An Arabinoglucoglycan in Chick-Pea Seed

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An arabinoglucoglycan, composed of L-arabinose and D-glucose in a ratio of about 3:7, was isolated in 0.8–1.2% yield from the seed of *Cicer arientinum*. Periodate degradation revealed that all units were attacked. Polarimetry and hydrolysis of the methylated polysaccharide showed the predominance of the α-(1 → 4)-linked glucopyranoside units. The optical rotation in cuprammonium is discussed.

Chick-pea, *Cicer arientinum*, is a leguminous plant which is widely grown in Middle Eastern countries. The seeds are of the size of peas and are borne in separate pods. They have high nutritional value[2] and are used as native food in different forms.

The polysaccharide was obtained in 0.8% or 1.2% yield from the ground seed by extraction with warm water or warm sodium hydroxide solution, respectively, and was purified *via* its copper complex. Hydrolysis of the chick-pea polysaccharide followed by chromatographic separation of the products yielded crystalline L-arabinose and D-glucose. A quantitative estimate of the molecular proportions of the two sugars indicated that they were present in an approximate ratio of 1:2. This ratio remained essentially the same when three different methods for isolating the polysaccharide were applied, indicating the homogeneity of the arabinoglucoglycan.

The polysaccharide then was exhaustively methylated with methyl sulfate and sodium hydroxide. The arabinoglucoglycan exhibited considerable resistance toward complete methylation and seven successive methylation processes were required to yield a methylated product containing 0.88 OCH_3 group per hydroxyl group.

Examination of the fission products of the methylated polysaccharide on paper chromatograms showed the presence of tri-*O*-methylglucose and smaller quantities of di-*O*-methylglucose and mono-*O*-methylarabinose. No tetra-*O*-methylglucose or tri-*O*-methylarabinose was detected. The procedure used for detection of methylated sugars on paper chromatograms is capable of detecting less than one part of tetra-*O*-methylglucose (indicative of end groups) in a hundred parts of tri-*O*-methylglucose[3] and, therefore, the methylated material has a chain length greater than one hundred units.

Separation of the fission products of the methylated polysaccharide by partition chromatography yielded 2,3,6-tri-*O*-methyl-D-glucose (65%), 2,3-di-*O*-methyl-D-glucose (5%), 3,6-di-*O*-glucose (7%), and 3-*O*-methyl-L-arabinose (17%). Comparison of the molecular ratio of methylated arabinose to methylated glucose (1:2.9) with that of arabinose to glucose (1:2) in the original polysacchride showed that some arabinose units were lost during methylation and hydrolysis. The large proportion of 2,3,6-tri-*O*-methyl-D-glucose showed a predominance of (1 → 4)-linkages between the glucose units while the high positive value for the specific rotation of the polysaccharide indicated α-linkages. The isolation of 2,3-3,6-di-*O*-methyl-D-glucose would suggest branching, forming (1 → 6)- and (1 → 2)-linkages. However, in view of the failure to detect any 2,3,4,6-tetra-*O*-methyl-D-glucose in the hydrolyzate of the methylated polysaccharide it is suggested that incomplete methylation of the polysaccharide and demethylation during hydrolysis might be the reason for the presence of all or most of the di-*O*-methyl derivatives. The isolation of 3-*O*-methyl-L-arabinose as the only L-arabinose derivative is suggestive of branching at positions 1, 2, and 4 of the arabinopyranoside. However, similarly the presence of only mono-*O*-methyl-L-arabinose may be due to incomplete methylation or to demethylation. Other authors[4,5] have previously made similar observations.

On oxidation by periodate, the arabinoglucoglycan consumed *ca.* one mole of oxidant per mole of sugar residue. This and the absence of arabinose in the hydrolyzate of the periodate oxidized polysaccharide indicated that the L-arabinose units were nonbranched.

The polysaccharide on reaction with Fehling's solution formed a copper complex which contained one-third atomic equivalent of copper per mole of sugar unit. Comparison of the specific rotation of the arabinoglucoglycan in dilute alkali (+385°) with that in cuprammonium (−12°) showed that a levorotatory complex was formed in cuprammonium. This has been shown[6] to occur in reactions involving the 2- and 3-hydroxyl groups of D-glucopyranoside as well as the 2- and 3- or the 3- and 4-groups of L-arabinopyranoside. Such shifts in the case of starch and glycogen where reaction involves all the sugar units are from +375° to −715° and from +366 to −597, respectively.[7] In the arabinoglucoglycan in chick pea, the copper complex isolated involves only one-third of the sugar units and the levorotatory shift is about one-third of that of starch or glycogen.

Experimental

All specific rotations are equilibrium values and were measured at room temperature. Chromatographic separations were carried out using the following solvent systems: (a) 1-butanol–ethanol–water (40:11:19 v./v.); (b) ethyl acetate–acetic acid–water (9:2:2 v./v.) for unsubstituted sugars; and (c) benzene–ethanol–water (190:50:5 v./v.) for methylated sugars. Separations were made by the descending technique on Whatman no. filter paper. Sugars were located on the paper by *p*-anisidine hydrochloride spray reagent.[8] Solutions were concentrated under reduced pressure.

Isolation of the Polysaccharide.—(a) The finely ground seeds (200 g.) were stirred with water on the water bath (4 × 500 ml.;

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ca. 1 hr. each time). After each extraction the residual seed material was separated on the centrifuge. The combined extracts were treated with diastase for 24 hr., then poured with stirring into alcohol (4 l.). The solid precipitate was washed with alcohol several times and dissolved in warm water (500 ml.). The solution was washed with chloroform (4 × 100 ml.) and it (solution A) was poured into ethanol (1 l.) yielding a white powder (1.6 g.).

Anal. Found: N, 1.95; sulfated ash, 2.7.

(b) The seeds (200 g.) were ground and heated on the water bath with 10% sodium hydroxide solution (2 l.) for 8 hr. with continuous stirring until a jelly-like mass was formed and no more ammonia was evolved. It was then poured with stirring into ethanol (4 l.). The precipitate was washed several times with ethanol, the polysaccharide extracted in warm 0.2% sodium hydroxide solution (4 × 500 ml.), treated with diastase for 24 hr., and reprecipitated from the solution (solution B) by the addition of ethanol (5 l.) yielding a white powder (2.4 g.).

Anal. Found: N, 0.94; sulfated ash, 4.4.

(c) To solution A or solution B (1 l.) was added Fehling's solution (200 ml.) and the mixture was allowed to stand for 7 days. The resulting copper complex (2–3 g.) was separated on the centrifuge, washed with alcohol several times, and dried in a desiccator.

Anal. Calcd. for $[(C_{17}H_{26.2}O_{14}\cdot 0.8\ Cu(NH_3)_2]_n$: Cu, 9.57. Found: Cu, 9.0.

The polysaccharide was recovered from the copper complex by treatment with *N* hydrochloric acid (100 ml.) with stirring and pouring into ethanol (150 ml.). The white precipitate was filtered off, washed successively with ethanol and ether, then dried under reduced pressure. It had $[\alpha]_D$ +385° (*c*, 2.1, 0.9% sodium hydroxide).

Anal. Found: N, 0.5; sulfated ash, 0.5.

Hydrolysis of the Polysaccharide and Identification of the Products.—The polysaccharide (1.65 g.) in *N* sulfuric acid (100 ml.) was heated on the water bath for 15 hr. The reaction mixture was neutralized with barium carbonate and filtered. The filtrate was concentrated to a sirup which on examination on paper chromatograms was observed to contain two components, R_f 0.32 and 0.20. The sirup was fractionated on a cellulose column using 1-butanol–water (10:1 v./v.) as the mobile phase. Two fractions were obtained.

Fraction I gave crystals (1.03 g.) of D-glucose with m.p. 146° $[\alpha]_D$ +51.6° (*c*, 5.6, water). It gave a crystalline phenylosazone with m.p. 205°, undepressed on admixture with an authentic specimen of D-glucophenylosazone.

Fraction II gave crystalline L-arabinose (0.46 g.) with m.p. 160°, $[\alpha]_D$ +104.8° (*c*, 2.2, water). It yielded crystalline L-arabinophenylosazone with m.p. and m.m.p. 166°.

Methylation of the Polysaccharide.—The arabinoglucoglycan (7 g.) was dissolved in sodium hydroxide solution (300 ml., 40%) and methyl sulfate (300 ml.) was added in portions during 5 hr. with vigorous stirring.[9,10] After being stirred overnight, the mixture was nearly neutralized with glacial acetic acid and dialyzed against tap water for 24 hr. The solution was concentrated to *ca.* 100 ml. and the methylation procedure was repeated using 50 g. of sodium hydroxide and 100 ml. of methyl sulfate, then 100 g. of sodium hydroxide and 200 ml. of methyl sulfate. The mixture was neutralized, dialyzed, concentrated, and methylated five times as described. The final reaction mixture was dialyzed for 48 hr. against tap water, concentrated, and extracted with chloroform. After removal of the chloroform under reduced pressure the partially methylated polysaccharide (4.8 g.) was obtained.

Anal. Calcd. for 63% methylation $[C_{17}H_{23}O_9(OCH_3)_5]_n$: OCH_3, 29.50. Found: OCH_3, 30.00.

This material was refluxed in methanol (10 ml.) with methyl iodide (50 ml.) and silver oxide (10 g.; added in portions) for 30 hr. The reaction mixture was then evaporated to dryness and the solid material extracted with chloroform. The chloroform extract was concentrated to a sirup which was treated with methyl iodide (15 ml.) and silver oxide (5 g.) as before, yielding a yellowish solid (4 g.).

Anal. Calcd. for 88% methylation $[C_{17}H_{21}O_7(OCH_3)_7]_n$: OCH_3, 39.2. Found: OCH_3, 39.00.

Identification of the Products.—The methylated polysaccharide (*ca.* 2 g.) was allowed to stand in 40% sulfuric acid (20 ml.), left at room temperature for 3 hr., then diluted again with water (40 ml.), and heated at 60° for 3 more hr. After further dilution with water (80 ml.), the reaction mixture was heated on the water bath for 14 hr. and neutralized with barium carbonate. The barium salts were filtered off and the filtrate concentrated to a sirup (1.42 g.) which on examination on paper chromatograms, using solvent c, was found to contain at least six components with R_f values of 0.0125, 0.075, 0.14, 0.336, 0.45, and 0.75. The sirup was fractionated by paper chromatography, using solvent c, yielding four fractions.

Fraction I yielded crystalline 2,3,6-tri-*O*-methyl-D-glucose (0.83 g.) which, after recrystallization from aqueous ethanol, had m.p. 123° $[\alpha]_D$ +70° (*c*, 1.25, methanol). Irvine and Hirst[11] record m.p. 121–123°, $[\alpha]_D$ +70° (water).

1,4-Di-*O*-acetyl-2,3,6-tri-*O*-methyl-α-D-glucose.—A mixture of 2,3,6-tri-*O*-methyl-D-glucose (fraction I) (*ca.* 0.1 g.), dry pyridine (2 ml.), and acetic anhydride (2 ml.) was allowed to stand at room temperature for 24 hr. and then poured into ice–water (*ca.* 20 ml.). The acetylated product was extracted with chloroform (3 × 20 ml.) and the combined extracts were washed successively with 2 *N* hydrochloric acid (2 × 20 ml.), saturated sodium hydrogen carbonate (2 × 20 ml.) and water, and dried (Na_2SO_4). Subsequent concentration gave a colorless sirup which crystallized on standing at 0° (yield, *ca.* 0.1 g.). After recrystallization from ethanol, the acetate had m.p. 67°. Micheel and Hess[12] record m.p. 67–68°.

Fraction II gave 2,3-di-*O*-methyl-D-glucose (0.069 g.) as crystals with m.p. 85°, $[\alpha]_D$ +50° (*c*, 1.0, methanol). Irvine and Scott[13] record m.p. 85–87°, $[\alpha]_D$ +48.3 (acetone).

Fraction III afforded 3-*O*-methyl-L-arabinose (0.215 g.) with $[\alpha]_D$ +96.5° (*c*, 1.15, water). Hirst, *et al.*,[14] record $[\alpha]_D$ +96° (water).

Anal. Calcd. for $C_6H_{12}O_5$: C, 43.90; H, 7.32. Found: C, 43.96; H, 7.03.

The compound (30 mg.) yielded the phenylosazone (45 mg.) as crystals with m.p. 163°. Smith[15] records m.p. 163°.

Fraction IV yielded crystalline 3,6-di-*O*-methyl-D-glucose (0.091 g.) with m.p. 113°, $[\alpha]_D$ +63° (*c*, 1.0, methanol). Percival and Duff[16] record m.p. 113–116°, $[\alpha]_D$ +61.5° (water). The compound (35 mg.) gave the phenylosazone as crystals (40 mg.) with m.p. 152°.

Periodate Oxidation.—Aqueous sodium metaperiodate, 0.3 *M*, (10 ml.) was added to the polysaccharide (*ca.* 0.1 g.) in water, the solution adjusted to 100 ml. with distilled water and stored in the dark. A blank was treated concurrently. At intervals the periodate uptake was estimated by transferring samples (5 ml.) from the oxidation mixture and from the blank into mixtures of phosphate buffer (pH 6.98; 25 ml.) and 20% potassium iodide (2 ml.), and the liberated iodine was titrated with 0.01 *N* sodium thiosulfate using starch as indicator.[17] Acid liberated during the oxidation was determined[18] by taking samples (5 ml.) from the oxidation mixture and from the blank, adding ethylene glycol (2 ml.), and, after 10 min., titrating with 0.01 *N* sodium hydroxide using methyl red screened with methylene blue as indicator. Formaldehyde was determined colorimetrically with chromatropic acid[19] using glucose as standard. The results, calculated in moles per mole of sugar unit are presented here in tabular form.

Time (hr.)	0.5	1	2	3	4
Uptake	0.34	0.52	0.70	0.74	0.78
Acid	0.06	0.07	0.09	0.10	0.12
CH_2O					
Time (hr.)		6	9	13	32
Uptake		0.85	0.90	0.91	0.95
Acid		0.14	0.14	0.15	0.15
CH_2O					nil

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In a separate experiment the polysaccharide (100 mg.) was oxidized with periodate[20] for 48 hr. as before. The excess periodate was destroyed by the addition of ethylene glycol and the solution was dialyzed against tap water for 72 hr. The nondialyzable material was recovered by concentration and hydrolyzed with N sulfuric acid. The reaction mixture was neutralized with barium carbonate, filtered, and concentrated. On chromatographic examination neither glucose nor arabinose was detected.

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An Investigation of the Hydrolysis of a Reduced 4-*O*-Methylglucuronoxylan[1]

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Uronic acid groups in an elm 4-*O*-methylglucuronoxylan were reduced by diborane without a decrease in degree of polymerization of the polymer. Partial hydrolysis of the reduced polymer gave a neutral hetero-trisaccharide fraction from which a new trisaccharide, *O*-α-4-*O*-methyl-D-glucopyranosyl(1 → 2)-*O*-β-D-xylopyranosyl(1 → 4)-D-xylopryanose, was isolated, and its crystalline phenylosazone was prepared and characterized. An authentic specimen of the new sugar was prepared from the ubiquitous aldotriouronic acid by an adaptation of the diborane procedure. An hypothesis based on conformational resistance was presented to account for the formation of the new trisaccharide and the aldotriouronic acid during partial hydrolysis of the reduced and unreduced 4-*O*-methylglucuronoxylan, respectively.

The 4-*O*-methylglucuronoxylans obtainable from most hardwoods consist of chains of 1 → 4 linked β-D-xylopyranose units with single 4-*O*-methyl-D-glucopyranosiduronic acid units attached as side chains on C-2 of the xylose units[4] as shown in Fig. 1.

Partial hydrolysis of such hemicelluloses in aqueous acid results in the formation of a polymer-homologous series of β-1–4 xylodextrins[5] and a closely related acidic series (Fig. 2). The linkage (α-1–2) between E and B, Fig. 1, is especially resistant, and the amorphous aldobiouronic acid, therefore, is the chief acidic product of the acid hydrolysis of these polymers. The crystalline aldotriouronic acid (EBC)[6,7] is the second most abundant product in the acidic series, but all efforts to find the isomeric acid (ABE) have failed. To account for these facts Hamilton and Thompson[6] suggested that the uronic acid carboxyl "stabilized" the linkages B–E and B–C through an inductive effect. Marchessault and Rånby[8] supported the stabilization hypothesis,[9,10] and further suggested that, simultaneously, the linkage A–B was "activated."

In order to test these hypotheses, the carboxyl groups in a 4-*O*-methylglucuronoxylan, isolated from American elm sapwood (*Ulmus americana*), were reduced to primary hydroxyl groups.[11] Then upon partial hydrolysis of the 4-*O*-methylglucoxylan in dilute aqueous acid, the products of the reduced and the unreduced polymers were compared. As expected, the 4-*O*-methylglucoxylan afforded two series of neutral, reducing oligosaccharides as shown in Fig. 2.

The hetero-trisaccharide component of the hydrolyzate was isolated by a gradient elution technique on a carbon–Celite column[12] followed by preparative paper chromatography. Although the trisaccharide

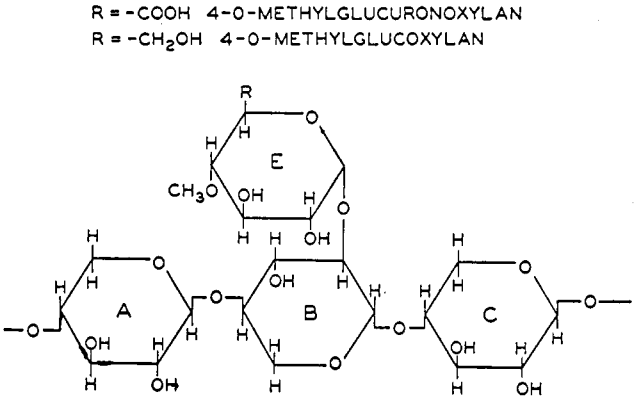

Fig. 1.—Principal linkages in 4-*O*-methylglucuronoxylans.

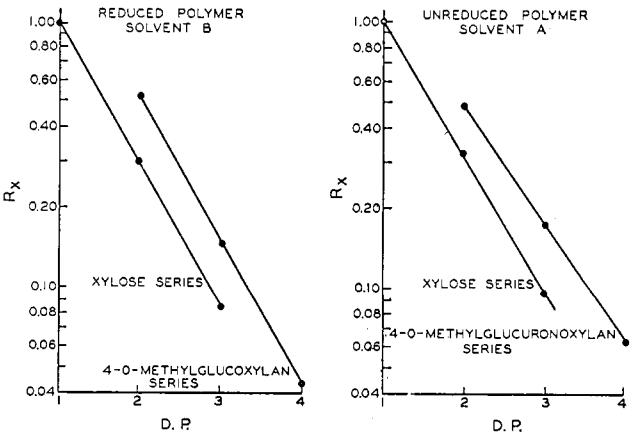

Fig. 2.—log R_x *vs.* D.P. for saccharides from the partial acid hydrolysis of reduced and unreduced 4-*O*-methylglucuronoxylan.

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