acetone then ether and dried *in vacuo*. The product was taken up in water, a trace of barium phosphate removed by centrifugation and the organic phosphates reprecipitated by the addition of ethyl alcohol and treated further as above to give a yield of 2.3 g. (*ca.* 55%). (The yield in different runs varied between 50–60%.) Descending paper chromatography in the solvent system isopropyl alcohol–ammonia–water (70-10-20, v.v.) showed a single spot ($R_f$ 0.16) corresponding to ribofuranose 1-phosphate. Ribose ($R_f$ 0.65), inorganic phosphate ($R_f$ 0.08) and ribopyranose 1-phosphate[1] ($R_f$ 0.11) all were absent.

**The Preparation and Fractional Crystallization of Dicyclohexylammonium α-D-Ribofuranose 1-Phosphate.**—The barium salt (1.98 g.), as prepared above, was dissolved in *ca.* 10 ml. of water, freed from a small amount of insoluble barium phosphate,[13] and the clear solution passed through a column (10 cm. × 1.5 cm. diameter) of Amberlite 1R-120 cyclohexylammonium form. The combined effluent and water wash was concentrated to dryness *in vacuo* at 40° and the residue dissolved in 20 ml. of methyl alcohol. Ether was added to turbidity and the mixture set aside at 0°. The crystalline product which separated overnight was collected by filtration and washed with ether containing 25% methyl alcohol; yield 1.5 g., $[\alpha]^{20}D$ +40.3° (*c* 2.37, water). *Anal.* Calcd. for $C_{17}H_{37}O_8N_2P$: C, 47.6; H, 8.70; N, 6.54; P, 7.23. $C_{17}H_{37}O_8N_2P \cdot 1H_2O$: C, 45.74; H, 8.81; P, 6.94. Found in air dried material: C, 45.38; H, 8.82; P, 7.4. After drying in a high vacuum at room temperature over phosphorus pentoxide; C, 47.06; H, 9.17; N, 6.51; P, 7.8.

A small sample (10 mg.) of this material was converted to the pyridinium salt, using an ion-exchange resin, and brought into reaction with an excess (25 mg.) of dicyclohexylcarbodiimide in 80% aqueous pyridine at 0°. Paper chromatography[10] showed the complete conversion of the starting material to faster travelling products, thus indicating the complete absence of β-D-ribofuranose 1-phosphate[1] in this material.

The mother liquor from the above crystalline cyclohexylammonium salt gave on concentration and dilution with ether two small crops of crystals, weighing 0.25 and 0.2 g. (highly colored) with $[\alpha]^{20}D$ −2.4° and −11.3°, respectively. Recrystallization of the first of these crops afforded pure dicyclohexylammonium β-D-ribofuranose 1-phosphate ($[\alpha]^{20}D$ −13.6° (*c* 2.0, ethyl alcohol)). No cyclic phosphate formation could be detected when the latter was treated with dicyclohexylcarbodiimide.[10]

(13) The barium salts tend to decompose in the solid state, even on storage at low temperature, to form barium phosphate.

**D-Ribofuranose 1-Phosphates Using 3,5-Di-*O*-benzoyl-D-ribofuranosyl Halides.** (a) **Chloride**[7].—3,5-Di-*O*-benzoyl-D-ribofuranosyl chloride (1.04 g.) in 5 ml. of dry benzene was treated at room temperature with a benzene solution containing one equivalent of triethylammonium dibenzyl phosphate. Triethylamine hydrochloride, which began to separate after 10 minutes, was removed after a total period of 3 hours by filtration (0.299 g., 78%) and the filtrate concentrated *in vacuo* at room temperature. The resulting thick syrup was hydrogenated in 50 ml. of anhydrous methyl alcohol at 0° in the presence of freshly prepared palladium catalyst. After about 1 hour, when the hydrogen uptake was complete, the catalyst was removed, 15 ml. of water added and the solution brought to and maintained at *p*H 11.3 with 1 *N* sodium hydroxide. After 3 hours at room temperature an excess of the pyridinium form 1R-120 ion-exchange resin (10 ml.) was added and the mixture well stirred for 10 minutes. The resin was then removed and washed with water and the combined solution and washings were concentrated *in vacuo* to 5 ml. with occasional additions of a few drops of pyridine. The product was isolated as the barium salt (0.454 g.) and converted to the crystalline cyclohexylammonium salt in the manner described above. The specific rotation of this sample, $[\alpha]^{20}D$ +16.4° (*c* 2.7, water) and a study of its reaction with dicyclohexylcarbodiimide showed it to be a mixture of the α- and β-D-ribofuranose 1-phosphates.

(b) **Bromide**.—3,5-Di-*O*-benzoyl-D-ribofuranosyl bromide[6] (0.59 g.) gave, in the above method, 0.140 g. of the barium salt of a product which again was a mixture of the α- and the β-D-ribofuranose 1-phosphates.

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VANCOUVER 8, B. C., CANADA

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

# The Structure of Chagual Gum. I. Composition of the Gum and Isolation of 2-*O*-(D-Glucopyranosiduronic Acid)-D-xylose[1]

BY J. K. HAMILTON, D. R. SPRIESTERSBACH AND F. SMITH

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Chagual gum, an exudate from species of *Puya* produced as a result of damage by insects, is the neutral salt of the polysaccharide acid which is shown by hydrolysis to be composed of arabinose, xylose, galactose and glucuronic acid. Graded hydrolysis of the gum gives an aldobiouronic acid, the structure of which has been shown to be 2-O-(D-glucopyranosiduronic acid)-D-xylose.

Chagual gum is obtained from species of *Puya* (*P. chilensis*, *P. lanuginosa* and *P. lanata*), the best known of which is *Puya chilensis* found on the slopes of the Andes in South America. The gum exudate, produced as a result of damage by the larvae of the insect *Kastnia elegans*, forms clear pale yellow globules which are only partly soluble in water, the remainder swelling to form jelly-like masses.[2] This gum is of interest not only because it is generated by the plant after injury but also because unlike other plant gum exudates[3] it contains a relatively large amount of xylose and a small amount of arabinose.

Chagual gum has been reported to contain galactose since it gave mucic acid upon oxidation with nitric acid and the presence of pentose sugars was revealed by the formation of furfural when the gum was treated with hydrochloric acid. Moreover acid hydrolysis furnished xylose and galactose and it was further suggested that some of the galactose belonged to the L-series.[4]

The native gum ($[\alpha]_D$ −30° (NaOH)) investigated in this work was only partially soluble in water but

(1) Paper No. 3536 Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota. This paper forms part of a thesis submitted by D. R. Spriestersbach to the Graduate School of the University of Minnesota in partial fulfillment for the degree of Ph.D., 1954.

(2) F. N. Howes, "Vegetable Gums and Resins," Chronica Botanica Company, Waltham, Mass., 1949.

(3) J. K. N. Jones and F. Smith, *Advances in Carb. Chem.*, **4**, 243 (1949).

(4) E. Wintersteiner, *Ber.*, **31**, 1571 (1898).

the free acid form of the gum, chagualic acid, dissolved completely in water (equiv. wt. 1,030 by direct titration, $[\alpha]_D -31°$). Hydrolysis of the gum acid, chagualic acid, with *N* sulfuric acid yielded arabinose (7%), xylose (31%), galactose (36%) and 2-*O*-(D-glucopyranosiduronic acid)-D-xylose (27% approx.) as shown by quantitative paper chromatographic analysis.[5] Contrary to a previous report,[4] the galactose component, separated by partition chromatography, was found to belong to the D-series.

Autohydrolysis and hydrolysis with dilute mineral acid showed there was no preferential liberation of arabinose.

The structure of the aldobiouronic acid which is relatively stable to acid hydrolysis is based upon the following experimental facts. Vigorous hydrolysis of this acid, whose equivalent weight corresponded to a disaccharide composed of a hexuronic acid and a pentose sugar, yielded D-glucuronic acid, recognized as its *p*-nitroanilide, and D-xylose, identified as the characteristic crystalline di-*O*-benzylidene dimethylacetal.[6]

Further proof that the aldobiouronic acid was composed of glucuronic acid and xylose was deduced from the observation that reduction of the methyl ester methyl glycoside of the aldobiouronic acid with lithium aluminum hydride[7] gave a neutral disaccharide which upon hydrolysis furnished glucose and xylose.

Treatment of the aldobiouronic acid first with methyl sulfate and alkali, then with silver oxide and methyl iodide gave the fully methylated derivative which upon methanolysis yielded methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid)-uronate and methyl 3,4-di-*O*-methyl-D-xyloside. The former was identified as the crystalline amide of methyl-2,3,4-tri-*O*-methyl-D-glucopyranosiduronic acid[8] while the latter was transformed into the characteristic crystalline δ-lactone.[9] Further support for the presence of a 1,2-biose linkage in the aldobiouronic acid was provided by the observation that when the acid was treated with methanolic hydrogen chloride, under mild conditions, an ester glycoside was produced which upon complete methylation followed by hydrolysis gave, in addition to 2,3,4-tri-*O*-methyl-D-glucuronic acid, a mixture of 3,4-di- and 3,5-di-*O*-methyl-D-xylose.

The high positive rotation, $[\alpha]_D +108°$ (methanol), of the methylated aldobiouronic acid indicated that the acid contained a biose linkage of the α-type and hence it is designated as 2-*O*-(α-D-glucopyranosiduronic acid)-D-xylose. Final proof of the structure of the aldobiouronic acid follows from the observation that acetylation of the methyl ester methyl glycoside of the acid gave the characteristic crystalline methyl 2-*O*-methyl-(2,3,4-tri-*O*-acetyl-D-glucopyranosid)-uronate-3,4-di-*O*-acetyl-β-D-xylopyranoside, the structure of which has been established.[10] This same aldobiouronic acid also has been found in corn cobs,[11] in corn hull gum[12] and in oat hulls.[13]

(5) M. Dubois, J. K. Hamilton, K. A. Gilles, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(6) L. J. Breddy and J. K. N. Jones, *J. Chem. Soc.*, 738 (1945).

(7) M. Abdel-Akher and F. Smith, *Nature*, **166**, 1037 (1950).

(8) F. Smith, *J. Chem. Soc.*, 1723 (1939).

(9) Sybil P. James and F. Smith, *ibid.*, 739 (1945).

(10) R. Montgomery, F. Smith and H. C. Srivastava, THIS JOURNAL, **78**, 6169 (1956).

## Experimental[14]

**Properties and Composition of Chagual Gum.**—The gum (19.6 g.) was dissolved with stirring in water (250 ml.). The solution was filtered and added dropwise with stirring to ethanol (3500 ml.). The flocculent precipitate was separated (centrifuge), was dissolved in water, and precipitated with alcohol as before. After a third precipitation the gum was washed successively with ethanol (5 times), ether (3 times) and dried *in vacuo* (yield 14 g.).

The white amorphous powder obtained in this way showed $[\alpha]^{23}_D -30°$ in *N* sodium hydroxide (*c* 0.6) and *p*H 4.5 (*c* 1.0 in water). The gum did not reduce Fehling solution.

A solution of the purified gum (1 g.) in water (100 ml.) was passed through a column of cation exchange resin. The aqueous solution was concentrated and poured with stirring into ethanol (6 vols.) to give chagualic acid as a white amorphous precipitate which was separated and dried by solvent exchange as described above. The chagualic acid (0.9 g.) was a white powder, $[\alpha]^{21}_D -31°$ in water (*c* 2.2), *p*H 2 in water (*c* 1.0); equiv. wt. 1030, by direct titration with 0.1 *N* sodium hydroxide; and equiv. wt. 690, by adding an excess of 0.1 *N* sodium hydroxide and back titrating with 0.1 *N* sulfuric acid.

When a solution of the gum (54 mg.) in *N* sulfuric acid (10 ml.) was boiled, it showed $[\alpha]_D -30°$ (initial value), +46° (after 1 hr.), +43° (after 2 hr.) constant for a further 2 hr. Neutralization ($BaCO_3$) of the solution, filtration, passage through the cation exchange resin and concentration gave a sirup which was found by paper chromatography to contain arabinose, xylose, galactose and 2-*O*-(D-glucopyranosiduronic acid)-D-xylose (see below).

Quantitative chromatography on Whatman No. 1 filter paper, using 1-butanol:ethanol:water (4:1:5)[15] for the separation of the sugars, *p*-anisidine as the spray reagent[16] and the phenol–sulfuric acid method[5] for the determination of the sugars, showed that the hydrolyzate contained arabinose (7%), xylose (31%), galactose (36%) and, in a separate experiment (see later), the amount of 2-*O*-(D-glucopyranosiduronic acid)-D-xylose was found to be 27%.

By paper chromatography it was found that autohydrolysis[17] of chagualic acid or hydrolysis of the gum with dilute mineral acid, *p*H 2, at 95° did not effect preferential liberation of arabinose since both xylose and galactose were also detected in the early stages of the hydrolysis.

**Isolation of 2-*O*-(D-Glucopyranosiduronic Acid)-D-xylose.**—When a solution of chagual gum (43.5 g.) in *N* sulfuric acid (260 ml.) was boiled under reflux the following changes in rotation were observed: $\alpha^{22}_D +2.30°$ (0.5 dm. tube) after 2 hr.; +3.25°, 4 hr.; +3.63°, 6 hr.; and +3.65° (8 hr.), corresponding to $[\alpha]^{22}_D +43.5°$. The solution was neutralized ($BaCO_3$) while still hot, treated with a little charcoal and filtered. The colorless filtrate was passed through the cation resin and then through the anion resin to remove the aldobiouronic acid component. The eluate containing the mixture of the neutral sugars, arabinose, xylose and galactose (tested by paper chromatography) was concentrated to give a sirup (yield 19.9 g.).

Separation of the three sugars by sheet paper chromatography using 1-butanol:ethanol:water followed by polarimetric examination of the sirupy galactose component, obtained as an anomeric mixture, $[\alpha]^{25}_D +80°$ in water (*c* 1), showed that it belonged to the D-series. After crystallization from aqueous alcohol the D-galactose showed m.p. and

(11) R. L. Whistler and L. Hough, *ibid.*, **74**, 4918 (1953).

(12) R. Montgomery and F. Smith, *J. Agric. and Food Chem.*, **4**, 716 (1956).

(13) E. L. Falconer and G. A. Adams, *Can. J. Chem.*, **34**, 338 (1956).

(14) Unless stated otherwise (a) all concentrations were carried out *in vacuo* at temperatures not exceeding 45–50° and (b) the cation resin used was Amberlite IR120 obtained from Rohm and Haas, Philadelphia, Pa., while the anion resin was Duolite $A_4$, from the Chemical Process Co., Redwood City, Calif.

(15) S. M. Partridge and R. G. Westall, *Biochem. J.*, **42**, 238 (1948).

(16) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(17) F. Smith, *ibid.*, 744 (1939).

mixed m.p. 166°, $[\alpha]^{25}_D$ +82.5° equilibrium value in water (*c* 1).

The aldobiouronic acid absorbed on the anion resin was displaced with 0.5 *N* sodium hydroxide. The alkaline eluate was passed through the cation exchange resin as before and concentrated to dryness to give 2-*O*-(D-glucopyranosiduronic acid)-D-xylose as a colorless glassy solid (yield 11.5 g.), $[\alpha]^{23}_D$ +95° in water (*c* 2.0). *Anal.* Calcd. for $C_{11}H_{18}O_{11}$: equiv. wt., 326. Found: equiv. wt. 326; $OCH_3$, nil.

Paper chromatography of the hydrolyzate of the aldobiouronic acid, obtained by boiling the latter with *N* sulfuric acid for 10 hr., revealed the presence of xylose, glucuronic acid and unchanged aldobiouronic acid. It was evident therefore that the aldobiouronic acid was composed of xylose and glucuronic acid.

**Methanolysis of 2-*O*-(D-Glucopyranosiduronic Acid)-D-xylose.**—A solution of the aldobiouronic acid (0.9 g.) in 8% methanolic hydrogen chloride (6 ml.) was heated (sealed tube) for 24 hr. at 120°. The solution was cooled, neutralized ($Ag_2CO_3$), filtered and concentrated. A solution of the sirupy product in 0.3 *N* barium hydroxide was heated for 1.5 hr. at 60°, neutralized (Dry Ice), filtered and concentrated to a sirup. Addition of ethanol precipitated the barium (methyl D-glucopyranosid)-uronate which was separated (centrifuge) and washed with ethanol. The supernatant solution and washings containing methyl D-xylopyranoside were combined (solution A).

A solution of a portion of the barium salt in water (10 ml.) was passed through the cation exchange resin and concentrated to give methyl-D-glucopyranosiduronic acid (40 mg.) as a sirup. Hydrolysis of the latter by heating for 12 hr. with *N* sulfuric acid on a boiling water-bath, followed by neutralization ($BaCO_3$), treatment with Amberlite IR120 cation exchange resin and concentration gave D-glucuronic acid as a sirup (23.5 mg.). After lactonization to give D-glucofuranuronic acid-6,3-lactone (D-glucurone), by heating for 1 hr. at 80°, the sirupy product was boiled for 15 min. with methanol (0.1 ml.) containing *p*-nitroaniline (20 mg.) and 0.01% hydrochloric acid.[18] Slow evaporation of the solvent by exposure to air gave crystalline D-glucopyranuronic acid *p*-nitroanilide, m.p. 129–130° alone or in admixture with an authentic specimen, prepared as described below.

Concentration of solution A (see above) containing methyl D-xyloside gave a sirup (0.13 g.) which was hydrolyzed by heating on a boiling water-bath with *N* sulfuric acid (5 ml.) for 24 hr. Neutralization (Duolite $A_4$), filtration and concentration gave D-xylose (69 mg.). Treatment of the latter with 1% methanolic hydrogen chloride (1.5 ml.) containing 1.5% benzaldehyde gave the characteristic di-*O*-benzylidine dimethyl acetal[8] m.p. and mixed m.p. 210° (after recrystallization from chloroform–petroleum ether); literature[6] m.p. 211°.

**Methyl 2-*O*-(Methyl-D-glucopyranosiduronate)-D-xylopyranoside.**—When a solution of the aldobiouronic acid (1.1 g.) in 1% methanolic hydrogen chloride (15 ml.) was allowed to stand at room temperature it showed $[\alpha]_D$ +99° (initial value), +100° (0.5 hr.), +101° (1.5 hr.), +101° (2.5 hr.), +99° (24 hr. constant value). After 24 hr. the solution no longer reduced Fehling solution.

Neutralization with ethereal diazomethane and concentration gave methyl 2-*O*-(methyl D-glucopyranosiduronate)-D-xyloside as a thick sirup (0.79 g.), $[\alpha]^{22}_D$ +98.5° in methanol (*c*, 3.9). *Anal.* Calcd. for $C_{13}H_{22}O_{11}$: $OCH_3$, 17.5; equiv. wt., 354. Found: $OCH_3$, 17.0; equiv. wt. 304.

Treatment of the above ester glycoside of the aldobiouronic acid with methanolic ammonia in the usual way afforded an amide, $[\alpha]^{22}_D$ +95°, in methanol (*c* 3.7), which could not be crystallized. *Anal.* Calcd. for $C_{12}H_{21}O_{11}N$: $OCH_3$, 9.2. Found: $OCH_3$, 10.9.

Reduction of the methyl ester glycoside (20 mg.) with lithium aluminum hydride in anhydrous tetrahydrofuran in the usual way[7,19] gave the corresponding neutral disaccharide methyl 2-O-α-D-glucopyranosyl-α- and β-D-xylopyranoside as a sirup. Hydrolysis of the product gave glucose and xylose as shown by paper chromatography.

In a second experiment a solution of the 2-*O*-(D-glucopyranosiduronic acid)-D-xylose (1.5 g.) in 1% methanolic hydrogen chloride (20 ml.) was allowed to stand at room temperature for 72 hr. and then refluxed for 2 hr. The solution, which showed $[\alpha]^{22}_D$ +93°, was neutralized ($Ag_2CO_3$), filtered, concentrated to remove solvent and then subjected to several Purdie methylations. Methanol was used in the initial treatments until the product became soluble in methyl iodide, after which 4 additional methylations were applied with methyl iodide and silver oxide alone. The fully methylated product (0.92 g.), obtained as a pale yellow fairly viscous liquid, was purified by distillation, b.p. (bath temp.) 180° (0.01 mm.). Treatment of this product with 10% methanolic hydrogen chloride in a sealed tube for 24 hr. at 100°, neutralization ($Ag_2CO_3$) and concentration gave a sirupy product consisting of the methyl glycosides of methyl 2,3,4-tri-*O*-methyl-D-glucopyranosiduronate, 3,4-di-*O*-methyl-D-xylose and 3,5-di-*O*-methyl-D-xylose.

Saponification by heating the sirupy mixture with 0.3 *N* barium hydroxide as described above, neutralization with carbon dioxide (Dry Ice), filtration and successive passage through the cation and the anion resins gave a solution containing the methyl glycosides of 3,4-di- and 3,5-di-*O*-methyl-D-xylose. When this mixture was isolated and hydrolyzed by heating for 8 hr. on a boiling water-bath with *N* sulfuric acid, the corresponding two di-*O*-methyl sugars were obtained; they were provisionally identified by paper chromatography using methyl ethyl ketone:water azeotrope[20] as the solvent and the authentic samples of these two di-*O*-methyl sugars as reference compounds; $R_f$ values: 3,5-di-*O*-methyl-D-xylose, 0.58; 3,4-di-*O*-methyl-D-xylose, 0.47.

The two di-*O*-methyl sugars were separated by chromatography on large sheets (18 × 22 inches) of Whatman No. 1 filter paper using methyl ethyl ketone:water azeotrope as the solvent. Extraction of the appropriate areas of the papers furnished the two di-*O*-methyl sugars as sirups.

Oxidation of the slower moving component, 3,4-di-*O*-methyl-D-xylose, with bromine as described below afforded 3,4-di-*O*-methyl-D-xylono-δ-lactone, m.p. and mixed m.p. 68° after purification by sublimation in *vacuo*; literature[9] m.p. 68°.

These preliminary results indicated that the biose link in the D-glucopyranosyluronic acid-D-xylose involved position 2 of the xylose residue.

**Isolation of Methyl 2-*O*-(Methyl 2,3,4-Tri-*O*-acetyl-α-D-glucopyranosiduronate)-3,4-di-*O*-acetyl-β-D-xylopyranoside.**—A solution of the aldobiouronic acid, 2-*O*-(D-glucopyranosiduronic acid)-D-xylose (100 mg.), was boiled with 0.5% methanolic hydrogen chloride (10 ml.) for 8 hr. The solution was neutralized ($Ag_2CO_3$), filtered and freed from solvent to give methyl 2-*O*-(methyl-D-glucopyranosiduronate)-D-xylopyranoside as a colorless viscous liquid. The latter was dissolved in pyridine (10 ml.), treated with acetic anhydride (10 ml.) and the mixture heated for 3 hr. at 60–70°. After keeping overnight at room temperature, the excess solvent was removed and the product triturated with methanol whereupon it crystallized spontaneously. After recrystallization from chloroform–ethanol, the methyl 2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosiduronate)-3,4-di-*O*-acetyl-β-D-xylopyranoside had m.p. 250° alone or in admixture with an authentic specimen,[10] $[\alpha]^{23}_D$ +101° in chloroform (*c* 0.5); literature[10] m.p. 257°, $[\alpha]_D$ +103° ($CHCl_3$).

*Anal.* Calcd. for $C_{22}H_{32}O_{18}$: C, 48.9; H, 5.7. Found: C, 48.7; H, 5.5.

**Isolation of Methyl 2-*O*-(Methyl-2,3,4-tri-*O*-methyl-D-glucopyranosiduronate)-3,4-di-*O*-methyl-D-xylopyranoside.**—The aldobiouronic acid (5 g.) was methylated in the usual way with methyl sulfate (100 ml.) and 45% potassium hydroxide (275 ml.). The reaction was commenced at room temperature until the solution no longer reduced Fehling solution and completed at 55–60°. After heating at 90° for 20 minutes, the solution was cooled, neutralized with 6 *N* sulfuric acid, filtered and concentrated to about 150 ml. and then remethylated as before. The reaction mixture was cooled, acidified with 6 *N* sulfuric acid (tested with congo red) and extracted 5 times with chloroform. The combined chloroform extracts were washed twice with an almost saturated solution of sodium sulfate, dried ($Na_2SO_4$) and concentrated to a sirup (2.1 g.). This sirup was subjected to two methylations with silver oxide and methyl iodide in the usual way, the product being isolated by means of acetone. Removal of the solvent gave methyl 2-*O*-

(18) F. Weygand, W. Perkow and P. Kuhner, *Ber.*, **84**, 594 (1951).
(19) F. Smith, *J. Chem. Soc.*, 2646 (1951).
(20) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

(methyl-2,3,4-tri-*O*-methyl-D-glucopyranosid uronate)-3,4-di-*O*-methyl-D-xylopyranoside (2.0 g.), b.p. 175–180° (0.01 mm.), $n^{24}D$ 1.4638, $[\alpha]^{22}D$ +105° in methanol (*c*, 4). *Anal.* Calcd. for $C_{18}H_{32}O_{11}$: $OCH_3$, 51.2; equiv. wt., 424. Found: $OCH_3$, 48.8; equiv. wt. (by saponification), 438.

**Methanolysis of the Methylated Aldobiouronic Acid and Isolation of 2,3,4-Tri-*O*-methyl-D-glucuronic Acid and 3,4-Di-*O*-methyl-D-xylose.**—A portion (0.27 g.) of the methylated aldobiouronic acid, obtained in the previous experiment, was dissolved in 10% methanolic hydrogen chloride (3 ml.) and the solution heated (sealed tube) for 24 hr. on a boiling water-bath. Neutralization ($Ag_2CO_3$), filtration and concentration gave a sirup (92 mg.). The low yield of product is probably due to decomposition during methanolysis.

The sirupy methanolysis product, consisting of a mixture of methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosiduronate) and methyl 3,4-di-*O*-methyl-D-xylopyranoside, was heated for 1 hr. at 80° with 0.3 *N* barium hydroxide (10 ml.); the solution was neutralized (Dry Ice), filtered and passed successively over the cation and the anion resins. The neutral eluate was concentrated to give sirupy methyl 3,4-di-*O*-methyl-D-xyloside (22 mg.) which was heated for 20 hours with *N* sulfuric acid (1 ml.) on a boiling water-bath. Neutralization ($BaCO_3$), filtration and concentration gave 3,4-di-*O*-methyl-D-xylose, $[\alpha]^{23}D$ +22° in methanol (*c* 1.0). This di-*O*-methyl-D-xylose was readily distinguished from the 2,3- and the 3,5-di-*O*-methyl isomers by paper chromatography using methyl ethyl ketone:water azeotrope. Oxidation of the di-*O*-methyl-D-xylose (18 mg.) in water (1 ml.) with bromine (0.1 ml.) for 4 days at room temperature gave 3,4-di-*O*-methyl-D-xylonic acid. Aeration to remove the excess of the bromine, neutralization ($Ag_2CO_3$), passage of the filtered solution through the cation exchange resin followed by concentration gave sirupy 3,4-di-*O*-methyl-D-xylonic acid. Purification by extraction with ether and heating *in vacuo* at 50° gave crystalline 3,4-di-*O*-methyl-D-xylono-δ-lactone[9] (17 mg.) which after sublimation had m.p. and mixed m.p. 66°, $[\alpha]^{23}D$ −55°, initial value in water (*c* 0.2) changing to −19° (equilibrium value).

The methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid uronic acid was displaced from the anion resin column with 0.5 *N* sodium hydroxide and recovered by passing the alkaline eluate through the cation resin. Concentration gave a sirup which was dissolved in ether and treated with ethereal diazomethane. Removal of solvent and treatment of the methyl ester with methanolic ammonia afforded the crystalline amide[8] of methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside uronic acid, m.p. and mixed m.p. 183°, $[\alpha]^{22}D$ +140° in water (*c* 0.4); literature[8] m.p. 183°, $[\alpha]D$ +138° in water.

**D-Glucuronic Acid *p*-Nitroanilide.**—D-Glucurone (1 g.) and *p*-nitroaniline (1.05 g.) were dissolved in the minimum of methanol containing 0.01% hydrochloric acid[18] and the solution refluxed for 15 minutes. On allowing to stand, the *p*-nitroanilide of D-glucuronic acid separated in the form of plates (0.7 g.), m.p. 129–130°, $[\alpha]^{23}D$ +265° in pyridine (*c* 0.4) (after filtration and washing with ice-cold methanol).

Crystallization of the plates from methanol furnished needles, m.p. 129–130°, $[\alpha]^{23}D$ +261° in pyridine (*c* 0.5). *Anal.* Calcd. for $C_{17}H_{12}O_7N_2.H_2O$: C, 46.8; H, 4.5; N, 8.9. Found (for the plates): C, 46.2; H, 5.0; N, 8.9; Found (for the needles): C, 46.8; H, 4.9; N, 8.8.

When either the plates or the needles of the *p*-nitroanilide were crystallized from hot ethanol, cubic crystals of the anhydrous *p*-nitroanilide of D-glucuronic acid were obtained, m.p. 160–163° dec., $[\alpha]^{23}D$ +272° in pyridine (*c* 0.2). *Anal.* Calcd. for $C_{12}H_{12}O_7N_2$: C, 48.7; H, 4.1; N, 9.5. Found: C, 48.8; H, 4.2; N, 9.5.

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ST. PAUL, MINNESOTA

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

# The Carbohydrates of Gramineae. IX. The Constitution of a Glucofructan of the Endosperm of Wheat (*Triticum vulgare*)[1,2]

BY R. MONTGOMERY AND F. SMITH

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The constitution of a glucofructan from wheat flour has been investigated by methylation studies. The methylated glucofructan gives upon hydrolysis 2,3,4,6-tetra-*O*-methyl-D-glucose (1 mol. prop.), 1,3,4,6-tetra-*O*-methyl-D-fructose (3 mol. props.), 1,3,4-tri-*O*-methyl-D-fructose (2 mol. props) and 3,4-di-*O*-methyl-D-fructose (2 mol. props.). The structural significance of these findings, which are different from those reported by other investigators for a wheat glucofructan, is discussed.

The glucofructans of wheat represent a group of non-reducing compounds which extend in molecular size from sucrose to polysaccharides with molecular weights of around 2000.[3–6]

The present paper is concerned with the determination of the structure of one member of this homologous series of compounds.

The glucofructans of wheat flour were isolated by extraction with 70% ethanol after preliminary inactivation of the enzymic constituents with boiling 82% ethanol. Further purification was achieved by precipitation of the glucofructans as the barium hydroxide complexes, which, after liberation of the free carbohydrates with sulfuric acid, were acetylated and the complex mixture subjected to fractional precipitation. In this manner 12 fractions of acetate were obtained varying in optical rotation in chloroform from $[\alpha]D$ − 22° to $[\alpha]D$ + 46° with three principal fractions showing $[\alpha]D$ + 3°, $[\alpha]D$ + 26° and $[\alpha]D$ + 31° (Table I). Deacetylation of the main fraction, $[\alpha]D$ + 3°, afforded the corresponding free carbohydrate as a hygroscopic, amorphous solid, $[\alpha]D$ − 21° in water, which was non-reducing to Fehling solution; these properties were similar to those de-

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