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

A New Crystalline Trisaccharide from Partial Acid Hydrolysis of Guaran and the Structure of Guaran[1]

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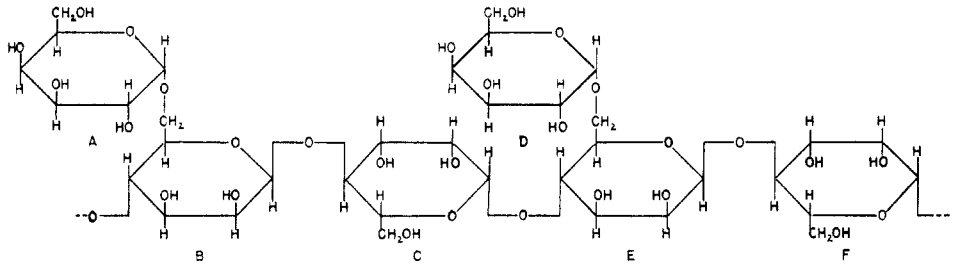
RECEIVED MARCH 10, 1952

From an acid hydrolysate of guaran a new crystalline trisaccharide has been isolated and characterized. Through periodate oxidation and partial hydrolysis the sugar has been established as O-α-D-galactopyranosyl-(1 → 6)-O-β-D-mannopyranosyl-(1 → 4)-β-D-mannopyranose. From a correlation of the available structural data, it is evident that the guar polysaccharide consists of a chain of D-mannopyranose units linked β-1 → 4 and, on the average, there is a D-galactopyranose unit linked α-1 → 6 to every other D-mannose unit.

Further investigation of the products from partial acid hydrolysis of guaran has led to the isolation of a new crystalline trisaccharide. The sugar is composed of D-mannose and D-galactose in the ratio 2:1, respectively. Its structure has been established through periodate oxidation and partial hydrolysis with identification of the products. The data presented identify the trisaccharide as O-α-D-galactopyranosyl-(1 → 6)-O-β-D-mannopyranosyl(1 → 4)-β-D-mannopyranose.

With the isolation of this trisaccharide and with information in hand concerning other fragments from guaran, it becomes feasible to correlate the existing data on the structure of the guaran macromolecule. This polysaccharide, which constitutes the major component of guar seed endosperm,[2] is an industrially valuable gum.[3] The polysaccharide, guaran, is for the most part chemically homogeneous and consists of D-mannose and D-galactose in the approximate ratio of 2:1, respectively.[2,4] Existence of numerous branches is suggested from the measurement of the large amount of formic acid produced during quantitative periodate oxidation.[5] Tosylation experiments[6] also indicate a branched structure with branching taking place through a 1 → 6 linkage. Stress–strain measurements on films of guaran triacetate[7] as well as X-ray investigation on films of crude guaran[8] reveal that the molecules are highly anisodimensional and that the branches must be very short in length. Methylation of the polysaccharide with subsequent hydrolysis and separation of products leads to the isolation of 2,3,4,6-tetra-O-methyl-D-galactose,[9,10] 2,3,6-tri-O-methyl-D-mannose[10] and 2,3-di-O-methyl-D-mannose.[10] These results, confirmed by others,[11] show that D-galactopyranose units occur as non-reducing terminal units. Since the branches must be short, the most likely structure of guaran, based on evidence to this point, is that shown.



Section of guaran molecule.

Further evidence for this structure and indication for the anomeric configurations have been obtained by fragmentation of the guaran molecule using partial acid hydrolysis in one case and enzymatic hydrolysis in another. By these processes there are obtained in crystalline form the definitive fragments 4-O-β-D-mannopyranosyl-β-D-mannopyranose,[12,13] O-β-D-mannopyranosyl-(1 → 4)-O-β-D-mannopyranosyl-(1 → 4)-β-D-mannopyranose,[14] 6-O-α-D-galactopyranosyl-β-D-mannopyranose,[13] and the trisaccharide described herein as O-α-D-galactopyranosyl-(1 → 6)-O-β-D-mannopyranosyl-(1 → 4)-β-D-mannopyranose. The mannobiose and mannotriose could be derived from any two- or three-unit combinations of B, C, E or F in the guaran structure. The galactomannose disaccharide could be derived from the units A, B or D, E in the indicated structure while the galactomannose trisaccharide could be derived from the units A, B, C or D, E, F in the guaran structure. The trisaccharide fragment theoretically derivable from the combination of units C, D, E has not been found. Nevertheless, the isolation in crystalline form of the four fragments listed above, when considered in the light of previous information, is very strong indication that the guaran molecule consists of a linear chain of D-mannopyranose units which are uniformly linked in β-1 → 4 fashion and that, on the average, every other D-mannose unit bears a side chain consisting of one D-galactopyranose unit joined by an α-1 → 6 linkage.

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Experimental

Acid Hydrolysis of Guaran.—The conditions employed were the same as previously reported for the isolation of disaccharides.[13] A homogeneous 2% solution of guaran was hydrolyzed at 80° with 0.5 *N* hydrochloric acid. Hydrolysis was followed iodimetrically and allowed to proceed to 79% of completion. The solution was filtered, cooled and neutralized with sodium bicarbonate. The neutral hydrolysate was adsorbed on columns of Darco G-60 and Celite and desorption was carried out with water, 5, and 15% ethanol in succession.[15] The 5% ethanol desorbed the disaccharide fraction.[13] The 15% ethanol effluent contained the trisaccharide fraction of the hydrolysate. Chromatographic separation was performed by placing the hydrolysate equivalent to 10 g. of guaran on a 44 × 265 mm. column or that from 120 g. on a 75 × 780 mm. column. In the latter case, fractionation was effected by use of an automatic fraction collector[16] and the effluents concentrated by use of a long-tube evaporator.[17] The yield of trisaccharide fraction was 13.1% of the weight of guaran hydrolyzed.

Isolation of Trisaccharide.—Analysis of the trisaccharide fraction by paper chromatography[18] revealed that the material was a mixture of a fast-moving component and other components which were only slightly displaced after 48 hours. Separation of the mixture was studied on a small scale before use of a large column. On a pre-wet column 44 × 265 mm., 1.60 g. of the mixture was adsorbed and the developers for separation were water, 5 and 8% ethanol. Two liters of each of the first two developers were used. None of the effluents had optical activity. The 8% aqueous ethanol effluent was collected in separate 200-ml. fractions until the optical activity returned to zero. Each of these fractions was concentrated and upon analysis by paper chromatography was shown to contain one compound.

All of the trisaccharide mixture was chromatographed on 75 × 780-mm. columns with the automatic fraction collector. The 8% effluents were concentrated separately and combined after paper chromatographic analysis had indicated their purity. In a sample separation, 5.77 g. of the pure sugar was obtained from 21.98 g. of the mixture.

The dry amorphous solid was taken up in absolute ethanol by refluxing several days. The solution was filtered and cooled slowly. A partially crystalline precipitate was obtained which, when recrystallized from 80–90% aqueous ethanol, yielded the sugar in the form of prisms. After two more recrystallizations from the same solvent, m.p. was 228.0–229.0° and $[\alpha]^{25}D$ +93.3 → +98.4° (12 hours), (*c* 1.22, water). Analyses indicated that the sugar was a trisaccharide which crystallized as the monohydrate and was composed of two parts mannose and one part galactose. Heating *in vacuo* at 100° for 48 hours yielded the anhydrous sugar which in 18 hours reverted to the hydrate when exposed to air. The yield was 3.4% of the weight of guaran hydrolyzed. Oxidation with iodine was used to determine the equivalent weights.

Anal. Calcd. for $C_{18}H_{32}O_{16}\cdot H_2O$: C, 41.38; H, 6.55; equiv. wt., 261.2. Found: C, 41.2; H, 6.6; equiv. wt., 270.8.

Characterization of Trisaccharide.—The structure of the sugar was established through periodate oxidation and by partial hydrolysis with isolation and characterization of products. One hundred 30-mg. samples were oxidized by periodate by the procedure of Halsall, Hirst and Jones,[19] and the determination made of periodate consumption and formic acid produced. In addition, formaldehyde production was determined by the method of Reeves.[20] These data suggested that the linkages in the sugar were 1 → 6 and 1 → 4 since per mole of sugar 7.08 moles of periodate was consumed with the production of 4.04 moles of formic acid and 0.98 mole of formaldehyde.

The position of the sugar residues in the trisaccharide molecule was determined by partial hydrolysis followed by isolation and characterization of the products. Hydrolysis was first performed on a small scale and its progress followed by paper chromatography. The sugar (81.1 mg.) was dissolved in 4 ml. of 0.5 *N* hydrochloric acid and the solution heated at 80°. At intervals ten-drop samples were removed, neutralized and placed on the base line of a paper chromatogram. After developing 36 hours with a mixture of 3:6:4 of water:butanol-1:pyridine,[18] the chromatogram was dried and sprayed with ammoniacal silver nitrate.[21] Upon heating there appeared a series of spots which graphically indicated the progress of hydrolysis. The trisaccharide steadily disappeared; two disaccharides appeared, increased and then decreased; and two monosaccharides appeared and increased until hydrolysis was complete. The distances moved by the spots corresponded to those for D-mannose, D-galactose, mannobiose,[12,13] galactomannose[13] and intact trisaccharide.

For the quantitative determination, 4.000 g. (1 mole) of the trisaccharide was hydrolyzed with 160 ml. of 0.5 *N* hydrochloric acid for 2 hours, the time indicated by the small run as yielding the maximum amount of disaccharides. The hydrolysate was quickly cooled, diluted to 1 l. and immediately adsorbed on a pre-wet 44 × 265-mm. column of carbon–Celite (1:1). Although disaccharides can be desorbed by hydrochloric acid,[15] the concentration and volume used in this experiment were such as not to affect fractionation. The separation was effected by water, 2, 4 and 8% aqueous ethanol in succession in sufficient volume to produce an effluent with no optical activity. Each of these effluents was concentrated to dryness *in vacuo* before characterization.

The water fraction yielded 1.7601 g. of dry sugar which, upon analysis in the usual manner, was found to contain 11.1% D-galactose (0.137 mole) and 88.6% D-mannose (1.091 moles).

The 2% ethanol effluent amounted to 0.6348 g. (0.234 mole) and crystallized from water:methanol:butanol-1 mixture. The sugar, in the form of tufts of needles, had a m.p. 194.0–195.5° and $[\alpha]^{25}D$ −7.7 → −2.2 (final), (*c* 2.27, water). This sugar was shown to be identical with the mannobiose isolated from guaran hydrolysate[12,13] by mixed m.p. (193.5–195.0°) and comparison of the X-ray diffraction patterns of the two sugars. Isolation of the mannobiose, *O*-β-D-mannopyranosyl-β-D-mannopyranose established the position and the configuration of the 1 → 4 linkage in the trisaccharide.

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

The 4% ethanol effluent contained 0.4428 g. of sugar (0.163 mole) which crystallized as cubes from the same solvent as the mannobiose. Determination of m.p. and mixed m.p. (both 201.0–203.0°), $[\alpha]^{25}D$ +120.8 → +124.5° (final), (*c* 1.18, water) and comparison of the X-ray diffraction pattern with that of the galactomannose isolated from guaran hydrolysate[13] identified the sugar as 6-O-α-D-galactopyranosyl-β-D-mannopyranose. The structure of this product established the position of the galactose residue, the position of the 1 → 6 linkage and its configuration in the trisaccharide.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.0; H, 6.6.

From the 8% ethanol effluent was recovered 1.3387 g. (0.355 mole) of the intact trisaccharide. The identity was established by m.p., mixed m.p. and specific rotation after crystallization from 90% ethanol.

The data presented above established the trisaccharide as O-α-D-galactopyranosyl-(1 → 6)-O-β-D-mannopyranosyl-(1 → 4)-β-D-mannopyranose.

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

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