usual way with methyl sulfate (162 ml.) and sodium hydroxide (405 ml. of 30%). The reagents were added in tenths during 2 hr. The methylation was completed by heating the reaction mixture for 30 min., after which it was cooled, mixed with chloroform (200 ml.) and filtered to remove sodium sulfate, the latter being washed with small amounts of chloroform. The chloroform layer was separated and the aqueous phase re-extracted twice with chloroform (50 ml.). The combined chloroform extracts were dried (sodium sulfate), freed from the solvent and the residual liquid distilled giving: 1,2;3,4-di-*O*-isopropylidene-6-*O*-methyl-D-galactopyranose, a colorless liquid, b.p. 125° (bath temp.), 0.001 mm., $n^{25}\text{D}$ 1.4525, $[\alpha]^{25}\text{D}$ −64° in chloroform (*c* 5) (yield almost quantitative); literature[17] $[\alpha]\text{D}$ −63.2° in tetrachloroethane.

Hydrolysis of the 1,2;3,4-di-*O*-isopropylidene-6-*O*-methyl-D-galactopyranose (30 g.) was described previously[18] with aqueous acetone containing hydrochloric acid gave 6-*O*-methyl-D-galactose (10.3 g.), m.p. and mixed m.p. 127.5° (after recrystallization from aqueous ethanol); literature[18] m.p. 128° and $[\alpha]^{22}_{5780}$ +114° → +77° in water.

A solution of 6-*O*-methyl-D-galactose (5 g.) in 2% methanolic hydrogen chloride (25 ml.) was refluxed for 13 hr. when the rotation had become constant. Removal of acid (Ag_2CO_3) and solvent (*in vacuo*) gave a sirupy product which crystallized spontaneously. Recrystallization from methanol gave methyl 6-*O*-methyl-α-D-galactopyranoside, m.p. 138°, $[\alpha]^{23}\text{D}$ +165° in water (*c* 1). *Anal.* Calcd. for $C_8H_{16}O_6$: OCH_3, 29.8. Found: OCH_3, 29.8.

Oxidation of Methyl 6-*O*-Methyl-α-D-galactopyranoside with Periodate.—Methyl 6-*O*-methyl-α-D-galactopyranoside (1 g.) was oxidized with periodic acid in the usual way to give the sirupy dialdehyde, D′-methoxy-D-methoxymethyldiglycolic aldehyde, $[\alpha]^{25}\text{D}$ + 145° in water (*c* 1.5) changing in 40 hr. to +119°. The dialdehyde was treated with hydrogen in the presence of palladium–charcoal as described in the previous experiments. The sirupy product (0.74 g.) thus obtained appeared to consist only of unchanged dialdehyde recognized by its characteristic mutarotation, $[\alpha]^{25}\text{D}$ +153° in water (*c* 1.5) changing in 50 hr. to +116°.

Bromine oxidation of the product from the hydrogenation reaction in the presence of barium carbonate yielded the barium salt of the corresponding dicarboxylic acid. Hydrolysis of this dicarboxylic acid and chromatography of the hydrolyzate using 1-butanol:acetic acid:water (2:1:1) revealed glyoxylic acid and 3-*O*-methyl-D-glyceric acid. The former was characterized as glyoxylic acid–2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 192–193°, while the latter was recognized by comparison of its R_f value with that of an authentic specimen. No 3-*O*-methyl-D-glycerol, which would arise by reduction of the C_4 aldehydic group as in the case of methyl α-D-glucopyranoside, was found.

Identification of D′-Methoxy-D-hydroxymethyldiglycolic Acid. (a) The Glyoxylic Acid Moiety.—To a solution of the strontium salt of D′-methoxy-D-hydroxymethyldiglycolic acid (0.2005 g.)[19] in water (15 ml.) was added concentrated sulfuric acid (0.5 ml.) and the precipitated strontium sulfate was filtered off. The filtrate was warmed on the water-bath at 70–75° for 1.5 hr. until it was no longer optically active and treated with a solution of 2,4-dinitrophenylhydrazine (0.2509 g., or two equivalent proportions) in water (10 ml.), containing concentrated sulfuric acid (1.5 ml.), for 10 min. at 70–75° as described above. After 48 hr. at room temperature a large crop of long, silky, yellow needles had separated along with a small amount of a microcrystalline orange-colored precipitate. These two crystalline products were separated by dissolving the yellow needles in water at 60–65°; the insoluble orange crystals were filtered off. On cooling the aqueous extract it afforded glyoxylic acid–2,4-dinitrophenylhydrazone (yield 0.1186 g., or 54.5% of the theoretical) m.p. 192°. *Anal.* Calcd. for $C_8H_6O_6N_4$: C, 37.8; H, 2.4; N, 22.0. Found: C, 38.1; H, 2.4; N, 22.0.

It would seem that the above procedure is more advantageous for the identification of small amounts of D′-(or L′)-methoxy-D-(or L)-hydroxymethyldiglycolic acid than the method used by Jackson and Hudson[4] who identified the glyoxylic acid moiety of the dicarboxylic acid, after hydrolysis of the latter, by oxidation with bromine to give oxalic acid. Thus for example, starting with 15 g. of the hydrated strontium salt of the dicarboxylic acid, these authors report a yield of 5.7 g. of barium oxalate. Experiments with 0.2–0.4 g. of the strontium salt of the dicarboxylic acid furnished negligible amounts of barium oxalate. On the other hand, according to the procedure described here, 0.4 g. of the hydrated strontium salt of D′-methoxy-D-hydroxymethyldiglycolic acid yielded approximately 0.24 g. of glyoxylic acid 2,4-dinitrophenylhydrazone and about 0.01 g. of the 2,4-dinitrophenylhydrazone of pyruvic acid–2,4-dinitrophenylhydrazide, amounts that were adequate for identification purposes.

(b) The Glyceric Acid Moiety.—When the orange, water-insoluble crystals, separated from the glyoxylic acid 2,4-dinitrophenylhydrazone in the preceding preparation, were recrystallized from hot ethyl acetate the crystalline 2,4-dinitrophenylhydrazone of pyruvic acid 2,4-dinitrophenylhydrazide was obtained m.p. 316° dec. From two experiments, each starting with 0.2 g. of the strontium salt of D′-methoxy-D-hydroxymethyldiglycolic acid, a total of 0.01 g. was obtained. The pyruvic acid is formed from glyceric acid by a rearrangement of the pinacol type.[20] *Anal.* Calcd. for $C_{15}H_{12}O_9N_8$: C, 40.2; H, 2.7; N, 25.0. Found: C, 40.6; H, 2.8; N, 25.3.

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(17) K. Freudenberg and R. M. Hixon, *Ber.*, **56**, 2119 (1923).

(18) K. Freudenberg and K. Smeykal, *ibid.*, **59**, 104 (1926).

(19) Prepared from methyl α-D-glucopyranoside by the method of Jackson and Hudson.[4]

(20) E. Erlenmeyer, *Ber.*, **14**, 321 (1881).

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

Structure of Corn Hull Hemicellulose. Part III. Identification of the Methylated Aldobiouronic Acid Obtained from Methyl Corn Hull Hemicellulose[1,2]

BY R. MONTGOMERY AND F. SMITH

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Corn hull hemicellulose has been methylated and the methyl derivative hydrolyzed. The cleavage fragments were separated by means of ion-exchange resins into a neutral and an acidic component. The latter was esterified and the resulting ester reduced to give methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3-*O*-methyl-D-xylopyranoside, m.p. 166–168°, $[\alpha]^{25}\text{D}$ +85° in water. This proved that the aldobiouronic acid cleavage fragment of the methylated hemicellulose was methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranoside and that the D-glucuronic acid end groups are linked directly to the main structural xylan framework of the polysaccharide.

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(1) Paper No. 3556, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota.

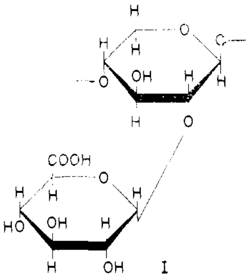
(2) This research was done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service. Presented at the 129th National Meeting of the A.C.S., Dallas, Texas, April, 1956.

It has been shown previously[3,4] that the graded hydrolysis of corn hull hemicellulose gives rise to 2-*O*-(-α-D-glucopyranosyluronic acid)-D-xylopyranose. This aldobiouronic acid has also been reported to be present in corn cob hemicellulose B,[5] chagual gum[6] and oat hulls.[7] Its presence in these and other acidic polysaccharides is easily demonstrated by hydrolysis and formation of the readily crystallizable acetate of the methyl ester methyl glycoside.[3,4,6]

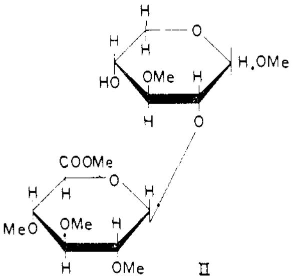
The present paper deals with the manner in which the aldobiouronic acid residues, 2-*O*-(α-D-glucopyranosyluronic acid) - D - xylopyranose, are linked to the polysaccharide. The corn hull hemicellulose appeared to be essentially homogeneous.[8] Thus, about 65% of the polysaccharide was precipitated when a 0.6% aqueous solution was gradually saturated with ammonium sulfate and this fraction had almost the same specific rotation as that of the fraction which remained in solution. Also, the acetyl derivative was fractionally precipitated from chloroform–ether or acetone–ether solutions with petroleum ether and the series of fractions so obtained appeared to be similar. The hemicellulose does not form an insoluble copper complex with Fehling solution, a property which is in direct contrast to the hemicellulose from corn cobs and by which means the latter was purified.[9]

In order to determine the mode of union of the component sugars and of the aldobiouronic acid, the hemicellulose acetate was methylated with 45% potassium hydroxide and methyl sulfate. The methylated hemicellulose was converted to the free acid by precipitation from solution in 1,4-dioxane with *N* hydrochloric acid and then freed from the latter by reprecipitation from 1,4-dioxane with water. The resulting material, showing $[\alpha]^{25}\mathrm{D}$ −129° in 1,4-dioxane and $-OCH_3$ 38.6, tenaciously retained water which was rapidly reabsorbed if exposed to the air after vigorous drying.

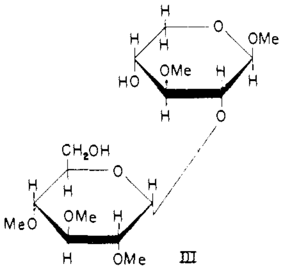
Methanolysis of the methylated hemicellulose with 3% methanolic hydrogen chloride gave a mixture of glycosides, the uronic acid ester groups in which were then saponified with 0.15 *N* barium hydroxide. Since losses of fully methylated sugar glycosides easily occur upon prolonged evaporation of their aqueous solutions, these volatile cleavage fragments were extracted with petroleum ether from the alkaline saponification solution before separation into the acidic and neutral sugar components on ion exchange resins was applied. The neutral methylated cleavage fragments in the petroleum ether extract and in the ion-exchange resins eluate were combined and investigated separately; these findings will be reported in another communication. The acidic cleavage fragment was esterified and then reduced with lithium aluminum hydride[10,11] to give crystalline methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-β-D-xylopyranoside (III).[12] The characterization of the latter, together with the previous identification of the residue, 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose[3,4] in the polysaccharide proved that the acidic cleavage fragment, resulting from methanolysis of the methylated corn hull hemicellulose (I), was methyl 2-*O*-[methyl-(2,3,4-tri - *O* - methyl - α - D - glucopyranosyl) - uronate]-3-*O*-methyl-D-xylopyranoside (II). It also proved that the units of D-glucuronic acid occupied terminal positions and were linked directly to the main structural xylan framework of the polysaccharide as in I.[13]



I



II



III

Experimental

The crude corn hull hemicellulose was isolated by the Northern Utilization Research Branch of the Agricultural Research Service. It gave a viscous, turbid solution in cold water and a clear, light yellow solution in *N* sodium hydroxide; these solutions did not reduce Fehling solution. The polysaccharide did not form an insoluble copper complex nor did it give a blue color with iodine. The ash-free hemicellulose, prepared by two precipitations in the cold with alcohol from acidified (HCl) aqueous solutions showed $[\alpha]^{24}\mathrm{D}$ −78° (*c* 1) in 0.5 *N* sodium hydroxide.

Acetylation of the Corn Hull Hemicellulose.—The crude hemicellulose (153 g.) was dissolved in formamide (1450 ml.) and to the brown viscous solution was added pyridine (1030 ml.). With external cooling and vigorous stirring, the solution was treated with acetic anhydride (750 ml.). After keeping the reaction mixture for 24 hr. at room temperature, it was poured into water with stirring. The acetate formed a stringy precipitate which was successively washed with water, methanol, ethanol–ether and ether and dried (yield 236 g.). The acetate was soluble in acetone, chloroform and pyridine and showed $[\alpha]^{28}\mathrm{D}$ −79° in pyridine (*c* 1.3).

Homogeneity of Corn Hull Hemicellulose.—Since only limited information concerning the chemical homogeneity

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(3) R. Montgomery, F. Smith and H. C. Srivastava, This Journal, **78**, 2837 (1956).

(4) R. Montgomery, F. Smith and H. C. Srivastava, *ibid.*, **79**, 698 (1957).

(5) R. L. Whistler and L. Hough, *ibid.*, **75**, 4918 (1953).

(6) J. K. Hamilton, F. Smith and D. R. Spriestersbach, *ibid.*, **79**, 443 (1957).

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

(8) R. L. Whistler and J. N. BeMiller, This Journal, **78**, 1163 (1956).

(9) I. Ehrenthal, R. Montgomery and F. Smith, *ibid.* **76**, 5509 (1954).

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

(11) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(12) G. G. S. Dutton and F. Smith, This Journal, **78**, 2505 (1956).

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

of a polysaccharide can be obtained by its fractional precipitation from aqueous solution with alcohol, the following approaches were made to the problem.

(a) Ash-free hemicellulose (2.5 g.) was dissolved in water (400 ml.) and the solution was gradually saturated with ammonium sulfate, at which point some material was precipitated. The precipitate was redissolved in water, dialyzed until free of sulfate, the solution concentrated *in vacuo* and poured into ethanol (4 vol.). The precipitated material was dried by solvent exchange in the usual manner (yield 1.6 g.) and showed $[\alpha]^{24}\mathrm{D}$ $-79°$ (*c* 1) in 0.5 *N* sodium hydroxide. The fraction remaining in the saturated ammonium sulfate solution was isolated in a similar way and showed $[\alpha]^{24}\mathrm{D}$ $-73°$ (*c* 1) in 0.5 *N* sodium hydroxide (yield 0.6 g.).

(b) To the hemicellulose acetate (10 g.) dissolved in chloroform (300 ml.) was added ether (100 ml.) followed by increasing amounts of petroleum ether. The principal fraction (7.0 g.), $[\alpha]^{26}\mathrm{D}$ $-81°$ in pyridine (*c* 1.0), was precipitated between the addition of 240 and 260 ml. of petroleum ether. The other minor fractions showed $[\alpha]^{26}\mathrm{D}$ -75 to $-86°$ in pyridine.

From these results it appeared that the hemicellulose was essentially homogeneous.

Methylation of the Corn Hull Hemicellulose.[8]—Hemicellulose acetate (20 g.) was dissolved in acetone (500 ml.) and methylated by the dropwise addition of 45% potassium hydroxide (600 ml.) and methyl sulfate (200 ml.) in aliquot tenths during 2 hr. at 40–48°. After the addition of the third aliquot of the reagents the viscous reaction mixture was diluted with benzene (100 ml.). When the addition of the reagents had been completed, the temperature was maintained at 45° and the stirring continued for 3 hr. after which time additional 45% potassium hydroxide (100 ml.) and methyl sulfate (50 ml.) were added dropwise. The reaction mixture finally was heated on a boiling water-bath and the methylated product separated out as granular particles. These were separated by decantation of the hot supernatant liquid and after dissolving the product in acetone the methylation was repeated as before, solution being maintained when necessary by the addition of 1,4-dioxane. A total of nine methylations were applied in this way, and after the final methylation the product was washed with boiling water to remove inorganic salts.

The methylated product was dissolved in 1,4-dioxane (300 ml.) and precipitated by the addition of water (150 ml.). The precipitate was redissolved in 1,4-dioxane and reprecipitated with *N* hydrochloric acid. After two more similar reprecipitations from 1,4-dioxane with water, the product was dissolved in chloroform and the solution washed with water to remove any residual hydrochloric acid. The chloroform solution (without drying with anhydrous salts since this has often been found to introduce inorganic impurity) was finally evaporated to dryness under reduced pressure, the residue redissolved in chloroform and precipitated as a colorless, amorphous product by pouring the chloroform solution into an excess of petroleum ether (yield 14 g.). The methylated derivative showed $[\alpha]^{25}\mathrm{D}$ $-129°$ in 1,4-dioxane (*c* 1.0) and OMe, 38.6%.

Methanolysis and Preliminary Separation of the Methylated Cleavage Fragments of the Methylated Hemicellulose.—When a solution of methylated hemicellulose (4.000 g.) in 3.0% methyl alcoholic hydrogen chloride (160 ml.) was boiled, it showed $[\alpha]^{25}\mathrm{D}$ $+25°$ (final value) after 8 hr., constant for a further 4 hr. The solution was cooled, the acid neutralized with silver carbonate, and after filtration the solution was evaporated *in vacuo* at room temperature. The residue of sirupy glycosides (4.424 g.) was treated with 0.15 *N* barium hydroxide (60 ml.) for 5 hr. at 60° and the more fully methylated volatile neutral glycosides removed from the alkaline solution by continuous extraction for 24 hr. with petroleum ether. The residual aqueous solution was then passed successively through columns of "Amberlite IR 120"[14] cation- and "Duolite A4"[15] anion-exchange resins. The neutral methyl sugar glycosides in the aqueous effluent from the "Duolite A4" anion-exchange column were isolated as a sirup (2.383 g.) by evaporation *in vacuo* at 30–35° (bath temperature) while those extracted by the petroleum ether, being volatile, were recovered by evaporation *in vacuo* with no heat applied (yield 1.631 g.) and then combined with the other fraction.

The methyl aldobiouronoside fragment, absorbed on the "Duolite A4" anion-exchange resin, was eluted with *N* sodium hydroxide and the free acid immediately regenerated with "Amberlite IR 120" cation-exchange resin. The aqueous solution of the methylated acid fragment was evaporated to dryness under reduced pressure at 30–35° (bath temperature) to give a thick sirup (0.455 g.).

Hydrolysis of the Neutral Methylated Sugar Glycoside Fractions.—The combined neutral glycosides (4.014 g.) were dissolved in *N* sulfuric acid (100 ml.) and the solution heated on a boiling water-bath for 10 hr. when the final constant observed rotation was α_{D} (2 dm.) $+0.12°$. The solution was neutralized ($BaCO_3$), filtered, the residue was well washed with aqueous alcohol and the combined filtrates evaporated to dryness under reduced pressure at 25–30° (bath temperature). The sirupy residue (3.62 g.) consisting of a mixture of reducing methylated sugars, was investigated separately; these findings will be reported later.

Examination of the Methyl Aldobiouronoside Fraction.—The methyl aldobiouronoside fraction (0.455 g.) was treated with 3.0% methyl alcoholic hydrogen chloride (20 ml.) under reflux for 4 hr. The acid was neutralized with an ethereal solution of diazomethane and the solution evaporated under reduced pressure to give a sirupy methyl ester (0.44 g.). The ester was dissolved in anhydrous ether (30 ml.) and the solution added dropwise to ether (50 ml.) containing lithium aluminum hydride (0.9 g.). The reduction was carried out in the usual way[10] and the product isolated by acetylation.[12,16] The acetate (0.352 g.) was dissolved in ethanol (10 ml.) to which was added *N* sodium hydroxide (1 ml.). The solution was boiled for 3 hr. and then passed through columns of "Amberlite IR 120" cation- and "Duolite A4" anion-exchange resins. Evaporation of the neutral effluent to dryness gave a crystalline residue (0.236 g.). Recrystallization from ethyl acetate afforded methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-β-D-xylopyranoside as colorless rectangular prisms, m.p. 166–168°, undepressed on admixture with an authentic sample, m.p. 167°,[12] and showed $[\alpha]^{25}\mathrm{D}$ $+85°$ in water (*c* 0.5).

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(14) A product of the Rohm and Haas Chem. Co., Philadelphia, Pa.

(15) A product of the Chemical Process Co., Redwood City, Calif.

(16) H. J. Klosterman and F. Smith, THIS JOURNAL, **74**, 5336 (1952).