amount of undialyzed material being determined by evaporating and drying the contents of the dialysis sac. Originally 80% of the stage II lignosulfonic acid failed to dialyze under these conditions; after the first oxidation this value was 40%, and only 15% after the fourth.

A Crude Calcium Oxylignosulfonate.—Nitrogen gas was bubbled through 30 cc. of a clear, centrifuged aqueous solution of the oxidized lignosulfonic acid until any chlorine dioxide had been expelled. The solution was shaken with small increments of calcium hydroxide until *p*H 4.5 was attained, and at this acidity the precipitated calcium sulfate was removed. After the clear mother liquor had been concentrated, the addition of 10 volumes of ethanol caused the precipitation of the crude product.

Anal. Found: S, 3.1, 3.2; OCH_3, 3.9, 3.8; Cl, 6.6, 7.0; Ca, 13.3. (When corrected for 4% of calcium sulfate, the analysis became: S, 2.1; OCH_3, 3.7; Cl, 6.7; Ca, 12.1 %.)

One gram of the crude calcium oxylignosulfonate was kept dispersed for one week in 150 cc. of water containing 7.5 g. of sodium hydroxide and 1.5 g. of sodium carbonate, and the suspension was then adjusted from *p*H 11.8 to *p*H 7 with glacial acetic acid. The residue obtained by evaporating the suspension was extracted with ethanol, was dissolved in 15 cc. of water and was reprecipitated with ethanol. After a second reprecipitation, the powder was dried by treatment with alcohol, then benzene, and finally *in vacuo* over phosphorus pentoxide; yield 0.82 g. or 82%.

Anal. Found: S, 2.7; Cl, 0.6; Ca, 22.5, 22.4. Of the sulfur, 0.8% was present as free sulfate ion and 1.9% was combined; the apparent calcium content included a little sodium.

The replacement of the sodium hydroxide–sodium carbonate buffer with saturated calcium hydroxide solution yielded a product with 1.8% of combined sulfur and only 1.4% of chlorine.

Fractionation of a Zinc Oxylignosulfonate.—Zinc oxide, 5 g., was stirred with 200 cc. of a solution containing 3.36 g. of oxylignosulfonic acid, and free of chlorine dioxide, until the *p*H was 4.9. The oxylignosulfonic acid had 1.2% of combined sulfur and 1.4% was present as sulfate ion; that is, 40.3 and 47.0 mg., respectively. Next day the excess of zinc oxide, 0.32 g., was removed on the centrifuge; the clear liquor was concentrated to about 50 cc. and was poured into 200 cc. of ethanol. The precipitate of zinc "oxylignosulfonate" was washed twice with 200 cc. of acetone before being dried *in vacuo*; yield 2.4 g. or 70% by weight.

Anal. Found: S, 0.9, 0.9; Cl, 4.7, 4.5; Zn, 21.8, 20.2.

The free chloride and sulfate content corresponded to the presence of 0.13 g. and 0.07 g. of the respective zinc salts. Correction for these impurities gave the composition of the zinc oxylignosulfonate as S, 0.0; Cl, 3.6; and Zn, 19.3.

The ethanolic mother liquors and the acetone washings, when mixed, deposited a second fraction which was recovered and dried; yield 0.71 g. or 22%.

Anal. Found: S, 0.5; Cl, 6.2; Zn, 23 (cor. for 1% of zinc sulfate and 10% of zinc chloride).

The analyses of aliquots showed that the mother liquors contained 45 mg. of sulfur as sulfate ion, or 96% of the original sulfate. The residue from these liquors was not analyzed because it was extremely deliquescent, and also exploded when attempts were made to dry it by heat. Since only 3.6 mg. of combined sulfur had been recovered in the second fraction of zinc oxylignosulfonate, the residue contained (40.3−3.6) mg., or about 37 mg. The organic material in the residue weighed about 1 g., and therefore contained about 3.7% of combined sulfur.

Isolation of a Barium Oxylignosulfonate.—A solution, 30 cc., containing 0.62 g. of oxylignosulfonic acid (S, 2.9% or 18 mg.) but no chlorine dioxide, was mixed with 10 cc. of cold, redistilled 47% hydriodic acid to reduce the chloric acid present. After extracting the liberated iodine with ether, the aqueous solution was shaken with moist silver carbonate, freshly prepared from 11 g. of the nitrate and 5.5 g. of potassium carbonate. The insoluble silver salts were removed on the centrifuge, and were extracted with dilute hydrochloric acid. Since the extract gave no precipitate with aqueous barium chloride, the salts included no sulfate. The supernatant solution, now containing silver oxylignosulfonate and silver sulfate, was mixed with an exact equivalent of dilute hydrochloric acid, and the precipitated silver chloride removed.

Concentrated aqueous barium hydroxide was added to the resulting solution of free oxylignosulfonic and sulfuric acids until the acidity was reduced to *p*H 4.8. The precipitated barium sulfate was removed and after ignition weighed 46.5 mg., corresponding to 6.4 mg. of sulfur or to 35% of the original amount. After concentrating the mother liquor to 5 cc., the barium lignosulfonate was precipitated by the addition of 50 ml. of ethanol, and was dried *in vacuo*; yield 0.45 g.

Anal. Found: S, 1.7; Ba, 36.6.

The sulfur content, 7.6 mg., was 42% of the original amount. Evaporation of the alcoholic filtrate to dryness left 0.67 g. of salts.

Anal. Found: Ba, 43.4; S, 0.2. The sulfur content corresponded to 1.3 mg. (7%).

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[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH COUNCIL OF CANADA]

Uronic Acid Components of Jute Fiber Hemicellulose[1,2]

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Jute fiber hemicellulose produces upon partial hydrolysis a number of neutral and acidic sugars and oligosaccharides. The acidic components isolated by ion exchange and cellulose-column chromatography have been identified and shown to be 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose and, tentatively, *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.

The jute plant (*Corchorus olitorius* and *C. capsularis*) gives, after a proper treatment of retting, long glossy fibers which are used for making burlap and sacking materials. Associated with cellulose, the jute fiber has a considerable quantity of hemicellulose (about 30%).[4] The structure of this hemicellulose has been studied by Sarkar, *et al.*[5−7] On the basis of periodate oxidation data

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(2) A preliminary report of this paper has appeared in *Chemistry & Industry*, 920 (1958).

(3) Postdoctorate Fellow of the National Research Council.

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and chromatographic evidence these authors concluded that the jute fiber hemicellulose was composed of a chain of 1,4′-linked D-xylopyranose residues and that to C_3 of every sixth D-xylose unit there was attached a residue of 3-*O*-methyl-D-glucuronic acid. They also claimed to have isolated an aldobiouronic acid to which was assigned the structure 3-*O*-(3-*O*-methyl-D-glucuronopyranosyl)-D-xylose. The aldobiouronic acid component of many wood and cereal hemicelluloses has been identified as 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose.[8-20] The identification of 3-*O*-methyl-D-glucuronic acid and 3-*O*-(3-*O*-methyl-D-glucuronopyranosyl)-D-xylose as components of jute fiber hemicellulose would thus indicate unique biosynthetic processes in the jute plant. A reinvestigation of the problem seemed desirable, an added reason being that the structural proofs offered by Sarkar and co-workers[5-7] for the presence of 3-*O*-methyl-D-glucuronic acid were not based upon the identification of crystalline compounds.

This paper is concerned with the isolation and identification of 4-*O*-methyl-D-glucuronic acid (I), 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose (II) and *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose (III) produced upon partial depolymerization of the jute fiber hemicellulose.

Jute fibers were hydrolyzed with 0.5 *N* sulfuric acid for 7 hr. when a mixture of neutral and acidic sugars and oligosaccharides was produced. Four acid components (I–IV) were isolated from the mixture by ion-exchange and cellulose column chromatography.

The identification of component I as 4-*O*-methyl-D-glucuronic acid is based upon the following facts. Component I, which had $[\alpha]^{25}D$ + 48° and equivalent weight 197, was converted to the methyl ester methyl glycoside derivative by boiling with methanolic hydrogen chloride and then was reduced with lithium aluminum hydride[21,22] to produce the glycoside of the neutral sugar. The glycoside gave upon acid hydrolysis 4-*O*-methyl-D-glucose which was identified by specific optical rotation, paper chromatography, paper electrophoresis and by transforming it into the corresponding phenylosazone. No 3-*O*-methyl-D-glucose was found.

The structure of the aldobiouronic acid (component II), 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose, was established in the following manner. Component II, which had $[\alpha]^{25}D$ + 97°, was converted to the methyl glycoside of the corresponding neutral disaccharide as described above. The latter gave upon hydrolysis D-xylose and 4-*O*-methyl-D-glucose. The mode of linkage between the two sugar residues was established by methylation studies. The methyl glycoside of the neutral disaccharide was methylated first with dimethyl sulfate and alkali and then with silver oxide and methyl iodide. The fully methylated disaccharide gave upon hydrolysis approximately equimolecular proportions of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose; the former was obtained in a crystalline form and the latter was characterized as 3,4-di-*O*-methyl-D-xylonolactone.[23] These data prove that in the aldobiouronic acid (II) the 4-*O*-methyl-D-glucuronic acid residue is joined by its reducing end to C_2 of the xylose moiety. The α-configuration of the biose linkage is indicated by the high positive rotation. A direct chemical proof for the configuration of the biose link in II has been provided recently by Gorin and Perlin.[24]

The aldotriouronic acid (component III), which was obtained in a crystalline form, had m.p. 180–183° and showed $[\alpha]^{25}D$ + 58°. Partial hydrolysis of the aldotriouronic acid with *N* sulfuric acid and examination of the hydrolyzate by paper chromatography and paper electrophoresis showed the presence of D-xylose and another component which had the mobility of 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose.

The aldotriouronic acid (III) was methylated in the following manner: III was converted to its corresponding methyl ester methyl glycoside derivative (V) by treatment with methanolic hydrogen chloride at room temperature. Reduction of V with lithium aluminum hydride[21,22] produced the neutral trisaccharide methyl glycoside (VI) which was methylated with methyl iodide and silver oxide to give a fully methylated trisaccharide which gave upon hydrolysis equimolecular proportions of 2,3-4,6-tetra-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-xylose and 3,4-di-*O*-methyl-D-xylose. 2,3,4,6-Tetra-*O*-methyl-D-glucose was characterized as *N*-phenyl-D-glucosylamine 2,3,4,6-tetramethyl ether,[25] 2,3-di-*O*-methyl-D-xylose as *N*-phenyl-D-xylopyranosylamine 2,3-dimethyl ether[26] and 3,4-di-*O*-methyl-D-xylose as 3,4-di-*O*-methyl-D-xylonolactone.[23] On the basis of evidence outlined above, the aldotriouronic acid (component III) is tentatively assigned the structure *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose. The X-ray diffraction pattern of component III was identical with that of the aldotriouronic acid isolated from western hemlock[17] to which was assigned the structure *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose. It may be pointed out that the data presented by Hamilton and Thompson[17] for the constitutional proof of the aldotriouronic acid did not allow the formulation of an unique structure for the com-

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pound. Further studies on the constitution of the aldotriouronic acid are now in progress in these laboratories.

The identification of 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose and *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose among the products of hydrolysis of jute hemicellulose[27] indicates that this hemicellulose bears resemblance to many other hemicelluloses especially those from western hemlock[17] and white spruce[28] which give the same three uronic acid components upon partial hydrolysis.

Experimental

Chromatographic separations were carried out by the descending method on Whatman No. 1 paper using the following solvents: (A) ethyl acetate–water–acetic acid–formic acid (20:4:3:1), (B) ethyl acetate–pyridine–water (2:1:2), (C) benzene–ethanol–water–ammonia (200:47:14:1), (D) 2-butanone–water (2:1) and (E) 2-butanone–acetic acid–water (9:2:2). The sugars and their methyl ethers were detected by *p*-anisidine hydrochloride. R_x and R_g values represent the rate of movement of sugars on paper relative to D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose, respectively.

All specific rotations are equilibrium values unless otherwise stated and all melting points are corrected.

Preparation of Uronic Acids.—Jute fiber (500 g.) was milled in a Wiley mill and then screened to give a "through" 40 mesh "on" 100 mesh fraction. This milled fraction was soaked for 16 hr. in 0.5 *N* sulfuric acid (7 l.) and the mixture was then heated with stirring on a boiling water-bath for 8 hr. The reducing power of the hydrolyzate[29] was then constant and the insoluble residue was removed by filtration. The filtrate and washings were brought to *p*H 6.0 by careful addition of barium hydroxide and barium carbonate. Barium sulfate was removed by filtration and the excess barium ions were removed during passage of the solution through a column of Amberlite IR-120 (30 × 150 mm.). The uronic acid components were then absorbed on a column of Dowex 1-X4 (30 × 355 mm., acetate form). The resin column was washed with water until free of sugars (anthrone test) and the uronic acids were displaced by 0.5 *N* formic acid. Six fractions were collected which, after drying, weighed 2.26, 8.86, 0.28, 0.11, 0.12 and 0.02 g., respectively. Chromatographic examination (solvent A) showed that all fractions were mixtures of uronic acids. The major fractions (1 and 2) each contained four components with R_x values 0.50, 0.65, 0.93 and 1.18. The latter three values corresponded to those for 4-*O*-methyl-D-glucuronic acid, an aldobiouronic acid and an aldotriouronic acid.

Separation of Uronic Acid Components.—The major uronic acid fraction 2 (5.0 g.) was separated further on a cellulose column (solvent A) into three main fractions and a fourth which was obtained in only small amounts (see Table 1). Apart from these four fractions a number of other fractions were collected, but since they were mixtures they were not examined further. Final purification of

TABLE I

Fraction number	Weight, g.	R_x value (solvent A)	$[\alpha]^{25}D$
I	0.20	1.18	+48.1°
II	1.23	0.93	+97
III	0.97	0.65	+57
IV	0.026	0.50	+49

the uronic acid components was achieved by repeated chromatography on paper using solvent A. The small amount of fraction 4 which was isolated in pure form precluded structural investigations.

Identification of 4-*O*-Methyl-D-glucuronic Acid.—Chromatographic comparison of fraction 1 with 4-*O*-methyl-D-glucuronic acid in solvent A showed that the two substances had equal rates of movement. Its neutralization equivalent (197) and methoxyl content (14.9%) agreed satisfactorily with the calculated value for $C_7H_{12}O_7$ (neut. equiv., 208; OCH_3, 14.9%). Identification of the uronic acid was made as follows. The uronic acid (167 mg.) was converted to the methyl ester methyl glycoside by heating at 80° in a sealed tube with 2.5% methanolic hydrogen chloride (7 ml.) for 8 hr. The product (168 mg.) had $[\alpha]^{25}D$ +94.5° in methanol (*c* 2). The methyl ester methyl glycoside (105 mg.) was dissolved in dry tetrahydrofuran (15 ml.) and the solution added dropwise over a period of 1 hr. to a stirred suspension of lithium aluminum hydride (200 mg.) in tetrahydrofuran (20 ml.). The reaction mixture was refluxed gently for one hour, cooled and the excess lithium aluminum hydride was decomposed by cautious successive addition of ethyl acetate and water. After filtration, concentration and deionization with Amberlite IR-4B and IR-120, the solution was evaporated *in vacuo* to yield the neutral methyl glycoside as a glassy solid (97 mg.). Hydrolysis with *N* sulfuric acid (5 ml.) by heating at 98° for 15 hr. yielded a yellow colored sirup (81 mg.), $[\alpha]^{25}D$ +55° in water (*c* 1) [lit.[30] value for 4-*O*-methyl-D-glucose +53°]. Chromatographic examination (solvent B) showed the presence of 4-*O*-methyl-D-glucose (R_x 1.14) (R_x value of 3-*O*-methyl-D-glucose, 1.2). Electrophoresis of the sirup on strips of glass paper [1] in borate buffer (*p*H 9.2) using authentic standards showed that the neutral methylated sugar derived from fraction 1 moved at the same rate as 4-*O*-methyl-D-glucose (R_g = 0.3 cm.).[32] 3-*O*-Methyl-D-glucose moved at a much faster rate (R_g = 4.4 cm.) and none could be found in the product derived from fraction 1.

Identification of 4-*O*-methyl-D-glucose was provided by preparation of its crystalline phenylosazone derivative, m.p. and mixed m.p. 159–160° (after recrystallization from 30% aqueous acetone), $[\alpha]^{25}D$ −33° (initial) → 0.0° (24 hr.).[33,34]

4-*O*-Methyl-D-glucuronic acid failed to give a crystalline *p*-nitro-*N*-phenylglycosylamine.[35]

Identification of 2-*O*-(4-*O*-Methyl-α-D-glucuronopyranosyl)-D-xylose.—Fraction 2 showed $[\alpha]^{25}D$ +97° and had an equivalent weight of 309 and methoxyl content of 9.1% [calcd. for 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose, equiv. wt. 340; OCH_3, 9.1%]. Aldobiouronic acid (10 mg.) was hydrolyzed with *N* sulfuric acid in a sealed tube at 100° for 12 hr. Neutralization with barium carbonate followed by removal of the barium ions by ion exchange resin and chromatographic analysis (solvent A) showed the presence of D-xylose and 4-*O*-methyl-D-glucuronic acid.

The aldobiouronic acid (1.12 g.) was converted to the methyl glycoside of the corresponding neutral disaccharide (yield 1.28 g.) in the manner described above for 4-*O*-methyl-D-glucuronic acid.

Methyl 2-*O*-(2,3,4,6-Tetra-*O*-methyl-α-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xyloside.—The disaccharide methyl glycoside (1.16 g.) was dissolved in 20% sodium hydroxide (10 ml., w./w.) and methylation carried out by the dropwise addition of 45% sodium hydroxide (55 ml.) and dimethyl sulfate (35 ml.) over a 3-hr. period. Similar methylations were repeated twice more without recovering the product. The reaction mixture was heated at 95° for 1 hr. to decompose excess dimethyl sulfate, acidified with 2 *N* sulfuric acid (congo red indicator) and extracted continuously with chloroform for 24 hr. The partially methylated disaccharide was recovered as a clear yellow sirup (0.921 g.). An infrared spectrum analysis showed the presence of free hydroxyl groups so a further methylation with Purdie reagents (silver oxide, 6 g., methyl iodide 40 ml.) was performed. The recovered product was a colorless sirup (0.835 g.), $[\alpha]^{25}D$ +109° in chloroform (*c* 1).

(27) According to a private communication from Dr. G. O. Aspinall, our results are in conformity with the methylation studies carried out on this hemicellulose by the Edinburgh group.

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Anal. Calcd. for $C_{18}H_{32}O_{11}$: OCH_3, 51.2. Found: OCH_3, 51.6.

A solution of the methylated disaccharide (800 mg.) in 8% methanolic hydrogen chloride (20 ml.) was heated in a sealed tube at 80° for 18 hr. After removal of the methanol, 0.5 *N* hydrochloric acid (10 ml.) was added and the solution was hydrolyzed for 8 hr. at 100°. Acid was removed with silver carbonate and the solution, after deionization with Amberlite IR-120 and 1R-4B ion-exchange resins, was evaporated *in vacuo* to give a sirup (682 mg.). Chromatographic examination in solvents C and D showed two spots corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The mixture of methylated sugars was separated on an alumina column (20 × 45 mm.).[36] The tetra-*O*-methylglucose component (411 mg.) was eluted with chloroform after which the di-*O*-methylxylose (265 mg.) was displaced with methanol.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—On removal of the solvent the sugar crystallized spontaneously. Recrystallization from diethyl ether–light petroleum ether (1:1) yielded fine white needles, $[\alpha]^{25}D$ +83 ± 2° in water (*c* 1), m.p. and mixed m.p. 96°. The 2,3,4,6-tetra-*O*-methyl-D-glucose (100 mg.) in methanol (5 ml.) containing aniline (60 mg.) was refluxed for 2 hr. On partial removal of the solvent the anilide crystallized. Recrystallization from ethanol yielded 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine, m.p. 134–136°, $[\alpha]^{25}D$ +233 ± 1° in chloroform (*c* 1).[25]

Identification of 3,4-Di-*O*-methyl-D-xylose.—On chromatographic examination in solvent C this sugar had an R_g value of 0.38, identical with that of 3,4-di-*O*-methyl-D-xylose and was distinguished from 2,3-di-*O*-methyl-D-xylose (R_g 0.31) and 2,4-di-*O*-methyl-D-xylose (R_g 0.27).

Anal. Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.6.

The sugar (80 mg.) dissolved in water (2 ml.) containing barium carbonate (70 mg.) was oxidized with bromine (10 drops) in the dark for 60 hr. The bromine was removed by aeration and the solution extracted exhaustively with chloroform. The chloroform extract was evaporated *in vacuo* to give a sirup (66 mg.) which was distilled, b.p. (bath temp.) 120–160° (0.02–0.015 mm.). The distillate crystallized on standing and after recrystallization from ether yielded 3,4-di-*O*-methyl-D-xylonolactone, m.p. and mixed m.p. 66–67°, $[\alpha]^{25}D$ −22 ± 1° in water (*c* 1).[23]

Characterization of *O*-α-4-*O*-Methyl-D-glucuronopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.—The aldotriouronic acid (fraction 3) was dissolved in the minimum amount of water and to the solution a few drops of 1-butanol was added. Upon slow evaporation, the solution deposited needles which were filtered and recrystallized from aqueous methanol. The crystals had m.p. 180–183° and showed $[\alpha]^{26}D$ +58° in water (*c* 1).[17]

Anal. Calcd. for $C_{16}H_{23}O_{14}(OCH_3)\cdot{}^1/_2H_2O$: C, 42.4; H, 6.03; OCH_3, 6.43. Found: C, 42.4; H, 5.90; OCH_3, 6.34.

An X-ray diffraction pattern of the crystals was identical with that obtained from a sample of *O*-α-4-*O*-methyl-D-glucuronopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.[17]

Partial Hydrolysis of the Aldotriouronic Acid.—The aldotriouronic acid (50 mg.) was dissolved in *N* sulfuric acid (5 ml.) and the solution heated (100°) in a sealed tube for 8 hr. The hydrolyzate was neutralized with barium carbonate and after filtration the solution was deionized with Amberlite 1R-120 and then concentrated *in vacuo* to a thin sirup. Paper chromatographic analysis of the hydrolyzate using solvents A and E showed the presence of D-xylose and another component which had the same R_x as 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose. Examination of the sirup by paper electrophoresis using formate buffer, *p*H 3.5[37] (600 volts for 2.5 hr.) and sodium bicarbonate buffer (0.025 *M*)[38] (700 volts for 2.5 hr.), also indicated that the hydrolyzate was composed of D-xylose and 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose.

Methyl [*O*-α-4-*O*-Methyl-D-glucopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)]-D-xyloside.—The aldotriouronic acid (133 mg.) was converted to the methyl ester methyl glycoside derivative (151 mg.) by standing in 1% methanolic hydrogen chloride (10 ml.) at room temperature until the rotation became constant (6 hr.). Further treatment with ethereal diazomethane assured complete esterification of the carboxyl groups. Reduction with lithium aluminum hydride[21,22] in tetrahydrofuran yielded the glycoside of the neutral trisaccharide which showed no carbonyl absorption upon infrared spectrum analysis. Acid hydrolysis and quantitative chromatographic analysis[39] of the hydrolyzate showed a molar ratio of xylose:4-*O*-methyl glucose of 1.96:1.

Methyl [*O*-α-2,3,4,6-Tetra-*O*-methyl-D-glucosyl-(1 → 2)-*O*-β-3,4-di-*O*-methyl-D-xylosyl-(1 → 4)]-2,3-di-*O*-methyl-D-xyloside.—The trisaccharide methyl glycoside (151 mg.) was dissolved in methanol (2 ml.) and methyl iodide (20 ml.) was added. After addition of silver oxide (3 g.) (1 g. each hour), the mixture was heated under reflux overnight. The reaction product was recovered by filtration and evaporation of the solution *in vacuo*. After three such methylations, the partially methylated trisaccharide was completely soluble in methyl iodide. Two further methylations with methyl iodide and silver oxide gave a clear sirup (114 mg.), $[\alpha]^{25}D$ +31° in methanol (*c* 2).

Anal. Calcd. for $C_{25}H_{46}O_{14}$: OCH_3, 48.9. Found: OCH_3, 49.1.

Hydrolysis of Methyl [*O*-α-2,3,4,6-Tetra-*O*-methyl-D-glucosyl-(1 → 2)-*O*-β-3,4-di-*O*-methyl-D-xylosyl-(1 → 4)]-2,3-di-*O*-methyl-D-xyloside and Identification of Components.—The fully methylated trisaccharide (93 mg.) was heated in a sealed tube at 80° with 8% methanolic hydrogen chloride for 12 hr. Methanol was removed and hydrolysis effected by heating with 0.5*N* hydrochloric acid for 8 hr. on a boiling water-bath. The acid was removed with Amberlite 1R-45 and the methylated sugars were recovered as a sirup (88 mg.). Chromatographic examination (solvents C and D) showed the presence of three components which corresponded in color reaction and rate of movement to authentic samples of 2,3-di-*O*-methyl-D-xylose, 3,4-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The mixture of the methylated sugars was resolved on filter paper using solvent C and the components were isolated in pure form by elution of appropriate portions of the paper. In this manner 2,3,4,6-tetra-*O*-methyl-D-glucose (16 mg.), 2,3-di-*O*-methyl-D-xylose (14 mg.) and 3,4-di-*O*-methyl-D-xylose (12 mg.) were obtained.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—The sirupy tetra-*O*-methyl-D-glucose was refluxed with ethanolic aniline to afford *N*-phenyl-D-glucosylamine 2,3,4,6-tetramethyl ether, m.p. and mixed m.p. 134–135°, $[\alpha]^{25}D$ +243° in acetone (*c* 0.7) (after recrystallization from ether).[25]

Identification of 2,3-Di-*O*-methyl-D-xylose.—The 2,3-di-*O*-methyl-D-xylose component, which was obtained as a sirup, showed $[\alpha]^{25}D$ +21° in methanol (*c* 1).

Anal. Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.2.

The sirup (10 mg.) was refluxed for 2 hr. with aniline (12 mg.) and methanol (2 ml.). On removal of the solvent, the anilide crystallized and was recrystallized from ethyl acetate. The *N*-phenyl-D-xylopyranosylamine-2,3-dimethyl ether had m.p. and mixed m.p. 123–124° and showed $[\alpha]^{25}D$ +188° in ethyl acetate (*c* 0.4).[26]

Identification of 3,4-Di-*O*-methyl-D-xylose.—The chromatographically pure 3,4-di-*O*-methyl-D-xylose component had $[\alpha]^{25}D$ + 25° in methanol (*c* 1).

Anal. Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.4.

It was transformed into 3,4-di-*O*-methyl-D-xylonolactone, as described previously, which had m.p. and mixed m.p. 66–67° and showed $[\alpha]^{25}D$ −23° (initial) in water (*c* 1) (after recrystallization from ether).[23]

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