product in ethyl ether was decolorized with activated carbon. Evaporation of the ether yielded a crystalline product which was refined by recrystallization from ethyl ether–petroleum ether to produce crystalline pentadecaacetylmaltotetraitol, m.p. 113–113.5° (uncor.), $[\alpha]^{25}D$ +52.6° (*c* 0.49, chloroform).

Anal. Calcd. for $C_{54}H_{74}O_{36}$: C, 49.92; H, 5.74. Found: C, 49.87; H, 5.84.

3-[1′(*O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl)-*d*-*erythro*-trihydroxypropyl]-1-phenylflavazole.[13]—To 2.52 g. (0.005 mole) of maltotriose in 50 ml. of water were added 0.55 g. of freshly crystallized *o*-phenylenediamine (0.005 mole), 2.7 g. of distilled phenylhydrazine (0.025 mole) and 4.2 g. of acetic acid (0.07 mole). The solution was refluxed under carbon dioxide for nine hours. On cooling, the product was obtained crystalline in a crude form; yield 0.4 g. (12%). Recrystallization from glacial acetic acid produced the flavazole as a yellow material, crystalline, as verified by X-ray diffraction studies, m.p. 180–182°, $[\alpha]^{25}D$ +87.0° (*c* 0.5, pyridine).

Anal. Calcd. for $C_{30}H_{36}O_{13}N_4$: N, 8.48. Found: N, 8.18.

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LAFAYETTE, INDIANA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

A Crystalline Galactobiose from Acid Hydrolysis of Okra Mucilage[1]

BY ROY L. WHISTLER AND H. E. CONRAD

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By acid hydrolysis of okra mucilage there is obtained a crystalline galactobiose which is shown to be 4-*O*-D-galactopyranosyl-D-galactopyranose. This is the first isolation of a crystalline galactobiose. Its occurrence as a hydrolytic fragment of okra mucilage suggests that 1 → 4′-glycosidic linkages are present between some of the D-galactose units in the polysaccharide.

Common okra, *Hibiscus esculentus*, which is widely grown in the southern United States for food, is known for the viscous, mucilagenous solutions which result when it is crushed and extracted with water. It is this mucilagenous property which led to its examination for blood volume expansion.[2]

Aqueous extraction of defatted okra yields a solution of a polysaccharide composed of D-galactose, D-galacturonic acid and L-rhamnose. In view of the clinical interest in the mucilagenous polysaccharide an examination of its structure has been undertaken in this Laboratory. Here is reported the isolation and characterization of a crystalline galactobiose obtained upon incomplete acid hydrolysis of okra mucilage. Evidence is also presented for the existence of two additional galactobioses from the same hydrolyzate.

The disaccharide is obtained by stopping the hydrolysis of okra mucilage at the appropriate stage of maximum yield. On neutralization of the hydrolyzate it is fractionated using charcoal chromatography.[3] After washing the column with water to remove monosaccharides, the disaccharides are eluted with 5% ethanol. This eluate, which contains a mixture of oligosaccharides, as shown by paper chromatography, is further fractionated on a column of cellulose[4] to yield three distinct neutral disaccharide components. Paper chromatographic analyses made during the acid hydrolysis of each of the three neutral disaccharides show D-galactobiose as the only product. Consequently, the assumption may be drawn that the three neutral disaccharides are galactobioses differing only in the manner in which the D-galactose units are linked to each other.

The disaccharide obtained in highest yield has been crystallized and its physical constants have been determined. On methylation and hydrolysis of the completely methylated product there is obtained 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-galactose. Periodate oxidation of the unmethylated disaccharide in alkaline solution yields formaldehyde, which is evidence that the C-5 of the reducing moiety of the disaccharide cannot be involved in the glycosidic linkage. Thus, the galactobiose is 4-*O*-D-galactopyranosyl-D-galactopyranose. Its high positive rotation suggests an alpha configuration at the anomeric carbon involved in the linkage. This is the first reported isolation of an unsubstituted crystalline galactobiose. Since subjecting crystalline D-galactose to the same conditions as those used in the partial hydrolysis of the mucilage does not give rise to this disaccharide, the galactobiose must arise as a true fragment of the polysaccharide.

Experimental

Material.—Fresh okra pods were cut into small pieces and macerated with 95% ethanol in a Waring blendor. After filtration on a large büchner funnel the residue was washed twice with 95% ethanol and twice with acetone. The air-dried product was then extracted with water in a Waring blendor. Insolubles were removed in an International Centrifuge and were re-extracted with water. The mucilagenous extracts were combined and passed through a Sharples Supercentrifuge at 40,000 r.p.m. to remove the last traces of insoluble material. To the centrifugate was added an equal volume of 95% ethanol to precipitate the mucilage, which was filtered on cheese cloth and dried by washing in a Waring blendor once with 95% ethanol and twice with fresh portions of absolute ethanol. It was finally washed with ether and placed over calcium chloride in a vacuum desiccator. The yield was approximately 1.5% of the dry material estimated to be present in the fresh okra pods. That the mucilage is located in the pods and not in the seeds was shown by carefully separating the seeds from the pods and finding the mucilage could be extracted only from pods.

Hydrolysis of Okra Mucilage.—To 50 g. of okra mucilage homogeneously dispersed in 2 l. of water at 80° there was

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added 500 ml. of a sulfuric acid solution to give a 5% acid solution. Hydrolysis proceeded at 80° with stirring for 1 hour at which time the maximum yield of disaccharide was attained, as previously shown by semi-quantitative paper chromatography. The cooled hydrolyzate was neutralized with 750 g. of barium hydroxide. After removal of the barium sulfate by filtration the solution was suitable for chromatographic separation.

Chromatography of Hydrolyzate.—The neutral filtrate was chromatographed on a 44 × 265 mm. Darco:Celite (1:1) column.[3] Monosaccharides were removed with 8 l. of water and were discarded. The disaccharide fraction was eluted with 4 l. of 5% ethanol, concentrated *in vacuo* to a sirup and rechromatographed on a 44 × 265 mm. column of powdered cellulose[4] using as eluant a mixture of butanol-1, pyridine and water (6:4:3). Fractions from this column were collected by an automatic fraction collector.[5] These were concentrated *in vacuo* and analyzed qualitatively by paper strip chromatography using the same developing solvent as above. Fractions containing chromatographically identical materials were combined and concentrated to sirups. In this manner three components were obtained in pure form from the disaccharide fraction.

Characterization of Disaccharide Components.—The three purified components moved at distinctly different rates on paper chromatograms. R_{gal} values [ratio of distance traveled by unknown on a paper chromatogram to distance traveled by galactose] in butanol-1, ethanol, water solvent (5:1:4, top layer) were 0.66, 0.41 and 0.25. A small amount of each of these sirups was taken up in 3 ml. of water in a test-tube and 1 drop of concentrated sulfuric acid was added to each tube which was held at a temperature of 100°. At 15-min., 30-min. and 60-min. periods after the start of hydrolysis several drops of hydrolyzate were withdrawn from each tube and neutralized with barium carbonate. Chromatograms of these aliquots showing the progress of hydrolysis for each of the three components show in each case no intermediate spots between the spot for the original material and the single monosaccharide spot, galactose. Thus, each of the components obtained in the 5% ethanol eluate from the carbon column is a disaccharide composed solely of galactose.

The galactobiose obtained in largest yield (R_{gal} 0.41) was crystallized from water–methanol–butanol[6] and was recrystallized several times to constant m.p. (210–211°) and $[\alpha]^{25}D$ +177°. A crystalline X-ray pattern was obtained for this material. *Anal.* Calcd. for $C_{12}H_{22}O_{11}$: C, 42.1; H, 6.5. Found: C, 42.05; H, 6.52. This is the material characterized as described below.

Methylation of Galactobiose.—Crystalline galactobiose (0.35 g.) was dissolved in water (5 ml.), and dimethyl sulfate (5 ml.) was added. Aqueous sodium hydroxide (7.5 ml., 40%) was added dropwise with stirring over an 8-hour period at 0°. The mixture was stirred an additional 15 hours and solid sodium hydroxide (5 g.) was added. Dimethyl sulfate (12 ml.) was added dropwise over an 8-hour period with stirring and cooling as before. When the solution had been stirred an additional 15 hr. it was acidified with dilute hydrochloric acid and extracted continuously with chloroform for 24 hours. The extract was concentrated *in vacuo* to a sirup (0.46 g.) which was twice methylated with methyl iodide (5 ml.) and silver oxide (2 g.); yield 0.42 g., $[\alpha]^{25}D$ +116°.

Hydrolysis and Identification of Products.—The methylated sirup (0.39 g.) was refluxed with 4% methanolic hydrochloric acid (15 ml.) for 6 hours. Water (15 ml.) was added, the methanol was boiled off, and the aqueous solution was heated at 100° for an additional 3 hours. The solution was cooled and neutralized with Amberlite IR-4B resin. The resin was filtered off and the filtrate was concentrated to a sirup (0.376 g.). Paper chromatography of this hydrolyzate in comparison with authentic methylated galactoses suggested that the two components released on hydrolysis were 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,6-tri-*O*-methyl-D-galactose. These sugars were separated on 6 large sheets of Whatman No. 1 paper (18.5 × 22.5 cm.) using butanol-1, ethanol, water (4:1:5, top layer) as the developing solvent.

The 2,3,4,6-tetra-*O*-methyl-D-galactose (0.144 g.) was characterized as its anilide derivative. The melting point and mixed melting point were 192°, $[\alpha]^{25}D$ −76° (*c*, 0.98 in acetone). *Anal.* Calcd. for $C_{16}H_{25}O_5N$: N, 4.5. Found: N 4.5.

The tri-*O*-methyl-D-galactose was oxidized with bromine water to 2,3,6-tri-*O*-methyl-D-galactonolactone which was crystallized from ether–heptane. After recrystallization the crystals had m.p. 100.5° and $[\alpha]^{25}D$ −40° (*c*, 1.03 in water). *Anal.* Calcd. for $C_9H_{16}O_6$: C, 49.1; H, 7.3. Found: C, 49.11; H, 7.35.

Qualitative Periodate Oxidation.—Several crystals of galactobiose were dissolved in 2 drops of water in a small test-tube and 2 drops of saturated sodium bicarbonate solution was added. Two drops of 0.3 *N* sodium periodate was then introduced and the oxidation proceeded 1 hour in the dark. A strong positive test for the liberated formaldehyde[7] was obtained, indicating that the linkage in the galactobiose could not be through C-5 or C-6 of the reducing portion of the disaccharide. Maltose, lactose and melibiose, run as controls simultaneously with galactobiose, gave the results expected on the basis of their structure. This evidence, in conjunction with the methylation data, indicates that the galactobiose is 4-*O*-D-galactopyranosyl-D-galactopyranose.

Test for Reversion.—On paper chromatography of a D-galactose solution which had been subjected to the same conditions used in the hydrolysis of okra mucilage no oligosaccharide spots were observed. This indicated that the three galactobioses produced during the acid hydrolysis of okra mucilage are not reversion products arising from the recombination of D-galactose molecules.

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