[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

The Isolation and Characterization of Two Crystalline Disaccharides from Partial Acid Hydrolysis of Guaran[1,2]

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Partial acid hydrolysis of guaran leads to the isolation of two crystalline disaccharides. One of these, 4-(β-D-mannopyranosyl)-β-D-mannopyranose is identical with the mannobiose isolated from enzymatic hydrolysis of guaran. The other disaccharide is a new sugar and is reported here for the first time. It is 6-(α-D-galactopyranosyl)-β-D-mannopyranose.

An investigation of the products of partial acid hydrolysis of guaran was undertaken to obtain more information concerning the structure of this galactomannan. Periodate oxidation[3] and methylation studies[3,4] combined with X-ray studies on films of guaran triacetate[5] and of guaran[6] have indicated that the molecule consists of a chain of D-mannose units of which every other one, on the average, bears a single D-galactose side unit. From the X-ray data[6] it was deduced that the main chain contained β-1,4′ linkages; this has recently been confirmed by the isolation and characterization of a crystalline mannobiose[2,7] containing this linkage.

From partial acid hydrolysis of guaran, two crystalline disaccharides have been isolated whose structures aid in establishing the nature of the linkages in the molecule. A solution of guaran was hydrolyzed to produce a maximum yield of disaccharide fraction. The hydrolyzate was fractionated by charcoal chromatography[8] to separate the disaccharide fraction from salt, monosaccharides and oligosaccharides. From this material two crystalline components were isolated by further application of the carbon column technique. Two per cent. ethanol desorbed a mannobiose 4-(β-D-mannopyranosyl)-β-D-mannopyranose identical with that isolated from enzymatic hydrolysis of guaran.[7] Four per cent. ethanol desorbed a galactomannose disaccharide whose structure was determined by periodate oxidation and by characterization of its osazone. The data establish the sugar as 6-(α-D-galactopyranosyl)-β-D-mannopyranose. This is the first reported isolation of a galactomannose disaccharide with an α-1,6′-linkage. Other disaccharides composed of D-mannose and D-galactose have been synthesized by Bergmann,[9] Haworth[10] and Freudenberg.[11]

Experimental

Hydrolysis of Guaran.—Guaran was prepared as previously reported.[12] The rate of hydrolysis of the polysaccharide was studied using hydrochloric acid in concentrations of 0.5, 1.0 and 2.0 *N*. In each case, 3.0 g. of guaran were added to 125 ml. of water and heated at 80° with stirring until completely dissolved. For these small amounts, a heating period of 45 minutes was sufficient. Hydrolysis was initiated by adding concentrated acid and water (25 ml. total) to produce a 2% solution with acid of the desired strength. Progress of hydrolysis was followed by an iodimetric procedure based on that of Ingles and Israel.[13]

Of the acid concentrations studied, 0.5 *N* hydrochloric acid was selected since it brought about rapid hydrolysis without decomposition. Hydrolysis was complete in 11 hours and further reaction caused no increase in reducing power. In the same way, guar flour was hydrolyzed with 0.5 *N* acid and the rate and the extent of hydrolysis were identical with those of guaran.

For the isolation of the desired oligosaccharides, guaran was hydrolyzed in 30-g. batches. The material was dispersed in 1350 ml. of water in 4 portions using a Waring blendor and mixing for a very short time. The suspension was poured into a 3-l. flask and, while stirring vigorously, was heated at 80° overnight, *i.e.*, 12 hours or more to ensure complete solution. Sixty ml. of concentrated hydrochloric acid diluted with water to 150 ml. was added and the hydrolysis followed iodimetrically. The maximum yield of disaccharide fraction was obtained in approximately 3.5 hours when the reducing value of the hydrolyzate was 79% of the final value. When this value was attained, the hydrolysate was filtered, cooled and neutralized with sodium bicarbonate. In the course of this work, both guaran and guar flour were used.

The following experiment was carried out to show that the sugars isolated were not synthesized from monosaccharides. A mixture of 2 parts mannose and 1 part galactose was dissolved in sufficient 0.5 *N* hydrochloric acid to give a 2% sugar solution. While heating at 80°, optical rotations and iodimetric titrations were determined at intervals for 24 hours and showed no change. Subsequent fractionation yielded only monosaccharides.

Fractionation of Hydrolysate.—The neutral hydrolysate from 30 g. of guaran was fractionated by use of the charcoal chromatography technique.[8] A dry mixture of Darco G-60 and Celite 535 (1:1) was packed into a column 44 × 265 mm. and washed with at least one column length of water. On each of three columns was placed one-third of the hydrolysate. Separation was effected by the use of water, 5 and 15% aqueous ethanol in succession. In each case, the next more powerful developer was added when the optical rotation of the effluent became zero. The 5% ethanol effluent was concentrated to dryness *in vacuo*. The yield was 5.4% of the weight of guaran used.

In other cases, hydrolyzates from 120 g. of guaran were fractionated by use of a 75 × 780 mm. column of Darco G-60 and Celite 535 (2:3) in conjunction with the automatic fraction collector devised for this purpose.[14] Separation was ensured by checking the optical rotation of the effluents in the successive receivers as the fractionation progressed. The relatively large volume of effluents was conveniently reduced *in vacuo* by use of an all-glass long tube evaporator.[15]

Refractionation of Disaccharide Material.—Analysis by paper chromatography[16] indicated that the 5% ethanol (disaccharide) fraction contained 2 components. A small amount (1.5 g.) of the mixture was adsorbed on a 44 × 265

(1) Journal Paper No. 524 of the Purdue Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry at the 117th Meeting of the American Chemical Society at Detroit, April, 1950.

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mm. column of carbon and Celite (1:1), and the column was developed with 2 l. each of water, 2 and 4% ethanol. The ethanol effluents were collected in separate 200-ml. volume each of which was concentrated to dryness, dissolved in a few drops of water and placed as successive spots along the base of a paper chromatogram. The separation was highly satisfactory with only a little overlapping, which was later remedied by increasing the amount of 2% ethanol developer.

Using the automatic fraction collector[14] fitted with a 75 × 780 mm. column of carbon–Celite (2:3), 7.80 g. of the disaccharide mixture was completely separated into its components with 2 and 4% ethanol as developers. To ensure the preparation of pure materials, each of the effluents was concentrated separately to a small volume *in vacuo* and analyzed by paper chromatography.[16] The proper solutions were combined and dried. The yields were 5.65 g. of 2% effluent and 2.11 g. of 4% effluent.

Characterization of 2% Effluent.—The chromatographically pure amorphous solid was dissolved in a minimum of water and a large volume of methanol was added. The solution was heated and filtered. To the cool filtrate was added a volume of butanol-1 equal to that of methanol. The solution was evaporated on a steam-bath to slight turbidity. Upon cooling, the sugar crystallized as tufts of long thin needles. After two recrystallizations from the same solvent mixture, m.p. was 193.2–194.0° and $[\alpha]^{25}D$ −7.7 → −2.3 in 20 hours (*c* 2.5, water). The equivalent weight by iodimetric titration was 175 and indicated the sugar was a disaccharide. The physical constants were identical with those reported for the mannobiose isolated from an enzymatic hydrolysate of guaran.[2,7] The mixed melting point with a specimen of the known sugar showed no depression, 192–193°. In addition, the X-ray diffraction patterns of the two samples were identical. These data established the structure of the disaccharide as being 4-(β-D-mannopyranosyl)-β-D-mannopyranose. The yield was 3.2% of the weight of guaran used.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.1; H, 6.5.

Characterization of 4% Effluent.—The amorphous solid, chromatographically pure, was crystallized from the same solvents as used for the mannobiose and with the same technique. The sugar appeared to crystallize in the form of cubes. The equivalent weight by iodimetric titration was 176.5 and indicated that this material was a disaccharide. Mannose[12] and galactose determinations[12] indicated the presence of one mannose and one galactose unit. After three recrystallizations from the methanol–butanol mixture, the m.p. was 201.0–201.5° and $[\alpha]^{25}D$ +120.9 → +124.6, 36 hours, (*c* 2.15, water). The yield was 2.2% of the weight of guaran used.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.1; H, 6.5.

The structure of this disaccharide was established through periodate oxidation and by comparison of its osazone with melibiosazone.[17] Periodate oxidation[18] was carried out on approximately 100-mg. samples, and the periodate consumption and formic acid production were determined. Its mode of reaction was identical with that of melibiose and the data indicated the presence of 1,6′ linkage.[19]

The osazone was prepared by dissolving 0.77 g. of the crystalline sugar, 1.46 g. of phenylhydrazine hydrochloride and 2.10 g. of sodium acetate hydrate in 10 ml. of water and heating for 1 hour on a boiling water-bath. Upon cooling, a yellow crystalline precipitate was obtained. This was recrystallized from 50% aqueous ethanol and then from 95% ethanol. The yield was 0.26 g. The m.p. was 175.0–176.0° and $[\alpha]^{25}D$ +43.1 (*c* 1.3, pyridine). This osazone in admixture with an authentic specimen of melibiosazone gave no depression in m.p., 174.5–175.5.[20] In addition the X-ray diffraction patterns of the two osazones were identical.

Anal. Calcd. for $C_{24}H_{32}O_9N_4$: C, 54.4; H, 6.2; N, 10.76. Found: C, 54.3; H, 6.3; N, 10.74.

These data indicate that the disaccharide is 6-(α-D-galactopyranosyl)-β-D-mannopyranose.

Acknowledgment.—The authors wish to thank the General Mills, Inc., for supplying the guar flour used in this work.

(17) Suggestion of C. S. Hudson before the Division of Sugar Chemistry at the 117th Meeting of the American Chemical Society at Detroit, April, 1950.

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LAFAYETTE, INDIANA RECEIVED MARCH 5, 1951

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

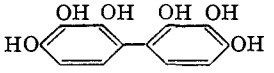
The Oxidation of Di-t-Butylpyrogallol by Oxygen in Alkaline Solution

BY TOD W. CAMPBELL

The autoxidation of di-*t*-butylpyrogallol in alkaline media with air gives an orthoquinone, which is partially cleaved to α-hydroxy-β,Δ-di-*t*-butylmuconic acid, and partially rearranged and oxidized to 2,4-di-*t*-butylcyclopentanedione-1,3 and 2,4-di-*t*-butylcyclopentanetrione-1,3,5.

The reaction of oxygen with polyphenolic antioxidants, especially in alkaline media, gives rise to extremely complex mixtures of poorly defined compounds. Pyrogallol in alkaline solution has been used for many years to remove oxygen from gaseous systems, since it reacts with oxygen so avidly. The oxidized solution of pyrogallol, however, is not amenable to a careful investigation, since the principal product is a black, intractable tar of complex nature. A number of small molecules are also formed.[2]

However, Harries[3] was able to isolate hexahydroxy-biphenyl (I) by oxidizing pyrogallol under mild alkaline conditions, while Nierenstein[4] was able to isolate a compound which he formulated as hexahydroxyterphenoquinone (II).



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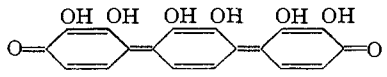

II

Erdtmann,[5] using conditions prescribed by Harries,[3] converted 4-ethylpyrogallol to diethylhexahydroxybiphenyl. However, he obtained only unidentifiable resins from 4,6-diethylpyrogallol. He

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

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