyzed reversion of D-glucose.

Hydrol, the residual mother liquor in the production of D-glucose by acid hydrolysis of corn starch, is recognized as a complex mixture containing D-glucose and its related oligosaccharides. The latter arise partly from incomplete hydrolysis of the starch and partly from the acid-catalyzed "reversion" of D-glucose. In recent years, the application of chromatographic techniques has resulted in the identification of the principal disaccharides both of hydrol and of simple acid reversion mixtures from D-glucose. Of the eleven possible disaccharides that can be formed by substitution of an α- or β-D-glucopyranosyl residue on the free hydroxyl groups of D-glucopyranose, eight have been identified previously in these mixtures. The present report describes the identification in hydrol and acid-treated solutions of D-glucose of 5-O-β-D-glucopyranosyl-D-glucose, one of the two possible disaccharides resulting from substitution of a D-glucopyranosyl residue on the hydroxyl group at carbon-5 of D-glucofuranose.

Hydrol was chromatographed on carbon–Celite[2] using water and 3, 5 and 15% ethanol as successive elution solvents. The following sugars, in the order of their appearance off the chromatogram, were identified: D-glucose, isomaltose (6-O-α-D-glucopyranosyl-D-glucose), 5-O-β-D-glucopyranosyl-D-glucose, gentiobiose and cellobiose. The D-glucose, gentiobiose and cellobiose were crystallized directly from the appropriate fractions after concentration. Isomaltose was characterized as its crystalline octaacetate and 5-O-β-D-glucopyranosyl-D-glucose as the crystalline *p*-tosylhydrazone through comparison with a known synthetic sample.[3]

In a parallel experiment, an acid reversion mixture from D-glucose also was examined. A preliminary fractionation of this mixture was made on carbon–Celite with water and 5, 15 and 25% ethanol as successive developers.[4] Rechromatography of the fractions using aqueous ethanol of various concentrations then yielded, in the order of their appearance off the chromatograms, maltose, α,α-trehalose, isomaltose, gentiobiose and cellobiose. The maltose, α,α-trehalose and gentiobiose were isolated by direct crystallization, whereas the isomaltose and cellobiose were identified as their crystalline octaacetates. Acetylation of a portion of the "isomaltose fraction," followed by chromatography on Magnesol–Celite,[5] established the presence in this fraction of a minor amount of 5-O-β-D-glucopyranosyl-D-glucose.

The occurrence of 5-O-β-D-glucopyranosyl-D-glucose in a starch hydrolysate is of interest because of the suggestion by Blom and Schwarz[6] that a 1 → 5-linkage may be preformed in starch. However, isolation of the sugar from hydrol is in no way confirmatory of its pre-existence in the starch structure, since it occurs also in simple acid reversion mixtures from D-glucose.

The disaccharides that now have been identified in hydrol and acid reversion mixtures from D-glucose are listed in Table I. It seems likely that the remaining D-glucose disaccharides of pyranosidic structure also are present in these complex mixtures, although the concentration of certain members may be extremely small due to a combination of factors unfavorable to their formation. It has been pointed out[4] that steric considerations probably explain the preponderance of isomaltose and gentiobiose in the disaccharide fraction. In addi-

TABLE I

DISACCHARIDES FROM HYDROL AND ACID REVERSION OF D-GLUCOSE

Disaccharide	Reference: Hydrol	Reference: D-Glucose reversion
α-D-Glucopyranosyl-α-D-glucopyranoside (α,α-trehalose)	7	8
β-D-Glucopyranosyl-β-D-glucopyranoside (β,β-trehalose)	..	4
2-O-β-D-Glucopyranosyl-D-glucose (sophorose)	..	4
3-O-α-D-Glucopyranosyl-D-glucose (nigerose)	..	4, 9
4-O-α-D-Glucopyranosyl-D-glucose (maltose)	7	10
4-O-β-D-Glucopyranosyl-D-glucose (cellobiose)	8, 11	4
5-O-β-D-Glucopyranosyl-D-glucose	8	8
6-O-α-D-Glucopyranosyl-D-glucose (isomaltose)	7	12
6-O-β-D-Glucopyranosyl-D-glucose (gentiobiose)	13	10

(1) Corn Industries Research Foundation Fellow, 1951–1954.

(2) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

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tion, since the reversion reaction is reversible, the comparative stability to hydrolysis of the various disaccharides is a contributing factor in determining the concentration of each that may accumulate. This could explain the failure, so far, to isolate any member of the additional family of D-glucose disaccharides in which an α- or β-D-glucofuranosyl residue is substituted on a hydroxyl group of D-glucopyranose or D-glucofuranose, since the furanosides are known to be hydrolyzed much more readily by acids than are the pyranosides.

Experimental

Isomaltose.—A 37.2-g. aliquot of the sample of hydrol described previously[11] was diluted to 100 ml. with water and the solution was introduced onto a 300 × 75 mm. bed of washed, wet carbon–Celite.[2] D-Glucose was removed from the column by the passage of water (6–7 l.) under slight suction at a rate of 8–900 ml./hr. until the effluent was negative to Benedict reagent. Aqueous 3% ethanol[14] then was passed through in the same manner and each 100 ml. of effluent was examined for optical activity and reducing action toward Benedict reagent. The initial 1600 ml. of effluent following introduction of the 3% ethanol at the top of the column was discarded and the next 1400 ml. yielded, on concentration,[15] 2.35 g. of amorphous residue. Acetylation[16] of 2 g. of this material produced 1.6 g. of β-isomaltose octaacetate, m.p. 144–145° and $[\alpha]^{25}D$ 98° (*c* 1 in chloroform).

Several unsuccessful attempts were made to obtain crystalline isomaltose[17] from the purified octaacetate by deacetylation in methanol solution with either barium methoxide or sodium methoxide. Slow evaporation of ethanol solutions of the free sugar thus obtained yielded solid material that showed m.p. 119–122° (after slight shrinkage at 85–90°) and $[\alpha]^{25}D$ 121° (*c* 0.6 in water). Examination by X-ray, however, showed these preparations to be noncrystalline.

5-*O*-β-D-Glucopyranosyl-D-glucose.—Following the isomaltose fraction, 100 ml. of effluent from the carbon–Celite column was discarded and the subsequent 1500 ml. was concentrated to yield 220 mg. of sirup showing $[\alpha]^{25}D$ −22.3° (*c* 1 in water). The reported[3] specific rotation for sirupy 5-*O*-β-D-glucopyranosyl-D-glucose is −23.8° in water. Treatment[3] of the sirup with *p*-toluenesulfonylhydrazine provided the corresponding hydrazone of 5-*O*-β-D-glucopyranosyl-D-glucose, m.p. and m.m.p.[18] 180–181° dec.; $[\alpha]^{25}D$ −22° (*c* 1 in pyridine–water (4:1)).

Anal. Calcd. for $C_{19}H_{30}O_{12}N_2S$: C, 44.7; H, 5.92; N, 5.48; S, 6.65. Found: C, 45.3; H, 6.03; N, 5.28; S, 6.39.

X-Ray powder diffraction data for the hydrazone prepared from hydrol and for the synthetic sample[3] were identical: 11.8[19]–12,[20] 7.37–75, 6.01–38, 5.00–19, 4.78–9, 4.59–31, 4.31–100, 4.11–50, 3.98–6, 3.85–38, 3.66–12, 3.26–12, 2.96–25, 2.86–9, 2.76–3, 2.48–6, 2.40–9, 2.29–12, 2.24–3.

Acetylation of the sirupy 5-*O*-β-D-glucopyranosyl-D-glucose from hydrol yielded an amorphous product with $[\alpha]^{25}D$ −30.4° (*c* 0.5 in chloroform). A 50-mg. sample of the acetate was chromatographed[5] on a 200 × 24 mm. bed of Magnesol–Celite using 250 ml. of benzene–ethanol (250:1, v./v.) as developer. After being extruded and streaked with alkaline permanganate solution, the column showed the presence of a single zone of carbohydrate material at 50–105 mm. from the top. The amorphous acetate was recovered from the isolated zone by extraction with acetone and concentration, yield 46 mg., $[\alpha]^{25}D$ −31° (*c* 0.5 in chloroform). Deacetylation, with sodium methoxide in methanol, regenerated the sirupy disaccharide, $[\alpha]^{25}D$ *ca.* −20° in water.

Gentiobiose.—An additional 1 l. of 3% ethanol was passed through the carbon–Celite column, the effluent being discarded, and the developing solution then was changed to 5% ethanol. The initial 1600 ml. of this effluent was discarded and concentration of the next 2 l. yielded 2.21 g. of amorphous residue. Crystallization of this material from 3 ml. of warm methyl cellosolve (ethylene glycol monomethyl ether), according to the directions of Thompson and Wolfrom,[21] yielded 1.21 g. of gentiobiose, m.p. 191–192°; $[\alpha]^{20}D$ 10° (*c* 2, final in water). Acetylation of 100 mg. of the sugar gave 180 mg. of β-gentiobiose octaacetate, m.p. 193–194°.

Cellobiose.—After elution of the gentiobiose, 15% ethanol was passed through the carbon–Celite column. The initial 600 ml. of this effluent was discarded and concentration of the next 800 ml. then yielded 980 mg. of amorphous residue. Crystallization of this material from aqueous methanol provided 150 mg. of cellobiose, m.p. after recrystallization 229–230°; $[\alpha]^{25}D$ 34.3° (*c* 0.35, final in water). Acetylation of 50 mg. of the sugar yielded 60 mg. of β-cellobiose octaacetate, m.p. after recrystallization 199–200°; $[\alpha]^{25}D$ −13.8° (*c* 1.1 in chloroform).

Chromatography of D-Glucose Reversion Products

Preliminary Fractionation.—A solution of 100 g. of D-glucose in 100 ml. of 0.08 *N* hydrochloric acid was heated in a boiling water-bath for ten hours. After cooling, the acidity was removed by ion exchange and the solution was introduced, with slight suction, onto a 340 × 95 mm. column of washed, wet carbon–Celite. The column then was developed with water (13.5 l.), 5% ethanol (9.3 l.) and 15% ethanol (4.1 l.) until each effluent reacted negatively to Benedict reagent. Upon concentration, the water effluent yielded 70 g. of D-glucose by direct crystallization. The 5 and 15% ethanol effluents yielded on concentration 8.8 and 7.9 g., respectively, of amorphous residues.

Maltose and α,α-Trehalose.—The sirup (8.8 g.) from the 5% ethanol effluent was rechromatographed on a 400 × 75 mm. carbon–Celite column with 3 and 5% ethanol. The course of the fractionation was followed in the effluent by observing optical rotations and by testing with Benedict and Molisch reagents. The first liter of 3% ethanol effluent was discarded and the next 800 ml. was concentrated to a sirup weighing 500 mg. ("maltose-α,α-trehalose" fraction). The next 2.8 l. of 3% ethanol effluent yielded 3.8 g. of sirupy residue ("isomaltose fraction"). Finally, 1.2 l. of 5% ethanol was passed through the column and the effluent concentrated to a sirupy residue weighing 3.4 g. ("gentiobiose fraction").

The "maltose-α,α-trehalose fraction" (500 mg.) was rechromatographed on a 600 × 34 mm. carbon–Celite column with 5% ethanol. The first 800 ml. of effluent was discarded and the next 300 ml. was concentrated to a sirup. Crystallization of this sirup from ethanol yielded 165 mg. of maltose hydrate, m.p. 99–101°; $[\alpha]^{25}D$ 129° (*c* 2, final in water); octaacetate, m.p. 159–160°.

The next 1.2 l. of effluent from the column yielded, on concentration, a non-reducing sirup. Crystallization from ethanol provided 105 mg. of α,α-trehalose dihydrate, m.p. 96–97°; $[\alpha]^{25}D$ 177° (*c* 2 in water); octaacetate, m.p. 97–98°.

Isomaltose and 5-*O*-β-D-Glucopyranosyl-D-glucose.—The "isomaltose fraction" above (3.8 g.) was rechromatographed on a 750 × 40 mm. carbon–Celite column with 3% ethanol. The first 2 l. of effluent was discarded and the next 2.1 l. was collected in 100-ml. cuts which then were concentrated separately. The residues from the first fifteen of these fractions showed specific rotations in water in the range 110–

(14) All aqueous ethanol developing solutions were prepared on a w./w. basis.

(15) All concentrations were performed at water aspirator pressure.

(16) All acetylations were performed with hot acetic anhydride and sodium acetate.

(17) E. M. Montgomery, F. B. Weakley and G. E. Hilbert, THIS JOURNAL, **71**, 1682 (1949).

(18) The reported melting point[3] of this derivative is 193–194° dec. However, the material sent to us by Professor Freudenberg melted alone or admixed with the sample prepared from hydrol at 180–181°, when the rate of heating was moderate. Rapid heating raised the melting-decomposition point to approximately 190°, whereas samples maintained at 170° melted with decomposition after several minutes. The crystals appeared to undergo a reversible weight loss of approximately 3% when dried at room temperature in high vacuum over phosphorus pentoxide. Due to lack of sufficient material, the possibility of hydrate formation has not been further explored.

(19) Interplanar spacing, Å., CuKα radiation. We are indebted to Mr. Eugene McLaren of this Department for the X-ray data.

(20) Relative intensities estimated by multiple film technique.

(21) A. Thompson and M. L. Wolfrom, THIS JOURNAL, **75**, 3605 (1953).

120°. They were combined and acetylated to yield 3.2 g. of β-isomaltose octaacetate, m.p. 145–146°.

The residues from the last six 100-ml. fractions above, which showed specific rotations in water in the range 80–105°, were combined and acetylated. Fractional crystallization from ethanol then yielded 50 mg. of β-gentiobiose octaacetate, m.p. and m.m.p. 194–195°, and 240 mg. of β-isomaltose octaacetate, m.p. 144–145°; $[\alpha]^{25}D$ 98° (*c* 1 in chloroform). The sirupy residue from these crystallizations, in two 40-mg. portions, then was chromatographed on 200 × 24 mm. columns of Magnesol–Celite, using 200 ml. of benzene–ethanol (250:1, v./v.) as developer. Streaking of the extruded columns with alkaline permanganate showed two well-separated zones at 1–55 and 80–105 mm., respectively, from the top. Precisely similar zones were observed when a mixture of known β-isomaltose octaacetate and 5-O-β-D-glucopyranosyl-D-glucose octaacetate prepared from hydrol were chromatographed in the same manner. The combined top zones, after elution with acetone and concentration, provided 53 mg. of β-isomaltose octaacetate, m.p. 145–146°. The combined lower zones yielded 21 mg. of sirupy 5-O-β-D-glucopyranosyl-D-glucose octaacetate, $[\alpha]^{25}D$ −29 ± 1° (*c* 0.8 in chloroform).

Gentiobiose.—The "gentiobiose fraction" above (3.4 g.) yielded, by direct crystallization from methyl cellosolve, 1.0 g. of gentiobiose, m.p. 192–193°; $[\alpha]^{25}D$ 10.2° (*c* 0.5, final in water); octaacetate, m.p. 194–195°.

Cellobiose.—The residue (7.9 g.) of the 15% ethanol effluent from the preliminary fractionation was rechromatographed on a 750 × 40 mm. carbon–Celite column with 8% ethanol. The first 1.8 l. of effluent was concentrated to an amorphous residue weighing 160 mg. Acetylation yielded 80 mg. of β-cellobiose octaacetate, m.p. and m.m.p. 200–201°.

An additional 7.5 g. of carbohydrate material was desorbed from this column with 3 l. of 15% ethanol. Acetylation of this fraction yielded no crystalline material.

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

The Constitution of the Hemicellulose of Western Hemlock (Tsuga heterophylla). I. Determination of Composition and Identification of 2-O-(4-O-Methyl-D-glucopyranosiduronic acid)-D-xylose[1]

BY G. G. S. DUTTON AND F. SMITH

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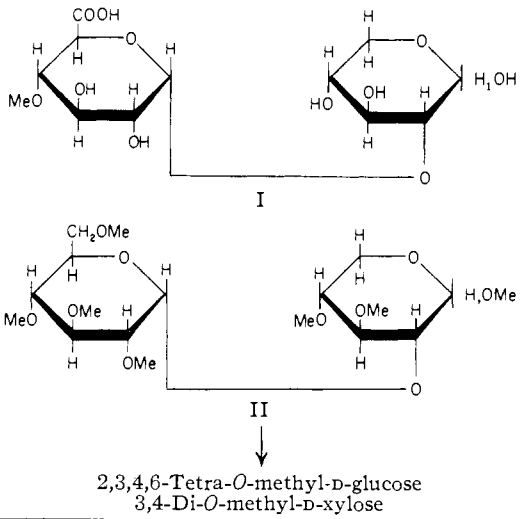
The hemicellulose of Western Hemlock (*Tsuga heterophylla*) gave upon hydrolysis arabinose, xylose, galactose, glucose, mannose and an aldobiouronic acid (I). The aldobiouronic acid (I) has been identified as 2-O-(4-O-methyl-α-D-glucuronosyl)-D-xylose.

Western Hemlock hemicellulose obtained from delignified sawdust by alkaline extraction gave upon hydrolysis a mixture of an aldobiouronic acid (I), arabinose, xylose, galactose, glucose and mannose. The acidic component I, which forms the subject of this communication, was separated from the hydrolysate by the use of an anion-exchange resin. The methoxyl content of I and its equivalent weight indicated that it was composed of a methoxyuronic acid and a pentose sugar, a deduction further substantiated by the observation that vigorous hydrolysis furnished D-xylose and 4-O-methyl-D-glucuronic acid.

Cleavage of the aldobiouronic acid (I) with 8% methanolic hydrogen chloride at 105° for 10 hr., followed by treatment of the cleavage products with ammonia, yielded the crystalline amide of methyl 4-O-methyl-α-D-glucopyranosiduronic acid.[2]

After removal of this uronic acid derivative hydrolysis of the neutral sugar glycoside gave crystalline D-xylose. The point of attachment of the 4-O-methyl-D-glucuronic acid unit to the D-xylose residue was determined from a study of the fully methylated disaccharide II obtained by lithium aluminum hydride reduction[3,4] and methylation of the aldobiouronic ester. Hydrolysis of II and chromatographic separation of the components on a cellulose–hydrocellulose column[5] using methyl ethyl ketone–water azeotrope as the irrigating solvent[6] yielded 3,4-di-O-methyl-D-xylose[7] and 2,3,4,6-tetra-O-methyl-D-glucose.[8] The former was identified as the crystalline 3,4-di-O-methyl-D-xylono-δ-lactone[7] and the latter both as the crystalline sugar[8] and as the crystalline anilide.[9]



(1) Paper No. 3480, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota. This paper was presented at the 128th A.C.S. meeting in Minneapolis, September, 1955.

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